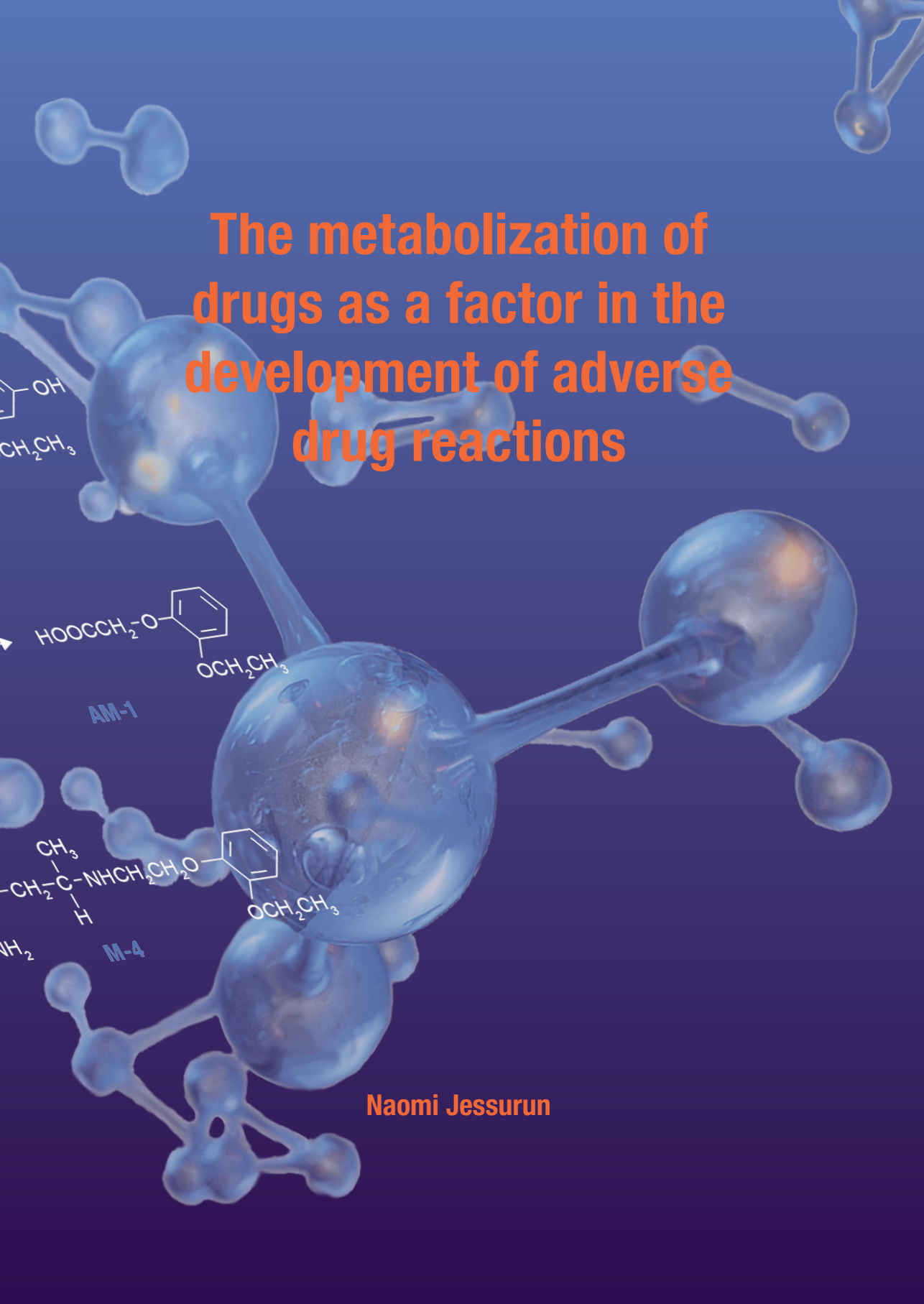
The metabolization of drugs as a factor in the development of adverse drug reactions



Naomi Jessurun

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The metabolization of drugs as a factor in the development of adverse drug reactions

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Introduction

PART I

Chapter 1

General introduction

General introduction

Mrs V. (76 years old) is suffering from severe depressive symptoms, so she is treated with 75 mg nortriptyline once daily. The therapeutic range to achieve the desired effect of nortriptyline is from 50 to 150 microgram per litre (µg/l). Mrs V.'s serum level is, however, 32 µg/l and thus below the therapeutic level. To improve the outcomes of her pharmacotherapy, an increased dosage of nortriptyline is indicated. However, in Mrs V.'s case, the enzymes that metabolize the nortriptyline and enhance the elimination of the drug have a high metabolic activity and fast action. Notably, the serum level of one of the drug's metabolites, hydroxynortriptyline, is already 351 µg/l, above the 200 µg/l upper level allowed, putting her at risk of prolonged QTc interval and other adverse drug reactions. In accordance with current guidelines, she should discontinue this treatment regime by withdrawing the nortriptyline. As there are no other treatment options for Mrs V, this is undesirable.

We often assume that the original active substance, the parent drug, is responsible for both the effectiveness and adverse drug reactions. However, after ingestion, the parent drug undergoes chemical changes and drug metabolites are formed. These metabolites may have a different pharmacokinetic and pharmacodynamic profile and may cause effects that are wrongly attributed to the original compound.[1] Although these metabolites sometimes add to the intended pharmacological effect – for example, morphine is a metabolite of codeine with a 200 times higher affinity for the intended opioid receptor – they usually cause unwanted adverse effects. The formation of drug metabolites is influenced by intrinsic factors like age, sex, and variations in genes encoding the drug-metabolizing enzymes, as well as by extrinsic factors such as concomitant drug use and smoking.[2,3] Adverse drug reactions induced by drug metabolites are further determined by their chemical characteristics and their concentrations in various body compartments and fluids.[4] Figure 1.1 shows a proposed diagram of drug metabolization, the formation of drug metabolites and the mechanisms of the adverse drug reactions. Despite the importance of drug metabolites in the development of adverse drug reactions, little attention has been paid to their role during post-marketing drug research and pharmacovigilance activities. This results in a gap of knowledge in this field. And if knowledge is available, it is fragmented across different disciplines and underutilized.[5]
Considering the role of drug metabolites in adverse drug reactions will provide us with more options for tailoring drug use to achieve the optimal treatment for a specific individual patient, with the ultimate aim of reducing, as much as possible, the burden of adverse drug reactions and promoting the safe use of drugs.

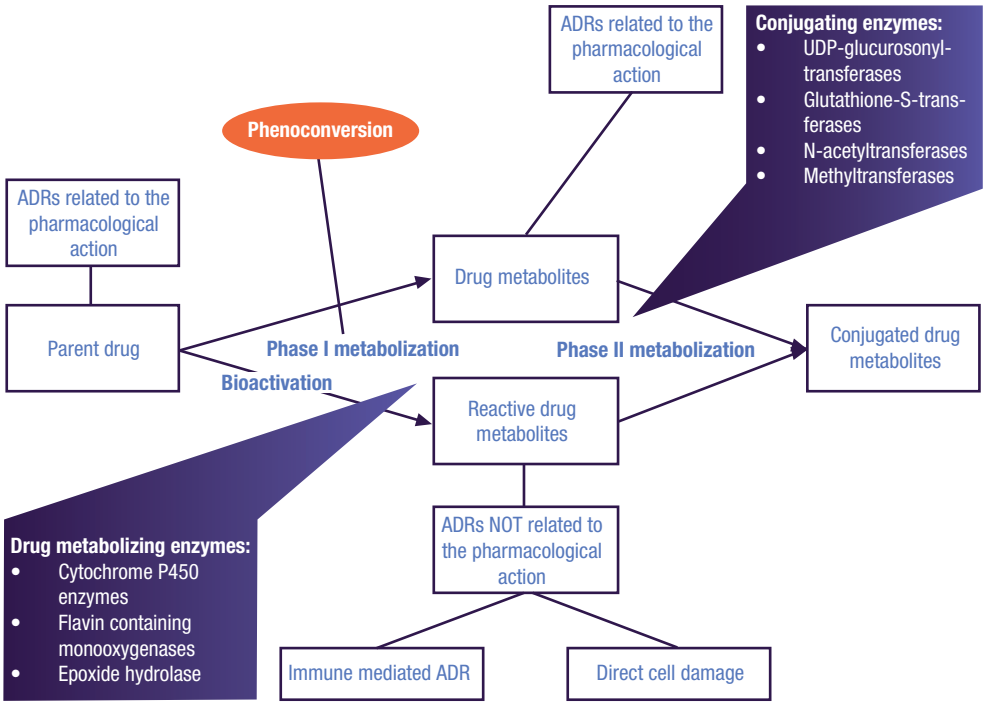

Figure 1.1 Conceptual diagram of drug metabolisation, drug metabolites and adverse drug reactions. ADRs = adverse drug reactions, UDP = uridine diphosphate.

Using information from drug development for pharmacovigilance purposes

Pharmaceutical companies and regulatory agencies do assess safety issues concerning drug metabolization and drug metabolites before new drugs are approved for marketing. These safety evaluations include genetic toxicology assessments, and screenings for the induction or inhibition of cytochrome P450 enzymes. Moreover, assays are performed to evaluate a compounds' potential to undergo bioactivation, with the aim of minimizing reactive metabolite formation, under the assumption that reactive metabolites increase the risk of certain toxicities. Furthermore, new drug molecules are assessed for structure-toxicity relationships, taking into account all the sites for metabolization in the molecule and other metabolization pathways.[6] The wealth of information on metabolites thus generated is, however, not often used for post-marketing pharmacovigilance, even though they may provide useful insights. Handoko *et al.*, for example, showed that anticonvulsants with a particular chemical structure, in this case the aromatic ring, are associated twice as frequently with hypersensitivity reactions than drugs without this chemical structure. The metabolization of this moiety to reactive metabolites and subsequent covalent binding to proteins is indicated as the main potential cause.[7]

Data sources to assess the role of drug metabolization and drug metabolites in adverse drug reactions

The cornerstone of pharmacovigilance and post-marketing surveillance is the spontaneous reporting system that relies on the motivation of patients and healthcare professionals to report adverse drug reactions to national and international drug authorities and to marketing authorisation holders.[8] The discovery of previously unknown, rare, adverse drug reactions has long relied on analysis of these reported cases.[8] Spontaneous reports are excellent for generating hypotheses and signals of previously unknown and rare adverse drug reactions, but when it comes to elucidating the role of drug metabolization and drug metabolites in adverse drug reactions, they often lack the required clinical information, such as laboratory data and data on therapeutic drug monitoring. Other data sources may be more suitable to assess these relationships.

Not all data sources will be appropriate to assess the association between drug metabolization, drug metabolites and adverse drug reactions, as specific information is required. Available post-marketing data sources include risk management plans (RMPs), patient registries, electronic health records and biobanks, each with their own advantages and disadvantages. Although not fully publicly accessible, RMPs should, for instance, include a summary of information on significant clinical and non-clinical safety findings regarding toxicity and pharmacology. These may be used to interpret findings described in reports on adverse drug reactions or to focus on adverse drug reactions that may be expected. Information to obtain a better idea of drug metabolization and drug metabolites might be provided by patient registries, electronic health records and biobanks, although not in a structured form or readily available. The importance of data collection on drug metabolization and drug metabolites for the assessment of possible associated adverse drug reactions is acknowledged in the drug development and lead optimization phases, whereas after marketing authorization, data are usually not collected for this purpose. To elucidate and confirm these associations, available data sources should be explored and their appropriateness should be assessed.

Harm-preventing interventions during drug development

The importance of clarity concerning the relationship between drug metabolization, drug metabolites and adverse drug reactions lies in the possibilities to design measures to avert drug toxicity and enhance the safe use of drugs.

In the drug development phase, several kinds of interventions, based on knowledge about drug metabolization and the formation of drug metabolites, are available to improve the safety of drugs. Two of them are adjusting the chemical structure by adding chemical moieties that, for example, release protective mediators, and

inserting chemical structures to slow down metabolic processes that lead to toxicity.[9,10] The latter intervention is exemplified by the addition of just one methyl group to the hepatotoxic ibufenac (see Figure 1.2), which was withdrawn from the market shortly after release. This resulted in the much safer and still used ibuprofen.[11-14] There are, however, more examples where knowledge about metabolizing pathways and drug metabolites has contributed to drug safety. For example, a minor adjustment of the chemical structure of sudoxicam and alpidem to slow down an unwanted metabolization pathway resulted in meloxicam and zolpidem, which are both less toxic than their predecessors and are still used in clinical practice.[3]

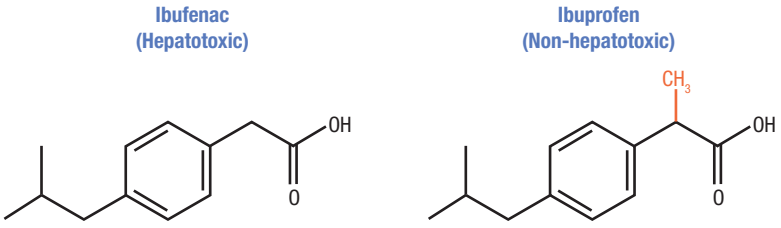

Figure 1.2 The introduction of a methyl group (-CH_3) to ibufenac slows down acyl glucuronide formation, which explains the difference in toxicity between the two drugs.[11]

Harm-preventing interventions after marketing authorization

In clinical practice, several measures are employed to enhance the safe use of drugs. Even the addition of another drug is well accepted, such as the additional use of proton pump inhibitors by NSAID users at risk of gastro-intestinal bleedings, and the addition of folinic acid and folic acid to methotrexate therapy.[15,16] The introduction of intentional drug-drug interactions to intervene in the metabolization pathways of drugs is also one of the measures to, on the one hand, improve drug effectiveness and, on the other, reduce adverse drug reactions. An example is the addition of ritonavir to lopinavir (Kaletra®, 2000 USA).[17] The addition of low dose ritonavir inhibits the metabolization of lopinavir and results in increased and sustained lopinavir serum drug levels.

The most promising intervention concerning drug metabolization, the formation of drug metabolites and the prevention of adverse drug reactions is phenoconversion (see Figure 1.1). When adverse drug reactions are a result of skewed drug metabolism and there is excess formation of unwanted drug metabolites, the introduction of an intentional drug-drug interaction to temporarily phenoconvert drug metabolizing enzyme activity to normal drug metabolism is one of the options to improve the outcome of the treatment.[18,19,20] Co-prescription of allopurinol, a xanthine oxidase inhibitor, to patients using thiopurine who exhibit thiopurine hypermethylation

reduces the number of adverse drug reactions and improves the outcome of the therapy.[18,21]
Intervening in metabolization pathways to optimize drug treatment and to increase the formation of the preferred drug metabolite, or to prevent the formation of less active or more toxic drug metabolites, offers a range of treatment and research options with the ultimate goal to find better and more personalized treatment. This is, however, currently not widely applied and not fully researched.

Aims and outline of the thesis

Approval of drugs for the market is always accompanied by uncertainties, as pre-approval studies involve limited numbers of patients, cover a short follow-up period, and apply strict inclusion criteria. This results in uncertainties regarding rare and long-term adverse drug reactions, as well as uncertainties regarding the benefit-risk profile in patients with specific characteristics. Data collection and pharmacovigilance activities after marketing authorization are a prerequisite to complete the adverse drug reaction profiles of drugs.
The goals of pharmacovigilance are to identify new information about hazardous associations with medicines and to prevent harm to patients treated with drugs in clinical practice. Considering drug metabolization and drug metabolites in observed adverse drug reactions represents a valuable addition to the field.
Four steps can be identified in pharmacovigilance research: detection of a possible association between the drug used and the observed adverse reaction, confirmation of this association, quantitative assessment of the possible association and preventing the occurrence of the adverse drug reactions.[22] These four steps are also applicable when assessing the role of drug metabolization and drug metabolites in adverse drug reactions.

Aims of the thesis

The aims of the research underlying this thesis were:

1. to further extend our knowledge about the role of drug metabolization and drug metabolites in adverse drug reactions using information from drug development,
2. to explore available data sources after marketing authorization to assess the role of drug metabolization and drug metabolites in adverse drug reactions, and
3. to investigate phenoconversion as a method to avert metabolite-induced adverse drug reactions.

Outline of the thesis

This thesis describes seven studies, divided over three parts, followed by a general discussion chapter presenting the main findings, recommendations for pharmacovigilance, clinical practice, and future research.

PART II:

To further extend our knowledge about the role of drug metabolization and drug metabolites in adverse drug reactions using information from drug development

Although the role of drug metabolization and drug metabolites in adverse drug reactions is acknowledged, it is often hard to link knowledge from drug development with clinical observations. **Part II** discusses three studies that investigated whether drug metabolizing enzymes and the formation of drug metabolites are able to explain observed adverse drug reactions. The study discussed in chapter 2.1 compared reported suspected metabolite-associated hepatoxicity in NSAID users with hemorrhage, an adverse reaction to NSAIDs that is not associated with the formation of reactive metabolites. Chapter 2.2 assesses the role of relevant enzymes in the metabolization of tamsulosin and suspected tamsulosin-associated interstitial lung disease. Chapter 2.3 discusses a literature review to explore the role of pharmacogenetics in drug metabolization and cytotoxic mechanisms that may lead to interstitial lung disease (ILD).

PART III:

To explore data sources after marketing authorization to assess the role of drug metabolization and drug metabolites in adverse drug reactions

Part III presents two studies that explored the association between drug metabolites and known adverse drug reactions. Chapter 3.1 tests the hypothesis that norclozapine plays a role in body weight gain, using data collected from patients of a clozapine outpatient clinic. Chapter 3.2 assesses the role of variations in genes encoding drug metabolizing enzymes and drug transporters, as well as the concomitant use of other drugs, in simvastatin-associated pulmonary toxicity, using data collected in a biobank of an ILD centre of excellence.

PART IV:

To investigate phenoconversion as a method to avert metabolite-induced adverse drug reactions

After the role of drug metabolites in specific adverse drug reactions has been confirmed, clinical measures are applied to avert the metabolite-induced toxicity. **Part IV** describes the prevention of the formation of an unfavorable drug metabolite

in patients with a disbalance regarding a pharmacologically active parent compound and an unwanted drug metabolite. Chapter 4.1 assesses the introduction in routine practice of an intentional drug-drug interaction to achieve phenoconversion, reducing the metabolic activity of CYP2D6 by adding low-dose paroxetine to nortriptyline treatment in patients with high hydroxynortriptyline serum levels. This study was succeeded by a prospective pharmacokinetic study discussed in chapter 4.2.

PART V:

Part V, the general discussion, presents and discusses the main findings presented in this thesis, followed by recommendations for pharmacovigilance activities, clinical practice, and future research.

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Drug metabolization, drug metabolites, and adverse drug reactions

PART II

Chapter 2.1

Relationship between structural alerts in NSAIDs and idiosyncratic hepatotoxicity: an analysis of spontaneous report data from the WHO database

Naomi T. Jessurun, Eugène P. van Puijenbroek

Drug Safety 2015;38(5):511-515

Abstract

Background
Idiosyncratic drug reactions such as hepatotoxicity and blood dyscrasias represent one of the major causes of drug withdrawal from the market. According to the reactive metabolite (RM) concept, this may be due to the metabolic activation of structural alerts (SAs), functionalities in the drug molecule that are susceptible to bioactivation resulting in RMs. The relationship, however, between metabolic activation of SAs in drugs with in vivo toxicity measured as disproportionate reporting of adverse drug reactions (ADRs) to the WHO VigiBase™ database has never been studied.

Objective
The objective of this study was to investigate whether reported associations of hepatotoxicity between NSAIDs with SAs and NSAIDs with mitigated SAs are disproportionately present in the ADR reporting VigiBase™ database of the WHO collaborating center (the Uppsala Monitoring Centre). The extent of disproportionality of these associations is compared with associations of NSAIDs and hemorrhage, an ADR not associated with the forming of RMs.

Methods
We calculated the reporting odds ratios for five NSAIDs [bromfenac (withdrawn), lumiracoxib (withdrawn), diclofenac, ibuprofen, and naproxen] associated with the MedDRA preferred terms: hepatic failure, hepatic function abnormal, hepatic necrosis, and hepatitis. The disproportionality of the association of these ADRs is compared with the preferred term hemorrhage.

Results
The results show that hepatotoxicity is more disproportionately reported in the WHO database for NSAIDs with SAs (bromfenac, lumiracoxib, diclofenac) than for NSAIDs where SAs are mitigated (ibuprofen and naproxen). This difference in reporting between NSAIDs with SAs and with mitigated SAs is not observed for the ADR hemorrhage, an ADR not associated with the forming of RMs.

Conclusions
This study shows that although spontaneous reports have many limitations, the findings are in line with previous research on the reactive metabolite concept. Whether SAs and the number of SAs in the NSAIDs actually play a role in the observed hepatotoxicity must be investigated in future studies.

Introduction

Adverse drug reactions (ADRs) are the most common cause of pharmaceutical product recalls and labeling changes. They are categorized as predictable and unpredictable (idiosyncratic) reactions. Idiosyncratic ADRs cannot be explained by the known pharmacology of the drug and although they are dose dependent in susceptible individuals, they can occur at any dose within the usual therapeutic range. Certain ADRs are not recognized as potential medical problems prior to approval due to the insufficient number of patients in clinical trials as the incidence rate can be extremely low.[1] Some drugs are known to elicit ADRs prior to metabolism. However, most drugs that elicit an ADR are first metabolized to proximate and ultimate toxic species, a process referred to as metabolic activation or bioactivation.[1,2]

It is generally thought that reactive, electrophilic compounds, formed either from the parent drug (e.g., a reactive quinone-imine from paracetamol) or as a consequence of increased cellular production of reactive oxygen and and/or nitrogen species (hydroxyl radical, superoxide, and peroxynitrite) are responsible for initiating toxicity.[3,4]

The NSAID bromfenac was withdrawn in 1998 after less than a year on the market. The US FDA received 20 reports of serious hepatotoxicity; of these reports, four patients died of liver failure and eight required liver transplants.[5] Bromfenac possesses arylacetic acid, aniline, and bromobenzene motifs that through enzymatic activation processes can undergo bioactivation to reactive epoxides, quinone metabolites, reactive nitroso compounds, acyl glucuronides and acyl-coenzyme A (CoA) thioesters.[6]

NSAIDs are a widely used drug class and a major class of drugs associated with toxicity. Depending on the NSAID structure, both cytochrome P450 (CYP)-dependent and glucuronosyltransferase-dependent metabolic pathways may be involved in the formation of metabolites that can react with proteins.[7]

Although the chemical structures of NSAIDs differ considerably, many of them contain arylacetic acid, 2-arylpropionic acid, or anthranilic acid derivatives. A number of examples of metabolic activation of carboxylic acid drugs have been documented that may serve as circumstantial evidence with regard to the toxicological relevance of acyl glucuronides and acyl-CoA thioesters.[3,8,9]

Lumiracoxib is an arylacetic acid derivative whose metabolism in humans is mostly catalyzed by CYP enzymes.[10] Hydroxylumiracoxib, the major circulating metabolite of lumiracoxib in humans, is oxidized to a reactive quinoneimine intermediate in human liver microsomes that can be trapped by glutathione.[11,12] Lumiracoxib is structurally related to diclofenac, a drug itself known to induce a rare but severe hepatotoxicity in exposed patients. Diclofenac undergoes CYP-catalyzed hydroxylation at the 4' and 5' positions, the products of which are also oxidized to reactive quinoneimine intermediates and characterized as their corresponding thiol adducts in humans.[13]

Carboxylic acids with a methyl substitution at the α-carbon of the arylacetic group, such as in ibuprofen and naproxen, exhibit lower reactivity with protein nucleophiles probably due to steric hindrance; these two drugs belong to the safest NSAIDs.[14] Although there are countless examples of drugs that are hepatotoxic or cause idiosyncratic drug toxicity for which bioactivation pathways are described, not all drugs possessing functionalities susceptible to bioactivation are bioactivated and, in addition, not all drugs that are bioactivated lead to toxicity.[1]

The careful use of structural alerts (SAs) within new chemical entities is one approach to minimize drug-induced toxicity; minimizing body burden is another. Drugs containing SAs might be considered safer if the dose does not exceed 100 mg/day.[14] Drug-induced hepatotoxicity and drug-induced autoimmune disease are more frequently associated with compounds administered at a high daily dose: for two compounds possessing the same SA, it is frequently the case that the low-dose compound will not cause toxicity, whereas a higher-dose compound will.[1]

The WHO Global Individual Case Safety Report (ICSR) database, VigiBase™, contains over 8.5 million spontaneously reported ADRs.[15] Although limited details about each suspected adverse reaction are sent, this is the largest database of spontaneously reported drug toxicity. Despite the evidence that metabolic activation of SAs leads to ADRs manifested as in vivo toxicity being well established[4], the reactive metabolite (RM) concept has never been linked to in vivo toxicity measured as spontaneously reported ADRs reported to the WHO database.

The objective of this research was to study whether reported associations of hepatotoxicity between NSAIDs with SAs (the bromobenzene ring, the arylacetic acid group, and the aniline ring) and NSAIDs with mitigated SAs (introduction of a methyl group on the α-C-atom in the arylacetic acid group) are disproportionally present in the ADR reporting VigiBase™ database of the WHO collaborating center [the Uppsala Monitoring Centre (UMC)]. The extent of disproportionality of these associations is compared with associations between NSAIDs and hemorrhage, an ADR not associated with the formation of RMs.

Methods

Study data were obtained from the WHO Global ICSR database, VigiBase™, which is maintained by the UMC. As of May 2014, this database contained over 8.5 million case reports of suspected ADRs.[15] For this study, all suspected ADRs reported to VigiBase™ were taken into account. To study the relationship between SAs in NSAIDs we determined the crude reporting odds ratios (RORs) for five NSAIDs (bromfenac, lumiracoxib, diclofenac, ibuprofen, and naproxen) associated with four *Medical Dictionary for Regulatory Activities* (MedDRA®) Preferred Terms (PTs) for hepatotoxicity: hepatic

failure, hepatic function abnormal, hepatic necrosis, and hepatitis. Choices for PTs are based on expert opinion. Three NSAIDs (bromfenac, lumiracoxib, diclofenac) contain SAs, whereas in ibuprofen and naproxen the arylacetic SA is mitigated. The strength of the association of these ADRs is compared with the crude RORs for the same five NSAIDs and the PT hemorrhage, an ADR not associated with the forming of RMs. The chemical structures, SAs, and daily dose of the NSAIDs are summarized in Figure 2.1.1.

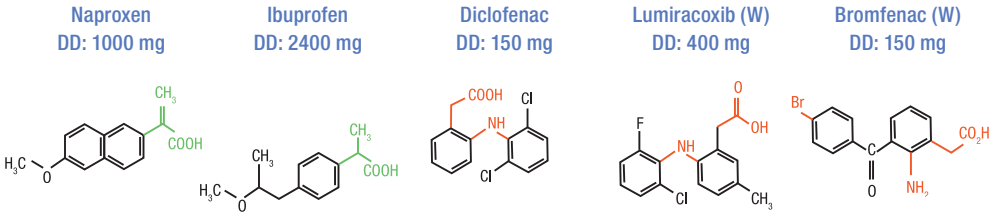

Figure 2.1.1 Researched NSAIDs, structural alerts, and daily dose. Structural features *orange* are chemical structures within the drug molecules prone to be bioactivated into reactive features and finally reactive metabolites. Structural features *green* are mitigated structural alerts that are less prone to be bioactivated into reactive features and reactive metabolites. DD = daily dose, W = withdrawn.

Results

Based on the reported ADRs in VigiBase™, the WHO database, associations with NSAIDs with SAs (bromfenac, lumiracoxib, diclofenac) seem to be reported more disproportionately in three out of four PTs representing hepatotoxicity than in drugs with mitigated SAs (ibuprofen and naproxen). This difference in disproportionate reporting is not observed for the ADR hemorrhage, which is not associated with the forming of RMs (see Figure 2.1.2). NSAIDs, PTs, and the RORs of the separate associations are shown in Table 2.1.1.

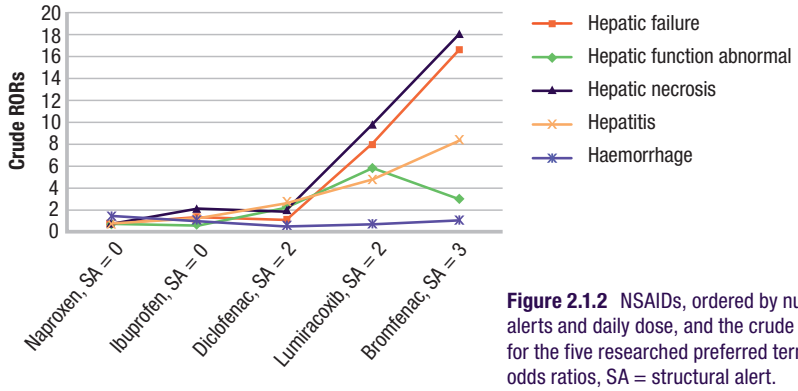

Figure 2.1.2 NSAIDs, ordered by number of structural alerts and daily dose, and the crude reporting odds ratio for the five researched preferred terms. RORs = reporting odds ratios, SA = structural alert.

Table 2.1.1 Number of reports and crude reporting odds ratios for the studied preferred terms.

NSAID	Hepatic failure	Hepatic function abnormal	Hepatic necrosis	Hepatitis	Hemorrhage
Naproxen	51 (0.61 [0.5–0.8])	169 (0.59 [0.5–0.7])	21 (1.00 [0.7–1.5])	177 (0.73 [0.63–0.85])	272 (1.29 [1.15–1.46])
Ibuprofen	114 (1.2 [1.0–1.5])	203 (0.63 [0.6–0.7])	50 (2.1 [1.6–2.8])	304 (1.13 [1.0–1.3])	252 (0.95 [0.83–1.08])
Diclofenac	95 (1.11 [0.91–1.36])	672 (2.4 [2.2–2.6])	47 (2.2 [1.7–3.0])	623 (2.6 [2.4–2.8])	146 (0.67 [0.57–0.79])
Lumiracoxib (Withdrawn)	13 (8.10 [4.7–14.0])	31 (5.78 [4.0–8.3])	4 (9.86 [3.7–26.3])	22 (4.80 [3.1–7.3])	3 (0.73 [0.24–2.28])
Bromfenac (Withdrawn)	58 (16.7 [12.8–21.6])	35 (2.92 [2.1–4.1])	16 (18.0 [11.0–29.5])	83 (8.4 [6.7–10.4])	10 (1.11 [0.6–2.07])

Data are presented as *n* (ROR [95 % CI]). ROR = reporting odds ratio.

Discussion

The outcomes of this study show a difference in disproportionality in reporting of hepatotoxicity between NSAIDs with SAs and NSAIDs with mitigated SAs in the WHO database, which is in line with the RM concept. Since the number of reported adverse reactions says not that much about the risk of a certain association, measures of disproportionality have been developed that basically compare the numbers of reports on a certain association with the number of reports that would have been expected based on chance. Still, this may not be an accurate indication of risk. Spontaneous reports have many limitations: amongst others, they may not contain sufficient pathological information or the reports may be on patients that are highly vulnerable to adverse reactions due to their existing disease state and multiple drug therapies, any or all of which may contribute to the observed liver damage in the reports we included. In addition, ADR spontaneous reports may be subject to various forms of bias and confounding, so the correlation between reported ADRs and the suspected drug does not necessarily have to be based on a truly causal relationship. Beside these limitations, adverse event identification and reporting rates may be higher if there have been warnings about a drug ('notoriety bias') or specific surveillance recommendations.[16] The impact of the withdrawal of bromfenac and lumiracoxib from the market on the disproportionality of the associations of these drugs is unknown. On the other hand, some drugs in our study seem to have a protective effect on the studied ADRs, such as naproxen for hepatitis and diclofenac for hemorrhage, which are well-known adverse effects of NSAIDs. Expected ADRs may have a lower rate of reporting than unexpected, unlabeled serious reactions, which may lead to under-reporting of such cases to pharmacovigilance centers and, consequently, to VigiBase™.[17,18]

So far, this is one of the first linkages between the RM concept and in vivo toxicity measured as spontaneously reported ADRs in VigiBase™. In this study, the disproportionalities of the associations of NSAIDs seem to increase with the number of SAs and the daily dose for three out of four PTs representing hepatotoxicity. However, no definitive evidence on the relationship between the number of SAs and increased risks can be concluded merely based on the results of this study. A relationship between the number of SAs and the ability to cause toxicities has never been established and needs further research.

Conclusion

The outcomes of this study show that the associations between NSAIDs with SAs susceptible for bioactivation and hepatotoxicity are more disproportionally reported than the associations of NSAIDs with mitigated SAs. This difference in reporting is not observed in the reporting of hemorrhage, an ADR not related to the forming of RMs. Additionally, the outcomes show that although spontaneous reports have many limitations, the outcomes are as expected with regard to previous studies on the RM concept. Whether SAs and the number of SAs in the NSAIDs actually play a role in the observed hepatotoxicity must be investigated in future studies.

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Chapter 2.2

Tamsulosin associated with interstitial lung damage in CYP2D6 variant alleles carriers

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Abstract

Background
Drugs are serious but underestimated causative agents of interstitial lung disease (ILD). Both cytotoxic and immune mechanisms may be involved in drug-induced ILD (DI-ILD).

Objective
We aimed to investigate whether polymorphisms of relevant CYP enzymes involved in the metabolization of tamsulosin might explain the pathologic mechanism of the DI-ILD in the cases with suspected tamsulosin DI-ILD.

Methods
We collected 22 tamsulosin-associated DI-ILD cases at two ILD Expertise Centers in the Netherlands between 2009 and 2020. CYP2D6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 single nucleotide polymorphisms were genotyped and compared with a control group of 78 healthy Caucasian male volunteers.

Results
Nine cases were phenotyped as CYP2D6 poor metabolizers and 13 as CYP2D6 intermediate metabolizers. The phenotypes of the cases differed significantly from those of the healthy controls, with more poor metabolizers. After withdrawal of tamsulosin, the pulmonary condition of three cases had improved, six patients had stabilized, and one patient stabilized after reducing the tamsulosin dose.

Conclusions
The described 22 cases suggest that an association between the presence of CYP2D6 allelic variants and tamsulosin-associated ILD is highly likely. These cases highlight the importance of both clinical and genetic risk stratification aimed to achieve a more accurate prevention of DI-ILD in the future and enhance the quality of life of patients.

Introduction

Nowadays, it is well recognized that genetic polymorphisms in genes coding for enzymes responsible for drug metabolism and drug disposition are of great importance for the efficacy and toxicity of medicines.[1] It is generally agreed that the cytochrome P450 (CYP) superfamily of enzymes, with more than 1000 isoenzymes, five of which (CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP1A2) metabolize 90% of all drugs, contributes greatly to the metabolization of drugs in the human body. Identifying polymorphisms of these CYPs is mostly done to predict or explain drug target serum levels, e.g., a reduced CYP metabolism leads to increased serum drug levels and to increased toxicity. The drug metabolites formed are sometimes assessed for their pharmacological activity, but the toxic characteristics are rarely acknowledged or recognized.

Drug metabolite toxicity is best illustrated by the example of acetaminophen. It is metabolized by several CYP enzymes to its reactive metabolite, N-acetylparabenzoquinone-imine (NAPQI) which depletes the scavenger glutathione and binds to liver proteins, leading to liver injury. Although the phrase acetaminophen-induced hepatotoxicity is used in the case of intentional auto-intoxications, it is NAPQI that determines the final hepatotoxicity.[2]

Considering CYP polymorphisms and adverse drug reactions (ADRs) of expected drug metabolites is not current clinical practice. Even less common is the use of pharmacogenetic knowledge to assess the impact on shifts in drug metabolization pathways and the formation of unexpected toxic metabolites.[3] The potential importance of this assessment is illustrated in this paper by 22 cases of tamsulosin-associated interstitial lung disease (ILD). ILD is a group of heterogeneous disorders that diffusely involve the lung parenchyma. There is an ever-increasing number of drugs that can produce variegated patterns of drug-induced ILD (DI-ILD), and virtually all are histopathologic patterns of interstitial pneumonia. However, drugs are underestimated as serious causative agents of ILD, and elucidating the causative drug is challenging.[4,5] At present, more than 350 drugs are known to cause injury to the lung, and new causative drugs are regularly being identified.[6] Furthermore, previous studies showed that DI-ILD is associated with reduced metabolic capacity.[7] The association between tamsulosin and DI-ILD has not been described before. We aimed to investigate whether polymorphisms of relevant CYP enzymes involved in the metabolization of tamsulosin and the subsequent formation of drug metabolites (Figure 2.2.1) might explain the pathologic mechanism of the DI-ILD thus incurred.

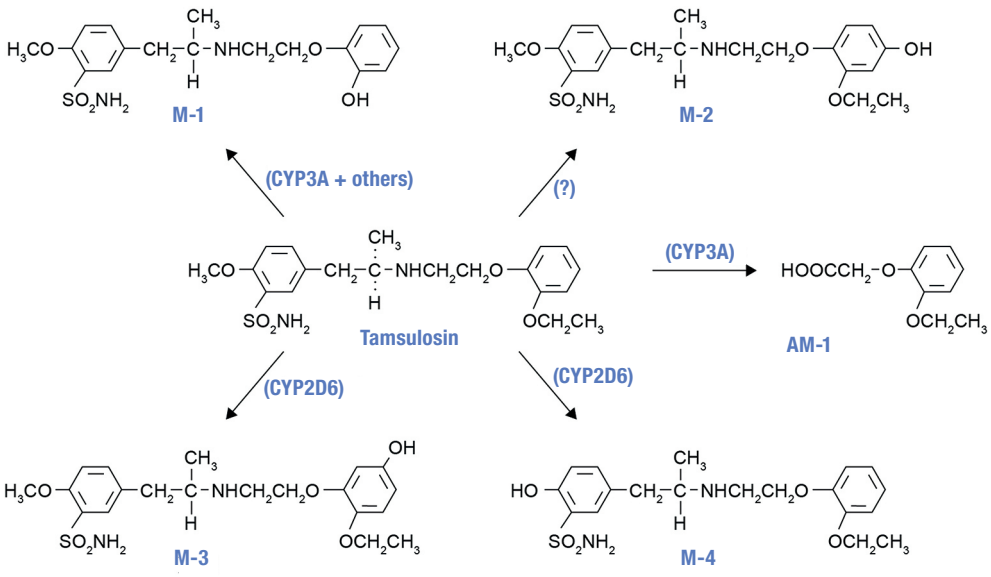

Figure 2.2.1 Tamsulosin is metabolized to five known metabolites by cytochrome P450 enzymes, mainly CYP3A4/5 and CYP2D6. After oral administration, M-1 is the major product (15.7% of the dose), followed by AM-1 (7.5% of the dose) and M-3 (6.4% of the dose). The formation of tamsulosin metabolites M-1 and AM-1 is mainly catalyzed by CYP3A isoforms (CYP3A4/5). The formation of M-3 is mainly catalyzed by CYP2D6.[8,9]

Results

Description of the cases

Data of 22 male patients who were taking tamsulosin (0.4 mg daily, orally) and developed side-effects were collected during a 12-year period. The patients experienced various manifestations of hypersensitivity while taking the medication. They recalled a history of progressive dyspnoea and exercise limitation for six months to four years prior to the referral, depending on when tamsulosin was started. High resolution computed tomography (HRCT) showed features of either non-specific interstitial pneumonia (NSIP) or idiopathic pulmonary fibrosis (IPF; end-stage pulmonary fibrosis). The diagnosis was confirmed by an experienced radiologist. Other causes of these ILDs were excluded, such as connective tissue diseases related interstitial lung disease (CTD-ILD) and familial IPF (FIPF). In 11 cases, the diagnosis was NSIP, and the other 11 patients were eventually diagnosed with IPF. All patients were male, with a mean age (SD) of 78.5 (6.3) years; range 68–93 (Table 2.2.1). The median latency period till diagnosis was six months (range three months to seven years). Eight patients used metoprolol concomitantly, which is also a substrate for CYP2D6. Tamsulosin had been withdrawn in almost all patients. After withdrawal

Table 2.2.1 Characteristics of the 22 patients with suspected tamsulosin-induced ILD. Abbreviations: NSIP = non-specific interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; AS = activity score (range between AS = 0 [poor metabolizer] and AS > 2 [ultra-rapid metabolizer]); n/a = not available; PM = poor metabolizer; IM = intermediate metabolizer; # half of the dossier every other day.

Patient	Age (years)	Diagnosis, condition after withdrawal of tamsulosin	Genotype							Concomitant drugs and the most important metabolizing cytochrome P450 isoenzyme for them		
			CYP2D6 (including phenotype)			CYP2C9	CYP3A4	CYP3A5	CYP2C19	CYP2D6	CYP2C9	CYP2C19
1	70	NSIP, stabilized	*4/*41	AS: 0.5	(IM)	*1/*2	*1A/*1A	*3/*3	*1/*1			
2	84	NSIP, stabilized	*4/*4	AS: 0.0	(PM)	*1/*2	*1A/*1A	*3/*3	*1/*1	Metoprolol	Valsartan	
3	79	NSIP, stabilized	*4/*6	AS: 0.0	(PM)	*2/*3	*1A/*1A	*3/*3	*1/*1			
4	93	IPF, stabilized	*4/*4	AS: 0.0	(PM)	*1/*1	*1A/*1A	*3/*3	*1/*2	Metoprolol		
5	87	IPF, stabilized	*3/*4	AS: 0.0	(PM)	*1/*1	*1A/*1B	*1/*3	*1/*1			
6	79	IPF, stabilized	*4/*4	AS: 0.0	(PM)	*1/*2	n/a	n/a	*1/*1	Metoprolol		
7	76	IPF, stabilized#	*1/*4	AS: 1.0	(IM)	*1/*2	*1A/*1A	*3/*3	*1/*2			
8	83	NSIP, improved	*1/*4	AS: 1.0	(IM)	*1/*1	n/a	n/a	*1/*1			
9	72	NSIP, improved	*1/*4	AS: 1.0	(IM)	*1/*2	*1A/*1A	*3/*3	*1/*1			
10	77	NSIP, improved	*1/*4	AS: 1.0	(IM)	*1/*1	n/a	*1/*3	*1/*1	Metoprolol		
11	71	IPF, died from pneumonia	*4/*4	AS: 0.0	(PM)	*1/*1	*1A/*1A	*3/*3	*1/*2			
12	79	IPF, died from cardiac failure	*5/*5	AS: 0.0	(PM)	*1/*2	*1A/*1A	*3/*3	*1/*1		Losartan	Clopidogrel
13	79	IPF, died from respiratory failure	*1/*4	AS: 1.0	(IM)	*1/*1	*1A/*1A	*3/*3	*2/*2			
14	68	IPF, died from respiratory failure	*1/*4	AS: 1.0	(IM)	*1/*1	*1A/*1A	*3/*3	*2/*2			
15	74	IPF, died from respiratory failure	*4/*6	AS: 0.0	(PM)	*1/*2	*1A/*1A	*3/*3	*1/*1	Metoprolol	Rosuvastatin	
16	80	NSIP, died from cardiac failure	*1/*3	AS: 1.0	(IM)	*1/*1	*1A/*1B	*1/*3	*1/*1	Metoprolol		
17	78	NSIP, died from cardiac failure	*1/*6	AS: 1.0	(IM)	*1/*1	*1A/*1B	*3/*3	*2/*2	Metoprolol		
18	90	NSIP, died from cardiac failure	*1/*6	AS: 1.0	(IM)	*1/*2	*1A/*1A	*3/*3	*1/*1			
19	77	NSIP, died from lung carcinoma	*1/*4	AS: 1.0	(IM)	*1/*1	*1A/*1A	*3/*3	*1/*1			
20	72	NSIP, no follow-up data yet	*1/*4	AS: 1.0	(IM)	*1/*3	*1A/*1A	*3/*3	*1/*1	Metoprolol		
21	79	IPF, no follow-up data yet	*4/*4	AS: 0.0	(PM)	*1/*1	*1A/*1A	*3/*3	*1/*1			
22	80	IPF, no follow-up data yet	*1/*5	AS: 1.0	(IM)	*1/*1	*1A/*1A	*3/*3	*1/*1			

of tamsulosin, the pulmonary condition of three cases (AS 1.0) had improved, six patients had stabilized, and one patient with an AS of 1.0 stabilized after reducing the tamsulosin dose (half of the dosage every other day, see also Table 2.2.1). Of the remaining 12 cases, six had died of comorbidities and three (all suffering from IPF) had died of respiratory failure, and of three cases follow-up data were lacking. The outcome for the causality score of the individual cases using the Naranjo probability scale was 'probable' in all 22 cases.

Genotyping

Nine patients (41%) were phenotyped as CYP2D6 poor metabolizers (PMs) and 13 patients (59%) as IMs. The phenotypes of the cases differed significantly from those of the healthy controls ($P<0.001$), with particularly more PMs and fewer extensive metabolizers (EMs) than in the controls (Table 2.2.2). Seventeen patients were genotyped as CYP3A5 non-expressors (the most common genotype [wild type] in a Caucasian population) and three appeared to be heterozygote expressors, producing functional CYP3A5 enzyme.

Table 2.2.2 CYP2D6 phenotype frequencies in the interstitial lung disease cases and healthy male volunteers.[7]

CYP2D6 Phenotypes	Cases (n=22)	Healthy volunteers (n=78)
Poor metabolizer	9 (41%)	8 (10.3%)
Intermediate metabolizer	13 (59%)	19 (24.4%)
Extensive metabolizer	0 (0%)	51 (65.3%)
Ultra-rapid metabolizer	0 (0%)	0 (0%)

Discussion

This paper is the first to describe a case series of DI-ILD associated with tamsulosin use. The association is supported by a mechanistic approach based on CYP2D6 and CYP3A enzyme metabolism and the formation of metabolites. The possible role of pharmacogenetics is also illustrated by the substantial differences in the CYP2D6 phenotypes frequencies between the 22 tamsulosin-associated ILD cases and the healthy volunteers.

The CYP2D6 and CYP3A enzymes involved are abundantly expressed in the human liver and lung. Additionally, just as in the liver, the pathogenesis of drug-associated cell injury in the lung may involve immune and cytotoxic mechanisms of action in which pharmacogenetics, reactive oxygen species, and reactive drug metabolites may play a role.[10,11]

Considering all the available knowledge on characteristics and polymorphisms of CYP2D6 and CYP3A and the drug metabolization pathway of tamsulosin, two important questions remain to be answered. The first one is whether we could have predicted these tamsulosin-associated ILD cases by applying pharmacogenetics, and the second one is what lesson we can learn from these cases. Knowledge of the CYP2D6 phenotype or activity score for these patients could have predicted a possible shift in metabolization to the CYP3A pathway, resulting in more of the M-1, M-2, and AM-1 metabolites (see Figure 2.2.1). The most toxic metabolite formed is AM-1 (o-ethoxyphenoxy acetic acid), containing a carboxylic acid moiety. Several non-steroidal anti-inflammatory drugs (NSAIDs) such as ibufenac, bromfenac, zomepirac, benoxaprofen, and pirprofen with this carboxylic acid moiety have been withdrawn from the market due to rare, mostly hepatic, ADRs.[12–14] A search in the World Health Organization's Global Individual Case Safety Report database (VigiBase®), maintained by the Uppsala Monitoring Centre in Sweden, showed that several reports of ILD have been received for these NSAIDs, indicating that drugs and metabolites with this moiety have previously been associated with the occurrence of ILD. Bioactivation of this carboxyl moiety forms reactive metabolites, namely coenzyme A thioesters and acyl glucuronides, representing an early step in the pathogenesis of ensuing adverse effects. Acyl glucuronides can covalently modify proteins via a simple transacylation reaction, or through an acyl migration within the β-O-glucuronide unit to a reactive R-hydroxy-aldehyde intermediate, which can react with proteins.[15]

Acyl-Coenzyme A (acyl-CoAs) thioesthers of the carboxylic acid moieties in drugs possess sufficient electrophilicity for nucleophilic reactions with amino acids and are able to form covalent adducts with proteins. Just like cytochrome P450 enzymes, the cofactor for the acyl glucuronidation, uridine diphospho glucuronic acid (UDPGA), is mostly expressed in the liver, but also in extra-hepatic tissues such as the skin and the lungs. Although liver and skin reactions are well-known and have previously been related to the bioactivation of the carboxylic moiety in drugs, this is the first

publication in which lung reactions have been associated with this moiety. Furthermore, despite their reactivity, these reactive metabolites are sufficiently stable to be transported out of the cell into the circulation. Although the evidence for the formation of these acyl glucuronides, their reaction with proteins and the potential clinically relevant ADRs in vivo is limited, there is a large body of in vitro data. Additionally, it is widely acknowledged that carboxylic drugs and carboxylic drug metabolites are prone to forming reactive metabolites that have the potential to play a mechanistic role in ADRs associated with the therapy concerned.[15]

Three patients appeared to be heterozygote expressors of CYP3A5, producing functional CYP3A5, an enzyme abundantly expressed in the lung.[10] This induces a faster metabolization of tamsulosin, but there are no indications that it affects the outcome of the ILD.

Only after all other possible causes have been excluded can the diagnosis of DI-ILD be made.[16,17] However, the differences in CYP2D6 phenotypes between the 22 cases and the controls suggest a role for genetics in the development of tamsulosin-associated ILD. Increased understanding of genetic variants in drug-metabolizing enzymes, followed by stratification based on these genetic variants and their possible relation with the proposed cytotoxic drug metabolites, may offer an opportunity to prevent the often serious DI-ILDs.[7] A lesson to be learnt from this might be that taking full advantage of pharmacogenetics in clinical practice requires more effort and more expertise than is currently being applied.

This deduction from the available knowledge, suggesting a positive association between tamsulosin- associated DI-ILD and decreased CYP2D6 activity, has many limitations. Although tamsulosin was the most suspected drug for the DI-ILD—in that after withdrawal the condition stabilized in most cases and improved in a few—no hard causal relationship can be established between the polymorphisms, the drug, and the DI-ILD. In addition, evidence regarding tamsulosin drug levels is lacking because in our cases of DI-ILD, the suspected drugs were withdrawn before serum levels were determined or conclusions were drawn. According to the Naranjo algorithm, which is still the most widely used causality method, all individually assessed cases were 'probable'. Unfortunately, no tamsulosin and/or metabolite serum levels were available of the presented cases and rechallenge was not considered, though this would have strengthened our observation. Moreover, we realize that the Naranjo algorithm probably does not cover all types of ADRs and might need adjustments.[18,19] A search for more cases yielded multiple reports of tamsulosin-associated DI-ILD in EudraVigilance (the system for suspected ADRs in the European Economic Area) and in Vigibase®, but unfortunately, information on genotyping was lacking for these cases. However, a few patients in the EudraVigilance database concomitantly used CYP2D6 inhibitors such as paroxetine, which reduces the metabolic activity of CYP2D6 even in low doses, and this probably turned these patients into poor or intermediate CYP2D6 metabolizers.[20] So far, no studies have

measured the impact of CYP2D6 and CYP3A polymorphisms on the formation of tamsulosin metabolites and cytotoxic reactions. Although the present case series shows a possible important role for pharmacogenetics, drug metabolization pathways and drug metabolites in the development of DI-ILD, it is observational and descriptive. Further research should confirm the suggested relationships.

A 'one-size-fits all' approach to drug prescription is based on broad population averages, whereas personalized medicine offers more effective and safer drug therapy that is tailor-made for individual patients. The growing understanding of pharmacogenetics and pharmacogenomics offers many advantages in terms of customizing drug use, which may result in better disease outcomes, less drug wastage, lower drug costs, safer drug prescriptions, and more effective treatments. The case series we present shows that genetic variations in metabolizing enzymes should be considered in the development of DI-ILD. NSIP and IPF are both regarded as chronic interstitial fibrosis or idiopathic interstitial pneumonia (IIP) of unknown cause. Compared to other IIPs, IPF has a significantly worse prognosis. The prognosis of NSIP is variable. Some patients improve, others remain stable or improve on treatment, but some evolve to end stage fibrosis IPF and finally die of the disease.[16] Therefore, it is of great clinical relevance to identify agents likely to be involved in the initiation and/or progression of the fibrotic process. One of these agents/triggers might be drugs, as was the case in our 22 presented cases. In earlier studies we found an association of certain gene variants with the appearance of DI-ILD.[7,21–26] This paves the way for a potential use of personalized medicine by genotyping, aiming to improve efficacy, tolerability, and drug safety.

Methods

Patients and methods

Patients presented with suspected tamsulosin-associated ILD (either NSIP or IPF (end-stage pulmonary fibrosis)) at the ILD Center of Excellence at St. Antonius Hospital, Nieuwegein, and at the Maastricht University Medical Centre (MUMC), both in the Netherlands (2009–2020), were selected. A multidisciplinary team confirmed the diagnosis based on clinical presentation, including dyspnea and hypoxia, pulmonary function impairment, exercise intolerance, and high-resolution CT-scan abnormalities, including multifocal areas of ground-glass opacity with intralobular interstitial thickening. Other possible causes, such as infections and other drug use, were meticulously excluded. The control group regarding the distribution of allele variants in the general population consisted of 78 healthy Caucasian male volunteers (average age 38 years) who did not use any medication nor had any relevant medical history.

All healthy volunteers were MUMC hospital employees.[7] The study was performed in accordance with the Declaration of Helsinki and its amendments. The protocol was approved by the local Medical Ethics Board of the MUMC. The medical ethics review committee of the MUMC approved the study (MAC# - METC 11-4-116, 9 November 2011). Written informed consent for participation in this study was obtained from all subjects. Demographic information of the cases (gender, age), tamsulosin dosage and all available concomitant medication data were gathered from (electronic) patient records. Since the patients had been referred to the two above centers, the tamsulosin treatment had already been stopped or reduced. Hence, as the determination of tamsulosin and/or its metabolites is not standard practice in the Netherlands, no serum drug levels were available or could be obtained. The causality score of the individual cases was assessed using the Naranjo Probability Scale.[27]

Genotyping

DNA was obtained from all subjects from venous EDTA-anticoagulated blood. Genotyping of CYP2D6, CYP3A4, and CYP3A5 single nucleotide polymorphisms (SNPs) was done by real-time PCR Fluorescence Resonance Energy Transfer (FRET) assays on the LightCycler (Roche Diagnostics, Mannheim, Germany). We used the CYP2C9 and CYP2C19 Mutation Detection Kits (Roche Diagnostics, Mannheim, Germany), and CYP3A4 and CYP3A5 FRET primer-probe mixes (TIB MOLBIOL, Berlin, Germany), according to the manufacturer's instructions. The CYP2D6 SNPs were genotyped using the Luminex xTAG CYP2D6 Kit v3 and the LX200 (Luminex, Austin, TX, USA), according to the manufacturer's instructions. According to conventional classification systems, individuals were phenotyped as poor metabolizer (PM) if they carried two non-functional alleles; as intermediate metabolizer (IM) if they carried one non-functional allele or two reduced activity alleles; as extensive metabolizer (EM) if they carried one allele associated with reduced activity and one functional allele or two functional alleles, and as ultra-rapid metabolizer (UM) if they carried at least two copies of a functional allele plus a reduced activity allele or three copies of a functional allele.

Statistical analysis

Statistically significant differences between CYP2D6 phenotype frequencies in cases and controls were assessed using a Fisher exact test in R (version 3.5.1, Vienna, Austria).[28] A p-value less than 0.05 was considered statistically significant.

Conclusion

Tamsulosin has so far not been recognized in general clinical practice as an agent that might be associated with the development and/or progression of lung damage. Although the sample size is rather low, the described 22 cases suggest that an association between the presence of CYP2D6 allelic variants and tamsulosin-associated ILD is highly likely. We acknowledge that the sample size is rather small as to allow us to calculate an effect size for the CYP2D6 phenotype on the occurrence of tamsulosin-associated ILD. However, the CYP2D6 phenotype of the 22 presented cases differed significantly from that of a control population of healthy male volunteers, in that there were more poor and intermediate metabolizers than among the controls. This may support the idea of an alternative metabolization route via CYP3A and the formation of the reactive metabolite (AM-1), which we associated with lung toxicity. These cases show the importance of including genetic risk stratification (pharmacogenomics) in the work-up of patients with suspected drug-induced (lung) toxicity, and the advantages of genotyping prior to drug prescription. This may be clinically useful for the prediction and prevention of ADRs in general and in our cases for drug-induced pulmonary toxicity, in particular by reducing the risk of development or progression of end-stage pulmonary fibrosis. Furthermore, genotyping and phenotyping drug metabolizing enzymes prior to prescription has the potential to contribute to safe drug use in patients using multiple drugs. Lack of familiarity with this approach may lead to causative factors being ignored and to unnecessary delays in their recognition. Both clinical and genetic risk stratification may lead to a more accurate prevention of drug-induced lung damage in the future and enhance the quality of life of the patients.

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Chapter 2.3

Drug-induced interstitial lung disease: role of pharmacogenetics in predicting cytotoxic mechanisms and risks of side effects

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Abstract

Purpose of review

The diagnosis of drug-induced interstitial lung disease (DI-ILD) is challenging and mainly made by exclusion of other possible causes. Toxicity can occur as a cause of drug(s) or drug–drug interactions. In this review, we summarize the possible role of pharmacogenetics of metabolizing enzymes in DI-ILD.

Recent findings

Knowledge of the genetic predispositions of enzymes involved in drug metabolization and their relation with proposed cytotoxic mechanisms of DI-ILD, in particular direct cell toxicity and free oxygen radical production is increasing. The cytochrome P450 enzyme family and other enzymes play an important role in the metabolism of all sorts of ingested, injected, or inhaled xenobiotic substances. The liver is the major site for metabolism. Metabolic cytotoxic mechanisms have however also been detected in lung tissue. Polymorphisms in genes coding for enzymes that influence metabolic activity may lead to localized (toxic) reactions and tissue damage. This knowledge may be helpful in preventing the risk of DI-ILD.

Summary

Drug toxicity can be the consequence of absence or very poor enzyme activity, especially if no other metabolic route is available. In the case of reduced enzyme activity, it is recommended to reduce the dose or to prescribe an alternative drug, which is metabolized by a different, unaffected enzyme system to prevent toxic side effects. However, enhanced enzyme activity may lead to excessive formation of toxic and sometimes reactive metabolites. Therefore, knowing a patient's drug-metabolizing profile before drug prescription is a promising way to prevent or explain DI-ILD.

Introduction

Diffuse or interstitial lung diseases (ILD) can involve various patterns and the causes vary.[1,2] An ever increasing number of drugs can produce variegated patterns of ILD, virtually all histopathologic patterns of interstitial pneumonia, including cellular and fibrotic nonspecific interstitial pneumonia, pulmonary infiltrates, and eosinophilia (PIE), organizing pneumonia, lymphocytic interstitial pneumonia, desquamative interstitial pneumonia (a condition in which both the interstitium and the alveolar space are involved), a pulmonary granulomatosis-like reaction, and a common interstitial pneumonia-like pattern.[3] Moreover, the presentation can be more or less subclinical, with only an alveolitis pattern in the cellular profile of bronchoalveolar lavage fluid (see also Figure 2.3.1).[4] Moreover, drug-induced pulmonary toxicity can present with varying patterns on chest computed tomography imaging (see also Figure 2.3.2).

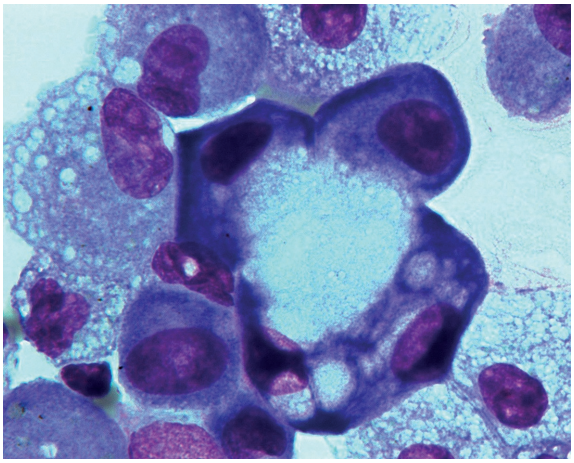

Figure 2.3.1 Reactive pneumocyte type II cell (central) present in bronchoalveolar lavage fluid of a patient with cocaine drug-induced interstitial lung disease (DI-ILD) (see also Figure 2.3.2).

Drugs in one therapeutic class may collectively produce the same pattern of involvement. Few drugs are known to produce more than one pattern of ILD.[5]
The diagnosis of drug-induced ILD (DI-ILD) essentially rests on the temporal association between exposure to the drug and the development of pulmonary infiltrates. Thus, the diagnosis of DI-ILD is mainly made by the meticulous exclusion of all other possible causes.[4,6] The striking individual susceptibility for drug-induced lung injury, however, suggests a genetic background. Increased understanding of the genetic predispositions of enzymes involved in drug metabolization and their relation with proposed cytotoxic mechanisms of drug-induced lung injury, in particular direct cell toxicity and free oxygen radical production, offers the possibility to prevent the frequently serious DI-ILD from occurring.[7]

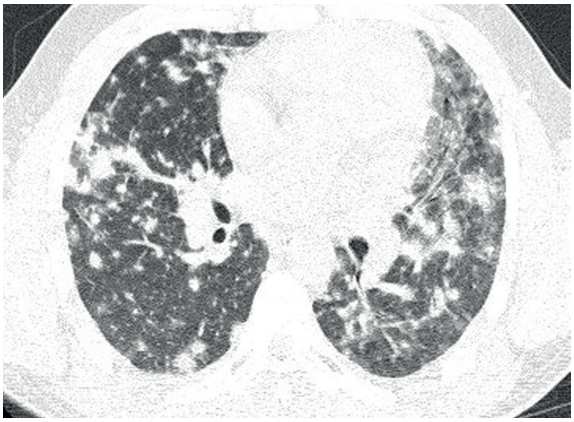

Figure 2.3.2 High-resolution computed tomography scan through the midlung zones shows scattered ill-defined nodulas and ground-glass opacity in the lingular segment of a patient with cocaine-induced ILD (a carrier of CYP2C and VKORC1 variant alleles).

Mechanisms of drug induced lung injury

Both cytotoxic and immune mechanisms may be involved independently or in combination, in the initiation and propagation of DI-ILD.[8] The lungs have the potential of metabolizing many foreign compounds, including pharmaceutical products. The so-called biotransformation is the process by which cells modify xenobiotics with the ultimate goal of facilitating the elimination of lipophilic substances. To increase the water solubility of xenobiotics, a broad set of enzymes capable of introducing new functional groups (phase I) or conjugating with internal cell's molecules (phase II) is involved. Sometimes, however, these enzymes transform an otherwise harmless product in a reactive intermediate, a process called bioactivation (Figure 2.3.3). Biotransformation can result in the formation of reactive electrophilic species such as epoxides, quinones, quinoneimines, methylene-imines, and acyl radicals which react with cell biomolecules, modifying them or forming covalent adducts and causing direct cell toxicity.[9] In addition to this mechanism, the production of free oxygen radicals and alteration of the oxidant-antioxidant balance is one of the mechanisms (Figure 2.3.3) of iatrogenic pneumonitis.[10] Redox cycling leads to the formation of superoxide anion free radicals ($O_2^{\bullet -}$), which may transform into other reactive oxygen species (ROS) such as H_2O_2 and $\bullet OH$ (Figure 2.3.3).[8,11] These ROS may directly or indirectly lead to lung damage. Interestingly, direct activation of lung fibroblasts is initiated via the influx of superoxide anion radicals through chloride channels. Activated fibroblasts result in the production of transforming growth factor beta-1 and collagen.[12] This finding makes it conceivable that redox cycling drugs lead to lung fibrosis. A similar mechanism has been suggested to occur in hepatic stellate cells, which has led to the suggestion that this may lead to liver fibrosis.[13] The non-commercial website Pneumotox provides a list of drugs that have shown or suggested to cause lung damage.[14] The website ranks the reported cases by 1–5 stars ranging, indicating the degree of plausibility that the drug is causative for lung damage.

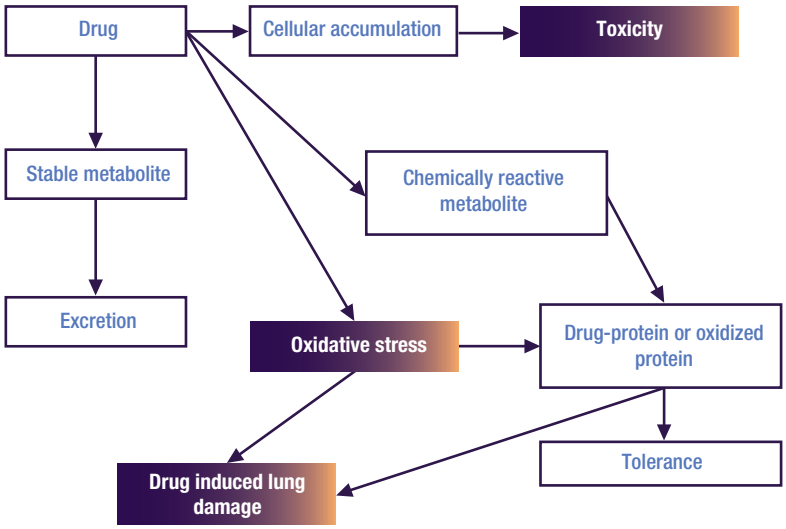

Figure 2.3.3 Overview of molecular mechanisms leading to drug-induced lung damage. Drugs are enzymatically degraded to stable metabolites (e.g. via cytochrome P450) and excreted. A decreased metabolic activity may cause accumulation of drugs, which may lead to toxicity. Biotransformation may also lead to the formation of chemically reactive metabolites. These metabolites (haptens) can form an adduct with proteins. This leads to tolerance or (sometimes), in combination with a costimulatory signals (e.g. oxidative stress, or a viral or bacterial infection) to a toxic immune reaction and subsequent lung damage.[15] Finally, there are strong indications that oxidative stress, for example via redox cycling of drugs, is involved in drug-induced lung damage.

Enzymes with genetic variation and involvement in drug metabolization possibly leading to interstitial lung disease

There are more than 30 families of drug-metabolizing enzymes in humans. Essentially all of the major human enzymes responsible for modifications of functional groups (phase I) or conjugation with endogenous substituents (phase II) exhibit common polymorphisms at the genomic level, many of which translate into functional changes in the encoded proteins and thereby determine the efficacy and toxicity of medications. In many cases, a polymorphism is associated with reduced activity of the encoded protein (e.g. glucose-6-phosphate dehydrogenase, G6PD), but there are also examples where the allelic variant encodes proteins with enhanced activity (e.g. CYP iso-enzyme 2C19*17).[16,17] It is now well recognized that adverse drug reactions may be caused by specific drug-metabolizer phenotypes, such as severe and potentially fatal hematopoietic toxicity that occurs when thiopurine methyltransferase-deficient patients are treated with standard dose of the thiopurines, azathioprine or 6-mercaptopurine.[18] Another example is dihydropyrimidine dehydro-

Table 2.3.1 Drug metabolizing enzymes, drugs metabolized by these enzymes and the current state of information of their relation with ILD

Enzymes involved in drug metabolism	Polymorphisms phenotype	Drugs metabolized by the particu-lar enzymes	Proposed mechanism of causing pulmonary toxicity	All if available: Rating in Pneumotox [14] * < 10 cases; ** 10-50 cases; *** 50-100 cases; **** 100-200 cases; ***** > 200 cases	Quality of the studies (very low – high) n/a = not included in the latest systematic review [1]
Phase I enzymes					
Glucose-6-phosphate dehydrogenase (G6PD)	Decreased activity	Nitrofurantoin	Generation of free oxygen radicals [11]	*****	Low
		Cocaine		*****	n/a
		Primaquine		*	n/a
		Flutamide		*	n/a
		Dapsone		**	n/a
		Sulfacetamide		*	n/a
Cytochrome P450			Release of toxic oxygen radicals and reactive metabolites [7]		
CYP2D6	Decreased (poor and intermediate metabolizer) Increased function (rapid metabolizer)	Tamoxifen		*	n/a
CYP3A		Acetaminophen		***	n/a
		Amiodaron		*****	Very low – low
		Dasatinib		****	n/a
		Fentanyl		*****	n/a
		Fluticasone		****	n/a
		Imatinib		***	Low
		Sirolimus		****	Very low
		Everolimus		*****	Very low – moderate
		Erlotinib		***	Low-moderate
		Gefitinib		*****	Low-moderate
		Methadone		*****	n/a

Table 2.3.1 - Continued

Enzymes involved in drug metabolism	Polymorphisms phenotype	Drugs metabolized by the particular enzymes	Proposed mechanism of causing pulmonary toxicity	All if available: Rating in Pneumotox [14] * < 10 cases; ** 10-50 cases; *** 50-100 cases; **** 100-200 cases; ***** > 200 cases	Quality of the studies (very low – high) n/a = not included in the latest systematic review [1]
CYP2C8	Decreased activity	Amiodaron		*****	Very low – low
		Paclitaxel		*****	n/a
CYP2C9	Decreased activity	Cyclophosphamide		****	n/a
CYP2C19	Decreased activity	Warfarine		**	n/a
Dihydropyrimidine dehydrogenase (DPD)	Decreased activity	5-FU capecitabine	Decreased detoxification of pyrimidine-based antimetabolite analogues [15]	*** *	n/a
Phase II enzymes					
N-Acetyltransferases	Decreased activity				
NAT2	Decreased activity	Isoniazid	Increased oxidative stress [16]	**	n/a
Thiopurine S-methyltransferase (TPMT)	Decreased activity	Azathioprine	ROS generation, causing oxidative DNA damage and mitochondrial dysfunction [15]	***	n/a
		Mesalazine		***	n/a
		6-Mercaptopurine		*	n/a
		6-Thioguanine		No results	n/a
UDP Glucuronyltransferases (UGTs)					
UGT1	Decreased activity	Irinotecan	Less scavenging of toxic and reactive metabolites [9]	**	low
	Increased activity		Instable acyl derivatives leading chemical protein adducts with electrophilic chemical reactivity [9]		
UGT2					

genase deficiency and 5-fluorouracil toxicity leading to hematological and gastrointestinal toxicities.[19] A large number of associations have been identified between drug-induced toxicity and genetic variations in their metabolizing enzymes.[20] The focus in this review, however, is on drug-genetic variant enzyme combinations, which are most important for the causation of DI-ILD.
Considering the mechanisms of lung injury and the (by-)products of drug metabolization, it is expected that polymorphism(s) of metabolizing enzymes enhancing or leading to the formation of reactive drug metabolites and ROS, may increase the chances of the occurrence of DI-ILD. In this review, the current state of research on the association between genetic variations in phase I and phase II enzymes involved in drug metabolization and their distinct role in the mechanisms of drug-induced lung damage is assessed and merged. The results of the analysis are summarized in Table 2.3.1. Except for the enzymes belonging to the CYP superfamily, most polymorphisms, lead to enzyme deficiencies. In the sections later, involvement of polymorphisms of *G6PD*, *CYP* and thiopurine S-methyltransferase (*TPMT*) in DI-ILD are further explored. Some clinical cases are included as an example.

Glucose-6-phosphate dehydrogenase

A single genotype seems to play a crucial role in the protection against ROS-induced lung damage, viz. G6PD, a critical enzyme in the pentose phosphate pathway. In this pathway a supply of NADPH is generated via the G6PD catalyzed conversion of glucose-6-phosphate to 6-phosphogluconolactone. NADPH is necessary for adequate generation of protective intracellular thiols, which are needed to protect against the damaging effects of ROS. An important protective thiol is the tripeptide glutathione (GSH). GSH itself is an antioxidant and acts as a cofactor in glutathione dependent antioxidant enzymes.[21,22] A diminished activity of G6PD thus increases the risk of a lack of intracellular antioxidant protection, and increases oxidative stress because it hampers the regeneration of the reduced protective form of GSH (Figure 2.3.4).

In many cases clinical manifestations of G6PD deficiency will not be observed. Other antioxidants such as mitochondrial antioxidant systems will take over the protection against ROS. Erythrocytes lack mitochondria and thus strongly depend on the cytosolic pentose phosphate pathway for NADPH and are therefore particularly vulnerable for oxidative damage in case of G6PD deficiency. Hemolytic anemia may be the result. Other tissues besides erythrocytes may also become damaged more easily in cases of oxidative stress. This can be a direct ROS damage or an indirect damage via the toxicity of iron, which is known to cause a fibrotic interstitial trigger in lung tissue. G6PD deficiency is a very common enzymopathy and is estimated to affect 400 million people especially in areas in Africa.[23] It is thought that G6PD deficiency offered an evolutionary advantage because it weakens the erythrocyte

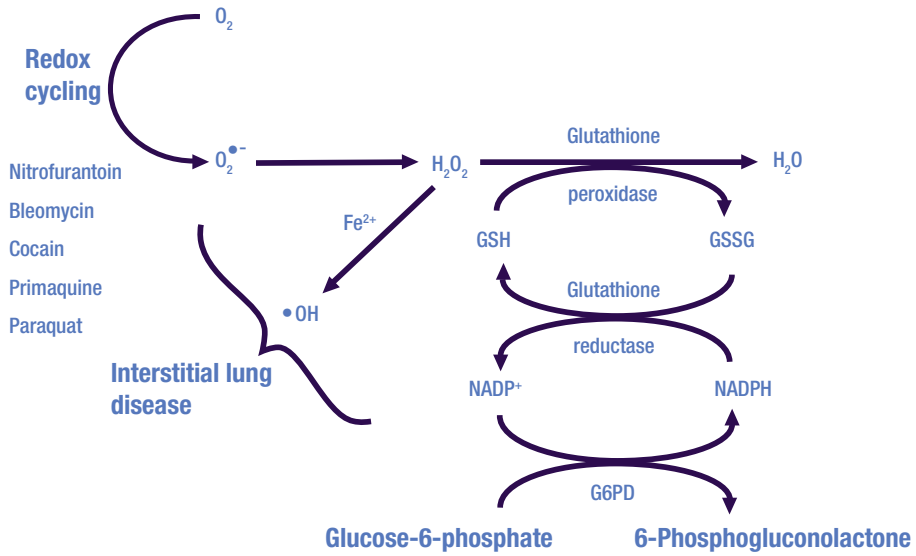

Figure 2.3.4 Redox cycling compounds generate superoxide anion free radicals ($O_2^{\bullet -}$). Upon dismutation $O_2^{\bullet -}$ is converted into hydrogen peroxide (H_2O_2). In the presence of Fe^{2+} (which may originate from haem, upon red blood cell haemolysis) H_2O_2 can be transformed into the very reactive damaging hydroxyl radical (•OH). H_2O_2 reduces to water H_2O by glutathione peroxidase. In this reaction glutathione (GSH) provides the reducing equivalents and glutathione disulphide (GSSG) is formed. GSSG is reduced to GSH by glutathione reductase, which obtains its reducing equivalents from NADPH originating from the pentose phosphate pathway. In this pathway glucose-6-phospate dehydrogenase (G6PD) plays a crucial role.

membrane, the host cell of the malaria parasite (*Plasmodium falciparum*). This makes it difficult for the parasite to have productive growth in the erythrocyte. Numerous medications and some oxidative food products like fava beans (*Vicia faba*) should be avoided by G6PD-deficient patients.

Redox cycling compounds generate superoxide anion radicals and subsequently various other ROS. Redox cycling compounds include the antibiotic nitrofurantoin,

Table 2.3.2 Drugs to be avoided by G6PD deficient patients.[17]

Drugs	Indication
Diaminodiphenyl sulfone (Dapsone)	Leprosy
Flutamide	Prostate cancer
Furazolidone	Largely forbidden as human antibiotic
Methylene blue	Methemoglobinemia
Nitrofurantoin	Urinary tract infections (amongst others)
Phenazopyridine	Analgesic
Primaquine	Malaria
Rasburicase	Excess uric acid
Sulfacetamide	Infections
Sulfanilamide	Infections

the cytostatic bleomycin (which is even used as model compound in animal or cell research to reliably induce pulmonary fibrosis), cocaine, and the antimalarial drug primaquine (Table 2.3.1).[24–27]
ILD induced by redox cycling drugs is based on this mechanism and G6PD deficiency, by increased oxidative stress, could increase the occurrence of ILD. Widely used drugs that should be avoided in G6PD deficiency are associated with DI-ILD (Table 2.3.2).[28] Figure 2.3.5a and 2.3.5b show an example of DI-ILD: a case of nitrofurantoin induced pneumonitis.

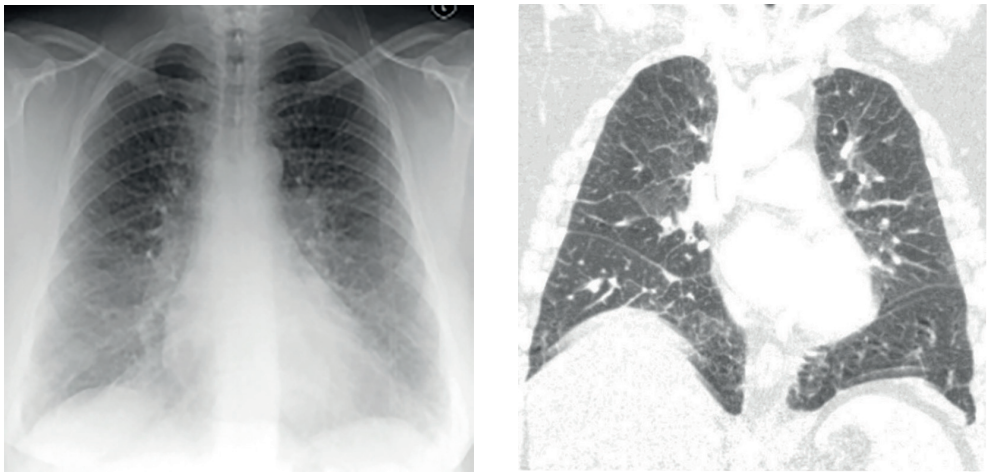

Figure 2.3.5 An example of nitrofurantoin-induced pneumonitis: (A) chest X-ray shows a diffuse reticular pattern: (B) High-resolution computed tomography (coronal slice) confirms this reticular pattern, which is caused by thickening of the interlobular septa.

Cytochrome P450 enzymes

Members of the CYP family are responsible for the metabolism of endogenous substrates, and for pharmaceuticals. CYP enzymes are involved in the biotransformation of chemicals like drugs. CYP activity frequently (but not always!) reduce or alter the pharmacological activity of many drugs while facilitating their elimination. The CYP enzymes are largely polymorphic and variant alleles together with host and environmental factors result in, normal (extensive metabolizer, EM), increased (ultra-rapid metabolizer), decreased (intermediate metabolizer), or no enzyme activity (poor metabolizer), and thus four possible metabolizing phenotypes. Therefore, an ultra-rapid metabolizer phenotype leads to accelerated drug metabolism of the parent drug resulting in low parent drug serum levels accompanied by sometimes less efficacy and the formation of (in)active and even toxic or reactive metabolites.

Intermediate metabolizer and poor metabolizer phenotypes lead to the accumulation of the parent drug, or a push to another sometimes less favourable metabolization pathway.[29] Because the various CYP enzymes have different metabolic activity and are not evenly distributed in organs, knowledge on the characteristics of CYP enzymes in the lung and drug metabolism pathways may have value in recognizing the causative agent in patients presenting with DI-ILD.[20] The most important enzymes for drug metabolism are CYP1A2(+), CYP2C9(++), CYP2D6(++), CYP3A4(+++), and CYP3A5(+++). Their presence in the lung is noted with for + low, ++ for intermediate, and +++ for high presence.[30] Review of www.pneumotox.com and the latest literature review show (Table 2.3.1) that drugs associated with pulmonary toxicity are more often metabolized by CYP enzymes that have high presence or activity in the lung compared to drugs that are solely metabolized by CYPs with low presence in the lungs.[1,14]

Acetaminophen/paracetamol

Although acetaminophen (APAP) is mostly known for causing hepatotoxicity, the wide use of this drug justifies drawing the attention for its rare pulmonary toxicity. It is a commonly used medicine for relieving pain and reducing fever in adults and children.[31] The majority of APAP is metabolized in the liver and after glucuronidation and sulfation safely excreted. However, a fraction of APAP is metabolically activated in the liver by CYP2E1, CYP3A4, and CYP1A2, to the pro-oxidant metabolite N-acetyl-p-benzoquinone-imine (NAPQI).[32] These CYPs are expressed in the respiratory tract, suggesting that similar metabolic activation as in the liver may also occur in the lungs.[33] Polymorphisms that accelerate the forming of NAPQI may lead to enhanced toxicity.[34] There are two hypotheses for the mechanisms of lung injury by NAPQI. The first one suggests that because of NAPQI is highly reactive, it causes cellular oxidative stress, and may covalently bind to cellular macromolecules.[35] The second hypothesis suggests a more specific mechanism of APAP-induced lung disease and proposes neurogenic inflammation. Nassini *et al*. suggested that inflammation develops in the lungs after APAP treatment because of activation of the transient receptor potential ankyrin 1 (TRPA1) channel in peptidergic neurons by NAPQI. The TRPA1 hypothesis may be more biologically plausible; however, the evidence for this hypothesis is also preliminary and both models should be further explored.[36–40]

Thiopurine S-methyltransferase

TPMT polymorphisms lead to an almost 50-fold variation in enzyme activity between individuals. TPMT catalyzes the transfer of the methyl-group of S-adenosylmethionine to the thiol-group on the thiopurine molecule. Methylation of thiopurines is one of the detoxification reactions in thiopurine metabolism. Variations in response

to thiopurine drug therapy are mainly caused by TPMT polymorphism. Adverse effects of the thiopurines, 6-mercaptopurine, and azathioprine include bone marrow suppression, which is of major concern, occurring in 2–5% of inflammatory bowel disease patients. The risk of thiopurine induced myelosuppression is increased in patients with TPMT deficiency. Liver toxicity occurs in 3–10% of azathioprine exposed patients with hypersensitivity, an idiosyncratic cholestatic reaction, or endothelial cell damage and results in drug withdrawal. A number of different factors have been reported to be linked to thiopurine-induced hepatotoxicity including higher concentrations of methylated metabolites and mitochondrial injury associated with glutathione depletion. Thiopurines are known to induce oxidative stress, especially in mitochondria, resulting in mitochondrial dysfunction and activation of stress activated protein kinase pathways. Azathioprine-induced oxidative stress causes tricarboxylic acid cycle dysfunction by depleting crucial mitochondrial enzymes. The metabolite 6-thioguanine nucleotide (6-TGN), a thiopurine metabolite, is also known to incorporate into mitochondrial DNA (mtDNA), where it is rapidly oxidized and inhibits mtDNA replication causing decreased mitochondrial protein concentrations and loss of mitochondrial function. A recent study in cultured human lymphoblasts proposed ROS generation, resulting in oxidative DNA damage and mitochondrial dysfunction as the mechanism responsible for thioguanine induced cytotoxicity. Thiopurine induced alterations in the expression of genes involved in protein and ATP-biosynthesis. When mice were treated with 6-mercaptopurine, significant alterations were observed in the expression of genes associated with abnormal lipid metabolism, inflammatory responses, oxidative stress, ATP depletion, and cell death.[41] Although several cases of azathioprine induced ILD are known, so far, in only one case TPMT deficiency has been associated with pulmonary toxicity.[14,42]

In case of an azathioprine indication, also used as treatment for certain ILDs, testing TPMT variants involved in azathioprine metabolism is advised before starting treatment.[42–44] In the Unites States of America, drug labels for azathioprine now include information on TPMT polymorphisms and recommend determining patients' phenotype or genotype prior to drug treatment.[45]

Discussion

Genetic variations in drug metabolizing enzymes may enhance the causation of DI-ILD by inducing the forming of ROS or reactive metabolites (phase I) or by reducing the scavenging of these ROS or reactive metabolites (phase II). We cautiously want to mark that drugs that are solely metabolized by CYP1A (low presence in the lungs), and known for other idiosyncratic adverse drug reactions, such as clozapine, are less associated with DI-ILD than drugs that are metabolized by CYPs with high presence in the lungs such as CYP3A. It was expected that drugs that undergo redox cycling would be well known for causing DI-ILD, but besides nitrofurantoin and cocaine, no other drugs that should be avoided in G6PD-deficiency had a five-star code in www.pneumotox.com.[14] We must, however, keep in mind that nitrofurantoin and cocaine are far more widely used than the other drugs. Although our findings may point to an association between genetic variation of metabolizing enzymes and the occurrence of DI-ILD it has thus far not been extensively researched, resulting in low grades of evidence and enforcing us to review older publications, however, from a novel perspective.

Both genetic and nongenetic information is important in the susceptibility, development, cause, and treatment response of diseases. The more we know about a patient's genes and context, the better disease management decisions can be made [46]. The ability to identify individuals who are susceptible to adverse drug reactions has the potential to reduce the personal and population costs of drug-related morbidity and the potential to attribute to the patients' safety. Genotyping should be considered to identify patients that might be at risk of severe toxic responses to environmental, pharmacological, herbal remedy, and/or nutritional stimuli, in order to guide appropriate individual dosage(s).[47] Some patients will continue to react unpredictably to therapy even though, according to obtained test results, problems were not expected. This variability in drug response among patients is multifactorial and include extrinsic factors like environmental aspects, comedication, nutritional status, smoking and alcohol consumption, and intrinsic factors that affect the disposition (absorption, distribution, metabolism, and excretion) of individual drugs.

There are an increasing number of examples where pharmacogenetic studies have indicated that genetic testing prior to treatment may be useful either for setting the individual dose or in choosing a certain drug.[48,49,50] Genetic screening prior to drug prescription may potentially prevent serious adverse effects such as diffuse alveolar hemorrhage (DAH) or DI-ILD.[51,52] The results obtained by genetic testing appeared to be useful in disease management, because of the prognostic value of the absence or presence of specific polymorphisms. An association with vitamin K epoxide reductase complex 1 (*VKORC1*) and/or *CYP2C9* variant alleles might even be a risk factor for the development or exacerbation of idiopathic pulmonary fibrosis.[51] Furthermore, it was accentuated that in DAH cases early recognition of the presence of one of

the studied polymorphisms is important, because of a potential lethal outcome and the fact that simple vitamin K supplementation can be life-saving.[52] Genetic variations are, of course, not limited to drug metabolizing enzymes like the substrates of these enzymes are not limited to drugs. Needless to say, many more substances are associated with the occurrence of ILD. The redox cycling herbicide paraquat is well known and another striking example is 4-ipomeanol, a toxin produced by moldy sweet potatoes (*Ipomoea batatas*) caused by postharvest diseases, the most common is infection with the fungus Fusarium solani. Ingested molded sweet potatoes by livestock causes interstitial pneumonia. The extrahepatic CYP enzyme CYP4B1, present in lung tissue, activates 4-ipomeanol to a reactive intermediate that reacts with nitrogen or sulfur nucleophiles and leads to toxicity.[53–55]
It has also been suggested that in the treatment of IPF, clinical meaningful precision medicine might be possible with the antioxidant N-acetylcysteine by taking into account polymorphism within TOLLIP.[56]

Conclusion

Although genetic variations in drug metabolizing enzymes may play an important role in the individual response on drug medication, there are many other factors involved such as age, renal and liver function, concomitant diseases, nutritional status, smoking and alcohol consumption. A 'one-size-fits-all' approach to medicine is based on broad population averages. The advent of personalized medicine is moving us closer to more precise, predictable, and powerful healthcare that is customized for the individual patient. Growing understanding of genetics and genomics provide many advantages in tailoring healthcare to each person's unique genetic make-up which may result in better disease prevention, more accurate diagnoses, safer drug prescriptions, and more effective treatments. It appears that genetic variations in metabolizing enzymes are able to enhance the drivers of DI-ILD. This paves the way for the potential usefulness of personalized medicine by genotyping and aiming to improve efficacy, tolerability, and drug safety. With this, knowledge on pharmacogenetics may finally serve as a predictor of toxicity and clinical response.
There is still a need for well designed prospective clinical trials that measure patient-oriented outcomes of selected genomic applications, and studies that evaluate the role of genomic variations in disease susceptibility, predicting prognosis, treatment response, and in tailoring drug treatment for individual patients. These investigations are aimed to help bridge the gap between 'personalized' and 'evidence-based' medicine.

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Assessing the role of drug metabolization and drug metabolites in adverse drug reactions

PART III

Chapter 3.1

Body weight gain in clozapine-treated patients: is norclozapine the culprit?

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Abstract

Background
The antipsychotic drug clozapine is associated with weight gain. The proposed mechanisms include blocking of serotonin ($5\text{-}HT_{2a/2c}$), dopamine (D_2) and histamine (H_1) receptors. Clozapine is metabolized by cytochrome P450 1A2 (CYP1A2) to norclozapine, a metabolite with more $5\text{-}HT_{2c}$ receptor and less H_1 blocking capacity.

Objective
We hypothesized that norclozapine serum levels correlate with body mass index (BMI), waist circumference and other parameters of the metabolic syndrome.

Methods
We performed a retrospective cross-sectional study in 39 patients (female n=8 (20.5%), smokers n=18 (46.2%), average age 45.8 ± 9.9 years) of a clozapine outpatient clinic in the Netherlands between 1 January 2017 and 1 July 2020.

Results
Norclozapine concentrations correlated with waist circumference (r=0.354, *P*=0.03) and hemoglobin A1c (HbA1c) (r=0.34, *P*=0.03). In smokers (smoking induces CYP1A2), norclozapine concentrations correlated with waist circumference (r=0.723, *P*=0.001), HbA1c (r=0.49, *P*=0.04) and BMI (r=0.63, *P*=0.004).

Conclusions
Elucidating the relationship between norclozapine and adverse effects of clozapine use offers perspectives for interventions and treatment options.

Introduction

Patients with schizophrenia have a higher prevalence of the metabolic syndrome and higher risk of cardiovascular disease mortality compared to the general population. Being overweight and obese are particular problems in these individuals, especially when second-generation antipsychotics, such as clozapine, are used.[1] The mechanism behind clozapine-induced body weight gain (BWG) has not been elucidated yet, but it is hypothesized that the blocking of serotonin (5-$HT_{2a/2c}$) receptors by clozapine plays an important role.[2]

In addition to the parent drug, drug metabolites should also be considered as a cause for BWG. Clozapine is metabolized by cytochrome P450 1A2 (CYP1A2) to norclozapine, a metabolite with more serotonin (5-HT_{2c}) receptor blocking capacity, more D_2 and D_3 agonis tic, and less H_1 receptor blocking capacity than clozapine.[3] Previous studies point towards a more important role for norclozapine than for clozapine in clozapine-associated BWG. Lu *et al.* showed that norclozapine serum levels and not clozapine serum levels were associated with increases in body weight, serum glucose and triglyceride serum levels. They compared two randomly assigned patient groups receiving either clozapine or clozapine with fluvoxamine, a CYP1A2 inhibitor that lowers the norclozapine/clozapine ratio. The patients with fluvoxamine addition had lower norclozapine serum levels and less BWG, body mass index (BMI), glucose and triglyceride serum levels. However, the authors could not rule out a contribution from fluvoxamine itself in the observed results.[4] In a retrospective audit, Lau *et al.* showed that clozapine users who smoke gained significantly more weight in 3 to 12 months compared to nonsmokers (+5.1% versus +1.2%).[5] Polycyclic aromatic hydrocarbons in cigarettes induce CYP1A2 and so advance the formation of norclozapine.[6] Lau *et al.* hypothesized that norclozapine should be the culprit but they had not measured norclozapine serum levels and could not confirm this pos ited relationship.[5]

More insight into the role of norclozapine in clozapine-associated BWG opens up treatment possibilities and interventions such as the introduction of phenoconversion to lower its formation. To further unravel the mechanism behind clozapine-induced BWG we hypothesized that higher norclozapine serum levels result in higher BMI and larger waist circumference, a parameter of the metabolic syndrome whose correlation with norclozapine serum levels has not been assessed before. The primary aim of this research was to assess the correlations between norclozapine serum levels and BMI and waist circumference. Furthermore, we aimed to acquire more insight into the correlation between norclozapine serum levels and other parameters of the metabolic syndrome, such as triglycerides and high-density lipoprotein (HDL)-cholesterol serum levels. As smoking induces CYP1A2 and hence impacts the metabolization of clozapine and the formation of norclozapine, the outcomes are stratified for smokers and nonsmokers.

Methods

Design and study population

We conducted an observational, retrospective cross-sectional study to assess the correlation between norclozapine serum levels and BMI, waist circumference and other parameters of the metabolic syndrome (triglycerides, HDL-cholesterol, fasting glucose and HbA1c serum levels) stratified for smokers and nonsmokers. The study population consisted of patients visiting a specialised clozapine outpatient clinic from the Reinier van Arkel Mental Health Institute, 's-Hertogenbosch, the Netherlands. For this study we included information of the last registered outpatient clinic visit with data on norclozapine serum levels and the metabolic parameters between 1 January 2017 and 1 July 2020. All patients visiting this clozapine outpatient clinic were eligible. Inclusion criteria were 18 years or older, valid measurements of norclozapine serum levels above the detection limit, and measurements of BW and waist circumference within 1 month prior to or after measurement of the norclozapine and clozapine serum levels. For the other parameters of the metabolic syndrome the time interval was set at 3 months prior to or after measurements of the serum drug levels. In case parameters were measured several times within the interval, the measurements nearest to the drug level measurement were included. In addition, patients were stratified into smokers and nonsmokers.

The study was approved by the local Medical Research Ethics Committee of the Reinier van Arkel Academy, 's-Hertogenbosch and received a waiver for the Dutch Medical Research Involving Human Subjects Act.

Sample size calculation

We calculated the sample size with an expected r=0.5 based on a type I error rate (α, two tailed) of 0.05, a type II error rate (β) of 0.20 and previously found correlation coefficients for norclozapine serum levels and BWG varying between r=0.16 and r=0.89.[7] Based on this calculation the study population should preferably comprise at least 29 patients.

Outcomes

For all eligible patients the following data were collected: gender (F/M), age (years), height (m), body weight (kg), smoking (yes/no/unknown), norclozapine and clozapine serum levels (µg/L), waist circumference (m) and other parameters of the metabolic syndrome triglycerides (mmol/L), HDL-cholesterol (mmol/L), fasting glucose (mmol/L) and HbA1c serum levels (mmol/mol). BMI was calculated with body weight (kg) and height (m) (weight [kg]/height [m]^2).

Determinant

Serum levels of norclozapine (µg/L) and clozapine (µg/L) were determined by high-performance liquid chromatography with ultraviolet detection (Hitatchi). The intra-assay and inter-assay coefficients of variation were <10% for clozapine and norclozapine. The lower limit of detection was 45 µg/L for clozapine and 55 µg/L for norclozapine. The serum levels of triglycerides (mmol/L), HDL-cholesterol (mmol/L), fasting glucose (mmol/L) and HbA1c (mmol/mol) were measured by Advia Chemistry XPT (Siemens) according to routine laboratory practice.

Data analysis

Patient characteristics are presented as mean with standard deviation (SD), median with range or frequency with percentage where appropriate and are stratified for smoking behaviour and gender. The Pearson correlation coefficient was assessed for norclozapine, clozapine and the ratio norclozapine/clozapine serum levels (µg/L) and BMI (kg/m^2), waist circumference (m) and the other selected parameters of the metabolic syndrome (triglycerides [mmol/L], HbA1c [mmol/mol], and HDL-cholesterol serum levels [mmol/L]) using IBM SPSS Statistics version 22. Furthermore, the Pearson correlation coefficients for norclozapine and clozapine serum levels and the parameters of the metabolic syndrome were calculated separately for smokers and nonsmokers using IBM SPSS Statistics version 22. A P value of less than 0.05 was considered statistically significant. No corrections for multiple tests were applied, as there were underlying hypotheses for stratification in smokers and nonsmokers.

Results

During the study period the clozapine outpatient clinic comprised 44 patients. Five patients did not meet the inclusion criteria: two patients had norclozapine serum levels below the detection limit of 55 µg/L, one patient had no body weight (BW) measurement within the set time interval, one patient had no waist circumference measured within the set time interval and one patient refused blood tests.
Therefore, the study population comprised 39 patients (female n=8, 20.5%) aged from 22 to 62 years (mean ± SD 45.8 ± 9.8 years). The characteristics of the 39 included patients are summarized in Table 3.1.1. None of the patients were “underweight” (BMI lower than 18.5 kg/m^2). The BMI of 13 patients (33.3%) was “normal” (18.5-24.9 kg/m^2), the BMI of 18 patients (46.2%) was categorized as “overweight” (25.0-29.9 kg/m^2), the BMI of seven patients (17.9%) was categorized as “obese” (higher than 30.0-39.9 kg/m^2) and one patient (2.6%) was categorized as morbid obese (higher than 40.0 kg/m^2). Seven out of eight females (87.5%) had a waist circumference over 0.88 m and 17 out of 31 males (54.8%) had a waist circumference over 1.02 m.

Correlation of norclozapine and clozapine serum levels with BMI and waist circumference

The Pearson correlation coefficients for norclozapine and clozapine serum levels and parameters of the metabolic syndrome are summarized in Table 3.1.2. When taking all patients together, norclozapine serum levels correlated with waist circumference (r=0.354, *P*=0.03; Figure 3.1.1), but did not correlate with BMI (r=0.282, *P*=0.08). After stratification for smoking behaviour, smokers showed a positive and significant correlation between norclozapine serum levels and BMI (r=0.63, *P*=0.005) and the correlation with waist circumference was stronger (r=0.723, *P*=0.001). In addition, the ratio norclozapine/clozapine serum levels correlated positively and significantly with waist circumference in smokers (r=0.488, *P*=0.04).

Correlation of norclozapine and clozapine serum levels with other parameters of the metabolic syndrome

Norclozapine serum levels correlated positively and significantly with HbA1c (r=0.34, *P*=0.03). In smokers norclozapine serum levels correlated more strongly with HbA1c (r=0.49, *P*=0.04).

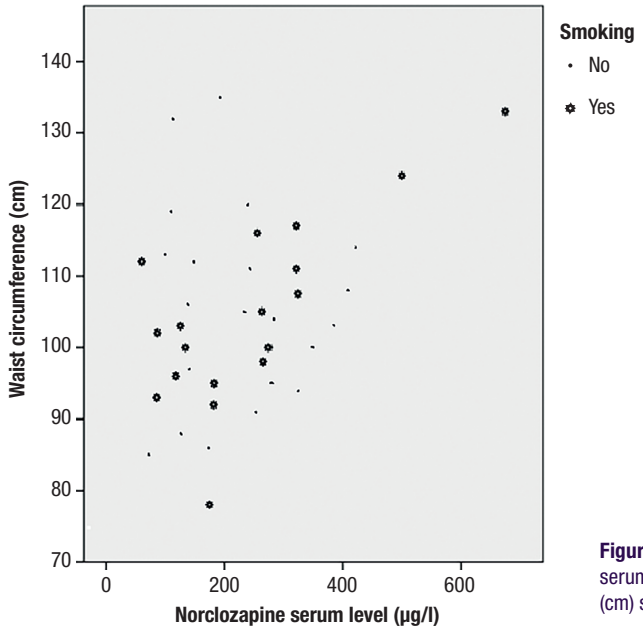

Figure 3.1.1 Scatterplot for norclozapine serum levels (µg/L) versus waist circumference (cm) stratified for smokers and nonsmokers.

Table 3.1.1 Patient characteristics.

n=39	
Female	8 (20.5 %)
Age (mean years ± S.D.)	45.8 ± 9.9
Smokers	18 (46.2%)
Diagnosis	
Schizophrenia	16 (41.0%)
Therapy resistance schizophrenia	16 (41.0%)
Schizophrenia affective disorder	6 (15.4%)
Bipolar disease type 1	1 (2.6%)
Mean norclozapine serum level (µg/l) (± S.D.)	232.3 ± 130.1
Female (n=8)	248.3 ± 123.2
Male (n=31)	228.1 ± 133.4
Smokers (n=18)	241.2 ± 155.4
Nonsmokers (n=21)	224.7 ± 107.2
Mean clozapine serum level (µg/l) (± S.D.)	340.7 ± 182.4
Female (n=8)	452.1 ± 225.6
Male (n=31)	311.9 ± 161.6
Smokers (n=18)	326.9 ± 190.6
Nonsmokers (n=21)	352.4 ± 179.0
Mean ratio norclozapine / clozapine serum level (µg/l) (± S.D.)	0.76 ± 0.38
Female (n=8)	0.60 ± 0.26
Male (n=31)	0.80 ± 0.40
Smokers (n=18)	0.86 ± 0.46
Nonsmokers (n=21)	0.68 ± 0.27
Mean body mass index (kg/m^2)	27.8 ± 5.5
Female (n=8)	29.8 ± 8.2
Male (n=31)	27.3 ± 4.6
Smokers (n=18)	27.7 ± 4.9
Nonsmokers (n=21)	27.9 ± 6.0
Mean waist circumference (m), 1 unknown	1.05 ± 0.13
Female (n=8)	1.07 ± 0.17
Male (n=31)	1.05 ± 0.13
Smokers (n=18)	1.05 ± 0.13
Nonsmokers (n=21)	1.06 ± 0.14
Mean triglycerides (mmol/l)	2.0 ± 1.4
Mean HDL-cholesterol (mmol/l)	1.3 ± 0.4
Mean fasting blood glucose (mmol/l), one unknown	6.0 ± 1.8
Mean HbA1c (mmol/mol)	37.9 ± 12.6

Table 3.1.2 Pearson correlation coefficients of norclozapine and clozapine serum levels and parameters of the metabolic syndrome

n = 39	Correlation coefficients norclozapine serum levels (µg/l)			Correlation coefficients clozapine serum levels (µg/l)		
Parameters of the metabolic syndrome	All patients	Smokers (n=19)	Non-smokers (n=21)	All patients	Smokers (n=19)	Non-smokers (n=21)
Body Mass Index (kg/m^2)	r=0.282 (P=0.08)	r=0.627 (P<0.01)	r=-0.035 (P=0.88)	r=0.039 (P=0.82)	r=0.318 (P=0.20)	r=-0.167 (P=0.47)
Waist circumference (cm)	r=0.354 (P=0.03)	r=0.723 (P<0.01)	r=-0.043 (P=0.85)	r=0.099 (P=0.55)	r=0.289 (P=0.24)	r=-0.065 (P=0.78)
Triglycerides (mmol/l)	r=0.058 (P=0.72)	r=0.221 (P=0.38)	r=-0.130 (P=0.57)	r=-0.098 (P=0.55)	r=-.042 (P=0.87)	r=-0.133 (P=0.57)
HDL-cholesterol (mmol/l)	r=-0.219 (P=0.18)	r=-0.411 (P=0.09)	r=-0.049 (P=0.83)	r=0.117 (P=0.48)	r=-0.143 (P=0.57)	r=0.271 (P=0.24)
Fasting glucose (mmol/l)	r=0.093 (P=0.58)	r=0.098 (P=0.71)	r=0.101 (P=0.66)	r=0.082 (P=0.62)	r= 0.033 (P=0.90)	r=0.120 (P=0.60)
HbA1c (mmol/mol)	r=0.340 (P=0.03)	r=0.493 (P=0.04)	r=0.122 (P=0.60)	r=0.054 (P=0.75)	r=-0.182 (P=0.47)	r=0.311 (P=0.17)

Discussion

This is one of the first studies addressing the relation between the clozapine metabolite norclozapine and BWG in patients with schizophrenia. We hypothesized that higher norclozapine serum levels result in higher BMI and larger waist circumference.

In our study population, norclozapine serum levels correlated with waist circumference but not with BMI. Waist circumference is considered a valuable predictor for metabolic syndrome and found to be the single best anthropometric surrogate for predicting insulin resistance in nondiabetic clozapine users.[8,9] This is the first study assessing the correlation between norclozapine serum levels and waist circumference and so it provides a first estimate of this relationship. Norclozapine serum levels did not correlate with assessed parameters of the metabolic syndrome except HbA1c.

After stratifying for smoking behaviour, norclozapine serum levels correlated with waist circumference, HbA1c and BMI in smokers but not in nonsmokers. Smoking induces CYP1A2 and enhances the formation of norclozapine. Lau *et al*. showed that smoking induces more BWG and hypothesized that the formation of norclozapine should be the culprit.[5] Other studies showed the reverse and found that norclozapine serum levels correlated best with BWG (r=0.89, *P*=0.046) in a very small sample of nonsmokers.[7] The positive and significant correlation between the norclozapine/clozapine serum level ratio and waist circumference in smokers points to a possible relationship between CYP1A2, norclozapine serum levels, smoking and parameters

of the metabolic syndrome. Although the exact relationships are not completely clarified, most studies, including ours, shows a better correlation between norclozapine serum levels and parameters of the metabolic syndrome than for clozapine serum levels and these parameters.[4,5,7]

The limitations of our study are the cross-sectional design, the rather small sample size and risk factors of clozapine-associated BWG that were not taken into account in our analysis. The cross-sectional design does not allow for establishing causal relationships. However, that was not the aim of our study and is suggested for follow-up research. The number of patients in our study is rather small but even this small sample shows that norclozapine serum levels correlate with important parameters of the metabolic syndrome. Furthermore, in our analysis we did not include known risk factors of BWG in clozapine users, such as low baseline BMI, female gender and negative symptoms. Although this information was obtainable, further stratification would not provide any conclusions due to the small number of patients.

Although this study illuminates the relationship between norclozapine serum levels and BWG only slightly, the correlation with waist circumference is of clinical relevance as it justifies more attention for high norclozapine serum levels in daily practice. Even though additional studies will be needed to confirm this relationship, researching the impact of interventions to decrease the formation of norclozapine, such as smoking cessation and CYP1A2 inhibition, on waist circumference is now conceivable. Further efforts in unravelling the mechanism of clozapine-induced body weight gain are important for clinical practice as clozapine is the most effective drug for treatment-resistant schizophrenia and adherence despite weight gain is a major challenge for patients.

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Chapter 3.2

Role of drug-gene interactions and pharmacogenetics in simvastatin-associated pulmonary toxicity

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Abstract

Introduction
Simvastatin has previously been associated with drug-induced interstitial lung disease. In this retrospective observational study, cases with non-specific interstitial pneumonia (NSIP) or idiopathic pulmonary fibrosis (IPF) with simvastatin-associated pulmonary toxicity (*n*=34) were evaluated.

Objective
To identify whether variations in genes encoding cytochrome P450 (CYP) enzymes or in the *SLCO1B1* gene (Solute Carrier Organic anion transporting polypeptide 1B1 gene, encoding the organic anion transporting polypeptide 1B1 [OATP1B1] drug transporter enzyme), and/or characteristics of concomitantly used drugs, predispose patients to simvastatin-associated pulmonary toxicity.

Methods
Characteristics of concomitantly used drugs and/or variations in the *CYP* or *SLCO1B1* genes and drug–gene interactions were assessed. The outcome after withdrawal of simvastatin and/or switch to another statin was assessed after 6 months.

Results
Multiple drug use involving either substrates and/or inhibitors of CYP3A4 and/or three or more drugs with the potential to cause acidosis explained the simvastatin-associated toxicity in 70.5% (*n*=24) of cases. Cases did not differ significantly from controls regarding CYP3A4, CYP2C9, or OATP1B1 phenotypes, and genetic variation explained only 20.6% (*n*=7) of cases. Withdrawal of simvastatin without switching to another statin or with a switch to a hydrophilic statin led to improvement or stabilization in all NSIP cases, whereas all cases who were switched to the lipophilic atorvastatin progressed.

Conclusion
Simvastatin-associated pulmonary toxicity is multifactorial. For patients with this drug-induced pulmonary toxicity who need to continue taking a statin, switching to a hydrophilic statin should be considered.

Introduction

Simvastatin is one of the most successful representatives of the cholesterol-lowering HMG-CoA reductase inhibitors, the class of drugs known as statins.[1] The drug is usually well tolerated, although statin therapy is associated with several adverse effects on hepatic, renal, and muscular systems.[2] Adverse reactions in skeletal muscle have been described and range from myalgia (pain) and myopathy (pain with evidence of muscle degradation) to rhabdomyolysis (severe muscle damage which may sometimes cause acute kidney injury).[3] The widespread use of simvastatin has also revealed several rare and sometimes severe toxicities in patients. So far, pulmonary toxicity has been reported mainly in case reports or case series.[4-7] The mechanisms behind simvastatin-associated pulmonary toxicity have not yet been elucidated.

A literature review revealed several isolated cases of statin-associated interstitial lung disease (ILD) involving simvastatin-associated pulmonary toxicity. The authors reported idiopathic pulmonary fibrosis (IPF), a severe form of ILD, and non-specific interstitial pneumonia (NSIP).[4-7] Moreover, Xu *et al.* reported that statin use was associated with ILD among smokers in the COPDGene study.[8]

The mechanisms and pathophysiology of toxicity associated with statins are still not fully clear, but include both patient-related factors such as age and pharmacogenetics, and factors that impact the pharmacologic, physicochemical, and pleiotropic characteristics of statins.[9] Statins are administered in lactone (e.g. simvastatin, lovastatin) and hydroxy acid forms (e.g. atorvastatin, fluvastatin, pravastatin) and have a complex metabolic pathway (see Figure 3.2.1). After intake, the interconversion of the two forms depends on the pH in the environment.[9-11] The lactone forms of statins are more cytotoxic to muscular cells than the hydroxy acid forms, and shifts in the ratio between the lactone and hydroxy acid forms may have an effect on pharmacological and toxicological response.[9] After absorption, statins are transported into hepatocytes by organic anion transport polypeptides (OATPs) and metabolized by cytochrome P450 (CYP) enzymes.[12] Genes encoding drug transporters and drug metabolizing enzymes are subject to polymorphisms, which may affect pharmacokinetics and serum drug levels, and subsequently have an impact on the degree of efficacy and toxicity.[12,13]

Genome-wide association studies showed that polymorphisms in the Solute Carrier Organic anion transporting polypeptide 1B1 gene (*SLCO1B1*, the gene encoding the OATP1B1 transporter) were associated with a higher risk of simvastatin toxicity.[14] Moreover, recommended change(s) in the medical management of simvastatin include testing the *SLCO1B1* genotype.[15] Previously, Li *et al.* demonstrated that genotype-guided statin therapy may improve patients' perceptions of statins and physician behavior, promoting higher statin adherence.[16] Furthermore, concomitantly used drugs may inhibit and/or compete for the available enzymes, which may also

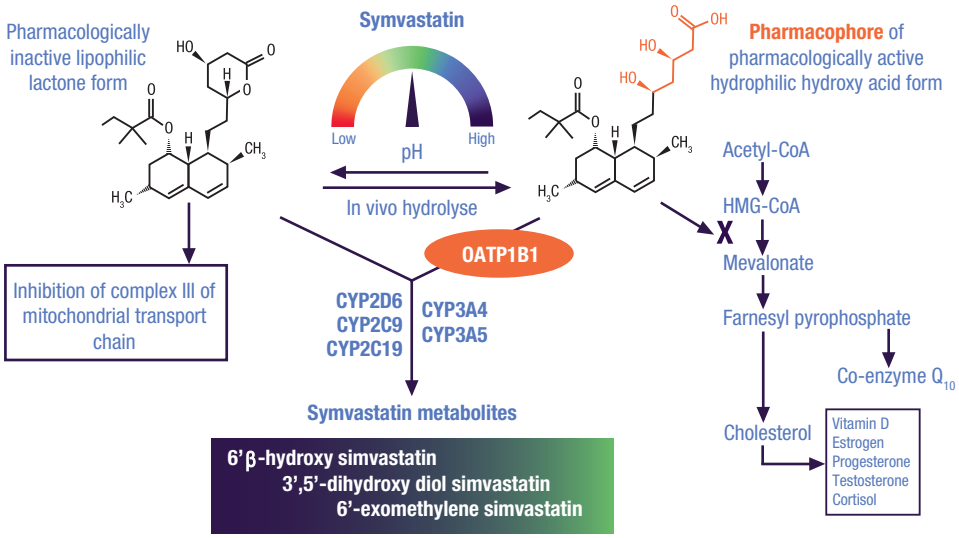

Figure 3.2.1 Simvastatin is administered in the pharmacologically inactive lactone form. In vivo, the lactone hydrolyzes to the corresponding cholesterol-lowering hydroxy acid form. The lactone form is the more lipophilic form and is predominantly present in an acidic environment. An acid–base imbalance may shift the lactone/hydroxy acid ratio.[9–11] Organic anion transport polypeptide 1B1 (OATP1B1) is an uptake transporter expressed on the sinusoidal (basolateral) side of hepatocytes. Simvastatin, like several other statins, is a substrate for this transporter. In hepatocytes, various cytochrome P450 iso-enzymes play a role in the biotransformation of simvastatin into various metabolites. It has been suggested that the lactone form inhibits complex III in the mitochondrial electron transport chain. In addition to inhibition of cholesterol formation, statins also hamper the biosynthesis of co-enzyme Q10, which is also critically involved in mitochondrial respiration.

affect the risk/benefit ratio. Inhibitors of drug metabolizing enzymes, such as paroxetine for CYP2D6, are able to transiently convert the phenotype of patients (so-called phenoconversion) and impact the pharmacokinetics of drugs metabolized or transported by the enzymes concerned.[17,18] So far, none of these mechanisms and factors in statin toxicity have been extrapolated to explain pulmonary toxicity.

Aim

The aim of the present retrospective observational study was to assess the possible involvement of concomitantly used drugs, as well as genetic variations in drug metabolizing enzymes and/or drug transporters involved in the metabolism of simvastatin, in patients with simvastatin-associated pulmonary toxicity. In addition, we evaluated the effect of withdrawal of simvastatin and/or its replacement by either a hydrophilic statin (pravastatin, rosuvastatin, fluvastatin) or a lipophilic statin (atorvastatin), on the outcome and course of pulmonary toxicity.

Methods

Study design and ethical statement

In this retrospective observational study of genotyped patients with NSIP or IPF, we assessed whether characteristics of concomitantly used drugs and/or variations in genes encoding CYP enzymes or in the *SLCO1B1* gene predispose patients to simvastatin-associated pulmonary toxicity. The study was conducted according to the principles of the Declaration of Helsinki (version 5, 2004) and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO). The protocol was approved by the Medical research Ethics Committees United (MEC-U) of the St. Antonius Hospital (approval R05-08A). Written informed consent for participation in this study was obtained from all subjects.

Selection of patients and controls

Patients who were referred between 2010 and 2019 to the ILD Center of Excellence at St. Antonius Hospital, Nieuwegein, the Netherlands (a tertiary referral center) with established ILD —either NSIP (*n*=233) or IPF (*n*=276)— and had been genotyped were considered for this observational study (Figure 3.2.2). For patients with unexplained ILD and a history of drug use, genotyping of certain specified genes was included in the diagnostic work-up. A multidisciplinary team confirmed the diagnosis based on clinical presentation, including dyspnea and hypoxia, pulmonary function impairment, exercise intolerance, and high-resolution computed tomography (HRCT) scan abnormalities, including multifocal areas of ground-glass opacity with intralobular interstitial thickening.[19]

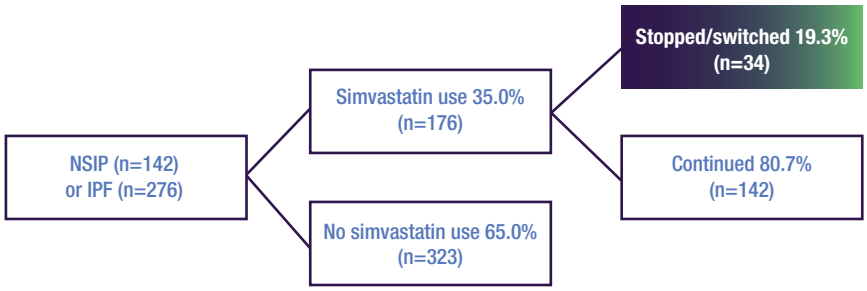

Figure 3.2.2 Flowchart of case selection: cases with non-specific interstitial pneumonitis (NSIP) or idiopathic pulmonary fibrosis (IPF) were divided into those who did not use simvastatin (not included) and those who used simvastatin. Those who used simvastatin were further divided into those who had stopped or switched to another statin (included, *n*=34) and those who continued simvastatin (not included, *n*=142).

Table 3.2.1 Summary of characteristics and metabolic pathways of concomitantly used drugs.[26–29]

	CYP2D6 INH	CYP2D6 IND	CYP2D6 SUB	CYP3A4 INH	CYP3A4 IND	CYP3A4 SUB	OATP1B1 INH	OATP1B1 SUB	Acidosis potential
Analgesics									
Codeine	0	0	1	0	0	0	0	0	0
Diclofenac	0	0	0	0	0	0	0	0	1
Morphine	0	0	1	0	0	0	0	0	1
Oxycodone; tramadol	0	0	1	0	0	1	0	0	0
Antacids									
(es)Omeprazole; pantoprazole	0	0	0	0	0	1	0	0	0
Antibiotics									
Sulfamethoxazole/ trimethoprim	0	0	0	0	0	0	0	0	1
Antidiabetics									
Gliclazide; glimepiride; tolbutamide	0	0	0	0	0	0	0	0	1
Antihypertensives									
Bisoprolol; formoterol	0	0	1	0	0	0	0	0	1
Enalapril	0	0	0	0	0	0	0	1	1
Losartan	0	0	0	0	0	1	0	0	0
Metoprolol; timolol	0	0	1	0	0	1	0	0	0
Nifedipine	0	0	1	0	0	1	0	0	1
Spironolactone	0	0	0	0	0	1	0	0	1
Valsartan	0	0	0	0	0	0	0	1	0
Verapamil	0	0	0	1	0	1	0	0	0
Antilipemics									
Ezetimibe	0	0	0	0	0	0	0	1	0
Simvastatin	0	0	0	0	0	1	0	1	0
Antiparkinson agent									
Ropinirole	0	0	0	0	0	1	0	0	0
Antithrombotics									
Acenocoumarol; phenprocoumon	0	0	0	0	0	1	0	0	0
Corticosteroids									
Prednisolone; prednisone	0	0	0	0	0	1	0	0	0
Psychotropics									
Amitriptyline	0	0	1	0	0	0	0	0	1
Escitalopram	0	0	1	0	0	1	0	0	0
Haloperidol	0	0	1	0	0	1	0	0	1
Temazepam; zolpidem	0	0	0	0	0	1	0	0	0
Others									
Flecainide	0	0	1	0	0	0	0	0	0
Tacrolimus	0	0	0	0	0	1	0	0	1
Tamsulosin	0	0	1	0	0	1	0	0	0

INH = inhibitor; IND = inducer; SUB = substrate.

Inclusion criteria

Patients who used simvastatin (*n*=176) were considered for this study. Only those who stopped or switched statin treatment were included (*n*=34). Patients stopped without replacement or switched to a hydrophilic statin or a lipophilic statin. They had already stopped simvastatin use or stopped simvastatin use shortly after referral.

Exclusion criteria

Patients who continued simvastatin, as well as those with other possible causes of pulmonary damage, such as infections or sarcoidosis, were meticulously excluded.
To compare the distribution of the allelic variants between cases (all Caucasian) and the general Caucasian population, controls were collected from the literature.[20-25]

Data collection

Demographic information on the included cases (i.e. gender, age at diagnosis, and concomitant drug use at diagnosis) were collected where necessary. Data on drug use were recorded and retrospectively supplemented with pharmacy data recorded in the electronic health records of the hospital. Concomitantly used drugs were classified according to their metabolic pathways as inhibitors and/or substrates for the enzymes studied. All cases were treated with standard dosages as used in clinical practice. No patient was treated with an unusually high dosage of any of the drugs used. Furthermore, the potential of the concomitantly used drugs to acidify the blood was assessed. The characteristics of the metabolic pathways of the concomitantly used drugs are summarized in Table 3.2.1. Appendix 3.2.A provides a more extensive summary of these characteristics.
The outcome or course of the ILD three months after diagnosis was assessed and recorded. Since the cases had been referred to the ILD Center of Excellence, the simvastatin treatment had already been stopped or reduced in most cases. Moreover, as determining serum simvastatin concentration is not standard practice in the Netherlands, no serum drug levels were available or could be obtained.

Genotyping

In all subjects, genomic DNA had been isolated from venous EDTA-anticoagulated blood. *CYP2C9* (*CYP2C9*2* [C430T], *CYP2C9*3* [A1075C]), *CYP2C19* (*CYP2C19*2* [G681A], *CYP2C19*3* [G636A], *CYP2C19*17* [C806T]), *CYP2D6* (*CYP2D6*3* [A2549del], *CYP2D6*4* [G1846A], *CYP2D6*6* [T1707del], *CYP2D6*5* [del] and *CYP2D6* copy number variation), *CYP3A4* (*CYP3A4*1B* [A-392G], *CYP3A4*22* [C522-191T]), and *SLCO1B1* (*SLCO1B1*5* [T521C]) alleles were identified by real-time PCR using the StepOnePlus™ Real-Time PCR System and TaqMan GTXpress Master/ Drug Metabolizing Genotyping Assay mixes

(Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

In accordance with conventional classification systems, individuals were classified (*CYP2D6, CYP2C9, CYP3A4*, and *CYP2C19*) as poor metabolizers (PMs) if they carried two non-functional alleles; as intermediate metabolizers (IMs) if they carried one non-functional allele; as normal metabolizers (NMs) if they carried one allele associated with reduced or increased activity and one functional allele or two functional alleles, and as ultra-rapid metabolizers (UMs) if they carried at least two copies of a functional allele plus a reduced activity allele, two copies of an increased function allele (*CYP2C19*17*) or three or more copies of a functional allele. *SLCO1B1* [521T/T] was classified as having normal transporter capacity, *SLCO1B1* [521T/C] as reduced transporter capacity, and *SLCO1B1* [521C/C] as very low transporter capacity. The laboratory that performed the tests is certified (ISO 15189:2012).

Data analysis

Concomitantly used drugs were evaluated to assess whether they were substrates, inhibitors or inducers of CYP3A4, CYP2D6, CYP2C19, and CYP2C9, and also whether they were OATP1B1 inhibitors or substrates. In addition, their potential to acidify blood was also evaluated. The characteristics of the concomitantly used drugs were categorized in terms of risk factors. Risk factors for simvastatin toxicity were based on previous research and expert opinion and were defined as using two or more drugs that are CYP3A4 substrates; using at least one drug that inhibits CYP3A4;[18] being a PM or IM of CYP3A4; carrying one or two *SLCO1B1* 521C alleles;[14] and using three or more drugs that have the potential to cause acidosis.[9] We assessed the concomitantly used drugs, the pharmacogenetic profile, and the number of risk factors for each case.

To compare the distribution of the allelic variants between cases (all Caucasian) and the general Caucasian population, historical controls were collected from the literature.[21-23,25]

To assess the effect of withdrawal of simvastatin after six months, we retrospectively evaluated the medical records of the 34 included cases.

Those patients who had used simvastatin (n=34) and stopped or switched to another statin were considered for this observational study (Figure 3.2.2). Patients were categorized into those who stopped using simvastatin without replacement and those who switched to a hydrophilic statin or to a lipophilic statin. Improvement was considered to have occurred if the forced vital capacity (FVC) had increased by 10% or more and/or the HRCT had improved. Stabilization was considered to have occurred if the FVC was stable (<10% increase or decrease), while deterioration/progression was considered to have occurred if FVC had decreased by >10% and the HRCT demonstrated progression of the features.[30,31]

Table 3.2.2 Demographics, genetic data and concomitantly used drugs of the studied population with non-specific interstitial pneumonitis (NSIP) or idiopathic pulmonary fibrosis (IPF).

	NSIP	IPF	Total
Number of cases (%)	16 (47.1%)	18 (52.9%)	34 (100%)
Age, years (± S.D.)	68.6 (± 9.5)	69.7 (± 7.1)	69.2 (± 8.2)
Gender, female (%)	5 (31.2%)	2 (11.1%)	7 (20.6%)
Simvastatin stopped, no switch to other statin (%)	7	5	12 (35.3%)
Improvement	6	0	6
Stable	1	5	6
Progression	0	0	0
Simvastatin stopped, switch to hydrophilic statin (%)	6	6	12 (35.3%)
Improvement	3	0	3
Stable	3	5	8
Progression	0	1 (stable, later progression)	1
Simvastatin stopped, switch to lipophilic statin (%)	3	7	10 (29.4%)
Improvement	0	0	0
Stable	0	0	0
Progression	3	7	10
SLCO1B1 polymorphisms (reduced transporter capacity or very low transporter capacity) (%) [not analysed]	3[3]	3[5]	6 (23.1%)
Concomitant use of OATP1B1 inhibitors/substrates	0	0	0
CYP3A4 polymorphisms (IM) (%)	1	1	2 (5.9%)
Concomitant use of CYP3A4 inhibitors/substrates	1	1	2
CYP2D6 polymorphisms (IM or PM) (%)	7	10	17 (50.0%)
Concomitant use of CYP2D6 inhibitors/substrates	5	5	10
CYP2C9 polymorphisms (IM or PM) (%)	8	8	16 (47.1%)
Concomitant use of CYP2C9 inhibitors/substrates	6	5	13
CYP2C19 polymorphisms (IM or PM) (%)	6	5	11 (32.4%)
Concomitant use of CYP2C19 inhibitors/substrates	4	2	6
Concomitant use of medication that induces acidosis (%)	17	15	32 (94.1%)
Patients using ≥ 3 drugs that induce acidosis	4	9	13

CYP = cytochrome P450, IM = Intermediate metabolizer, OATP1B1 = organic anion transporting polypeptide 1B1, PM = poor metabolizer, S.D. = standard deviation, SLCO1B1 = solute carrier organic anion transporting polypeptide 1B1.

Statistical analysis

Differences in *CYP3A4, CYP2D6, CYP2C9, CYP2C19*, and *SLCO1B1* genotype frequencies between cases and controls were assessed using a Fisher exact test in R (version 3.5.1, Vienna, Austria).[32] Actual allele distributions were compared with the expected frequencies that were calculated using the Hardy–Weinberg (HW) equilibrium. Deviations from HW were analyzed using the chi-squared test. A Bonferroni correction was applied to adjust for multiple comparisons; a P value <0.01 was considered to be statistically significant.

Results

In a sample of NSIP and IPF cases (n=499; average age [± S.D.]: 69.0 [± 9.5] years; 342 [68.5%] men; 157 [31.5%] women), a total of 176 cases (136 [77.3%] men; 40 [22.7%]) women) were identified as using simvastatin. Of these 176 cases, 34 (19.3% [34/176]; including seven women [20.6%]), with an average age (± S.D.) of 69.2 (± 8.2) years, were found to have stopped simvastatin use due to suspected pulmonary toxicity (i.e., deterioration occurred after simvastatin was started). There was no age difference between the original sample of NSIP and IPF cases (*n*=499) and the 34 identified cases that had stopped simvastatin. Although overall fewer women used simvastatin, the percentage of women who developed pulmonary toxicity did not differ from that of the male patients. The percentage of cases who had stopped simvastatin due to simvastatin-associated pulmonary toxicity did not differ between men (19.6%) and women (17.5%). The period of drug use varied from six months to 10 years, with a median of five years. None of the cases had used simvastatin in a dosage above 40 mg daily. Based on the available diagnostic tests and exclusion of other possible diagnostic options, the diagnosis was highly likely.
Diagnoses and outcomes of the 34 cases are summarized in Table 3.2.2.

Drug–gene Interactions and pharmacogenetics

Information on the concomitantly used drugs and phenotypes of the cases is summarized in Tables 3.2.2, 3.2.3, and 3.2.4. As regards drug interactions and pharmacogenetics, the majority of cases (82.4%) had several possible risk factors assumed to be associated with simvastatin toxicity, and only six cases (17.6%: cases 4, 8, 11, 14, 29, and 30) had none of these risk factors (Table 3.2.3). These cases all concomitantly used two drugs inhibiting CYP3A4, which might have played a role in the development of simvastatin toxicity. The OATP1B1 activity of three cases was unknown. Multiple use of drugs that are substrates and/or inhibitors of CYP3A4, and/or three or more drugs that have the potential to cause acidosis, explained the simvastatin-associated toxicity in 70.5% of cases. Genetic variation causing a reduced OATP1B1 activity and/or being an IM of CYP3A4 explained toxicity in 20.6%. In two cases, the only risk factor was reduced OATP1B1 activity, whereas in one case it was being an IM of CYP3A4. The risk factors assessed are summarized in Table 3.2.5.

All four cases who used a drug inhibiting CYP3A4 concomitantly used two or more other drugs, and up to seven drugs that are CYP3A4 substrates. Six cases had reduced OATP1B1 activity, but none of them used a substrate or inhibitor of the drug transporter, apart from simvastatin. Two of these cases used three or more CYP3A4 substrates (see Table 3.2.3).
The actual allele distributions of the cases were in HW equilibrium. However, the

Table 3.2.3 Individual cases, pharmacogenetics, possible mechanisms of interacting drugs and risk factors that may affect simvastatin-associated toxicity (marked in orange)

Nr.	Gender	Diagnosis	Age (years)	Course	2D6 INH	2D6 SUB	2D6	3A4 INH	3A4 SUB	3A4	2C9 INH	2C9 SUB	2C9	2C19 INH	2C19 SUB	2C19	OATP1B1 INH	OATP1B1 SUB	*SLCO1B1* T521C	Drugs with potential to acidify blood
Simvastatin stopped, no switch to another statin																				
1.	M	NSIP	79	improved	0	1	NM	1	5	NM	0	1	IM	1	1	NM	0	1	T/T	3
2.	F	NSIP	71	improved	0	0	PM	0	4	NM	0	2	IM	0	0	IM	0	2	n/a	2
3.	M	NSIP	68	improved	0	3	IM	0	3	NM	0	2	IM	1	1	NM	0	1	T/C	2
4.	F	NSIP	76	improved	0	0	IM	0	2	NM	0	0	IM	0	0	NM	0	1	n/a	1
5.	M	NSIP	69	improved	0	2	NM	0	3	NM	0	1	NM	0	0	NM	0	1	T/T	3
6.	M	NSIP	74	improved	0	0	NM	0	2	NM	0	1	NM	1	2	NM	0	2	T/T	0
7.	M	NSIP	86	stable	0	2	IM	0	5	NM	0	1	IM	1	2	NM	0	2	T/T	2
8.	M	IPF	70	stable	0	1	IM	0	2	NM	0	1	NM	0	0	IM	0	1	T/T	2
9.	M	IPF	75	stable	0	2	IM	0	3	NM	0	0	IM	0	0	NM	0	1	n/a	3
10.	M	IPF	58	stable	0	0	IM	0	1	NM	0	1	NM	0	0	PM	0	1	T/C	3
11.	F	IPF	82	stable	0	0	IM	0	1	NM	0	1	NM	0	0	NM	0	2	n/a	2
12.	M	IPF	69	stable	0	1	IM	0	4	NM	0	0	NM	1	1	NM	0	1	T/T	1
Simvastatin stopped, switched to a hydrophilic statin																				
13.	M	NSIP	63	rosuvastatin improved	0	2	IM	0	4	NM	0	2	NM	0	0	IM	0	1	T/T	2
14.	M	NSIP	68	pravastatin improved	0	1	NM	0	2	NM	0	0	NM	0	0	NM	0	2	T/T	1
15.	F	NSIP	67	fluvastatin improved	0	0	NM	0	2	NM	1	2	IM	1	1	IM	0	1	T/C	2
16.	M	NSIP	68	rosuvastatin stable	0	1	NM	0	6	NM	0	2	NM	1	1	NM	0	1	T/T	1
17.	F	NSIP	61	rosuvastatin stable	0	1	NM	1	2	NM	0	0	IM	0	0	NM	0	1	n/a	3
18.	M	NSIP	71	pravastatin stable	0	2	NM	2	7	IM	1	2	NM	1	2	IM	0	2	T/T	2
19.	M	IPF	72	rosuvastatin stable	0	1	IM	0	4	NM	0	1	NM	1	1	NM	0	1	T/T	2

Table 3.2.3 - Continued

Nr.	Gender	Diagnosis	Age (years)	Course	2D6 INH	2D6 SUB	2D6	3A4 INH	3A4 SUB	3A4	2C9 INH	2C9 SUB	2C9	2C19 INH	2C19 SUB	2C19	OATP1B1 INH	OATP1B1 SUB	*SLCO1B1* T521C	Drugs with potential to acidify blood
20.	M	IPF	75	rosuvastatin stable	0	0	IM	0	1	NM	0	1	PM	0	0	NM	0	1	T/T	3
21.	M	IPF	72	rosuvastatin stable	0	0	IM	0	1	NM	0	0	NM	0	0	NM	0	1	T/T	4
22.	M	IPF	65	rosuvastatin stable	0	1	IM	0	2	IM	0	0	NM	0	0	NM	0	1	T/T	2
23.	M	IPF	74	rosuvastatin stable	0	0	IM	0	1	NM	0	1	IM	0	1	NM	0	1	n/a	3
24.	M	IPF	72	rosuvastatin stable, later progression	0	1	NM	0	3	NM	1	1	IM	1	2	IM	0	1	T/T	3
Simvastatin stopped, switched to atorvastatin																				
25.	M	NSIP	58	progression	0	1	NM	0	2	NM	2	1	NM	0	1	IM	0	2	T/T	3
26.	F	NSIP	44	progression	0	1	IM	1	4	NM	0	1	IM	1	1	IM	0	2	T/T	1
27.	M	NSIP	74	progression	0	1	IM	0	4	NM	0	2	NM	1	3	NM	0	1	T/C	2
28.	M	IPF	78	progression	0	1	NM	0	4	NM	0	2	IM	0	1	NM	0	1	n/a	1
29.	F	IPF	69	progression	0	1	NM	0	2	NM	0	0	NM	0	0	NM	0	1	T/T	2
30.	M	IPF	58	progression	0	0	NM	0	2	NM	1	1	NM	0	1	UM	0	1	n/a	0
31.	M	IPF	63	progression	0	1	NM	0	3	NM	0	0	IM	0	0	IM	0	1	T/T	3
32.	M	IPF	72	progression	0	1	IM	0	3	NM	0	1	NM	1	1	NM	0	1	T/C	4
33.	M	IPF	56	progression	0	1	IM	0	2	NM	0	1	IM	0	1	IM	0	1	T/T	3
34.	M	IPF	75	progression	0	2	NM	0	2	NM	0	0	IM	0	1	IM	0	1	T/C	2

M = male; F = female; NSIP = non-specific interstitial pneumonitis; IPF = idiopathic pulmonary fibrosis; INH = number of concomitantly used drugs that inhibit the CYP enzyme; SUB = number of concomitantly used drugs that is metabolized by this enzyme; PM = poor metabolizer; IM = intermediate metabolizer; NM = normal metabolizer; UM = ultra-rapid metabolizer; OATP1B1 = organic anion transporting polypeptide 1B1; SLCO1B1 = solute carrier organic anion transporter 1B1; SLCO1B1 521T/T = wildtype; SLCO1B1 521T/C = heterozygous variant; n/a = not applicable.

cases tended to differ from the controls, with more PMs and/or IMs for *CYP2D6* (*P*=0.03) and *CYP2C19* (*P*=0.04). Cases did not differ significantly from controls in phenotypes of *CYP3A4* (*P*=0.45), *CYP2C9* (*P*=0.20), or *SLCO1B1* (*P*=0.88). More than half of the cases with reduced metabolic activity concomitantly used drugs that compete with and/or inhibit the metabolic enzymes concerned (see Table 3.2.4).

Outcomes after withdrawal of simvastatin

Twelve cases had stopped simvastatin use without continuing with another statin, while 12 cases had switched to a hydrophilic statin (rosuvastatin nine; pravastatin two; fluvastatin one) and 10 had switched to the lipophilic statin atorvastatin. Cases with NSIP who had stopped simvastatin without replacing it with another statin were more likely to improve (85.7%) than those who had simvastatin replaced by a hydrophilic statin (50.0%). The five IPF cases who stopped all stabilized, which in the case of IPF is the best that is achievable. No improvement was observed in cases who were switched to atorvastatin.

Table 3.2.4 Metabolic genotype frequencies of *CYP3A4, CYP2C9, CYP2D6, CYP2C19*, and *SLOC1B1* in cases and controls.[15–18]

	Cases (n = 34)	Historical controls (n = 235)[21]
CYP3A4 (P=0.45)		
Poor metabolizer	0 (0.0%)	0 (0.0%)
Intermediate metabolizer	2 (5.9%)	20 (8.5%)
Normal metabolizer	32 (94.1%)	215 (91.5%)
CYP2C9 (P=0.20)	Cases (n=34)	Controls (n=121)[22]
Poor metabolizer	1 (2.9%)	3 (2.5%)
Intermediate metabolizer	15 (44.2%)	36 (29.8%)
Normal metabolizer	18 (52.9%)	82 (67.8%)
CYP2D6 (P=0.03)	Cases (n=34)	Controls (n=765)[23]
Poor metabolizer	1 (2.9%)	42 (5.5%)
Intermediate metabolizer	18 (52.9%)	233 (30.3%)
Normal metabolizer	15 (44.2%)	490 (64.2%)
Ultra-rapid metabolizer	0 (0.0%)	0 (0.0%)
CYP2C19 (P=0.04)	Cases (n=34)	Controls (n=736)[23]
Poor metabolizer	1 (2.9%)	19 (2.6%)
Intermediate metabolizer	10 (29.4%)	163 (22.1%)
Normal metabolizer	22 (64.7%)	554 (75.3%)
Ultra-rapid metabolizer	1 (2.9%)	0 (0.0%)
SLCO1B1 (P=0.88)	Cases (n=26)	Controls (n=724)[25]
Very low transporter capacity	0 (0.0%)	12 (1.5%)
Reduced transporter capacity	6 (23.1%)	193 (26.5)

CYP = cytochrome P450; *SLCO1B1* = solute carrier organic anion transporting polypeptide 1B1.

Table 3.2.5 Extent to which cases (*n*=34) were exposed to possible risk factors that may affect simvastatin-associated toxicity.

Risk factors	Number of cases, n (%)
Using two or more other drugs that are CYP3A4 substrates	17 (50.0)
Using at least one drug that inhibits CYP3A4	4 (11.8)
Being an intermediate or poor CYP3A4 metabolizer	2 (5.9)
SLCO1B1 521T/C heterozygote or SLCO1B1 521C/C homozygous variant genotype (n=26, 8 not analyzed)	6 (23.1)
Using three or more drugs that have the potential to cause acidosis	13 (38.2)

CYP = cytochrome P450; *SLCO1B1* = solute carrier organic anion transporting polypeptide 1B1; *SLCO1B1521T/C* = heterozygote; *SLCO1B1 521C/C* = homozygous variant.

Discussion

In this retrospective study, we identified 34 cases with simvastatin-associated pulmonary toxicity. In our population, at least 34 out of 176 simvastatin users (19.3%) developed simvastatin-induced ILD. It is important, however, to realize that the number of simvastatin users in the general population is much larger, and our sample is influenced by selection bias. It is therefore hard to give a reliable indication of simvastatin-associated toxicity in the whole population of simvastatin users. However, the cases we studied showed a remarkable association. The fact that most of the NSIP cases (6/7) improved or stabilized (1/7) after withdrawal of the drug makes this association highly likely.

Drug interactions are a substantial cause of adverse effects, leading to hospitalization and sometimes to death.[33] Estimating the interaction potential of concomitantly used drugs is difficult. Drugs can be substrates, inhibitors or inducers of several drug metabolizing enzymes and drug transporters, and pathways may play major or minor roles in the biotransformation of the drug involved.[34] We assessed whether concomitantly used drugs could interact with the biotransformation of simvastatin and hence predispose patients to simvastatin-associated pulmonary toxicity. In the population studied, concomitant use of multiple drugs is quite common. Polypharmacy may lead to various drug–drug interactions. Moreover, we observed that almost all cases (90%) used at least one drug that was a substrate for CYP3A4, and that 35.3% used two or more drugs, while 50% used three or more. This is also considered the most important metabolic pathway for simvastatin. More than two-thirds of the cases (70%) used one or more drugs that may cause acidosis, which in turn may enhance the formation of the more toxic lactone form of simvastatin.

Simvastatin is available and administered in the lactone prodrug form. Depending on the local pH, simvastatin is primarily present in either the pharmacologically active hydroxy acid form or the pharmacologically inactive and more lipophilic lactone form.[9] Disturbances in the acid/base balance of the blood impact the interconversion

between the more lipophilic lactone and the more hydrophilic hydroxy acid form of simvastatin, and so have an effect on drug concentrations and the observed toxicity of simvastatin.[9,35] Various metabolites of simvastatin also contain the lactone moiety and will undergo a similar pH-dependent interconversion (Figure 3.2.1). Furthermore, the lactone form disturbs complex III in the mitochondrial transport chain. In an acidic environment (i.e. at a low pH), more of the lactone form is present, which is also associated with a higher incidence and severity of statin-induced myotoxicity.[10] There are various conditions that may lead to acidosis. Metabolic acidosis can occur when too much of the basic compounds is lost. This can be caused by diarrhea, kidney damage or the use of cholesterol-lowering agents. Extreme exercise can lead to lactate acidosis, and insufficiently controlled type 1 diabetes mellitus may also result in acidosis. Furthermore, acidosis may be caused by intoxication with alcohol, while dysfunctional lung physiology may lead to so-called respiratory acidosis. It is also known that the pH drops at inflammation sites. This means that more of the lactone form will be present at an existing inflammation site (e.g. in the lung).

The lipophilicity of simvastatin increases as the pH drops, as is indicated by the increase in log P octanol/water, which is 1.81, 2.06, and 3.62 at pH 7.4, 7.0, and 5.0, respectively.[35] Moreover, drug-induced acidification may also shift the lactone versus hydroxy acid equilibrium in favour of the toxic lactone form.[10] Drugs that cause metabolic acidosis can be grouped into four categories: drugs that represent exogenous acid loads (e.g. salicylates); drugs leading to loss of bicarbonate in the gastrointestinal tract or kidney (e.g. topiramate); drugs causing increased endogenous acid production (e.g. metformin or isoniazid, which may lead to lactic acid, or paracetamol and beta-lactam penicillin, leading to pyroglutamic acid); drugs that compromise renal acid secretion (e.g. drugs that act via inhibition of the renin-angiotensin-aldosterone system, or via impaired proximal or distal tubule H^+-secretion, as is the case with acetazolamide or lithium).[36] Most of our cases (94.1%) concomitantly used drugs that have the potential to at least slightly lower the blood pH. Although previous findings point to a relationship between the incidence and severity of statin-induced myopathy and a more acidic blood, or more lipophilic statins, it is too early to extrapolate this to statin-associated pulmonary toxicity.[9]

A major factor explaining statin-associated myotoxicity is the dosage, and consequently the concentration, of the drug.[37-40] Polypharmacy may influence the simvastatin concentration because of the possibility of drug–drug interaction. This may also apply to the pulmonary toxicity observed in our cases and is illustrated by the finding that after withdrawal of simvastatin without switching to another statin, the lung function of eight NSIP patients improved, while one of the two IPF patients also improved and the other remained stable. In the cases where the lipophilic simvastatin was replaced by a hydrophilic statin, the lung function either improved or remained stable. By contrast, replacing simvastatin by the lipophilic atorvastatin further worsened the clinical condition in all cases. This corresponds with what has

been found for statin-associated myotoxicity, which is related to the use of the more lipophilic statins simvastatin and atorvastatin.[41]

Genetic variation is also a risk factor, though to a lesser extent. Both *SLCO1B1* and *CYP3A4* appeared to be important in explaining simvastatin-associated DI-ILD. There are known risk factors for the more extensively investigated statin-associated myotoxicity, which include advanced age, female gender, drug interactions, genetic variability in drug metabolizing enzymes and transporters, lipophilicity of statins, coincident morbidities, and high doses of the statin used.[42] By contrast, risk factors for pulmonary toxicity are largely unknown.

We observed that variations in genes encoding enzymes known to play a role in simvastatin metabolism (CYP3A4 and/or OATP1B1) did not differ between cases and controls. Variations in genes encoding CYP enzymes and transport proteins influence the pharmacokinetics and toxicity of simvastatin, which is mainly biotransformed by CYP3A4 and transported into the hepatocytes by OATP1B1.[43] Using human liver microsomes, it has been shown by means of both immune inhibition and classical inhibitor studies that CYP3A4/5 is primarily involved (>80%) in the metabolism of simvastatin hydroxy acid. A minor contribution to the simvastatin metabolism (<20%) was found for CYP2C8.[44] The allelic frequencies of the two most important *CYP3A4* variants (*CYP3A4*22* and *CYP3A4*1B)* are low and have a limited role in the interindividual differences in CYP3A4 expression and activity.[45] Other sources, such as epigenetic factors, should also be considered. Furthermore, external factors such as medication (CYP3A4 inhibitors) and nutrition (grapefruit juice) may reduce the metabolic activity of CYP3A4, resulting in transient poor metabolism due to phenoconversion.[18] This is why we checked for *CYP3A4* polymorphism and co-administered drugs, which act either as competitive substrates or as inhibitors of CYP3A4. Although only two of our cases had *CYP3A4* polymorphisms, and the phenotypes did not differ significantly from healthy controls, most of our cases used several CYP3A4 substrates and inhibitors. One of the two cases who were genotyped as IMs used six other CYP3A4 substrates and two CYP3A4 inhibitors (patient 18). Such a situation may lead to increased simvastatin concentrations. Moreover, it is not only the number of drugs taken that is relevant, but also their dosages. A high dose of a single inhibitor may result in the same toxicity as a normal to low dose of multiple drugs metabolized by the same affected enzyme. The exact role of CYP2D6 in the biotransformation of simvastatin is not yet clear, but some studies point to a relationship between being a PM and having more Type A plasma-level dependent adverse drug reactions (ADRs), or between being a UM and having less cholesterol lowering effects.[46-48] Interestingly, 19 out of our 34 cases (55.9%) had *CYP2D6* polymorphisms (PM or IM), which is more than might be expected from data for the general population, and tended to differ from the controls.[49] However, this did not identify risk factors related to the use of drugs that have the potential to be a CYP2D6 substrate.

The protein OATP1B1 is not only important in transporting endogenous compounds

like bile acids, steroids, and hormones, but also for the transport of drugs like statins, ACE inhibitors, or methotrexate.[29] Polymorphisms in *SLCO1B1*, its encoding gene, result in altered transport of statins and their metabolites into the liver. Several studies have demonstrated that *SLCO1B1* T521C significantly affects simvastatin pharmacokinetics, causing decreased transport into hepatocytes, increased serum simvastatin levels and increased risk of myopathy.[50] Although several of our genotyped cases were carriers of *SLCO1B1* 521C (8/26), they did not differ statistically significantly from the controls in this respect.

In addition to the assessed role of concomitantly used drugs and pharmacogenetics, the mechanism behind statin-associated ILD should be further explored. One of the mechanisms that is associated with lung injury is the formation of reactive oxygen species (ROS) during biotransformation of xenobiotics including pharmaceuticals. This results in the formation of reactive electrophilic species such as epoxides and quinones, which react with cell molecules and cause direct cell toxicity.[51-53] Xu *et al.* showed that pravastatin and also atorvastatin treatment increases the formation of ROS, which results in increased immune response.[8] Furthermore, the generation of ROS during the metabolism of statins and the increase of oxidative stress are associated with well-known ADRs of statins, such as myopathy, nephrotoxicity, hepatotoxicity, and various diabetic complications.[54] The period during which simvastatin had been used varied from six months to 10 years. This underlines that simvastatin is not often recognized as a drug associated with pulmonary toxicity. Awareness of the pulmonary toxicity in addition to the better known toxicities like myopathies may avoid more progressive pulmonary damage and benefit patients' quality of life. One large cohort study has so far been unable to confirm that statins are a significant risk factor in the development of ILD.[7] However, Xu *et al.* found that statin use is associated with interstitial lung abnormalities among current and former smokers in the COPDGene study.[8] Statin use was positively associated with ILD (odds ratio 1.60; 95% confidence interval 1.03-2.50; *P*=0.04) after adjustment for covariates including a history of high cholesterol or coronary artery disease. Although we were unable to establish a causal relationship between the observed ILD and simvastatin use, we did observe that all cases who had stopped using simvastatin and had not switched to another statin improved, which is in line with other studies with well-documented cases.[4-6] In line with Xu *et al.*,[8] we acknowledge that although increased risks of ILD and radiologic features of pulmonary fibrosis are causes for concern, these risks likely do not outweigh the substantial benefits of statin therapy in patients with cardiovascular disease. Instead, we believe that clinicians should be aware that radiographic evidence of ILD, much like myopathy, can occur in some patients on statins.[3]

To verify whether there have been other reports of simvastatin-associated pulmonary toxicity besides what has been published,[4-7] we searched EudraVigilance (the system for suspected ADRs in the European Economic Area) for cases of pulmonary toxicity associated with simvastatin. This search yielded more than 200 reports of pulmonary

toxicity associated with simvastatin (164 ILD, four IPF, and 59 pulmonary fibrosis cases). Analyzing the data in these reports could contribute to knowledge about the association between simvastatin and ILD, and possibly provide further insight into the risk factors assessed.

Limitations of our study are the rather small number of cases and the fact we did not determine simvastatin serum levels. We deliberately selected only those who stopped simvastatin use, as in these cases the association between the observed toxicity and simvastatin use was acknowledged and was strongest. Because these cases were sent to a referral center, and in most instances simvastatin had already been stopped or stopped shortly after referral, we could not assess serum drug levels. In fact, in the case of simvastatin (hydroxy acid form), which has a very short plasma half-life (approximately 1.9 hours), assessing the serum drug level makes little sense and is also not standard practice in the Netherlands. Another limitation is that we only used a historical control group of Caucasians to compare polymorphism distribution. A more valid control group would be a sample of cases using simvastatin who did not develop pulmonary abnormalities. It would then be interesting to know which drugs were used concomitantly, especially drugs that may cause acidosis and/or those that affect the metabolic pathway of simvastatin, and to investigate whether the polymorphism distribution differed from that in our sample. Unfortunately, we do not have access to such a sample. Moreover, cases without pul monary symptoms will never be referred to our department.

Conclusion

We demonstrated that simvastatin may cause pulmonary toxicity. Simvastatin-associated pulmonary toxicity is complex, multifactorial, and under-recognized in clinical practice. Therefore, it is highly likely that the number of reported cases is underestimated. Multiple drug use, concomitantly used drugs that inhibit CYP3A4 or are metabolized by CYP3A4, and/or using three or more drugs that may acidify the blood, converting simvastatin to the more lipophilic and more toxic lactone form, explained the simvastatin-associated pulmonary toxicity in 70.5% of our patients.

Genetic variation, mostly resulting in a reduced OATP1B1 activity or in being an intermediate CYP3A4 metabolizer, explained toxicity in more than 20% of the cases. It is therefore essential to consider the metabolic properties of concomitantly used drug(s) in explaining toxicity, in addition to genetic variations. It should be realized that polypharmacy by itself may have a huge influence and that the drug interactions may be mistaken for genetic variation. Studies on concomitantly used drugs comparing cases with and without simvastatin-associated ADRs could be a topic for further research. Although we could not establish a firm relationship between the

use of simvastatin and the pulmonary toxicity observed in our cases, withdrawal of simvastatin without switching to another statin led to improvement in almost all NSIP cases. This not only points to a potential relationship, but also shows the best clinical strategy. If the use of a statin in patients with simvastatin-associated toxicity is essential, it appears that switching to a hydrophilic statin such as rosuvastatin yields better outcomes than switching to the lipophilic atorvastatin, which yielded the poorest outcomes. The advice that continuation with a hydrophilic statin is a better choice in case of simvastatin-associated toxicity applies not only to myotoxicities, but according to our cases, also to pulmonary toxicities.

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Appendix 3.2.A Characteristics and metabolic pathways of concomitantly used drugs. [26-29]

	CYP2D6 INH	CYP2D6 IND	CYP2D6 SUB	CYP3A4 INH	CYP3A4 IND	CYP3A4 SUB	CYP2C9 INH	CYP2C9 IND	CYP2C9 SUB	CYP2C19 INH	CYP2C19 IND	CYP2C19 SUB	OATP1B1 INH	OATP1B1 SUB	ACIDOSIS POTENTIAL
(es)Omeprazole	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0
Acenocoumarol	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0
Allopurinol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amitriptyline	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1
Amlodipine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atenolol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Azathioprine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bisoprolol	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Budesonide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carbasalate calcium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Celiprolol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Chlorthalidone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Citalopram	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Clopidogrel	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Codeine	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Colchicine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colecalciferol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dabigatran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Danazol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dexmethasone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diclofenac	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
Dipyridamole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Doxazosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enalapril	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Escitalopram	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0
Ezetimibe	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Appendix 3.2.A - Continued

	CYP2D6 INH	CYP2D6 IND	CYP2D6 SUB	CYP3A4 INH	CYP3A4 IND	CYP3A4 SUB	CYP2C9 INH	CYP2C9 IND	CYP2C9 SUB	CYP2C19 INH	CYP2C19 IND	CYP2C19 SUB	OATP1B1 INH	OATP1B1 SUB	ACIDOSIS POTENTIAL
Flecainide	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Formoterol	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1
Fosinopril	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Furosemide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gabapentin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gliclazide	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Glimeperide	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Haloperidol	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Hydrochlo-rothiazide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hydrokinine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Insulin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isosorbide dinitrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isosorbide mononitrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Levothyroxine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lisinopril	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Losartan	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
Lutein	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metformin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Metoprolol	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Morphine	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Naproxen	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Nifedipine	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Nitrofurantoin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxazepam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxycodone	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0

Appendix 3.2.A - Continued

	CYP2D6 INH	CYP2D6 IND	CYP2D6 SUB	CYP3A4 INH	CYP3A4 IND	CYP3A4 SUB	CYP2C9 INH	CYP2C9 IND	CYP2C9 SUB	CYP2C19 INH	CYP2C19 IND	CYP2C19 SUB	OATP1B1 INH	OATP1B1 SUB	ACIDOSIS POTENTIAL
Pantoprazole	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Paracetamol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Perindopril	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenprocoumon	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
Prednisolone	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Prednisone	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ramipril	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Risedronic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ropinirole	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Salbutamol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Simvastatin	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Sotalol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Spironolactone	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Sulfamethoxazole/ Trimethoprim	0	0	0	0	0	0	2	0	1	0	0	0	0	0	1
Tacrolimus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Tamsulosin	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Temazepam	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Timolol	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Tolbutamide	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1
Tramadol	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Valsartan	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Verapamil	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Vitamin K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zolpidem	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0

INH = inhibitor; IND = inducer; SUB = substrate.

Phenoconversion as a method to prevent drug metabolite induced adverse drug reactions

PART IV

Chapter 4.1

Inhibition of CYP2D6 with low dose (5 mg) paroxetine in patients with high 10-hydroxynortriptyline serum levels: a review of routine practice

Naomi T. Jessurun, Eugène P. van Puijenbroek, Leila S. Otten, Oenone Mikes, Annemiek Vermeulen-Windsant, Rob J. van Marum, Koen Grootens, Hieronnymus J. Derijks

British Journal of Clinical Pharmacology 2017;83(5):1149-115

Abstract

Background
Nortriptyline, a tricyclic antidepressant with selective noradrenergic reuptake inhibitor and little anticholinergic characteristics, is metabolized by CYP2D6 to the less active and more cardiotoxic 10-hydroxy(OH-)nortriptyline.

Objective
The aim of this review of routine practice was to retrospectively assess the pharmacokinetic impact of once daily low dose 5 mg paroxetine addition on nortriptyline and 10-OH-nortriptyline serum levels in patients with high 10-OH-nortriptyline serum levels.

Methods
Patients treated with nortriptyline with at least one high 10-OH-nortriptyline serum level above 200 µg/L and to whom paroxetine 5 mg was prescribed for phenoconversion were eligible for the assessment. To measure the impact of paroxetine on nortriptyline metabolism, the last nortriptyline and 10-OH-nortriptyline serum levels before, and the first nortriptyline and 10-OH-nortriptyline serum levels after reaching the steady state situation, which is one week after start of paroxetine, were evaluated. Patients with co-medication that influences CYP2D6 metabolic activity were excluded.

Results
A total of four patients met de inclusion criteria. Before the start of paroxetine administration, three patients had nortriptyline serum levels in the therapeutic range and one patient had a nortriptyline serum level below the therapeutic range. After the addition of 5 mg paroxetine, all subsequent nortriptyline serum levels fell within the therapeutic range and three out of four of the subsequent 10-OH-nortriptyline serum levels decreased to below 200 µg/L.

Conclusion
The addition of 5 mg paroxetine lowers 10-OH-nortriptyline serum levels and may allow treatment with nortriptyline for patients who have few other treatment options. Further prospective research is needed to address these options.

Introduction

Nortriptyline, a tricyclic antidepressant (TCA) with selective noradrenergic reuptake inhibitor and little anticholinergic characteristics, is metabolized by CYP2D6 to active metabolites, E-10-hydroxy(OH-)nortriptyline and Z-10-hydroxy(OH-)nortriptyline (see also Table 4.1.1).
Severe depression and depression with psychotic features in the elderly are treated with TCAs. Nortriptyline is preferred in the Netherlands, because it causes the least adverse drug reactions.[1] The therapeutic range of nortriptyline serum levels for anti-depressive treatment lies between 50 µg/L and 150 µg/L.[2] The 10-OH-nortriptyline serum level is preferably kept below 200 µg/L as higher levels are associated with increased occurrence of side effects (e.g. increase in QRS duration and Q-Tc intervals).[3] So far, only one prospective pharmacokinetic study in five healthy volunteering ultra-rapid metabolizers has been published describing the effects of the addition of 10-20 mg paroxetine (for CYP2D6 inhibition) to 50 mg nortriptyline in order to phenoconvert ultra-rapid metabolizers into poor metabolizers.[4] In the Reinier van Arkelgroep (RvA), 's-Hertogenbosch, the Netherlands, a mental health institution, the addition of low dose (5 mg) paroxetine once daily to patients with high 10-OH-nortriptyline (above 200 µg/L) serum levels is applied ad hoc to carefully lower the 10-OH-nortripyline level and maintain patients on nortriptyline therapy.
The aim of this review of routine practice was to retrospectively assess the pharmacokinetic impact of this once daily 5 mg paroxetine addition on nortriptyline and 10-OH-nortriptyline serum levels in patients with high 10-OH-nortriptyline serum levels.

Material and methods

Patients treated with nortriptyline in the RvA between 1 July 2011 and 1 July 2015 were considered; patients with at least one high 10-OH-nortriptyline serum level and to whom paroxetine 5 mg was prescribed for phenoconversion are described. Patients with co-medication that influences CYP2D6 activity (such as bupropion, cinacalcet, fluoxetine, quinidine, duloxetine, sertraline, terbinafine, amiodarone, cimetidine, dexamethasone, rifampin[5]) were disregarded. Nortriptyline and unconjugated 10-OH-nortriptyline were measured in serum by high-performance liquid chromatography with photodiode array detection. To assess the impact of paroxetine on nortriptyline metabolism, the last nortriptyline and 10-OH-nortriptyline serum levels before, and the first nortriptyline and 10-OH-nortriptyline serum levels after reaching the steady state situation, which is one week after start of paroxetine, were evaluated.

Table 4.1.1 Impact of 5 mg paroxetine on nortriptyline serum levels and hydroxyl(OH)-nortriptyline serum levels in five patients

Patient	1	2	3	4	Average (± s.d.)
F/M	F	F	F	F	
Age (years)	74	83	44	68	67.3 ± 16.7
Nortriptyline once daily dose (mg)	75	50	100	50	
Nortriptyline serum levels before (µg/l)	74	65	56	43	59.5 ± 13.2
OH-nortriptyline serum levels before (µg/l)	351	224	226	215	254.0 ± 64.8
Nortriptyline serum levels after (µg/l)	117	98	75	66	89.0 ± 23.0
OH-nortriptyline serum levels after (µg/l)	308	109	137	77	157.8 ± 103.1
% decrease in OH-nortriptyline serum levels	12%	51%	39%	64%	41.5% ± 22.0%
% increase in nortriptyline serum levels	58%	51%	34%	53%	49.0% ± 10%
Before paroxetine nortriptyline / OH-nortriptyline	0.20	0.29	0.24	0.20	0.23 ± 0.04
After paroxetine nortriptyline / OH-nortriptyline	0.37	0.89	0.54	0.85	0.66 ± 0.25

Results

Four patients received 5 mg paroxetine for phenoconversion. Before the start of paroxetine administration, three patients had nortriptyline serum levels in the therapeutic range and one patient had a nortriptyline serum level below the therapeutic range. All patients had 10-OH-nortriptyline serum levels above 200 µg/L. After the addition of 5 mg paroxetine, all subsequent nortriptyline serum levels fell within the therapeutic range and three out of four of the subsequent 10-OH-nortriptyline serum levels decreased to below 200 µg/L. The effect of the low dose paroxetine on nortriptyline and 10-OH-nortriptyline serum levels are summarized in Figure 4.1.1.

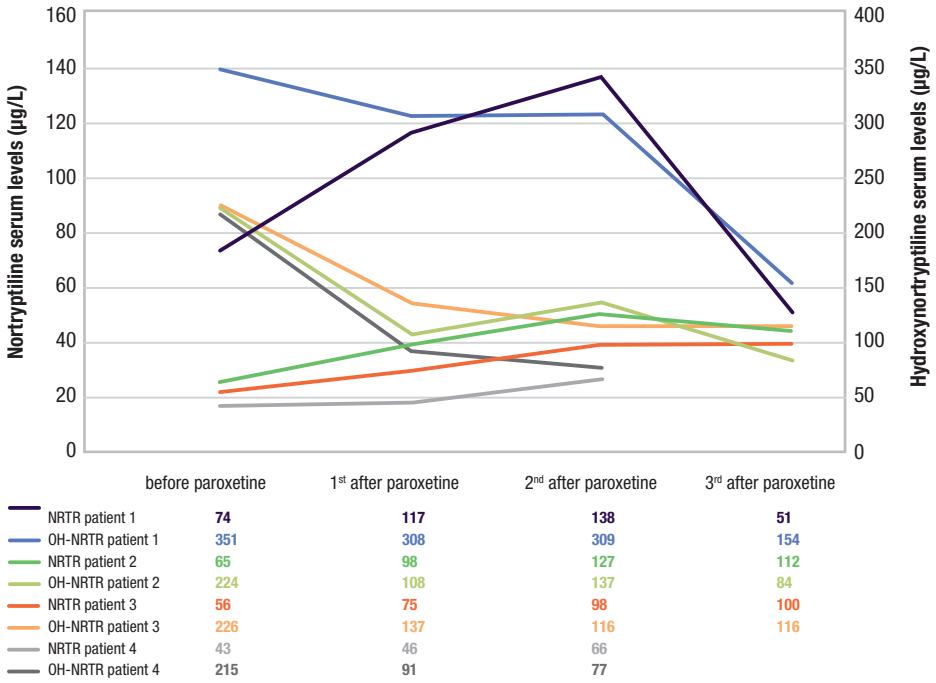

Figure 4.1.1 Increase of nortriptyline and decrease of hydroxynortriptyline serum levels (µg/L) after the addition of 5 mg paroxetine to the four patients. (Patient 1 had a dose reduction from once daily 75 mg to once daily 40 mg after the second follow-up nortriptyline/OH-nortriptyline serum level).

Discussion

This study suggests that the addition of low dose (5 mg) paroxetine to nortriptyline treatment is able to slow down nortriptyline metabolism. The increase in the ratio between nortriptyline/hydroxynortriptyline serum levels after the addition of paroxetine in all patients supports this. The outcomes are comparable with previous research which showed a decrease of 40% in 10-OH-nortriptyline serum levels after addition of paroxetine.[4] However, the retrospective design does have limitations; for example, the relatively small decrease of 10-OH-nortriptyline serum level in patient 1 could not be explained with the retrieved data. The intentional introduction of a drug-drug interaction to normalize skewed drug metabolism to optimize drug use is well known. Addition of allopurinol to thiopurine use in patients with high thiopurine methyltransferase activity and the addition of ritonavir to lopinavir use are both comparable interventions that are included in standard care.[6,7] No adverse drug reactions and changes in tolerability are recorded during the addition, and although both paroxetine and nortriptyline inhibit serotonine reuptake, none of the patients reported signs of serotonine syndrome which would be a possible adverse drug interaction. However, the dose of paroxetine is so low that despite the complex metabolism of this drug, with autoinhibition, the 5 mg once daily dosage will not lead to high paroxetine levels or CYP2D6 saturation.[4]

Conclusion

In conclusion, according to the outcomes of this study, the addition of 5 mg paroxetine lowers 10-OH-nortriptyline serum levels and may make treatment with nortriptyline possible for patients who have few other treatment options. To further address these possibilities, the research will be continued in a prospective design.

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Chapter 4.2

Inhibition of CYP2D6 with low dose (5 mg) paroxetine in patients with high 10-hydroxynortriptyline serum levels: a prospective pharmacokinetic study

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British Journal of Clinical Pharmacology 2021;87(3):1529-1532

Abstract

Background
The antidepressant nortriptyline is metabolized by cytochrome P450 2D6 (CYP2D6) to the less active and more cardiotoxic drug metabolite, 10-hydroxynortriptyline.

Objective
High serum levels of this metabolite (>200 µg/L) may lead to withdrawal of nortriptyline therapy. Adding CYP2D6 inhibitors reduce the metabolic activity of CYP2D6 (phenoconversion) and so decrease the forming of hydroxynortriptyline.

Methods
In this study, 5 mg paroxetine is administered to patients with high hydroxynortriptyline concentrations (>200 µg/L). The shift in number of patients to therapeutic nortriptyline (50-150 µg/L) and safe hydroxynortriptyline (<200 µg/L) concentrations, and the degree of phenoconversion, expressed as the change in ratio nortriptyline/hydroxynortriptyline concentrations before and after paroxetine addition, are prospectively observed and described.

Results
After paroxetine addition, 12 patients (80%) had therapeutic nortriptyline and safe hydroxynortriptyline concentrations. Hydroxynortriptyline concentrations decreased in all patients. The average nortriptyline/hydroxynortriptyline concentrations ratio increased from 0.32 to 0.59.

Conclusion
This study shows that addition of 5 mg paroxetine is able to lower high hydroxynortriptyline serum levels to safe ranges.

Introduction

Tricyclic antidepressants (TCAs) are important options for the treatment of severe depression and depression with psychotic features. Nortriptyline, a selective noradrenergic reuptake inhibitor, is the preferred TCA for elderly in the Netherlands because of its favourable adverse drug reaction (ADR) profile compared to other TCAs.[1] Nortriptyline is metabolized by cytochrome P450 isoenzyme 2D6 (CYP2D6) to the less active and more cardiotoxic drug metabolite, 10-hydroxynortriptyline.[2]
Nortriptyline serum levels should be kept in the therapeutic range (50–150 µg/L) and E-10-hydroxynortriptyline serum levels must be in the safe range (<200 µg/L) as higher levels are associated with cardiotoxicity.[3] Genetic polymorphisms of CYP2D6 can significantly influence the efficacy and safety of nortriptyline. Ultrarapid CYP2D6 metabolizers may have higher levels of the metabolite hydroxynortriptyline and are prone to ADRs, therapeutic failures and withdrawal of their treatment.[4] Reducing the metabolic activity of CYP2D6, phenoconversion, with CYP2D6 inhibitors such as paroxetine could be an effective strategy for rapid metabolizers to keep nortriptyline and hydroxynortriptyline serum levels within preferable ranges.[5] So far, there are only two published studies of this intended drug–drug interaction, both with a very small number of participants. One is a prospective pharmacokinetic study in five healthy volunteering ultrarapid metabolizers and the other is a retrospective review of routine practice (case-series) conducted in four female patients using nortriptyline, all with high E-10-hydroxynortriptyline serum levels above 200 µg/L.[5,6] Considering the addition of 5 mg paroxetine in patients with high hydroxynortriptyline serum levels belongs to standard care in in the mental health institute, Reinier van Arkel, 's-Hertogenbosch, the Netherlands.
The aim of this research was to prospectively observe the effects of adding paroxetine to nortriptyline therapy on nortriptyline and hydroxynortriptyline serum levels in patients with high (>200 µg/L) hydroxynortriptyline concentrations.

Methods

An observational prospective pharmacokinetic study was conducted between September 2016 and September 2019 in patients treated with nortriptyline and low dose, 5 mg, paroxetine addition within the mental health institute Reinier van Arkel, 's-Hertogenbosch, the Netherlands. The study received a waiver for the Dutch Medical Research Involving Human Subjects Act (WMO) by the regional medical ethics committee Brabant (#NW2016–05).
Patients were selected if they were treated with nortriptyline and had at least one high hydroxynortriptyline serum level (above 200 µg/L) for which paroxetine 5 mg

once daily was prescribed. Exclusion criteria were: comedication that influences CYP2D6 activity as defined by Flockhart[7] and renal function disorders defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.[8] Eligible patients were asked informed consent to participate in the study. For all participating patients, the last nortriptyline serum level before paroxetine addition and the first hydroxynortriptyline serum level within 1 to 4 weeks after paroxetine addition, were collected.
Nortriptyline and E-10-hydroxynortriptyline were measured in serum by high-performance liquid chromatography with photodiode array detection in the laboratory of the Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands.
The following information in the electronic health record was collected: nortriptyline dose before and with paroxetine addition, and concomitant drugs that interact via CYP2D6.[7]
We calculated the prevalence of patients with both nortriptyline therapeutic serum levels (50–150 µg/L) and safe hydroxynortriptyline (<200 µg/L) serum levels after paroxetine addition.
The impact of paroxetine addition for all observed patients is expressed by the decrease in hydroxynortriptyline serum levels (range, average, %). The change in metabolic activity, phenoconversion, is expressed as the change in ratio nortriptyline/hydroxynortriptyline serum levels before and after the addition of paroxetine.

Statistical analysis

As serum levels of nortriptyline and hydroxynortriptyline are not normally distributed, non-parametric tests are used for statistical analysis. A P-value <0.05 was considered statistically significant.

Results

A total of 17 patients received 5 mg paroxetine per day for phenoconverting nortriptyline metabolism by CYP2D6 inhibition. One patient stopped because of experiencing an increase in depressed mood. Another patient was excluded because nortriptyline and hydroxynortriptyline serum levels were not measured between 1 and 4 weeks after 5 mg paroxetine addition. The effects of addition of 5 mg paroxetine on hydroxynortriptyline and nortriptyline serum levels in the 15 remaining patients are summarized in Table 4.2.1.

Overall effect of 5 mg paroxetine addition on hydroxynortriptyline and nortriptyline serum levels

Before paroxetine addition, hydroxynortriptyline serum levels of the 15 observed patients ranged from 204 to 407 µg/L (average 264 µg/L) and 2 of these patients had nortriptyline serum levels below the therapeutic range. After paroxetine 5 mg addition, 12 out of 15 patients (80.0%) had nortriptyline and hydroxynortriptyline serum levels within the preferred ranges. Hydroxynortriptyline serum levels decreased in all patients (ranging now from 96 to 270 µg/L, average 173 µg/L) and 13 patients reached hydroxynortriptyline serum levels below 200 µg/L. One patient (patient 14) with a low nortriptyline serum level before paroxetine addition, kept nortriptyline serum levels below the therapeutic range.
The impact of paroxetine on CYP2D6 metabolic activity is further showed by the increase in average nortriptyline/hydroxynortriptyline serum level ratio from 0.32 to 0.59 ($P<0.01$), which expresses the introduced phenoconversion even more.

Effect of 5 mg paroxetine addition on nortriptyline and hydroxynortriptyline serum levels for patients without nortriptyline dose changes

Nine patients had no changes in their nortriptyline dose. Their average hydroxynortriptyline serum level decreased with 25.5% from 250.6 to 186.7 µg/L and their average nortriptyline serum level increased with 19.8% from 87.9 to 105.3 µg/L. Seven out of 9 patients reached hydroxynortriptyline serum levels <200 µg/L. All patients kept or reached therapeutic nortriptyline serum levels.

Table 4.2.1 Impact of 5 mg paroxetine once daily on nortriptyline and hydroxynortriptyline (OH-nortriptyline) serum levels. Therapeutic nortriptyline serum levels: 50–150 µg/L, safe OH-nortriptyline serum levels: <200 µg/L.

Patient	F/M	Age	Nortriptyline dose before (mg)	Nortriptyline dose with paroxetine addition (mg)	Nortriptyline serum level before (µg/L)	Nortriptyline serum level after (µg/L)	OH-nortriptyline serum level before (µg/L)	OH-nortriptyline serum level after (µg/L)	Ratio paroxetine nortriptyline/OH-nortriptyline before	Ratio paroxetine nortriptyline/OH-nortriptyline after
Patients without nortriptyline dose changing										
1	F	68	50	50	41	106	215	182	0.19	0.58
2	F	65	100	100	64	87	204	186	0.31	0.47
3	F	45	125	125	120	121	226	169	0.53	0.72
4	F	54	250	250	86	142	240	206	0.36	0.69
5	M	54	100	100	141	67	361	270	0.39	0.25
6	F	80	50	50	75	96	230	188	0.33	0.51
7	F	71	100	100	78	121	261	199	0.30	0.61
8	F	80	75	75	100	130	297	184	0.34	0.71
9	F	75	75	75	86	78	221	96	0.39	0.81
Patients with concomitant nortriptyline dose changes										
Patients with nortriptyline dose increase										
10	M	72	75	175	97	115	246	183	0.39	0.63
11	M	65	50	100	112	149	276	178	0.41	0.84
Patients with nortriptyline dose decrease										
12	F	73	75	50	58	92	221	151	0.26	0.61
13	F	73	100	50	70	58	407	110	0.17	0.53
14	F	46	150	100	36	33	280	154	0.13	0.21
15	M	68	100	75	93	103	277	139	0.34	0.74
Average (±S.D.)		66.8 ± 11.4	98.3 ± 50.4	98.3 ± 53.8	83.8 ± 28.5	99.9 ± 33.0	264.1 ± 56.5	173.0 ± 45.4	0.32 ± 0.1	0.59 ± 0.18

Discussion

This study shows that adding low dose (5 mg) paroxetine to nortriptyline treatment in patients with high hydroxynortriptyline serum levels is able to attain safe hydroxynortriptyline and therapeutic nortriptyline serum levels. This outcome is in line with a previously published case series of this addition in four patients of whom three reached preferred serum levels for both nortriptyline and hydroxynortriptyline.[5]
Adding a new drug with possible ADRs should always be carefully considered. Although ADRs are not specifically researched in this study, it is not expected that the low dose (5 mg) paroxetine once daily will lead to substantial ADRs, to high paroxetine levels or to CYP2D6 saturation.[9]

A limitation of this pharmacokinetic study is the observational design and there were no measures to improve adherence to the intended treatment and prescribed medication. The changes in serum drug and metabolite levels of Patient 5, for example, are not consistent with changes in other patients and not with what is expected. Furthermore, the nortriptyline dose was not fixed and prescribers were free to adjust the nortriptyline dose to what they expected best for their patients. Although, Patient 14 had low nortriptyline serum levels before paroxetine addition, the nortriptyline dose was reduced and, in this case, paroxetine addition was not able to increase the nortriptyline serum level to therapeutic ranges. In this study, the subpopulations of patients with nortriptyline dose unchanged, increased and decreased with paroxetine addition are too small to draw conclusions on advice for nortriptyline dosing when adding paroxetine 5 mg. Further research on nortriptyline dosing with paroxetine addition should be conducted.

Conclusion

In conclusion, despite the rather small number of patients, this study shows that paroxetine addition to nortriptyline therapy in patients with high hydroxynortriptyline serum levels as a result of high CYP2D6 metabolic activity, such as in CYP2D6 ultrarapid metabolizers, allows for the attainment of safe hydroxynortriptyline serum levels.

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General discussion and summaries

PART V

Chapter 5.1

General discussion

Introduction

There are various ways in which drugs may lead to adverse drug reactions. The best known reactions are those that arise from the mechanism of action of the drug itself and that are dose-dependent and reversible. Less common and less well-known are adverse drug reactions that arise due to the formation of toxic metabolites in the metabolic pathways.

The aims of the research underlying this thesis were:

1. to further extend our knowledge about the role of drug metabolization,
2. to explore data sources after marketing authorization to assess the role of drug metabolization and drug metabolites in adverse drug reactions, and
3. to investigate phenoconversion as a method to avert metabolite-induced adverse drug reactions.

In this general discussion chapter, the main findings presented in this thesis are put in the broader perspective of the current need to optimize the utilization of available knowledge, and ends with recommendations for pharmacovigilance activities, clinical practice, and future research.

A role for drug metabolization and drug metabolites in adverse drug reactions

It is well-known that metabolites of ingested or otherwise administered drugs may exert other adverse effects than the parent drug. These adverse effects may relate to an altered pharmacological mechanism or to bioactivation of the parent drug to form reactive metabolites. More knowledge about the role of drug metabolism and drug metabolites, and utilization of this knowledge in research into the development of adverse drug reactions, may help to understand the mechanism of adverse drug reactions.

Using historical pharmacovigilance information on drug metabolization to predict adverse drug reactions

Historical pharmacovigilance information from previously marketed structurally related drugs can provide insight into a possible role of drug metabolism and drug metabolite formation and so predict associated adverse drug reactions. Both bromfenac (1998) and lumiracoxib (2007) have been withdrawn from the market for oral administration in Europe and the United States because of acute liver failure, leading to liver transplantations and to fatal fulminant hepatitis.[1,2] However, when bromfenac was approved for marketing authorization in 1997 and lumiracoxib in 2003, there was already a mounting body of evidence that many acidic nonsteroidal anti-inflammatory drugs (NSAIDs) are metabolized to reactive (acyl glucuronide)

metabolites, which are associated with hepatotoxicity.[3,4] NSAIDs with similar acidic chemical structures had previously been withdrawn from the market shortly after release due to rare but severe hepatotoxicity, which in some cases was eventually fatal. Examples are ibufenac (market authorization 1966 – withdrawal 1968), benoxaprofen (1980 – 1982) and pirprofen (1982– 1990).[5-11] Without claiming that severe hepatotoxicities due to the use of bromfenac and lumiracoxib could easily have been predicted, examining the available information on drug metabolization and drug metabolites might have pointed to important drug safety information.

Part II explored the role of drug metabolization in observed liver and lung toxicity. In the study discussed in chapter 2.1, the theory that bioactivation leads to reactive metabolites which cause adverse drug reactions was tested using real world cases from the WHO's Vigibase™ database. We compared the reported hepatotoxicity between NSAIDs that are metabolized to reactive drug metabolites and NSAIDs less prone to such metabolization.[5-7,11] Hepatotoxicity was more often reported for NSAIDs in the first group (bromfenac, lumiracoxib, diclofenac) compared to those in the second group (ibuprofen, naproxen).[12] No differences in reports were observed for hemorrhage, an adverse drug reaction not related to the forming of reactive metabolites.[12] These findings are in line with the theory that bioactivation of certain chemical structures leads to adverse drug reactions that are unrelated to the pharmacological activity of the drug.

Using information on drug metabolization to explain adverse drug reactions

Interstitial lung disease is not a well-known adverse drug reaction of tamsulosin and has so far only been seen in a small subset of users. Chapter 2.2 discusses a series of male patients with lung toxicity who all used tamsulosin and were assessed to see whether the metabolic activity of drug-metabolizing enzymes and the metabolization of tamsulosin in these patients could explain the observations.[13] The phenotypes of the identified cases differed significantly from that of a control population of healthy male volunteers, in that all of the study cases had low CYP2D6 metabolic activity and were either CYP2D6 poor metabolizers or CYP2D6 intermediate metabolizers. This, together with alternative metabolization pathways and the formation of drug metabolites prone to bioactivation, point to a role for drug metabolization as a factor in the onset of the observed pulmonary toxicities.

While cases were selected between 2009 and 2020, the first patient to present with interstitial lung disease in whom tamsulosin was the suspected drug was seen long before in 2002. However, it took more than 10 years to elucidate only a small part of what is seen in clinical practice by using research published in 1998 on the metabolization of tamsulosin and the identification of the drug metabolites formed.[14]

Why the role of drug metabolization in adverse drug reactions is still underrecognized

Both studies discussed in Part II of this thesis (Chapters 2.1 and 2.2) illustrate an enduring gap between the available information on drug metabolization and drug metabolites and adverse drug reactions observed in clinical practice when the drug is used. The studies show that it is not easy to close this gap by establishing or confirming a relationship between drug metabolism and drug metabolites in the development of adverse drug reactions.

First, the best way to confirm a causal relationship between drug metabolization, drug metabolites and adverse drug reactions would be to demonstrate a relationship between the suspected metabolites formed by drug metabolization and the occurrence of the adverse effects, supported by drug level measurements and clear outcomes. However, data on serum drug levels are generally lacking in clinical practice. Neither serum levels of NSAIDs, nor those of tamsulosin, nor those of their drug metabolites were measured in our studies. In our cases, only clinical improvement or at least stabilization after withdrawal of the drug indicated a causal relationship. Another option is provocation after cessation, so-called rechallenge, but this is rarely considered ethical, given the potential risks and severity of adverse drug reactions. Second, investigating rare adverse drug reactions requires large numbers of patients, which makes intervention studies expensive and almost impossible. With only observational research available, the confirmation of a causal relationship between drug metabolization, drug metabolites and adverse drug reactions is often difficult. Third, although some drugs on the market do contain chemical structures that theoretically may cause adverse drug reactions after metabolization, this fortunately does not always happen in practice. Numerous drugs on the market that contain chemical structures which are theoretically prone to form reactive metabolites do not cause the expected adverse drug reactions. Canagliflozin, an antidiabetic drug, contains the thiophene chemical structure that could theoretically lead to bioactivation, but the expected toxicity has not been reported yet.[15] Likewise, rivaroxaban, which is used to prevent blood clots, also contains the thiophene and dianilide chemical structures that are prone to bioactivation, but it is not associated with the expected hepatotoxicity.[15] Both drugs are metabolized by other pathways, which fortunately do not result in toxicity and make the safe use of these drugs possible. This shows that if we abstained in advance from prescribing drugs with chemical structures prone to bioactivation, we would be deprived of countless life-saving drugs, so we may conclude that structural alerts alone will not accurately predict adverse drug reactions.

Although there are many obstacles in the assessment of the role of drug metabolization and drug metabolites in adverse drug reactions, both our studies show that including information from medicinal chemistry and drug development research helps to predict and explain observed adverse drug reactions, and may ultimately assist in approaches to circumvent them.

A need for more and different data sources

When a drug is allowed on the market, post-marketing safety surveillance starts. Before market authorization, the drug has only been used by small groups of healthy volunteers or carefully selected patients. After market authorization, it is widely available for patients who may have multiple conditions and use multiple drugs. The cornerstone of post-marketing drug surveillance is spontaneous reports of adverse drug reactions sent to national and international drug authorities by patients and healthcare professionals.[16]

To extend our knowledge about the role of drug metabolization and drug metabolites in adverse drug reactions, spontaneous reports are less appropriate, as the collected data does not contain the required information. Healthcare professionals often perceive the reporting of adverse drug reactions to national pharmacovigilance centres as a tedious exercise outside the health care process. Focus group studies among physicians working in a tertiary teaching hospital showed that the workload in the hospitals and the resulting time constraints hamper this reporting.[17] Consequently, information on the genetic variety or phenotypes of drug metabolizing enzymes is rarely reported. Likewise, serum drug levels of parent drugs, let alone those of drug metabolites, are rarely reported. In addition, the data collected in spontaneous reporting databases are subject to many biases such as notoriety bias, indication bias or protopathic bias.[18]

Available data sources after marketing authorization

Extending our knowledge about adverse drug reactions in which a role for drug metabolizing enzymes and drug metabolites is suspected may thus require other data sources. We used data collected in a clozapine outpatient clinic to assess the correlation between norclozapine serum levels and body weight gain (Chapter 3.1). To gain more in-depth knowledge of the association between simvastatin and pulmonary toxicity (Chapter 3.2), we used data in the biobank of the ILD Center of Excellence of the Department of Pulmonology of the Sint Antonius Hospital, Nieuwegein, the Netherlands.[19,20]

There are far more data sources available for pharmacovigilance purposes. They provide real-world information on adverse drug reactions which is useful for elucidating and strengthening associations between drug metabolization, drug metabolites and adverse drug reactions. As adverse drug reactions impact on the treatment pathways and the quality of life of patients, patient tracking systems such as electronic health record (EHR) systems and patient registries often provide entry fields for recording adverse drug reactions. The ability to share data on adverse drug reactions in EHR repositories of hospitals, pharmacies and general practitioners' practices in the near future offers great potential. It will make far more data available, including

information on therapeutic drug monitoring and pharmacogenetics, to assess patient-related factors in the development of adverse drug reactions.[21,22] Although these data sources may collect important drug safety information, there will always be a trade-off between the best methods to answer the research question and the limitations of the collected data.[21-28] Both our studies show that although highly specific data is required for assessing and strengthening the role of drug metabolization and drug metabolites in adverse drug reactions, these data sources do exist and have proved to be suitable to extend our knowledge.[18,29]

Predicting and preventing

The ultimate aim of exploring the role of drug metabolization and drug metabolites is to prevent drug toxicities. Once the pathway of toxicity has been elucidated, steps to prevent harm can be taken. When drug metabolism results in an excess of unwanted drug metabolites and predicts adverse drug reactions, phenocoversion is a possible intervention to prevent toxicity, an intervention that is currently not often utilized.

Chapter 2.3 discusses a literature review we conducted to explore the role of pharmacogenetics in predicting cytotoxic mechanisms and risks of adverse effects. It shows that genetic variation in metabolizing enzymes is able to enhance the drivers of drug-induced lung toxicity. Absent or very poor enzyme activity and enhanced enzyme activity may both result in drug toxicity, showing that skewed drug metabolism due to genetic variation may result in adverse drug reactions, as is seen with the use of thiopurines, fluoropyrimidines, clopidogrel, and codeine.[30-38]

Once a skewed drug metabolism has been identified as a key factor in the risk of experiencing adverse drug reactions, and there are very few or no other therapeutic options, phenoconversion should be considered as an intervention. In Chapters 4.1 and 4.2 we showed that adding low-dose paroxetine to nortriptyline therapy in patients with high hydroxynortriptyline serum levels allows safe drug and drug metabolite serum levels to be attained, and that phenoconversion is an option to keep patients on their nortriptyline therapy.

Although the introduction of a new drug increases the risk of new adverse drug reactions, phenoconversion has already proven its viability in reducing the risk of adverse drug reactions and improving drug response. The addition of low-dose allopurinol, a xanthine oxidase inhibitor, to lower the levels of the unwanted drug metabolite methylmercaptopurine led to better drug response and fewer adverse effects of thiopurines.[39] This is a very well-known and popular example of an intentional drug-drug-interaction which successfully led to better drug response; it has been referred to over and over again.[34,39,40]

Implementing phenoconversion

Before phenoconversion of drug metabolizing enzyme activity can be regarded as the optimal intervention and is worth investigating, it must be certain that drug metabolization and the drug metabolites formed play a consistent and substantial role in the observed adverse effects. There is a multitude of factors, both intrinsic patient-related ones and extrinsic ones, that may blur the relationship. Intrinsic patient-related factors such as higher age, cancer and inflammation result in reduced metabolic activity, while concomitant use of CYP450-inhibiting drugs is one of the extrinsic factors that result in lower metabolic activity. Smoking and CYP450 inducers are extrinsic factors that result in higher metabolic activity.[41,42,43] It is important to take these factors into account in advance and to supervise this intervention together with a pharmacist, as therapeutic drug monitoring is the most appropriate method to monitor the pharmacokinetic effects.

Despite all possible complicating factors, optimizing pharmacotherapy with phenoconversion by reducing the metabolic activity of CYP2D6 through the addition of low-dose paroxetine, as described in the review of clinical practice (Chapter 4.1) and implemented in the resulting pharmacokinetic study (Chapter 4.2) did result in more favourable drug serum levels of nortriptyline and its metabolite.[44,45] Moreover, the implementation was easy and effective, and none of the expected adverse drug reactions that could be attributed to the use of paroxetine were observed. The addition of low-dose paroxetine provided an opportunity for the included patients to continue their best pharmacotherapeutic option, and shows that phenoconversion is a valuable contribution to more personalized medicine.

The main findings of the studies presented in this thesis are:

1. Although the role of drug metabolization and drug metabolites in adverse drug reactions is acknowledged in general, there is still much ground to be gained. Closing the gap between available relevant information from, for example, previously marketed structurally related drug, medicinal chemistry and drug development studies and the application of this information in assessments of possible adverse drug reactions will increase our understanding of drug toxicities.
2. To extend our knowledge and to assess the relationship between drug metabolization, drug metabolites and adverse drug reactions, common data sources for pharmacovigilance should be expanded. Data collected in clinical practice and data derived from a disease-related biobank proved to be appropriate.
3. When drug metabolism results in an excess of unwanted drug metabolites, phenoconversion is a valuable intervention to prevent drug metabolite-induced adverse drug reactions. Its application should be explored more extensively.

Recommendations for pharmacovigilance activities, clinical practice, and future research

Based on the studies in this thesis, implications for pharmacovigilance and clinical practice are presented below, while recommendations for future research are also offered. They may help to improve pharmacovigilance activities and ultimately enhance the safe use of drugs.

Recommendations for pharmacovigilance activities

1. **Create awareness of the role of drug metabolization and drug metabolites in drug safety**

The goals of pharmacovigilance are to identify new information about hazardous associations with medicines and to prevent harm to patients treated with drugs in clinical practice. The studies discussed in this thesis show that there is a role for drug metabolization and drug metabolites in the development of adverse drug reactions, and that knowledge about this topic may even predict and prevent adverse drug reactions in the future. Therefore, drug metabolization and drug metabolites should be more prominently included in the assessment of adverse drug reactions.

2. **Include historical pharmacovigilance information and information collected in drug development research more actively in pharmacovigilance**

Historical pharmacovigilance information and outcomes of drug development research offer a wealth of information which is useful for pharmacovigilance. The desire to predict and prevent adverse drug reactions was in fact the inspiration for the research in this PhD project. Literature research pointed to structural alerts, metabolization pathways, drug metabolites, and concepts such as 'reactive metabolites', 'haptenization' and 'phenoconversion', which are all suitable for generating hypotheses. Testing of these hypotheses can contribute to the ability to predict adverse drug reactions more proactively. So far, pharmacovigilance centres have rarely captured this kind of information in their assessments of reported adverse drug reactions. However, this wealth of information from medicinal chemistry and drug development should be used to proactively monitor adverse drug reactions once drugs have received marketing authorization and are used in clinical practice. Including this information more actively in pharmacovigilance activities will enhance the learning loop between data collected during drug development, data collected after marketing authorization, and pharmacovigilance activities, and will ultimately contribute to the safe use of drugs.

3. Include more and different data sources for pharmacovigilance purposes

More and different data sources are needed to utilize knowledge from previously marketed structurally related drugs and from drug development for pharmacovigilance. Although spontaneous reports have proved to be a very important information source for drug safety and have led to adjustments in the information on drug use and sometimes even to suspension or withdrawal of drugs all over the world, the information provided is often too limited to assess the role of drug metabolization and drug metabolites in adverse drug reactions. Other data sources are available to examine associations between drug metabolization, drug metabolites and adverse drug reactions, and have proven to be very useful.[13,19]

Recommendations for clinical practice

1. Draw more attention to drug metabolization and drug metabolites in clinical practice

Morbidity related to drug metabolization and drug metabolites may present in disguise. For example, the metabolization of a drug which is used for a long time without noticeable adverse effects changes due to inhibition or induction of drug metabolizing enzymes by comedication or changes in lifestyle. Therapeutic drug monitoring and pharmacogenetics are both important to assess a possible role of drug metabolizing enzymes, drug transporters, and drug metabolites in observed adverse effects. For an optimal assessment, however, a more holistic approach to risk stratification is recommended. Considering other factors besides drug serum levels and genetic variation, such as intrinsic patient-related factors like age, weight, renal and hepatic function, and extrinsic factors like concomitantly used drugs, smoking, and alcohol use, will result in a balanced, more accurate estimate, of the relationship between the observed adverse drug reactions and the use of a specific drug or specific drugs.

2. Educate health care professionals about drug metabolization and drug metabolites

Recognizing, confirming, quantifying and preventing adverse drug reactions suspected of being associated with drug metabolites requires knowledge about the chemical structure, pharmacodynamics and pharmacokinetics of both the original compound and the drug metabolites, together with information about important patient factors such as comorbidities, pharmacogenetics, concomitantly used drugs, drug exposure and serum levels of drug metabolites and parent drugs.[46-49] This requires a combination of knowledge and data sources that is often not readily available in a pharmacovigilance centre or an individual hospital department, making multidisciplinary collaboration essential.

As it is hard to combine all relevant knowledge in one discipline and there is no specific discipline for drug-induced diseases, a multidisciplinary and holistic approach

to assessing adverse drug reactions should be considered. Moreover, drug metabolites are often not seen as separate chemical substances with their own pharmacological profile, pharmacokinetics and toxicities. More attention and education is required regarding possible adverse drug reactions in general, but they are particularly important for adverse reactions due to drug metabolization and metabolites. Presentation and discussion of suspected cases of drug-induced morbidity in multidisciplinary teams, including clinical pharmacologists, is a good starting point.[30]

3. Simplify the registration of adverse drug reactions and corresponding relevant information

Before we can utilize data sources, health care professionals should register adverse drug reactions they observe, and specify the relevant clinical data, systematically and in the appropriate entry fields of the electronic systems. In view of the burden of registration on health care professionals, registration of adverse drug reactions and the corresponding relevant information should be as simple as possible and should be limited to adverse effects that are important to share with other health care providers. These include adverse drug reactions that are serious, lead to changes in the treatment, have an unexpected course, and have not previously been associated with the drugs currently being used and clinical circumstances of patients using the drug. For adverse drug reactions suspected to be associated with drug metabolization and drug metabolites, registration of therapeutic drug monitoring and the activity of drug metabolizing enzymes and drug transporters should be facilitated.

Recommendations for future research

When patients use drugs, drug-induced harm should be watched out for, although it is sometimes hard to establish a relationship between drugs used and adverse symptoms observed. This thesis has shown that it is even harder to identify drug metabolization and drug metabolites as the culprits of adverse drug reactions. Once the role of drug metabolization and drug metabolites in adverse drug reactions has been established, actions can be taken to enhance the safe use of drugs, avert the adverse drug reactions, and keep the best pharmacotherapy options open for individual patients.

1. Explore the role of drug metabolites in known adverse drug reactions

Future research should reconsider known and listed adverse drug reactions which have a high impact on patients' quality of life and which are currently attributed to the parent drug, to see whether they are actually caused by the parent drug or by drug metabolites. If drug metabolization and drug metabolites are playing a part, this should be further investigated, with the ultimate goal of designing measures to avert these adverse drug reactions and improve the use of the drugs in question.

2. Explore phenoconversion as a measure to avert adverse drug reactions

Once a possible relationship between drug metabolite and adverse drug reaction has been established, phenoconversion is one of the measures that are worth exploring. An example is the addition of fluvoxamine (CYP1A2 inhibitor) to clozapine to prevent the formation of norclozapine which is associated with the increase in waist circumference in clozapine users.

3. Explore more and different data sources regarding the assessment of the role of drug metabolization in adverse drug reactions

Extending and enriching the pharmacovigilance discipline by adding drug metabolization and drug metabolites to adverse drug reaction assessments requires new data sources to be explored. Options for this include data collected in clinical practice, such as the drug and drug metabolite serum levels available from therapeutic drug monitoring, as well as patient characteristics such as information on the pharmacogenetics of drug metabolizing enzymes and drug transporters.

Summary of recommendations for pharmacovigilance activities, for clinical practice and for future research:

1. Recommendations for pharmacovigilance activities
   - Create awareness of the role of drug metabolization and drug metabolites in drug safety.
   - Include historical pharmacovigilance information and information collected in drug development research more actively in pharmacovigilance.
   - Include more data sources for pharmacovigilance purposes.
2. Implications for clinical practice
   - Draw more attention to drug metabolization and drug metabolites in clinical practice.
   - Educate about drug metabolization and drug metabolites.
   - Simplify the registration of adverse drug reactions and relevant information.
3. Recommendations for future research
   - Explore the role of drug metabolites in known adverse drug reactions.
   - Explore phenoconversion as a measure to avert adverse drug reactions.
   - Explore more and different data sources regarding the assessment of the role of drug metabolization in adverse drug reactions.

Final remarks

The studies in this thesis showed that including drug metabolization and drug metabolites in assessments of adverse drug reactions, prevention of toxicities and provision of the best pharmacotherapy for patients should be more common. Since we took only small steps in the research discussed in this thesis, far more attention should be paid to relationships between drug metabolization, drug metabolites, and observed adverse drug reactions, before patients and clinical practice can gain maximum benefit.

We provided Mrs V. with low-dose paroxetine (5 mg), a CYP2D6 inhibitor that slows down the metabolization of nortriptyline to hydroxynortriptyline. At that time, this intervention had been described in one small study in five healthy volunteers, and was being cautiously implemented at the Reinier van Arkel mental health institution in 's-Hertogenbosch, to keep patients on nortriptyline.[50] After the addition of 5 mg paroxetine, Mrs V.'s nortriptyline serum levels increased to therapeutic levels, and her hydroxynortriptyline serum levels decreased to acceptable ranges. This turned out to be of great clinical benefit, as she was able to continue her nortriptyline therapy.

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Chapter 5.2

Summary

Introduction and aim of the thesis

While medicines bring huge benefits to society by reducing the impact or duration of diseases, adverse drug reactions burden health care systems and lead to therapy non-adherence, morbidity, and sometimes even mortality. Adverse drug reactions are usually attributed to the parent drug. However, after intake, drug metabolization starts, generating drug metabolites that may have their own pharmacokinetic and pharmacodynamic profiles.
This process, which is intended to prevent toxicity, can in itself lead to toxicity. Although we know a lot about the relationships between a drug's metabolism in the body and the adverse drug reactions that can arise, recognizing new associations between drug metabolites and adverse effects remains difficult. Once the associations are clear, steps can be taken to limit the occurrence of adverse drug reactions and so contribute to safer drug use. The aims of the studies in this thesis were to further extend our knowledge about the role of drug metabolization and drug metabolites in adverse drug reactions and to explore data sources available after marketing authorization to assess this role.
This thesis includes five studies exploring the role of drug metabolism and the development of adverse drug reactions, as well as two studies investigating ways of preventing these adverse drug reactions.

Drug metabolization, drug metabolites, and adverse drug reactions

The studies discussed in part II investigated the role of drug metabolism and drug metabolites in the development of hepatic and pulmonary adverse drug reactions. Chapter 2.1 shows that what is known in theory, namely that certain chemical structures are metabolized to reactive metabolites, is also reflected in the spontaneously reported adverse drug reactions in the WHO database. Hepatotoxicity is reported more frequently for drugs with these characteristic chemical structures than for NSAIDs without these structures. This difference is not seen for haemorrhage, an adverse drug reaction unrelated to the formation of reactive metabolites. Chapter 2.2 analyses the polymorphisms in cytochrome P450 (CYP) 2D6 enzymes from 22 men with interstitial lung disease who all used tamsulosin. It appeared that all affected men had a reduced metabolic activity of CYP2D6, which was much more frequent than in a control group of healthy men. This reduced CYP2D6 metabolic activity may lead to alternative metabolization pathways and to the formation of a metabolite that could cause lung damage. Chapter 2.3 is a literature review on the role of pharmacogenetics in predicting cytotoxic mechanisms and the development of interstitial lung disease. It appears that genetic variation in drug metabolizing enzymes may increase its development.

Assessing the role of drug metabolization and drug metabolites in adverse drug reactions

Relationships between drug metabolites and an adverse drug reactions are often hypothetical and will usually be based on a case series. There are several data sources available to assess the relationship we are interested in. Chapter 3.1 discusses a cross-sectional study in which, using data from a clozapine outpatient clinic, we assessed the correlation between serum levels of the main metabolite of clozapine, norclozapine, and the adverse drug reaction of body weight gain, measured as body mass index (BMI) and waist circumference. Norclozapine serum levels correlated with waist circumference, and in smokers with both waist circumference and BMI. This study showed that other data sources than spontaneous reports are useful and necessary to elucidate the role of drug metabolization and the formation of drug metabolites in adverse drug reactions. In the study discussed in chapter 3.2, data from a biobank was used to gain more in-depth knowledge of the association between simvastatin and the less well-known but labelled adverse drug reaction of pulmonary toxicity. Although we were unable to draw hard conclusions regarding the relationships between pharmacogenetics, the metabolic activity of drug metabolizing enzymes relevant for simvastatin, drug transporters and the occurrence of pulmonary toxicity, the availability of a wealth of data showed the multifaceted origin of pulmonary toxicity associated with simvastatin use.

Phenoconversion as a method to prevent drug metabolite induced adverse drug reactions

Once the role of drug metabolism in the development of adverse drug reactions has been established, steps can be taken to reduce the risk of their occurrence. In the studies discussed in part IV we investigated phenoconversion as a method to prevent drug metabolite induced adverse drug reactions. Chapter 4.1 assessed the addition of low dose (5 mg) paroxetine, a CYP2D6 inhibitor, in patients on nortriptyline with high hydroxynortriptyline serum levels in daily practice. This review of routine practice showed that the addition of paroxetine was indeed able to lower the level of this metabolite. Chapter 4.2 discusses a prospective pharmacokinetic study to confirm the previous findings. This study also showed that the addition of low-dose paroxetine is able to maintain blood hydroxynortriptyline levels within the desired range, and thereby offers a possibility for patients to continue their nortriptyline therapy.

General discussion

In chapter 5.1, the main findings presented in this thesis are put into the broader perspective of the current need to optimize the utilization of available knowledge. Increasing the understanding of mechanisms underlying adverse drug reactions requires that historical pharmacovigilance information from, for example, previously marketed structurally related drugs should be fully exploited, as many difficulties arise when assessing the role of drug metabolization and drug metabolites in adverse drug reactions. The studies in this thesis also showed that assessing drug metabolite induced adverse drug reactions requires other data sources than databases of spontaneously reported adverse drug reactions, as the clinical information provided with these reports is usually insufficient to assess these kinds of relationships. Once the role of drug metabolism in the development of adverse drug reactions has been established, steps can be taken to favourably influence this. Phenoconversion, by introducing an intended drug-drug interaction that impacts the metabolic activity of relevant drug metabolizing enzymes, is a promising option. Recommendations for clinical practice include education of health care professionals about drug metabolization and the formation of drug metabolites, and facilitating the registration of adverse drug reactions. Future research should focus on further exploration of the role of drug metabolites in known adverse drug reactions that have a high impact on patients' quality of life, with the aim of developing and investigating interventions that may circumvent them. Phenoconversion is such an intervention and has been shown to be a promising measure.

Chapter 5.3

Nederlandse samenvatting

Inleiding en doel van het proefschrift

Geneesmiddelen bieden de samenleving enorme voordelen doordat ze de impact en de duur van ziekten kunnen verminderen. Helaas kunnen geneesmiddelbijwerkingen aanleiding geven tot therapieontrouw, morbiditeit, en soms zelfs tot mortaliteit. Deze bijwerkingen worden meestal toegeschreven aan de werkzame stof in het geneesmiddel. Na inname begint echter de metabolisatie van het geneesmiddel, waarbij de werkzame stof wordt gemetaboliseerd en metabolieten kunnen ontstaan die een eigen farmacokinetisch en farmacodynamisch profiel hebben. Dit proces dat bedoeld is om toxiciteit te voorkomen, kan in uitzonderlijke gevallen juist tot toxiciteit leiden.
Hoewel er eerder verbanden zijn gelegd tussen het metabolisme van een geneesmiddel en bijwerkingen die kunnen optreden, blijft het moeilijk om nieuwe verbanden tussen geneesmiddelmetabolieten en ontstane bijwerkingen te herkennen en te bevestigen. Zodra de associaties duidelijk zijn, kunnen stappen worden ondernomen om het optreden van bijwerkingen te beperken en zo bij te dragen aan veiliger geneesmiddelengebruik. Het doel van de studies in dit proefschrift was om onze kennis over de rol van geneesmiddelmetabolisering en geneesmiddelmetabolieten in de ontwikkeling van bijwerkingen verder uit te breiden en om beschikbare databronnen die hiervoor gebruikt kunnen worden te verkennen. Dit proefschrift omvat vijf studies waarin de rol van het metabolisme van geneesmiddelen en de ontwikkeling van bijwerkingen van geneesmiddelen wordt onderzocht en twee studies waarin wordt nagegaan hoe deze bijwerkingen kunnen worden voorkomen.

Geneesmiddelmetabolisme, geneesmiddelmetabolieten en geneesmiddelbijwerkingen

In de onderzoeken in deel II wordt de rol van geneesmiddelmetabolisme en geneesmiddelmetabolieten bij het ontstaan van lever- en longbijwerkingen onderzocht. Uit hoofdstuk 2.1 blijkt dat wat in theorie bekend is, namelijk dat bepaalde chemische structuren worden gemetaboliseerd tot reactieve metabolieten, ook terug te vinden is in de spontaan gemelde bijwerkingen in de WHO-database. Hepatotoxiciteit wordt vaker gemeld voor NSAID's met deze karakteristieke chemische structuren dan voor NSAID's zonder deze structuren. Dit verschil wordt niet gezien voor bloedingen, een bijwerking die geen verband houdt met de vorming van reactieve metabolieten. In hoofdstuk 2.2 worden de polymorfismen in cytochroom P450 (CYP) 2D6 enzymen van 22 mannen met interstitiële longziekte geanalyseerd die allen tamsulosine gebruikten. Het bleek dat alle getroffen mannen een verlaagde metabole activiteit van CYP2D6 hadden en dit veel vaker voorkwam dan in een controlegroep van gezonde

mannen. Deze verminderde metabole activiteit van CYP2D6 kan leiden tot alternatieve metaboliseringspaden en tot de vorming van een metaboliet die de mogelijk longschade zou kunnen veroorzaken. Hoofdstuk 2.3 betreft een literatuuroverzicht over de rol van farmacogenetica bij het voorspellen van cytotoxische mechanismen en de ontwikkeling van interstitiële longaandoeningen. Het blijkt dat genetische variatie in geneesmiddelmetaboliserende enzymen een rol kan spelen in de ontwikkeling hiervan.

Het beoordelen van de rol van geneesmiddelmetabolisatie en geneesmiddelmetabolieten in geneesmiddelbijwerkingen

Relaties tussen geneesmiddelmetabolieten en geneesmiddelbijwerkingen zijn vaak hypothetisch en gebaseerd op case-series. Er zijn verschillende databronnen beschikbaar om de relatie waarin we geïnteresseerd zijn te beoordelen. Hoofdstuk 3.1 betreft een cross-sectionele studie waarin we, met gegevens van een clozapine polikliniek, de correlatie hebben beoordeeld tussen serumspiegels van de belangrijkste metaboliet van clozapine, norclozapine, en de bijwerkingen gewichtstoename, gemeten als body mass index (BMI), en buikomvang. Norclozapine serumspiegels correleerden met de buikomvang, en bij rokers met zowel de buikomvang als de BMI. Deze studie toonde aan dat andere databronnen nuttig en noodzakelijk zijn om de rol van geneesmiddelmetabolisatie, de vorming van geneesmiddelmetabolieten en het optreden van geneesmiddelbijwerkingen verder uit te werken. In de studie besproken in hoofdstuk 3.2, werden gegevens uit een biobank gebruikt om meer diepgaande kennis te verkrijgen over de associatie tussen simvastatine en de minder bekende maar gelabelde bijwerking pulmonale toxiciteit. Hoewel we geen harde conclusies konden trekken over de verbanden tussen de gelijktijdig gebruikte geneesmiddelen, de metabole activiteit van voor simvastatine metabolisatie relevante enzymen, drug-transporters en het optreden van pulmonale toxiciteit, is de mogelijke multifactoriële relatie tussen simvastatinegebruik en pulmonale toxiciteit verder uitgediept.

Fenoconversie als methode om door metabolieten geïnduceerde bijwerkingen te voorkomen

Zodra de rol van het metabolisme van geneesmiddelen bij het ontstaan van bijwerkingen van geneesmiddelen is vastgesteld, kunnen stappen worden ondernomen om het risico van het optreden van bijwerkingen te verminderen. In de studies die in deel IV zijn beschreven, hebben we fenoconversie onderzocht als methode om bij-

werkingen door geneesmiddelenmetabolieten te voorkomen. In hoofdstuk 4.1 werd de toevoeging van een lage dosis (5 mg) paroxetine, een CYP2D6 remmer, beoordeeld bij patiënten die nortriptyline gebruikten met hoge hydroxynortriptyline serumspiegels. Deze beoordeling van de dagelijkse praktijk toonde aan dat de toevoeging van paroxetine inderdaad in staat was om de spiegel van deze metaboliet te verlagen. Hoofdstuk 4.2 bevat een prospectieve farmacokinetische studie ter bevestiging van de eerdere bevindingen. Ook deze studie toonde aan dat de toevoeging van een lage dosis paroxetine in staat is om de hydroxynortriptyline-spiegels in het bloed binnen het gewenste bereik te houden en daarmee een mogelijkheid biedt voor patiënten om hun nortriptyline therapie voort te zetten.

Algemene discussie

In hoofdstuk 5.1 worden de belangrijkste bevindingen van dit proefschrift in het bredere perspectief van de huidige noodzaak om het gebruik van beschikbare data en kennis te optimaliseren geplaatst. Het vergroten van het inzicht in de mechanismen die ten grondslag liggen aan bijwerkingen van geneesmiddelen vereist dat historische geneesmiddelenbewakingsinformatie van bijvoorbeeld eerder op de markt gebrachte structureel verwante geneesmiddelen ten volle moet worden benut om zo bij te kunnen dragen aan het beoordelen van de rol van geneesmiddelmetabolisering en - metabolieten in geneesmiddelbijwerkingen. De studies in dit proefschrift hebben ook laten zien dat voor de beoordeling van door geneesmiddelmetabolieten veroorzaakte geneesmiddelbijwerkingen andere databronnen nuttig zijn dan databases van spontane bijwerkingen, omdat de klinische informatie in spontaan gemelde bijwerkingen meestal onvoldoende is om dit soort relaties te leggen en te beoordelen. Zodra de rol van het metabolisme van geneesmiddelen bij de ontwikkeling van geneesmiddelbijwerkingen is vastgesteld, kunnen stappen worden ondernomen om dit gunstig te beïnvloeden. Fenoconversie, het introduceren van een beoogde geneesmiddel-geneesmiddelinteractie die de metabole activiteit van relevante geneesmiddelmetaboliserende enzymen beïnvloedt, is een veelbelovende optie. Aanbevelingen voor de klinische praktijk zijn onder meer voorlichting van beroepsbeoefenaren in de gezondheidszorg over het metabolisme van geneesmiddelen en de vorming van geneesmiddelmetabolieten en het vergemakkelijken van de registratie van geneesmiddel-bijwerking associaties. Toekomstig onderzoek kan zich richten op verdere exploratie van de rol van metabolieten bij bekende geneesmiddelbijwerkingen die een grote impact hebben op de kwaliteit van leven van patiënten en op de ontwikkeling van interventies die het optreden van bijwerkingen door geneesmiddelmetabolieten kunnen omzeilen. Fenoconversie is zo'n veelbelovende interventie gebleken.

Dankwoord
Curriculum vitae
List of publications

PART VI

Dankwoord

Allereerst dank aan mijn promotieteam, aan Eugène, Rob, Jeroen en Koen. Eerlijk gezegd kan ik me niet meer herinneren wanneer het startschot van dit promotietraject werd gelost, maar na mijn opleiding tot klinisch farmacoloog en het afronden van het daarbij behorende wetenschappelijk onderzoek naar paroxetine additie bij nortriptyline en hoge hydroxynortriptyline serumspiegels waren we een team en gingen we van start. Zonder jullie methodologische, klinische en bij tijd en wijlen ook strenge input en begeleiding was dit promotietraject niet mogelijk geweest. Dank voor deze jaren van steun, inzichten, hulp en bijsturing. Eugène, dank voor deze vormende jaren bij Lareb. Je kennis over farmacovigilantie was onontbeerlijk in dit traject.

Marjolein, je zat niet in mijn officiële promotieteam maar je was al snel zeer betrokken. Zonder jou was dit boekje er in deze vorm niet geweest. We hebben elkaar gevonden in de farmacogenetica en toxiciteit op de longen en onze samenwerking heeft tot meerdere publicaties geleid waarvan een aantal behorend tot dit proefschrift. Jouw enthousiasme, drijvende kracht en energie zijn daarin terug te zien en ik ben blij dat we elkaar nog steeds op zeer regelmatige basis spreken. Veel dank voor alle tijd die we de afgelopen jaren met elkaar hebben doorgebracht, ik had het niet willen missen.

Leden van de expertcommissie van de Monitor biologische middelen, met name Mike, Bart en Harald. Jullie zijn niet betrokken geweest bij het tot stand komt van dit proefschrift maar hebben zeker bijgedragen aan mijn wetenschappelijke vorming door deel te nemen aan de Monitor en nu aan de Bijwerkingmonitor. Fijn dat we de samenwerking voort kunnen zetten en nu ook samen promovendi begeleiden.
Jette en Leanne, mijn partners in crime van het eerst uur, want de Monitor biologische geneesmiddelen was een kriem. Door al het onderzoek dat we hebben uitgevoerd voor dat project hebben jullie indirect bijgedragen aan dit proefschrift. Dank voor jullie vertrouwen. Ik hoop dat we nog vele mooie gezamenlijke onderzoeksjaren voor de boeg hebben.

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Lieve Javier en Anouk, lieve broer en zus. Grote delen van het jaar zijn we gescheiden van elkaar. Gelukkig kunnen we door de huidige stand van de technologie de afstand eenvoudig en goedkoop overbruggen. Gelukkig hebben we niet veel nodig, we weten van elkaar dat we er – ook op grote afstand - voor elkaar zijn.
Javier, helaas, in september ben je uit Suriname met je hele gezin naar Nederland overgekomen, maar was het proefschrift nog niet af en de verdediging niet in zicht. Excuus, je zus heeft een eigen tempo. Gelukkig is er nu livestream, ik zal even naar je knipogen.
Anouk, mijn niet meer zo kleine zusje die als frisse veertiger de bijna vijftiger up-to-date houdt. Dank dat je op deze dag zo dicht bij me kan zijn en mijn paranimf wilt zijn.

Lieve pa, jammer dat je dit niet meer mee kunt meemaken. Als je er nog was geweest had je kunnen zien dat het goed is gekomen met mijn zeer bewuste keus voor farmacie. Je had kunnen zien dat na bijna 25 jaar apotheker zijn, geneesmiddelen en hun molecuulstructuren mij nog steeds fascineren. Het zegt genoeg. Langs deze weg naar boven dank dat je me de stevige basis van zelfvertrouwen hebt meegegeven. Ik heb het nodig gehad.

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Curriculum vitae

Naomi Jessurun was born in Paramaribo, Suriname, on 16 October 1973. After completing her secondary school at the 'Vrije Atheneum' in Paramaribo in 1991, she started studying pharmacy at Utrecht University, The Netherlands (NL). She graduated in 1998 and became a pharmacist. After working for six months as a project pharmacist in the hospital pharmacy of what is now Gelre Hospital in Apeldoorn (NL) and at the Merwede Hospital in Dordrecht (NL), she started training as a hospital pharmacist at the Meander Medical Center in Amersfoort (NL) in the year 2000. After her registration as a hospital pharmacist (2003), she and her family left for Suriname, where she worked for just over four years at the Paramaribo University Hospital and then for several years in various positions for the Ministry of Health in Suriname. Since her return to the Netherlands (2011), she has been working at the Netherlands Pharmacovigilance Centre Lareb in 's-Hertogenbosch (NL), first as a scientific assessor, then as project leader of the Dutch Monitor Biologics (Monitor Biologische Geneesmiddelen) and its successor, the Adverse Drug Reactions Monitor (De Bijwerkingmonitor), head of harmonization and sharing pharmacovigilance data. She is now the head of the Lareb Medicine Team focussed on immunomodulating agents, hormones and drugs for treatment of sensory disorders. From 2014 to 2016, she trained as a clinical pharmacologist at the Jeroen Bosch Hospital, 's-Hertogenbosch (NL), which resulted in being registered as a clinical pharmacologist in 2016. The research project described in this thesis is a continuation of the work she did in her clinical pharmacology training, and was started in 2017, supervised by Prof. dr. Eugène van Puijenbroek, Prof. dr. Rob van Marum, Prof. dr. Koen Grootens and dr. Jeroen Derijks. She is a member of the Expert Group on Clinical Pharmacology at the Jeroen Bosch Hospital and a member of the ild care foundation research team and ILD Center of Excellence at the St. Antonius Hospital, Nieuwegein (NL). She has been a board member of the Royal Society of Pharmacists (KNMP) and a member of the advisory board of the Dutch Pulmonary Fibrosis Patients Association since 2022.

Since 2021, she has been a member of the PhD supervision team for Leanne Kosse, and since 2022 of the PhD supervision team for Jette van Lint. Naomi married Martijn de Haan and together they have a son Mischa and a daughter Lara.

List of Publications

This thesis

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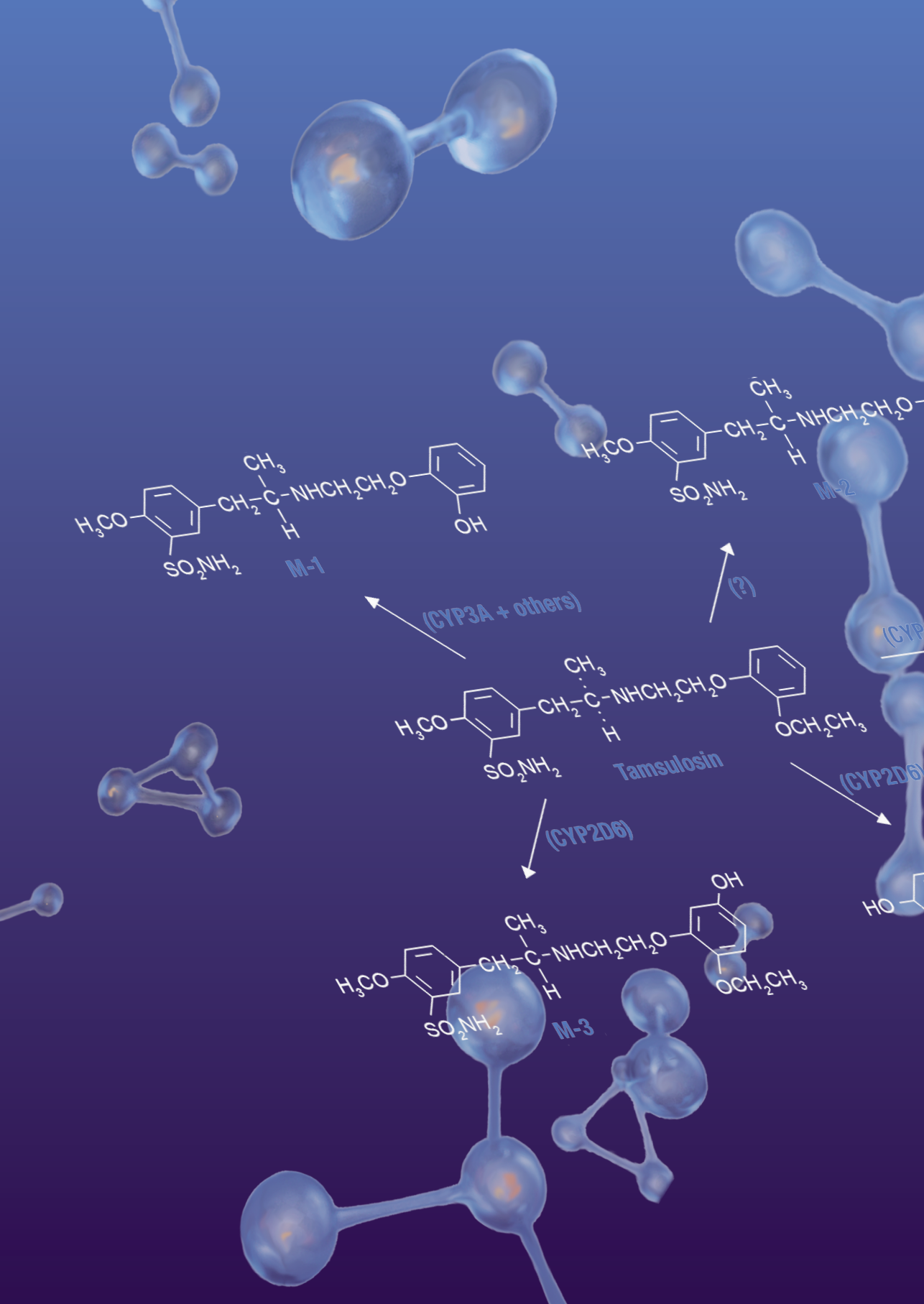
M-1
M-2
(CYP3A + others)
(?)
Tamsulosin
(CYP2D6)
(CYP2D6)
M-3