Drug-induced, occupational and environmental related diffuse lung diseases

Utrecht, 30 September 2004

Hotel Karel V; Utrecht

Drug-induced, occupational and environmental related diffuse lung diseases

Contents

Preface 1

Program 2

Curriculum Vitae

- Prof. dr. Paul J.A. Borm 3
- Prof. dr. Benoit Nemery 3
- Prof. dr. Aalt Bast 4
- Prof. dr. Philippe Camus 4
- Dr. Marjolein Drent 5

Abstracts

- Particles and pulmonary disease. *Prof. dr. Paul Borm*. 6
- Occupational and environmental factors in diffuse interstitial lung Diseases. *Prof. dr. Benoit Nemery* 7
- Drug-induced lung damage: a role for genomics in prevention. *Prof. dr. Aalt Bast* 8
- Drug-induced respiratory disease. *Prof. dr. Philippe Camus* 9

Appendix

- Mercaptopurine treatment and pharmacogenetic screening: never or ever? *Jaap Bakker* 10
- G6PD-deficiëntie. *Snjezana Rothkrantz-Kos* 12

References 16

Preface

Diffuse interstitial lung diseases (ILD) caused by exogenous factors can be divided into those that arise as a result of a progressive accumulation of (mainly) mineral particles in the lung parenchyma, and those that essentially result from a particular susceptibility of the host for specific agents. The former category consists mainly of silicosis, coal worker's pneumoconiosis and asbestosis, as well as other mineral pneumoconioses. Size, dose-rate, components of the particles and route of entry do matter with regard to toxicological effects of particles. The latter category contains extrinsic allergic alveolitis (EAA), which is mainly caused by the inhalation of bio-aerosols, chronic beryllium disease and other sarcoid-like diseases, and hard-metal or cobalt lung.

Individual susceptibility is based on a genetic tendency for becoming immunologically sensitized (demonstrated for beryllium) and/or possibly on a poor defense against oxidative stress. Moreover, in the inflammatory response cytokine production is involved. There is evidence that polymorphisms in the promoter region of genes affect the amount of production of certain cytokines, resulting in high producers and low producers.

Rational treatment of drug-toxicities in cases where the mechanism of toxicity is known is common clinical practice. However, often the connection with drug-use and the development of related inflammatory damage or idiosyncratic toxicities is hard to recognize and objectify, especially in those cases using multiple drugs. An ever increasing number of drugs can produce/reproduce variegated patterns of naturally-occurring ILD, including most forms of interstitial pneumonias, non specific interstitial pneumonias (NSIP)[1], alveolar involvement and, rarely, vasculitis. The diagnosis of drug-induced ILD is mainly one of exclusion, and this requires the meticulous exclusion of all other possible causes.

Clinically it is challenging trying to find predictors of the possibility that a single individual is at risk developing such reaction, especially to avoid rechallenge. Several theories have been postulated. Genetic polymorphisms have extensively been studied for drug metabolizing enzymes. The presence of certain polymorphisms can result in extensive or poor metabolizers for certain drugs. There is evidence that inherited variation is involved in drug metabolism. Therefore, genotyping could be considered to identify patients that might be at risk[2] of severe toxic responses to environmental, pharmacological, herbal remedies, and/or nutritional stimuli.

September 2004, Marjolein Drent

1 Drent M, Du Bois RM, Poletti R. Recent advances in the diagnosis and management of non specific interstitial pneumonia. *Curr Opin Pulm Med* 2003; 9: 411-7.

2 Drent M, Suveer Singh M, Gorgels APM, Hansell DM, Bekers O, Nicholson AG, van Suylen RJ, du Bois RM. Drug-induced pneumonitis and heart failure simultaneously associated with venlafaxine. *Am J Respir Crit Care Med* 2003; 167: 958-61.

Program

9.30-10.00	Registration, welcome with coffee or tea

Morning program

10.00-1015	Introduction by the chairman Prof. Dr. Paul J.A. Borm Hogeschool Zuyd, Centre of Expertise Life Sciences (CEL) Heerlen, the Netherlands, and Particle Research Core Institute for Environmental Research at the University of Düsseldorf, Germany
10.15-11.00	'Occupational and environmental factors in diffuse interstitial lung diseases' Prof. Dr. Benoit Nemery, Laboratory of Pneumology (Lung Toxicology), Catholic University. Leuven, Belgium
11.00-11.45	'Drug-induced lung damage: a role for genomics in prevention?' Prof. Dr. Aalt Bast, Department of Toxicology and Farmacology University Maastricht, the Netherlands
11.45-12.30	'Drug-induced respiratory disease' Prof. Dr. Philippe Camus, Department of Pulmonary and Intensive Care, Univ Med CTR, School of Medicine, and Université de Bourgogne at Dijon, France
12.30-13.45	Lunch break

Afternoon program

13.50-14.40	Workshop A (Group 1): P. Borm/B. Nemery: 'Occupational exposure: how can we make the relation plausible?' Workshop B (Group 2): A. Bast/Ph. Camus: 'Relevance of screening for genetic defects to prevent drug-induced damage. Dose and drug-targeting in clinical practice.'
14.40-15.00	Coffee or tea break
15.00-15.50	Workshop A (Group 2): P. Borm/B. Nemery: 'Occupational exposure: how can we make the relation plausible?' Workshop B (Group 1): A. Bast/Ph. Camus: 'Relevance of screening for genetic defects to prevent drug-induced damage. Dose and drug-targeting in clinical practice.'
16.00-16.45	Clinical round Prof. Dr. A. Bast; Prof Dr. P. Borm; Prof. Dr. Ph. Camus; Dr. M. Drent; Prof. Dr. B.Nemery
16.45-17.00	Closing remarks
17.00-18.00	Drinks

Curricumum Vitae

Prof. Dr. Paul J.A. Borm, PhD
Professor of Toxicology and Pharmacology, Lector Life Sciences
Centre of Expertise for Life Sciences, Hogeschool Zuyd, Heerlen, the Netherlands
tel: +31 45 4006800
e-mail: P.Borm@hszuyd.nl

Paul Borm is the head of the Centre of Expertise in Life Sciences at the Hogeschool Zuyd in Heerlen and Professor of Toxicology & Pharmacology at the University of Dusseldorf (Germany). He is recognized as an expert in the mechanisms of lung disease caused by particles and fibres and in this capacity has provided expert opinion and consultancy to the World Health Organisation, International Agency for Research on Cancer (Lyon France), WHO Air Quality and Health (Bonn, Germany), German and Dutch Health and Safety Executive. In relation to nanoparticles he is a member of many study panels regarding applications and risks of nanoparticles in medicine, occupational exposure and technological changes. He has acted as a consultant to various bodies on the risk from NPs such as European Science Foundation, European Union (DG Sanco), VDI, German Parliament. He has published over 170 scientific papers, is running a research programme into the cardiovascular effects of particles, and is now driven by technology transfer into education and European industry (SME).

Prof. Dr. Benoit Nemery, PhD
Professor of Pneumology and Occupational, Environmental and Insurance Medicine
Laboratory of Pneumology (Lung Toxicology)
Catholic University Leuven, Belgium
tel: +32 16 347121, fax::+32 16 347124
e-mail : ben.nemery@med.kuleuven.ac.be

Ben Nemery is holder of degrees in medicine (Leuven, 1977), occupational medicine (Leuven, 1980) and toxicology (MSc, University of Surrey, 1982; and PhD, Council for National Academic Awards, UK, 1986).
After four years research in the laboratory of cardiopulmonary function of the U.C.Leuven, he trained in experimental toxicology at the Medical Research Council's Toxicology Unit in Carshalton, Surrey, England.
He has been affiliated with the K.U.Leuven since 1987, heading the research unit of Lung Toxicology, which is a joint venture between the departments of Pneumology and Occupational, Environmental and Insurance Medicine. He was promoted to full professor in 1998.
His research involves experimental as well as clinical-epidemiological studies in the mechanisms of lung disease caused by occupational and environmental agents (metals, asbestos, synthetic chemicals, air pollutants). He holds a weekly outpatient clinic for occupational pulmonary disorders. He teaches toxicology and occupational medicine, mainly at postgraduate level.
He is a member of various national and international scientific bodies, including the European Respiratory Society, where he is the current head of the assembly "Occupation and Epidemiology". He has held positions in editorial boards of various journals, including the American Journal of Respiratory and Critical Care Medicine (associate editor, since 2004).

Prof. Dr. Aalt Bast, PhD
Professor of Human Toxicology
Department of Pharmacology and Toxicology, University Maastricht, the Netherlands
e-mail: a.bast@farmaco.unimaas.nl

Prof. Aalt Bast was born in 1953. He studied chemistry at the Free University in Amsterdam, where he graduated in 1978. His PhD study was done at the Erasmus University of Rotterdam (Faculty of Medicine) and at the University of Utrecht (Faculty of Pharmacy), and was finished in 1981. Subsequently he became Assistant Professor of Pharmacology at the University of Utrecht. He was appointed Associate Professor of Molecular Pharmacology at the Department of Pharmacochemistry at the Free University in Amsterdam in 1985, and became professor in Molecular Pharmacology in 1988. In July 1998 he started to work as professor of Human Toxicology at the Maastricht University. His main field of interest is the role of free radicals in drug toxicity and pathologies of the lung, the cardiovascular system and in diabetes. Moreover, the possibilities for therapeutic intervention with antioxidants (from food or as drugs) are explored. He is registered in the Netherlands as an experimental pharmacologist and toxicologist. He (co-)authored over 350 papers and book chapters. He is co-founder of the Dutch Heidelberg Appeal Foundation. A foundation that aims for a greater role for science and realistic risk analyses in influencing public opinion and policy decisions by providing sound scientific information. He is vice-chairman of the Dutch Toxicological Society, member of the Concilium Toxicologicum and member of the National Health Council, the scientific board of the Vitamine Information Bureau at TNO and the advisory board of the Dutch Pulmonary Fibrosis Patient Society.

Prof. Dr. Philippe Camus, MD
Professor and head of Department of Pulmonary and Intensive Care
Univ Med CTR, School of Medicine, and Université de Bourgogne at Dijon, France
e-mail: philippe.camus@chu-dijon.fr
http://www.pneumotox.com

Prof. Philippe Camus was born on April 24, 1950 in Strasbourg (France). He started his academic education on the Training School of Medicine at Dijon, Université de Bourgogne France. He graduated in 1978 at the Cardiology and Pulmonary board. Thereafter, he became Senior Resident in Pulmonology and Intensive Care Laval Hospital in Québec City, Canada 1978 to 1979. From 1980 to 1984 he worked as an Assistant Professor in Pulmonary Diseases at the University Medical Center and School of Medicine at Dijon. From 1984 to 1985 he was a Senior Research Associate at the Department of Pharmacology and Toxicology University of Mississippi Medical Center Jackson MS (isolated perfused lung, studies on amiodarone kinetics in lung). In 1985 he became a Full Professor of Pulmonary Medicine Université de Bourgogne in Dijon. Since 1998 till now he is Head of the Department of Pulmonary Diseases and Intensive/Critical Care, a 54-bed department with 6 ICU. His fields of intrests are: 1) drug-induced/iatrogenic respiratory disease; 2) respiratory disease in inflammatory bowel disease; 3) the input of websites in teaching and patient care: ex in drug-induced lung disease Pneumotox.com® and 4) the role of the lung in the pharmacokinetics metabolism and toxicity of drugs. He has published 73 articles, several book chapters, a book in french on diseases of the lung, and a website.

Dr. Marjolein Drent, MD
Department of Respiratory Medicine,
University Hospital Maastricht, the Netherlands
tel: +31 43 3877042
e-mail: m.drent@lung.azm.nl
http://www.pul.unimaas.nl

Marjolein Drent finished her study physiotherapy at the Academy of Physiotherapy of Arnhem (NL) in 1979. She ended her medical study in 1988 at the Catholic University of Nijmegen (NL). Her training as a pulmonologist at the St. Antonius Hospital (Nieuwegein, NL) was completed in 1994. Subsequently she started to work as a pulmonary physician at Department of Respiratory Medicine of the University Hospital Maastricht (NL). Currently, she is a consultant for interstitial lung diseases (ILD). Furthermore, she is secretary of the assembly 1.6 Clinical Pathobiology of the ERS (European Respiratory Society) and a member of the executive committee of the WASOG (World Association of Sarcoidosis and other Granulomatous diseases). She is a member of the editorial board of Sarcoidosis Vasculitis and Diffuse Lung Diseases and Current Opinion in Pulmonary Medicine. She collaborates with many international centres famous for ILD and related disorders. In this respect, she is adjunct senior scientist of the Lovelace Respiratory Research Institute (LRRI), Albuquerque, New Mexico, USA. Since 1995 she is a member of the medical advice board of the SBN (Dutch Sarcoidosis Society) as well as the Dutch Pulmonary Fibrosis Society. She (co-) authored over 100 papers and book chapters. Currently, she is involved in clinical and biochemical research into inflammation focussing on interstitial lung diseases, including occupational and environmental related disorders. She is the initiator and manager of the sarcoidosis management team and ILD management centre University Hospital Maastricht. This centre participates in several international trials (Centocor, InterMune, and Zambon) aimed to improve the therapeutic approach of patients suffering from idiopathic pulmonary fibrosis (IPF) or sarcoidosis. Furthermore, she is concerned with several educational assignments and organisation of postgraduate courses.

Particles and pulmonary disease

Prof. Dr. Paul Borm
Departments of Particle Research, IUF at the University of Dusseldorf, Germany and Centre of Expertise in Life Sciences, Zuyd University, Heerlen, the Netherlands

Ancient mining has probably been the first identified cause of interstitial lung disease (ILD) due to massive, cumulative inhalation of inert, micron size particles that lead to deposition and interstitialisation of grams of particles. In humans this is often associated with collagen deposition and local sites of inflammation, but malignant outcomes only occur in association to minerals such as quartz or asbestos. In contrast, inhalation studies in rats show that lung cancers (adenoma, squamous carcinoma, keratinizing cysts) readily occur even after inhalation of non-toxic particles such as titanium dioxide and carbon black. Current findings in animal toxicology have large implications for risk assessment, labeling and production/use of many particulate materials.
On the other hand environmental exposure to very low concentrations (around 20 micrograms/m^3) have been associated to acute effects in the general population, including respiratory and cardiovascular mortality and morbidity. Instead of particle mass, biomedical research is pointing here at particle numbers and surface of so-called ultrafine particles to explain the effects in humans and toxicological studies. Ultrafine particles have a large surface which seems associated to the acute inflammatory response, and subsequent systemic effects. This opens up a final avenue, it is the use of ultafine (= Nano) and ultralight particles in drug delivery including the treatment of respiratory diseases. Clearly, size, dose-rate and route of application do matter with regard to toxicological effects of particles and these concepts will be addressed.

Key references for the workshop

1 Borm PJA. Particle Toxicology: from coal mining to nanotechnology. *Inhalation Toxicol* 2002; 14: 101-14.
2 Höhr D, Steinfartz Y, Martra G, Fubini B, Borm PJA. The surface area rather than the surface coating determines the acute inflammatory response after instillation of fine and ultrafine TiO2 in the rat. *Int J Hyg Env Health* 2002; 205: 239-44.
3 Borm PJA, Schins RPF, Albrecht C. Inhaled particles and lung cancer- A review. part B-paradigms and risk assessment. *Intern J Cancer* 2004; 110: 3-14.
4 Beckett WS. Occupational Respiratory Diseases. *New Eng J Med* 2000; 342: 406-13.
5 Donaldson D, Stone V, Tran L, Kreyling W and Borm PJA. Nanotoxicology, a new frontier. *Occup Environ Med* 2004; in press.

Occupational and environmental factors in diffuse interstitial lung diseases

Prof. Dr. Benoit Nemery
Laboratory of Pneumology (Lung Toxicology)
Catholic University, Leuven, Belgium

The purpose of this review is to describe the present state of knowledge regarding host susceptibility factors that may determine the occurrence, development and severity of interstitial lung disease (ILD) caused by exogenous agents[1]. First, host susceptibility may pertain to differences in the delivery and/or persistence of the noxious agent in the lung. The deposition and clearance of inhaled particles or fibres may vary depending on innate anatomical or physiological characteristics, and on acquired changes, such as nasal disease or smoking-induced alterations. Genetically- or environmentally-induced interindividual differences in the expression of pulmonary biotransformation enzymes may form the basis for, or contribute to the risk of, drug-induced interstitial lung disease. Secondly, there are genetic and acquired variations in various enzymatic and nonenzymatic defence systems that protect cells and tissues against oxidative stress, which is often involved in the pathogenesis of interstitial lung disease caused by particles, fibres, metals, organic agents and drugs. Thirdly, the occurrence of immunological sensitization is dependent on both genetic and environmental factors. This has been demonstrated in chronic beryllium lung disease and in hypersensitivity pneumonitis. Fourthly, the propensity of individuals to develop particular types of inflammation, such as granulomas, is probably under genetic control. The regulation and resolution of inflammation and fibrogenesis caused by dust particles are also partly determined by genetic factors, involving cytokine networks and growth factors. Recent outbreaks of severe lung disease in textile workers who had been engaged in paint spraying ("Ardystil syndrome")[2], have illustrated that organizing pneumonia ("BOOP") may have an occupational origin. Similarly, the occurrence of interstitial lung disease in workers exposed to microfibers of synthetic materials such as nylon or polyethylene ("flock workers's lung")[3] and possibly man made mineral fibers (MMMF) show that new occupational causes of lung diseases may still occur. In conclusion, although the issue of genetics pervades the entire discussion of host susceptibility, genes are not the only determinants of health and disease. Environmental factors may be equally important in shaping host susceptibility. Therefore, research must be focused on both the genetic bases and the environmental determinants of interstitial lung disease, in order to provide mechanism-based prevention strategies, early detection of, and improved therapy for these conditions. Pulmonary physicians and pathologists have an important role in discovering and characterizing these conditions. One should remain vigilant for the occurrence of novel occupational causes of pulmonary disease.

Key references

1 Nemery B, Bast A, Behr J, Borm PJ, Bourke SJ, Camus PH, De Vuyst P, Jansen HM, Kinnula VL, Lison D, Pelkonen O, Saltini C. Interstitial lung disease induced by exogenous agents: factors governing susceptibility. *Eur Respir J* 2001; 32: S30-42.
2 Camus P, Nemery B. A novel cause for bronchiolitis obliterans organizing pneumonia: exposure to paint aerosols in textile workshops. *Eur Respir J* 1998; 11: 259-62.
3 Eschenbacher WL, Kreiss K, Lougheed MD, Pransky GS, Day B, Castellan RM. Nylon flock-associated interstitial lung disease. *Am J Respir Crit Care Med* 1999; 159: 2003-8.

Drug-induced lung damage: a role for genomics in prevention

Prof. Dr. Aalt Bast, PhD
Department of Pharmacology and Toxicology, Faculty of Medicine,
Maastricht University, Maastricht, the Netherlands

Rational treatment of drug-toxicities in cases where the mechanism of toxicity is known is common clinical practice. A well-known example is the hepatotoxicity that results from an overdose of paracetamol. It is known that the biotransformation of paracetamol produces a reactive N-acetyl-p-benzoquinone imine metabolite that reacts with intracellular thiol moieties thus resulting in cell death. Administration of high doses of N-acetylcysteine replenishes thiol groups thus alleviating the toxicity. Another example is the cardiotoxicity of the antitumor drug doxorubicin. Redox cycling of the drug produces oxygen free radicals. In addition free iron worsens the free radical damage. As expected, based on the molecular mechanism, an antioxidant with iron chelating activity offers adequate protection.
Clinically more challenging is the development of possibilities to predict idiosyncratic drug toxicities. In other words how to find the single individual who is at risk? Several theories have been postulated to explain idiosyncratic toxicities.
The *hapten hypothesis* proposes that reaction of a protein with a reactive metabolite creates a 'foreign' (immunogenic) protein, which in some cases, leads to an immune-mediated adverse reaction. There are shortcomings with this hypothesis. A full immune response to foreign proteins generally requires an adjuvant as second signal. In the *danger hypothesis*, it is therefore suggested that a reactive metabolite could initiate cellular damage, leading to transient elevations of intracellular enzymes, thus producing the second signal.
With these hypotheses it is possible to envision individual toxic responses to a drug. The most common DNA variations in the human genome are called single nucleotide polymorphisms (SNPs). Genetic polymorphism has extensively been studied for the so-called phase I drug metabolizing cytochrome P-450 enzymes. This can result in extensive or poor metabolizers for certain drugs. Inherited variation[1,2] has also been established for phase II drug metabolizing enzymes (*e.g.* N-acetyltransferase, thiopurine S-methyltransferase).
A recent example[3] involves a patient diagnosed with sarcoidosis with an inherited glucose-6-phosphate dehydrogenase deficiency. It is known that these patients are not able to maintain adequate formation of NADPH and thereby reduced intracellular glutathione. The cardiac failure associated with the disease was treated with the non-selective β-adrenoceptor blocker carvedilol. The successful treatment was ascribed to the antioxidant action of the drug. In order to prevent worsening of the disease specific food restrictions were advised.
Genotyping could be considered to identify patients that might be at risk of severe toxic responses to environmental / pharmacological / nutritional stimuli.

Key references

1 Weinshilboum R. Inheritance and drug response. *New Engl J Med* 2003; 348: 529-37.
2 Nebert W, Russell. Clinical importance of the cytochromes P450. *Lancet* 2002; 360: 1155-62.
3 Drent M, Gorgels AP, Bast A. Cardiac failure associated with G6PD deficiency. *Circulation Res* 2003; 93 (8): e75.

Drug-induced respiratory disease

Prof. Dr. Philippe Camus, MD
Professor and head of Department of Pulmonary and Intensive Care
Univ Med CTR, School of Medicine, and Université de Bourgogne at Dijon, France

An ever increasing number of drugs can produce/reproduce variegated patterns of naturally-occurring infiltrative lung disease (ILD), including most forms of interstitial pneumonias, alveolar involvement and, rarely, vasculitis[1]. Drugs in one therapeutic class may collectively produce the same pattern of involvement. A few drugs can produce more than one patterns of ILD. The diagnosis of drug-induced ILD rests on the temporal association of exposure to the drug, and the development of pulmonary infiltrates. The histopathological features of drug-induced ILD generally are consistent, rather than suggestive or, even less often, specific for the drug etiology. Thus, the diagnosis of drug-induced ILD is mainly one of exclusion, and this requires the meticulous exclusion of all other possible causes. Drug dechallenge produces measurable improvement in symptoms and imaging in the majority of patients, whereas corticosteroid therapy is indicated if symptoms are present or drug dechallenge is without an effect. Rechallenge is justified in a minority of patients, and is discouraged for diagnostic purpose only. Many other patterns of respiratory involvement are described, involving the upper and lower airways, pleura, and neuromuscular system.
Illicit drugs and herbal remedies also cause respiratory adverse effects.
www.pneumotox.com ® provides updated information in drug-induced respiratory disease.

Key reference

1 Camus P, Fanton A, Bonniaud P, Camus C, Fouchier P. Interstitial lung disease induced by drugs and radiation. *Respiration* 2004; 71: 301-26.

Mercaptopurine treatment and pharmacogenetic screening: never or ever?

Jaap Bakker[1,2], Jörgen Bierau[2] and Albert van Gennip[2]
[1] Dept. of Clinical Chemistry and [2]Laboratory of Biochemical Genetics, Dept. of Clinical Genetics, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, the Netherlands.
e-mail: j.bakker@klinchem.azm.nl

Adverse drug reactions (ADR) in immunosuppresive and cytostatic therapy are mostly due to defects in drug metabolising enzyme systems like the Cytochrome P_{450} (CYP) and the multidrug resistance protein (MRP). These defects are either genetic or acquired.
In this abstract we focus on the genetic causes of mercaptopurine toxicity, which is becoming more relevant because of the use of these drugs in several fields of medicine: pulmonology, oncology, gastroenterology, rheumatology and paediatrics.
Mercaptopurines like thioguanine and 6-mercaptopurine are used in the treatment of leukaemia, its prodrug azathioprine is used as an immunosuppressant. These drugs need to be activated by the Nucleotide Salvage pathway into their active thioguanine nucleotides, which will be incorporated into DNA, causing cell death.
Besides activation of the mercaptopurines a mechanism of inactivation is active. This is accomplished by the action of xanthine oxidase/dehydrogenase (XDH) and by thiopurine methyl transferase (TPMT). The first enzyme being the less important, although the use of allopurinol as medication against hyperuricemia (gout) causes an ADR in patients treated with mercaptopurines through inhibition of XDH. The enzyme TMPT is far more important in the catabolism of mercaptopurines and genetic polymorphisms or true mutations cause (severe) ADR in patients treated with these (pro)drugs. Patients with complete TMPT deficiency are at high risk for severe myelosuppression induced by therapy with thiopurines.
Recently a third enzyme involved in the metabolism of mercaptopurines was described: there is an association with polymorphisms in the gene encoding for inosine triphosphate pyrophosphatase and ADR in patients treated with azathioprine. [The pharmacogenetic consequences of this enzyme in mercaptopurine therapy was already predicted by A.H. van Gennip during the workshop on Purines and Pyrimidines in Zurich in 2002].
It is beneficial that before starting with mercaptopurine therapy activity measurement of the enzymes involved in inactivation of these drugs is performed in red blood cells. When there is a reduction in enzyme activity the therapeutic dose can be lowered to prevent ADR. Mutation analysis can establish the exact nature of the enzyme defect, which can be important for the therapeutic regimen of familiar forms of malignant disease.
Further pharmacogenetic defects are described in 5-fluorouracil toxicity: mutations in the genes for dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and dihydropyrimidinase (DHP) cause severe ADR in these patients. Newer developments in the field of pharmacogenomics describes the role of (mitochondrial) nucleotide metabolizing enzymes, like thymidine kinase and deoxyguanosine kinase, in cytotoxity for chemotherapeutics and virostatics. Recently started research will focus on the role of these and other mitochondrial enzymes involved in purine and pyrimidine metabolism.

Measurement of the involved enzymes can be done on red blood cells (TPMT, ITPase, XDH) or peripheral lymphocytes, (DPD, TP, DHP). Mutation analysis will be performed in the case of lowered enzyme activity. In addition mutation analysis of the CYP

complex can also be accomplished to rule out other causes of ADR. 10 ml EDTA-blood has to be shipped at room temperature to the laboratory, except Friday.

Please contact the laboratory-staff before sending the samples. Precaution: no blood transfusion in the last 90 days

Sample	10 ml EDTA Blood
Storage conditions	Room temperature, ***not*** centrifuged
Adress	Laboratorium Erfelijke Metabole Ziekten Afd. Klinische Genetica Academisch Ziekenhuis Maastricht Joseph Bechlaan 113 6229 GR Maastricht
Laboratory staff	Dr. A.H. van Gennip albert.vangennip@gen.unimaas.nl Dr. J. Bierau jorgen.bierau@gen.unimaas.nl
Telephone	043 387 78 35

References

1** Baker DE. Pharmacogenomics of azathioprine and 6-mercaptopurine in gastroenterologic therapy. *Rev Gastroenterol Disord* 2003; 3: 150-7.

2** Nebert DW, Russell DW. Clinical importance of the cytochromes P450. *The Lancet.* 2002/10/12 2002;360(9340): 1155-62.

3** O'Kane DJ, Weinshilboum RM, Moyer TP. Pharmacogenomics and reducing the frequency of adverse drug events. *Pharmacogenomics* 2003; 4: 1-4.

4** Relling MV, Dervieux T. Pharmacogenetics and cancer therapy. *Nat Rev Cancer* 2001; 1: 99-108.

5* Tapner MJ, Jones BE, Wu WM, Farrell GC. Toxicity of low dose azathioprine and 6-mercaptopurine in rat hepatocytes. Roles of xanthine oxidase and mitochondrial injury. *J Hepatol* 2004; 40: 454-63.

6** Oscarson M. Pharmacogenetics of drug metabolising enzymes: importance for personalised medicine. *Clin Chem Lab Med* 2003; 41: 573-80.

7* Hirano Y, Kageyama S, Ushiyama T, Suzuki K, Fujita K. Clinical significance of thymidine phosphorylase and dihydropyrimidine dehydrogenase expression in transitional cell cancer. *Cancer Chemother Pharmacol* 2003; 51: 29-35.

8* van Kuilenburg AB, Meinsma R, Zonnenberg BA, et al. Dihydropyrimidinase deficiency and severe 5-fluorouracil toxicity. *Clin Cancer Res* 2003; 9: 4363-67.

9** Van Kuilenburg AB, Vreken P, Abeling NG, et al. Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Hum Genet* 1999; 104: 1-9.

10* Marinaki AM, Ansari A, Duley JA, et al. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). *Pharmacogenetics* 2004;14: 181-7.

11* van der Eb MM, Geutskens SB, van Kuilenburg AB, et al. Ganciclovir nucleotides accumulate in mitochondria of rat liver cells expressing the herpes simplex virus thymidine kinase gene. *J Gene Med.* Dec 2003; 5: 1018-27.

12* Lotfi K, Mansson E, Peterson C, Eriksson S, Albertioni F. Low level of mitochondrial deoxyguanosine kinase is the dominant factor in acquired resistance to 9-beta-D-arabinofuranosylguanine cytotoxicity. *Biochem Biophys Res Commun* 2002; 293: 1489-96.

** Articles of interest on the topic of pharmacogenetics

* Articles going into the depth of the field of pharmacogenetics.

Appendix

G6PD-deficiëntie

Snjezana Rothkrantz-Kos
Department of Clinical Chemistry, University Hospital Maastricht,
PO Box 5800, 6202 AZ Maastricht, the Netherlands
e-mail: skos@klinchem.azm.nl

Inleiding

Een verkorting van de levensduur van de erythrocyten (hemolyse) kan een gevolg zijn van extracellulaire of intracellulaire factoren. Een van de intracellulaire oorzaken kunnen enzymstoornissen zijn, zoals glucose-6-fosfaat-dehydrogenase (G6PD)-deficiëntie.
G6PD-deficiëntie komt in verschillende vormen voor. In Nederland zijn het Mediterrane type en het A(-) type de meest voorkomende. Het Mediterrane type G6PD-deficiëntie komt vooral voor bij mensen (oorspronkelijk) afkomstig uit landen rond de Middellandse Zee, het A(-) type bij de negroïde bevolking. Ook bij mensen afkomstig uit het Verre Oosten komt regelmatig G6PD-deficiëntie voor. Geen van deze afwijkingen leidt tot chronische hemolytische anemie; wel kan hemolyse geïnduceerd worden door diverse farmaca, door infecties en door acidose. Tevens kan het een leiden tot oxidatieve stress in de longen en uiteindelijk ook extra schade veroorzaken zoals bij interstitiële longaandoeningen inclusief sarcoïdose[1].
Tabel 1 geeft een lijst met geneesmiddelen die vermeden moeten worden door G6PD-patiënten.
Tabel 2 geeft een overzicht van geneesmiddelen die in therapeutische doseringen veilig gegeven kunnen worden aan G6PD-deficiënte personen zonder chronische niet-sferocytaire hemolytische anemie.
Bij het Mediterrane type kan bovendien een hemolytische crisis geïnduceerd worden door het eten van tuinbonen (favisme). In de neonatale periode kan icterus voorkomen. Diagnose van G6PD-deficiëntie is belangrijk om hemolytische crises door tuinbonen of geneesmiddelgebruik te kunnen voorkomen.

Tabel 1. Gecontraïndiceerde geneesmiddelien bij alle vormen van G6PD-deficiëntie

Acetanilide[1]	Primaquine
Fenazopyridine[1]	Rasburicase
Furazolidon[1]	Sulfacetamide
Glibenclamide	Succimer (dimercaptobarnsteenzuur)
Methyleenblauw[2]	Sulfanilamide[1]
Nalidixinezuur[1]	Sulfapyridine[1]
Niradazol[1]	Tolonium[1]
Nitrofurantoïne	Trinitrotolueen (TNT)[1]

[1] niet in Nederland in de handel
[2] hoewel geen geneesmiddel, moet men voorzichtig zijn met het gebruik van methyleenblauw bij onderzoek naar de doorgankelijkheid van eileiders tijdens laparoscopie

Diagnostiek

Morfologisch worden alleen afwijkingen in een bloeduitstrijkje gezien als de cellen met oxiderende reagentia zoals acetyifenylhydrazine zijn behandeld. In G6PDdeficiënte cellen worden dan vaak zogenaamde 'Heinz bodies' gezien: neerslagen van gedenatureerd eiwit.

Voor de diagnose is de directe biochemische bepaling van de G6PD-activiteit betrouwbaarder. In het laboratorium bloedcelchemie van Sanquin Diagnostiek wordt dit met een standaard spectrofotometrische methode gedaan. Tevens wordt altijd een tweede enzym gemeten om te kunnen corrigeren voor de aanwezigheid van jonge rode cellen (reticulocyten) in de circulatie. Jonge cellen hebben altijd hogere enzymactiviteiten dan oudere cellen en kunnen een enzymdeficiëntie daardoor versluieren. In feite wordt dus de verhouding bepaald tussen de activiteit van G6PD en die van een ander enzym (glutathionreductase of pyruvaatkinase).
Aangezien het G6PD-gen op het X-chromosoom is gelegen, uit een deficiëntie zich bij mannen door een zeer geringe enzymactiviteit in de rode bloedcellen of het totaal ontbreken daarvan. Vrouwen kunnen heterozygoot (draagster) voor deze afwijking zijn, met G6PD-activiteiten van vrijwel normaal tot praktisch nul. Het vaststellen van heterozygotie is belangrijk omdat de bij deze vrouwen voorkomende subpopulatie G6PD-deficiënte erytrocyten tijdens stress kunnen lyseren, waardoor ook een hemolytische crisis ontstaat.
Minstens zo belangrijk is het om draagsterschap vast te stellen bij zwangeren, zodat een bij de pasgeborene optredende geelzucht in het licht van deze kennis kan worden behandeld. Diagnose van draagsterschap op basis van G6PD-activiteit is moeilijk vanwege de grote spreiding in de enzymactiviteit bij heterozygoten. Een extra aanwijzing voor het al of niet voorkomen van draagsterschap voor G6PD kan worden verkregen met behulp van de zogenaamde 'chroominhibitie test'. Hiervoor wordt bloed opgevangen in door Sanquin Diagnostiek toegezonden buizen met natriumchromaat. In normale cellen zorgt G6PD voor een hoog gehalte aan NADPH; hierdoor worden vele eiwitten, waaronder glutathionreductase, in gereduceerde toestand gehouden. Het gereduceerde glutathionreductase wordt door chromaat geïnactiveerd. In G6PD-deficiënte cellen, ook bij draagsters, wordt glutathionreductase niet volledig gereduceerd gehouden, waardoor het niet volledig vatbaar is voor chromaatremming. Bij deze personen wordt derhalve nog een aanzienlijke glutathionreductase activiteit gevonden in chromaatbehandelde erytrocyten, in tegenstelling tot normale cellen, waarbij deze activiteit tot vrijwel nul daalt door de chromaatbehandeling. Ongeveer driekwart van de draagsters wordt herkend met de chroominhibitietest[1]. Uitsluitsel omtrent draagsterschap kan worden verkregen door DNA-onderzoek, na telefonisch overleg.

Tabel 2 Middelen die waarschijnlijk veilig gegeven kunnen worden in therapeutische dosering aan G6PD-deficiënte personen zonder chronische niet-sferocytaire hemolytische anemie

Acetylsalicylzuur	Paracetamol
Aminobenzoëzuur	Probenecide
p-Aminosalicyizuur[1]	Procaïnamide
Aminopyrine	Proguanil
Antazoline	Pyrimethamine
Ascorbinezuur	Streptomycine[1]
Chlooramfenicol	Sulfacliazine
Chloroquine	Sulfafurazol
Colchicine	Sulfaguanidine[1]
Difenhydramine	Sulfamerazine[1]
Fenazon[1]	Sulfamethoxazol
Fenylbutazon	Sulfamethoxypyridazine
Fenytoïne	Tiaprofeenzuur
Fytomenadion	Trimethoprim
Lsoniazide	Tripelennamine
Kinine	Trihexyfenidyl
Levodopa	

1 niet in Nederland in de handel

Voor een volledige lijst met doseringen wordt verwezen naar het artikel van H.J.Guchelaar: Glucose-6-fosfaat dehydrogenase deficiëntie als contra-indicatie. Welke geneesmiddelen zijn veilig? Pharma Selecta 1996; 12:62-65.

G6PD deficiëntie draagsterschaponderzoek

ALGEMEEN	
Benaming op aanvraagformulier	G6PD deficiëntie draagsterschaponderzoek
CLB-aanvraagcode	B912
CLB-afkorting	G6PDpak.
CLB-aanvraagformulier	! Niet op CLB-aanvraagformulier
Vademecum 2003 hoofdstuk	5

TESTEN	Techniek	Normaalwaarden
Glucose-6-fosfaat dehydrogenase (+ referentie enzym)	Spectrofotometrische activiteit bepaling	Zie opmerkingen **< 1,1 IE/g Hb**
G6PD (mbv GSR act) vóór en nà chroominhibitie	Spectrofotometrische activiteit bepaling	Geen mutatie
DNA-analyse tbv G6PD deficiëntie typering	PCR (Polymerase Chain Reaction)	

AFNAME	Hoeveel	Minimaal	Temperatuur
Alléén na telefonische afspraak			4°C

BIJZONDERHEDEN	
Verzenden	PTT: CLB-standaardverzendverpakking CLB-bode: CLB-doos (etiket zwarte band)
Temperatuurslimiet	geen scherpe temperatuurslimiet
Tijdslimiet	geen scherpe tijdslimiet
Materiaalcode	4CB

TARIEF	CTG	Cluster	Aantal	Prijs (€)
Bloedcelchemie, bijzonder	79006	XIII	1	34,72
Bloedcelchemie, bijzonder	79006	XIII	2	34,72
Bloedcelchemie, bijzonder	79007	XVI	2	94,07

DESKUNDIGEN	
Laboratorium	Bloedcelchemie, Tel. 020-512 3346
Deskundigen	prof. dr D. Roos, biochemicus, E-mail: **d.roos@sanquin.nl** hr R. van Zwieten, hoofdanalist, E-mail: **r.vanzwieten@sanquin.nl** mw H.A. Vuil, hoofdanalist, E-mail: **h.vuil@sanquin.nl**

OPMERKINGEN	
	-> Glucose-6-fosfaat dehydrogenase (+ referentie enzym): : opmerkingen bij uitvoering onderzoek/normaalwaarden Bij de bepaling van één enzym dient altijd een referentie-enzym meebepaald te worden. Indien twee enzymen zijn aangevraagd is de één de referentie van de ander. Bij de bepaling van G6PD kan het referentie-enzym glutathion reductase of pyruvaatkinase zijn. Normaalwaarden G6PD per 01-01-2004: 0 - 3 mnd: 5,0 - 7,1 IE/g Hb > 3 mnd: 3,8 - 5,4 IE/g Hb Normaalwaarden glutathion reductase per 01-01-2004: 0 - 3 mnd: 3,1 - 6,6 IE/g Hb > 3 mnd: 2,7 - 5,8 IE/g Hb Normaalwaarden pyruvaatkinase per 01-01-2004: 0 - 3 mnd: 4,3 - 9,6 IE/g Hb > 3 mnd: 3,7 - 8,2 IE/g Hb Bij de interpretatie van de uitslag zijn behalve de normaalwaarden ook de leeftijd én een eventuele combinatie met andere afwijkingen belangrijk.
INDICATIE VOOR ONDERZOEK	
	Tuinbonen geïnduceerde hemolyse G6PD-deficiëntie, mediterrane vorm Favisme Hemolytische anemie Congenitale G6PD deficiëntie

VERWIJZING	
Andere trefwoorden	Glucose 6 fosfaat dehydrogenase deficiëntie draagsterschap Chroominhibitietest

laatste wijziging: 5 september 2000, van http://www.sanquin.nl

Reference

1 Rothkrantz-Kos S, Drent M, Vuil H, De Boer M, Bast A, Wouters EFM, Roos D, van Dieijen-Visser MP. Decreased redox state in red blood cells from patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2002; 19: 114-20.

References

Baker DE. Pharmacogenomics of azathioprine and 6-mercaptopurine in gastroenterologic therapy. *Rev Gastroenterol Disord* 2003; 3: 150-7.

Beckett WS. Occupational Respiratory Diseases. *New Eng J Med* 2000; 342: 406-13.

Camus P, Fanton A, Bonniaud P, Camus C, Fouchier P. Interstitial lung disease induced by drugs and radiation. *Respiration* 2004; 71: 301-26.

Drent M, Du Bois RM, Poletti R. Recent advances in the diagnosis and management of non specific interstitial pneumonia. *Curr Opin Pulm Med* 2003; 9: 411-7.

Nebert W, Russell. Clinical importance of the cytochromes P450. *Lancet* 2002; 360: 1155-62.

Nemery B, Bast A, Behr J, Borm PJ, Bourke SJ, Camus PH, De Vuyst P, Jansen HM, Kinnula VL, Lison D, Pelkonen O, Saltini C. Interstitial lung disease induced by exogenous agents: factors governing susceptibility. *Eur Respir J* 2001; 32: S30-42.

Weinshilboum R. Inheritance and drug response. *New Engl J Med* 2003; 348: 529-37.

Notes

Drug-induced, occupational and environmental related diffuse lung diseases