Species and strain differences in drug metabolism in liver and intestine

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Chapter 8

Summary

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Summary

The research described in this thesis was aimed to investigate interspecies differences in drug metabolism. For this, *in vitro* model systems, combined with sensitive analytical techniques and with analysis of gene expression were developed and compared to the *in vivo* situation.

Animal studies are commonly used to predict metabolism and toxicity of potential new human drugs. However, it is important to realize that humans differ from animals in isoform composition, expression and catalytic activities of enzymes involved in drug metabolism. In fact, even small changes in the amino acid sequences of these enzymes can give rise to profound differences in substrate specificity and catalytic activity. Therefore, differences in expression between species of the most important family of drug metabolizing enzymes, the cytochrome P450s (CYPs) are a major cause of species differences in drug metabolism. Therefore, **chapter 1** is focused on the description of the main CYPs involved in drug metabolism and an extensive comparison of the different isoforms among animal species commonly used for drug research and man is made.

In **chapter 2**, species differences in metabolism of two model compounds were investigated using liver slices of several experimental animal species. The method of organ slices was selected because slices can be easily made, not only from several tissues, but also from different species, including man. In addition, the effect of cryopreservation was tested in perspective to the formation of a liver slice bank from rat, mouse, monkey, dog and human, allowing the easy comparison of qualitative differences in metabolic profiles (both phase I and phase II mediated metabolism) of potential drug candidates among different species, including human. It was found that species differences in metabolic patterns and rates for both phase I and phase II reactions were detected and were well maintained after cryopreservation. Moreover, phase I and phase II metabolic activities were less affected by cryopreservation than some of the viability parameters in all investigated species.

Drug induction of metabolism is also markedly different between species and is the result of species dependent gene expression regulation. Therefore, it was explored whether induction of mRNA expression of CYP genes could be studied *in vitro* by using slices in combination with real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). In **chapter 3 and chapter 4**, induction of CYPs in two rodent species (rat and mouse) was investigated in slices from liver and intestine. It was concluded that liver and intestinal slices are a useful and predictive tool to study CYP induction, because both qualitatively, as well as quantitatively, induction profiles observed *in vitro* were quite similar to those *in vivo*. In addition, it was demonstrated that the RT-PCR method is a suitable and sensitive technique to assess changes in CYP mRNA expression in liver and intestinal tissue slices with relative low amount of tissue. For example, CYP1A1 which is constitutively expressed at extremely low levels in the rat liver and poorly detected using conventional Western Blot, was readily measured by RT-PCR.

In **chapter 5**, drug metabolism and expression of efflux transporters was investigated in different organs in CD-1 and nude mice. CD-1 mice are commonly used in drug discovery and development to examine drug metabolism and toxicity properties of new chemical entities, whereas athymic nude mice are often used in oncology research to investigate

tumour growth inhibition. Therefore, there is a need to understand similarities and differences in drug disposition of these mouse strains with respect to drug disposition. Hepatic phase I and phase II metabolism of a set of well known substrates in microsomes and/or liver slices demonstrated close similarity between the two strains. Moreover, the mRNA levels of the major CYP isoforms and drug efflux proteins, investigated by real time RT-PCR, in several organs, such as liver, kidney, intestine and adrenal glands of both strains, confirmed similarities in drug metabolism properties. Therefore, it was concluded that the potential error in extrapolating pharmacokinetic data from CD-1 to nude mice, or *vice versa*, is expected to be minimal.
In **chapter 6**, intestinal slices, as *in vitro* technique, were explored further in order to predict the role of the intestine in drug metabolism. An important finding was that the intestine was highly active in the metabolism of drugs. The formation of metabolites of several human CYP3A substrates by liver and intestinal slices from rat and mouse was compared. The results show that liver slices exhibited a higher metabolic rate for the majority of the studied substrates, but some metabolites were produced at a higher rate by intestinal slices, when compared with liver slices. Another important finding was that co-incubation with ketoconazole inhibited the metabolic conversion in intestinal slices almost completely but inhibition was variable in liver slices, demonstrating that metabolism of these substrates in the liver can be attributed to several CYP isoforms, some of which are not or weakly active in the intestine. This was also supported by the different expression in CYP3A isoforms between liver and intestine, as described previously in rats, which may explain the differences in formation rates of CYP3A metabolites between liver and intestine. To be able to better interpret the metabolic fate in the mouse, the expression of different CYP3A isoforms in mouse liver and intestine was investigated by RT-PCR, because in this species CYP3A expression has not been well described. It was concluded that similar to rats, mouse CYP3A isoenzymes are expressed differently between liver and intestine.

In conclusion, *in vitro* models provide an opportunity to screen the disposition of new drugs and provide rapid initial information on potential drug-drug interactions. Furthermore, *in vitro* models, such as organ slices, are important tools to enable the comparison of experimental animal species and man in order to be able to understand and predict potential differences of non-clinical and clinical drug discovery and development. This allows a reduction of the number of laboratory animals needed for research and facilitates the design of better animal and human *in vivo* experiments.

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