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CLINICAL STUDIES OF CYP3A4 AND P-GLYCOPROTEIN

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”Try to learn something about everything and everything about something.”

Thomas Huxley

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Clinical studies of CYP3A4 and P-glycoprotein

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ABSTRACT

In the field of pharmacokinetics, cytochrome P450 enzymes have been extensively studied. The CYP450 enzyme with the most drug substrates is CYP3A4, but despite its significance, administration of a specific substrate to subjects is required to determine its activity, by measuring the metabolic ratio. For reasons of feasibility and safety it would be preferable to have an endogenous biomarker instead of a probe drug. The activity of CYP3A4 varies considerably as it can be induced and inhibited by certain compounds. For example, it is induced by ligands to the Pregnane X receptor (PxR). Activation of this receptor also induces the drug transporter P-glycoprotein. Regarding genetic variability, it is well known that *CYP3A4* does not display genetic polymorphism in activity, but results from studies of P-gp in that aspect have been inconclusive.

In the first study of this thesis, which was also aiming to evaluate the inductive property of rifampicin on four other CYP450 enzymes, a probe drug and an endogenous biomarker were compared regarding their properties for measuring CYP3A4 activity. Induction was achieved by administration of rifampicin in three different doses (20-500 mg once daily) to healthy volunteers. The endogenous biomarker 4 β -hydroxycholesterol had a linear relationship with the metabolic ratio of the CYP3A4 probe drug quinine. In this study four other probe drugs were also used simultaneously, each specific for a different CYP450 enzyme. All enzymes except CYP2D6 were induced by rifampicin. This cocktail had been designed not to cause any drug-drug interactions among the probes, which are also specific for each enzyme.

In the second study, 4 β -hydroxycholesterol was used to investigate whether exposure to triclosan in the doses reached by normal use of toothpaste can induce the enzyme. Triclosan activates PxR *in vitro*, but the results from our clinical study indicate that this effect is not relevant when subjects are exposed in the dose range achieved by normal use of toothpaste.

In the third clinical study two common haplotypes of *MDR1*, which encode P-gp, were compared by phenotyping with the substrate digoxin. The haplotypes were chosen because they are common and because their clinical relevance has been under discussion for many years. Digoxin is the most commonly used drug for investigating P-gp activity as it is not metabolized and because it is a known substrate of this transporter. The difference in plasma digoxin kinetics was not statistically significant in our study and the study does not support that genetic variability in *MDR1* has an impact on P-gp activity in the haplotypes investigated. Ligands to PxR also induce the drug transporter P-glycoprotein, which has largely overlapping substrate specificity with CYP3A4. Drug transporters have been considerably less studied than CYP450 enzymes but the knowledge is rapidly increasing and the focus has been mainly on P-gp because of its broad specificity. Even though *CYP3A4* does not have polymorphic activity, it is well known that some CYP450 enzymes do, and the data on whether P-gp has such variability has been inconclusive.

LIST OF SCIENTIFIC PAPERS

- I. Kanebratt KP, Diczfalusy U, Bäckström T, Sparve E, Bredberg E, Böttiger Y, Andersson TB, Bertilsson L. Cytochrome P450 induction by Rifampicin in healthy subjects. *Clinical Pharmacology and Therapeutics* 2008; Nov; 84 (5): 589-594
- II. Allmyr M, Panagiotidis G, Sparve E, Diczfalusy U, Sandborg-Englund G. Human exposure to triclosan via toothpaste does not change CYP3A4 activity or plasma concentrations of thyroid hormones. *Basic and Clinical Pharmacology and Toxicology*. 2009; Nov; 105 (5): 339-44
- III. Sparve E, Aklillu E, Fukasawa T, Bertilsson L, Panagiotidis G. In vivo digoxin kinetics in two different MDR1 haplotypes. Manuscript.

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LIST OF ABBREVIATIONS

ABC	Adenosine-triphosphate binding cassette
ABCB1	ATP-binding cassette B1
ADME	Absorption, distribution, metabolism, elimination
BRCP	Breast cancer related peptide
CNS	Central nervous system
CYP	Cytochrome P450
CYP3A	Cytochrome P450 3A
CYP3A4	Cytochrome P450 3A4
GCP	Good clinical practice
MDR1	Multi drug resistance 1
P-gp	Permeability-glycoprotein
PK	Pharmacokinetics
PxR	Pregnane X receptor
SLC	Solute carrier
TDM	Therapeutic drug monitoring

1 INTRODUCTION

It is well known that the same drug may have different effects in different individuals. It is also known that the same drug, and the same dose of that drug, may affect the same person differently on different occasions. The reasons for this are several. Pharmacology is usually divided into how the drug affects the body (pharmacodynamics) and how the body affects the drug (pharmacokinetics). Variability in pharmacokinetics (PK) may be due to genetic, constitutional and environmental factors such as the number of gene copies of an enzyme, renal function and concomitant medications to name but a few.

Drug metabolism has been extensively studied and there are methods of approximating the activity of different cytochrome P450 enzymes (CYPs). A practical limitation of performing such investigations is, however, that they require administration of exogenous compounds. A less studied field is that of drug transporters, even though knowledge is rapidly increasing in that area. The most important drug transporter is Permeability-glycoprotein (P-gp).

1.1 PHARMACOKINETICS

PK is divided into absorption, distribution, metabolism and elimination (ADME). All of these parameters demonstrate inter- and intraindividual variability. Absorption may be affected by e.g. the food ingested. Differences in distribution may be affected by e.g. concomitant medications (1). The metabolism of certain drugs may depend on genetics (2) and in clinical practice the doses of several drugs are routinely adjusted for renal function, as the elimination can vary significantly.

PK often refers only to plasma concentrations of the drugs, even though the drug concentration at the target organ is more relevant. The reason for this is practical – it is much easier to measure the plasma concentration of for example a psychotropic drug than to measure how much that actually reaches the brain.

1.2 CYTOCHROME P450 SYSTEM

The cytochrome P450 enzymes were named in 1961 (3) and are most abundant in the liver, even though for example CYP3A4 is present in significant amounts also in enterocytes. The impact of genetics is large in some of the CYPs, such as *CYP2D6*, while *CYP3A4* does not demonstrate polymorphic activity. Even though a CYP does not have a variable metabolic capacity due to genetics, there may be a large inter- and intraindividual variability also in those depending on interactions with drugs and other exogenic compounds.

The CYP with the most drug substrates is CYP3A4, while other CYPs such as CYP2C9 has considerably fewer substrates. The same drug is often a substrate of several CYPs and some drugs, such as donepezil, is cleared from the body both by CYPs and in unchanged form by the kidneys (4). Drugs with several routes of elimination show less variability within and between individuals as other routes may compensate if one of the routes is inhibited. For example sertraline has a very linear relationship between dose and plasma concentrations, according to data extracted from the TDM database at the Karolinska Hospital (Unpublished data). The drugs that are developed today are seldom specific substrates of CYP2D6 for example, because of the large genetic variability which cannot be known without genetic testing of patients.

1.3 DRUG TRANSPORTERS

Drug transporters are usually divided into passive (non energy demanding) and active (demanding energy). Since passive transporters do not consume energy, they do not transport their substrates against electrochemical gradients but simplify the flux from a high concentration compartment to a low concentration compartment. There is a large family of passive transporters named solute carriers (SLC) (5).

There are at least 48 different ABC transporters which can transport drugs between different compartments and they often have overlapping specificity (6). Therefore it is not uncommon that the same compound may be transported by e.g. breast cancer related peptide (BCRP) and P-gp.

Drug transporters are present in the cell membranes in different barriers in the body, such as the intestinal wall, the blood-brain barrier, the testes and the placenta. The same transporter is often localized in several barriers, thereby affecting not only distribution but also absorption from the intestine and elimination via the kidneys or the bile.

Inhibition of P-gp in the blood-brain barrier, where it normally pumps its substrates out of the central nervous system (CNS), and in the enterocytes, where it prevents absorption, has been suggested to allow for a central opioid effect of loperamide (1).

1.4 PHARMACOGENETICS

The area of pharmacogenetics includes both pharmacodynamics and pharmacokinetics. The genetic influence on PK has been focused on CYP enzymes for several decades, and many clinically relevant variations have been discovered during this period. Genetic variability in drug transporters is less studied, but in that field the main focus has been on *MDR1* because

of its many substrates and the potential impact genetic polymorphism could have on a number of clinical outcomes. The technical evolution makes genotyping cheaper and easier at a great pace, affecting clinical routines and drug development.

1.5 CYP3A4

The CYP3A4 enzyme does not show polymorphic activity, even though a very similar enzyme (CYP3A5) may or may not be active, and contribute to metabolism, depending on genetics. While not being polymorphic in activity, the activity of CYP3A4 varies up to 10-fold due to interactions with drugs and other exogenous compounds. The most potent inducer of CYP3A4 that is known is rifampicin, which induces several proteins, and one of the most potent inhibitors is ketokonazol (7). CYP3A4 is present not only in the liver but also in enterocytes, where it can be inhibited also by consumption of different fruit juices (8).

CYP3A4/5 are the cytochrome P450 enzymes with the most substrates and has been estimated to participate in the metabolism of about half the drugs on the market (9). Because of the many substrates and the potential of inhibition and induction, a situation may occur when the same drug is an inducer, a competitive inhibitor and a substrate of this enzyme which makes the pharmacokinetics very difficult to predict (10, 11). Further complicating this scenario, it may also be a substrate and an inducer of P-gp. A well documented marker of CYP3A4 activity is quinine (12), but an endogenous marker would be useful in the clinic and in the development of new drugs.

1.6 P-GLYCOPROTEIN

P-gp was originally named Multi Drug Resistance 1 (MDR1) since it was discovered because of its ability to prevent cytostatics from entering cancer cells (13). It has since been shown to have a very large number of drug substrates and to be present in several barriers in the body. As CYP3A4, it is highly inducible and can also be inhibited by e.g. verapamil (14) but whether genetic polymorphism is a relevant factor has been under discussion. Interestingly, both CYP3A4 and P-gp can be induced by the same drugs by activating the PXR receptor, indicating that they interact in limiting the exposure to possible toxins. The large gene encoding P-gp is called ATP-binding cassette B1 (*ABCB1*) or *MDR1* and consists of 200 000 base pairs. There are at least 124 known SNPs in Caucasians where different nucleotides may be present, but no functional variation has yet been determined with certainty (15).

P-gp has substrates in many different therapeutic areas with a large potential of clinically important impact. It is present in several locations (figure 1) and affects not only absorption from the intestine but also distribution, as it is present in several barriers, and elimination

since it pumps substrates into the urine and the bile. The most common marker of P-gp activity is digoxin, because it is not metabolized and because it is a well known substrate of P-gp (16). A possible limitation of digoxin as a probe drug for P-gp activity is that it may be a substrate also of other transporters (17).

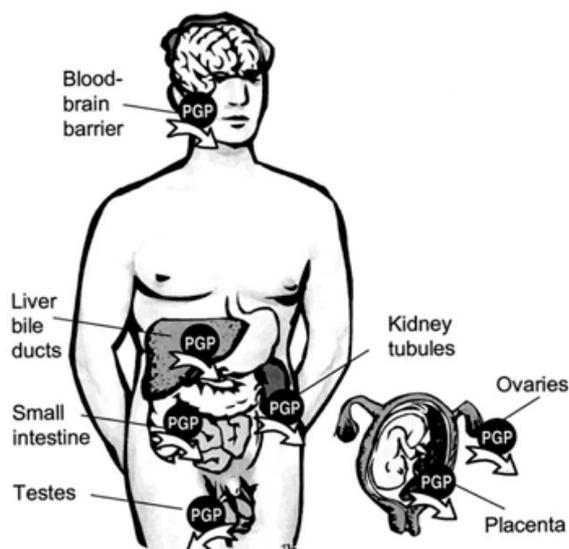


Figure 1.

P-gp is located in different barriers and actively eliminates substrates via the kidneys and the bile ducts.

Marzolini C, Paus E, Buclin T, Kim RB (2004). Polymorphisms in human MDR1 (P-glycoprotein): Recent advances and clinical relevance. Clin Pharm Ther 75:13-33.

1.7 P-GP AND CYP3A4

P-gp and CYP3A4 interact in protecting the body from toxic compounds, but may also complicate drug treatment. Both proteins can be induced and often by the same drugs and both can be inhibited by concomitant medication and by certain beverages (grape fruit juices). Rifampicin may induce both proteins via the P_xR receptor (18, 20). Both P-gp and CYP3A4 are present in the intestinal wall, where P-gp in the cell membrane actively transports substrates back into the gut lumen. Theoretically, this could prolong the exposure of substrates for intracellular CYP3A4 enzyme.

1.8. CLINICAL TRIALS

Clinical studies are performed in humans, either in healthy volunteers or in patients. The outcomes are usually measured in terms of effects and adverse events. In purely pharmacokinetic studies, however, the goal is to obtain reliable plasma concentrations with the lowest possible risk of adverse events. As most side effects of drugs are dose dependent, the lower plasma concentrations that can be measured with the laboratory method used, the lower the risk of adverse events. The clinical studies need to fulfill ethical requirements as

evaluated by an Ethics committee and should be performed according to standards of Good Clinical Practice (GCP).

2 THE PRESENT STUDY

2.1 AIMS OF THE STUDY

- To characterize an endogenous biomarker of the activity of the drug metabolizing enzyme CYP3A4.
- To investigate whether the antibacterial compound triclosan can induce CYP3A4 using the endogenous biomarker previously characterized
- To investigate whether there is a functional difference between two common MDR1 haplotypes using the probe drug digoxin.

2.2 MATERIALS AND METHODS

2.2.1 Study populations

In the first study (paper 1), 24 healthy Caucasian Swedish adults participated. Their ages were in the range 23-61 years (mean 34 years) and body weights were between 52-99 kg (mean 78 kg for men and 66 kg for women). Subjects were ascertained to be healthy by medical history, physical examination and laboratory testing including virology (Hepatitis B, hepatitis C, HIV) and routine tests for kidney function, liver function and hematology. Female subjects had negative tests for pregnancy. Subjects expressing CYP3A5 were excluded by genotyping.

In the second study (paper 2), 12 healthy Caucasian Swedish adults participated. Five were men and seven were women. Subjects were ascertained to be healthy by medical history, physical examination and routine laboratory testing. The exclusion criteria were pregnancy, ongoing infection, intake of medication including natural remedies (for example St John's wort), within two months prior to starting the study, except for paracetamol, and active drug or alcohol abuse.

In the third study (paper 3), 14 healthy Caucasian Swedish adults participated. Nine were men and five were women. Their ages were in the range 20-62 years (mean 32 years) and body weights were between 64-93 kg (mean 74 kg). The inclusion criteria were normal GFR (70-120 ml/min) as measured by Cockcroft Gault, normal P-potassium and normal ECG including heart frequency above 50/min and normal findings in the physical examination. The exclusion criteria were pregnancy, ongoing infection, intake of medication including natural remedies (for example St John's wort), within one month prior to starting the study, except for paracetamol, and active drug or alcohol abuse. Subjects had either the haplotype

1236/2677/3435 CGC-CGC (six subjects) or the haplotype TTT-TTT (eight subjects) in MDR1.

2.2.2 Phenotyping

In the first study (paper 1), phenotyping was performed with five probe drugs and the endogenous biomarker 4 β -hydroxycholesterol. The probes were caffeine for CYP1A2, losartan for CYP2C9, omeprazole for CYP2C19, debrisoquine for CYP2D6 and quinine for CYP3A4.

In the second study (paper 2), phenotyping for CYP3A4 was performed with the endogenous biomarker 4 β -hydroxycholesterol.

In the third study (paper 3), phenotyping for P-gp was performed with the probe drug digoxin.

2.2.3 Statistical analysis

For all statistical analysis p-values less than 0.05 were regarded as statistically significant, using the two-sided Student's *t*-test.

2.2.4 Laboratory analysis

Plasma concentrations of the probe drugs in the first study (paper 1) was performed according to the methods described in Christensen et al (12) and Hultman et al (19).

In the second study (paper 2), triclosan was analysed using liquid-liquid extraction. An isotope dilution method with ¹³C-labelled triclosan as the internal standard was applied for quantification, using gas chromatography/electron capture negative ionisation/mass spectrometry. The entire method is described in Allmyr et al. (20). 4 β -Hydroxycholesterol was determined by isotope dilution gas chromatography-mass spectrometry using a deuterium-labelled internal standard as described by Bodin et al (21).

In the third study (paper 3), digoxin was quantified by DRI ® Digoxin Assay, which is the method used in clinical routine. This method was modified by adding an additional standard concentration of 0.13 nmol/L.

3 RESULTS AND DISCUSSION

3.1 PAPER 1

The main findings of the first study were that the probe drugs and the endogenous biomarker 4 β -hydroxycholesterol may be used to establish the induction properties of a compound. 4 β -hydroxycholesterol was dose dependently induced within the investigated dose range of 20-500 mg rifampicin once daily (Figures 2 and 3).

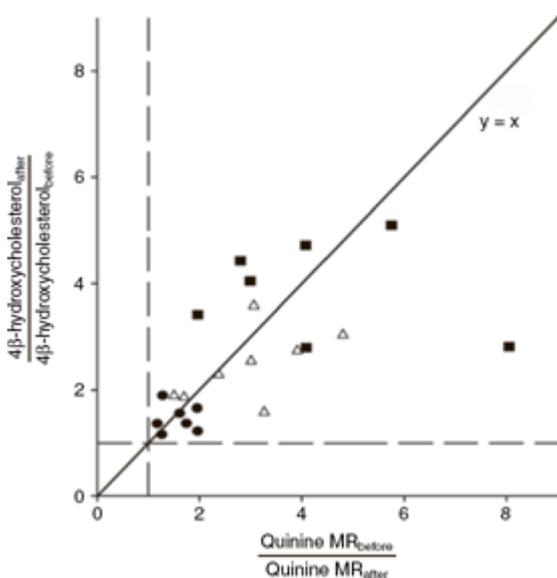


Figure 2. Correlation of induction ratios for plasma concentrations of 4 β -hydroxycholesterol with quinine:3'-hydroxyquinine metabolic ratio after three different daily doses of rifampicin.

Closed circles = 20 mg

Open triangles = 100 mg

Closed squares = 500 mg

(Paper 1)

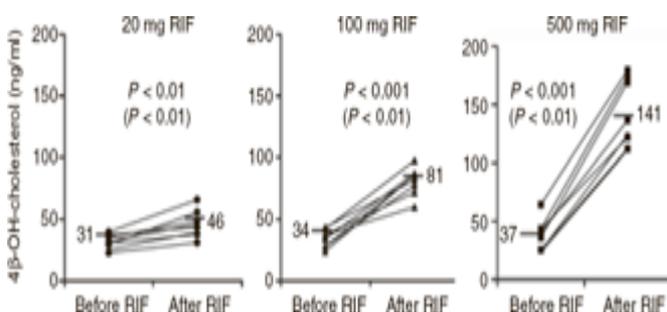


Figure 3. Induction of CYP3A4 was dose dependent within the investigated dose range of 20-500 mg rifampicin once daily.

(Paper 1)

CYP3A4, CYP1A2, CYP2C9 and CYP2C19 were induced by rifampicin, as demonstrated by the use of exogenous substrates. CYP2D6 seems not to be inducible. As the probe drugs are specific for each enzyme and do not interact with each other, this "cocktail" may be used for phenotyping the enzyme activities at the same time. It was demonstrated that the endogenous marker 4 β -hydroxycholesterol indicates the activity of CYP3A4.

In the highest dose group (500 mg o.d.) one subject developed an eczema which resolved within two months. No other significant adverse events occurred.

3.2 PAPER 2

The second study showed that even though triclosan activates PXR in vitro, the exposure via toothpaste in normal use (Fig 4) is too low to result in a measurable induction of CYP3A4. The structure of triclosan resembles the ones of thyroid hormones and previous studies have shown that triclosan poses thyroid-disrupting properties. It was recently shown that orally administered triclosan caused a dose-dependent decrease of circulating serum levels of thyroid hormones in rats. However, thyroid hormonal levels were also unaffected, indicating that the exposure to triclosan via toothpaste does not have a significant effect on these parameters (Figures 4 and 5).

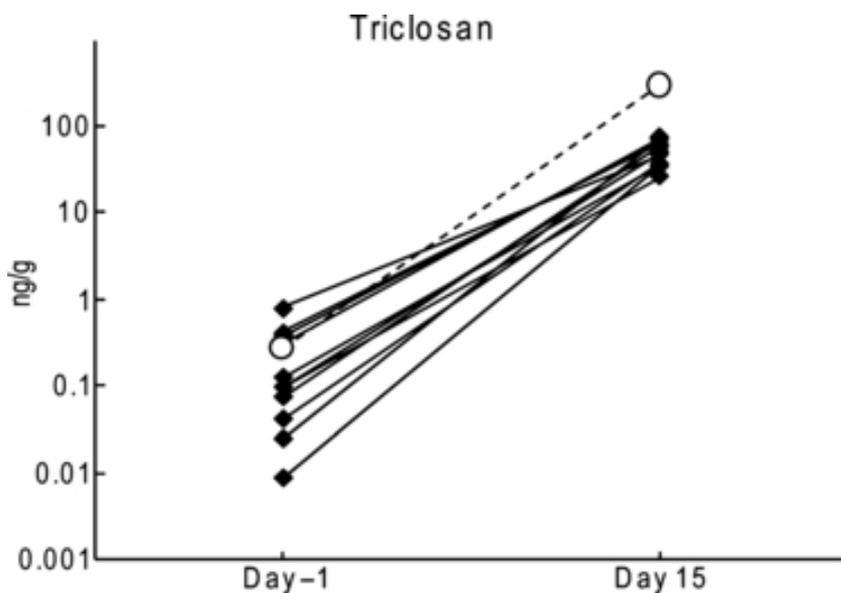


Figure 4. The levels of triclosan in plasma increased during the study, as expected.

(Paper 2)

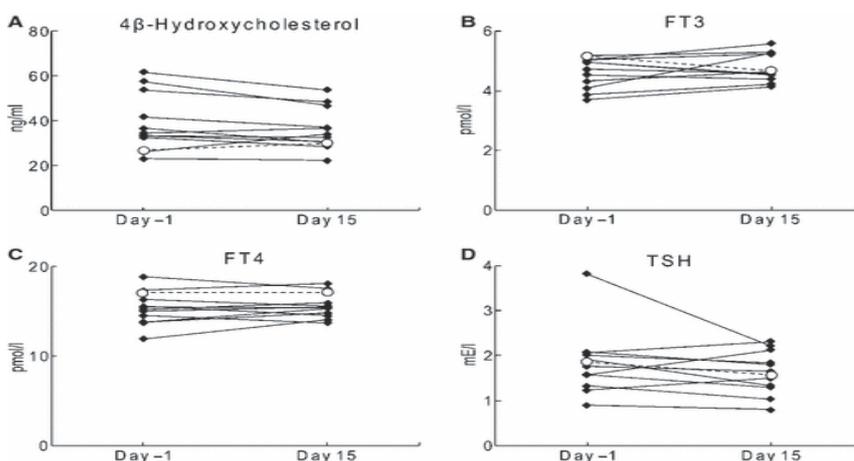


Figure 5. 4β-hydroxycholesterol and thyroid hormone levels were unaffected by the increase in triclosan.

(Paper 2)

There was an expected and quantifiable increase in triclosan plasma concentrations during exposure to tooth paste, while thyroid hormonal levels and 4 β -hydroxycholesterol remained unaffected.

3.3 PAPER 3

The third study demonstrated no statistically significant difference in AUC 0-24h, C_{max} or T_{max} between the two haplotypes in this study. Our result indicates no significant impact of the two *ABCB1* haplotypes on P-gp transporter activity, as measured by digoxin kinetics.

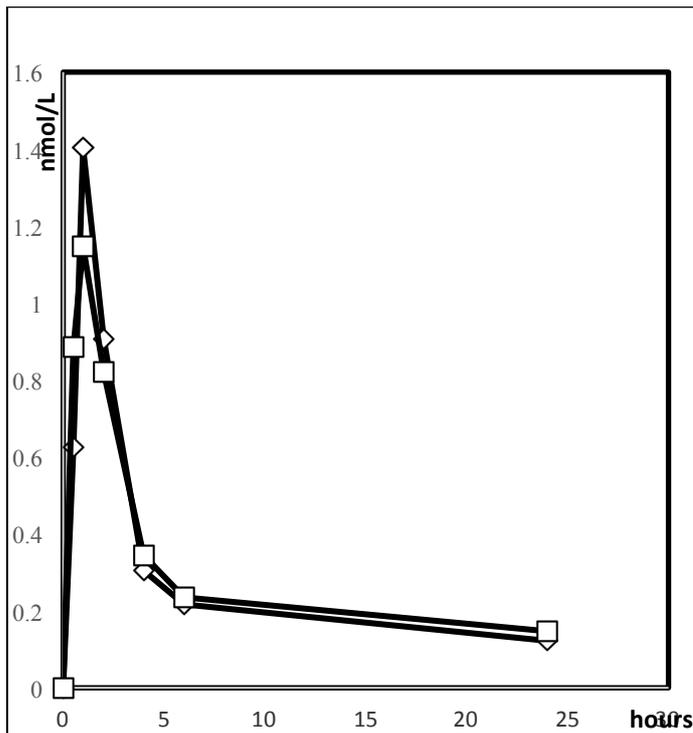


Figure 6. Average plasma concentrations of digoxin in the 1236/2677/3435 CGC-CGC and TTT-TTT groups. Diamonds are CGC. Rectangles are TTT.

(Paper 3)

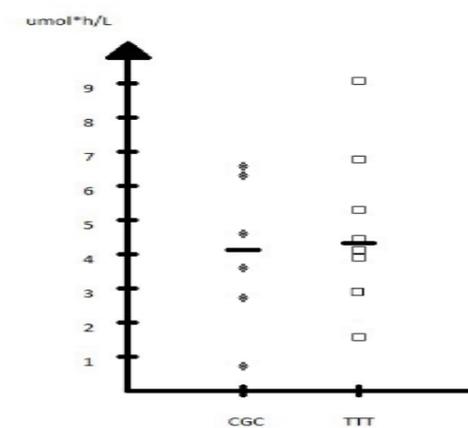


Figure 7. AUC 0-24 hours of digoxin in the 1237/2677/3435 CGC-CGC and TTT-TTT groups. The bold lines represent mean values in each group. (Paper 3)

Despite a large number of studies testing potential phenotypic associations of the three most common *MDR1* SNPs on drug disposition with digoxin, the literature is inconclusive. The first report regarding the impact of c.3435T on P-gp activity was published in 2000 by Hoffmeyer et al (22), in which several SNPs in the *MDR1* gene were examined. Expression levels of P-gp in intestinal enterocytes were determined by biopsies in healthy subjects and in patients and correlated to different genotypes. The c.3435TT genotype correlated with a 2-fold lower expression of the *MDR1* gene. Some later studies have suggested an impact of position c.3435 on digoxin kinetics (23, 24, 25).

The c.3435 C>T variant is part of a haplotype including also the positions c.1236 and c.2677. Since the c.3435 variation does not change the amino acid sequence in the protein two main hypotheses emerged to account for the decreased P-gp activity related to the 3435T SNP: either the variation at c.3435 resulted in a decreased translation efficiency (26), or the finding was due to the incomplete linkage disequilibrium with the positions 1236 and 2677. Several studies have been conducted focusing on position 3435 since 2001, but the results have been inconsistent (27, 28, 29) regarding the impact on the well-known, non-metabolized P-gp substrate digoxin (30) in vivo, which would suggest the second explanation to be more plausible as the linkage disequilibrium is incomplete. Neither the variation in position 3435 nor the variation in position 1236 results in amino acid substitution. On the other hand, each of the three possible nucleotides G, T or A in position 2677 encodes a different amino acid.

Digoxin was quantifiable in plasma with good reliability until 24 hours post-dose. We found no statistically significant difference (Student's *t*-test, two-sided) in the mean digoxin plasma exposures as measured by the trapezoidal method from 0-24 hours (TTT group 4.82 $\mu\text{mol}\cdot\text{h}/\text{L}$ versus CGC group 4.13 $\mu\text{mol}\cdot\text{h}/\text{L}$, $p=0.58$), adjusting for different doses by dividing concentrations in the CGC group by two. C_{max} was 1.34 and 1.48 $\mu\text{mol}/\text{L}$ ($p=0.50$), respectively, and T_{max} was 1.19 and 1.25 hours ($p=0.84$), respectively. As presented in figure 7 the variability was large in both groups. Average plasma concentrations in the two groups over time are presented in figure 6 and AUC 0-24 hours is presented in figure 7.

4 CONCLUSIONS

4.1 PAPER 1

Although considerably less complicated to use, the endogenous biomarker has some limitations compared to phenotyping with the probe drug. The half-life of 4 β -hydroxycholesterol is 17 days (30), which makes the method less suited for measuring rapid changes in enzyme activity.

Another difference between phenotyping with quinine and with 4 β -hydroxycholesterol is that the latter may only reflect CYP3A4 activity in the liver. As the enzyme exists in significant amounts also in enterocytes, some important information could be missing if no probe drug is used to measure the activity level.

However, 4 β -hydroxycholesterol is still a useful addition to the toolbox in the clinic and in drug development because it is far more practical to measure with a simple blood sample than to administer a drug only for the purpose of quantifying enzyme activity.

4.2 PAPER 2

Triclosan is a common chemical in tooth paste and because of the *in vitro* findings of the inductive properties of the compound, there was a need for investigating whether the effect had clinical significance when the exposure to tooth paste is in the range achieved by normal use. The results of this study indicate no such effect.

As studies in rats indicated a potential decrease in thyroid hormonal levels, this was also investigated in the present, but no such effect could be observed.

4.3 PAPER 3

There have been conflicting results in the studies of a potential impact of genetic variability in the *MDR1* gene on digoxin kinetics. As previous studies have focused on the silent variation in position 3435, we chose to investigate subjects with the haplotypes TTT-TTT versus CGC-CGC as 2677 is a non silent variation. If a difference had been established, this would have explained the conflicting results in studies focusing on position 3435. However, the variability was large in our study and there was no statistically significant difference in

plasma kinetics between the two groups. Theoretically, the haplotypes may still have clinical relevance but the results in our study do not support genetic variability to be of importance for P-gp activity.

4.4 SUMMARY

Personalized medicine has to consider within and between subjects variations in pharmacokinetics. In that area, there is considerably more knowledge about drug metabolizing enzymes than about drug transporters. It is not disputed that variability in CYP3A4 activity has clinical relevance. Therefore, there is a value in being able to determine this activity as easily as possible in order to estimate correct dosing with better reliability. In this thesis, it has been demonstrated that an endogenous biomarker may be used for this purpose. The advantages of an endogenous biomarker, compared to administration of a probe drug, are twofold. Firstly, there is no risk of adverse events due to administration of a foreign compound. Secondly, the feasibility is significantly increased as there is no need for detailed planning regarding the time of blood sampling. There are, however, limitations that the endogenous biomarker has compared to using the probe drug. 4 β -hydroxycholesterol has a long half-life and is therefore not suitable for measuring rapid changes in CYP3A4 activity. Moreover, it may not reflect the total metabolic capacity of CYP3A4 as the enzyme is not only present in the liver but also in enterocytes. Despite these limitations, the significant advantages make 4 β -hydroxycholesterol useful both in the clinic and in research.

As CYP3A4 has been extensively studied, it is well known that its variable activity is due to interactions with drugs and beverages. One of the most potent inducers is rifampicin, which was used in the first study, but several compounds may increase or decrease the activity of the enzyme. In vitro findings indicated that triclosan, a compound which is present in common tooth paste, may induce CYP3A4. As this effect could potentially affect a large number of drugs, we performed a second study where subjects brushed their teeth in a normal fashion while measuring the activity of CYP3A4. In this second study we used the endogenous biomarker which had been characterized in the first study to quantify the enzyme activity. The result was that there was no effect on CYP3A4 activity by exposure to triclosan when using tooth paste normally. It is likely that the effect is dependent on the exposure to

triclosan, which was lower in vivo. Studies in rats had also indicated that exposure to triclosan could decrease thyroid hormonal levels, but nor could such effect be observed in our study.

As enzymes have been extensively studied, it is well known that several enzymes may participate in elimination of a drug. Drugs may also be eliminated renally and/or with the bile. Further complicating the fate of an administered drug, there are drug transporters which may affect distribution, uptake and elimination of their substrates. These transporters are also known to have overlapping substrate specificity. The most studied drug transporter is P-glycoprotein because of its many substrates and clinical relevance. As CYP3A4, it can be induced and inhibited by administration of various compounds, but whether genetic variability contributes to affecting its function is uncertain. In this large gene, the focus in the literature has been mainly on the positions 1236, 2677 and 3435. By administration of digoxin to healthy volunteers, we investigated a potential difference in activity between two groups. However, no statistical difference was observed and our study does not support that genetic factors in *MDR1*, which encodes P-gp, are of importance for its activity. Even though digoxin is the most commonly used probe for phenotyping P-gp activity, it is possible that other drug transporting proteins are also involved in the fate of the compound.

In conclusion, the complexity of pharmacokinetics is often humbling and the resulting levels in the plasma and in different compartments when administering a drug depends on several factors, which involve many areas in medicine.

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