Pharmacogenetics (1994) 4, 209–218

Original Article

The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes*

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Received 15 November 1993 and accepted 14 April 1994

The impact of gender, use of oral contraceptive steroids (OCS), coffee consumption and of smoking on the metabolism of sparteine, caffeine, and paracetamol was studied in 194 randomly selected subjects (98 male and 95 female). Thirty-eight of the male volunteers were cigarette smokers, 40 of the female subjects were smokers and/or users of OCS. The metabolic ratio of sparteine oxidation (MR_s) showed a trimodal distribution. 7.7% of the subjects had a $MR_s > 20$ and thus were poor metabolizers (PMs). Within the extensive metabolizer (EM) subjects, a distinct subgroup accounting for 11% was observed with $20 > MR_s > 1.2$. Six of the 15 phenotypical PMs were heterozygous EMs by genotyping. This indicates the existence of one or several CYP2D6 mutations which cannot be identified by the currently employed genotyping methods. In each subgroup, i.e. smokers/OCS and non-smokers/non-OCS, the cumulative frequency distribution of the heterozygous (wt/B) phenotype caused a shift to higher MR_s compared with the wild-type homozygotes (wt/wt). Thus, for the *in vivo* activity of CYP2D6, genetic determinants prevail over environmental factors. Smoking, use of oral contraceptive steroids, caffeine consumption, or gender had no influence on sparteine metabolism. The distribution of the paracetamol glucuronide/paracetamol metabolic ratio appeared to be unimodal although skewed. Glucuronidation capacity was clearly affected by gender, OCS use and smoking. It was higher in male than in female subjects. Male smokers had the highest, and female non-smokers/non-OCS users the lowest metabolic ratio. CYP1A2 activity, as determined by a caffeine metabolic ratio ((AFMU + 1X + 1U)/1,7U), was multimodally distributed and was clearly increased in smokers. It was significantly correlated to paracetamol glucuronidation in male heavy smokers ($r = 0.85$), suggesting an element of co-regulation of CYP1A2 and of paracetamol conjugating UDP-glucuronosyltransferase isozymes, including UGT1.6.

Introduction

Drug-metabolizing enzymes are of paramount importance in the inactivation and activation of drugs, chemicals and carcinogens. Thus, interindividual variability in the activity of these enzymes can influence individual drug response, susceptibility to adverse drug reactions, the occurrence of drug-induced diseases and certain types of chemically-induced cancers. Since both genetic and environmental factors have been shown to be relevant determinants for the activity of drug-metabolizing enzymes, it is important to identify the factors which determine and modulate their activity. In the case of drugs it has been demonstrated that interindividual differences, e.g. on a genetic basis, in the activity of drug-metabolizing enzymes determine the intensity and duration of drug action and toxicity (Brøsen and Gram, 1989; Eichelbaum & Gross, 1990).

*The nomenclature of cytochromes P450 and of UDP-glucuronosyltransferases used in this report is that suggested by Nelson *et al.* (*DNA Cell Biol* 1993: 12, 1–51) and by Burchell *et al.* (*DNA Cell Biol* 1991: 10, 487–494), respectively.

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§Abbreviations: AIC, Akaike's information criterion (Yamaoka *et al.*, 1978); CYP, cytochrome P450; DQ, debrisoquine; EM, extensive metabolizer of debrisoquine/sparteine; MR, metabolic ratio(s); MR_p, MR of paracetamol metabolism; MR_s, MR of sparteine metabolism; MR_c, MR of caffeine metabolism; MSQ, mean sum of squares; OCS, oral contraceptive steroids; PAH, polycyclic aromatic hydrocarbons; PCR, polymerase chain reaction; PM, poor metabolizer of debrisoquine/sparteine; RFLP, restriction fragment length polymorphism; RRSW, Rosin-Rammler-Sperling-Weibull function (Rosin *et al.*, 1933; Weibull, 1951); UGT, UDP-glucuronosyltransferase; wt, wild-type.

Data which link interindividual differences at the level of expression of enzymes activating carcinogens with increased cancer risk are still equivocal. For example, the tobacco smoke nitrosamine 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone is activated by CYP2D6, although other cytochromes P450 also metabolize this compound (Crespi *et al.*, 1991). In addition, the relationship between the phenotype of the debrisoquine/sparteine polymorphism and cancer is not unequivocal (Ayesh *et al.*, 1984; Cartwright *et al.*, 1984; Kaisary *et al.*, 1987; Roots *et al.*, 1988; Benítez *et al.*, 1990; Caporaso *et al.*, 1990; Sugimura *et al.*, 1990). Whether, in addition to genetic factors, environmental factors such as smoking, OCS§ use, or coffee consumption could alter sparteine oxidation capacity at least in the EM phenotype has not been studied in greater detail.

The inducing effect of several xenobiotics on the activity of CYP2D6 has been studied in EMs and PMs. No induction was observed in PMs. This is not surprising since CYP2D6 is not expressed in PM individuals. In EMs, only minor effects of rifampicin and phenobarbital were observed (Eichelbaum *et al.*, 1986). Caffeine consumption was found to correlate with the metabolic ratio of debrisoquine, a predominant but not exclusive substrate of CYP2D6. However, the correlation was only weak ($r = -0.19$; $p < 0.01$) (Steiner *et al.*, 1985). Thus, the genetic background appears to be predominant in the determination of DQ metabolism.

Environmental factors prevail in the case of regulation of the expression of other drug-metabolizing enzymes such as UDP-glucuronosyltransferases (Nash *et al.*, 1984; Miners & Mackenzie, 1991). For example, evidence has been obtained that CYP1A2 activity (determined by caffeine oxidation; Campbell *et al.*, 1987a; Kalow & Tang, 1993) and glucuronidation of paracetamol (Mucklow *et al.*, 1980; Bock *et al.*, 1987), 1-naphthol (Fleischmann *et al.*, 1986) and propranolol (Walle *et al.*, 1987) are increased in cigarette smokers. However, in other situations such as OCS use the two enzyme reactions are affected differently. For example, OCS use inhibits caffeine oxidation (Callahan *et al.*, 1983; Rietveld *et al.*, 1984; Abernethy & Todd, 1985; Campbell *et al.*, 1987b) whereas paracetamol glucuronidation is induced (Miners *et al.*, 1983; Mitchell *et al.*, 1983; Bock *et al.*, 1988). The effect of smoking on glucuronidation remains controversial (Miners & Mackenzie, 1991) probably because of the small increase in glucuronide formation and large interindividual variation.

Therefore it was of interest to study in a larger population the influence of gender, OCS use and smoking habit on sparteine oxidation (reflecting CYP2D6 activity), caffeine oxidation (reflecting CYP1A2 activity) and paracetamol glucuronidation (reflecting in part UGT1.6 activity), reactions which are predominantly influenced by genetic and environmental factors, respectively. Furthermore, paracetamol glucuronidation was compared with caffeine oxidation (reflecting CYP1A2 activity) in male heavy smokers to assess the extent of co-regulation of the two reactions by smoking.

Materials and methods

Subjects

One hundred and ninety-four Caucasian volunteers (98 male, 96 female), as a random population sample, were recruited for the study by public announcement. They were healthy by medical history, physical examination and laboratory tests, in particular creatinine clearance. The mean age was 29 years (range: 18–58 years) in all subjects, 30 years (range: 18–58 years) in males, and 29 years (range: 18–55 years) in females. The mean body weight was 67 kg (range: 47–100 kg) in all subjects, 73 kg (range: 60–100 kg) in males, and 64 kg (range: 47–82 kg) in females. They were not taking any drugs, with the exception of oral contraceptives. Exclusion criteria were pregnancy, liver or kidney disease, or an allergy to the probe drugs. Smokers (38 male) specified their cigarette consumption (5–20 cigarettes per day). Those subjects consuming >10 cigarettes per day are referred to as heavy smokers. Of the female subjects 40 were smokers and/or users of OCS. It was also recorded whether or not the volunteer was a regular consumer of caffeine (coffee or tea). The protocol was approved by the ethical committee of the Robert Bosch Hospital, and each subject gave written informed consent.

Drug administration

After voiding their bladder at 3 p.m., the subjects took 200 mg of caffeine (one tablet Coffeinum 0.2 g Comprette® Cascan) and collected their urine for 6 h in a bottle containing 50 ml of 1 M sodium citrate-HCl buffer, pH 3.0. At 9 p.m., the subjects took 100 mg of sparteine sulfate (1 tablet of Depasan®) and 1000 mg of paracetamol (two tablets of Paracetamol-ratiopharm® 500) and urine was collected for 10 h. It had been shown in pilot experiments that the intake of paracetamol did not affect the phenotyping with sparteine and vice versa. After measurement of the urine volumes, aliquots were stored at −20° C until analysed. A sample (30 ml) of venous blood was obtained and anticoagulated with EDTA for DNA

preparation from leukocytes. The use of caffeine and alcohol was not allowed from the evening prior to the test until the end of urine collection.

Determination of the sparteine metabolizer phenotype

The phenotype of individuals was determined by using a single oral dose of 100 mg sparteine (Eichelbaum *et al.*, 1979; Osikowska-Evers & Eichelbaum, 1986). PMs are defined as subjects with a urinary metabolic ratio (MR_s) of greater 20 ($\log_{10} MR_s > 1.3$; Eichelbaum *et al.*, 1982).

DNA-RFLP analysis

Genomic DNA was prepared from leukocytes isolated from whole blood samples (Neitzel, 1986). DNA was digested to completion with *Xba I* restriction endonuclease, and Southern hybridization with a CYP2D6 cDNA was used to determine the RFLP. The genotype was assigned according to the presence of fragments of differing length (Skoda *et al.*, 1988).

Amplification test by PCR

Genotyping was also performed using allele-specific PCR amplification (Heim & Meyer, 1990; Broly *et al.*, 1991). The DNA samples were tested for the CYP2D6-A and CYP2D6-B mutations. The CYP2D6-D mutation, i.e. the deletion of the entire *CYP2D6* gene, was identified by RFLP analysis (Skoda *et al.*, 1988; Gaedigk *et al.*, 1991). For the test of the D6-C mutation, the same method as used by Broly & Meyer (1993) was employed (Tyndale *et al.*, 1991).

Determination of paracetamol glucuronidation (MR_p)

Urinary paracetamol and paracetamol glucuronide was analysed by a modification of the HPLC method of Howie *et al.* (1977), as described (Bock *et al.*, 1987; Kietzmann *et al.*, 1990). Paracetamol glucuronide was identified with ^{14}C-paracetamol glucuronide, synthesized *in vitro* with ^{14}C-paracetamol, UDP-glucuronic acid and liver microsomes. Due to interfering peaks some urine samples could not be analysed. Therefore, MR_p data are given for 162 subjects (78 female; 84 male).

Determination of caffeine oxidation (MR_c)

Caffeine metabolites were analysed as described (Campbell *et al.*, 1987; Kalow & Tang, 1993). The metabolites were identified by spiking with the respective compounds: AFMU (5-acetylamino-6-formylamino-3-methyluracil), 1U (1-methyluric acid), 1X (1-methylxanthine) and 1,7U (1,7-dimethyluric acid). The ratio of (AFMU + 1X + 1U)/1,7U was used to measure caffeine oxidation.

Statistical calculations

Metabolic ratios for paracetamol (MR_p) and sparteine (MR_s) were sorted into females and males. Females were further sorted into subjects who neither smoked nor used OCS (non-smoker/non-OCS) and those who smoked and/or used OCS (smokers/OCS); males were sorted in smokers and non-smokers. In addition, metabolic ratios for sparteine (MR_s) were sorted for different genotypes. Wild-type homozygotes (wt/wt) and wt/B heterozygotes were further sorted in females and males and, in addition, in non-smokers/non-OCS users and smokers and/or OCS users. Due to the small numbers of subjects, the remaining subgroups of genotypes (wt/A and B/B) were not analysed in this way. Cumulative frequencies $F(MR) = n/N$ (with n = number of subjects with a MR equal or below a certain MR_x, and N = total number of subjects) were plotted versus MR_p and MR_s, respectively. Differences between the cumulative frequency functions for subgroups were tested with the Kolmogorov-Smirnov test at $\alpha = 0.05$. Additionally, cumulative frequencies were analysed by a probit plot of untransformed MR_p values and $\log_{10}$-transformed MR_s data. A sum of RRSW functions (Rosin *et al.*, 1933; Weibull, 1951) was adjusted to the cumulative frequency data for both drugs:

$$f(\mathrm{MR}) = \sum_j a_j (1 - e^{-(\lambda_j \cdot (\mathrm{MR} - \tau_j))^{\beta_j}})$$

with

a_j = fraction of the total number of subjects assigned to the j-th distribution;
λ_j = a scaling factor;
β_j = slope factor;
τ_j = shift of the RRSW function on the MR axis.

This adjustment was performed for pooled data and also separately for the subgroups. The HOEGIP-PC software (Brockmeier & Lückel, 1991), according to the least-square principle (Gauss, 1809), was used for fitting. Additionally, each adjustment was performed with β_j fixed to 3.57, for which the RRSW function reached a good approximation to normal distribution function (Moyer *et al.*, 1962). To support the appropriateness of model selection, the AIC (Yamaoka *et al.*, 1978) was calculated for each adjustment. Furthermore, fitting results were compared by the F test with the quotient of the MSQ computed for each fitting.

Results

Phenotypes of sparteine oxidation

Among the 194 subjects, 15 had a $MR_s > 20$ and thus were PMs (Table 1). This corresponds to a prevalence

Table 1. Distribution of phenotypes of sparteine metabolism among the genotypes of CYP2D6

Genotype	*Phenotype*			*Total*
	EM with $MR_s < 1.2$	*EM with* $20 > MR_s > 1.2$	*PM with* $MR_s > 20$	
wt/wt	100	6	0	106
wt/A	2	2	0	4
wt/B	46	10	4	60
wt/D	10	3	2	15
B/B	0	0	8	8
B/D	0	0	1	1
total	158	21	15	194

of the PM genotype of 7.7%. Twenty-one volunteers (10.8% of the total population, 11.7% of all EMs) had $20 > MR_s > 1.2$. The metabolic ratio of sparteine oxidation showed a clear trimodal distribution (Fig. 1A). In addition to the PM group (with $MR_s > 20$), a bimodality within the group of EMs was apparent. The probit plot of the $\log_{10}$-transformed MR_s was curvilinear with three phases.

For the adjustment of the RRSW function to the cumulative frequency data, $\log_{10}$-transformed MR_s data were used since adjustment was significantly improved compared to using untransformed MR_s values. The adjustment of a single RRSW function to the MR_s data was inappropriate. The best adjustment (assessed by AIC and F-test) was obtained with three RRSW functions. Fig. 2A shows the distribution of the MR_s data using three RRSW functions with the slope parameters β_j fixed to 3.57 (quasi-normal distribution). In addition, the corresponding density functions for the cumulative RRSW functions are shown. The first and second distribution slightly overlapped each other whereas the third distribution was clearly separate from the others.

Comparing the cumulative frequency distribution for the four subgroups (male-smoker, female-smoker/OCS, etc.), the curves were nearly superimposable, and no significant differences could be observed (graph not shown). The curves differed only in the PM part, since PMs were not equally distributed among the four subgroups (see below). Therefore, gender, smoking, and OCS use did not significantly influence sparteine metabolism. Sparteine metabolism was not faster in caffeine consumers than in abstinents of caffeine. In EMs, MR_s was 0.84 ± 1.59 in consumers ($N = 157$) and 0.40 ± 0.24 ($N = 18$) in abstinents of caffeine (not significant). Considering EMs with $MR < 1.2$ only, MR_s was 0.43 ± 0.22 in consumers ($N = 137$) and 0.40 ± 0.24 ($N = 18$) in abstinents of caffeine (values = means $\pm$ SD; not significant).

Genotypes of CYP2D6

Using the combination of RFLP and allele-specific PCR, nine of the 15 PMs had the derived genotype of a PM (4.6% of the total population, 60% of PMs) since no wt allele was found (Table 1). Among the 194 subjects, 106 (55%) were homozygotes for wt/wt and thus had a derived EM phenotype. All of them were indeed EMs. Seventy-nine subjects (41%) were heterozygotes carrying one wt allele and one mutant allele. Among these wt-heterozygotes, however, there were six PMs. Also, EMs with $MR_s > 1.2$ were more frequent among wt-heterozygous EMs (21%) than in wt-homozygous EMs (6%).

Fig. 3 shows the cumulative frequency distributions for the two subgroups, smokers/OCS and non-smokers/non-OCS, of the genotypes wt/wt and wt/B.

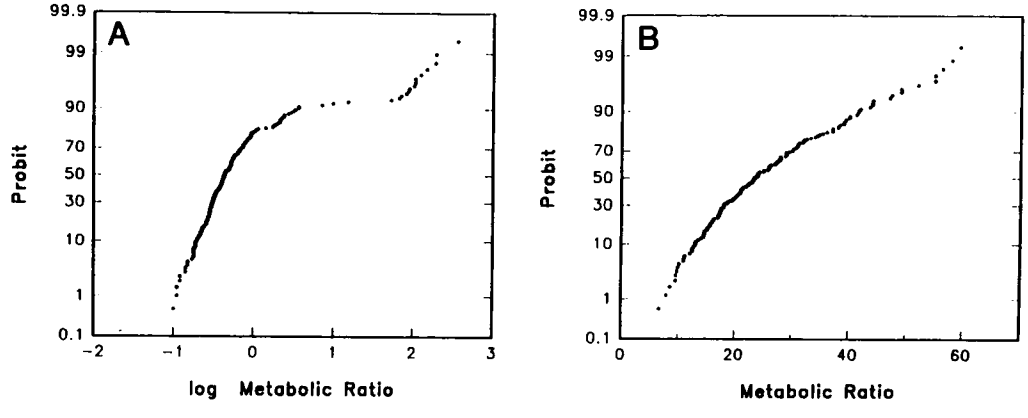

Fig. 1. Probit plots for metabolic ratios of sparteine oxidation (MR_s; A) and of paracetamol glucuronidation (MR_p; B). The cumulative frequencies of $\log_{10}$-transformed MR_s and of untransformed MR_p of all subjects were transformed to probits. For MR_s three phases, for MR_p two phases are evident.

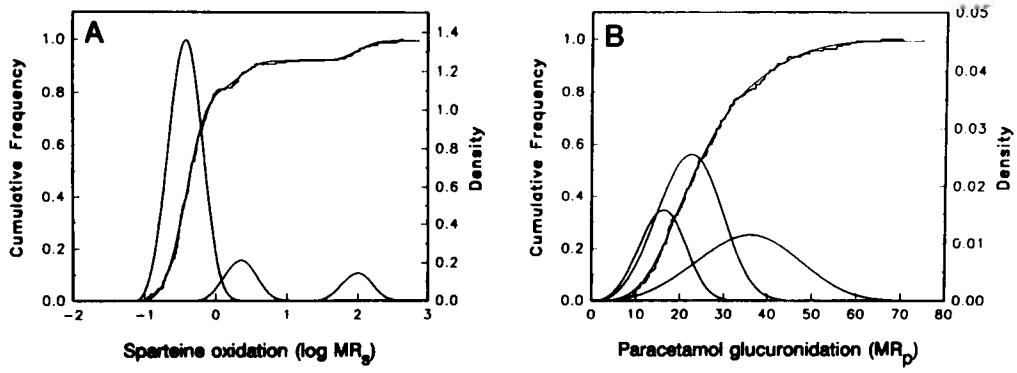

Fig. 2. Cumulative frequencies of metabolic ratios of sparteine oxidation (MR_s; A) and of paracetamol glucuronidation (MR_p; B). Cumulative frequencies (left y-axis) of $\log_{10}$-transformed MR_s and of untransformed MR_p of all subjects were adjusted by a sum of three cumulative RRSW-functions. These three RRSW-functions are shown separately in terms of probability density functions (right y-axis). By fixing the slope factors of the three RRSW-functions to 3.57, the RRSW-functions closely approximate the Gaussian distribution.

In each subgroup the heterozygous phenotype (wt/B) caused a shift to higher MRs as compared to the wild-type homozygotes (wt/wt). For the distribution within the genotype wt/wt, no differences between smokers/OCS and non-smokers/non-OCS could be observed. The two distributions were almost superimposable. The same holds true for the heterozygous EMs, wt/B, up to a MR_s of about 0.7. The cumulative frequency distributions for males and females with the genotype wt/wt were almost superimposable. When the two distribution curves for the genotypes wt/wt and wt/B were not further stratified, the difference was significant (graph not shown). This indicates that the genotype has a clear influence on MR_s.

Paracetamol glucuronidation (MR_p)

The cumulative frequency distribution of MR_p data did not reveal evidence for a polymorphism of paracetamol glucuronidation (Fig. 1B). Adjustment of RRSW functions to MR_p data (similar to those adopted for the analysis of MR_s data) showed three overlapping functions (Fig. 2B). Some factors (gender, hormones, smoking habit) were evident when cumulative frequencies for the subgroups female non-smokers/non-OCS, female smokers/OCS, male smokers, male non-smokers

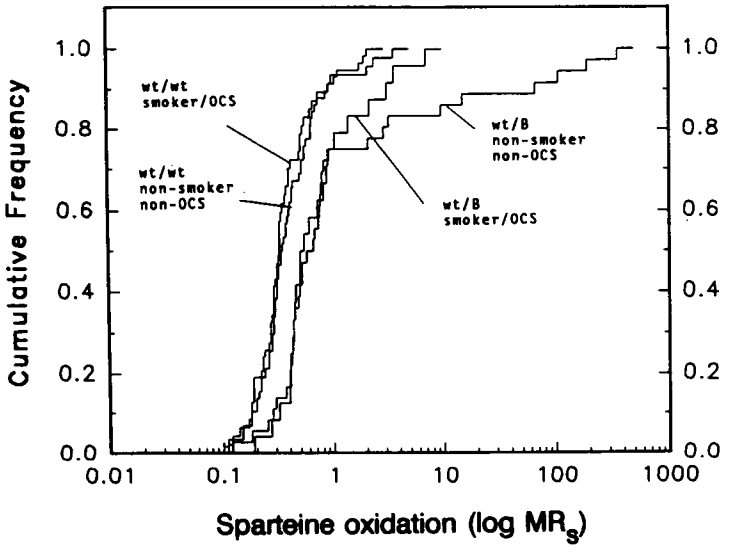

Fig. 3. Distribution of metabolic ratios of sparteine oxidation (MR_s). The $\log_{10}$-transformed MR_s for the two subgroups with genotype wt/wt and wt/B were further classified into smokers/OCS users and non-smoker/non-OCS users. In the subgroup with the genotype wt/wt, the two distributions are virtually superimposable.

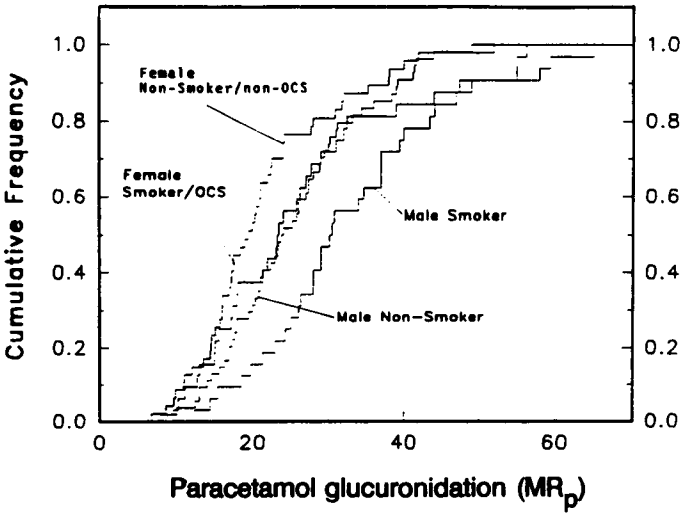

Fig. 4. Distribution of metabolic ratios of paracetamol glucuronidation (MR_p). The MR_p for the different subgroups are shown as cumulative frequencies. The difference between male non-smokers and male smokers is significant.

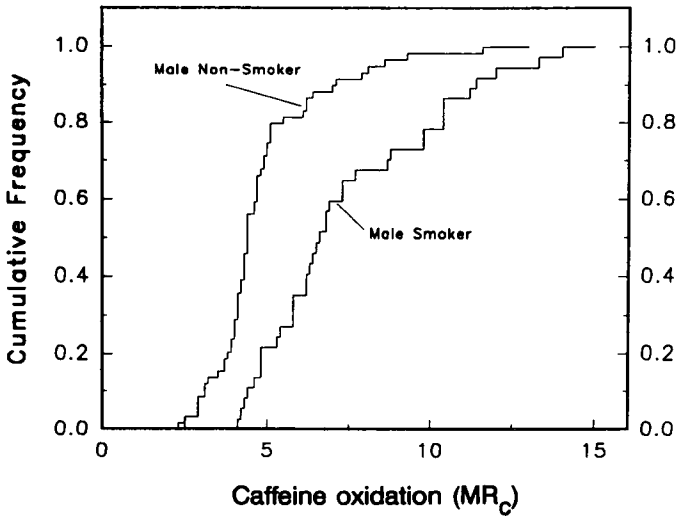

Fig. 5. Distribution of metabolic ratios of caffeine oxidation (MR_c). The MR_c for male heavy smokers and non-smokers are shown as cumulative frequencies, the difference being significant.

were analysed (Fig. 4). The glucuronidation capacity was higher in males than in females. Male smokers had the highest and female non-smokers/non-OCS the lowest MR_p.

Comparison of paracetamol glucuronidation with caffeine oxidation

The frequency of distribution of MR_c of caffeine oxidation appeared to be multimodally distributed and markedly shifted to the right male smokers (Fig. 5). Caffeine oxidation appeared to be a more sensitive indicator of PAH-type induction than paracetamol glucuronidation (compare Fig. 5 with Fig. 4). Multimodal distribution was substantiated by analysis of RRSW functions (D. Brockmeier, unpublished results). A detailed study of the distribution of CYP1A2 activity is currently being prepared. When caffeine oxidation (MR_c) was compared with paracetamol glucuronidation (MR_p) in all subjects no significant correlation was found (not shown). However, there was a high correlation ($r = 0.85$) in the subgroups of male heavy smokers (Fig. 6). No significant correlation was seen in male non-smokers ($r = 0.35$).

Discussion

Gender, OCS use and smoking are known to affect drug-metabolizing enzymes. Therefore we have explored the impact of these factors on the *in vivo* activities of CYP2D6, CYP1A2 and paracetamol conjugating UGTs. Recently, it has been demonstrated in studies with cell-expressed recombinant UGTs that human UGT1.6 represents one major UGT isozyme conjugating paracetamol (Bock *et al.*, 1993; see below). In the case of CYP2D6 activity, genetic factors are known to prevail whereas hormonal and environmental factors appear to predominate in the case of paracetamol glucuronidation. Caffeine oxidation was compared with paracetamol glucuronidation in a subgroup of male heavy smokers because of the known influence of the smoking habit on CYP1A2 activity (Campbell *et al.*, 1987; Kalow & Tang, 1993). Female subjects have not been included because of the opposing effects of OCS on the two reactions.

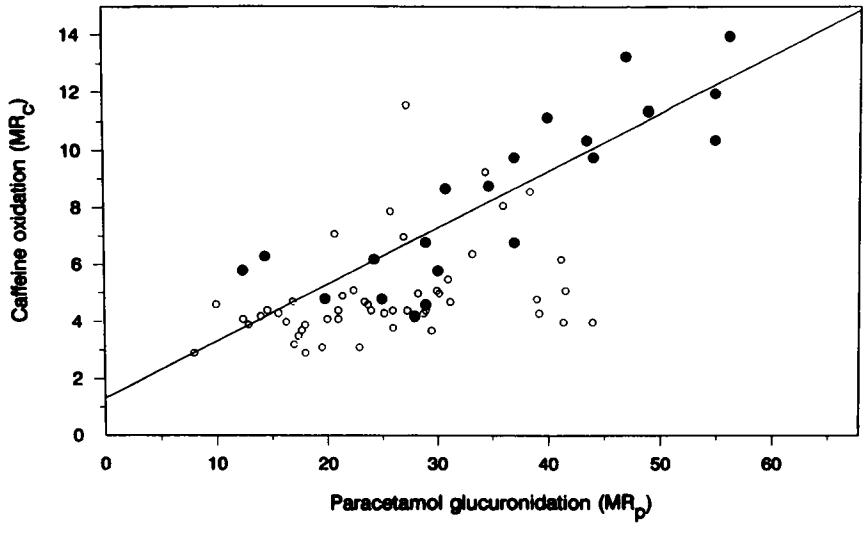

Fig. 6. Comparison of paracetamol glucuronidation (MR_p) with caffeine oxidation (MR_c). A high correlation ($r = 0.85$) between MR_p and MR_c is found in the subgroup of male heavy smokers (●) and no correlation ($r = 0.38$) in the subgroup of male non-smokers (○).

Sparteine oxidation

The reason for choosing sparteine as the *in vivo* probe for CYP2D6 activity was the following: In the metabolism of debrisoquine (DQ), several metabolites are formed. While the metabolism to 4-hydroxy-DQ is catalysed by CYP2D6, additional phenolic metabolites, such as 5-, 6-, 7- and 8-hydroxy-DQ, are formed. However, it is unknown whether or not CYP2D6 or other cytochrome P450 isozymes are involved in their formation. The influence of genetic and environmental factors on DQ metabolism has been studied; it could be demonstrated that 79% of the variability of DQ metabolism is genetic and that coffee intake correlates inversely ($r = -0.19$; $p < 0.01$) with $ln(MR_{DQ})$ (Steiner *et al.*, 1985). Smoking had no effect. Since several metabolites were formed and multiple enzymes are involved (in addition to CYP2D6) it could not be decided, if those changes were due to modulation of CYP2D6 activity. Furthermore, unequivocal identification of heterozygous EMs was not possible since at that time no genotyping was available. Sparteine is a pure CYP2D6 substrate and therefore a more appropriate probe for differentiating genetic and environmental bases of variability.

The present data confirm our previous observation of a gene-dose effect on the metabolism of sparteine among EMs (Broly *et al.*, 1991). Heterozygous EMs formed less metabolite and hence had significantly higher MR_s. An interesting finding of this study is the clear trimodal distribution of MR_s, with 11% of the subjects having a $20 > MR_s > 1.2$. Although more heterozygous EMs are among this subgroup than among EMs with $MR_s < 1.2$, 29% are wt homozygotes.

The observation of PMs who, by RFLP and PCR genotyping, carry a wt allele can be explained by the existence of one or several other CYP2D6 mutations which have not yet been characterized. The presence of the mutation D6-C in these subjects is unlikely since it has recently been demonstrated that the mutation D6-C does not cause the PM phenotype (Broly & Meyer, 1993). We tested these PM subjects for the presence of the mutation D6-C and did not observe it in any of them (not shown).

The lack of an accelerating effect of caffeine on sparteine metabolism supports the predominant genetic regulation of CYP2D6. The lower MR of debrisoquine (DQ) which was observed in high consumers of caffeine (Steiner *et al.*, 1985) may be due to the fact that DQ is also metabolized by CYP isozymes other than CYP2D6.

Paracetamol glucuronidation

Allelic variants of *UGT1A* or phenol/bilirubin *UGT* gene complex are known as the Crigler-Najjar syndromes characterized by congenital non-hemolytic unconjugated hyperbilirubinemia (Owens & Ritter, 1992). The isozymes of this large gene complex (including UGT1.6 and two bilirubin UGTs) are formed by differential splicing of unique exon 1 sequences (coding for the substrate specificity) to common exons 2–5. Hence, mutations of the common region of UGT DNA not only affect bilirubin but also paracetamol glucuronidation, as far as its glucuronidation is catalysed by phenol UGTs of this gene complex such as UGT1.6. The cumulative frequency distribution of paracetamol glucuronidation did not reveal evidence for polymorphisms of UGTs conjugating paracetamol (Fig. 1B). However, several factors (gender, OCS use and tobacco smoke) clearly affected paracetamol glucuronidation. Dietary cabbage and brussels sprouts (known to contain precursors of PAH-type inducers) have been reported to increase paracetamol glucuronidation (Pantuck *et al.*, 1983). In the present study the distribution of the paracetamol glucuronide/paracetamol ratio appeared to be unimodal although skewed. In previous studies the distribution of paracetamol glucuronidation has been interpreted as bimodal (Critchley *et al.*, 1986; Patel *et al.*, 1992).

Paracetamol is not an ideal probe drug, mainly because it is an overlapping substrate of several UGT isozymes. Recently, it has been demonstrated that paracetamol is conjugated by UGT1.6 (=HlugP1) with higher affinity than by HlugP4 (which also belongs to the *UGT1A* gene complex) and by other liver microsomal UGTs (Bock *et al.*, 1993). These findings were obtained in kinetic studies using cell-expressed recombinant HlugP1 and HlugP4. Hence, UGT1.6 may be a major contributor to paracetamol glucuronidation *in vivo*. Interestingly, human UGT1.6 is considered to be orthologous to the known 3-methylcholanthrene-inducible rat phenol UGT (Iyanagi *et al.*, 1986). Hence, increased paracetamol glucuronidation in smokers is consistent with regulation of human UGT1.6 by PAHs, similar to the rat model.

Suggestive evidence for coordinate regulation of CYP1A1/1A2 and UGT1.6 by the AhR has been reported in mice (Owens, 1977) and in rats (Bock *et al.*, 1990; Bock, 1991). The MR_c for caffeine oxidation mainly reflects caffeine 3-demethylase activity catalysed by CYP1A2 (Campbell *et al.*, 1987; Butler *et al.*, 1989; Kalow & Tang, 1993). MR_c appears to be more sensitive for PAH-type induction of human liver enzymes than paracetamol glucuronidation (compare Figs 4 and 5). Moreover, the large interindividual variation in responses of paracetamol glucuronidation to smoking has been substantiated. Nevertheless, the high correlation between caffeine oxidation and

paracetamol glucuronidation in male smokers (Fig. 6) supports the existence of elements of co-ordinate regulation of the two reactions in man, irrespective of the fact that in many instances the two enzymes are independently regulated.

In conclusion, the present population study substantiates that genetic factors prevail in the regulation of CYP2D6, also in genotypes such as wt/B. Moreover, it suggests that paracetamol glucuronidation (catalysed in part by UGT1.6) is clearly affected by factors such as gender, OCS use and smoking. The high correlation between CYP1A2 activity and paracetamol glucuronidation in male smokers suggests that the Ah receptor may be an element of co-regulation of CYP1A2 and paracetamol UGTs, presumably UGT1.6.

Acknowledgements

The skilful technical assistance of Ms Sabine Hödl, Ms Brigitte Körner, Mrs Magda Müh-Zange and Mrs Silvia Vetter is gratefully acknowledged. We thank Dr Dieter Teschner for help with the computerized data analysis. A sample of the caffeine metabolite AFMU was a generous gift of Dr W. Kalow (Department of Pharmacology, University of Toronto). The authors also thank the Robert Bosch Foundation (Stuttgart) and the Deutsche Forschungsgemeinschaft for financial support.

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