Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein

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Sex-related differences in drug pharmacokinetics can generally be classified as being caused by one of several factors, including absorption, distribution, metabolism, and elimination. Sex-related differences in the pharmacokinetics and pharmacodynamics of drugs have been extensively reviewed by Harris et al[1] and, more recently, by Beierle et al.[2] We focus on one of the most frequently observed pharmacokinetic sex-related differences—that observed for metabolic drug disposition.

Oxidative drug metabolism by cytochrome P450 (CYP) enzymes is a major pathway for drug elimination. Of the numerous CYP enzymes found in the liver, CYP3A4 is the isoform that is most important for human drug disposition. It is the most abundant isoform in the liver, and among CYP substrates it is responsible for metabolizing more than 50% of drugs currently on the market. The observation that the clearances of drugs metabolized by CYP3A4 are frequently higher in women than men is a classic example of a well-known metabolic sex-related difference.[1] This difference was initially assumed to be the result of higher CYP3A4 protein expression in women compared with men, but there is limited evidence to support that hypothesis. In a study that compared the CYP3A4 metabolic activity of human liver microsomes from men and women (by use of erythromycin demethylation), Hunt et al[3] found a 24% higher CYP3A activity in female liver microsomes than in male liver microsomes. However, studies by Schmucker et al,[4] Shimada et al,[5] and George et al[6] that examined CYP3A4 protein content and function from human livers were not able to show any significant sex-related differences. One proposed explanation for the observed in vitro–in vivo discrepancy in women is the presence of the female sex steroids estrogen and progesterone, which are known substrates of this enzyme system and may therefore play a role in modulation of this sex-related difference in vivo.[1] However, extensive studies of midazolam and alfentanil pharmacokinetics performed in female patients at different phases of the menstrual cycle failed to demonstrate any differences in the midazolam or alfentanil clearances with hormonal fluctuations.[7,8]

We present an alternate hypothesis for this sex-related difference. Observed clearance differences may be attributable to the interaction of the drug with a plasma membrane-bound transporter, P-glycoprotein, which could in turn influence the interaction of the drug with the enzyme—an interplay that would not be present in an in vitro microsomal experiment.

In 1995, Wacher et al[9] reported a striking overlap in the drugs that were metabolized by CYP3A4 and those that were substrates or inhibitors of the multidrug efflux transporter P-glycoprotein. There is extensive diversity in the classes of drugs that are metabolized by CYP3A4 and transported by P-glycoprotein, including immunosuppressants, anticancer drugs, and calcium channel blockers. Schuetz et al[10] proposed that the large interindividual variability in the clearance of CYP3A4 substrates found in humans (10-fold) may in part be attributable to the interindividual variability in P-glycoprotein levels. A study of P-glycoprotein expression in liver biopsies from normal liver and secondary hepatic neoplasms found a 55-fold variation across the population.[10] Furthermore, in normal livers, men had approximately 2.4-fold higher P-glycoprotein

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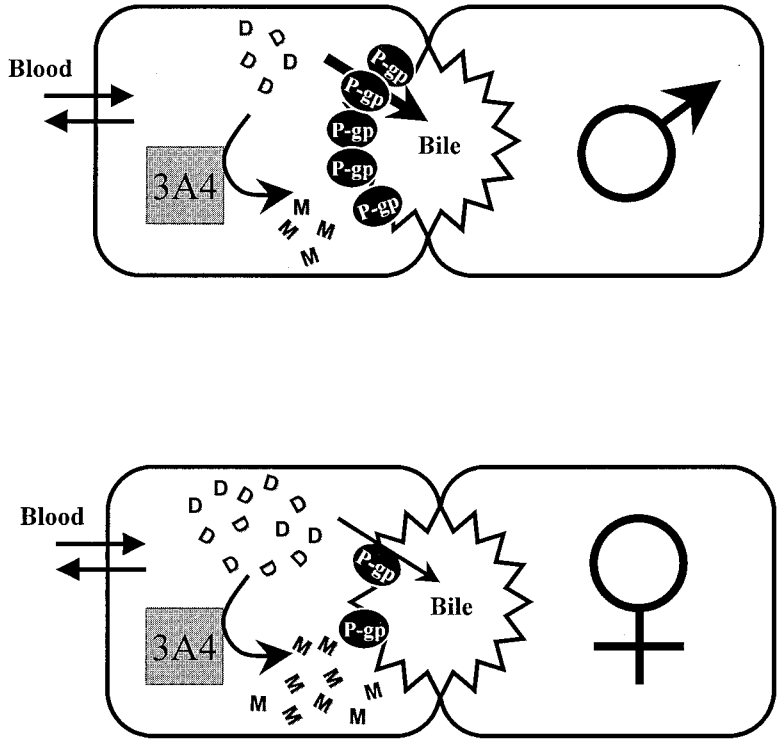

Fig 1. Schematic diagram of P-glycoprotein (P-gp) and CYP3A4 in hepatocytes from representative men *(top)* and women *(bottom)* to illustrate the proposed hypothesis. Drug in the blood enters the hepatocyte from the basolateral membrane. The lower expression of P-glycoprotein in women compared with men could result in higher intracellular drug (D) levels and consequently higher metabolite (M) levels, even though there is no sex-related difference in the CYP3A4 enzyme expression.

levels compared with women.[10] This sex-related difference could be the link to explain the in vitro–in vivo discrepancy observed for CYP3A4 substrates. In vivo, drugs delivered to the liver from the blood cross the sinusoidal membrane of the hepatocyte, enter the cell, and have an opportunity to interact with CYP3A4, and then be actively excreted into the bile by P-glycoprotein located on the bile canalicular membrane. P-glycoprotein efflux can effectively lower the intracellular levels of a drug and thereby indirectly modulate CYP3A4 metabolism. In women (who have less P-glycoprotein), one might therefore expect higher intracellular hepatic levels and consequently greater metabolism and higher clearance, even though the enzyme protein levels are similar between men and women. This hypothesis is illustrated schematically in Fig 1.

KEY EXAMPLES

Erythromycin. The erythromycin breath test (ERMBT) developed by Watkins et al[11] is commonly used to study the metabolic activity of liver CYP3A4. The test requires the intravenous administration of a ^{14}C-labeled dose of erythromycin and the subsequent measurement of expired $^{14}CO_2$ derived from the demethylation of erythromycin. The test is suggested to measure primarily liver CYP3A4 activity in an individual because erythromycin demethylation activity correlated best with CYP3A4 when compared with a panel of other liver enzymes.[12] Of interest to the hypothesis, many investigators have reported a higher clearance in women than in men who were administered the ERMBT.[11,13,14] In 1998, Schuetz et al[15] discovered that erythromycin was a substrate for P-glycoprotein and postulated that the ERMBT may be measuring the combined role of CYP3A4 and P-glycoprotein on erythromycin disposition. This finding also helped to explain the discordance between the breath test method for probing CYP3A4 liver activity versus other noninvasive methods, including midazolam hydroxylation.

Midazolam. The sedative hypnotic midazolam is considered to be a classic CYP3A4 probe substrate and is metabolized by CYP3A4 to one main metabolite, 1-hydroxymidazolam. An interesting feature of midazolam is that it is not a substrate for P-glycoprotein,[16] unlike many other drugs metabolized by CYP3A4 that share extensive substrate overlap with this transporter. Several investigators have studied midazolam pharmacokinetics with respect to sex and have not found any significant differences in the intravenous clearances between men and women.[7,14,17-20] It is interesting that 2 studies[21,22] with intravenous midazolam have shown increased clearance in women. However, each of those 2 studies tested only 3 female subjects, whereas the larger studies that compared 4 to 10 female subjects each revealed no sex-related difference in midazolam clearance. In the 1986 study,[21] the authors even concluded that the sample sizes were not large enough to allow meaningful analysis of sex stratification. Intramuscular administration of midazolam also appears to provide no sex-related difference in the total clearance[23]; however, oral administration has yielded conflicting results, with one study reporting the oral clearance of midazolam in women to be greater than in men[19] and another indicating no sex-related difference in oral clearance.[18] This discrepancy may be attributed to the increased complexity of midazolam metabolism after an oral dose as a result of extensive intestinal metabolism by CYP3A4. Several investigators have shown that there is no correlation in the expression of CYP3A in the liver and the intestine.[18,24-26] In addition, although there is some evidence that a sex-related difference exists in the intestinal CYP3A levels, with women having higher levels than men,[26,27] that observation was not replicated in a study of renal transplant patients, where no sex-related difference in intestinal CYP3A4 was found.[25] In general, clinical studies that probe liver CYP3A activity by administration of an intravenous dose of midazolam have failed to show a sex-related effect. According to the proposed hypothesis, because midazolam is not a P-glycoprotein substrate, no sex-related difference would be expected in its hepatic clearance.

Verapamil. The calcium channel blocker verapamil was one of the first well-characterized P-glycoprotein substrates.[28] At one time it was considered to be a promising candidate as a P-glycoprotein reversal agent to inhibit P-glycoprotein–mediated efflux of cancer therapeutics.[29] Verapamil is also a well-known CYP3A4 substrate that undergoes extensive first-pass intestinal and hepatic metabolism. Women appeared to have higher total clearance values than men after intravenous administration of verapamil.[30-33] However, studies that examined the pharmacokinetics of orally administered verapamil in regular or extended-release formulations revealed no sex-related differences[33,34] or the opposite trend, with men having higher oral clearances than women.[31,35,36] These combined results clearly suggest a sex-related difference in CYP3A or P-glycoprotein in the gut that counterbalances the sex-related effect in the liver. Despite the formulation-related differences in the pharmacokinetic parameters of verapamil, the trend observed for intravenous clearance (women > men) was the same as that found for erythromycin, another CYP3A and P-glycoprotein substrate.

EXPERIMENTAL EVIDENCE

In vitro studies. There is considerable experimental evidence to support the hypothesis that decreased expression or function of P-glycoprotein can result in increased CYP3A4-mediated drug metabolism. To approximate hepatic metabolism in vitro, our laboratory has used a polarized epithelial cell line (CYP3A4-transfected Caco-2 cells) that expresses both P-glycoprotein and CYP3A4.[37] This cell line, originally derived from a human colon carcinoma cell, was found by confocal microscopy to express P-glycoprotein on the apical brush-border membrane (representative of the bile canalicular membrane) and CYP3A4 on the endoplasmic reticulum (as observed in hepatocytes).[37] A drug that is administered to the monolayer of cells from the basolateral side serves to mimic the appearance of the drug from the blood into the hepatocyte. By using this model, we could study whether decreasing the efflux rate of drug from the cell (using chemical inhibitors of P-glycoprotein) would result in an increase in the extent of drug metabolism. We recently published data using this in vitro system with which the metabolism of two CYP3A4 substrates, felodipine (a calcium channel blocker) and K11777 (*N*-methyl piperazine-Phe-homoPhe-vinylsulfone phenyl; a cysteine protease inhibitor in preclinical development), was examined.[38] K11777 was found to be a very good substrate for P-glycoprotein when tested across P-glycoprotein overexpressing cell lines, whereas felodipine was not transported at all. Inhibition studies were performed with these substrates in CYP3A4-transfected Caco-2 cells with use of cyclosporine (INN, ciclosporin) as a dual inhibitor of CYP3A4 and P-glycoprotein and GG918 as an inhibitor of P-glycoprotein only. Table I summarizes the results obtained after a basolateral dose and displays the potential role that P-glycoprotein can play in the liver in

Table I. Changes in intracellular distribution and metabolism of K11777 and felodipine in the presence of the P-glycoprotein inhibitors cyclosporine (INN, ciclosporin) and GG918 after a basolateral dose that represented drug delivery to the liver*

Substrate†	*Intracellular amount of parent drug (pmol)*	*Effluxed amount (basolateral to apical) (pmol)*	*Total metabolites formed (pmol)*	*Extraction ratio (%)‡*
K11777	260 ± 6	2660 ± 160	193 ± 8	6.2 ± 0.4
Plus cyclosporine	703 ± 39	1670 ± 120	100 ± 5	4.0 ± 0.3
Plus GG918	681 ± 39	1450 ± 130	278 ± 11	12 ± 1
Felodipine	951 ± 47	91 ± 10	335 ± 16	24 ± 2
Plus cyclosporine	1050 ± 60	101 ± 18	147 ± 15	11 ± 1
Plus GG918	1060 ± 10	101 ± 12	418 ± 19	27 ± 1

These data were from the last time point obtained for each drug (3 hours for K11777 and 36 minutes for felodipine) from Cummins et al.[38]

*Data are represented as mean values ± standard deviation from n = 3.

†Both drugs were studied at concentrations of 10 μmol/L. The inhibitors were added to both sides of the monolayer at 10 μmol/L for cyclosporine and 200 nmol/L for GG918.

‡Calculated with use of equation 1.

affecting intracellular drug concentrations, as well as the extent of metabolism by CYP3A4.

When K11777 was incubated in the presence of the P-glycoprotein inhibitor GG918, there was a 2.6-fold increase in the intracellular level of K11777 and a 42% decrease in the efflux (basolateral to apical) transport across the cell. In contrast, there was no change in the amount of felodipine inside or across the cell in the presence of GG918. The increased intracellular K11777 resulted in a 44% increase in the amount of metabolites formed when P-glycoprotein was inhibited. The extent of metabolism for each drug was measured by calculation of an extraction ratio across the CYP3A4-transfected Caco-2 cells (equation 1). This parameter measured the fraction of the drug that was metabolized after transit across the cell, with the amount of drug that was inside the cell and therefore able to interact with CYP3A4 taken into account.

$$\text{Extraction ratio} = \frac{\sum \text{amount of metabolism}_{(\text{all chambers})}}{\sum \text{amount of parent}_{(\text{apical, cellular})} + \sum \text{amount of metabolites}_{(\text{all chambers})}} \quad (1)$$

For K11777, there was a 2-fold increase in the extraction ratio when P-glycoprotein was inhibited (Table I). This finding was in agreement with the hypothesis that lower levels of P-glycoprotein result in higher clearances. Cyclosporine decreased the total metabolite formation and extraction ratios for both K11777 and felodipine through direct inhibition of CYP3A4. It is not surprising that there was no change in the extraction ratio for felodipine when incubated in the presence of GG918 (because it is not a P-glycoprotein substrate). This finding also showed that GG918 did not affect CYP3A4 metabolism at the concentration tested (200 nmol/L). These results supported the hypothesis that decreased levels of P-glycoprotein can result in greater metabolism in the hepatocyte (represented by the basolateral dose).

In vivo studies. In vivo data in support of the proposed hepatic interplay between P-glycoprotein and CYP3A4 was obtained from elegant studies performed in P-glycoprotein knockout mice in which a gene dose response between the *mdr1* allele expression and erythromycin metabolism was observed.[39] This study showed that, although *mdr1a/1b(+/+), mdr1a(−/−),* and *mdr1a/1b(−/−)* mice had similar levels of CYP3A by immunoquantitation, the amounts of expired $^{14}CO_2$ collected after an intravenous dose of [^{14}C]erythromycin were 51% and 91% higher in the single and double knockout mice relative to the wild type. The amounts of total radioactivity in the livers of the *mdr1a(−/−)* and *mdr1a/1b(−/−)* mice at the end of the study were 41% and 80% greater than in wild-type mice, supporting our in vitro results that showed that P-glycoprotein can alter intracellular drug levels.

Opposition to this view of the role of P-glycoprotein in hepatic metabolism has been raised by Chiou et al[40] on the basis of a review of literature data on the pharmacokinetics of various drugs in P-glycoprotein knockout mice. Drugs such as tacrolimus,[41] paclitaxel,[42] and vinblastine[43] have shown decreased total clearances in *mdr1a(−/−)* mice relative to the control mice after an intravenous dose. These results are contrary to what we would have predicted on the basis of the

Table II. Comparison of metabolites excreted from bile-cannulated *mdr1a(+/+)* and *mdr1a(−/−)* mice (90 minutes after an intravenous dose)

Substrate, dose, and genotype	*Parent drug in gut (% dose)*	*Parent drug in bile (% dose)*	*Metabolites in gut (% dose)*	*Metabolites in bile (% dose)*	*Extraction ratio (%)**	*Reference*
Paclitaxel (10 mg/kg)						Sparreboom et al[42]
mdr1a(+/+)	10.8 ± 0.3	4.8 ± 0.3	0.4 ± 0.1	4.9 ± 0.4	25 ± 2	
mdr1a(−/−)	2.5 ± 0.9	2.6 ± 0.6	0.4 ± 0.2	3.8 ± 0.4	45 ± 7	
Vinblastine (1 mg/kg)						van Asperen et al[44]
mdr1a(+/+)	6.7 ± 0.7	1.1 ± 0.2	0.62 ± 0.04	0.19 ± 0.02	9 ± 1	
mdr1a(−/−)	3.3 ± 0.6	0.38 ± 0.06	0.39 ± 0.05	0.12 ± 0.02	12 ± 2	
Doxorubicin (5 mg/kg)						van Asperen et al[44]
mdr1a(+/+)	10.5 ± 0.5	13 ± 2	0.37 ± 0.07	0.53 ± 0.09	3.6 ± 0.5	
mdr1a(−/−)	10.0 ± 0.4	2.4 ± 0.3	0.8 ± 0.4	0.20 ± 0.05	7.3 ± 0.3	

Data are reported as mean values ± SEM.
*Signifies the fraction of excreted radiolabeled drug that represents known metabolites (calculated with use of equation 1).

hypothesis that a decrease in P-glycoprotein would increase CYP3A metabolism (through decreased biliary secretion and consequently higher intracellular drug levels). To explain these seemingly contrary results, Chiou et al[40] suggested that the clearance was decreased as a result of decreased hepatic elimination caused by metabolite inhibition. This interpretation requires the assumption that the clearance calculated from an intravenous dose is measuring only hepatic clearance. Yet for some drugs there is evidence for significant drug secretion occurring from the intestine as a result of P-glycoprotein (we estimated that about 30% of the paclitaxel dose and about 20% of the vinblastine dose were excreted directly into the intestine from mice studies performed by Sparreboom et al[42] and van Asperen et al,[43,44] respectively) so that hepatic clearance may not be correctly estimated from calculation of the total clearance after an intravenous dose.

The elimination that occurs in a knockout mouse may be markedly different from that of a normal mouse because it may need to rely on secondary transporters and alternate excretion routes to help with elimination. This was observed for digoxin in which the major pathway for elimination went from primarily fecal in normal mice to primarily urinary in *mdr1a/1b(−/−)* mice.[45] In addition, *mdr1* knockout data are often complicated by the measurement of total radioactivity or nonspecific assays that assume the signal observed is mainly from the parent drug when pharmacokinetic parameters are calculated. When a comparison was made between the actual amounts of parent drug and known CYP-derived metabolites excreted in *mdr1a(−/−)* versus *mdr1a(+/+)* for intravenous doses of paclitaxel, vinblastine, and doxorubicin (Table II), it was found that the fraction metabolized was actually greater in the P-glycoprotein knockout mice, a finding consistent with the data obtained for erythromycin in *mdr1a/1b(−/−)* mice. Furthermore, in a study of vinblastine intravenous kinetics, the total tissue level of deacetylvinblastine (measured at 4 hours) was found to be 5-fold higher in *mdr1a(−/−)* mice compared with control mice, even though the plasma levels of vinblastine in the *mdr1a(−/−)* mice were only 1.7-fold greater than the levels in control mice.[43]

Although it is true that the clearance of many P-glycoprotein substrates is decreased in *mdr1a* knockout mice, the overall fraction metabolized appears to be increased. This can be rationalized by the decreased clearance of the drug from other routes of elimination (ie, biliary, intestinal, and renal; note the decreased elimination of parent drug in the gut and bile in knockout mice for the 3 drugs in Table II) while the metabolic clearance is increased. In this way, more drug is metabolized over time, even though the total drug clearance is reduced. This explanation is consistent with the hypothesis that more metabolism occurs when liver P-glycoprotein is decreased. Our analysis explains the previously considered inconsistency of the study by Lan et al[39] that was noted by Chiou et al[46]: that greater erythromycin metabolism was found in the knockout animals but measured erythromycin plasma concentrations were higher, implying lower clearance in the knockout animals. It should be noted that the interpretation of data obtained from *mdr1a(−/−)* mice was complicated by the changes in other drug transporters *(mdr1b)* and enzymes (CYP3A) that can occur after disruption of the *mdr1a* gene.[47,48] CYP3A expression has also been shown to be influenced by different types of laboratory chow and bedding used in the United States versus The Netherlands.[48]

In summary, although the total clearance of many P-glycoprotein and CYP3A substrates was reduced in the *mdr1a(−/−)* mice, the fraction of excreted drug that was metabolized was greater. To isolate the role of the liver in overall drug clearance and limit the possibility for confounding factors, it would be ideal to conduct isolated perfused liver studies in the presence of P-glycoprotein inhibitors and to quantitate drug and metabolite levels with use of specific analytical assays, studies that are currently ongoing in our laboratory.

CLINICAL EVIDENCE

Literature survey of pharmacokinetic studies considering sex as a covariate. To determine whether P-glycoprotein could be contributing to the sex-related difference in the clearance of CYP3A4 substrates in humans, we performed a literature survey and collected clearance data from clinical trials that included both male and female subjects. Combinations of the key words *female, sex, gender, P450, pharmacokinetics,* and *disposition* were used to obtain published articles from the MEDLINE (1966-2001) and PubMed databases. All articles that described human clinical trials in which clearances were compared between men (M) and women (F) were classified according to the results of the clearance (CL) data as follows: $CL_F > CL_M$, $CL_F = CL_M$, and $CL_M > CL_F$. Each drug was classified according to whether it was metabolized by CYP3A4 or transported by P-glycoprotein. Only drugs that were known to be either a P-glycoprotein or a CYP3A4 substrate (or both) were included in the survey. Clinical data that met these criteria are summarized in Table III,* with intravenous data identified and reported separately from oral data. If the groups were subdivided into young and elderly men and women, the data were reported for young men versus young women and elderly men versus elderly women.

According to the proposed hypothesis, higher P-glycoprotein in men would cause lower intracellular hepatocyte levels of a drug compared with women (Fig 1). After an intravenous dose, therefore, we would predict higher clearances from women for drugs that were substrates of both proteins and no sex-related difference for substrates of only CYP3A4 and not P-glycoprotein. The expression of P-glycoprotein in the intestine with respect to sex has not been thoroughly examined; it would therefore be difficult to predict a priori how clearance would change. However, if P-glycoprotein in the gut were also greater in men than in women, we would predict the opposite trend for intestinal clearances ($CL_M > CL_F$). We recently used an in vitro cell culture model to show that after an apical dose (representative of an oral dose) greater metabolism can occur with higher P-glycoprotein expression, presumably through repeated cycles of absorption and efflux.[38] Because of the potential confounding effect of the intestine (for orally administered drugs), the compounds were further subdivided on the basis of their dosing regimens (intravenous [Table IV*] or oral [Table V†]) to better determine whether there was agreement with the hypothesis for intravenously administered drugs.

The liver biopsy specimens that yielded the 2.4-fold greater P-glycoprotein expression in men compared with women were obtained from white and black donors. The biopsy specimens were obtained from men who were from 5 to 67 years old and from women who were from 21 to 49 years old.[10] Because the difference in the expression of P-glycoprotein between men and women was known only for this demographic group, the drugs listed in Tables IV and V include only compounds that had corresponding data from black or white male and female donors who were <65 years old.

There is reasonable evidence from functional[2] and expression[5] data that CYP1A2 expression is higher in white men than in white women and that this enzyme is inducible with smoking.[6] Drugs that were substrates of CYP1A2, even if they were also substrates of CYP3A4, were not included in Table III because they could confound the effect of P-glycoprotein. Higher clearances were observed in men for the P-glycoprotein substrates ondansetron[89] and ciprofloxacin[90] after intravenous dosing. Because both compounds are substrates for CYP1A2, it would be difficult to conclude whether the sex-related difference was the result of greater P-glycoprotein efflux or the interaction with CYP1A2.

Intravenous dosing. In general, drugs that were P-glycoprotein and CYP3A4 substrates administered intravenously exhibited good concordance with the predicted higher clearances in women compared with men and with the predicted no sex-related difference for substrates of CYP3A4 and not P-glycoprotein. Data for cyclosporine, prednisolone, and verapamil supported greater clearances in women, but for each drug one study failed to find a sex-related difference. This seems to be reasonable because the clearance differences between men and women are often not as great as the

*References 7, 11, 13, 14, 17-23, 30-36, 49-88.

* References 7, 8, 11, 13, 14, 17-23, 30-33, 49-51, 55-57, 60-62, 64, 67, 72, 77, 78, 80, 81, and 84.

† References 18, 19, 31, 33, 35, 36, 52-54, 56, 58, 59, 63-65, 68-71, 73, 74, 76, 79, 85, 86, and 88.

Table III. Literature data from clinical studies that examined sex-related differences in CYP3A4 or P-glycoprotein substrates

Drug	*Dosage route*	*CYP3A4*	*P-glycoprotein*	*CL (L/h/kg)**				*No. of subjects*		*Reference*
				Female	*Male*	*P value*	*Comments*	*Female*	*Male*	
Alfentanil	Intravenous	Yes	No	0.37	0.22	$P < .001$†		10	6	Lemmens et al[49]
				0.19	0.22	$P = 0.247$‡		11	9	
				0.558	0.558	NS†	Sex-dependent values	5	5	Sitar et al[50]
				0.246	0.246	NS‡	not reported, but no difference between sexes written in text	5	5	
				0.217	0.32	$P = .054$†	Studies were performed by same author, but data for men and women were from consecutive publications	9	9	Kharasch et al[8,51]
Atorvastatin	Oral	Yes	Yes	116.3	103	ND§	Subjects not stratified by age (included young and elderly); significance not determined but data available suggest it is significant; CL reported in L/h (no weight data available)	16	16	Gibson et al[52]
Buspirone	Oral	Yes	No	1592	1752	NS†§‖	CL reported in L/h (no weight data available)	16	17	Sakr and Andheria[53]
Cerivastatin	Oral	Yes	Yes	0.242	0.179	ND†§	Significance not determined but data available suggest it is significant	9	8	Isaacsohn et al[54]
Cyclosporine	Intravenous	Yes	Yes	0.88	0.684	$P < .05$¶	Subjects not stratified by age (included young and elderly); age known to significantly affect CL	36	77	Kahan et al[55]
				0.246	0.210	$P = .28$ (white)†		4	7	Min et al[56]
				0.330	0.210	$P = .04$ (black)†		5	4	
				2.58	2.58	NS†¶	Sex-dependent values not reported, but no difference between sexes written in text	26	22	Yee et al[57]
Cyclosporine	Oral	Yes	Yes	0.570	0.582	$P = 1.0$ (white)†§		4	7	Min et al[56]
				1.04	0.606	$P = .025$ (black)†§		5	6	
				0.94	0.50	$P < .05$†§	CL value corrected for weight on the basis of average weight given for each group	25	33	Schroeder et al[58]
				1.59	1.23	NS†§¶	CL value corrected for weight on the basis of average weight given for each group	6	6	Bleck et al[59]
Diazepam	Intravenous	Yes	No	0.038	0.029	NS†		13	10	Ochs et al[60]
				0.031	0.023	ND†	Significance not determined but data available suggest it is significant	11	11	Greenblatt et al[61]
				0.024	0.032	NS†		5	4	MacLeod et al[62]
Diltiazem	Oral	Yes	Yes	2.95	1.74	$P = .051$†§		6	6	Saenz-Campos et al[63]
				4.38	2.30	$P = .182$†§		10	10	Yeung et al[64]
Ebastine	Oral	Yes	Yes	720	701	$P = .445$†§‖	CL reported in L/h (no weight data available)	4	8	Rohatagi et al[65]

Table III—Cont'd

Drug	Dosage route	CYP3A4	P-glycoprotein	CL (L/h/kg)*				No. of subjects		Reference
				Female	Male	P value	Comments	Female	Male	
Erythromycin	Intravenous	Yes	Yes	0.49	0.36	$P = .04$†		12	12	Wong et al[66]
				4.13	2.03	$P < .01$	Subjects not stratified by age (included young and elderly); erythromycin breath test—results expressed as percent $^{14}CO_2$ expired	11	19	Watkins et al[11]
				3.14	2.29	$P < .02$	Erythromycin breath test—results expressed as percent $^{14}CO_2$ expired; age of subjects not reported	12	35	Watkins et al[13]
				3.41	2.40	$P < .05$†	Erythromycin breath test—results expressed as percent $^{14}CO_2$ expired	10	10	Kinirons et al[14]
Etoposide	Intravenous	Yes	Yes	0.912	0.912	$P = .98$	Subjects not stratified by age (included young and elderly); age known to significantly affect CL; CL measured in L/h/m^2	90	101	Kaul et al[67]
Fexofenadine	Oral	No	Yes	2.36	1.93	$P = .445$†§	CL value corrected for weight on the basis of average weight given for each group	6	6	Hamman et al[68]
Lidocaine	Intravenous	Yes	No	0.86	0.75	NS†#		9	9	Wing et al[69]
Lidocaine	Oral	Yes	No	2.56	3.31	NS†§#		9	9	Wing et al[69]
Lovastatin	Oral	Yes	Yes	179	262	NS†§¶	CL reported in L/h (no weight data available)	9	9	Cheng et al[70]
Methadone	Oral	Yes	Yes	0.088	0.092	NS†§¶		9	11	de Vos et al[71]
Methylprednisolone	Intravenous	Yes	Yes	0.447	0.288	$P = .02$†		6	6	Lew et al[72]
Midazolam	Intravenous	Yes	No	0.563	0.465	NS†		10	10	Greenblatt et al[17]
				0.277	0.301	NS†		10	10	Kinirons et al[14] and Thummel et al[18]
				0.44	0.35	NS†#		8	8	Gorski et al[19]
				0.49	0.46	$P = .68$†		10	10	Kashuba et al[7]
				0.246	0.324	NS	Age of subjects not reported	4	13	Lown et al[20]
				0.612	0.378	$P = .047$†		3	6	Tsunoda et al[22]
				0.858	0.510	$P = .026$†**		3	5	Greenblatt et al[21]
	Intramuscular	Yes	No	0.404	0.344	NS†		7	8	Holazo et al[23]
	Oral	Yes	No	0.96	1.3	NS†§		10	10	Kinirons et al[14] and Thummel et al[18]
				1.9	1.0	$P < .05$†§#		8	8	Gorski et al[19]
Nefazodone	Oral	Yes	No	2.15	2.03	NS†§	CL value corrected for weight on the basis of average weight given for each group	12	12	Barbhaiya et al[73]
Nifedipine	Oral	Yes	No	1.25	1.12	NS†§#		6	6	Lobo et al[74]
				0.726	0.558	$P = .0021$‡§¶	Included a combination of white subjects and black subjects	51	176	Krecic-Shepard et al[75]
Pimozide	Oral	Yes	Yes††	0.00088	0.00147	NS†§‖		5	7	Desta et al[76]
Prednisolone	Intravenous	Yes	Yes	0.20	0.18	NS†	CL calculated from mean body surface area and mean weights of subjects	5	8	Boekenoogen et al[77]
				0.193	0.164	$P = .022$†		4	4	Meffin et al[78]
Prednisolone	Oral	Yes	Yes	0.181	0.178	NS (white)†§		8	8	Magee et al[79]
				0.165	0.172	NS (black)†§		8	8	
Simvastatin	Oral	Yes	Yes	207	307	NS†§¶	CL reported in L/h (no weight data available)	9	9	Cheng et al[70]

Continued on p. 482

Table III—Cont'd

Drug	*Dosage route*	*CYP3A4*	*P-glyco-protein*	*CL (L/h/kg)**				*No. of subjects*		*Reference*
				Female	*Male*	*P value*	*Comments*	*Female*	*Male*	
Tacrolimus	Intravenous	Yes	Yes	Baseline CL higher in women versus men†¶			Included a combination of white subjects and black subjects	8	11	Tuteja et al[80]
Tirilazad	Intravenous	Yes	Yes††	0.841	0.474	$P < .05$†		6	6	Hulst et al[81]
				0.493	0.445	$P = .335$‡		6	6	
				0.435	0.426	NS†	Postmenopausal women included (menopause known to significantly decrease CL)	8	7	Fleishaker et al[82]
				0.365	0.336	$P = .56$‡¶	Postmenopausal women included (menopause known to significantly decrease CL)	8	12	Fleishaker and Peters[83]
Trazodone	Intravenous	Yes	No	0.169	0.139	NS†		13	12	Greenblatt et al[84]
Triazolam	Oral	Yes	No	0.276	0.219	NS†§‖		5	5	Smith et al[85]
				0.330	0.219	NS‡§‖		5	5	
				0.528	0.372	$P = .062$†§**		8	8	Greenblatt et al[86]
				44.2	25.0	$P < .02$†**	CL reported in L/h (no weight data available)	12	12	Greenblatt et al[87]
				0.522	0.330	$P > .1$†§‖		8	10	Greenblatt et al[88]
Verapamil	Intravenous	Yes	Yes	0.984	0.720	$P < .005$**§§	Subjects not stratified by age (included young and elderly)	26	12	Schwartz et al[32]
				1.02	0.66	$P < .001$†#		12	12	Dilger et al[33]
				0.822	0.756	$P = .076$#	Subjects not stratified by age (included young and elderly); age known to significantly affect CL; included a combination of white subjects and black subjects	42	42	Krecic-Shepard et al[31]
				0.942	0.834	$P = .305$†		14	6	Schwartz et al[30]
				0.720	0.462	$P < .002$‡§§		11	5	
Verapamil	Oral	Yes	Yes	2.27	1.65	NS†§#		12	12	Dilger et al[33]
				3.50	4.96	$P = .076$§#	Subjects not stratified by age (included young and elderly); age known to significantly affect CL; included a combination of white subjects and black subjects	42	42	Krecic-Shepard et al[31]
				33	13	NS†§**‡‡§§		6	6	Sasaki et al[34]
				1.6	1.0	NS†§**‡‡‖‖		6	6	
				($AUC_{female} > AUC_{male}$ not corrected for weight)†				14	16	Gupta et al[35]
				2.57	4.51	$P < .05$§	Subjects not stratified by age (included young and elderly)	6	7	Krecic-Shepard et al[36]

CL, Clearance; NS, not significant; ND, significance not determined (see "Comments"); AUC, area under the plasma concentration–time curve.
*CL reported in liters per hour per kilogram or calculated from individual weight and CL data presented in the study unless otherwise noted.
†Young subjects (average age between 20 and 50 years old).
‡Older subjects (more than 65 years old); for alfentanil[49] more than 50 years old.
§Oral CL (CL/F) reported for drugs administered orally.
‖High intersubject variability.
¶Tested in patient population (cyclosporine, renal transplant patients[55,59] and marrow transplant recipients[57]; lovastatin and simvastatin, patients with hypercholesterolemia[70]; methadone, opiate addicts[71]; nifedipine, hypertensive patients[75]; tacrolimus, renal transplant patients[80]; tirilazad, patients who have had strokes[83]).
#Some females were taking oral contraceptives.
**Values estimated from graph.
††Known P-glycoprotein inhibitor; no data available on whether it is a substrate.
‡‡Japanese population.
§§*S*-Verapamil CL.
‖‖*R*-Verapamil CL.

Table IV. Comparison of relationship between sex and clearance in humans for intravenously or intramuscularly administered drugs that are metabolized by CYP3A4 or transported by P-glycoprotein

	$CL_F > CL_M$	$CL_F = CL_M$	$CL_M > CL_F$	*References*
Substrates of CYP3A4 and P-glycoprotein	Cyclosporine			Kahan et al[55] and Min et al[56]
	Erythromycin			Watkins et al,[11,13] Kinirons et al,[14] and Yeung et al[64]
	Methylprednisolone			Lew et al[72]
	Prednisolone			Meffin et al[78]
	Tacrolimus			Tuteja et al[80]
	Tirilazad			Hulst et al[81]
	Verapamil			Schwartz et al[32] and Dilger et al[33]
		Cyclosporine		Yee et al[57]
		Etoposide		Kaul et al[67]
		Prednisolone		Boekenoogen et al[77]
		Verapamil		Schwartz et al[30] and Krecic-Shepard et al[31]
Substrates of CYP3A4 and not P-glycoprotein	Alfentanil			Lemmens et al[49]
	Diazepam			Greenblatt et al[61]
	Midazolam			Greenblatt et al[21] and Tsunoda et al[22]
		Alfentanil		Kharasch et al[8,51] and Sitar et al[50]
		Diazepam		Ochs et al[60] and MacLeod et al[62]
		Lidocaine		Yeung et al[64]
		Midazolam		Kashuba et al,[7] Greenblatt et al,[17] Thummel et al,[18] Gorski et al,[19] Lown et al,[20] and Holazo et al[23]
		Trazodone		Greenblatt et al[84]

Shaded areas represent areas in which the drugs would be expected to fall according to the hypothesis if administered intravenously.

standard deviation in the sample set; therefore the results are not statistically significant unless large numbers of subjects are tested. Etoposide was an exception: no sex-related difference was observed for this drug.

Recent reports have suggested that the data obtained from the ERMBT may not accurately reflect a true sex-related difference in clearance because the rate of CO_2 exhalation could be different between men and women and the correction factor used to calculate the results of the ERMBT does not take that into account.[46,91] Although there may be some sex-related differences in CO_2 production,[92] there is still convincing evidence for a sex-related difference in erythromycin clearance, independent of CO_2 production, from a pharmacokinetic study of erythromycin in which plasma concentrations were measured and the same sex-related difference in clearances was observed.[93]

Two CYP3A4 substrates that are not transported by P-glycoprotein (alfentanil and diazepam) each had data in 1 study that showed greater clearance in women. As listed in Table III, both drugs also had multiple studies that showed either no difference with sex or the tendency for clearances to go in the opposite direction (men > women) that did not reach statistical significance. When these findings are taken together, we believe that there is not a true sex-related difference for these drugs.

Oral dosing.

For drugs that were substrates of both CYP3A4 and P-glycoprotein, there was a preponderance of compounds that showed no difference with sex after oral dosing (Table V). Because it is unknown whether there are biochemical differences between men and women for CYP3A4 or P-glycoprotein in the intestine, it is difficult to predict whether oral clearances will be different. The data from Table V are consistent with the in vitro findings[38] that the intestine and liver may play opposite roles in affecting clearance, thereby canceling each other and resulting in no sex-related difference overall. Depending on the compound, if the intestine played a more dominant role in oral clearance, it would be possible to see the opposite trend with $CL_M > CL_F$, as observed with oral verapamil.

Table V. Comparison of relationship between sex and clearance in humans for orally administered drugs that are either metabolized by CYP3A4 or transported by P-glycoprotein or both

	$CL_F > CL_M$	$CL_F = CL_M$	$CL_M > CL_F$	*References*
Substrates of CYP3A4 and P-glycoprotein	Atorvastatin			Gibson et al[52]
	Cerivastatin			Isaacsohn et al[54]
	Cyclosporine			Min et al[56] and Schroeder et al[58]
		Cyclosporine		Min et al[56] and Bleck et al[59]
		Ebastine		Rohatagi et al[65]
		Diltiazem		Saenz-Campos et al[63] and Yeung et al[64]
		Lovastatin		Cheng et al[70]
		Methadone		de Vos et al[71]
		Pimozide		Desta et al[76]
		Prednisolone		Magee et al[79]
		Simvastatin		Cheng et al[70]
		Verapamil		Krecic-Shepard et al[31] and Dilger et al[33]
			Verapamil	Gupta et al[35] and Krecic-Shepard et al[36]
Substrates of CYP3A4 and not P-glycoprotein	Midazolam			Gorski et al[19]
	Triazolam			Greenblatt et al[87]
		Buspirone		Sakr and Andheria[53]
		Lidocaine		Wing et al[69]
		Midazolam		Thummel et al[18]
		Nefazodone		Barbhaiya et al[73]
		Nifedipine		Lobo et al[74]
		Triazolam		Smith et al[85] and Greenblatt et al[86,88]
Substrates of P-glycoprotein and not CYP3A4		Fexofenadine		Hamman et al[68]

Shaded areas represent areas in which the drugs would be expected to fall according to the hypothesis if administered intravenously.

The clearance values in Table III were reported (when possible) with respect to body weight to correct for differences in body composition between men and women. Although sex-related differences in drug clearances are frequently attributed to body weight, the analysis we performed showed that sex-related differences generally persisted for intravenously administered drugs that were P-glycoprotein substrates. Of all the studies listed in Table III, only 6 of the 61 studies that reported clearance values did not correct for weight. If the general assumption that women weigh less than men were true in these studies, we hypothesized that 3 of the compound values that were not corrected for body weight (atorvastatin, ebastine, and triazolam) would have accentuated sex-related differences in their clearance values after correction and 3 would have diminished the sex-related difference (lovastatin, simvastatin, and buspirone). Therefore the paradigm that sex-related differences in clearance values disappear after weight correction cannot be generally applied.

Future considerations. A number of compounds that exhibited sex-related differences in their oral clearances (ie, metronidazole[94] and modafinil[66]: $CL_F > CL_M$) could not be included until further data obtained from in vitro studies could confirm whether they were P-glycoprotein or CYP3A4 substrates. Most evidence suggested that the clearance values of the CYP3A4 substrates cocaine[95] (intravenous) and alprazolam[96-98] (oral) were not dependent on sex, but because no information was available on whether these drugs are P-glycoprotein substrates, they were omitted from Table III. Kuehl et al[99] recently showed that the polymorphic enzyme CYP3A5 is differentially expressed across ethnic groups and suggested that this may be a contributing factor in the overall variability observed for the

metabolism of CYP3A4 substrates. Further studies to examine the substrate specificity of CYP3A5 are needed before any conclusions can be made about the impact of this enzyme on overall CYP3A metabolism.

The hypothesis presented here requires that substrates of P-glycoprotein primarily interact with this transporter, and this is not necessarily the case. For example, it has been reported that fexofenadine, a P-glycoprotein substrate, is also a substrate of the uptake transporter OATP (organic anion transporting polypeptide).[100] It is not yet known whether sex-related differences exist for other efflux and uptake transporters that could also affect overall hepatic metabolism. Although sex-related differences in pharmacokinetics are interesting from a scientific perspective, they are rarely considered to be of clinical importance. Most sex-related differences in clearance values are on the order of 20% to 30% and are not usually found with low-therapeutic-index drugs. One important exception was tirilazad, for which the higher clearance value in women resulted in lower efficacies for treatment of subarachnoid hemorrhage.[101] Nevertheless, because of the frequent lack of clinical significance for sex-related differences, there may be several other examples of these modest sex-related differences in clearance values that have never been explicitly reported.

CONCLUSIONS

The literature survey revealed that for drugs administered intravenously there was strong concordance with the hypothesis that sex-related differences in drug clearances for CYP3A4 substrates may be attributable to interactions with P-glycoprotein. Drugs that are substrates for both CYP3A4 and P-glycoprotein typically had higher clearance values in women, whereas drugs that were metabolized by CYP3A4 but not transported by P-glycoprotein did not exhibit sex-related differences. In vitro data obtained from cell culture studies, which showed that increased intracellular drug levels were obtained with inhibition of P-glycoprotein efflux transport, also correlated well with clinical data. P-glycoprotein knockout mice data were more difficult to interpret because many routes of elimination were changing at once and because the severe impairment of overall drug excretion might have caused alternate routes of elimination to become important. The inclusion of women in clinical trials, as recommended by the US Food and Drug Administration in 1993, has led to the emergence of more clinical data for the investigation of sex-related differences in pharmacokinetics and pharmacodynamics for new compounds. In addition, sex is now more commonly being examined in pharmacokinetic studies that test bioequivalence. The sex-related differences revealed in these studies frequently postulated enzymatic differences between men and women, but it is now important to consider that transporters may be responsible for mediation of these different metabolic outcomes. This realization could lead to a redefinition of what intrinsic clearance truly reflects, which we will address in a subsequent publication, because access of the drug to the metabolizing enzyme must also be considered—a parameter that is easily altered by uptake and efflux transporters.

While this article was being reviewed, Meibohm et al[102] published a general review on sex-related differences in pharmacokinetics in which they also suggest that the difference in the clearance of CYP3A4 substrates may be attributable to the sex-related difference in P-glycoprotein expression. However, Meibohm et al[102] did not provide the in-depth analysis given in this article.

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