# Effect of Gonadectomy and Hormones on Sex Differences in Ketoprofen Enantiomer Glucuronidation and Renal Excretion of Formed Glucuronides in the Rat

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***Purpose.*** To investigate the sex hormone dependency of phase II metabolism using S-ketoprofen (S-KT) urinary excretion (ΣXu) as a marker in the rat.

***Methods.*** The effect of surgical gonadectomy, with or without concomitant estradiol or testosterone treatment, on the ΣXu of glucuronidated S-KT was studied in male and female rats. Hepatic and renal glucuronidation of KT enantiomers was also determined using microsomal preparations from these animals.

***Results.*** A controlling effect of testosterone was demonstrated by a rapid increase in ΣXu of glucuronidated S-KT in castrated males (27.9 ± 9.0%) compared to control males (7.2 ± 3.9%). This approximated control female excretion (40.5 ± 11.6%). Treatment of ovarectomized females with testosterone resulted in a steady reduction in ΣXu of glucuronidated S-KT with time (13.4 ± 5.4% at end point). Hepatic glucuronidation of S-KT by male rat liver microsomes was significantly higher than that of female, whereas renal glucuronidation of S-KT by female rat kidney microsomes was significantly higher than that of male. Significant correlations were found between hepatic ($r = -0.78$) or renal ($r = 0.83$) glucuronidation and ΣXu of glucuronidated S-KT.

***Conclusions.*** Urinary excretion of S-KT-GC is sex hormone–dependent. This metabolite may have utility as a marker or probe for sex hormone–dependent studies of phase II metabolism.

**KEY WORDS:** glucuronidation; gonadectomy; ketoprofen; nonsteroidal anti-inflammatory drug; sex differences.

## INTRODUCTION

Sex, phase of menstrual cycle, oral contraceptive use, menopause, or andropause may contribute to intra- and intersubject variability in pharmacokinetics and pharmacodynamics of drugs (1,2). Although recognized as potential sources of variability, the specific factors or pathways underlying sex differences in disposition of drugs are often difficult to elucidate as the result may be due to a number of underlying physiologic processes that can occur simultaneously or sequentially. It is therefore often necessary to perform specific mechanistic studies in order to determine how the disposition of a drug is under control of the sex hormones. Though there has been extensive investigation in both humans and animals into sex differences in cytochrome P-450–mediated metabolism (3,4) and membrane transporters (5,6), there is little information in the literature on such differences in phase II metabolism or route(s) of elimination of drugs (7,8).

We have previously reported a marked sex difference in the clearance of the S-enantiomer of ketoprofen (KT), a racemic 2-arylpropionic acid nonsteroidal anti-inflammatory drug (NSAID), in the rat (9). A pronounced sex difference in S-KT disposition was observed in female rats compared to male rats evidenced by significantly higher plasma concentrations of S-KT and a significantly greater fraction of the administered dose excreted in the urine of females as the phase II metabolite, S-KT glucuronide conjugate (GC). The underlying mechanism for the sex difference was unknown, but thought to be due in part to differences in sex hormone–dependent metabolism and/or elimination.

Sex differences in the urinary excretion of GC metabolites in rats have previously been linked to renal uridine 5′ diphosphate glucuronosyltransferase (UDPGT) activity toward various substrates (10). In addition, differences in the excretion of GC metabolites have also been attributed to sex differences in hepatic glucuronidation as male rats are reported to have higher levels of uridine 5′ diphosphate glucuronic acid (UDPGA) and UDPGT activity in the liver than female rats (7,10–12). The testes and/or androgens, either directly or indirectly through their linkage with growth hormones, are known to be dominant factors in determining sex variations in drug metabolic enzymes and function as important regulators in the adult period as well as in imprinting during neonatal exposure (4,13,14). Past attempts to identify the specific hormone(s) responsible for sex differences in metabolism and elimination have mainly focused on ablation of endocrine organs and/or appropriate sex hormone replacement (15–18).

It is realized that although pronounced sex differences in the disposition of a drug may be observed in an animal model (i.e., rat), they may not be reproducible in humans. Furthermore, even when such sex differences are evident in humans, the therapeutic importance of the observations are often unclear. Nevertheless, studies describing sex differences in drug disposition provide important confirmatory information regarding sources of intra- and intersubject variability in experimental data.

In the current report, we sought to determine the sex hormone dependency of S-KT clearance in the rat by performing specific mechanistic studies. We studied the effect of surgical gonadectomy, with or without concomitant sex hormone treatment, on the excretion pattern of S-KT-GC in male and female rats. We also investigated the renal and hepatic glucuronidation of KT enantiomers in microsomal preparations obtained from these animals. By undertaking these experiments, we have shown that the urinary excretion of S-KT-GC can be used as a convenient marker or probe for sex hormone–dependent studies of phase II metabolism.

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**ABBREVIATIONS:** GC, glucuronide conjugate; KT, ketoprofen; NSAID, nonsteroidal anti-inflammatory drug; UDPGA, uridine 5′ diphosphate glucuronic acid; UDPGT, uridine 5′ diphosphate glucuronosyltransferase.



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## MATERIALS AND METHODS

### Materials

Ketoprofen (rac-2-[3-benzoylphenyl]-propionic acid) and internal standard, indoprofen (rac-*p*-[1-oxo-2-isoindolinyl]-hydratropic acid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). In addition, all kits used for the determination of albumin and total microsomal protein concentrations and all reagents and chemicals used for the microsomal incubations were purchased from Sigma Chemical Co. Estradiol valerate (Delestrogen) was purchased from Squibb (Montreal, Quebec, Canada) and testosterone cypionate (DepoTestosterone) was purchased from Upjohn (Don Mills, Ontario, Canada). All chemicals and solvents used were of analytical grade.

### Gonadectomy

Age-matched adult female and male Sprague-Dawley rats weighing between 250 and 330 g were used for the study. All surgeries were done under methoxyflurane anesthesia (Pitman-Moore Ltd., Mississauga, Ontario, Canada) following preoperative cleansing (after shaving) with chlorhexidine gluconate 4% (Hibitane; Ayerst, Montreal, Quebec, Canada) and povidone iodine 7.5% (Betadine; Purdue-Frederick, Inc., Toronto, Ontario, Canada). Bilateral ovarectomy in female rats was done via small midline dorsal incisions with ovaries severed at the junction of the fallopian tube and uterine horn and periovarian fat and blood vessels following ligatation (19). The muscular layer was sutured and the skin clipped with 9-mm wound clips (Autoclips; Clay Adams, Parisippanny, NJ, USA), which were removed after 3 days. In the male rats, castration was done via a small median incision through the scrotal skin and then testicular sacs. The testis accompanied by the vas deferens and spermatic blood vessels were severed distal to a single ligature and removed (19). The remaining incisions were closed using Nexaband Liquid (Tri-Pont Medical, Raleigh, NC, USA). Rats were allowed to recover for 1 week prior to dosing with racemic KT. Animals were weighed on the same day each week throughout the experiment. Percent weight gain was calculated as the incremental gain in body weight from week 1 multiplied by 100.

### Dosing and Sample Collection

Following surgery, female rats (n = 4/group) were divided into ovarectomized and ovarectomized + testosterone groups. A control group was added to the set. Similarly, male rats (n = 4/group) were also divided as follows: control; castrated and castrated + estradiol. Rats were dosed with either estradiol valerate (1 mg/kg) or testosterone cypionate (10 mg/kg) via the i.m. route immediately following surgery and then on the same day each week throughout the experiment. Racemic KT (10 mg/kg), dissolved in polyethylene glycol 400, was administered by the i.p. route once a week on the same day for 4 weeks and then again at 11 weeks. Rats were individually housed in plastic metabolic cages with food and water *ad libitum* during each dosing experiment. Total cumulative urine output from 0 to 24 h was collected after KT dosing on weeks 1, 2, 3, 4, and 11 into containers previously rinsed with 1 M HCl to prevent spontaneous cleavage of the GC metabolites. All specimens were frozen at –20°C until analysis.

### Microsomal Preparation

Hepatic and renal microsomes were prepared from the rats used in the gonadectomy and sex-hormone dosing portion of the study following completion of the experiment. Rats were anesthetized with diethyl ether (Caledon Laboratories, Georgetown, Ontario, Canada), and all 5 lobes of the liver and both kidneys were excised from each animal via midline incision and immediately placed in ice-cold 1.15% KCl solution. Individual tissues from the 4 rats in each group were pooled and homogenized with 2 volumes of ice-cold 100 mM buffer (pH 7.4) containing 250 mM sucrose, all the while keeping the tissue samples on ice. The homogenized tissue was centrifuged at 5°C for 20 min at 10,000 × *g* in a Beckman model L855 centrifuge (Beckman, Palo Alto, CA, USA). The supernatant was then centrifuged at 5°C for 60 min at 105,000 × *g*. The supernatant was discarded and the solid pellet resuspended in 2 volumes of phosphate:sucrose buffer and again centrifuged at 5°C for 60 min at 105,000 × *g*. The supernatant was again discarded and the resulting pellet resuspended in 2 volumes of phosphate:sucrose buffer and frozen at –20°C until use. The liver and kidney microsomal protein concentration of each group was determined in triplicate by the method of Lowry *et al.* (20) (Protein Assay Kit No. P5656, Sigma Diagnostics, St. Louis, MO, USA).

### Microsomal Incubations

Stereospecific glucuronidation of 50 μg/ml racemic KT was determined in triplicate in each group as this concentration is in accordance to what is seen in plasma following 10 mg/kg racemic doses given i.v. to rats as per our previous work (9). For each incubation, 2.0 mg of liver or kidney microsomal protein was incubated with reaction mixtures containing 20 mM UDPGA, 5 mM $MgCl_2$, and 0.05% Triton-X in 100 mM phosphate and 250 mM sucrose buffer (pH 7.4) for 10 min at 37°C in a shaking water bath. Preliminary work indicated that glucuronidation was linear under these conditions with respect to time and KT, UDPGA and protein concentrations. Reactions were started by addition of racemic KT (50 μg/ml in reaction mixture) and run for 60 min; total incubation volume was 1.0 ml. Samples (100 μl) were taken at 5, 15, 30, and 60 min and transferred to clean test tubes containing 100 μl 1 M NaOH (hydrolyzed) or 150 μl 0.6 M $H_2SO_4$ (unhydrolyzed). To the basified tubes, 200 μl of 0.6 M $H_2SO_4$ was added to enable extraction, and then all samples were immediately analyzed. The difference between acidified and basified/acidified samples was taken to be the amount of R- or S-KT enantiomer metabolized to GC.

### Quantitation of KT Enantiomers and GC

Concentrations of R- and S-KT and R- and S-KT-GC in rat urine and microsomal fractions were determined using a previously reported stereospecific reversed-phase HPLC method (21). The amount of R- and S-KT-GC were measured following alkaline hydrolysis with the amount of enantiomer GC taken to be the difference between the hydrolyzed and unhydrolyzed sample. The percent of the dose recovered as

GC in urine was calculated as the amount of R- or S-KT-GC obtained in the 24-h collection divided by the R- or S-KT enantiomer dose (1/2 the racemic dose) and multiplied by 100.

### Data Analysis

For the gonadectomy/sex-hormone dosing portion of the study, significant differences between treatment groups and within treatment groups with time were examined by use of the two-way, repeated-measures ANOVA in female and male rats, respectively. For the microsomal glucuruonidation portion of the study, significant differences in the data were examined by use of the one-way ANOVA. In either case, where significant differences were found, the Duncan's multiple range test was used to compare means. All tests were conducted at the $\alpha = 0.05$ level of significance. Results are reported as mean ± SD.

## RESULTS

### Gonadectomy and Sex-Hormone Dosing

As expected, the urinary excretion of KT enantiomers was significantly stereoselective in control rats of both sexes, consistent with our earlier report (9). At week 1, 7.2 ± 3.9% and 40.5 ± 11.6% of the S-KT dose was recovered over 24 h in the urine as S-KT-GC from control male and female rats, respectively. In both sexes, the small amount of R-KT-GC recovered in urine (<3%) was considered to be negligible. These results confirmed that S-KT-GC was the more appropriate marker to use to detect relative differences in sex hormone dependency in the study. No detectable concentrations of unconjugated R- or S-KT were found in urine. All groups demonstrated normal and consistent weight gain over the duration of the experiment with the exception of castrated male rats treated with estradiol, as this group failed to gain any significant body weight during the entire 11 week period (Fig. 1).

In female rats, ovarectomy did not appear to have a significant effect on the urinary excretion of S-KT-GC when compared with control female rats at any time point (Fig. 2). In contrast; however, treatment of ovarectomized female rats with testosterone resulted in a steady and significant reduction in S-KT-GC excretion into urine over time resulting in only 13.4 ± 5.4% of the S-KT dose being recovered in the urine at week 11. No differences in the negligible urinary recovery of R-KT-GC was found in any group.

In male rats, castration resulted in a rapid and significant increase in the urinary excretion of S-KT-GC as early as week 1 postsurgery (Fig. 2). At week 1, 27.9 ± 9.0% of the S-KT-GC dose was recovered in castrated rats vs. 7.2 ± 3.9% in control rats. The amount recovered in the castrated males progressively increased to 57.3 ± 16.2% of the S-KT dose by week 11. Consistent with findings in the female rats, there was no treatment effect on the negligible recovery of R-KT-GC in any group.

### Renal Microsomal Glucuronidation

The highest renal glucuronidation of both KT enantiomers was observed in kidney microsomes prepared from control female rats (R-KT: 6.0 ± 0.4; S-KT: 5.9 ± 1.2 $\mu mol \cdot h^{-1} \cdot mg^{-1}$ protein). In comparison, renal glucuronidation from kidney microsomes prepared from control male rats was as follows: R-KT, 3.8 ± 1.9; S-KT, 3.8 ± 0.6 $\mu mol \cdot h^{-1} \cdot g^{-1}$ protein (Fig. 3). Microsomes prepared from ovarectomized rats and ovarectomized rats treated with testosterone displayed significantly lower renal glucuronidating activity than those prepared from control female rats. A significant positive correlation ($r = 0.83$) was found between renal glucuronidating activity toward S-KT and the 24 h urinary recovery of S-KT-GC as percent of S-KT dose in both male and female rats (Fig. 4).

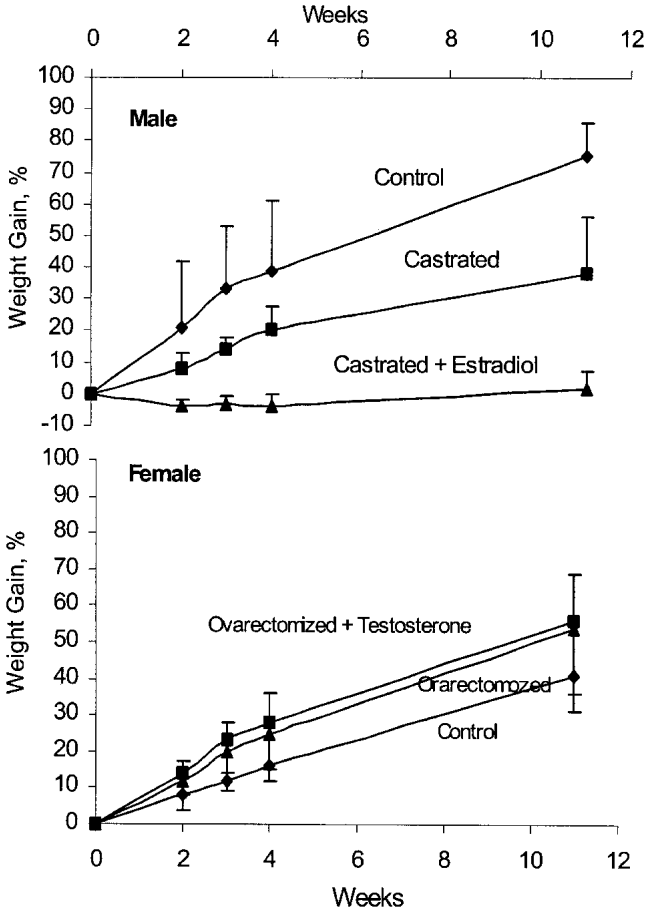


**Fig. 1.** Percent weight gain from week 1 following gonadectomy in male and female rats. Data are presented as the mean ± SD (n = 4/group). Weight gain in castrated + estradiol treated male rats not significantly different from week 1, $\alpha = 0.05$.

### Hepatic Microsomal Glucuronidation

The highest hepatic glucuronidation of KT enantiomers was observed in liver microsomes prepared from control male rats (R-KT, 25.3 ± 5.4; S-KT, 26.5 ± 5.4 $\mu mol \cdot h^{-1} \cdot mg^{-1}$ protein). In comparison, hepatic glucuronidation from liver microsomes prepared from control female rats was as follows: R-KT, 16.6 ± 2.5; S-KT, 14.3 ± 2.7 $\mu mol \cdot h^{-1} \cdot g^{-1}$ protein (Fig. 5). In control males, the amount of S-KT-GC formed was significantly higher than all other male groups. There was significantly less hepatic glucuronidation of both R- and S-KT in microsomes prepared from castrated males when compared with control male rats. A significant negative correlation ($r = -0.78$) was found between hepatic glucuronidating

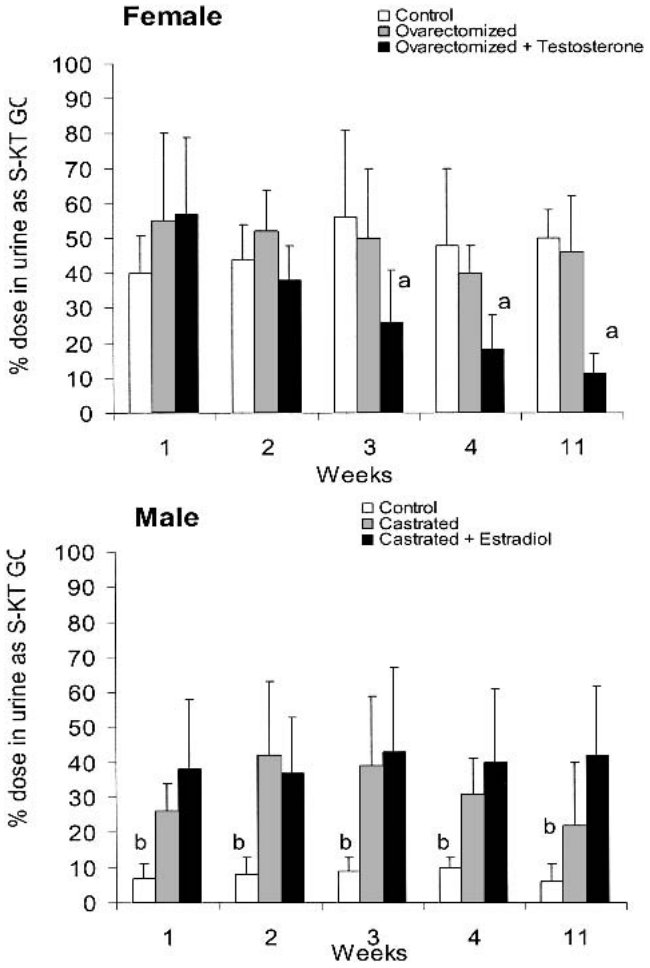


**Fig. 2.** Percent of S-KT dose recovered in 24-h urine collection as S-KT-GC with time from gonadectomy in female and male rats. Data are presented as the mean ± SD. Key: a, significant different from week 1; b, significantly different from control female group and other male groups; $\alpha = 0.05$.

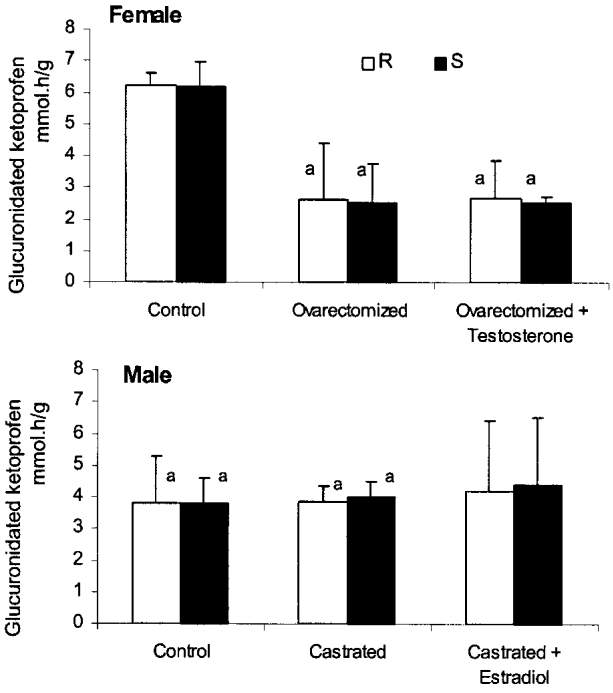


**Fig. 3.** *In vitro* glucuronidation of R-KT and S-KT by kidney microsomes prepared from female and male rats. Data are presented as the mean ± SD (n = 3 incubations). Key: a, significantly different from control female rats; $\alpha = 0.05$.

activity toward S-KT and the 24-h urinary recovery of S-KT-GC as percent of S-KT dose in both male and female rats (Fig. 4).

## DISCUSSION

The current data confirm that sex differences in the urinary excretion of S-KT-GC in the rat are indeed under control of the sex hormones. It appears that estradiol may play a lesser role when compared to testosterone in this regard, as ovarectomy alone did not significantly alter the urinary excretion of S-KT-GC from that of control female rats (Fig. 2). In contrast, testosterone did markedly influence the urinary excretion pattern of S-KT-GC. This is evidenced by the rapid and significant increase in urinary excretion of S-KT-GC in castrated male rats to a level approaching that of control female rats at week 1 (Fig. 2). In addition, treatment of ovarectomized female rats with testosterone resulted in a steady and significant reduction over time in the urinary excretion of the S-KT-GC metabolite to an extent approximating that of control male rats at study end-point (Fig. 2).

A controlling effect of testosterone on urinary excretion of drugs has been previously reported. Tanaka *et al.* (22) have shown that treatment of gonadectomized male and female rats with testosterone results in suppression of the urinary excretion of the aldose reductase inhibitor, zenarestat, to a pattern resembling that of male rats. Similar to our observation with S-KT, ovarectomy had little effect in their study, whereas castration alone resulted in a female urinary excretion pattern of zenarestat. An inhibitory action of testosterone has also been demonstrated in the investigation of the renal toxicity of 2-aminoanthraquinone in the rat (17). Male rats and testosterone-treated ovarectomized female rats were protected from renal toxicity of the compound whereas control female rats developed nephrotoxicity. Recently, Kudo *et al.* (6) have reported significantly greater urinary excretion of perfluorooctanoic acid by female and castrated rats as compared with intact male rats. The observed sex-difference in urinary excretion was attributed to sex-controlled expression of renal organic anion transporters. Castration resulted in a reduction in the level of mRNA of organic anion transporter 1 and the kidney specific-organic anion transporter K.

Our finding that the urinary excretion of S-KT-GC was positively associated with renal glucuronidation of S-KT by kidney microsomes is notable (Fig. 4). This would support that sex differences in urinary excretion of S-KT-GC are due, in part, to sex-dependent phase II metabolism of KT. The higher glucuronidating activity in the female kidney thus has a direct effect on the amount of S-KT-GC available for subsequent excretion into the urine. Interestingly, we found the highest renal glucuronidating activity of any group to be in kidney microsomes prepared from control female rats (Fig. 3), whereas activity in ovarectomized and ovarectomized females treated with testosterone was found to be significantly less when compared with control females. Manipulation of testosterone levels in male rats did not have a substantive

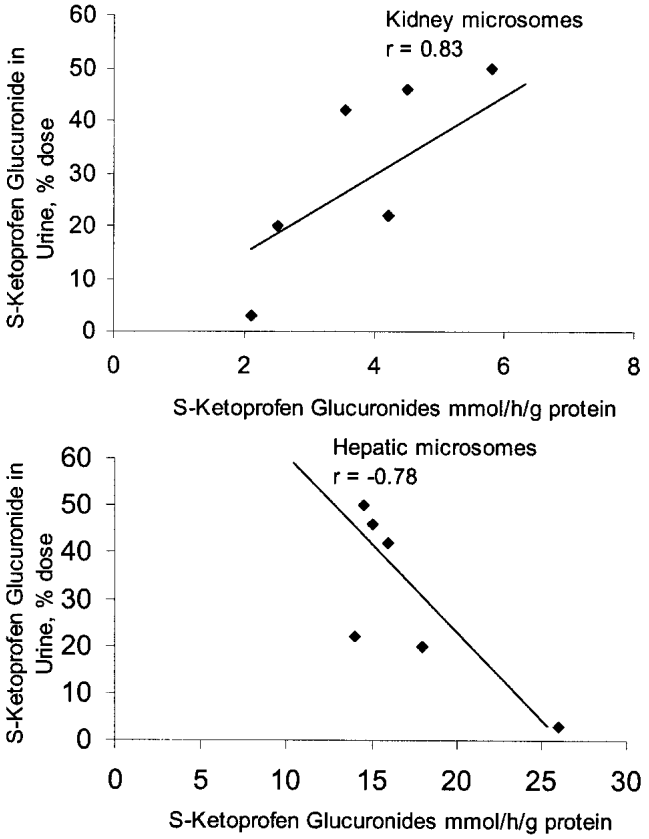


**Fig. 4.** Relationships of mean percent of S-KT dose recovered in 24-h urine collection as S-KT-GC 11 weeks postsurgery (n = 4 rats/group) and mean amount of S-KT-GC formed by kidney or liver microsomes (n = 3 incubations) prepared from the same animals.

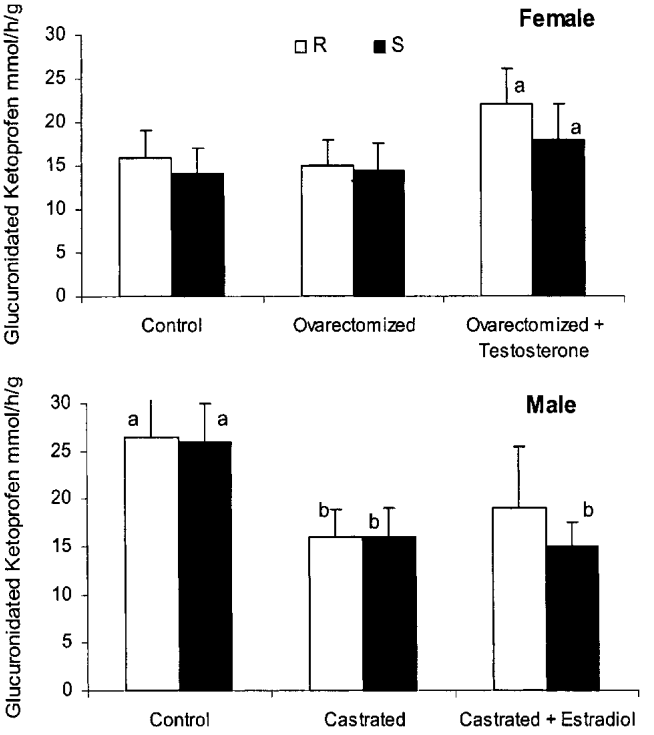


**Fig. 5.** *In vitro* glucuronidation of R-KT and S-KT by liver microsomes prepared from female and male rats. Data are presented as the mean ± SD (n = 3 incubations). Key: a, significantly different from control female rats; b, significantly different from control female rats; $\alpha = 0.05$.

impact on renal glucuronidation, as activity in castrated male rats and castrated male rats treated with estradiol did not differ from that of control male rats. There did appear to be a trend toward higher renal activity in castrated male rats treated with estradiol; however, the difference was not statistically significant (Fig. 2). These observations support that estradiol or female sex hormones may play a role in the regulation of renal glucuronidation of KT. This is supported by renal microsomal activity being diminished in ovarectomized females compared to that of control females. However, the administration of exogenous estradiol to castrated males was not sufficient to bring about the activity seen in control females. This suggests that the changes brought about by ovarectomy go beyond the lack of estradiol and that in fact, a balance of hormones may be involved.

Higher renal glucuronidating activity in female rat kidney microsomes compared with those of male rats has been reported elsewhere (10,11). Correlations between renal excretion of glucuronide metabolites and renal microsomal UDPGT activity have been reported for various substrates. An example of this is the finding that female rats excrete significantly more *p*-nitrophenol glucuronide in urine than males and have higher renal microsomal UDPGT activity toward *p*-nitrophenol than male rats despite males having higher hepatic microsomal activity (10).

The pattern of S-KT glucuronidation by liver microsomes was opposite to that observed with kidney microsomes, as we found that urinary excretion of S-KT-GC was negatively associated with hepatic glucuronidation of S-KT (Fig. 4). The highest hepatic S-KT glucuonidating activity of any group was found in liver microsomes prepared from control male rats. Treatment of ovarectomized rats with testosterone did result in a trend toward numerically greater hepatic glucuronidation of S-KT, but the results failed to reach statistical significance. Castration of male rats and treatment of castrated rats with estradiol did significantly reduce glucuronidation of S-KT compared with control males to a level that was not significantly different from that of control female rats. We have some preliminary data suggestive of a more rapid excretion of KT-GC into bile in the male rat than the female (23). The finding of enhanced hepatic activity toward KT enantiomers in the male rat is consistent with more GC being available for elimination via the biliary route. These data support a controlling or regulatory function of testosterone that is consistent with the findings of others that hepatic microsomes prepared from male rats have higher glucuronidating activity toward a variety of aglycones (10–12).

Androgens are known to be important regulators and may influence phase II conjugation reactions through control of cofactor availability, tissue dependent UDPGT activity, active transport processes, and sequential metabolism of the glucuronidates (7). With specific regard to glucuronidation, testosterone is reported to regulate the cofactor UDPGA levels in the intact cell rather than alter UDPGT levels (12,18). Others report that the effect of testosterone is closely related to its anabolic action and/or state of activation of UDPGT in different organs (11). A controlling effect of testosterone has also been suggested by Iatsimirskaia *et al.* (24), who reported the oral bioavailability of a GnRH receptor antagonist (AG-

045572) to be 8% in intact male rats but 24% in both intact female and castrated male rats. The authors attributed their observation to suppression of testosterone by AG-045572 and hormonal dependency of the metabolism by CYP3A.

The differences in glucuronidation of S-KT between male and female rat kidney and liver microsomes and the ensuing relationships between glucuronidating activity and urinary excretion of S-KT-GC following manipulation of the sex hormones, confirms the sex hormone dependency of S-KT clearance in the rat. These results corroborate our preliminary observation (23) that in the male rat, the primary route of elimination of S-KT-GC is via the biliary route. This is likely due to greater hepatic glucuronidation of S-KT in the male rat resulting in more GC being available for this route of elimination. Because the biliary route is so efficient in the male rat, elimination of S-KT-GC via the urine becomes a minor pathway. Due to a reduced efficiency for hepatic glucuronidation in female rats, a reduced clearance and, thereby, a greater availability for renal glucuronidation and subsequent elimination may be occurring. This may explain why the primary route of elimination in the female rat is thus via the urine. Such a compensatory relationship between biliary and renal elimination has been described previously (10,11), and our data support that the relative contribution of each pathway in the clearance of S-KT in the rat may be sex dependent

We did not find any significant sex differences in the amount of R-KT-GC recovered in urine of any experimental group when compared with controls. This is likely due to the major route of elimination of R-KT being through unidirectional bioinversion to the active S-antipode (25).

An interesting phenomenon in our study was the observation that castrated male rats treated with estradiol failed to gain weight at a rate in accordance with other groups (Fig. 1). This may be an indirect reflection of the absence of the anabolic action of testosterone, as liver and kidney weight have been found to correlate with levels of testosterone (16,17). All other groups continued to gain weight at a rate similar to control animals.

In this study we have found that the processes controlling renal or hepatic formation of and/or urinary excretion of S-KT-GC have shown marked sex hormone dependency in the rat, although the exact mechanism by which the hormones exert their action remains unknown. The effect of administering exogenous hormones as opposed to naturally occurring hormones also remains unknown, and ultimately it may be that the balance of estrogenic:androgenic hormones are crucial to overall KT enantiomer clearance in this animal model.

Sex differences in drug disposition are often more evident in rats as opposed to humans, and as a result of this, interspecies extrapolation of data may not be meaningful. Nonetheless, sex-dependent pharmacokinetic and pharmacodynamic differences are not uncommon in humans. For example, citalopram (26), propranolol (27), and verapamil (28) all demonstrate greater plasma concentrations in human females than males. Piroxicam steady-state plasma concentrations are twice as high in aged women than those in age-matched males (29). In addition, it has been shown that female patients have a lower pain threshold (30) and respond better to analgesic therapy than do males (30,31). The therapeutic importance of sex-differences in drug disposition may be a subject of debate; however, such differences may contribute substantively to the intra- and intersubject variability observed in experimental designs that include both sexes.

Given the confirmed sex-dependency of urinary excretion of S-KT-GC in the rat that we report in this study, the urinary excretion of this metabolite may have utility as a convenient marker or probe for sex hormone–dependent studies of phase II metabolism. Furthermore, because a substantial fraction of the administered dose of KT is excreted in the urine after glucuronidation in humans (32), the female rat may be a more appropriate animal model than the male rat to study various experimental effects on the pharmacokinetics of this NSAID.

## ACKNOWLEDGMENTS

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