Short Communication

Androgen Regulation of Renal Uridine Diphenylglucuronosyltransferase 1A1 in Rats

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ABSTRACT:

Many phase I and II enzymes are under hormonal regulation, resulting in sex-related expression patterns. This sex-related enzyme expression can result in differential metabolism of physiologically active endogenous substances, altered xenobiotic clearance, and differences in susceptibility to drug toxicities. Treatment of female Sprague-Dawley (SD) rats with 5 mg testosterone propionate/kg/day, 2 ml/kg s.c. for 8 days resulted in induction of renal uridine diphenylglucuronosyltransferase (UGT) 1A1, as determined by immuno-
blot and probe substrate activity. Glucuronidation activity for mycophenolic acid, a substrate for rat UGT1A1, 1A6, and 1A7, was significantly elevated approximately 2-fold in renal microsomes from testosterone propionate-treated animals. Protein expression of rat UGT1A1 was also dramatically increased, whereas 1A6 and 1A7 remained unchanged as a result of treatment. Male SD rats were determined to express greater renal UGT1A1 than age-matched female rats. These data support the androgen regulation of rat renal UGT1A1.

Glucuronidation mediated by uridine diphenylglucuronosyltransferases (UGTs) is the dominant phase II, or conjugative, metabolic pathway for both xenobiotics and endogenous compounds. These enzymes catalyze the addition of glucuronic acid from the uridine diphenylglucuronamide cosubstrate to a nucleophilic functional group of an aglycone acceptor, including hydroxyl, carboxylic acid, amine, thiol, and carbon (Radomska-Pandya et al., 1999). The addition of the sugar group can dramatically increase the hydrophilicity and solubility of the aglycone, facilitating its biliary or renal excretion. Although glucuronidation is primarily considered a detoxification pathway, glucuronidation can in some cases be considered a bioactivation pathway. Acyl glucuronides, in particular, are reactive and potentially toxic (Stachulski, 2007). Because glucuronidation is the primary metabolic pathway for many compounds, and altered expression can result in significant changes in clearance and toxicity, a thorough understanding of the regulation of UGT expression is important.

Androgens have been shown to regulate many rat UGT isoforms in a tissue-specific manner. For example, expression of hepatic UGT2B1 and 2B3 (Strasser et al., 1997) and Sertoli cell UGT1A1 (Magnanti et al., 2000) show responsiveness to androgens. This present study investigates the androgen regulation of rat renal UGT1A1. Because both human and rat UGT1A1 glucuronidate a wide range of substrates, including anthraquinolones, coumarins, estrogens, flavonoids, phenolic, and opioid compounds (King et al., 1996), hormonal control of renal UGT1A1 may have important physiological and pharmacological consequences. Testosterone, an endogenous androgen found in all mammalian species, was selected for these enzyme regulation studies conducted in Sprague-Dawley rats.

Materials and Methods

Materials. Mycophenolate (≥98% pure) and suprofen were purchased from Sigma-Aldrich (St. Louis, MO). Mycophenolate-glucuronide reference standard was prepared and characterized as described previously (Wiwattana-ongsma et al., 2001). Solvents used for sample preparation and high-performance liquid chromatography (HPLC) were obtained from Fisher Scientific Co. (Pittsburgh, PA). Electrophoresis, gel/membrane transfer boxes, and immunoblot reagents were obtained from Bio-Rad (Hercules, CA), unless otherwise stated. All other chemicals for this study were of the highest purity possible purchased from Sigma-Aldrich.

Animals and Treatments. Male and female Sprague-Dawley rats (3–5 months old) were housed in a temperature and humidity controlled facility, with 12-h light/dark cycles. Animals were provided rodent chow and water ad libitum. Animals were allowed to acclimate to housing conditions for at least 1 week prior to initiation of experiments. For the testosterone induction study, female rats, five per group, were treated s.c. with 5 mg testosterone propionate/2 ml/kg/day or peanut oil vehicle for 8 consecutive days. All animal procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee.

Renal Microsome Preparation. Rat renal microsomes were prepared by differential centrifugation on study day 9, 24 h after the last dose administration (Tallman et al., 2005). Briefly, the kidney cortex was homogenized in 4 volumes of ice-cold 0.25M sucrose with EDTA (1 mM), dithiothreitol (0.1 mM), and phenylmethylsulfonyl fluoride (0.25 mM) and centrifuged at 100,000 g for 20 min. The supernatant was then spun at 100,000g for 1 h. The resulting pellet was reconstituted in 250 mM sucrose containing phenylmethylsulfonyl fluoride (0.25 mM) and leupeptin (10 mM). Protein concentrations were determined by the Bradford method, using albumin as a standard.

In Vitro Mycophenolic Acid Glucuronidation Assay. The microsomal incubation conditions were 0.05% Brij 35/mg protein, 10 mM D-saccaric acid-1,4-lactone, 2 mM UDP glucuronic acid, 10 mM MgCl2, 2.0 mM mycophenolate, and 0.25 mg of microsomal protein in a total reaction volume of 1 ml Tris-HCl (pH 7.4, 37°C). The reaction was terminated after 10 to 30 min by the addition of 4 volumes of ice-cold acetonitrile. The reaction proceeded at...
2-fold increased renal microsomal glucuronidating activity toward mycophenolic acid in comparison with vehicle-treated females (5.5 ± 0.5 versus 2.5 ± 0.2 nmol MPAG/min/mg, p ≤ 0.05). In contrast, no changes in hepatic or intestinal UGT1A1 protein expression or microsomal glucuronidating activity toward mycophenolic acid were observed in response to testosterone treatment (data not shown). The rat recombinant UGT study of Miles et al. (2005) demonstrated that mycophenolic acid is a substrate for rat UGT1A1, 1A6, and 1A7, with respective $K_m$ values of 0.208, 1.28, and 0.034 mM and $V_{\text{max}}$ values of 1.7, 4.8, and 10.3 nmol/min/mg. Because treatment-related changes in renal UGT1A6 and 1A7 protein expression were not observed in response to testosterone treatment (Fig. 1A), the differences in renal mycophenolate glucuronidation activity can be attributed to the induction of UGT1A1. Differences in male and female renal UGT expression were also identified in the present study (Fig. 1B); males had higher UGT1A1 and UGT1A common region reactive protein (1ACR) expression, females had slightly higher UGT1A7 expression, and expression of UGT1A6 was comparable between the sexes. Despite these differences in UGT protein expression, renal microsomes from male and female Sprague-Dawley rats have been shown previously to have similar mycophenolate glucuronidation activities (1.6 versus 1.3 nmol/min/mg for male and females, respectively) (Stern et al., 2007). This lack of gender differences in mycophenolate glucuronidation activity may be attributable to offsetting changes in two or more UGTs able to catalyze mycophenolic acid glucuronidation, e.g., females have higher renal microsomal UGT1A7 but lower UGT1A1 levels. Although these data clearly support the androgen regulation of renal UGT1A1 in the rat, indirect effects of testosterone on UGT1A1 expression could also explain the resulting data.

In mice, sex differences in mRNA expression of several UGT isoforms, including UGT1A1, have been observed (Buckley and Klaassen, 2007). In the rat, however, no marked sex differences in transcription of any UGT isoforms were noted, although UGT1A1 mRNA was found to be ubiquitously identified in all major tissues, including kidney (Shelby et al., 2003). Nonetheless, several UGTs in the rat have been shown to undergo androgen-regulated transcription in vitro. For example, transcription of UGT1A1 and 1A6 mRNA in rat Sertoli cells is up-regulated in response to testosterone treatment (Magnani et al., 2000). Additionally, there are many examples of sex differences in rat glucuronidation, such as for mycophenolic acid glucuronidation in vivo (Stern et al., 2007), phenol red glucuronidation in vivo (Hart et al., 1969), p-nitrophe- nol glucuronidation in vivo and in renal microsomes (Russell et al., 1983), and 17β-estradiol in hepatic microsomes (Rao et al., 1977). In some cases these sex differences may be due to factors other than UGT isoform transcription, such as differences in post-translational UGT modification, substrate absorption, co-substrate abundance, competing metabolic pathways, or metabolite excretion. Human UGTs have also been shown to undergo androgen regulation. Experiments in human prostate epithelial cells also have demonstrated androgen-regulated expression of UGT2B11 and 2B15 (Chouinard et al., 2006). There are reasons to believe that rat and human UGT1A1 may be similarly regulated, as has been shown for aryl hydrocarbon-mediated regulation of UGT1A1 in rat and human liver (Munzel et al., 1994; Yueh et al., 2003).

Rat and human UGT1A1 are considered orthologous enzymes, sharing a high degree of sequence homology and substrate specificity (King et al., 1996). Rat and human UGT1A1 have both been shown to glucuronidate a wide range of substrates, including anthraquinolines, coumarins, estrogens, flavonoids, and phenolic and opioid compounds (King et al., 1996). The most clinically significant substrate of human UGT1A1, bilirubin, is also glucuronidated by rat UGT1A1, with similar high affinity (low micromolar concentration) (King et al., 1996). Despite these similarities, the tissue distribution of human and rat UGT1A1 appears to be divergent.

**Results and Discussion**

Testosterone treatment of female rats resulted in increased renal UGT1A1 and UGT1A common region expression (Fig. 1A) and
Although UGT 1A1 mRNA was found in a wide variety of rat tissues (Shelby et al., 2003), studies evaluating human UGT1A1 mRNA report limited distribution and are inconsistent. All studies reported human UGT1A1 mRNA transcript in the liver and gastrointestinal tract, and reports of kidney transcript were mixed (McDonnell et al., 1996; Strassburg et al., 1997, 1998; King et al., 1999; Valle et al., 2001). Because the sexes of the human tissues used in the studies evaluating renal UGT1A1 mRNA expression were not identified (King et al., 1999; Valle et al., 2001), there is the potential that inconsistencies in study results could be explained by an androgen-regulated expression pattern. However, a study evaluating human UGT1A1 glucuronidation using male renal microsomes did not observe UGT1A1 bilirubin glucuronidation activity in any of the samples tested (McGurk et al., 1998). This is in agreement with the study of Sutherland et al. (1993), who also were unable to detect microsom al bilirubin glucuronidation activity in human kidney samples. In contrast, a separate study found human UGT1A1 estradiol-3-glucuronidation activity in kidney tissue (Fisher et al., 2000), and a recent study demonstrated human UGT 1A1 immunoreactivity in primary proximal tubular cells (Lash et al., 2008). Clearly, there are significant discrepancies regarding renal UGT1A1 expression in man.

Androgen-regulated expression of renal rat UGT1A1 may result in important sex-related differences in renal metabolism. Previous studies have posited that human UGTs may play a role in renal homeostasis by suppressing responses to endogenous mediators, including eicosanoids and aldosterone, based on localization of these enzymes in the macula densa, medullary collecting ducts, and loop of Henle (Knights et al., 2005; Gaganis et al., 2007). For example, prostaglandin E2 and 20-hydroxyeicosatetraenoic acid have important vasodilatory and vasoconstrictive activities in the kidney (Hao and Breyer, 2007), respectively, and both of these eicosanoids, including arachidonic acid itself, are metabolized by human UGT1A1 (Little et al., 2004). The renal UGT enzymes may also mediate drug-induced toxicities by formation of reactive metabolites, namely acyl glucuronides (Kuehl et al., 2006; Dickinson et al., 1994; Liu et al., 1996) or by protective conjugative metabolism. Because renal UGT metabolism can have these potential influences on renal physiology and xenobiotic disposition, it is important to thoroughly characterize the mechanisms of renal enzyme expression.

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