ABSTRACT:

Egualen sodium (sodium 3-ethyl-7-isopropyl-1-azulenesulfonate 1/3 hydrate) is a new antiulcer drug. There has been no difference observed in absorption between male and female rats, the relative amount of metabolites in male plasma has been higher than that in females, and the excretion ratios of metabolites in males have been significantly higher than those in females. However, the plasma concentration profile of total radioactivity in males has been higher than that in females. To clarify this discrepancy, the renal clearances and plasma concentrations of the unchanged drug and its metabolites were determined. The renal clearance of the unchanged drug in males was 21 times lower than that in females, and the urinary excretions in males and females were 2.1 and 39.5% of dose, respectively. This indicates that the major factor in the sex-related difference observed in the plasma concentration of total radioactivity is due to the difference in the renal clearance of the unchanged drug between the sexes. The results of treatments with probenecid in normal and gonadectomized rats revealed that egualen sodium was mainly excreted into urine by secretion through the renal tubule. Furthermore, the results of treatments with testosterone in rats revealed that the excretion of egualen sodium was highly affected by androgens. These facts indicated that the sex-related difference observed in the plasma concentration of total radioactivity can be attributed to the inhibition of renal tubular secretion of the unchanged drug by androgens. This is the first example of sex-related differences in both metabolism and excretion.

SEX-RELATED DIFFERENCES IN URINARY EXCRETION OF EGUALEN SODIUM IN RATS

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A number of reports have been published on the sex-related differences in drug pharmacokinetics that are found extensively in both phase I and II metabolism. The cytochrome P-450s of male and female rats have been purified and their regulation mechanism has been investigated in detail (Kato, 1974; Kato and Kamataki, 1982; Kato and Yamazoe, 1992). There were only four known compounds that exhibit a sex difference in excretion: γ-aminobutyric acid (Riggs and Walder, 1963); perfluoro-octanoate (Jannsen et al., 1976); 1-aminocyclohexanecarboxylic acid, which is a major metabolite of cyclacillin, a semisynthetic penicillin (Hanijarvi et al., 1982); and zenarestat, which is an aldose reductase inhibitor (Tanaka et al., 1991a). These compounds were not metabolized, except for zenarestat. However, zenarestat was not observed to determine a sex-related difference in metabolism.

Egualen sodium (Fig. 1) was synthesized in our laboratories as a drug candidate (Yanagisawa et al., 1988) that exhibits a potent antiulcer activity (Sekiguchi et al., 1986) and has an excellent therapeutic effect in clinical phase III trials (Miyoshi et al., 1990). The pharmacokinetics of this drug have been investigated in mice, rats, dogs, and humans (Ebihara et al., 1990; Sato et al., 1990a,b). The drug was mainly excreted into urine in each species; however, the urinary excretion of metabolites was negligible in mice, dogs, and humans. In rats, there was a marked sex-related difference in the excretion ratios of the unchanged drug and the metabolites. The relative amount of metabolites in male plasma was higher than that in female plasma at a monitoring point, and the excretion ratios of metabolites in males were significantly higher than those in females (Sato et al., 1990a). In general, metabolites are easily eliminated from the plasma into the urine or bile compared with the unchanged drug. For [14C]egualen sodium, therefore, it seems plausible that the plasma concentration of total radioactivity in males should be lower than that in females after oral administration. However, the plasma concentration profile of total radioactivity in males was higher than that in females, despite there being no sex-related differences in absorption, protein binding, and biliary excretion (Sato et al., 1990a,b,c). Thus, we report that the sex-related difference observed in the plasma concentration of total radioactivity is attributable to the sex-related difference in the excretion process (Sato et al., 1990a). To clarify the mechanism, we assayed the plasma concentration profiles and excursions of total radioactivity for the unchanged drug and its major metabolites sodium 3-(2-hydroxyethyl-7-isopropyl-1-azulenesulfonate 1/3 hydrate (M1) and sodium 3-ethyl-7-(2-hydroxy-1-methylethyl)-1-azulenesulfonate 1/3 hydrate (M2) (Fig. 1).

Thus, in the present study, we determined the renal clearances of egualen sodium and its metabolites with the infusion method and the plasma concentration profiles after oral administration of egualen sodium in rats. Furthermore, we investigated the effect of sex-related hormones on the urinary excretion of egualen sodium and its metabolites in rats.

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2 Abbreviations used are: M1, sodium 3-(2-hydroxyethyl-7-isopropyl-1-azulenesulfonate 1/3 hydrate; M2, sodium 3-ethyl-7-(2-hydroxy-1-methylethyl)-1-azulenesulfonate 1/3 hydrate; RID, radioisotope detector; AUC, area under the curve; GFR, glomerular filtration rate.
Materials and Methods

Chemicals. [14C]Egalen sodium (Fig. 1) was synthesized in our laboratories (Shimada et al., 1994). The specific activity was 1.98 GBq/mmol (6.47 MBq/mg), and the radiochemical purity was found to be 99.9%. The labeled compound was used after diluting with the unlabeled compounds. 14C-labeled compounds of metabolites M1 and M2 were purified and isolated from male rat urine after oral administration of [14C]Egalen sodium (20 mg/67 µmol/7.41 MBq/kg) as previously described (Sato et al., 1990a), and were used after diluting with the corresponding unlabeled compounds. The radiochemical purity of each metabolite was found to be >95.9%. The specific activities were 17.0 MBq/mmol (53.9 kBq/mg) for M1 and 21.8 MBq/mmol (69.1 kBq/mg) for M2. [3H]Inulin was purchased from Amersham (Little Chalfont, UK). The specific activity was 470 GBq/mmol (9.03 MBq/mg) and its radiochemical purity was 97.2%. Each labeled compound was used after diluting with the corresponding unlabeled compounds.

Animals. Male and female Wistar rats were purchased from NRC Haruna Co., Ltd. (Agatsuma, Japan). Rats were used at 10 to 12 weeks old (males, 322–384 g; females, 278–340 g) for renal clearance and at 7 weeks old (males, 170 g; females, 150 g) for the standard method (Jannsen et al., 1976), the glomerular filtration rate (GFR) calculation, and the urinary excretions (0–24 h) were 57.4% of the dose for [14C]Egalen sodium. The specific activity was 1.98 GBq/mmol (6.47 MBq/mg). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h after administration. The heparinized blood was centrifuged for 10 min at 3000 rpm at 4°C and plasma was obtained. The plasma was divided into two samples; one was used to determine total radioactivity, and the other was analyzed for unchanged drug and metabolite concentrations from the urinary and plasma concentrations. Furthermore, the renal clearance of total radioactivity, unchanged drug, and metabolites were calculated from the urinary and plasma concentrations. The radioactivity in the plasma as found to be 43.2 µg eq/ml for males and 353 µg eq/ml for females, respectively. The AUC was 540 µg eq·h/ml for males and 353 µg eq·h/ml for females. Although there was no significant difference in the Cmax between the sexes, there was a significant difference in AUC between the sexes (P < .05; Fig. 2). In contrast, from the previously described results (Sato et al., 1990a), the urinary excretions (0–24 h) were 57.4% of the dose for males and 70.4% of the dose for females. The urinary excretion in males was significantly lower than that in females (P < .01). After i.v. constant infusion dosing of [14C]Egalen sodium to rats at the rate of 4 µg/min, the renal clearances were 0.139 ml/min/kg for males and 0.358 ml/min/kg for females. The ratio of males to females was 1:2.6.
and the renal clearance of males was significantly lower than that of females ($P < .05$; Fig. 2).

**Unchanged Drug in Infusion and Oral Administration Study.** After oral dosing of 20 mg/kg $[^{14}C]$egualen sodium to rats, the $C_{\text{max}}$ of the unchanged drug in the plasma was 34.2 $\mu$g eq/ml for males and 36.7 $\mu$g eq/ml for females. The AUC was 397 $\mu$g eq $\cdot$ h/ml for males and 326 $\mu$g eq $\cdot$ h/ml for females, respectively. There were no significant differences in the $C_{\text{max}}$ and AUC of the sexes (Fig. 3). However, from the previously described results (Sato et al., 1990a), the urinary excretion of the unchanged drug (0–24 h) was 2.1% of the dose for males and 39.5% of the dose for females. The urinary excretion in males was significantly lower than that in females ($P < .01$). After i.v. constant infusion dosing of $[^{14}C]$egualen sodium to rats at the rate of 4 $\mu$g/min, the renal clearances were 0.009 ml/min/kg for males and 0.193 ml/min/kg for females. The ratio of males to females was 1:21 and the renal clearance of males was greatly significantly lower than that of females ($P < .01$) (Fig. 3).

**Total Metabolites in Infusion and Oral Administration Study.** After oral dosing of $[^{14}C]$egualen sodium to rats at 20 mg/kg, the $C_{\text{max}}$ of total metabolites in the plasma was 11.3 $\mu$g eq/ml for males and 2.6 $\mu$g eq/ml for females. The AUC was 143 $\mu$g eq $\cdot$ h/ml for males and 27 $\mu$g eq $\cdot$ h/ml for females. There were significant differences in the $C_{\text{max}}$ and AUC values between the sexes ($P < .01$) (Fig. 4). From previously described results (Sato et al., 1990a), the urinary excretions (0–24 h) were 52.2% of the dose for males and 29.9% of the dose for females. Contrary to our expectation, the urinary excretion in males was significantly higher than that in females ($P < .01$). However, after i.v. constant infusion dosing of $[^{14}C]$egualen sodium to rats at a rate of 2 $\mu$g/min, the renal clearances were 1.490 ml/min/kg for males and 2.469 ml/min/kg for females. The ratio of males to females was 1:1.7. As for total radioactivity and the unchanged drug, the renal clearance of the metabolites in males was significantly lower than that in females ($P < .01$) (Fig. 4).

**M1 in Infusion and Oral Administration Study.** After oral dosing of $[^{14}C]$egualen sodium to rats at 20 mg/kg, the $C_{\text{max}}$ of M1 in the plasma was 3.1 $\mu$g eq/ml for males and 0.8 $\mu$g eq/ml for females. The $C_{\text{max}}$ in males was significantly higher than that in females ($P < .05$) (Fig. 5). The AUC was 37 $\mu$g eq $\cdot$ h/ml for males and 8 $\mu$g eq $\cdot$ h/ml for females. The AUC in males also was significantly higher than that in females ($P < .01$). Sato et al. (1990a) found that the urinary excretions (0–24 h) of M1 were 26.4% of the dose for males and 15.6% of the dose for females. The urinary excretion of M1 in males was significantly higher than that in females ($P < .01$). After equimolar i.v. constant infusion dosing of $[^{14}C]$M1 and M2 to rats at a rate of 2 $\mu$g/min, the renal clearances of M1 were 0.813 ml/min/kg for males and 1.248 ml/min/kg for females. The ratio of males to females was 1:1.5. Similar to findings for total radioactivity, unchanged drug, and total metabolites, the renal clearance in males was significantly lower than that in females ($P < .05$).

**M2 Infusion and Oral Administration Study.** The pharmacokinetics profile of M2 was similar to that of M1. After oral dosing of $[^{14}C]$egualen sodium to rats at a dose of 20 mg/kg, the $C_{\text{max}}$ of M2 was 7.6 $\mu$g eq/ml for males and 1.6 $\mu$g eq/ml for females. The AUC in males was significantly higher than that in females ($P < .01$) (Fig. 5). The AUC was 98 $\mu$g eq $\cdot$ h/ml for males and 15 $\mu$g eq $\cdot$ h/ml for females. The AUC in males also was significantly higher than that in females ($P < .01$). From previous results (Sato et al., 1990a), the urinary excretions (0–24 h) of M2 were 22.5% of the dose for males and 12.2% of the dose for females. The urinary excretion of M2 in males was significantly higher than that in females ($P < .01$). After equimolar i.v. constant infusion dosing of $[^{14}C]$M1 and M2 to rats at a rate of 2 $\mu$g/min, the renal clearances of M2 were 0.677 ml/min/kg for males and 1.089 ml/min/kg for females. The ratio of males to females was 1:1.6. The renal clearance in males was significantly lower than that in females ($P < .05$).

**M1 and M2 in Both Sexes.** In both sexes, the plasma concentrations of M2 were higher than those of M1 (Fig. 5). These results reflected well in the renal clearance in both sexes. In both metabolites, the urinary excretions of M1 and M2 in males were significantly higher than those in females ($P < .01$), but the renal clearances of M1 and M2 in males were significantly lower than those in females ($P < .05$). These results reflected the difference in plasma concentrations of the metabolites between the sexes.

**Excretion Route in Infusion Study.** The renal clearances of the unchanged drug were determined by administration of probenecid and $[^{14}C]$egualen sodium to males and females to confirm the pathway of urinary excretion. Renal clearance was significantly inhibited by the treatment with probenecid ($P < .01$) in females with high renal clearance (Table 1). In the males with low renal clearance, the renal clearance of unchanged drug was increased from 0.009 to 0.031 ml/min/kg by the probenecid treatment, although there was no statistical significance (Table 1).

**Inhibition Factors in Tubular Secretion in Infusion Study.** The renal clearances of the unchanged drug were measured with gonadec-
tomized males and females to examine the effect of sex hormones on tubular secretion. There was no significant differences in the renal clearances of the unchanged drug between nontreated and gonadectomized females (Table 2). In contrast, the renal clearance of the unchanged drug in males was significantly increased by the gonadectomy ($P < .05$) equal to that in nontreated females (Table 2).

In gonadectomized males, there was no significant difference in the renal clearance of unchanged drug between probenecid treatment and nontreatment. However, renal clearance was decreased from 0.158 to 0.055 ml/min/kg by the probenecid treatment (Table 3) as well as that in nontreatment females (Table 1). Therefore, it was confirmed that the unchanged drug was excreted through the renal tubular secretion. Furthermore, testosterone treatment significantly inhibited the renal tubular secretion of the unchanged drug in gonadectomized males and females ($P < .05$) (Table 3).

**Discussion**

For egualen sodium, there has been no difference in absorption between male and female rats, the relative amount of metabolites in male plasma has been higher than that in females at a monitoring point, and the excretion ratios of metabolites in males have been significantly higher than those in females (Sato et al., 1990a). However, the plasma concentration profile of total radioactivity in males has been higher than that in females. To clarify this apparent discrepancy, the renal clearances and plasma profiles of the unchanged drug and its metabolites were determined.

In the study of the sex-related differences in excretion of an unmetabolized drug, the use of conventional methods may be enough to simply measure the total radioactivity of the labeled compound. However, for sex-related differences in excretion of a drug being metabolized, it is necessary to use a method suitable for quantifying the unchanged drug and its metabolites separately, as well as total radioactivity.

We have found a sex-related difference in metabolism in the plasma level after oral dosing of $[^{14}C]\text{egualen}$ sodium in rats (Sato et al., 1990a). In the present study, we used a specific and highly sensitive method of RID-HPLC that enabled us to quantify the unchanged drug and its metabolites in plasma and urine separately. In addition, $[^{14}C]\text{M1}$ and $[^{14}C]\text{M2}$ obtained from male rat urine after oral dosing of $[^{14}C]\text{egualen}$ sodium with the previous method (Sato et al., 1990a) were used for the determination of renal clearances.

Regarding the relationship between urinary excretion and renal
clearance of total radioactivity, we clarified that the AUC of total radioactivity in male rat plasma was significantly higher than that in females, and that renal clearance of total radioactivity in males was significantly lower than that in females (Fig. 2). Furthermore, the renal clearances of the unchanged drug in males was markedly lower than that in females by a factor of 21. In addition, from the previously described results of Sato et al. (1990a), the urinary excretions of total radioactivity were 57.4% of the dose for males and 70.4% of the dose for females, and the excretions of the unchanged drug were 2.1% of the dose for males and 39.5% of the dose for females. The above-mentioned findings strongly suggest that the difference in excretion between the sexes is due to the difference in renal clearance between the sexes (Fig. 3).

Despite the fact that there was no significant difference in absorption between the sexes (Sato et al., 1990a), the plasma concentration of the total radioactivity in males was higher than that in females. In addition, the relative amount of metabolite in male plasma also was higher than that in females, although in general, the metabolite is more easily eliminated from the plasma to urine or bile compared with the unchanged drug. This fact suggests a sex-related difference in the renal clearance. Thus, the remarkably low renal clearance of the unchanged drug in males results in the reduction of its urinary excre-
Thus, the reabsorption of the unchanged drug may be inhibited by probenecid in males, although there was no significant difference. Secretion through the renal tubule. However, the renal clearance of total metabolites in males was higher than that in females. Each plasma concentration profile of total metabolites in males was significantly lower than that in females. Therefore, the assumption would be that the plasma concentration of total metabolites in males was lower than that of females. However, the plasma concentration profile of total metabolites in males was higher than that in females (Fig. 4). Thus, this unexpected finding may be due to the low renal clearance of total metabolites of males, as well as that of the unchanged drug.

To clarify the difference between the metabolites, the plasma concentration, urinary excretion, and renal clearances of total metabolites were compared between the sexes (Fig. 4). Despite the lack of a significant sex-related difference in the absorption of the unchanged drug, the quantity of total urinary metabolites in males was larger than that in females due to a typical sex-related difference in metabolism. Therefore, the assumption would be that the plasma concentration of total metabolites of males would be lower than that of females. However, the plasma concentration profile of total metabolites in males was higher than that in females (Fig. 4). This, thus unexpected finding may be due to the low renal clearance of total metabolites of males, as well as that of the unchanged drug.

Moreover, to clarify the difference, the plasma concentrations, urinary excretions, and renal clearances of total metabolites were compared between the sexes (Fig. 4). Despite the lack of a significant sex-related difference in the absorption of the unchanged drug, the quantity of total urinary metabolites in males was larger than that in females due to a typical sex-related difference in metabolism. Therefore, the assumption would be that the plasma concentration of total metabolites of males would be lower than that of females. However, the plasma concentration profile of total metabolites in males was higher than that in females (Fig. 4). Thus, this unexpected finding may be due to the low renal clearance of total metabolites of males, as well as that of the unchanged drug.

To clarify the difference between the metabolites, the plasma concentration, urinary excretion, and renal clearance of the major metabolites M1 and M2 were compared (Fig. 5). In both sexes, the plasma concentration of M2 was higher than that of M1, and the urinary excretion of M2 was lower than that of M1. For the sex-related differences, the urinary excretions of M1 and M2 in males were significantly higher than those in females, whereas the renal clearance in males was significantly lower than that in females. Each plasma concentration of M1 and M2 was well reflected in the renal clearance. The above-mentioned results indicated that the sex-related difference in the plasma profiles was due to the renal clearances of unchanged drug and metabolites.

We also investigated the effect of sex-related hormones on the urinary excretion of equalen sodium and its metabolites in rats. In females with high renal clearance, the renal clearance of the unchanged drug was significantly decreased by treatment with probenecid, an inhibitor of active secretion in the renal tubule (Table 1). These results suggest that equalen sodium is mainly excreted by secretion through the renal tubule. Then, the renal clearance of the unchanged drug was measured after gonadectomy in males to confirm the results. As expected, the renal clearance of gonadectomized male rats was significantly lower than that of nontreated males and comparable to nontreated females (Table 2). In addition, treatment with probenecid significantly inhibited secretion in the gonadectomized males (Table 3) equal to secretion in nontreated females (Table 1). These findings support the idea that equalen sodium is mainly excreted by secretion through the renal tubule. However, the renal clearance of the unchanged drug was increased by treatment with probenecid in males, although there was no significant difference. Bowman and Raud (1980) reported that probenecid blocks the active reabsorption of uric acid in the proximal tubule in sufficient dosage. Thus, the reabsorption of the unchanged drug may be inhibited by treatment with probenecid in the same manner as uric acid, but this hypothesis still needs to be confirmed.

Next, we examined sex hormones as a factor that inhibits this secretion. The renal clearance of the unchanged drug was measured after gonadectomy was carried out in both sexes. The treatment with testosterone in gonadectomized males decreased their renal clearance of equalen sodium (Table 3). This result indicates that the excretion of equalen sodium was highly affected by androgens. The hormonal regulation of cytochrome P-450-dependent metabolism among testes, ovaries, hypothalamus, pituitary gland, and liver was reported in rats (Mugford and Kedderis, 1998). We suggest that the hormonal regulation system in renal tubular secretion among kidney and other organs needs to be investigated to clarify whether androgens directly affect the receptor in kidney or not.

In conclusion, the sex difference observed in the plasma concentrations of total radioactivity after oral dosing of equalen sodium in rats is attributed to the inhibition of renal tubular secretion, which is regulated by androgens. In addition, the high plasma concentration of total metabolites as well as the high urinary excretion of the metabolites in males indicate the occurrence of a sex difference in metabolism. This is the first example of sex-related differences in both excretion and metabolism. It is suggested that enhancement of the metabolism in males after oral dosing of equalen sodium may compensate for the decreased renal clearance of the unchanged drug with androgens.

For other actively secreted anionic drugs like equalen sodium, the sex-related differences in renal clearance may be common in rats due to the regulation of androgens. However, the difference in renal clearance may be masked by extensive metabolism. Furthermore, the magnitude of the differences in humans may be much less pronounced than that in rats due to large individual variations. The detailed mechanisms involved in the hormonal regulation system remain to be investigated further.

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