Gender Differences in the Antidiarrheal Effect of Zaldaride Maleate in Rats

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The amelioration of secretory diarrhea has been reported after the administration of zaldaride maleate (ZAL), a selective calmodulin inhibitor, to male rodents. In this study, the antidiarrheal effect of ZAL in female rats was compared with that in male rats. In female and male rats, ZAL significantly ameliorated 16,16-dimethyl prostaglandin E₂-induced diarrhea at doses of 1 and 3 mg/kg (p.o.), respectively, with ID₅₀ values of 0.7 mg/kg (p.o.) in the females and 10.3 mg/kg (p.o.) in males. In castor oil-induced diarrhea, ZAL also significantly reduced the incidence of diarrhea in female and male rats at doses of 10 and 30 mg/kg (p.o.), respectively. When the same dose of ZAL was given orally to female and male rats, the maximum plasma level of this compound was approximately 3 times higher in female rats than in male rats. In contrast, after intravenous administration of the same dose of ZAL to female and male rats, the total clearance of this compound was similar. In an Ussing chamber experiment, the inhibitory action of ZAL on vasoactive intestinal polypeptide-induced ion secretion in the colon showed no difference between female and male rats. In conclusion, the antidiarrheal effect of ZAL in female rats is more potent than that in males, and could be due to the difference in plasma levels of this compound between female and male rats after oral administration.

Key words zaldaride maleate; gender; diarrhea; castor oil; prostaglandin E₂

Gender differences in the potency of certain drugs such as barbiturates and strychnine are well known. Zaldaride maleate (ZAL), 1,3-dihydro-1-[(4-methyl-1H,6H-pyrrrolo-[1,2-a][4,1]-benzoxazepine-4-yl)methyl]-4-piperinyl]-2H-benzimidazol-2-one-maleate, is a highly selective and potent inhibitor of calmodulin (CaM), and has been reported to ameliorate secretory diarrhea in male rodents. Its effects have not been reported in female rodents. For clinical development, it is necessary to clarify if gender affects the antidiarrheal efficacy of ZAL. In this study, we compared the antidiarrheal effect of ZAL in female and male rats using two different secretory diarrhea models. In addition, the antisecretory action of this compound was studied in colon excised from female and male rats using an Ussing chamber.

MATERIALS AND METHODS

Experimental Animals Female and male Sprague-Dawley rats (Charles River, Atsugi, Japan), 8—9 weeks old, were used in the present study. They were housed in a controlled environment at 22—24°C and 50—60% humidity with light from 7:00 a.m. to 7:00 p.m. Commercial rat chow and water were provided prior to the studies. The present studies were conducted in compliance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the experimental protocols were approved by the Ethical Committee of the Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.

Materials ZAL was a gift from Novartis Consumer Health, Inc. (Nyon, Switzerland). Castor oil and vasoactive intestinal polypeptide (VIP) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and 16,16-dimethyl prostaglandin E₂ (dmpPGE₂) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of analytical grade. ZAL was dissolved in dimethylsulfoxide or in distilled water containing 20% (v/v) polyethylene glycol 400 and was suspended in distilled water containing 0.2% (w/v) methyl cellulose 400. dmpPGE₂ was diluted in saline solution. ZAL was administered orally to rats at a volume of 5 ml/kg or was applied to the bath perfusion solution at a volume of 1 ml/l. The vehicle was administered or added at the same volume.

Methods dmpPGE₂-Induced Diarrhea: The dmpPGE₂ diarrhea model has been described elsewhere. Non-fasted female and male rats were screened to exclude animals with preexisting diarrhea, and chosen were placed in individual cages for 3 h. ZAL or vehicle was administered to male and female rats. One hour later, dmpPGE₂ (500 µg/kg, i.p.) was administered to the rats. After 1 h, the rats were inspected for the presence or absence of diarrhea, and the evacuated fecal pellets were scored according to an arbitrary scoring criteria as follows: hard stool or no defecation, score 0; ordinary stool, score 1; wet but formed stool, score 2; unformed stool, score 3; severe watery stool, score 4. The average of scores was defined as the diarrhea index, and the ID₅₀ value for diarrhea was calculated.

Castor Oil-Induced Diarrhea: The castor oil diarrhea model was implemented according to the previously described method. Female and male rats were fasted for 18—20 h prior to the experiments but had free access to drinking water. On the day of the experiments, they were screened to exclude animals with preexisting diarrhea, and were acclimated in individual cages for 1—2 h. ZAL or vehicle was administered to rats 1 h before the oral administration of castor oil (10 ml/kg). After 1 h, the cages for each rat were inspected for the presence or absence of diarrhea.

Ussing Chamber Experiments: In the Ussing chamber experiments, non-fasted female and male rats were used. Animals were sacrificed by cervical dislocation, and the distal colon was removed carefully without stretching. Mucosal preparations were obtained by stripping away the serosa and smooth muscle of the distal colon. Mucosal preparations were mounted in Ussing chambers (surface area 0.693 cm²), and 10 ml of Kreb's Henseleit solution was added to each

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side of the preparations. The composition of this physiological solution was as follows (mmol/l): NaCl 119.0; KCl 4.7; MgSO₄·7H₂O 1.2; KH₂PO₄ 1.2; CaCl₂·2H₂O 1.8; NaHCO₃ 24.9, glucose 11.1 and ascorbic acid 0.05 (pH 7.4). This solution was maintained at 37°C and gassed with carbogen (5% CO₂ in 95% O₂). The change in short-circuit current (A Ir), an indicator of chloride ion secretion, was measured continuously as a response to added VIP. After a 30-min equilibration period, ZAL or vehicle was added to the bath on the serosal side of the preparation, and 30 min later, VIP (30 nmol/l) was applied to the serosal side of the preparation.

Plasma Concentration: Male and female rats were fasted for 18–24 h but had free access to water prior to the experiments. ZAL was administered to female and male rats at doses of 1, 3, 10 and 30 mg/kg (p.o.) or 1 mg/kg (i.v.), and venous blood samples were collected under light anesthesia at the following times: 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 30 and 48 h. The concentration of ZAL was measured by high-performance liquid chromatography with a fluorescence detector (Ex. 280 nm, Em. 310 nm), and the area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The total plasma clearance was calculated by dividing the dose by the AUC. Bioavailability (BA) was calculated by the following formula:

\[ BA = \frac{[AUC_{p.o.}/dose (p.o.)]}{[AUC_{i.v.}/dose (i.v.)]} \]

**Statistical Analysis** Data for the castor oil diarrhea experiments are expressed as the ratio of the number of animals with diarrhea to the total number of 10 animals for each group. Statistical significance between the vehicle-treated group and the test compound-treated groups was analyzed using Fisher’s exact test. The data for the dmPGF₂α diarrhea experiments are shown as the mean±S.E.M. of 10 animals for the groups. Statistical significance between the vehicle-treated group and the test compound-treated groups was analyzed using the Kruskal–Wallis test followed by the Steel test. The ID₅₀ values were calculated by the Probit (L) method. The data from the Ussing chamber experiments are expressed as the mean±S.E.M. of 6 experiments for the groups in the experiment. Statistical significance between the vehicle-treated group and the test compound-treated groups was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s test. A p-value of less than 0.05 is regarded as statistically significant and was denoted by an asterisk.

**RESULTS**

ZAL at doses of 1 mg/kg and higher (p.o.) significantly improved dmPGF₂α-induced diarrhea in female rats (Fig. 1), whereas doses of 3 mg/kg and higher (p.o.) significantly ameliorated the dmPGF₂α-induced effect in male rats (Fig. 1). The ID₅₀ value for female rats was 1 mg/kg (p.o.) and, for male rats was 10 mg/kg (p.o.) (Table 1). In the vehicle group, the diarrheal action of dmPGF₂α showed no difference between female and male rats (Fig. 1).

In castor oil-induced diarrhea, ZAL at doses of 10 mg/kg and higher (p.o.) significantly inhibited the incidence of diarrhea in female rats (Fig. 2). In male rats a dose of 30 mg/kg ZAL significantly inhibited the onset of diarrhea (Fig. 2). The ID₅₀ values for female and male rats were not calculated.

![Fig. 1. Effect of ZAL on dmPGF₂α-Induced Diarrhea in Female and Male Rats](image1)

Each column represents the mean±S.E.M. of 10 animals. □, female rats; ■, male rats. *p<0.05, **p<0.01; statistically significant vs. the value of the vehicle control group. N.S.: not significant.

![Fig. 2. Effect of ZAL on Castor Oil-Induced Diarrhea in Female and Male Rats](image2)

Each column represents the ratio of rats with diarrhea to 10 animals in each group. Ten animals were used in each group. □, female rats; ■, male rats. *p<0.05; statistically significant vs. the value of the vehicle control group. N.S.: not significant.

<table>
<thead>
<tr>
<th>Gender</th>
<th>ID₅₀ (mg/kg, p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0.7 (0.6–0.8)</td>
</tr>
<tr>
<td>Male</td>
<td>10.3 (9.2–11.5)</td>
</tr>
</tbody>
</table>

ID₅₀, 50% inhibitory dose; ( ), confidential limits.

In the vehicle group, the incidence of diarrhea induced by castor oil was no different between female and male rats (Fig. 2).

ZAL at a concentration of 30 μmol/l significantly decreased the VIP-induced increase in Iₑ in female rat colon, and at 10 μmol/l and higher significantly inhibited the VIP-induced effect in male rat colon (Fig. 3). In the vehicle group, the Iₑ response to VIP was no different between female and male rat colon (Fig. 3).

After intravenous administration of 1 mg/kg ZAL to fe-
male and male rats, the total plasma clearance of ZAL was similar: 2.33 l/h/kg, female; 2.45 l/h/kg, male (Table 2). In male rats, orally delivered ZAL at doses of 1, 3 and 10 mg/kg reached a maximum plasma level, and at 30 mg/kg, plasma levels up to 12 h decreased slowly compared with those of other doses. The values for BA at 1, 3 and 10 mg/kg were calculated to be 1.50, 2.32 and 6.45%, respectively (Table 3), and at 30 mg/kg it was not calculated because data did not allow the calculation of a half life. In female rats, ZAL given orally at doses of 1 and 3 mg/kg increased the plasma level rapidly, and reached a maximum. At doses of 10 and 30 mg/kg, the plasma levels from 0.5 to 6 h were essentially constant. The maximum concentrations of this compound in female rats were 3—4 times higher than those in males (Table 3). The BA values in female rats at doses of 1, 3, 10 and 30 mg/kg were 17.26, 17.73, 36.03 and 91.45%, respectively (Table 3).

**DISCUSSION**

In this study, the CaM inhibitor ZAL inhibited dmPGE₂ and castor oil-induced secretory diarrhea in female and male rats. CaM is localized in the intestinal tract and plays an important role in ion secretion in the intestinal mucosa. DmPGE₂, a stable analog of prostaglandin E₂, stimulates adenylyl cyclase activity through prostaglandin receptors, which then activate ion secretion and inhibit ion absorption in the intestinal tract. The activity of some adenylyl cyclase isoforms is regulated by CaM. We expect that ZAL inhibits dmPGE₂-induced diarrhea in male and female rats via the inhibition of adenylyl cyclase activity. Castor oil or its active ingredient, ricinoleic acid, causes diarrhea, which is induced by indomethacin, a cyclooxygenase inhibitor, and N⁶-nitro-L-arginine methyl ester, a nitric oxide synthase (NOS) inhibitor, in rats. It has been reported that castor oil-induced diarrhea is caused by the activation of constitutive NOS and the production of prostaglandins. CaM regulates arachidonic acid biosynthesis and constitutive NOS activity. This suggests that ZAL may inhibit castor oil-induced diarrhea by the inhibition of constitutive NOS activity and arachidonic acid biosynthesis, and in part, by the reduction of adenylyl cyclase activity.

ZAL may ameliorate secretory diarrhea without inhibiting gastrointestinal propulsive motility. In this study, the anti-secretory action of this compound in female and male rat colon was similar. However, based on the minimum effective dose of this compound on both dmPGE₂ and castor oil-induced diarrhea, the antidiarrheal effect of ZAL in female rats was approximately 3 times more potent than that in males. The maximum plasma concentrations of ZAL after oral administration to female rats at the same doses were also approximately 3 to 4 times higher than those in males, and the BAs in female rats were higher than those in males. We believed that the gender difference in the antidiarrheal effect of ZAL is related to the difference in plasma concentrations in female and male rats.

After intravenous administration of ZAL to female and male rats, the total plasma clearances were almost the same. Intravenous administration of ZAL did not alleviate secretory diarrhea in male rats. We suggest that ZAL may not act systemically, and that the difference in plasma concentrations of ZAL in female and male rats after oral administration is caused by a difference in the absorption ratio and metabolic rate during the absorption phase in intestinal tissue.

In conclusion, in at least one animal model, the antidiarrheal effect of ZAL in females is superior to that in males, and this may be related to the plasma concentrations and intestinal tissue accumulation of this compound. Further studies using other animals are required to determine more precisely whether the antidiarrheal effect of ZAL is pharmacologically different between females and males.

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**Table 2. Pharmacokinetic Parameters of ZAL after Intravenous Administration to Female and Male Rats**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Dose (mg/kg)</th>
<th>AUC&lt;sub&gt;0-12h&lt;/sub&gt; (ng·h/ml)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>Clearance (l/h/kg)</th>
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<tbody>
<tr>
<td>Female</td>
<td>1</td>
<td>430.0</td>
<td>1.3 (β)</td>
<td>2.33</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>407.6</td>
<td>0.1 (α)</td>
<td>2.45</td>
</tr>
</tbody>
</table>

Each value was calculated by the mean of 4 animals.

**Table 3. Pharmacokinetic Parameters of ZAL after Oral Administration to Female and Male Rats**

| Gender | Dose (mg/kg) | C<sub>max</sub> (ng/ml) | T<sub>max</sub> (h) | AUC<sub>0-12h</sub> (ng·h/ml) | T<sub>1/2</sub> (h) | BA (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
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<tr>
<td>Female</td>
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<td>0.5</td>
<td>17.3</td>
<td>31.0</td>
<td>0.7</td>
<td>7.26</td>
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<td></td>
<td>3</td>
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<td>93.4</td>
<td>216.7</td>
<td>2.1</td>
<td>17.73</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>557.0</td>
<td>1545.9</td>
<td>4.2</td>
<td>36.03</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>1696.4</td>
<td>15653.8</td>
<td>7.8</td>
<td>91.45</td>
</tr>
<tr>
<td>Male</td>
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<td>4.0</td>
<td>5.9</td>
<td>0.6</td>
<td>1.50</td>
</tr>
<tr>
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<td></td>
<td>0.5</td>
<td>28.0</td>
<td>1.3</td>
<td>2.32</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>187.4</td>
<td>262.9</td>
<td>1.4</td>
<td>6.45</td>
</tr>
</tbody>
</table>
|        | 10           |                          | 377.8               | 1652.4                        | 4.7               | N.C.

Each value was calculated by the mean of 4 animals. N.C., not calculable; T<sub>max</sub>, maximum concentration time; C<sub>max</sub>, maximum concentration; AUC, area under the curve; T<sub>1/2</sub>, half life; BAs, bioavailabilities.
REFERENCES