EFFECT OF TROGLITAZONE ON THE LIVER OF A GUNN RAT 
MODEL OF GENETIC ENZYME POLYMORPHISM

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ABSTRACT — There is a possibility that serious liver dysfunction rarely observed in diabetic patients given troglitazone is attributable to idiosyncratic abnormalities in liver drug-metabolism. In addition, the results of blood biochemical examinations in serious cases of liver dysfunction showed a tendency for a high level of total bilirubin (T-Bil) over a long period compared with other indicators of liver dysfunction. Thus, we focused on genetic variation of UDP-glucuronosyltransferases (UGTs) that are involved in the conjugation of troglitazone and bilirubin. In this study, Gunn rats, which are hereditarily deficient in the UGT1 family of UGT isozymes, and Wistar rats, the parent strain of Gunn rats, were treated with troglitazone for 3 months at dose levels of 0, 100 or 400 mg/kg to investigate two possibilities: first, whether the genetic deficiency in UGT1s induces an alteration of the metabolic profile of troglitazone followed by liver dysfunction, and second, whether the dosing of troglitazone to Gunn rats which show hyperbilirubinemia result in liver dysfunction. As a result, the metabolic profile of troglitazone in Gunn rats was much the same as that of Wistar rats, suggesting that genetic deficiencies in UGT1s did not influence the metabolic profile of troglitazone. Moreover, no elevation of blood biochemical parameters, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), or histopathological liver injuries, such as hepatocellular degeneration and necrosis, were observed in either strain of rats, and hyperbilirubinemia in Gunn rats was not aggravated by the dosing of troglitazone. These results strongly suggest that troglitazone was not metabolized by UGT1s but by other UGT isozyme (s) in rats, and that glucuronidation of troglitazone did not compete with glucuronidation of bilirubin in vivo. Thus, it is suggested that high levels of total bilirubin in patients with liver dysfunction induced by troglitazone are attributable to hypofunction due to hepatocellular injury, not to metabolic competition of bilirubin with troglitazone. Moreover, it is also suggested that the deficiency in the UGT1 family of UGT isozymes itself may not be the cause of liver dysfunction associated with troglitazone treatment.

KEY WORDS: Troglitazone, Liver dysfunction, Genetic polymorphism, Gunn rat, Hyperbilirubinemia, Toxicokinetics

INTRODUCTION

Troglitazone is a curative medicine for type II diabetes mellitus that improves hyperglycemia by the enhancement of insulin action (Iwamoto et al., 1991 and 1996; Horikoshi et al., 1994; Mimura et al., 1994; Kelly and Killian, 1998; Plosker and Faulds, 1999). This agent was put on the market in March 1997 in both Japan and the U.S.A. as an epoch-making new drug that improved hyperinsulinemia and hyperlipidemia as well as hyperglycemia through a different mechanism from that of previous orally effective antidiabetic agents. However, while troglitazone was being widely used in the clinical field, serious cases of liver dysfunction, the cause of which was suggested to be the dosing of the agent, were reported. Although representing only a very small percentage, cases of death were also reported (FDA Talk Paper, 1997).

So far, the mechanism of liver dysfunction induced by troglitazone has not been clarified. Kuramoto et al.
(1998) investigated the pathogenic pattern of the liver dysfunction, the improvement after withdrawal, and the cases of death. Consequently, they speculated that the liver dysfunction induced by troglitazone was attributable to idiosyncratic abnormalities in liver drug-metabolism. On the other hand, in a number of nonclinical safety studies on troglitazone performed prior to its approval for clinical use (Matsunuma et al., 1993a and 1992b; Kimura et al., 1993; Tanase et al., 1993; Tanase and Hirose, 1993; Sudo and Mori, 1993; Shinkai et al., 1993; Hirano et al., 1993; Mayfield et al., 1993), no symptoms of liver dysfunction, which was rarely reported in humans, were observed. Thus, we concluded that this liver dysfunction is idiosyncratic, as it is extremely difficult to reproduce in normal experimental animals (Watanabe et al., 1999). Our conclusion based on the results of the nonclinical safety studies was consistent with the speculation of Kuramoto et al. (1998) based on their clinical observations. This led us to perform safety studies on troglitazone using an animal model which showed genetic abnormalities in drug-metabolism.

When selecting an animal model, we paid attention to the following points: first, that the elevation of T-Bil was considered to be an indicator of a poor prognosis in human patients with liver dysfunction (Kuramoto et al., 1998); second, that bilirubin was demonstrated to be mainly metabolized by UGT1A1 in humans and rats (Roy-Chowdhury et al., 1987; Bosma et al., 1994); and third, that troglitazone was reported to be metabolized in both humans and experimental animals by sulfation, glucuronidation and oxidation to a quinone-type metabolite (Shibata et al., 1993; Horikoshi et al., 1994; Kawai et al., 1997; Izumi et al., 1997). Considering these points, we selected Gunn rats, which are hereditarily deficient in the UGT1 family of UGT isozymes (Gunn, 1938; Iyanagi et al., 1989; Iyanagi, 1991; Roy-Chowdhury et al., 1991; Sato et al., 1993), and Wistar rats, the parent strain of Gunn rats. In Gunn rats, it was demonstrated that the metabolic profile of acetaminophen which, like troglitazone, is metabolized by sulfation, glucuronidation and oxidation to a quinone-type metabolite, was different from that of Wistar rats, and due to this difference, liver injury induced by acetaminophen was more serious in Gunn rats than in Wistar rats (de Morais and Wells, 1988 and 1989). Therefore, in the present study, we investigated the possibility that the genetic deficiency in UGTs, which is considered to be involved in the conjugation of both troglitazone and bilirubin induces an alteration of the metabolic profile of troglitazone followed by liver dysfunction, as well as the possibility that the dosing of troglitazone to Gunn rats which show hyperbilirubinemia results in liver dysfunction.

**MATERIALS AND METHODS**

**Chemicals**

Troglitazone and its two metabolites, troglitazone sulfate and troglitazone glucuronide, were synthesized in our laboratories. The chemical structures of these compounds are shown in Fig. 1. A solid dispersion of troglitazone containing 60.1% of troglitazone was used for the administration.

**Test animals and housing conditions**

Homozygous Gunn rats, which are hereditarily deficient in UGT1s, and Wistar rats, the parent strain of Gunn rats, were used for the present study. Both strains of male and female rats, purchased from Japan SLC., Inc. (Shizuoka, Japan), were transferred to the animal room at 6 weeks of age. At the initiation of drug administration, rats were 7 weeks old, and their body weights ranged from 161 to 188 g in male Gunn rats,

![Fig. 1. Chemical structures of troglitazone and its metabolites.](image-url)
Toxicity study of troglitazone using Gunn rats.

115 to 133 g in female Gunn rats, 189 to 206 g in male Wistar rats, and 128 to 135 g in female Wistar rats. The rats were kept in a controlled environment at a room temperature of 23 ± 2°C and a humidity of 55 ± 10% with an illumination period of 13 hr (6:00 to 19:00) per day. All animals were housed individually in bracket cages and fed ad libitum with a solid feed (NMF: Oriental Yeast Co., Ltd., Tokyo, Japan) sterilized by radiation (irradiated with a 60Co-γ ray of 30 kGy) and tap water supplied through the nozzles of an automatic water-supplying apparatus.

**Study design**

For both strains of rats, three groups were prepared: one control group and two troglitazone treatment groups. All groups were composed of five subjects from each sex; thus 60 animals in total were used for the present study. The solid dispersion of troglitazone was suspended in 0.5% carboxymethylcellulose solution (5 mL/kg body weight of each dose was given) for oral administration of 100 and 400 mg/kg of troglitazone once a day for 3 months (94 days). Control animals were given the vehicle (5 mL/kg body weight of 0.5% carboxymethylcellulose solution) alone.

**General observations**

The first day of administration was defined as Day 1 in the present study. Mortality and clinical signs were monitored daily during the treatment period.

**Toxicokinetics (TK) measurement**

TK measurement was performed using four of five animals from each group. Blood samples (0.3 mL, each) were taken immediately before administration on Days 1 and 14, and then 1, 2, 4, 8 and 24 hr after administration on both days. On Day 94, blood samples were taken only 1 hr after administration. Blood samples were centrifuged at 10,000 rpm for 5 min at 4°C to prepare plasma samples. Then, 0.4 mL of ethanol containing the internal standard (9-acetylanthracene) was mixed with 0.1 mL of each plasma sample and centrifuged at 10,000 rpm for 10 min at 25°C to deproteinize. The resultant supernatants were injected into a fully-automated high-performance liquid chromatograph (HPLC) system (Waters 2690 Separation Module System, Waters Co., MA, U.S.A.). Plasma concentrations of troglitazone, its sulfate and glucuronide were determined, and finally, AUC0-24 was calculated from these concentrations. The quinone-type metabolite, an oxidative metabolite of troglitazone, was demonstrated to be formed by CYP3A4 and CYP2C8 in human liver microsomes (Yamazaki et al., 1999). However, even if troglitazone had been administered to phenobarbital-pretreated Wistar rats, the quinone-type metabolite was not detected (unpublished data). Therefore, we did not analyze for the quinone-type metabolite in this study. The HPLC conditions that were applied for this analysis were as follows: column, ERC-ODS1262 (100 mm × 6.0 mm I.D., S-5 μm, ERC INC., Saitama, Japan) + YMC Pack ODS-AM 302 (150 mm × 4.6 mm I.D., S-5 μm, YMC Co., Ltd., Kyoto, Japan); column temperature, 40°C; mobile phase, CH3CN/H2O/H3PO4 = 53/47/0.1; flow rate, 1.0 mL/min; injection volume, 50 μL; and UV-detector wavelength, 230 nm.

**Blood biochemical examination**

On the day of autopsy, all surviving animals were anesthetized with ether, and blood samples for the biochemical examination were collected from the abdominal aorta. These samples were kept at room temperature for 30 min, and then centrifuged at 3,000 rpm for 10 min to obtain serum samples. AST, ALT, alkaline phosphatase (ALP), T-Bil, and direct bilirubin (D-Bil) were determined using these serum samples with a Hitachi 7250 system (Hitachi, Ltd., Tokyo, Japan).

**Pathological examination**

At autopsy, macroscopic observations on organs and tissues were performed. Histopathological specimens of each organ and tissue were also prepared according to routine procedures. These specimens were stained with hematoxylin-eosin and observed under a light microscope.

**Statistical analysis**

The mean value and standard deviation in each group were calculated for TK parameters and blood biochemical parameters. For the blood biochemical parameters, statistical analysis was performed by the means described below. The Bartlett’s test (Bartlett, 1937) was performed to analyze the homogeneity of variance (p<0.01). Then, the data were further analyzed by one-way ANOVA (Tukey, 1949; Kramer, 1956) or Kruskal-Wallis’s test (Kruskal and Wallis, 1952), respectively (p<0.01). In the cases in which the significant differences were observed in the one-way ANOVA or Kruskal-Wallis’s test, the data were further analyzed by Dunnett’s test or Dunnett’s rank test (Dunnett, 1955 and 1964), respectively (p<0.01 and 0.05).
RESULTS

General conditions

Throughout the administration period, no abnormalities in general conditions were found in any but one of the Gunn rats. The exception was a male Gunn rat given 400 mg/kg, which showed a decrease in defecation from Day 77, exhibited an irregular respiratory rate from Day 83, and died on Day 89, without pathological changes attributable to troglitazone-dosing in any organs including the liver (the details are described in the results of the pathological examinations). Thus, it was diagnosed that this rat died of hyperbilirubinemia. On the other hand, no deaths or abnormalities in the general conditions were observed in any Wistar rats.

TK measurement

Plasma concentrations of troglitazone and its metabolites in both strains of rats treated orally with 400 mg/kg of troglitazone are shown in Figs. 2 (data on Day 1) and 3 (data on Day 14). Plasma concentrations of each compound at 1 hr after administration on Days 1, 14 and 94 are listed in Table 1. AUC_{0-24} of 400 mg/kg-treated groups are also presented in Table 2. Each TK data in the 100 mg/kg-treated groups was lower than those in the 400 mg/kg-treated groups on both days (data not shown).

Plasma concentrations of troglitazone on Day 1 in male Gunn rats were much the same as those in male Wistar rats (Fig. 2-a). However, in the females, the plasma concentrations at all sampling points were about 2 times higher in Gunn rats than in Wistar rats (Fig. 2-d). In both sexes, plasma concentrations of sulfate on Day 1 in Gunn rats were about the same as those in Wistar rats (Figs. 2-b and 2-c). Surprisingly, the glucuronide was detected not only in Wistar rats but also in Gunn rats, which are hereditarily deficient in UGT1s. In the male rats, plasma concentrations of the glucuronide on Day 1 were higher in Wistar rats than in Gunn rats (Fig. 2-c). In the female rats, they were almost comparable in both strains (Fig. 2-f).

Plasma concentrations of troglitazone on Day 14 were much the same as those on Day 1, except that the concentrations in females were lower on Day 14 than on Day 1 in both strains (Figs. 3-a and 3-d). Although there were no strain differences in sulfate concentrations on Day 14 as well as on Day 1, each concentration was lower on Day 14 than on Day 1 (Figs. 3-b and 3-c). On the other hand, the glucuronide was also detected in Gunn rats on Day 14 as well as on Day 1. However, on Day 14 the concentrations of the glucuronide in male Gunn rats were about the same as those in male Wistar rats, and those in female Gunn rats were higher than those in female Wistar rats (Figs. 3-c and 3-f).

Plasma concentrations of troglitazone and its two metabolites at 1 hr after administration on Day 94 were much the same as those on Day 14 except for female Wistar rats (Table 1). Although the plasma concentration of troglitazone in female Wistar rats on Day 94 was approximately twice of that on Day 14, it was comparable to that on Day 1. On the other hand, plasma concentrations of sulfate and glucuronide in female Wistar rats on Day 94 were also twice those on Day 14, and about half of those on Day 1, respectively.

Comparison of AUC_{0-24} values between Gunn rats and Wistar rats showed that, although values of the sulfate and glucuronide in male Gunn rats on Day 1 were lower than the values in male Wistar rats, all other AUC_{0-24} values were higher in Gunn rats than in Wistar rats (Table 2). However, except for the female exposures of troglitazone between Gunn and Wistar rats (Gunn rats > Wistar rats), differences in exposure were not considerable within each strain. Finally, a comparison of exposure profiles between the male and female rats showed that the sulfate levels were higher in males than in females and troglitazone levels were higher in females than in males in both strains of rats.

Blood biochemical examination

The results of the blood biochemical examination are shown in Table 3. Both T-Bil and D-Bil were markedly higher in Gunn rats than Wistar rats because of the deficiency in UGT1s in Gunn rats. In Gunn rats, D-Bil was significantly elevated in the female groups given 100 and 400 mg/kg compared with the female control group; however, these changes were slight in nature (11.8 and 23.5% increase, respectively). Any other parameters, including AST, ALT and ALP, showed no significant changes in Gunn rats. In Wistar rats, the AST levels were significantly decreased in the male group given 100 and 400 mg/kg (35.8% and 36.3% decrease, respectively) and in the female group given 400 mg/kg (32.8% decrease). On the other hand, the ALP levels were significantly elevated in the male groups given 100 and 400 mg/kg (20.3 and 20.0% increase, respectively). No other parameters changed significantly in Wistar rats.

Pathological examination

In the macropathological and histopathological examinations, no abnormalities were observed in any
Toxicity study of troglitazone using Gunn rats.

Fig. 2. Plasma concentrations of troglitazone and its metabolites on Day 1 in rats treated orally with troglitazone at a dose level of 400 mg/kg. Each parameter represents the mean value. ● and ○ represent Gunn rat and Wistar rat values, respectively.

Fig. 3. Plasma concentrations of troglitazone and its metabolites on Day 14 in rats treated orally with troglitazone at a dose level of 400 mg/kg. Each parameter represents the mean value. ● and ○ represent Gunn rat and Wistar rat values, respectively.
of the Wistar rats. In Gunn rats, hyperplasia of bile ducts, acidophilic cytoplasmic change of hepatocytes and bile pigmentation in both the liver and kidney were observed in all animals, including the dead animal and control animals. All of these findings were diagnosed as being spontaneous lesions, and no additional findings were observed in the dead animals. Thus, no pathological changes attributable to troglitazone-dosing were observed in both Gunn rats and Wistar rats.

**DISCUSSION**

In the present study, the safety evaluation of troglitazone was performed using Gunn rats, which are

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**Table 1.** Plasma concentrations of troglitazone and its metabolites at 1 hr after administration in rats treated orally with troglitazone at a dose level of 400 mg/kg.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Days</th>
<th>Troglitazone</th>
<th>Sulfate</th>
<th>Glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gunn rat</td>
<td>Wistar rat</td>
<td>Gunn rat</td>
</tr>
<tr>
<td>Male</td>
<td>Day 1</td>
<td>5.3 ± 0.6</td>
<td>4.7 ± 1.1</td>
<td>61.1 ± 26.2</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>8.0 ± 2.4</td>
<td>3.4 ± 2.7</td>
<td>31.0 ± 17.8</td>
</tr>
<tr>
<td></td>
<td>Day 94</td>
<td>6.5 ± 2.1</td>
<td>4.5 ± 0.9</td>
<td>28.5 ± 11.0</td>
</tr>
<tr>
<td>Female</td>
<td>Day 1</td>
<td>36.8 ± 13.4</td>
<td>16.0 ± 1.2</td>
<td>27.8 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>17.8 ± 8.8</td>
<td>8.8 ± 5.1</td>
<td>7.7 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>Day 94</td>
<td>16.2 ± 7.1</td>
<td>16.8 ± 5.2</td>
<td>4.5 ± 2.9</td>
</tr>
</tbody>
</table>

**Table 2.** AUC_{0-24} of troglitazone and its metabolites in rats treated orally with troglitazone at a dose level of 400 mg/kg.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Days</th>
<th>Troglitazone</th>
<th>Sulfate</th>
<th>Glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gunn rat</td>
<td>Wistar rat</td>
<td>Gunn rat</td>
</tr>
<tr>
<td>Male</td>
<td>Day 1</td>
<td>45.7 ± 6.2</td>
<td>24.8 ± 4.3</td>
<td>196.7 ± 55.0</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>43.1 ± 9.7</td>
<td>19.3 ± 9.2</td>
<td>172.1 ± 48.7</td>
</tr>
<tr>
<td>Female</td>
<td>Day 1</td>
<td>510.9 ± 148.0</td>
<td>100.6 ± 16.1</td>
<td>145.5 ± 19.0</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>215.8 ± 63.3</td>
<td>77.3 ± 7.8</td>
<td>88.9 ± 26.8</td>
</tr>
</tbody>
</table>

**Table 3.** Blood biochemical parameters in rats treated orally with troglitazone for 3 months.

<table>
<thead>
<tr>
<th>Strain (Sex)</th>
<th>Dose (mg/kg)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>T-Bil (mg/dL)</th>
<th>D-Bil (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunn (Male)</td>
<td>0</td>
<td>175.0 ± 59.9</td>
<td>83.6 ± 30.9</td>
<td>654.4 ± 147.6</td>
<td>7.13 ± 1.01</td>
<td>0.64 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>113.0 ± 27.5</td>
<td>51.4 ± 15.6</td>
<td>627.2 ± 148.1</td>
<td>7.73 ± 1.33</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>107.8 ± 24.8</td>
<td>52.3 ± 14.7</td>
<td>830.5 ± 115.8</td>
<td>6.12 ± 1.18</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>Gunn (Female)</td>
<td>0</td>
<td>103.6 ± 13.7</td>
<td>49.6 ± 6.7</td>
<td>529.2 ± 38.4</td>
<td>6.26 ± 0.47</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>126.4 ± 35.8</td>
<td>65.2 ± 23.9</td>
<td>657.4 ± 119.6</td>
<td>5.45 ± 0.54</td>
<td>0.57 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>98.0 ± 12.2</td>
<td>54.2 ± 12.1</td>
<td>673.8 ± 192.2</td>
<td>6.05 ± 0.82</td>
<td>0.63 ± 0.05**</td>
</tr>
<tr>
<td>Wistar (Male)</td>
<td>0</td>
<td>127.4 ± 27.3</td>
<td>75.6 ± 19.0</td>
<td>756.6 ± 61.4</td>
<td>0.08 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>81.8 ± 9.5**</td>
<td>53.4 ± 9.3</td>
<td>910.4 ± 53.0**</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>81.2 ± 7.0**</td>
<td>52.0 ± 4.3</td>
<td>907.6 ± 24.3**</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Wistar (Female)</td>
<td>0</td>
<td>134.2 ± 24.3</td>
<td>88.8 ± 10.9</td>
<td>877.0 ± 116.2</td>
<td>0.07 ± 0.00</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>111.8 ± 11.8</td>
<td>72.2 ± 11.3</td>
<td>935.0 ± 110.1</td>
<td>0.07 ± 0.00</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>90.2 ± 5.7**</td>
<td>69.2 ± 11.9</td>
<td>990.8 ± 70.5</td>
<td>0.07 ± 0.00</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>
hereditarily deficient in UGT1s: Gunn rats and Wistar rats, the parent strain of Gunn rats, were treated orally with troglitazone for 3 months at dose levels of 0, 100 or 400 mg/kg, and TK measurement, blood biochemical examinations and pathological examinations were performed. Before we obtained the results of this study, we anticipated that the metabolic profile of troglitazone in Gunn rats would be different from that in Wistar rats, as indicated in the report regarding acetaminophen (de Morais and Wells, 1988 and 1989). We thought that low levels of troglitazone glucuronidation in Gunn rats would probably result in either elevating the existing levels of troglitazone or sulfate.

However, the glucuronide of troglitazone was unexpectedly detected in Gunn rat plasma. When the amounts of glucuronide in Gunn rats and Wistar rats were compared, that in Gunn rats was almost the same as or higher than in Wistar rats, except in male rats on Day 1. These findings suggest that the glucuronidation of troglitazone was catalyzed by UGT(s) other than those of UGT1s in Gunn rats. There was also little strain difference in the amounts of both troglitazone and its sulfate, except that the troglitazone in female Gunn rats was considerably higher than that in female Wistar rats. In addition, male plasma concentrations of troglitazone and sulfate were lower and higher, respectively, than female values in both strains of rats. These are thought to have resulted from the sex difference of sulfation reaction of troglitazone (male > female). Based on these results, we concluded that there was no strain difference between Gunn rats and Wistar rats in the metabolic profile of troglitazone.

As discussed above, the possibility that liver dysfunction may be induced by a change in the metabolic profile of troglitazone was practically abandoned by Day 14. However, the Gunn rat is an animal species that congenitally shows hyperbilirubinemia attributable to genetic deficiencies in UGT1s. The elevation of T-Bil was thought to be an indicator of a poor prognosis in liver dysfunction rarely reported in humans treated with troglitazone. Therefore, it is also very important to know whether or not aggravation of hyperbilirubinemia or remarkable elevation in biochemical markers of liver dysfunction is observed in the administration of troglitazone to Gunn rats for a long period of time. This led us to continue the administration to both strains of rats for up to 3 months.

Consequently, except for female Wistar rats, plasma concentrations of troglitazone and its sulfate and glucuronide at 1 hr after administration on Day 94 were much the same as those on Day 14. In female Wistar rats, plasma concentrations of each compound on Day 94 were approximately twice those on Day 14. This indicated that the same or greater exposure levels compared with Day 14 were maintained until Day 94 by the dosing of troglitazone. Nevertheless, no abnormalities were observed in the general condition, except that one of the male Gunn rats treated with 400 mg/kg died on Day 89 attributable to spontaneous hyperbilirubinemia. Blood biochemical examination performed on the day of the autopsy showed no remarkable elevation in any parameters, including AST, ALT and ALP, and hyperbilirubinemia in Gunn rats was also not aggravated by the long-term administration of troglitazone. In the histopathological examination of Gunn rats, hyperplasia of bile ducts, acidophilic cytoplasmic change of hepatocytes and bile pigmentation in both the liver and kidney were observed in all animals, including the control animals. However, these findings were thought to be attributable to hyperbilirubinemia, which is specific to Gunn rats. On the other hand, no abnormalities were found in the histopathological examinations of the Wistar rats. Thus, no liver injury showing a remarkable elevation of blood biochemical parameters, liver degeneration or necrosis was observed in Gunn rats or Wistar rats.

In the present study, the metabolic profile of troglitazone was unexpectedly similar in both strains of rats. Furthermore, hyperbilirubinemia in Gunn rats was not aggravated by troglitazone and liver dysfunction was not induced. These results strongly suggest that troglitazone was not metabolized by UGT1s but by other UGT isozyme(s) in rats, and that glucuronidation of troglitazone did not compete with glucuronidation of bilirubin in vivo. Thus, it is conceivable that high levels of total bilirubin in patients with liver dysfunction induced by troglitazone are attributable to hypofunction due to hepatocellular injury and not to metabolic competition of bilirubin with troglitazone. Moreover, the deficiency in UDT1s, an abnormality in drug-metabolism (idiosyncrasy), may be concluded to be not associated with troglitazone-induced liver injury.

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