Sex-Related Differences in Hepatic Drug-Oxidizing Capacity of Streptozotocin-Induced Diabetic Rats

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Male and female animal models of diabetes were prepared by treating rats with streptozotocin (STZ). Trimethadione (TMO) metabolism was depressed in male but increased in female diabetic rats. The insulin treatment normalized rats of both sexes. Serum dimethadione/TMO ratios at 2 h correlated with the elevated blood glucose levels in male and female control, the STZ-induced diabetic and the insulin-treated diabetic rats. Treatment with STZ in male rats affected the metabolism of antipyrine, decreasing urinary excretion of norantipyrine (NORA) urine but increasing elimination of 4-hydroxyantipyrine (OHA). In female diabetic rats, the amounts of the three major metabolites, NORA, OHA and 3-hydroxymethyl-3-norantipyrine and the total (conjugate + free) were increased compared to the control. In the insulin-treated groups, these changes were normalized.

In conclusion, our study showed that the effect of STZ-induced diabetes on drug metabolism varies with the sex and the drugs used. Insulin normalized all these diabetic changes.

Keywords — streptozotocin; diabetes; trimethadione; antipyrine; drug-oxidizing capacity

Introduction

A number of investigators have shown that the pattern of drug metabolism in diabetic animals varies with the species, strain, sex, and other experimental conditions, suggesting a complicated mechanism of drug metabolism.1—13) The multiplicity in cytochrome P-450 is considered to be a cause of this varied drug metabolism.11) A commonly used in vivo procedure to study this metabolic multiplicity is to investigate metabolism of antipyrine (AP).14—17) In a series of experiments carried out in rats by using trimethadione (TMO), we showed that the plasma or serum concentration ratio of dimethadione (DMO) to TMO measured at 2 h after oral administration of TMO correlated well with the hepatic microsomal drug-oxidizing capacity which was measured in vitro and in vivo.18—23) A similar correlation is seen in normal rats as well as in rats pretreated with certain chemicals such as hepatotoxic agents18—22) and inducers of an enzyme system.23) These two methods are quantitative and sensitive tests for estimating the liver microsomal function.

In the present study, male and female animal models of diabetes were prepared by treating rats with streptozotocin (STZ) which is frequently used to induce diabetes in rats. The microsomal drug-oxidizing capacity in these rats was estimated in terms of AP and TMO metabolism. In addition, the effect of insulin treatment was also investigated.

Materials and Methods

Chemicals — STZ and DMO were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), respectively, and TMO was obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan).

AP, phenacetin and insulin were purchased from Wako Pure Chemical Ind. (Osaka, Japan) and 4-hydroxyantipyrine (OHA), norantipyrine (NORA) and 3-hydroxymethyl-3-norantipyrine (HMA) were obtained from Chikoh Co., Ltd. (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

Animals and Treatment — Adult male and female Wistar rats (Doken, Ibaraki, Japan) weighing 200—240 g were used for most of the experiments. Before starting the experiment, the rats were kept in an air-conditioned room
Sex Differences in Diabetes

(25±1 °C, 50—60% humidity) with a 12-h light–dark cycle (8:00—20:00) and had free access to commercial rat chow (Oriental-MF, Tokyo, Japan) and water.

Diabetes was induced by i.p. injection of 80 mg/kg of STZ freshly dissolved in 0.05 M citrate buffer (pH 4.5). The rats in the control group received citrate buffer only. Starting on day 6, some diabetic animals were given daily s.c. injection of insulin (15 I.U./kg) in doses sufficient to normalize urinary glucose concentrations. Glucose in serum was assessed on day 10. Animals with blood glucose levels greater than 200 mg/dl were judged to be diabetic. Blood glucose levels were measured by a glucose oxidase method using a kit from the Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan).

Ten days after administration of STZ, the rats received AP (50 mg/kg, i.p.) or TMO (100 mg/kg, p.o.). The control and STZ-treated rats were fixed on a board (CFK Lab., Tokyo, Japan) only at the time (0.5, 1, 2, 4, 6, 8 and 12 h) of blood sampling from the jugular vein after p.o. administration of TMO. Serum fractions separated from the blood samples by centrifugation were stored at −20 °C until used for the determination of TMO and DMO levels. For collection of urine after AP administration, control rats and STZ-treated rats were individually placed in metabolic cages (Natsume Seisakusho Co., Ltd., Tokyo, Japan) and given only water without food, and 24-h urine samples were collected.

Preparation of the Liver Microsomal Fraction — The rats were sacrificed at 9:30 to 10:00. The liver was perfused in situ with cold 0.9% NaCl solution via the portal vein, removed and homogenized in 4 volumes of 1.15% KCl by using a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 9000 × g for 20 min. The resulting supernatant fraction was centrifuged at 105000 × g for 60 min. The microsomal pellet was washed once and resuspended in 0.05 M phosphate buffer (pH 7.6) containing 1 mM ethylenediaminetetraacetic acid.

Enzyme Assay — The cytochrome P-450 content was determined from the CO difference spectrum of dithionite-treated microsomes as described by Omura and Sato.24) The activity of aminopyrine N-demethylase was measured by determining the formaldehyde released according to the method of Nash.25) The activity of aniline hydroxylase was estimated by the method of Imai et al.26) The protein content was determined by the method of Lowry et al.27) using bovine serum albumin as the standard.

TMO and DMO Assay — Serum TMO and DMO levels were determined by gas-liquid chromatography using maleimide as an internal standard.28)

Assay of AP and Its Metabolites — Urine concentrations of unchanged AP and its 3 main metabolites (NORA, HMA and OHA) were carried out according to Teunissen et al.29) and Blyden et al.30)

Calculation — Concentration-time curves of TMO and DMO were drawn on semilogarithmic scales. The half-life ($T_{1/2}$) and the elimination rate constant ($K_{el}$) were calculated from the linear portion of the curve obtained by linear regression analysis. The apparent volume of distribution ($V_d$) was calculated from the rate of the dose to the serum concentration extrapolated to time zero. The area under the curve (AUC) was calculated by the trapezoidal rule or by integrating $C_t/K_{el}$ to infinite time, where $C_t$ is the last value of TMO concentration, and $K_{el}$ was calculated from the equation:

$$K_{el} = 0.693/T_{1/2}$$

Metabolic clearance (Cl) was calculated according to the following equation:

$$Cl = V_d \cdot K_{el}$$

Statistical Analysis — Each value of the results is expressed as the mean ± S.D. Overall differences among the treatment groups were assessed by the Newman–Keuls test after repeated measures analysis of variance (ANOVA).

Results

Characterization of Male and Female STZ-Induced Diabetic and Insulin-Treated Diabetic Rats
TABLE I. Characterization of Male and Female STZ-Induced Diabetic and Insulin-Treated Diabetic Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetics</td>
</tr>
<tr>
<td>Growth in 10 d a)</td>
<td>18.3±4.1</td>
<td>5.2±2.2 b)</td>
</tr>
<tr>
<td>Blood glucose b)</td>
<td>121±6</td>
<td>449±5 c)</td>
</tr>
<tr>
<td>Water consumption c)</td>
<td>32±4</td>
<td>75±6 c)</td>
</tr>
<tr>
<td>Wet liver weight d)</td>
<td>7.9±0.6</td>
<td>7.1±0.5</td>
</tr>
<tr>
<td>Relative liver weight e)</td>
<td>5.2±0.2</td>
<td>6.4±0.6 f)</td>
</tr>
</tbody>
</table>

STZ-induced diabetes lasted for 10 d.
b) mg/dl, c) ml/rat/24 h, d) g, e) % of body weight, f) p < 0.05 compared to control group.
g) p < 0.01 compared to control group.

Within 48 h after the injection of a single dose of STZ, all treated rats exhibited glucosuria in excess of 200 mg/dl. At the time of all experiments, the blood glucose levels in male and female diabetic rats were about 4 and 3 times higher than the control levels, respectively (Table I). Furthermore, in both male and female rats, diabetes was evident by polydipsia and failure to thrive, and these changes were normalized by insulin treatment (Table I). The wet liver weight was not affected by the diabetic state, but the relative liver weight was significantly increased above that of the controls in the diabetic and the insulin-treated diabetic rats.

**Time Course and Pharmacokinetics of Serum TMO and DMO in Male and Female STZ-Induced Diabetic and Insulin-Treated Rats**

Figure 1 represents the time course of serum TMO and DMO levels in the male and female control, the STZ-induced diabetic and the insulin-treated rats. The serum level of TMO in male and female control rats after oral adminis-

![Fig. 1. Time Course of Changes in Serum TMO and DMO Levels (Mean) in Male and Female STZ-Induced Diabetic and Insulin-Treated Diabetic Rats.](image-url)

Control: ○ --- ○, TMO; ○ --- ○, DMO; diabetics: ● --- ●, TMO; ● --- ●, DMO; diabetics + insulin: △ --- △, TMO; △ --- △, DMO.
TABLE II. Pharmacokinetic Parameters of Serum TMO in Male and Female STZ-Induced Diabetic and Insulin-Treated Diabetic Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetics</td>
<td>Diabetics + insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Diabetics</td>
<td>Diabetics + insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{1/2}$ a)</td>
<td>1.50±0.21</td>
<td>1.66±0.11 c)</td>
<td>1.49±0.12 c)</td>
<td>2.86±0.19</td>
<td>1.36±0.14 f)</td>
</tr>
<tr>
<td>Cl b)</td>
<td>0.067±0.008</td>
<td>0.041±0.009 f)</td>
<td>0.061±0.006 h)</td>
<td>0.031±0.004</td>
<td>0.059±0.005 f)</td>
</tr>
<tr>
<td>$V_d$ c)</td>
<td>0.146±0.015</td>
<td>0.099±0.008 c)</td>
<td>0.132±0.011 s)</td>
<td>0.130±0.013</td>
<td>0.116±0.009 e)</td>
</tr>
<tr>
<td>$AUC$ d)</td>
<td>395±11</td>
<td>453±12 e)</td>
<td>402±7 h)</td>
<td>606±18</td>
<td>300±14 f)</td>
</tr>
</tbody>
</table>

STZ-induced diabetes lasted for 10 d and after overnight fasting, TMO (100 mg/kg) was orally administrated. Each value is the mean±S.D. of 5 rats. a) h. b) l/h. c) l. d) μg/ml/h. e) $p<0.05$ compared to control group. f) $p<0.01$ compared to control group. g) $p<0.05$ compared to diabetics group. h) $p<0.01$ compared to diabetics group.

The concentration of TMO (100 mg/kg) reached its peak at about 1 h, and that of the metabolite, DMO, reached its peak at 8—12 h and then gradually decreased. TMO metabolism was depressed in male, but increased in female diabetic rats. In insulin-treated rats of both sexes, these changes were normalized by insulin treatment. As shown in Table II, the pretreatment with STZ in the male rats prolonged serum TMO $T_{1/2}$, increased the $AUC$, and decreased the Cl and $V_d$ values, whereas in the female rats pretreatment shortened serum TMO $T_{1/2}$, increased the Cl and decreased the $V_d$ values and $AUC$. In the insulin-treated male and female rats, these changes were normalized by insulin treatment.

The serum DMO/TMO ratios were compared between the control and the diabetic rats. As shown in Fig. 2, the serum DMO/TMO ratios at 2 h after TMO administration in the diabetic male rats were significantly decreased from those in the control rats (31% decrease: 0.84±0.05 vs. 0.58±0.07, mean±S.D., $p<0.01$), whereas in the female rats, these ratios were significantly increased from those in the control rats (360% increase: 0.59±0.06 vs. 2.12±0.16). In insulin-treated rats of both sexes, these changes were restored to control values (male: 0.84±0.05 vs. 0.80±0.12; female: 0.59±0.06 vs. 0.77±0.10). Further analysis revealed that there was a direct correlation between the serum DMO/TMO ratio at 2 h and the elevated blood glucose level in the male and female control, the STZ-induced diabetic and the insulin-treated diabetic rats (Fig. 3, male: $r = -0.935$; female: $r = 0.934$).

Changes in Hepatic Mixed Function Monooxygenase Enzymes in Male and Female STZ-Induced Diabetic Rats

As shown in Table III, the contents of cytochrome P-450 were 30% higher in the diabetic females than in the control females, but were unchanged in the diabetic males. The activities of aminopyrine N-demethylase and aniline hydroxylase in diabetic female rats were increased to 123% and to 205% of the control, respectively. In diabetic male rats, the activity of aniline hy-

![Fig. 2. The Serum DMO/TMO Ratios at 2 h after TMO Administration in Male and Female STZ-Induced Diabetic and Insulin-Treated Rats](image-url)
Fig. 3. Correlation between Serum DMO/TMO Ratios at 2 h and Blood Glucose Levels in Male and Female STZ-Induced Diabetic and Insulin-Treated Diabetic Rats

○, control; ●, diabetics; △, diabetics + insulin. Male, \( r = -0.935 \), female, \( r = 0.934 \).

**TABLE III.** Changes in Hepatic Mixed Function Mono-oxygenase Enzymes in Male and Female STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetics</td>
</tr>
<tr>
<td>Cytochrome P-450 a)</td>
<td>0.77±0.09</td>
<td>0.85±0.12</td>
</tr>
<tr>
<td>Aminopyrine N-demethylase activity b)</td>
<td>4.69±0.25</td>
<td>3.17±0.16 c)</td>
</tr>
<tr>
<td>Aniline hydroxylase activity b)</td>
<td>1.18±0.04</td>
<td>1.40±0.05 c)</td>
</tr>
</tbody>
</table>

STZ-induced diabetes lasted for 10 d and the animals were sacrificed. Each value is the mean±S.D. of 5 rats.

a) nmol/mg protein. b) nmol/mg protein/min. c) \( p < 0.05 \) compared to control group. d) \( p < 0.01 \) compared to control group.

**TABLE IV.** Excretion of AP and Its Metabolites Expressed as Percentage of Dose in 24 h Urine in Male and Female STZ-Induced Diabetic and Insulin-Treated Diabetic Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetics + insulin</td>
</tr>
<tr>
<td>AP</td>
<td>2.3±0.2</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>HMA</td>
<td>20.6±0.7</td>
<td>18.3±1.0</td>
</tr>
<tr>
<td>NORA</td>
<td>10.3±0.2</td>
<td>9.1±0.3 c)</td>
</tr>
<tr>
<td>OHA</td>
<td>21.2±0.8</td>
<td>22.7±1.1</td>
</tr>
<tr>
<td>Total</td>
<td>54.4±1.5</td>
<td>52.8±1.7</td>
</tr>
</tbody>
</table>

STZ-induced diabetes lasted for 10 d and AP (50 mg/kg) was i.p. administrated. Each value is the mean±S.D. of 4 rats.

a) \( p < 0.05 \) compared to control group. b) \( p < 0.01 \) compared to control group. c) \( p < 0.05 \) compared to diabetics group. d) \( p < 0.01 \) compared to diabetics group.
droxylase was increased by 19%, but the activity of aminopyrine N-demethylase was decreased by 32%.

Excretion Data of AP and Its Metabolites in 24-h Urine in Male and Female STZ-Induced Diabetic and Insulin-Treated Rats

As shown in Table IV, treatment with STZ in the male rats decreased the amount of NORA in 24-h urine, but urinary OHA was increased compared to the corresponding controls. In the female diabetic rats, the amounts of three main metabolites (NORA, HMA and OHA) and the total (conjugate + free) were increased compared to the controls. In the insulin-treated groups, these changes were normalized.

Discussion

The sex difference in drug metabolism has been recently described by Kamataki et al. Preceding published reports showed that the pattern of drug metabolism varied with the sex and the strain. An involvement of hormones in this metabolic difference has been suggested.

In the present in vivo study using male and female Wistar rats, experimental diabetes did not change the cytochrome P-450 content in the males, but was increased by 30% in the females. In the diabetic state, the activity of aminopyrine N-demethylase was 32% lower in the males and 23% higher in the females than in the corresponding controls. The activity of aniline hydroxylase was elevated by 19% in the males and by 105% in the females (Table III).

These results are somewhat similar to those reported in male or female rats of Sprague-Dawley strain by Kato and Gillette and other investigators. However, Chawalit et al. who used male rats of the Fisher strain reported a 29% decrease in aniline hydroxylase activity in the diabetic state. The difference in the strain of the rat and the difference in the dose or administration method of STZ may have been responsible for this discrepancy. From the biochemical point of view, however, a selective induction or a selective inhibition of particular forms of cytochrome P-450 might also be involved. Past and Cook who made male Sprague-Dawley diabetic rats by i.v. administration of alloxan (45 mg/kg) demonstrated an increase in the cytochrome P-450 heme protein with a molecular weight of 52000 which facilitated metabolism of a type II substrate (aniline).

We have so far used the serum concentration ratio of DMO, the only metabolite of TMO, to unchanged TMO at 2 h after TMO administration as an indicator of the severity of hepatic dysfunction or induction of the hepatic drug-metabolizing capacity. In the present in vivo experiments, diabetes decreased TMO metabolism in male rats but markedly increased it in females (Table II, Figs. 1 and 2). This result closely resembles the result of the aminopyrine breath test made by Zysset and Sommer in male and female SD rats.

We also performed the AP test and determined unchanged AP and its free and conjugated metabolites. There was a decrease in NORA and an increase in OHA in male diabetic rats (Table IV). In diabetic females, all three main metabolites (HMA, NORA, and OHA), but not the unchanged AP, were significantly increased from the controls (Table IV). These results suggest that NORA and TMO are metabolized by similar cytochrome P-450 isozymes.

Insulin treatment of diabetic animals antagonized all the abnormalities of mixed function mono-oxygenase enzymes that we have observed in diabetic animals. The mechanism of this normalization by insulin is not fully elucidated. Reinke et al. described that the primary action of insulin may be ascribed to the correction of the insulin-deficient state of diabetic animals.

In conclusion, our study showed that the effect of STZ-induced diabetes on drug metabolism varies with the sex of the rat and the substrate used. Insulin normalized all diabetic changes. Further studies of diabetic patients are needed to evaluate the relevance of our findings in animals to a possible human sex difference in the effect of diabetes in drug metabolism.

References
