Influence of Short-Term Water Deprivation on Kinetics of Trimethadione and Its Metabolite in Rats

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Abstract—The effects of acute (24-, 48- or 72-hr) water deprivation on the disposition kinetics of trimethadione (TMO) and its only metabolite, dimethadione (DMO), and on the microsomal hepatic drug-oxidizing enzyme activities were investigated in male rats. The DMO/TMO ratios in the serum at 2 hr after intravenous administration of 100 mg/kg TMO were significantly decreased in 48- and 72-hr water-deprived rats, but in 24-hr water-deprived rats, the DMO/TMO ratios were not changed as compared to controls and food restrictions. In the 48- and 72-hr water-deprived rats, contents of cytochrome p-450 and activities of aminopyrine N-demethylase were significantly decreased. On the other hand, activities of aniline hydroxylase in these rats were significantly increased as compared to controls and food restrictions. These results suggest that the effects of water deprivation on drug metabolism not only depend on the time of water deprivation but also vary with the indicator substrate.

Recently, the influence of water deprivation on drug disposition kinetics was investigated (1–4). In humans, dehydration occurs as a result of polyuria or diarrhea, a form of pathologically produced short-term water deprivation. Significant hormonal (5, 6), enzymatic (4), and physiological (7, 8) changes have been reported in the water-deprived state.

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 1 or 2 hr after oral administration of TMO correlated well with hepatic microsomal drug-oxidizing capacity which was measured *in vitro* and *in vivo* (9–13). A similar correlation is seen in normal rats as well as in rats pretreated with some chemicals such as hepatotoxic agents (9–13) and inducers of an enzyme system (14). The present study was undertaken to investigate the disposition kinetics of TMO and its only metabolite,

DMO, and to determine the changes in hepatic microsomal enzyme systems and changes in physiological parameters in rats deprived of water for 24, 48 or 72 hr. Because water deprivation causes a decrease in food consumption of rats, the influence of such a decrease in food intake was also investigated.

Materials and Methods

Chemicals: The TMO preparation used was a commercially available 66.7%-pure powder (Mino-Aleviatin®; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). Aminopyrine and aniline were obtained from Aldrich Chemical Co. (Wisconsin, U.S.A.) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. All other chemicals were of the highest grade commercially available.

Animals and treatment: Adult male Wistar rats (Doken, Ibaraki, Japan) weighing 200–240 g were used for most of the experiments. Before starting the deprivation experiments, the rats were kept in an air-conditioned room

($25 \pm 1^\circ\text{C}$, 50–60% humidity) with a 12-hr light-dark cycle (8:00–20:00) and maintained on free access to commercial rat chow (Oriental-MF, Tokyo, Japan) and water. The rats were randomly assigned to the following 2 series of experiments, each containing 4 groups of rats: (1) one group of control rats were allowed free access to food and water, and 3 groups of dehydrated rats, i.e., rats deprived of water for 24, 48 or 72 hr but allowed free access to food and (2) another group of control rats and 3 groups of pair-fed hydrated rats, i.e., rats allowed free access to water but not to food, the supply of which was restricted to an amount equal to that consumed by the rats undergoing 24-, 48- or 72-hr water deprivation. Water and food intakes and body weights were measured daily. These rats intravenously received 100 mg/kg doses of TMO from the jugular vein. Blood samples were obtained via the jugular vein of each rat without anesthesia at 2 hr after intravenous 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. The serum specific gravity and the hematocrit value were determined before TMO administration. The rats were individually placed in metabolic cages, and urine samples were collected. Weights of the body and the remaining food were measured at 9:30 to 10:00 every day.

Preparation of the liver microsomal fraction: Following the above-mentioned blood sampling, the rats were sacrificed 2 hr after TMO which was administered at 9:30 to 10:00. Then, the livers were 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 $9000 \times g$ for 20 min. The resulting supernatant fraction was recentrifuged at $105,000 \times g$ 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 (EDTA).

Enzyme assays: Microsomal cytochrome P-450 contents were determined by the method of Omura and Sato (15). The activity

of aminopyrine N-demethylase was measured by the procedure of Cochin and Axelrod (16), and the activity of aniline hydroxylase was determined by measuring the formation of p-aminophenol by the method of Imai et al. (17). Protein concentrations were determined by a modified Lowry method (18) using bovine serum albumin as the standard.

SDS-polyacrylamide gel electrophoresis:

Hepatic microsomal proteins from control and water-deprived rats were dissolved in a solution containing 2% sodium dodecyl-sulfate, 10% glycerol, 5% β -mercaptoethanol and 62.5 mM Tris-HCl (pH 6.8), and the thus prepared protein solution was heated at 100°C for 3 min before electrophoresis. Samples containing 0.01 mg protein were run on slab gels (2 mm thick) of 10% polyacrylamide in the presence of 0.1% sodium dodecylsulfate at 25 mA/slab for 15 hr according to the method of Laemmli (19). The gels were stained with 0.25% Coomassie brilliant blue R and dried on thick filter paper.

TMO and DMO assay: Serum TMO and DMO levels were determined by a gas-liquid chromatographic (GLC) method using paramethadione as an internal standard, as reported previously (9).

Statistical analysis: The results were statistically analyzed by Student's *t*-test.

Results

The serum DMO/TMO ratios were compared among the control, the water-deprived, and the food-restricted rats. As shown in Fig. 1, the serum DMO/TMO ratios at 2 hr after intravenous administration of 100 mg/kg TMO in the 48- and 74-hr (but not 24-hr) water-deprived rats were significantly decreased from those in the control rats by 33% (0.91 ± 0.03 vs. 0.61 ± 0.04 , $P < 0.01$) and by 57% (0.91 ± 0.03 vs. 0.39 ± 0.02 , $P < 0.01$), respectively. Food restriction of 48 and 72 hr (but not 24 hr) also significantly decreased those serum ratios from the controls by 24% (0.95 ± 0.05 vs. 0.72 ± 0.03 , $P < 0.05$) and by 46% (0.95 ± 0.05 vs. 0.51 ± 0.03 , $P < 0.01$), respectively. The serum DMO/TMO ratios in the 48- and 72-hr (but not 24-hr) water-deprived rats were significantly lower than those of the corresponding food-restricted rats by 15% (0.72 ± 0.03 vs. 0.61 ± 0.04 ,

Fig. 1. Serum DMO/TMO ratios at 2 hr after intravenous administration of TMO (100 mg/kg, i.v.) in water deprived (A) and food-restricted (B) rats. (1) non-treatment, (2) 24 hr treatment, (3) 48 hr treatment, (4) 72 hr treatment. Mean values $\pm$ S.E.M. * $P < 0.05$ and ** $P < 0.01$, in comparison to the control. + $P < 0.05$, in comparison to the food restricted rats. $n = 4$.

$P < 0.05$) and by 24% (0.51 ± 0.03 vs. 0.39 ± 0.02 , $P < 0.05$), respectively.

The status of the hepatic enzyme system in the water-deprived rats was compared with those in the control and the food-restricted rats. As shown in Tables 1A and 2A, in the 48- and 72-hr (but not 24-hr) water-deprived and food-restricted rats, the contents of cytochrome P-450 and activities of aminopyrine N-demethylase were significantly decreased as compared to the controls. Water deprivation of 48- and 72-hr (but not 24-hr) was significantly lower than those of the corresponding food-restricted rats. On the other hand, in the 48- and 72-hr (but not 24-hr) water-deprived and food-restricted rats, the activities of aniline hydroxylase were significantly increased as compared to the controls (Tables 1A and 2A). Water deprivation of 48- and 72-hr (but

Table 1. Effects of water deprivation on hepatic drug metabolism (A) and physiological parameters (B) in rats

(A) Hepatic drug metabolism parameters

	Control	24-hr water deprivation ⁱ	48-hr water deprivation ⁱ	72-hr water deprivation ⁱ
Microsomal protein ^a (%)	21.1 $\pm$ 0.45 (100)	21.8 $\pm$ 0.51 (103)	22.2 $\pm$ 0.65 (105)	20.8 $\pm$ 0.36 (98)
Content of cytochrome P-450 ^b (%)	0.81 $\pm$ 0.02 (100)	0.77 $\pm$ 0.03 (95)	0.61 $\pm$ 0.04** (75)	0.62 $\pm$ 0.03**** (62)
Aminopyrine N-demethylase ^c (%)	5.42 $\pm$ 0.31 (100)	5.11 $\pm$ 0.28 (94)	3.86 $\pm$ 0.13** (71)	2.52 $\pm$ 0.21**** (46)
Aniline hydroxylase ^c (%)	0.64 $\pm$ 0.08 (100)	0.66 $\pm$ 0.12 (103)	0.78 $\pm$ 0.21** (122)	0.83 $\pm$ 0.17**** (130)

(B) Physiological parameters

	Control	24-hr water deprivation ⁱ	48-hr water deprivation ⁱ	72-hr water deprivation ⁱ
Body weight ^d (%)	230 $\pm$ 5.2 (100)	221 $\pm$ 3.2 (96)	213 $\pm$ 2.4* (93)	200 $\pm$ 3.1* (87)
Urine volume ^e (%)	20.2 $\pm$ 3.2 (100)	15.2 $\pm$ 2.0 (75)	5.4 $\pm$ 0.8** (27)	2.3 $\pm$ 0.3** (11)
Serum specific gravity	1.042 $\pm$ 0.001 (100)	1.044 $\pm$ 0.001 (100)	1.048 $\pm$ 0.002* (101)	1.048 $\pm$ 0.001** (101)
Hematocrit value ^f (%)	40 $\pm$ 1.6 (100)	41 $\pm$ 1.2 (103)	43 $\pm$ 1.6* (108)	46 $\pm$ 0.8** (115)
Food intake ^g	30	18	9	5
Water intake ^h	37	0	0	0

Values are the means $\pm$ S.E.M., $n = 5$; * $P < 0.05$ and ** $P < 0.01$, in comparison to the control. + $P < 0.05$ and ** $P < 0.01$, in comparison to the corresponding data shown in Table 2. ^a mg/g liver, ^b nmol/mg of protein, ^c nmol/mg protein/min, ^d g, ^e ml/24 hr, ^f %, ^g g/24 hr, ^h ml/24 hr, ⁱ Food ad libitum.

Table 2. Effects of food restriction on hepatic drug metabolism (A) and physiological parameters (B) in rats

(A) Hepatic drug metabolism parameters

	Control	24-hr food restriction ¹	48-hr food restriction ¹	72-hr food restriction ¹
Microsomal protein ^a (%)	23.1±0.55 (100)	22.8±0.20 (99)	21.9±0.43 (95)	22.2±0.33 (96)
Content of cytochrome P-450 ^b (%)	0.78±0.05 (100)	0.75±0.08 (96)	0.71±0.04** (91)	0.59±0.10**** (76)
Aminopyrine N-demethylase ^c (%)	6.01±0.11 (100)	5.66±0.28 (94)	5.21±0.09** (87)	3.91±0.12**** (65)
Aniline hydroxylase ^c (%)	0.59±0.09 (100)	0.61±0.11 (103)	0.63±0.08** (107)	0.68±0.09**** (115)

(B) Physiological parameters

Body weight ^d (%)	220±1.8 (100)	211±2.2 (96)	205±1.9* (93)	196±3.6* (89)
Urine volume ^e (%)	21.3±2.8 (100)	16.2±3.2 (76)	5.8±1.1** (27)	2.8±0.5** (13)
Serum specific gravity	1.041±0.001 (100)	1.042±0.001 (100)	1.044±0.001* (100)	1.048±0.001** (101)
Hematocrit value ^f (%)	40±1.5 (100)	42±1.4 (102)	44±1.2* (107)	46±0.9** (112)
Food intake ^g	31	18	9	3
Water intake ^h	38	25	18	5

Values are the means±S.E.M., n=4; *P<0.05 and **P<0.01, in comparison to the control. *P<0.05 and **P<0.01, in comparison to the corresponding data shown in Table 1. ^a mg/g liver, ^b nmol/mg of protein, ^c nmol/mg protein/min, ^d g, ^e ml/24 hr, ^f %, ^g g/24 hr, ^h ml/24 hr, ⁱ pair-fed to equal food consumed by water-deprived rats.

not 24-hr) was significantly higher than those of the corresponding food-restricted rats. Microsomal protein contents of the liver were not changed by any of these water deprivation and food restriction treatments.

Physiological parameters of rats were also compared among the control, the water-deprived, and the food-restricted rats. As shown in Tables 1B and 2B, body weights and urine volumes in the 48- and 72-hr (but not 24-hr) water-deprived and food-restricted rats were significantly less than the controls, and their serum specific gravities and hematocrit values were significantly increased. However, there were no significant differences in these parameters between the water-deprived and food-restricted rats. Hepatic microsomal proteins of the control, water-deprived and food-restricted rats were

analyzed by electrophoresis. We found no significant difference in electrophoretic behavior of the proteins among these groups.

Discussion

The purpose of the present study was mainly to investigate the disposition kinetics of TMO and to determine the possible changes in microsomal enzyme systems in water-deprived rats. Preliminary experiments were conducted to determine the average food intake in water-deprived rats as a basis for finding the amount of food to be supplied to the pair-fed groups. Dehydration was produced by deprivation of water for 24, 48 or 72 hr. Since rats usually survive water deprivation as long as 10 to 14 days or until they lose approximately 50% of their initial body weight, 72 hr of water deprivation

did not seem to impose a fatal stress.

Recently, Prasad et al. (1, 2), Bakar and Niazi (3), and Baetjer and Rubin (4) have reported that water deprivation might alter the pharmacokinetic characteristics of antipyrine (1, 2), aspirin (3), salicylic acid (3), hexobarbital, (4) and aniline (4). It was reported that there was no significant change in the volume of distribution of antipyrine and aspirin following 36 hr of water deprivation in rats (1, 3). On the other hand, biological half-lives of antipyrine and aspirin were prolonged, and their total body clearance was decreased. Prasad et al. (2) reported that the disposition kinetics of antipyrine in rats deprived of water for 96 hr was altered significantly: the total body clearance and the volume of distribution decreased by 27.1 and 22.4%, respectively, as compared to the controls. There was a 51.4% decrease in the hepatic cytochrome P-450 content in water-deprived rats.

TMO is used as a model drug to assess the capacity of liver oxidative metabolism and the mixed-function oxidase system (9–14). The present study demonstrated that the DMO/TMO ratios, contents of cytochrome P-450, and activities of aminopyrine N-demethylase and aniline hydroxylase in the 24-hr water-deprived rats were not changed as compared to the controls and rats with food restrictions (Fig. 1 and Table 1). However, in the 48- and 72-hr water-deprived rats, the content of cytochrome P-450 and aminopyrine N-demethylase activity were significantly decreased as compared to the controls and rats with food restrictions, but aniline hydroxylase was significantly increased. Likewise, measured values of physiological parameters were significantly changed after 48- and 72-hr water deprivation as compared to the controls (Table 1). As shown in Table 2, in the food-restricted rats, the changes in hepatic drug metabolism and physiological parameter values were similar to those in water-deprived rats. However, the changes in hepatic drug metabolism were significantly different between the water-deprived and food-restricted rats. The reduction of TMO metabolism in the water-deprived rats appeared to be related with some alteration in the metabolic system,

e.g., reduction of cytochrome P-450, which occurred secondarily to the decrease in total and circulating blood volumes, the decrease in body weights, and the decrease in water contents of serum, all of which may have occurred in the water-deprived rats (2).

Baetjer and Rubin (4) reported that the rate of in vitro hexobarbital metabolism in the presence of the microsomal fraction prepared from either 24- or 48-hr water-deprived rats was significantly lower than the controls. They also reported that in vitro aniline metabolism by the microsome preparation from 24-hr water-deprived male rats was increased. This discrepancy between metabolism of hexobarbital and that of aniline may be explained by a difference in spectra of their binding with cytochrome P-450 (referred to as types I and II, respectively) (1). Thus, the results of our study suggest that different types of cytochrome P-450 are involved in the metabolism of TMO, aminopyrine and aniline and that the effect of water deprivation on drug metabolism not only depend on the time of water deprivation but also vary with the indicator substrate. Further studies are under way to confirm these results.

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