Cross-Physical Dependence of Several Drugs in Methaqualone-Dependent Rats

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Abstract—We investigated the characteristics of physical dependence on methaqualone. Rats were made physically dependent on methaqualone by the use of the drug-admixed food (DAF) method for 33 days. Pentobarbital, barbitol, ethanol and diazepam were cross-administered against methaqualone to evaluate the degree of suppression of methaqualone withdrawal signs as an index for the cross-physical dependence liability of these drugs to methaqualone. To evaluate the cross-physical dependence liability, we used AUC of body weight loss and withdrawal scores between the first cross-administration (9 hr after the withdrawal) and 27 hr after the withdrawal. AUC of weight loss was significantly suppressed by the four test drugs as compared to each control. Withdrawal scores were also significantly inhibited by the cross-administration of barbitol, ethanol and diazepam. Considering that the rats given barbitol or ethanol fell asleep after the cross-administration, diazepam seems to cause the strongest suppression of methaqualone withdrawal signs among the four test drugs. Thus, physical dependence on methaqualone may be similar by nature to that on benzodiazepines rather than barbiturates and alcohol.

Misuses of methaqualone, a quinazolinone derivative hypnotic, for non-medical purposes appear to be quite widespread as suggested by surveys carried out by various workers (1–4). Methaqualone has been reported to cause dependence in man (5–7). Physical dependence develops from the chronic use of methaqualone at doses of above 7.5 g daily. The doses of 2 g daily of methaqualone used for one month is sufficient to produce withdrawal seizures (8).

In laboratory animals such as dogs (9, 10), rats (11, 12) and mice (13), evidence for physical dependence on methaqualone has been reported. Severe physical dependence on methaqualone, as manifested by spontaneous convulsions after its withdrawal, has not been however documented, suggesting that physical dependence on methaqualone is a mild type. Moreover, Yanagita and Miyasato (14) found that methaqualone produced no physical dependence in rhesus monkeys,

but functioned as a reinforcer for monkeys in an intravenous self-administration procedure. However, Suzuki et al. (15) reported that severe physical dependence on methaqualone, which was characterized by spontaneous convulsions during withdrawal, was easily developed by the drug-admixed food (DAF) method in female and male rats.

Dependence-producing drugs are classified into 9 types (16). Methaqualone belongs to the barbiturate type. There are some reports of cross-physical dependence liability among sedative-hypnotics including methaqualone in dogs (9), monkeys (17), rats (18) and mice (19). In barbitol- or pentobarbital-dependent dogs, the degree of withdrawal signs after barbiturate withdrawal was lessened by methaqualone (9, 10).

The purpose of the present study was to evaluate the characteristics of physical dependence on methaqualone. We therefore investigated the degree of inhibition of

methaqualone withdrawal signs by cross-administration of several CNS depressants (barbital, pentobarbital, ethanol and diazepam).

Materials and Methods

Animals: Male Sprague-Dawley rats weighing 150–200 g at the beginning of the experiment were used. Animals were housed in individual cages under a 12-hr light-dark cycle with food and water continuously available. The room temperature was maintained at $22 \pm 1^\circ\text{C}$, and the relative humidity was maintained at $55 \pm 5\%$. The rats were allowed to adapt to their environment for a period of 1 week.

Development of physical dependence: For preparing the drug-admixed food, methaqualone was mixed with a normal powdered food (CA-1, Japan Clea, Tokyo) in a mortar (20, 21). Each rat was allowed to eat the methaqualone-admixed food and to drink tap water ad libitum. For developing the physical dependence on methaqualone, the rats were chronically treated with methaqualone according to the method of Suzuki et al. (15). The concentration of methaqualone in the food was gradually increased from 1 and 2 to 10 and 12 mg/g of food for 33 days.

ED50 of test drugs in rotarod performance test: Motor incoordination was evaluated by the rotarod performance test (9 cm in diameter, 5.3 rpm; Natsume Seisakusho Co., Tokyo) in naive rats. Motor incoordination was assessed by the complete inability of the animal to walk on the rotarod in each of three trials. Median effective doses (ED50) for motor impairment were calculated by the up and down method (22). Rats were examined by the rotarod performance test after administration of pentobarbital (at 15 min after i.p.), barbital (30 min after i.p.), ethanol (15 min after i.p.) and diazepam (45 min after p.o.).

Cross-physical dependence on test drugs: Withdrawal was conducted by substituting normal food for methaqualone-admixed food at 14:00 of the last day of the treatment in all rats. Cross-administration of test drugs was started from 9 hr after the methaqualone withdrawal. The dose of ED50 for motor impairment was used for cross-administration as the minimal dose of each drug.

Three doses of each test drug and saline or 0.5% CMC as the respective control were administered with the same schedule. Pentobarbital (25.4, 33.0 and 42.9 mg/kg, i.p.) was administered 8 times at 1.5-hr intervals until 16.5 hr after the withdrawal. Barbital (156, 203 and 264 mg/kg, i.p.) was administered only once at 9 hr after the withdrawal. Ethanol (2.18, 2.83 and 3.68 g/kg, i.p.) and diazepam (65.0, 84.5 and 109.9 mg/kg, p.o.) were administered twice at 9 and 15 hr after the withdrawal.

During and after the cross-administration, body weight and food intake were measured every 3 hr and withdrawal signs were observed. Suppression of body weight loss by the cross-administration was used as an index for the evaluation of cross-physical dependence liability to test drugs. The area under the curve (AUC) of weight loss was calculated by percent change in weight loss between the starting point of cross-administration of the test drugs (9 hr after the withdrawal) and 27 hr after the methaqualone withdrawal. Moreover, the following rating score for withdrawal signs was used according to the method of Tagashira et al. (23), with a minor modification. The withdrawal signs were classified into four grades: no abnormality (score 0), mild (score 1), intermediate (score 2) and severe (score 3).

Statistical analysis: Analysis for the incidence of withdrawal signs was performed by the chi-square (2×2) test. All other analyses were carried out using Student's *t*-test.

Results

Pentobarbital: The cross-administration with three doses (25.4, 33.0 and 42.9 mg/kg) of pentobarbital caused a slight inhibition of body weight loss by methaqualone withdrawal. However, body weight did not increase by pentobarbital injections and gradually kept decreasing (Fig. 1). AUCs of weight loss for 25.4, 33.0, 42.9 mg/kg of pentobarbital and the control were 69.38 ± 9.83 , 65.02 ± 21.14 , 88.01 ± 14.40 and 128.15 ± 18.04 , respectively (Fig. 2). The AUC of weight loss at the lowest dose of pentobarbital, 25.4 mg/kg, was significantly smaller than that in the control. Straub's tail reaction was significantly inhibited at 33.0 and 42.9

Fig. 1. Time course of changes in body weight after the cross-administrations of several drugs in methaqualone-dependent rats. Each test drug was cross-administered from 9 hr after the methaqualone withdrawal. The arrows indicate the cross-administration of the test drugs and saline or 0.5% CMC. The dotted line represents that one rat died.

Fig. 2. Effects of several sedative-hypnotics on body weight loss after the methaqualone withdrawal. Cross-physical dependence liability of these drugs was evaluated by AUC. AUC of weight loss was calculated by changes of body weight between the starting point of cross-administration (9 hr after the withdrawal) and 27 hr after the withdrawal. The numerals near the columns represent the doses of drugs. C: control.

mg/kg of pentobarbital as compared to the control ($P<0.05$, Table 1). Withdrawal scores for 25.4, 33.0 and 42.9 mg/kg of pentobarbital and the control were 16.0 ± 2.6 , 11.5 ± 3.2 , 14.7 ± 1.9 and 16.7 ± 2.4 , respectively. There was no significant difference in the withdrawal scores between any dose of pentobarbital and the control.

Barbital: The cross-administration with barbital inhibited body weight loss by methaqualone withdrawal (Figs. 1 and 2). The converse dose-related inhibition was observed. AUCs of weight loss for 156, 203, 264 mg/kg of barbital and the control were -36.76 ± 31.06 , 24.17 ± 23.00 , 59.39 ± 21.08 and 98.86 ± 19.44 , respectively. The cross-administration of the low and medium barbital doses significantly inhibited the weight loss by methaqualone withdrawal. In the case of the low dose (156 mg/kg), body weight after the cross-administration increased. Withdrawal scores for 156, 203, 264 mg/kg of barbital and the control were 2.3 ± 1.3 , 2.2 ± 0.6 , 0.8 ± 0.5 and 15.6 ± 2.0 , respectively (Table 2). By the cross-administration with all 3 doses of barbital, the withdrawal scores

showed a significant inhibition as compared to those in the control. During the cross-administration with 203 and 264 mg/kg, barbital-induced sleep was observed in 3 out of 5 and 4 out of 6 rats tested, respectively.

Ethanol: The cross-administration with the low (2.18 g/kg) and medium (2.83 g/kg) doses of ethanol failed to inhibit body weight loss by methaqualone withdrawal (Figs. 1 and 2). The high dose (3.68 g/kg) of ethanol significantly inhibited body weight loss as compared to the control ($P<0.01$). AUCs of weight loss for 2.18, 2.83, 3.68 g/kg of ethanol and the control were 47.98 ± 7.86 , 51.87 ± 6.04 , 4.27 ± 6.67 and 88.99 ± 18.72 , respectively. Withdrawal scores for 2.18, 2.83, 3.68 g/kg of ethanol and the control were 12.3 ± 2.5 , 7.3 ± 2.1 , 0.6 ± 0.2 and 14.8 ± 2.2 , respectively (Table 3). The withdrawal scores for 2.83 and 3.68 g/kg of ethanol were significantly smaller than that in the control. During the cross-administration with 2.83 and 3.68 g/kg of ethanol, ethanol-induced sleep was observed in 1 out of 6 and all the rats tested, respectively.

Diazepam: The cross-administration with

Table 1. Behavioral changes during pentobarbital cross-administration in methaqualone-dependent rats

Withdrawal signs (score)	Positive animals/Used animals			
	Pentobarbital (mg/kg)			Control (saline)
	25.4	33.0	42.9	
Weight loss (percent of AUC from control)				
0% > (0)	0/6	0/5	0/6	—
0–30 (1)	0/6	1/5	0/6	—
30–60 (2)	3/6	2/5	3/6	—
60% < (3)	3/6	2/5	3/6	6/6
Vocalization (2)	3/6	3/6	1/6	1/6
Irritability (2)	2/6	2/6	2/6	0/6
Aggression (2)	1/6	0/6	0/6	1/6
Muscle rigidity (2)	6/6	6/6	6/6	6/6
Straub's tail (2)	1/6	0/6*	0/6*	4/6
Ear-twitching (2)	5/6	3/6	5/6	5/6
Fascicular-twitch (3)	5/6	3/6	5/6	5/6
Tremor (3)	5/6	3/6	5/6	5/6
Convulsion (3)	5/6	3/6	5/6	5/6
Death (3)	0/6	1/6	0/6	1/6
Withdrawal scores	16.0 ± 2.6	11.5 ± 3.2	14.7 ± 1.9	16.7 ± 2.4

* $P<0.05$ vs. control. The chi-square (2×2) test was used in the statistical evaluation for each withdrawal sign. The withdrawal scores were calculated by the sum of the rating score per animal.

65.0, 84.5 and 109.9 mg/kg of diazepam completely inhibited body weight loss by methaqualone withdrawal (Figs. 1 and 2). Body weight at 65.0 and 84.5 mg/kg of diazepam

Table 2. Behavioral changes during barbitol cross-administration in methaqualone-dependent rats

Withdrawal signs (score)	Positive animals/Used animals			
	Barbitol (mg/kg)			Control (saline)
	156	203	264	
Weight loss (percent of AUC from control)				
0% > (0)	4/6	2/5	0/5	—
0–30 (1)	1/6	1/5	2/5	—
30–60 (2)	0/6	0/5	1/5	—
60% < (3)	1/6	2/5	2/5	5/5
Vocalization (2)	1/6*	1/5	0/6**	4/5
Irritability (2)	0/6	0/5	0/6	2/5
Aggression (2)	0/6	0/5	0/6	0/5
Muscle rigidity (2)	2/6*	1/5**	1/6**	5/5
Straub's tail (2)	2/6*	0/5**	0/6***	5/5
Ear-twitching (2)	0/6***	0/5**	0/6***	5/5
Fascicular-twitch (3)	0/6**	0/5**	0/6**	4/5
Tremor (3)	0/6**	0/5**	0/6**	4/5
Convulsion (3)	0/6*	0/5*	0/6*	3/5
Death (3)	0/6	0/5	1/6	1/5
Withdrawal scores	2.3±1.3 ^a	2.2±0.6 ^b	0.8±0.5 ^b	15.6±2.0

*P<0.05, **P<0.01, ***P<0.001 vs. control. The chi-square (2×2) test was used in the statistical evaluation for each withdrawal sign. ^bP<0.01, ^aP<0.001 vs. control. Analysis for the withdrawal scores was performed by Student's *t*-test. The withdrawal scores were calculated by the sum of the rating score per animal.

Table 3. Behavioral changes during ethanol cross-administration in methaqualone-dependent rats

Withdrawal signs (score)	Positive animals/Used animals			
	Ethanol (g/kg)			Control (saline)
	2.18	2.83	3.68	
Weight loss (percent of AUC from control)				
0% > (0)	0/6	0/6	2/5	—
0–30 (1)	1/6	0/6	3/5	—
30–60 (2)	3/6	5/6	0/5	—
60% < (3)	2/6	1/6	0/5	6/6
Vocalization (2)	3/6	2/6	0/5	3/6
Irritability (2)	0/6	0/6	0/5	0/6
Aggression (2)	0/6	0/6	0/5	0/6
Muscle rigidity (2)	6/6	5/6	0/5***	6/6
Straub's tail (2)	4/6	4/6	0/5***	6/6
Ear-twitching (2)	4/6	1/6	0/5*	4/6
Fascicular-twitch (3)	4/6	1/6	0/5	3/6
Tremor (3)	4/6	1/6	0/5	3/6
Convulsion (3)	1/6	1/6	0/5	2/6
Death (3)	0/6	0/6	0/5	3/6
Withdrawal scores	12.3±2.5	7.3±2.1 ^a	0.6±0.2 ^b	14.8±2.2

^aP<0.05, ^bP<0.01 vs. control. Other explanations are as in Table 2.

Table 4. Behavioral changes during diazepam cross-administration in methaqualone-dependent rats

Withdrawal signs (score)	Positive animals/Used animals			
		Diazepam (mg/kg)		Control (0.5% CMC)
	65.0	84.5	109.9	
Weight loss (percent of AUC from control)				
0%> (0)	3/6	2/6	4/6	—
0–30 (1)	1/6	2/6	0/6	—
30–60 (2)	1/6	2/6	1/6	—
60%< (3)	1/6	0/6	1/6	6/6
Vocalization (2)	5/6	5/6	4/6	3/6
Irritability (2)	0/6	1/6	0/6	2/6
Aggression (2)	0/6	0/6	0/6	0/6
Muscle rigidity (2)	5/6	4/6	3/6*	6/6
Straub's tail (2)	2/6*	2/6*	3/6*	6/6
Ear-twitching (2)	1/6*	1/6*	2/6	5/6
Fascicular-twitch (3)	1/6	1/6	2/6	4/6
Tremor (3)	1/6	1/6	2/6	4/6
Convulsion (3)	1/6	1/6	2/6	4/6
Death (3)	0/6	0/6	0/6	1/6
Withdrawal scores	7.0±2.1 ^b	6.8±2.3 ^a	7.8±2.4 ^a	16.8±2.2

Explanations are as in Table 3.

showed almost no decrease from the start of cross-administration. Body weight at 109.9 mg/kg increased rather significantly by diazepam cross-administration. AUCs for 65.0, 84.5, 109.9 mg/kg and the control were 1.79 ± 14.76 , 3.34 ± 19.51 , -35.82 ± 30.37 and 82.39 ± 6.08 , respectively. Withdrawal scores for 65.0, 84.5, 109.9 mg/kg and the control were 7.0 ± 2.1 , 6.8 ± 2.3 , 7.8 ± 2.4 and 16.8 ± 2.2 , respectively (Table 4). There were significant differences in the withdrawal scores between all the 3 doses of diazepam and the control.

Discussion

The cross-physical dependence test has been widely used to evaluate the physical dependence liability of sedative-hypnotics because it can be done quickly and economically (9, 17–19). In the present study, the cross-physical dependence test was used to investigate the characteristics of physical dependence on methaqualone. We made methaqualone-dependent rats by the DAF method and then examined the degree of suppression of the methaqualone withdrawal signs by cross-administration of test drugs. Barbitol as a long-acting barbiturate, pento-

barbital as a short-acting barbiturate, ethanol as alcohol and diazepam as a benzodiazepine were evaluated for the liability of cross-physical dependence. According to the classification of dependence-producing drugs by WHO, barbitol, pentobarbital and diazepam belong to the barbiturate type (16). However, ethanol belongs to a different type, the alcohol type, which nevertheless possesses cross-physical dependence liability to the barbiturate type (17).

The cross-administration of test drugs was performed from 9 hr after the termination of methaqualone treatment, because several signs of methaqualone withdrawal started to be observed from approximately 9 hr after the withdrawal.

The ED₅₀ for motor impairment was used for cross-administration as the minimal dose of each drug because there is high correlation between motor impairment by sedative-hypnotics and severity of withdrawal signs after the termination of drug treatment (24).

The present study showed that all the four test drugs (barbitol, pentobarbital, ethanol and diazepam) produced cross-physical dependence to methaqualone. AUCs of weight loss by methaqualone withdrawal were

significantly inhibited by cross-administration with all the test drugs, as compared to the control (Figs. 1 and 2). Withdrawal scores for barbitol at the doses of 156 and 203 mg/kg and ethanol at the doses of 2.83 and 3.68 g/kg were significantly smaller than that in the control. Barbitol at the dose of 156 mg/kg (cross-administration of minimal dose) caused strong suppression of the methaqualone withdrawal signs. Tagashira et al. (25) reported that the cross-administration with barbitol inhibited almost all mild to severe withdrawal signs of phenobarbital. They used 40 mg/kg $\times$ 4 and 80 mg/kg $\times$ 5 (p.o.) at 4–6 hr intervals as doses of cross-administration of barbitol. These doses were comparable low doses as compared with this study. The suppression of methaqualone withdrawal signs may result from the sleep induced by the cross-administration of high and medium doses of barbitol or ethanol. On the other hand, diazepam among the four test drugs indicated the clearest suppression of methaqualone withdrawal signs. These results demonstrate that high doses of diazepam are required for the development and/or maintenance of physical dependence, as compared to the usual doses used in general pharmacological studies. The present study suggests that physical dependence on methaqualone may be more similar to that on benzodiazepines than dependences on barbiturates and ethanol.

Tagashira et al. (25) cross-administered barbitol, pentobarbital, ethanol, diazepam, nitrazepam, methaqualone, etc. to evaluate cross-physical dependence liability in phenobarbital-dependent rats. From the mode of suppression and severity of relapsed withdrawal signs, these drugs were classified into 3 types. Barbitol belonged to type-I: drugs suppressing withdrawal signs of phenobarbital almost completely with the following relapse of severe withdrawal signs of phenobarbital. Ethanol, pentobarbital, diazepam and methaqualone belonged to type-II-a: drugs suppressing withdrawal signs of phenobarbital partially with the following relapse of moderate withdrawal signs of phenobarbital. Ifenprodil belonged to type-II-b: drugs suppressing withdrawal signs of phenobarbital partially with the following

relapse of only mild or practically no signs. Diphenylhydantoin, reserpine, clonidine and chlorpromazine belonged to type-III: drugs practically failing to suppress or rather aggravate withdrawal signs of phenobarbital. There are no data about type-III drugs in the present study, but the report of Tagashira et al. (25) showed that the type-III drugs did not suppress the withdrawal signs of phenobarbital. Type-III drugs such as reserpine, clonidine and chlorpromazine may have no cross-physical dependence liability to methaqualone. They therefore showed that barbitol possessed the best cross-physical dependence liability to phenobarbital. In the present study, our results suggest that diazepam among the drugs tested possesses the best cross-physical dependence liability to methaqualone.

Methaqualone exhibited a negligible effect on (^3H)GABA binding in vitro and inhibited (^3H)diazepam binding in vitro (26). (^{35}S)t-butylbicyclophosphorothionate binding in vitro was inhibited by methaqualone, and this inhibition was reversed by the bicuculline-like GABA antagonist R5135 (27). The effects of methaqualone might be expected to be mediated via GABA/benzodiazepine/barbiturate receptor complexes.

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