[Chem. Pharm. Bull. 31(4)1404—1407(1983)]

Effect of Temperature on the Metabolism of Aminopyrine

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(Received September 13, 1982)

We examined the effect of temperature on the fate of aminopyrine (AM) by using five male rabbits kept at 15 or 30°C. The rabbits kept at 15°C showed an increase in elimination of plasma AM compared with those at 30°C. The total urinary excretion of AM and its metabolites at 15°C was smaller than that at 30°C.

Keywords——effect of temperature; aminopyrine; metabolism; urinary excretion; plasma concentration

We previously examined the fate of aminopyrine (AM) in man by using gas chromatography to measure the concentrations of AM and its metabolites in healthy male volunteers from our laboratory.[1-3] The data collected over two years demonstrated a seasonal variation in the urinary excretion of AM and its metabolites.[4] Environmental temperature is though to be one of the factors which cause the variation.

As to the effect of environmental temperature on drug metabolism, Furner and Stitzel demonstrated that by subjecting rats to a cold environment for several days the metabolism of aniline or ethylmorphine was increased.[5] Kaplansky and Ben-Zvi demonstrated that the chronic exposure of rats to high environmental temperature caused a significant reduction in both hepatic *N*-demethylation of *p*-chloro-*N*-methylaniline and aniline hydroxylation.[6] However, the effect of environmental temperature remains to be investigated in detail. In the present work, by using rabbits kept in a cool room (15°C) or in a warm room (30°C), the effect of environmental temperature on the metabolism of AM was studied.

Experimental

Materials——Aminopyrine (AM), 4-monomethylaminoantipyrine (MAA), 4-formylaminoantipyrine (FAA) and 4-aminoantipyrine (AA) used in this work were obtained as described in the previous paper.[1] Deuterium-labeled AM (d_3-AM) was synthesized according to the method of Goromaru.[7]

Animal Experiment——Male albino rabbits weighing 2.6 to 3.5 kg were kept in a cool room or in a warm room for at least 20 d. The cool room was kept at a constant temperature of 15 ± 1°C under 60% humidity, and the warm room at a constant temperature of 30 ± 1°C under the same humidity. In both rooms, light (2000 lux) was on from 6 a.m. to 6 p.m. At each temperature, a crossover test was performed. Rabbits A, B and C were first placed in the room at 15°C and then moved to the room at 30°C. Rabbits D and E were first placed in the room at 30°C and then moved to the room at 15°C. The rabbits, which had been kept on commercial diet (Oriental Yeast Co., Ltd.), were fasted for 12 h prior to intravenous administration of 30 mg/kg of AM dissolved in 1 ml of saline. Blood samples were taken at 15, 30, 60, 120 and 180 min after the drug administration. Urine samples were collected for 24 h.

The Procedure for Blood Sample Preparation——A blood sample (4 ml) collected from a rabbit was immediately centrifuged at 3000 rpm, A portion of plasma (2 ml) was gently shaken in a stoppered centrifuge tube by hand for 10 s after the addition of 3 ml of phosphate buffer (pH 7.4) containing d_3-AM (30 μg) as an internal standard. Ammonium sulfate (4 g) was added to the mixture for deproteinization. The mixed solution was extracted twice with 20 ml of chloroform. The combined extracts were dehydrated with anhydrous sodium sulfate, and evaporated to dryness. One μl of the sample was injected into the gas chromatograph–mass spectrometer. Urine sample preparation was performed as described in the previous paper.[1]

Gas Chromatography–Mass Spectrometry (GC–MS)——The instrument used was a Shimadzu GC–MS 7000 equipped with a multiple ion detector peak matcher. A glass column (1 m × 3 mm i.d.) containing 1.5% OV-17 on Shimalite W (80—100 mesh) was used. The operating temperatures were as follows: injec-

tion port and separator, 250°C; column oven, 220°C. Mass spectrometer conditions were as follows: accelerating voltage, 3 kV; ionizing chamber temperature, 250°C. Mass fragmentography was employed in the analysis of AM. To determine the amount of AM, the relative height ratio of the molecular ion peak of AM at m/z 231 to that of d_3-AM at m/z 234 was calculated.

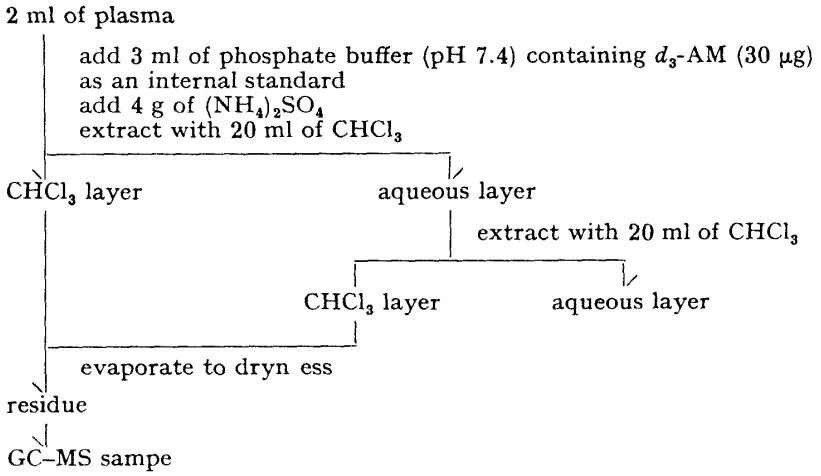

Chart 1. Sample Preparation for GC–MS Determination of AM from Rabbit Plasma

Results and Discussion

Fig. 1 shows the effect of environmental temperature on AM elimination in rabbit plasma after the intravenous administration of AM. The closed circles represent the plasma AM level at 30°C and the open circles that at 15°C. In all cases, a crossover procedure was used. The rabbits kept at 15°C showed a higher elimination rate of plasma than the rabbits kept at 30°C. The pharmacokinetic parameters are shown in Table I. There was a significant difference in the elimination rate of AM from rabbits at 30° and 15°C. The rate was 1.5 times higher in the rabbits kept at 15°C than in those at 30°C. Table II shows the amounts of the metabolites excreted in the 24 h urine after the intravenous administration of AM, MAA, AA, FAA and a small amount of AM were excreted. 4-Acetylaminoantipyrine, the major metabolite in man, could not be detected in urine from rabbits kept at 15° or at 30°C.

Thus, in the present study, a remarkable effect of temperature on the urinary excretion was observed. AM was detectable at 30°C, but not at 15°C. In the case of MAA, urinary excretion at 15°C was smaller than that at 30°C. Therefore, the total urinary excretion was dependent on the room temperature. In all cases except one (rabbit B), the total amount at 15°C was smaller than at 30°C.

TABLE I. Pharmacokinetic Parameters indicating the Disposition of AM

Rabbit	Temp. (°C)	$t_{0.5}$ (h)	K (h^{-1})	$K_{15°}/K_{30°}$
A	15	0.845	0.820	1.6
	30	1.352	0.513	
B	15	0.636	1.090	1.5
	30	0.929	0.746	
C	15	0.601	1.153	1.5
	30	0.877	0.790	
D	15	0.492	1.409	1.4
	30	0.695	0.997	
E	15	0.866	0.800	1.3
	30	1.115	0.623	

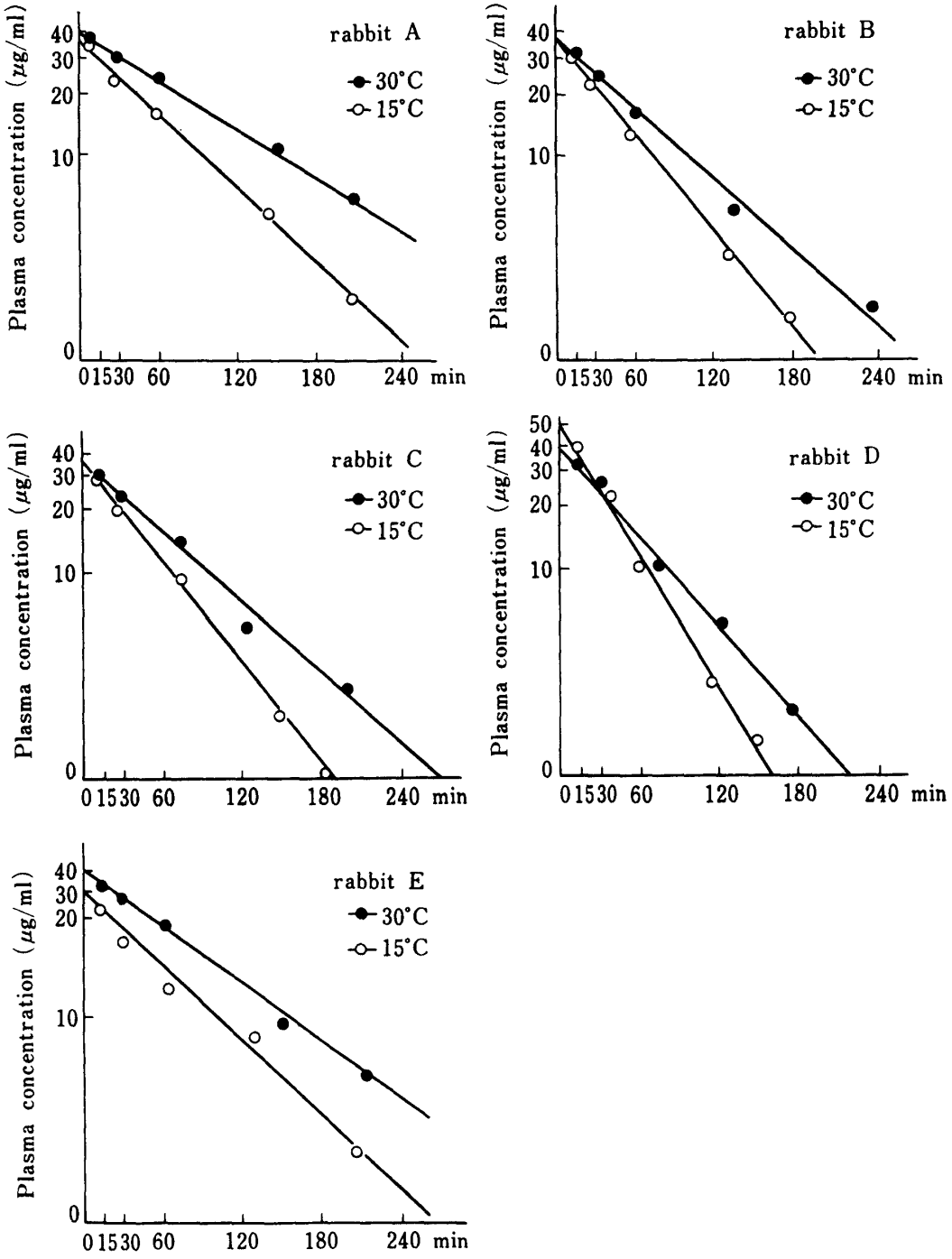

Fig. 1. Effect of Environmental Temperature on AM Elimination in Rabbit Plasma (30 mg/kg, *i.v.*)

In the previous paper,[4] we examined the fate of AM in man by means of GC, and the data collected over two years demonstrated a seasonal variation; the total urinary excretion in winter was smaller than in summer. Environmental temperature may be one of the factors which cause the seasonal variation.

Houston *et al.* reported that the total urinary excretion of AM and its metabolites decreased after phenobarbital pretreatment, though the demethylation step (AM to AA via MAA) increases.[8] Thus, when animals are kept in a cool environment, their drug-metabolizing

TABLE II. Excretion of AM and Its Metabolites in 24 h Urine following the Intravenous Administration of AM (30 mg/kg)

	Room temp. (°C)	Weight (kg)	AM	MAA	AA	FAA	Total mg (% to the dose)
A	15	3.0	—	2.74 (3.04)	7.94 (8.82)	9.56 (10.62)	20.24 (22.48)
	30	2.9	0.43 (0.49)	4.32 (4.97)	13.90 (15.98)	5.24 (6.02)	23.89 (27.46)
B	15	3.3	—	3.55 (3.59)	14.22 (14.36)	9.39 (9.48)	27.16 (27.43)
	30	3.1	0.13 (0.14)	3.79 (4.08)	13.29 (14.29)	7.55 (8.12)	24.76 (26.63)
C	15	2.9	—	0.83 (0.95)	5.29 (6.08)	4.72 (5.43)	10.84 (12.46)
	30	2.6	0.09 (0.12)	12.94 (16.59)	14.05 (18.01)	6.41 (8.22)	33.49 (42.94)
D	15	3.5	—	4.61 (4.39)	9.67 (9.21)	3.11 (2.96)	17.39 (16.56)
	30	3.0	0.06 (0.07)	11.71 (13.01)	11.63 (12.92)	3.86 (4.29)	27.26 (30.29)
E	15	3.3		2.44 (2.46)	7.03 (7.10)	5.94 (6.00)	15.41 (15.56)
	30	2.9	0.15 (0.17)	11.24 (12.92)	8.70 (10.00)	6.44 (7.40)	26.53 (30.49)

Each value has been converted into AM eq.
Values in parentheses represent percent of the dose.
— not detected.

enzyme activities may increase in a manner resembling phenobarbital induction. The relationship between the enzyme activity and the environmental temperature is under examination.

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