DIURNAL EFFECT ON CAFFEINE ACETYLATION PHENOTYPING: A PRELIMINARY REPORT

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ABSTRACT

The present study examined whether caffeine acetylation phenotype could be altered by its time of administration. Caffeine was given orally to nine healthy subjects at 10 a.m. and 10 p.m. and acetylation phenotype was determined by measuring the major metabolites of caffeine in urine. The results showed that acetylation phenotypes determined in the day trial were not different from those determined during the night trial.

KEY WORDS

diurnal effect, caffeine, acetylation phenotype

ABBREVIATIONS

AFMU	5-acetylamino-6-formylamino-3-methyluracil
1X	1-methylxanthine
1U	1-methyluric acid
17X	1,7-dimethylxanthine
17U	1,7-dimethyluric acid

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INTRODUCTION

It is well known that the rate of metabolism is genetically determined for several drugs, such as isoniazid, caffeine and debrisoquine /1,2/. Recent advances in chronopharmacology, however, have demonstrated daily variations in the activity of drug metabolizing enzymes /3/. Based on these observations, it is assumed that the rate of metabolism of a drug and its metabolic phenotype might also depend on its time of administration.

Ollagnier *et al.* found a time-dependent alteration in isoniazid acetylation phenotype in three out of six subjects /4/. In contrast, such an alteration was not demonstrated for debrisoquine oxidation phenotype /5,6/. It is interesting to examine, therefore, whether the time-dependent alteration in acetylation phenotype observed in isoniazid is common to other groups of drugs, including caffeine, which are metabolized by acetylation.

In the present study, caffeine was given orally to nine healthy subjects at 10 a.m. and 10 p.m. Acetylation phenotype during the day trial was compared to that during the night trial.

MATERIALS AND METHODS

Nine healthy subjects (6 men and 3 women), aged 27-37 years (mean age 30), were studied at the Medical College of Oita, Oita, Japan. None had a history of cardiovascular, renal or hepatic disease. All subjects gave informed consent to undergo caffeine phenotyping.

Subjects were admitted to a special hospital ward on two occasions with a one week interval. Subjects were randomly assigned to one of two treatment groups. Group I (n=5) received 300 mg of caffeine with 200 ml of water orally at 10 a.m. (day trial), and one week later had the same dose of caffeine with 200 ml of water at 10 p.m. (night trial). For group II (n=4), the night trial was first, and the day trial second. Two slices of toast and 500 ml of orange juice were served at 8 a.m. (day trial) or 8 p.m. (night trial). Water (200 ml) was given orally 1, 2, 3, 4 and 6 hours after administration of caffeine. Alcoholic beverages, coffee, tea, foods containing methylxanthine and smoking were not allowed for 3 days before and during the trial. Urine was collected for 8 hours following caffeine administration. Urine samples were stored at -20°C and were assayed within two weeks.

Acetylation phenotype was determined using urinary metabolites of caffeine as follows /2/:

$$\text{rapid acetylator} = \frac{\text{AFMU}}{\text{1X+1U+17X+17U+AFMU}} \geq 0.1$$

$$\text{slow acetylator} = \frac{\text{AFMU}}{\text{1X+1U+17X+17U+AFMU}} < 0.1$$

Urinary concentrations of these metabolites were measured by a HPLC method as previously described /7/. The sensitivity of this assay was 0.5 μg/ml. The covariance was 6.9%.

TABLE 1

The urinary amount of caffeine metabolites and the molar ratio of AFMU to 1X+1U+17X+17U+AFMU in the day and night trials. Caffeine (300 mg) was given orally at 10 a.m. (day trial) or 10 p.m. (night trial), and urine was collected for 8 hours after administration.

trial	subject	metabolite					ratio
		AFMU μmole	1X μmole	1U μmole	17X μmole	17U μmole	
day	1	43.2	11.8	36.5	70.4	34.7	0.22
	2	12.8	1.3	12.5	50.4	18.2	0.13
	3	80.0	68.9	63.2	79.4	33.9	0.25
	4	4.2	18.8	22.7	34.6	22.2	0.04
	5	25.6	23.6	43.7	55.1	34.5	0.14
	6	11.2	14.6	21.1	44.5	12.5	0.11
	7	0	24.0	24.9	41.5	19.4	0
	8	55.4	74.5	68.2	47.2	15.9	0.21
	9	22.1	12.1	23.0	25.3	11.4	0.24
night	1	18.8	1.7	24.1	39.5	19.6	0.18
	2	16.0	4.1	27.8	40.7	20.8	0.15
	3	65.1	93.9	72.6	37.9	32.2	0.22
	4	6.2	26.0	35.6	45.1	28.6	0.04
	5	13.0	12.9	20.2	38.1	23.5	0.12
	6	6.0	4.2	14.7	23.7	6.3	0.11
	7	0.1	10.1	20.5	30.5	16.2	0
	8	85.7	109.3	98.9	44.4	28.4	0.23
	9	8.9	4.6	14.3	17.2	5.4	0.18

RESULTS AND DISCUSSION

The urinary concentrations of metabolites of caffeine are shown in Table 1. The molar ratios of AFMU to the sum total of the major metabolites of caffeine were greater than 0.1 in the day and night trials in seven subjects. In contrast, these ratios in the other two subjects were smaller than 0.1 in both trials. A highly positive correlation was observed in these ratios between the day and night trials (Fig. 1).

These data indicate that caffeine acetylation phenotype determined in the day trial is not different from that determined during the night trial. Therefore, the time-dependent alteration in acetylation phenotype reported for isoniazid /4/ might not be common to other drugs which are metabolized by the acetylating pathway. Further studies involving a larger number of subjects (>30) are needed to evaluate this hypothesis.

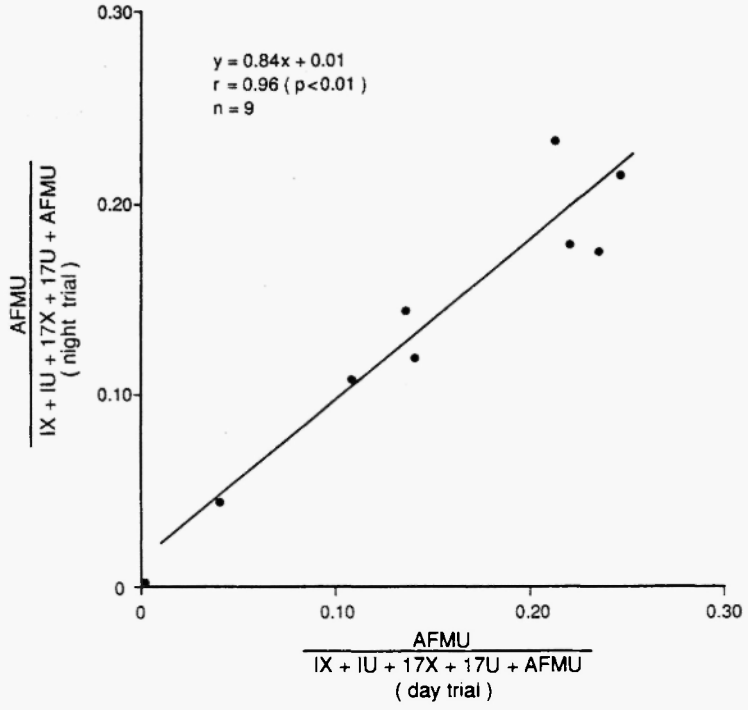

Fig. 1: Relationship between molar ratios of AFMU to 1X+1U+17X+17U+AFMU in the day trial and those in the night trial in nine healthy subjects

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