〈Regular Article〉

Methamphetamine concentrations in gastric contents and blood after parenteral and oral administration in rats

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ABSTRACT It is important, yet difficult, to distinguish the route of administration, parenteral or oral, of methamphetamine on postmortem examination. It might be possible to determine the route of administration by comparing the methamphetamine concentrations in the blood and stomach. However, the changes in methamphetamine concentrations in gastric contents and blood after oral and parenteral administration are unknown. Therefore, the time course of the ratio of methamphetamine concentration in gastric contents and blood (the GB ratio) was investigated using rats to which methamphetamine was administered subcutaneously (subcutaneous group) or orally (oral group). The maximum GB ratio and the maximum mean GB ratio in the subcutaneous group were 54.2 and 31.5, respectively. On the other hand, the GB ratio was 6.38-5,749 in the oral group. Therefore, it is difficult to distinguish the route of methamphetamine administration using the GB ratio alone. However, if the time from administration of methamphetamine to death can be proven, and it is less than 3 h, the route of administration could be clearly identified by measuring methamphetamine concentrations in the blood and gastric contents. Moreover, it might be suggested that the GB ratio would not exceed a certain value (54.2 was observed) after parenteral methamphetamine administration in rats.

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Key words：Methamphetamine, Administration route, Parenteral administration, Drug concentration in gastric content

INTRODUCTION

It is well known that some drugs in the blood are secreted into the urine and oral fluid[1]. Some drugs are absorbed from the small intestine into the blood in the portal vein, secreted into the bile, and reabsorbed from the small intestine, which is well known as the enterohepatic circulation[2]. Some cases indicated that the basic drugs accumulate in the stomach when they are administered intravenously[3, 4], but it is not clear that the drug is secreted into the stomach.

On the other hand, from the perspective of

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legal medicine, it is important to evaluate the effects of drugs administered to humans before death, not only to diagnose the cause of death, but also to judge the manner of death. However, on postmortem examination, the effects of drugs can only be determined by measurement of drug concentrations in body fluids and tissues, because symptoms and signs due to the effects of drugs cannot be observed and cannot be assessed by the gross appearance. In some cases, it is important to determine not only the effects of drugs, but also the route of administration. Methamphetamine is one of the most abused stimulant drugs in Japan, and some methamphetamine abusers say that they were given a drink containing methamphetamine by another person against their will, because they want to disguise the fact that they committed a crime.

There is no doubt that the drug concentration in gastric contents should be very high immediately after oral administration of a drug. In fact, some cases show that the drug concentrations in gastric contents are very high, and the total amount of a drug in gastric contents has been used to estimate the minimum amount of the drug taken[5].

On the other hand, there is a report of a case in which a decedent self-administered bath salts containing acetyl fentanyl and 4-methoxy PV8 intravenously. In this case, the ratios of drug concentrations in gastric contents/peripheral blood of acetyl fentanyl and 4-methoxy PV8 were 5.75 and 1.29, respectively[6].

We previously reported that some basic drugs accumulate in the stomach even if administered intravenously[4]. Nora *et al*. also showed that the methamphetamine concentration in gastric contents rose immediately and reached a peak 30 min after intravenous administration[7]. However, the changes in methamphetamine concentrations in gastric contents and blood after oral and parenteral administration are unknown.

It is very difficult to perform these analyses in humans, but relatively easy in animals. The purpose of this study was to clarify the relationships between methamphetamine concentrations in gastric contents and blood depending on the time after oral administration and subcutaneous administration in rats. This is one of the early basic animal studies to develop the methods for the identification of the route of drug administration for humans in the future.

MATERIALS AND METHODS

Reagents

Methamphetamine hydrochloride (Philopon) and methoxyphenamine hydrochloride were purchased from Sumitomo Dainippon Pharma Co., Ltd. (Osaka, Japan) and Sigma-Aldrich Japan (Tokyo, Japan), respectively. All other chemicals used were of reagent grade.

Animal experiments

Approximately 9-week-old, male, freely fed, Wistar/ST rats were used. Methamphetamine hydrochloride solution was administered at 1 mg/kg or 10 mg/kg either subcutaneously (subcutaneous administration group: SC administration group) or orally (oral administration group: PO administration group). The rats were then sacrificed at 0.5, 1, 2, 3, 6, or 12 h (SC administration group) or 3, 6, or 12 h (PO administration group) after methamphetamine administration in the free-feeding condition. Blood was collected from the right ventricle, and gastric contents were collected from the stomach. All samples were stored at -20℃ until methamphetamine measurement. Methamphetamine stability in gastric contents was not known, but it was reported that methamphetamine showed good stability in blood and other tissues[8, 9]. Therefore, based on these results, methamphetamine would be stable in gastric contents, at least under the conditions of this study.

The animal experiment protocol for this study was performed in compliance with the protocol

approved by the Animal Care and Use Committee of Kawasaki Medical School (No. 17-078 and No. 19-082).

Methamphetamine analysis

Methamphetamine concentrations in animal samples were determined by gas chromatography-mass spectrometry (GC/MS) using a GCMS-QP2010 chromatograph (Shimadzu, Kyoto, Japan) equipped with a 30 m × 0.25 μm Rxi-5Sil MS column (Restek, Bellefonte, PA, USA) with an internal diameter of 0.25 mm. For the methamphetamine analysis, blood and gastric contents were diluted with twice the weight of distilled water and then homogenized. Methamphetamine was refined from these samples by liquid-liquid extraction. In brief, samples (0.1-1.0 mL) spiked with 100 ng of methoxyphenamine as an internal standard (IS) were extracted with *tert*-butyl methyl ether under alkaline conditions. Hydrochloric acid (0.1 M) was mixed with the separated organic phase, which was then discarded. The water phase was alkalinized and mixed with *tert*-butyl methyl ether. The organic phase was gently evaporated under a stream of nitrogen gas. Methamphetamine was derivatized with trifluoroacetic anhydride and analyzed by GC/MS. The column oven temperature was initially 100℃, held for 2 min, then increased to 280℃ at a rate of 40℃/min and maintained for 10 min. Methamphetamine was monitored at ion m/z 154, 118, and 110. The IS was monitored at ion m/z 154, 148, and 110.

RESULTS

Animal experiments

The methamphetamine concentrations in blood and gastric contents with SC and PO administration are summarized in Tables 1 and 2 and Fig. 1 to 2. Methamphetamine concentrations in blood and gastric contents after SC administration of 1 mg/kg were 4.29-693 ng/mL and 4.05-1,920 ng/mL (Table 1 and Fig. 1A and B), respectively, and those after PO administration were 13.4-3,770 ng/mL and 100-32,500 ng/mL (Table 1 and Fig. 2A and B), respectively. Furthermore, with 10 mg/kg, the concentrations after SC administration were 13.4-3,770 ng/mL in blood and 100-32,500 ng/mL in gastric contents (Table 2 and Fig. 1D and E), and after PO administration, they were 66.1-696 ng/mL in blood and 31,500-590,000 ng/mL in gastric contents (Table 2 and Fig. 2D and E). In cases of SC administration, methamphetamine levels in gastric contents 30 min after administration were the same or higher than those in blood. The GB ratios after SC administration of 1 mg/kg and 10 mg/kg were 0.680-11.3 (Table 1 and Fig. 1C) and 0.484-54.2 (Table 2 and Fig. 1F), respectively, and those after PO administration were 6.38-1,050 at 1 mg/kg (Table 1 and Fig. 2C) and 244-5,749 at 10 mg/kg (Table 2 and Fig. 2F).

DISCUSSION

Even with SC administration, methamphetamine concentrations of gastric contents at 0.5 h were the same level or higher than those of blood. These results show that the methamphetamine in blood moved into the stomach quickly.

Methamphetamine concentrations in blood decreased quickly until 3 h, but they did not change thereafter after SC administration of 1 mg/kg (Table 1 and Fig. 1A). On the other hand, methamphetamine concentrations in blood were not very significantly different until 2 h after SC administration of 10 mg/kg, but they decreased quickly thereafter (Table 2 and Fig. 1D). Moreover, the peak GB ratio after SC administration of 10 mg/kg was delayed compared with the peak time of the GB ratio after SC administration of 1 mg/kg (Table 1, 2 and Fig. 1C and F). These results might show that the speed of methamphetamine moving from blood to gastric contents was affected by the blood methamphetamine concentration.

Tabel 1. Methamphetamine concentration in gastric contents and blood and those ratio administrated 1 mg/kg methamphetamine by S. C. and P. O.

	Time (h)	n	Methamphetamine concetnration				Ratio of methamphetamine concentration (Gastric contents/Blood)	
			Blood (ng/mL)		Gastric contents (ng/g)			
			mean	SD	mean	SD	mean	SD
S. C.	0.5	4	553.0	93.7	1,400.0	495.0	1.94	1.24
	1	4	205.0	89.3	1,210.0	340.0	7.08	3.85
	2	3	157.0	23.8	1,540.0	280.0	8.29	3.02
	3	5	27.6	7.73	147.0	47.7	5.33	2.57
	6	4	21.8	20.5	64.0	56.0	2.94	1.72
	12	3	26.0	36.4	41.0	59.0	1.58	0.69
P. O.	3	4	22.8	3.86	15,800.0	7,020.0	694.0	282.0
	6	4	49.0	39.8	5,660.0	4,210.0	116.0	336.0
	12	4	6.0	2.71	233.0	239.0	38.9	28.5

Tabel 2. Methamphetamine concentration in gastric contents and blood and those ratio administrated 10 mg/kg methamphetamine by S. C. and P. O.

	Time (h)	n	Methamphetamine concetnration				Ratio of methamphetamine concentration (Gastric contents/Blood)	
			Blood (ng/mL)		Gastric contents (ng/g)			
			mean	SD	mean	SD	mean	SD
S. C.	0.5	4	2,620.0	1,130.0	2,930.0	1,850.0	1.18	0.67
	1	4	2,700.0	923.0	15,000.0	10,700.0	5.41	2.63
	2	3	2,600.0	403.0	26,900.0	8,250.0	10.2	1.85
	3	4	521.0	64.8	12,200.0	4,450.0	23.6	8.22
	6	5	99.3	41.7	3,130.0	2,750.0	31.5	17.0
	12	5	20.1	6.26	379.0	299.0	18.8	9.93
P. O.	3	4	312.0	251.0	360,000.0	171,000.0	1,150.0	2,520.0
	6	3	322.0	83.5	523,000.0	63,900.0	1,620.0	359.0
	12	5	318.0	233.0	142,000.0	90,400.0	448.0	190.0

After SC administration of 1 mg/kg, the difference in methamphetamine concentrations between blood and gastric contents might have not reached the limit of the speed of movement; accordingly, methamphetamine concentrations in gastric contents were high enough 0.5 h after administration. Following SC administration of 10 mg/kg, the difference in methamphetamine concentrations between blood and gastric contents might have greatly exceeded that expected based on the speed of movement; consequently, the methamphetamine concentrations in gastric contents might not yet have reached maximum levels at 0.5 and 1 h after administration.

After PO administration of 1 mg/kg, blood methamphetamine concentrations rose in the early stage and then decreased (Table 1 and Fig. 2A). On the other hand, following PO administration of 10 mg/kg, methamphetamine concentrations in blood showed no significant differences from 3 h to 12 h after administration (Table 2 and Fig. 2D). These results might indicate that peristaltic movement of the gastrointestinal tract was strongly inhibited by 10 mg/kg of methamphetamine, but not very inhibited by 1 mg/kg of methamphetamine.

The maximum GB ratio with SC administration of 1 mg/mL was 11.3 (Table 1 and Fig. 1C); however, that with SC administration of 10 mg/mL was 54.2 (Table 2 and Fig. 1F), which was thought to be very high. This phenomenon could also be

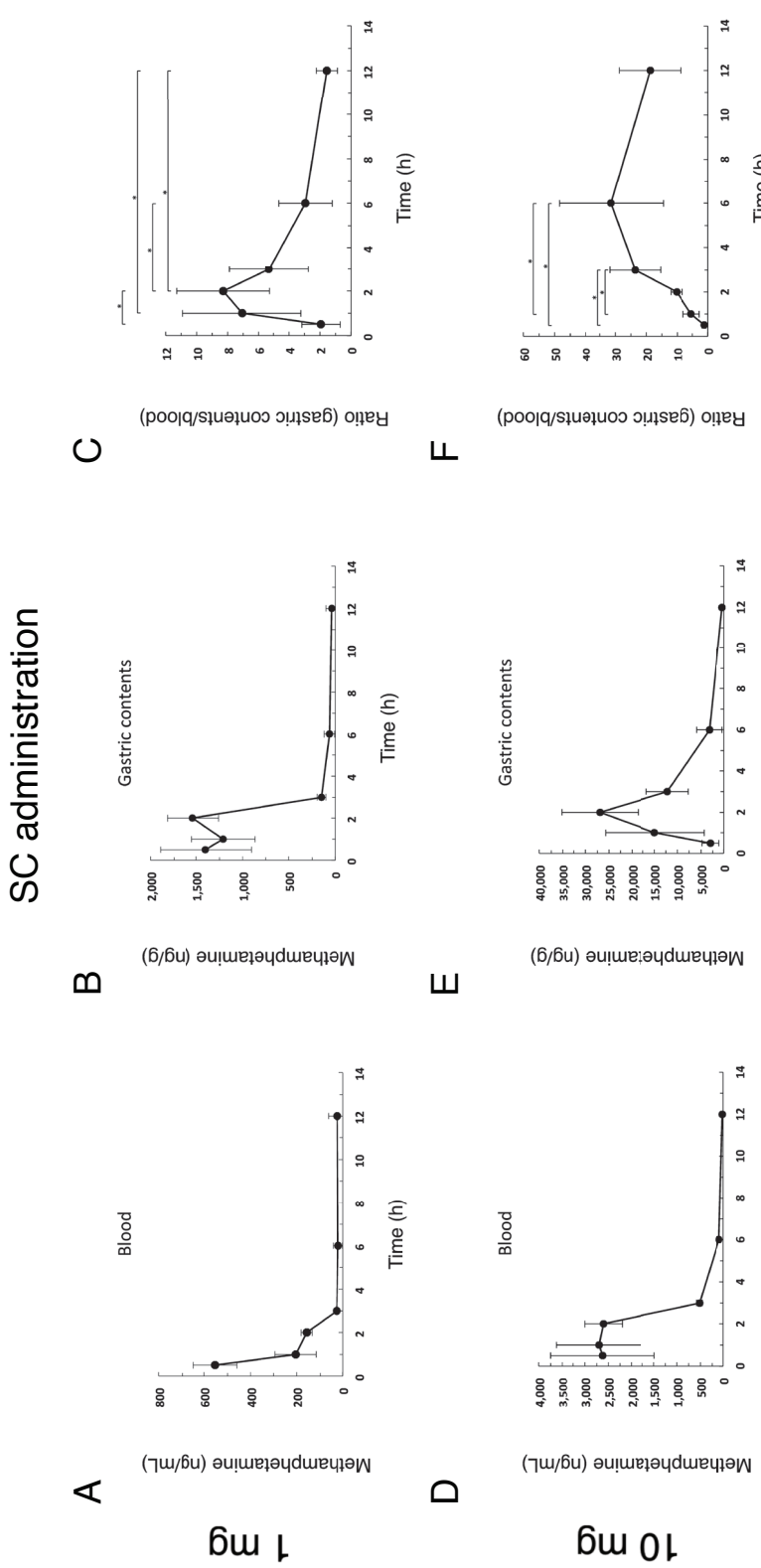

Fig. 1. Methamphetamine concentrations (ng/mL) in blood and gastric contents and the ratios (blood/gastric contents) when methamphetamine was administered by subcutaneous injection. A, B, and C show when 1 mg/kg of methamphetamine was administered. D, E, and F show when 10 mg/kg of methamphetamine were administered. A and D show methamphetamine concentrations in blood, B and E show methamphetamine concentrations in gastric contents. C and F show the ratios of methamphetamine concentrations in gastric contents to blood.
* $p < 0.05$ (one-way ANOVA/Tukey).

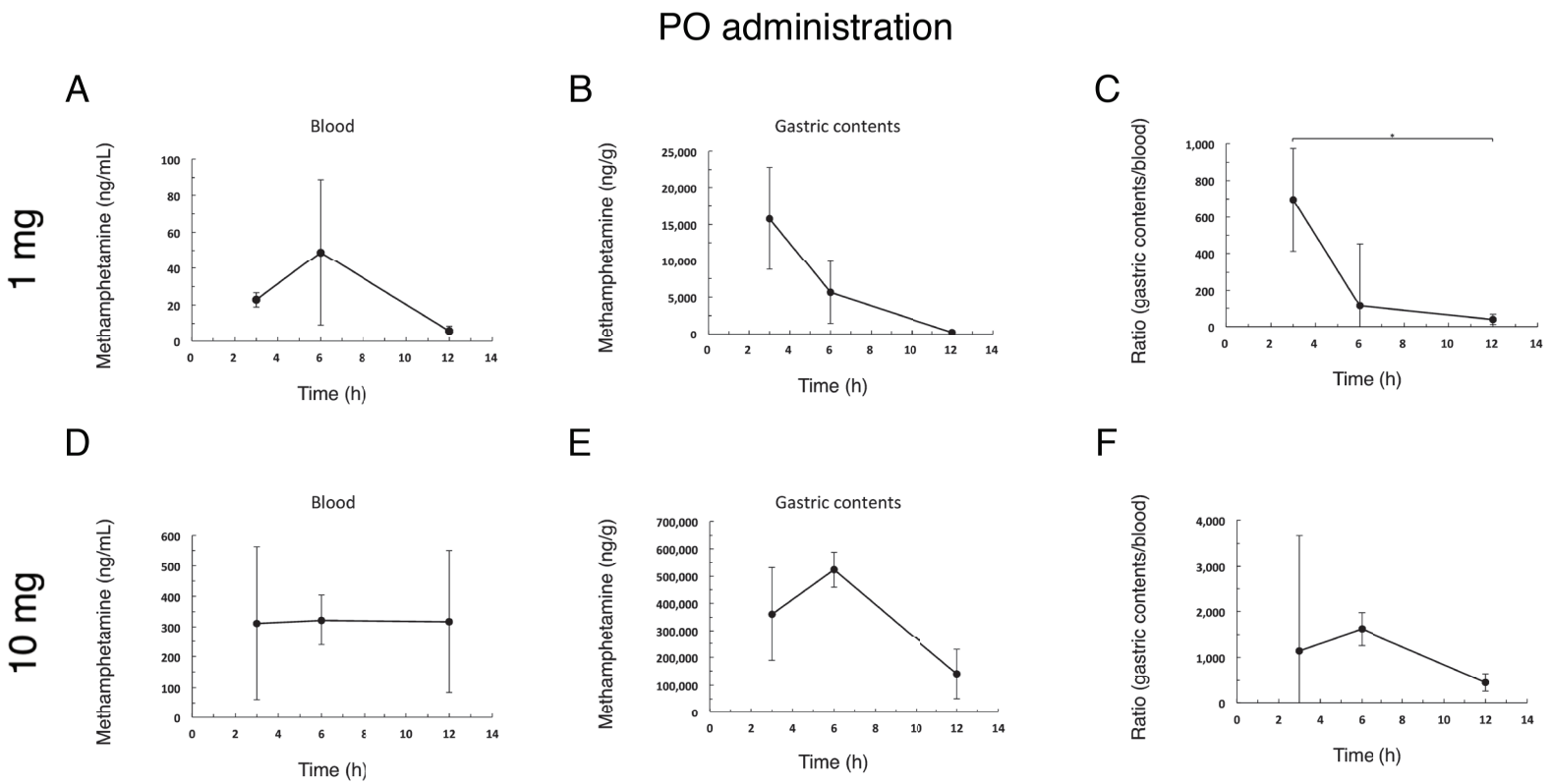

Fig. 2. Methamphetamine concentrations (ng/mL) in blood and gastric contents and the ratios (blood/gastric contents) when methamphetamine was administered orally. A, B, and C show when 1 mg/kg of methamphetamine was administered. D, E, and F show when 10 mg/kg of methamphetamine were administered. A and D show methamphetamine concentrations in blood, and B and E show methamphetamine concentrations in gastric contents. C and F show the ratios of methamphetamine concentrations in gastric contents to blood.
* $p < 0.05$ (one-way ANOVA/Tukey).

explained by the strong inhibition of peristaltic movement of the gastrointestinal tract by 10 mg/kg of methamphetamine, but not such strong inhibition by 1 mg/kg.

In the SC administration groups, it was natural that the methamphetamine concentrations in gastric contents increased at first, because methamphetamine was not present in gastric contents at baseline, and GB ratios also rose initially.

It was thought that the greater the time after administration, the more difficult it became to determine the route of administration, because even if the administration route was oral, stomach contents, which include a high concentration of drug, would be moved into the small intestine, lowering the methamphetamine concentration in stomach contents. Actually, in the reported case in which methamphetamine was administered at least 21 h before death, the GB ratio was only 39, despite the oral intake of methamphetamine[10]. However, under the conditions of the present study, if focusing on less than 3 h from administration to death, the maximum value of the GB ratio after SC administration was 32.2, but the minimum value after PO administration was 244, and these values were sufficiently different. Therefore, it was considered that the administration route of methamphetamine could be identified if the drug intake time was clear and less than 3 h before death. However, it is very rare that the intake time of the drug is clear. In actual cases, it has been previously reported that when the GB ratio was 36 or larger, a deceased could be diagnosed to have used methamphetamine orally[4]. However, it is quite difficult to assess the drug administration route and drug administration manner in actual cases.

This animal study showed that one could develop criteria to determine whether methamphetamine was administered orally or parenterally. Nevertheless, more studies are needed to clearly distinguish the route of administration. It was expected that the methamphetamine concentration in gastric contents would be diluted by a large amount of ingested water, as in a case of drowning. It may also be necessary to investigate the factors that affect movement of methamphetamine from the blood into the stomach.

This study was only one animal experiment, and future studies of human cases are needed to establish the standard to determine the administration route.

Amphetamine is one of the important metabolites of methamphetamine, and it is well known that its concentration in blood reaches 1/10 that of methamphetamine about 12 h after administration[11]. Unfortunately, amphetamine concentrations were not measured in the present study. Perhaps comparing the amphetamine/methamphetamine ratio in blood and gastric contents might be useful to determine the route of administration. In fact, in a previous case report of oral administration of methamphetamine, the GB ratio was 39, and no amphetamine was detected in gastric contents, despite the fact that amphetamine was detected in blood at 0.02 times that of methamphetamine[10]. Namely, a large difference in the amphetamine/methamphetamine ratio in blood and gastric contents might indicate that the route of methamphetamine intake was oral. However, additional studies are needed to test this hypothesis.

CONCLUSION

This study showed that methamphetamine concentrations of gastric contents rose quickly and were higher than those of blood at 0.5 h or more after subcutaneous administration. Therefore, even if the methamphetamine concentration in gastric contents was higher than that of blood, it did not mean that methamphetamine was taken orally. However, the GB ratio of the SC administration groups did not exceed 54.2 in the present study, even though a higher GB ratio was seen in the PO

administration groups. This fact suggested that a standard could be developed to distinguish between methamphetamine that was administered orally or by injection. If the time from administration to death were short enough (less than 3 h), then it might be possible to identify the route of administration (oral or parenteral) clearly by measuring methamphetamine concentrations in blood and gastric contents, at least under the condition that the administered doses of methamphetamine were 1 mg/kg to 10 mg/kg in rats.

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The authors have no conflicts of interest to declare.

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