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## CASE REPORT

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# Two Fatal Cases Involving Concurrent Use of Methamphetamine and Morphine

**ABSTRACT:** We report on two cases of simultaneous administration of methamphetamine (MAP) and morphine (MOR) with hyperthermia. The blood levels of MAP and MOR were toxic and putatively lethal, respectively, although hyperthermia is a known cause of intoxication due to MAP rather than MOR. In Japan, MAP is the most predominant cause of drug intoxication. The presented cases suggest that MOR may exert synergistic effect on hyperthermia due to the MAP intoxication, together with experimental findings.

**KEYWORDS:** forensic science, methamphetamine, morphine, multi-drug abuse, fatal poisoning, gas chromatography/mass spectrometry

Methamphetamine (MAP) and morphine (MOR) are stimulant and depressant, respectively, of the central nervous system. Though simultaneous abuse has not been reported in Japan, Yamamura et al. (1) reported that the synergism between alcohol addiction and MAP abuse, depressant and stimulant, respectively, might cause fatality. However, there is no case report on the simultaneous abuse of MAP and MOR with reference to the cause of death, although Moriya et al. (2) reported on a tissue distribution in such a case. Here we report on two fatal cases associated with the concurrent use of MAP and MOR and wrote the autopsy findings, chemical analyses, and speculations on the mechanism of the fatality.

### History and Autopsy Findings

*Case 1*

A 43-year-old musician and his wife intravenously injected the MAP at home around midnight on an April day. He vomited twice around 1:00 a.m. The next morning (8:30 a.m.) the wife found him dead in bed. By police investigation they were found to have abused MAP and cocaine biweekly. External examination showed no significant findings except for several needle scars in the left elbow bend. Internal examination showed mild edema in the brain (1350 g) and lungs (left: 350 g, right: 365 g). Histological examination showed fatty liver and chronic active HCV hepatitis. The wife did not remember whether MAP and MOR were injected simultaneously or successively. He was estimated to have died around 2:00 a.m. His rectal temperature (34.0°C at 1:30 p.m.) and the postmortem ambient temperature (11.4 to 22.5°C) showed his hyperthermia.

*Case 2*

A 21-year-old unemployed man was found dead in bed at home around 6:00 p.m. on a day in May. A disposable syringe (1 mL) nearby contained a solution with white crystals, which were identified as MAP. External examination showed no significant injury except for a needle mark in the left thigh. Internally, there was severe edema in the brain (1530 g) and the lungs (left: 620 g, right: 650 g). The tonsils and deep cervical lymph nodes were swollen bilaterally. There were no microscopic abnormalities except for mild tonsillitis. The police investigators estimated that MAP and MOR were injected shortly before midnight and early morning, respectively, when the death occurred. He was estimated to have died around 2:00 p.m. on the basis of the postmortem changes. The rectal temperature (34.0°C at 1:20 a.m. the next day) and the ambient temperature before the autopsy (16.0 to 17.3°C) strongly suggests antemortem hyperthermia.

### Postmortem Toxicological Analyses

*Triage™ Screening*

Triage™ (Biosite Diagnostics, San Diego, CA), an immunoassay for phencyclidine, benzodiazepines, cocaine, amphetamines, tetrahydrocannnabinol, opiates, barbiturates, and tricyclic antidepressants, was used for drug screening with urinary samples, according to the manufacturer's instruction.

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*Reagents*

Morphine hydrochloride, methamphetamine hydrochloride, and Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Takeda Chemical Industries Ltd. (Osaka, Japan), Dainippon Pharmaceutical Co. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO), respectively. Nalorphine hydrochloride was provided by Shionogi Pharmaceutical (Osaka, Japan). N-methylbenzylamine hydrochloride was prepared from N-methylbenzylamine by us. Other drugs of analytical grade were purchased from Nacalai Tesque (Kyoto, Japan).

*Biological Specimens*

All human samples obtained at autopsy were kept at −80°C until completing the analyses within a year. The anonymity of the victims was strictly protected, though informed consent was not obtained because of the prohibited contact of the autopsy operation with the bereaved due to criminal autopsy regulation in Japan.

*Conditions of GC/MS*

GC/MS analysis was performed on a Shimadzu GCMS-QP2000A (Kyoto, Japan) and was operated in electron impact (EI) mode at 70 eV with an ion source temperature of 250°C and *m*/*Z* range of 50 to 500 atomic mass units. The GC/MS was also run in total ion chromatogram (TIC) and selected ion-monitoring (SIM) modes. The fused silica capillary column (Shimadzu CBJ1-S30-100; 30 m by 0.32-mm inner diameter and 1 μm film thickness) was run, while the head pressure on the column and the temperature of the injection port being maintained at 49 kP and 250°C, respectively. The column oven temperature was initially maintained at 65°C for 3 min, but raised to 280°C at 10°C/min for MAP and 30°C/min for MOR after the split-less injection with a split-valve off-time of 3 min.

*Analysis of Methamphetamine*

We used the method of solid-liquid extraction. One mL of whole blood obtained from the intra-cardiac blood, urine, and the homogenate from 1 g of the liver, brain, and kidney were mixed with 2 μg of *N*-methylbenzylamine hydrochloride, an internal standard, and 2 mL of 0.05 *N* hydrochloric acid (3). The mixtures were shaken and centrifuged. The aqueous layer was aspirated and mixed with 1.5 mL of phosphate buffer (pH 6.0) and applied to a Bond Elute Certify column (Varian, Harbor City, CA), which had been activated by water and methanol. The column was washed successively with 2 mL each of water and methanol and eluted with 2 mL of methanol/chloroform (98:2). The elute was mixed with 50 μL of 1 *N* hydrochloric acid, evaporated under a stream of $N_2$ gas and derivatized with 200 μL of trifluoroacetic acid anhydride (TFA)/ethyl acetate (1:1) at 60°C for 20 min and evaporated. The residue was dissolved in ethyl acetate and the aliquot was injected into GC/MS to obtain EI mass spectrum by TIC. The calibration curve was prepared using whole blood containing MAP, which was extracted as described above. The standard curve was obtained by plotting the ratio of peak area of MAP-TFA *m*/*Z* 154 to that of *N*-methylbenzylamine-TFA 217 *m*/*Z* versus the amount of MAP by SIM.

*Analysis of Morphine*

The method of liquid-liquid extraction with back extraction was performed (4). Briefly, 1 mL or 1 g of blood, urine, or the homogenate from 1 g of the liver, brain, and kidney was mixed with 2 μg of nalorphine hydrochloride, an internal standard, and 1.5 mL of concentrated hydrochloric acid. The mixture was heated at 100°C for 1 h. After alkalinization, the extracted solvent was evaporated to dryness and the residue dissolved into diluted acid. After being washed with solvent, the acid layer was adjusted to basic pH and back-extracted. The extract was evaporated to dryness, and the residue was derivatized with BSTFA including 1% TMCS at 90°C for 30 min and evaporated. The residue was dissolved into ethyl acetate, from which the aliquot was subjected to GC/MS analyses. The calibration curves for morphine hydrochloride and nalorphine hydrochloride were prepared similarly as that for MAP. The standard curves were obtained by plotting the ratio of peak area of morphine-di TMS *m*/*Z* 429 to that of nalorphine-di TMS 455 *m*/*Z* versus the amount of morphine by SIM.

## Results

A Triage™ screening test was positive both for amphetamines and opiates, but negative for cocaine in the urine samples in both cases. GC/MS analyses showed that all the samples contain MAP and MOR by EI mass spectra in both cases. In Case 1, the blood MAP concentration was 0.55 μg/mL, while MOR concentration was 0.76 μg/mL. In Case 2, the blood concentrations of MAP and MOR were 2.64 and 0.50 μg/mL, respectively. In the specimens of urine, brain, liver, and kidney, both MAP and MOR were detected as shown in Table 1. The MAP concentrations in all samples from Case 1 were much higher than those in Case 2. The tissue MOR concentrations in Case 1 were slightly higher than those in Case 2 except for the blood. Ethanol was not detected by head-space gas chromatography in either case.

## Discussion

The two cases presented in this report showed the death due to synergism between methamphetamine (MAP) and morphine (MOR) through hyperthermia. Generally, once a significant level of MAP is detected upon autopsy, another drug would not be searched because MAP is the most prevalent abuse-related death in Japan. However, the presented cases prompted the forensic pathologists to further screening for MOR or other toxic drugs a priori even when a significant MAP level was found. Though MOR abuse has been rare in Japan, its abuse has increased through illegal imports or in-hospital crimes. In Case 1, despite the police information on cocaine abuse, the Triage™ screening was negative for cocaine. Therefore, we neglected the contribution of cocaine on the basis of the urine cut-off level of cocaine and its metabolite benzoylecgonine (550 and 300 ng/mL, respectively) and the lethal blood cocaine level (>900 ng/mL).

The blood MAP levels in both cases were below the lethal level (0.55 and 2.64 μg/mL in Cases 1 and 2 versus lethal concentration >4.5 μg/mL). Although hyperthermia is the most popular autopsy finding in MAP-related deaths, repeated intermittent administra-

TABLE 1—*Methamphetamine and morphine concentration.*

| | Case 1 | | Case 2 | |
|---|---|---|---|---|
| | Methamphetamine | Morphine | Methamphetamine | Morphine |
| Blood | 0.55 | 0.76 | 2.64 | 0.50 |
| Urine | 4.54 | 2.63 | 38.82 | 2.63 |
| Brain | 0.45 | 0.57 | 5.90 | 0.17 |
| Liver | 1.59 | 0.85 | 9.79 | 0.48 |
| Kidney | 1.02 | 1.15 | 4.63 | 0.40 |

Note: (μg/mL or μg/g)

tions of amphetamines enhance tachycardia, hypertension, as well as hyperthermia through sympathetic activation as we demonstrated in our experimental studies (5,6). Although the cardiovascular sensitization can explain the cause of death of our cases, the hyperthermia did exist. One explanation for the relatively low MAP concentration is the sensitization to MAP by repeated administration, though there was no evidence in our cases. By contrast, the MOR concentrations in our Cases 1 and 2 were 0.76 and 0.50 μg/mL, respectively, which exceed the putatively non-toxic concentration of MOR (0.3 μg/mL) (7). It was shown that a single exposure to MOR induces long-lasting behavioral sensitization (8). Although there was no supportive evidence in our cases, another explanation is that precedent MOR administration sensitized the victims to the subsequent administration of MOR, causing death primarily due to MOR. However, the latter is unlikely because the solitary MOR administration induces hypothermia but not hyperthermia (9). On the other hand, although the possible abuse of heroin should be examined in our cases, we could not analyze 6-acetylmorphine, a popular MOR metabolite, since its standard compound is not legally available in Japan.

There are some reports supporting the antagonism between MAP and MOR. Moriya and Hashimoto interpret their data on the tissue concentration and distribution in a case of concurrent use of MAP and MOR as the contribution of MOR to the MAP tolerance (2). They did not describe autopsy findings. Jores (9) reported a case of oral ingestion of large doses of 3,4-methylenedioxyethylamphetamine (MDEA) and heroin without any classic signs of intoxications despite high serum levels of both drugs. They assumed that antagonistic interaction of the two drugs prevented the patients from death. The difference in the effect can be explained by the difference in the effect of MDEA and MAP.

To the contrary of these observations, the data on our two cases suggest the synergism between MAP and MOR on hyperthermia and lethality. Therefore, we focus on the hypothesis that MOR enhances the hyperthermic effect of MAP. Two experimental studies strongly support our hypothesis. Funahashi et al. (10) showed that MAP-induced hyperthermia was enhanced by MOR in mice. Ginawi et al. (11) also reported that lethality and hyperthermia induced by MAP was augmented in MOR-addicted mice as compared with non-addicted mice. It has been reported that the enhanced toxicity in the combined administration of amphetamines and MOR is mediated through opioid receptor (12), $\alpha_1$b-adrenergic receptors (13), or dopamine-serotonin (14).

In our two cases, each of MAP concentration or MOR concentration was sub-lethal level, and the hyperthermia is likely to be associated with the cause of death. Taken together with the experimental studies, it is tempting to speculate that MOR enhances the hyperthermic effect of MAP and lethality.

In conclusion, we present two cases suggesting the synergism between MAP and MOR causes death primarily due to hyperthermia.

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