

CASE REPORT

The application of immunohistochemical findings in the diagnosis in methamphetamine-related death-two forensic autopsy cases-

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Abstract : Forensic autopsy cases detecting methamphetamine (MA) are usually diagnosed according to its toxicological concentration. It has been reported that the lethal blood concentration of MA is 4.48 µg/ml (3.0 µmol/dl). We autopsied two MA-detected cadavers, and immunohistochemical staining was performed on the skeletal muscle with an anti-myoglobin antibody, and on the kidney with an anti-the 70 kDa heat shock protein (HSP70) antibody. One case showed a high rectal temperature (40 °C). The toxicological examination revealed 0.75 µg/ml of MA in the blood, and 16.8 µg/ml in the urine. Myoglobin was negative and HSP70 was positive in the kidney immunohistochemically. From the toxicological and immunohistochemical findings, it was considered that the subject died of hyperthermia and acidosis caused by muscular hyperactivity. In another case, the autopsy revealed highly congested lungs, with dark-red bloody fluid and foam in the trachea and bronchus. MA (17.0 µg/ml) was detected in the blood. HSP70 was negative and myoglobin was positive immunohistochemically. It was thought that the subject died of acute MA intoxication based on the high MA concentration, although rhabdomyolysis was suspected. It is suggested that myoglobin and HSP70 immunostaining are useful to diagnose MA poisoning. *J. Med. Invest.* 50 : 112-116, 2003

Keywords : methamphetamine, kidney, myoglobin, the 70 kDa heat shock protein, immunohistochemistry

INTRODUCTION

It is considered that the sudden death of methamphetamine (MA) abusers is caused by acute MA intoxication and/or by hyperthermia, metabolic acidosis, reversion tolerance, or hypersensitivity (1). To diagnose the cause of death when MA is detected, the MA concentration in the blood is very important, and it has been reported that the lethal MA concentration in the blood is 4.48 µg/ml (3.0 µmol/dl) (2). If the MA concentration is more than

3.0 µmol/dl in the blood of the cadaver, the cause of the death can be diagnosed as acute intoxication. The literature describes that autopsy findings in cases of overdose are generally non-specific (3). There have been many studies about the relation between the cardiac lesions of chronic MA abusers and the cause of death (4, 5). However, hyperthermia can also cause death, so it is sometimes difficult to diagnose the cause of death in MA abusers.

We report two cases of methamphetamine poisoning in which the cause of the death was examined pathologically and toxicologically.

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CASE REPORT

Case 1 : A 22-year-old male was found and dead

in a rice field. The surrounding rice plants were flattened, and his clothes were scattered around. As a result of the police investigation, it was discovered that he lived close by. As the circumstance and cause of his death were uncertain, a forensic autopsy was performed.

Autopsy findings

His height was 177 cm, and he weighed 74kg. There were many abrasions and subcutaneous bleeding on the body. The rectal temperature was 40 at the postmortem examination. The brain was edematous, and various organs were congested. There was a blood-like solution and bubbles in the trachea and bronchus. Rigor mortis was relatively advanced. Table 1 shows the weight of the main organs. It was presumed from these findings that the postmortem time was about 15 hours.

Histological findings

Severe congestion was found in every organ, and it was especially remarkable in the lung, liver, kidney, and spleen. The lungs were edematous. In the heart, there were no abnormal findings except these. In the proximal tubules, the epithelia were swollen and their nuclei were enlarged (Fig. 1a).

Toxicological examination

In blood from the heart, 0.75 µg/ml of MA was detected by GC/MS (gas chromatography-mass spectrometry) analysis (7). It was 16.8 µg/ml in the urine, and 6.2 µg/ml in the stomach contents. Amphetamine was also detected in the blood by GC/MS analysis.

Immunohistochemical findings

We conducted an immunohistochemical study on the skeletal muscle with an antibody against myoglobin (1:200, Dako, Denmark), and on the kidney with an antibody against 70 kDa heat shock protein

(HSP70, 1 : 1,000, Sigma-Aldrich, USA). Immunostaining was carried out using the AutoProbe II™ staining kit (Biomedica, USA) and MicroProbe™ system (FisherBiotech, USA) according to the manufacturer's instructions based on streptavidin-biotin complex technology with diaminobenzidine chromogen. These samples were stained with positive and negative control slides.

The immunoreactivity of myoglobin was decreased in the skeletal muscle (Fig. 2 a). Myoglobin was negative, and HSP70 (Fig. 3a) was positive in the kidney.

Case 2 : An 18-year-old female. She had stayed at a hotel with her boyfriend over night. She took methamphetamine orally with him towards noon on the day of death. After a while, she groaned and fell into dyspnea for about 3 hours, and then

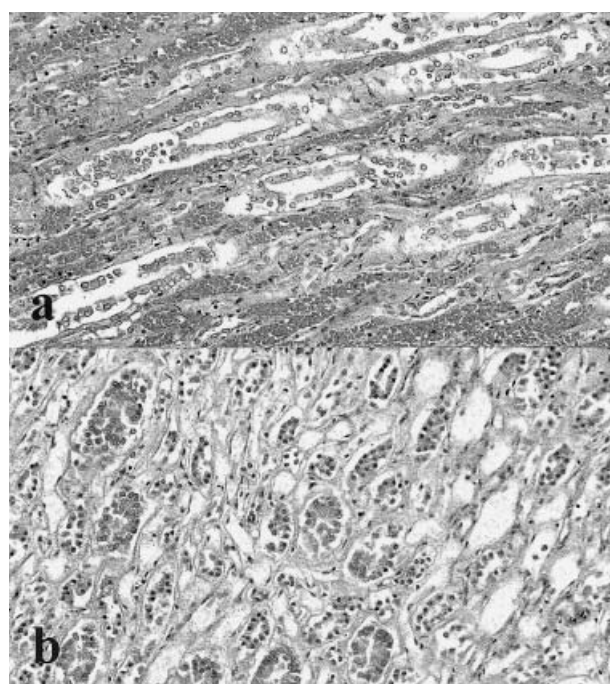


Fig. 1. HE staining of the kidney (×50). a ; case 1, b ; case 2

Table 1. The weight of the main organs (g).

organs	Case 1	(mean ± SD)	Case 2	(mean ± SD)
Brain	1680	(1480 ± 123)	1395	(1388 ± 87)
Heart	315	(309 ± 45.9)	210	(224 ± 23.5)
Lung	L 525	(431 ± 149.9)	490	(451 ± 98.4)
	R 570	(505 ± 178.8)	510	(500 ± 97.9)
Kidney	L 110	(149 ± 30.1)	105	(129 ± 19.3)
	R 155	(135 ± 23.3)	92	(125 ± 20.4)
Liver	1374	(1429 ± 263)	1140	(1271 ± 173)

L : left, R : right, Mean ± SD is the mean weight ± standard deviation from the data of normal Japanese (6).

she died.

Autopsy findings

Her height was 171 cm, and she weighed 53 kg. There were a few abrasions and subcutaneous bleeding on the body. Hemorrhaging was found at the lower points of attachment of the left sternothyroid muscle. The lungs were highly congested and were

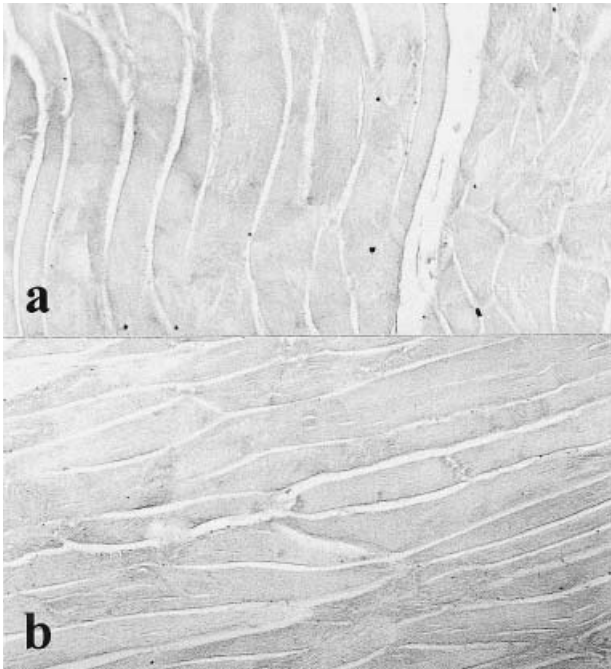


Fig. 2. Myoglobin staining of the skeletal muscle ($\times 50$). a ; case 1, b ; case 2

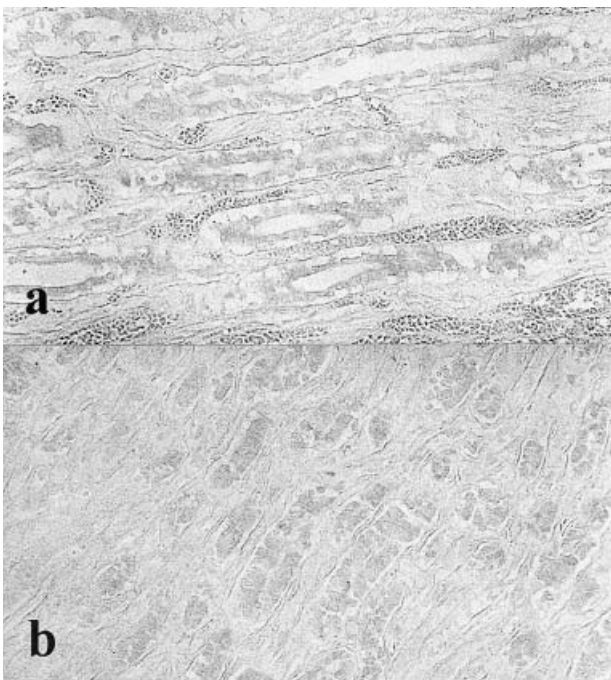


Fig. 3. Immunohistochemical staining of the kidney ($\times 50$). a ; HSP70 (case 1), b ; myoglobin (case 2)

ballooning. A relatively large amount of blood flowed from a section of the lung. A large amount of dark-red bloody fluid and foam were in the trachea and bronchus. Cyanosis was observed in the fingers and toes. Rigor mortis was relatively advanced. The rectal temperature was 32.5 at the postmortem examination. Table 1 shows the weight of the main organs. It was presumed that the postmortem time was about 17 hours from these findings.

Histological findings

The edema in the lungs was severe. The other observed findings were almost identical to case 1 (Fig. 1b).

Toxicological examination

The MA concentration was 17.0 $\mu\text{g/ml}$ in blood from the heart. The urine was tested qualitatively by Triage[®] Drugs of Abuse Panel plus Tricyclic Antidepressants kit (Biosite Diagnostics, Inc., USA), and "Amphetamine", amphetamine, methamphetamine, and related drugs, was positive. Methamphetamine and amphetamine were detected qualitatively in the stomach contents, and amphetamine was also detected in the blood by GC/MS analysis.

Immunohistochemical findings

Myoglobin immunoreactivity was also decreased in the skeletal muscle in this case (Fig. 2b). HSP70 was negative although myoglobin (Fig. 3b) was positive in the kidney.

DISCUSSION

It was recently reported that MA causes rhabdomyolysis (8-10), myoglobinuria, and acute renal failure (11-14).

Myoglobin is the heme protein in cardiac and skeletal muscle, and transports oxygen from blood to the tissues. When the muscle is injured (cardiac infarction, trauma, etc), it separates easily from the tissue, and comes out in blood and urine. Therefore, the measurement of myoglobin is used to diagnose muscle injury. Heat shock proteins (HSPs) are molecular chaperones induced by various stresses such as heat stress, inflammation, oxidative injury, and ischemia (15-19). 70 kDa HSP (HSP70) is particularly effective in protecting cells against heat stress. We used HSP70 in this study as an index of the presence of antemortem hyperthermia and cell

damage.

In case 1, the cause of death was not thought to be acute MA intoxication because the concentration of MA in the blood (0.75 µg/ml) did not reach a lethal level. No fresh track marks which seemed to be the intake route of MA were found at the autopsy. MA was detected in the stomach contents, and its concentration (6.2 µg/ml) was higher than in the blood. It was therefore considered that he had taken MA orally. On the other hand, it was suspected that he had acted abnormally immediately before his death from the condition of the rice field (the surrounding rice plants were flattened, and his clothes were scattered around). Moreover, the rectal temperature was 40 °C at the postmortem examination. HSP70, a heat stress marker, was positive-stained in the kidney. As myoglobin was decreased in the skeletal muscle, it was suspected that muscular hyperactivity caused his hyperthermia. It also causes fatal metabolic acidosis (1). Therefore, it was considered that he died of hyperthermia and metabolic acidosis caused by muscular hyperactivity. Myoglobin immunoreactivity was negative in the kidney. It has been reported that elevated urinary myoglobin after muscle damage is closely related to longer survival (20). It was suspected that his muscular damage occurred in the agonal stage.

In case 2, severe congestion was observed in every organ, macro- and microscopically. In particular, severe edema was observed in the lungs, and dark-red bloody fluid and foam were in the trachea and bronchus. The fingers and toes were cyanotic. These findings did not contradict the testimony that she had fallen into dyspnea. Myoglobin was decreased a little in skeletal muscle, and was positive in the kidney, and rhabdomyolysis was suspected. In the police investigation, she was alive for about 3 hours after taking MA. It was considered that myoglobin was detected because of her survival duration, longer than that of case 1 (20). In addition, the MA concentration in the blood was 17.0 µg/ml, which was over the lethal concentration. Therefore, the cause of her death was considered to be acute MA intoxication.

From these cases, we propose that not only toxicological analysis but also immunohistochemical staining is very useful in the diagnosis of methamphetamine poisoning.

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