Hepatic Injury and Gluconeogenesis After Subcutaneous Injection of Monochloroacetic Acid in Rats

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Abstract

Objective: Monochloroacetic acid (MCA) is corrosive to skin, and causes not only chemical injury but also fatal systemic poisoning. Little is known about the cause of death. We studied the acute toxicity of MCA before clinical symptoms appeared in fasting rats. **Methods:** Blood samples were analyzed 2 h after subcutaneous MCA injection (LD₉₀: 162 mg/ml kg body weight). Control rats were injected with saline. **Results:** Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were about 1.5-fold higher than in the controls, and mitochondrial AST (mAST) was 2-fold higher. Blood urea nitrogen and creatinine were significantly increased, while serum glucose was significantly decreased in the treated group. Lactate was 6-fold higher and pyruvate was 13-fold higher than in the controls. **Conclusions:** MCA caused injury to the liver and kidneys but these injuries were slight. However, the larger increase in mAST indicated that hepatocellular mitochondria were selectively targeted. Hepatocellular mitochondrial injury decreased gluconeogenesis and caused hypoglycemia and extremely high levels of lactate and pyruvate. Hypoglycemia and lactic acidosis were insidious before the critical symptoms appeared and this combination accelerated to death, affecting other organs such as the heart and brain. Nosotropic therapy of these abnormalities up to the appearance of symptoms may help to establish an early therapy for skin exposure to MCA.

Key words: monochloroacetic acid (MCA), chemical injury, acute lethal toxicity, hypoglycemia, lactic acidosis

Introduction

Monochloroacetic acid (MCA, ClCH_2COOH) is a colorless crystalline substance with the following physical and chemical properties: molecular weight: 94.50; pKa: 2.86; melting point: 50–63°C; boiling point: 189°C; and relative density (water=1): 1.6. MCA is highly soluble in water, and is also soluble in benzene, ethanol, and ethylether (1). MCA is formed as one of several byproducts during chlorination processes for the disinfection of drinking water (2). MCA is a metabolite of several chlorinated hydrocarbons. MCA has been widely used as an herbicide and an intermediate in the industrial synthesis of carboxymethylcellulose, phenoxyacetic acid, thioglycolic acid, glycine, and indigoid dyes (3). MCA is usually prepared as an 80% solution at 60°C for transport and industrial use (4).

Strong acids, such as hydrochloric acid, sulfuric acid, and nitric acid, generally cause discoloration of tissues and coagulative necrosis. The latter also tends to prevent acids from seeping further into deep tissues (5). MCA solution is corrosive to tissues. This property has led to dermatologic use as a treatment for viral warts (6). However, it has been reported that MCA caused lethal systemic poisoning after skin exposure at chemical plants (7, 8). Five of the seven reported cases of skin exposure to MCA were fatal, and occurred by CNS dysfunction leading to coma and involvement of the cardiovascular system within a few hours after exposure. In the case of one man who was splashed with an 80% MCA solution over 25 to 30% of his body surface, systemic poisoning including disorientation, agitation, cardiac failure, and coma developed into death within a day (4). Another accident happened at an MCA manufactory in Niigata, Japan, in 1999. A worker had skin contact over his leg with 80% MCA at 60°C after slipping and falling. The chemical injury, almost second degree and partly third degree, extended over 10% of the body surface. His vital signs were normal just after the accidental exposure, but his general condition suddenly worsened after 1 h. He became unconscious, hypotensive and anuric, and died 6 h after the exposure (9). As

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noted above, high concentrations of MCA in solution cause not only local injury but also severe systemic toxicity leading to death in as little as a few hours. It is interesting that the serious or fatal symptoms suddenly appear without prodromal symptoms within a few hours. This clinical course is evidently different from serious thermal injury. There have been some reports that investigated the tissue distribution and target organs by oral gavage or intravenous injection. However, no information was found in the literature regarding the toxicity of MCA after skin exposure. It was reported that two-carbon chloroacetate enters the tricarboxylic acid (TCA) cycle and forms chlorocitrate, which inhibits acetate oxidation in the TCA cycle (4). However, the mechanisms underlying death after skin exposure to MCA have not been established, and neither has an effective therapy. In the present study, we sought to determine the target organs, especially the liver, using the parameters of mAST and acute toxicity before the appearance of clinical symptoms after skin exposure. An emergency therapeutic approach is suggested after accidental skin exposure to MCA.

Materials and Methods

Chemicals

MCA (purity>99%) was purchased from Wako Pure Chemical Industries, Osaka, Japan. MCA was dissolved in saline (Otsuka Normal Saline, Tokyo, Japan: Na⁺ 154 mEq/L, Cl⁻ 154 mEq/L, pH 6.4). The concentration of MCA in solution was 243 mg/mL, and the pH was 3.0. The solution was colored purple with a small amount of crystal violet to stain the area of infiltration. Subcutaneous injection of MCA solution was performed with a 1-mL glass hypodermic syringe (Seijodo, Tokyo, Japan) and a 27-G needle (Terumo, Tokyo, Japan). Saline was colored with crystal violet for the controls.

Animals and diets

Twenty 10-week-old SPF male Sprague-Dawley rats were obtained from Japan SLC. They were allowed to acclimate to our animal facility for 7 days after arrival, with free access to tap water and standard food (Funabashi Farm, Chiba, Japan; MM-3). They were housed in groups of five in polycarbonate cages in an air-conditioned room (22±1°C) illuminated for 12 h/day from 8:00 AM to 8:00 PM. For the 18 hours before the experiment, they were given only tap water and no food. The average body weight was 304 g. All aspects of these studies were conducted under the guidelines recommended by the Osaka Medical College Ethical Association for Accreditation of Laboratory Animal Care.

Experimental design

In our previous study of skin exposure to MCA, the time from exposure to death was irregular. In another previous study using subcutaneous MCA injection (162 mg/kg body weight), all rats had died by 3 h after the injection. The LD₉₀ after 24 h toxicity in male Sprague-Dawley rats has been reported to be 162 mg/kg body weight s.c. of MCA (10). The general condition and vital signs such as the respiratory and heart rates did not differ between the MCA group and the saline group up to 2 h after the injection. However, differences subsequently appeared

between the two groups. All rats worsened and finally died in the MCA group. MCA has been reported to be readily absorbed into the subcutaneous tissues following corrosive injury to the skin (11). Therefore, subcutaneous injection was used as a means of substituting for skin exposure.

In the main study, the rats were divided into two groups of 10 (control and treated), and anesthetized with pentobarbital sodium (75 mg/kg body weight i.p.). Hair on the lateral side of the thigh was shaved off. A subcutaneous injection was performed above the biceps femoris. The injection volume of MCA solution was about 0.2 mL per rat (162 mg/kg body weight s.c.). All the rats in this study were sacrificed for total blood collection at 2 h after the injection via direct cardiac puncture by thoracotomy, since the blood samples had to be evaluated for laboratory data to exclude other secondary causes of the critical condition. Control rats were given a subcutaneous injection of saline (0.2 mL), and blood samples were collected at 2 h following the injection using the same method. Samples were centrifuged for 3 min at 3000 rpm at room temperature. Hemolysis was not observed in either group. Each serum supernatant was stored at -20°C until measurements were taken. To measure the lactate and pyruvate levels, samples were mixed with 0.8 N perchloric acid (HClO₄) and then centrifuged for 10 min at 3000 rpm at 4°C. The supernatant fluids were stored as described above. The following serum biochemical parameters were measured according to the producer's standard protocols: aspartate aminotransferase (AST, ultraviolet method), mitochondrial aspartate aminotransferase (mAST, MDH-ultraviolet method after inactivation of the serum cytoplasmic AST (cAST) isoenzyme by proteinase K, A-kokusai kit, International Reagents Corporation, Japan), alanine aminotransferase (ALT, ultraviolet method), blood urea nitrogen (BUN, urease-glutamic dehydrogenase method), creatinine (Cr, Jaffe method), lactate dehydrogenase (LDH, ultraviolet method), LDH isozyme (agarose gel electrophoresis cataphoresis method), alkaline phosphatase (ALP, ultraviolet method), ALP isozyme (cellulose acetate membrane electrophoresis cataphoresis method), sodium (Na, ion-selective electrode method), potassium (K, ion-selective electrode method), calcium (Ca, colorimetric method), chloride (Cl, ion-selective electrode method), aldolase (ultraviolet method), amylase (enzymatic method, CNP-G₃), lactate (enzymatic method, CNP-G₇), pyruvate (enzymatic method, CNP-G₇), citrate (enzymatic method, CNP-G₇) and glucose (glucoseoxidase method).

Statistical analysis

Differences within groups were analyzed by paired Student's t-tests, while differences between the control and treatment groups were analyzed by unpaired Student's t-tests if the variances were equal, and Welch's test if the variances were unequal (F-test) (p<0.01). Statistical analysis was performed with SPSS 10.0J software (SPSS, Chicago). Results were considered significant when the p value was less than 0.01. Data are expressed as the mean±SD.

Results

Systemic status. In our previous study, clinical symptoms

Fig. 1 Macroscopic photographs of skin over thigh 2 h after subcutaneous injection of MCA.

A. Black round discoloration was bordered with white.

B. A perpendicular section shows staining crystal violet and a white-layer overlying the muscle.

were observed from the injection to death. Irregular tachypnea and peripheral cyanosis were observed beyond 2 h after the injection. Overt nervous dysfunction was not observed. Watery diarrhea and a little bloody nasal discharge occurred in three of the ten rats. Hematemesis, hematochezia, and hematuria were not apparent. A little macroscopic hemorrhaging was observed in the lungs of all the rats.

Local status. In the treated group, neither erosion nor ulcers were observed on the epidermis at 2 h after the subcutaneous injection. Erythema and vesiculation were absent. A round, dark brown area of discoloration about 15 mm in diameter was surrounded by a circular white band about 5 mm wide (Fig. 1A). This lesion was elastic hard. On sections cut perpendicular to the skin surface, crystal violet staining was seen between the subcutaneous tissue and fascia of the biceps femoris muscle. A whitish layer of coagulative necrosis was observed on the superficial aspect of the muscle. Deeper muscle and the femur showed no macroscopic abnormalities (Fig. 1B). These findings were common to all the rats in the treated group. In the controls, no macroscopic abnormalities were present and the same area as in the treated group showed crystal violet staining. The crystal violet staining revealed the depth of injection of each

solution into the subcutaneous tissue.

Serum biochemical examinations. AST, ALT, and mAST were significantly increased in the treated group compared with those in the controls ($p < 0.01$). The levels of AST and ALT were about 1.5-fold higher than in the controls, while mAST was 2-fold higher. BUN and creatinine were significantly increased in the treated group ($p < 0.01$) (Table 1). LDH was significantly

Table 1 Laboratory data 2 h after s.c. injection in MCA-treated and control groups

	Control group (N=10)	Treated group (N=10)
AST (IU/L)	132.3±35.6	188.1±35.1 [†]
mAST (IU/L)	11.56±3.2	22.4±4.9 [†]
ALT (IU/L)	35.1±6.3	57.3±11.9 [†]
BUN (mg/dL)	18.3±3.6	23.3±22.0 [†]
Creatinine (mg/dL)	0.42±0.04	0.68±0.09 [†]
Glucose (mg/dL)	148±16.6	74.7±27.5 [†]
Lactate (mg/dL)	5.6±2.9	35.3±12.8 [†]
Pyruvate (mg/dL)	0.36±0.15	4.6±1.4 [†]
Citrate (μg/mL)	32.2±3.0	34.8±3.3

mean±SD, [†] $p < 0.01$.

Table 2 Laboratory data 2 h after s.c. injection in MCA-treated and control groups

	Control group (N=10)	Treated group (N=10)		Control group (N=10)	Treated group (N=10)
LDH (IU/L)	953±257	1832±737 [†]	Na (mEq/L)	144±1.5	145±2.1
LDH1 (%)	12.4±4.3	10.3±3.5	K (mEq/L)	4.4±0.3	3.5±0.3 [†]
LDH2 (%)	12.7±5.1	11.4±3.9	Cl (mEq/L)	102±2.0	103±2.2
LDH3 (%)	4.9±1.7	6.5±1.8	Ca (mEq/L)	10.4±0.2	9.6±0.3 [†]
LDH4 (%)	8.9±1.7	9.2±2.1	Aldolase (U/L)	60.0±17.4	68.8±27.2
LDH5 (%)	60.9±8.9	56.4±18.5	Amylase (IU/L)	550±84	585±20.4
ALP (IU/L)	383±58	431±59			
ALP2 (%)	8.5±1.4	10.0±2.2			
ALP3 (%)	85.0±1.9	81.3±5.3			
ALP5 (%)	6.4±1.2	8.6±3.3			

mean±SD, [†] $p < 0.01$.

increased in the treated group compared with that in the controls ($p < 0.01$). However, the increase showed no organic specificity for any LDH isozyme fraction. ALP, ALP isozyme fractions, Na, and Cl were not significantly different in either group. K and Ca were significantly decreased in the treated group ($p < 0.01$). Aldolase and amylase were not different in either group (Table 2). Lactate and pyruvate were remarkably increased in the treated group. The level of lactate was 6-fold higher than in the controls, and the level of pyruvate was 13-fold higher. Citrate was not significantly elevated compared with that in the controls. The serum glucose level was significantly decreased to half the level of the controls ($p < 0.01$) (Table 1). In our previous study, the serum glucose level was under 30 mg/dL just before death.

Discussion

The increases in AST and ALT indicate hepatic injury. The lack of significant change in ALP established that the hepatotoxicity of MCA was hepatocellular and not intrahepatic cholestatic toxicity. As with hepatocellular diseases, acute liver failure is characterized by large increases in serum AST and ALT. mAST is an indicator of mitochondrial injury and is important in the clinical assessment of severe liver injury (11). However, the hepatic injury was slight considering the rates of the increases in AST and ALT. AST has two isoenzymes, one associated with the cytoplasm (cAST) and the other with the mitochondria (mAST). The half-life of cAST in the body is about 15 h while that of mAST is no more than 2 h. mAST increases when hepatocellular injury occurs to the mitochondria. The larger increase in mAST shows that the hepatocellular mitochondria were selectively targeted by MCA. The increases in BUN and creatinine indicate kidney dysfunction. The liver and kidney were target organs for MCA subcutaneous injection but these injuries were not severe. Organs other than these two could not be considered from these laboratory data. The levels of aldolase, K and ALP3 could be used to argue against severe injury to the skeletal muscle and bone (12). It was also reported that cutaneous damage from MCA is limited to the superficial layer according to pathologic findings (13). Acute pancreatitis was also negated. The LDH isozyme fraction could not specify the target organs in the treated group. It is conceivable that the increase in serum LDH could constitute a part of the homeostatic mechanism of hyperlactemia. Electrolyte disorder is one of the causes of death from thermal injury (12, 14). However, serious electrolyte disorder was not observed. The low levels of serum potassium and calcium were within the normal ranges. The significant differences would be caused by the influence of pH and anions such as bicarbonate, citrate, phosphate, and lactate (15). The target organs clarified by subcutaneous injection were in agreement with previous literature using oral gavage and intravenous injection (16–18). However, there is a question

as to whether slight hepatorenal injury leads to sudden death.

The changes in serum glucose were converse despite the fasting of both groups. In general, physical stress is reported to cause hyperglycemia (19). Hyperglycemia is also induced in severe thermal injury (20). The hyperglycemia in the controls was caused by increased hepatic gluconeogenesis, since the hepatic glycogen is almost consumed by fasting for 18 h in this study, and therefore, the body could not obtain glucose from glycogen (21). In early starvation, gluconeogenesis largely occurs in the liver, and to a much smaller extent in the renal cortex. The serum glucose level is maintained by gluconeogenesis in the liver. Some reports have described that MCA causes hyperglycemia, while others have described hypoglycemia (2, 22). The difference depends on whether glycogen still exists in the liver. In the previous study, the glucose level did not decrease in feeding rats. The hypoglycemia is due to interference with gluconeogenesis in the treated group. MCA reportedly enters the TCA cycle like any other two-carbon-atom acetate and inhibits aconitase (23). Chloropyruvic acid was produced as a metabolite from MCA in the TCA cycle, and this has been reported to inhibit pyruvate carboxylase in gluconeogenesis (24).

The conspicuously high levels of lactate and pyruvate were due to interference with gluconeogenesis in the liver, since glucose is synthesized from lactate and pyruvate in the TCA cycle in early starvation. These clinical results gain support from a study showing that sodium monochloroacetate causes the release of lactate and pyruvate from cultured liver epithelial cells into the culture medium (22). Although lactic acidosis naturally arises in fatal conditions, MCA caused hyperlactemia in the early stage without prodromal symptoms.

Skin exposure to MCA causes a decrease in gluconeogenesis by interference with the TCA cycle. The present study indicates that hypoglycemia and lactic acidosis are closely related to the lethal toxicity of MCA. The clinical manifestations are sudden in onset, but hypoglycemia and lactic acidosis are insidious and this combination accelerates to death, affecting other organs such as the heart and brain. Hypoglycemia causes CNS disorder. Hyperlactemia reportedly causes bradycardia, a marked reduction in the ventricular contractile force and irreversible nerve cell injury in the brain (25, 26). Most previous reports indicate that the first clinical symptom is CNS involvement, such as disorientation, agitation, unconsciousness, and coma. These symptoms may be associated with encephalopathy from the hypoglycemia. Many antidotes for MCA have been studied, but there is no effective antidote to date. Correcting the hypoglycemia and lactic acidosis is a nosotropic therapy, but may be one of the emergency measures that should be taken just after skin exposure to MCA.

Further investigations are necessary to fully understand the lethal mechanism of MCA exposure. This research was partly presented at the 73rd Annual Meeting of the Japanese Society for Hygiene, in Oita, Japan, March 26 to 29, 2003.

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