〈*Original Article*〉

Acute Toxicity after Monochloroacetic Acid Exposure in Rats

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ABSTRACT

This study aimed to clarify the lethal toxicity in acute monochloroacetic acid (MCA) poisoning. MCA was subcutaneously injected in rats and blood was collected. We measured metabolites and hormones related to gluconeogenesis. The blood glucose level in the MCA-treated group was significantly reduced. Serum concentrations of acetoacetate and 3-hydroxybutyric acid were decreased and serum free fatty acid levels were increased in the MCA-treated group, whereas serum concentrations of carnitine were reduced. Concentrations of plasma ammonia in the treated group were more than 5 times higher than those in the control group. Serum concentrations of hyperglycemic hormones (catecholamine, ACTH, cortisol, glucagon) were significantly increased in the MCA-treated group. Serum concentrations of insulin were within the reference value in both groups. It was concluded that hypoglycemia caused by exposure to MCA is not due to the resultant hormonal disturbance, but might be partly induced by impaired beta-oxidation of fatty acid owing to the inadequate biosynthesis of carnitine. Hypoglycemia, hypoketonemia and hyperammonemia may play roles in MCA poisoning deaths.

Introduction

Monochloroacetic acid ($ClCH_2COOH$, MCA) is widely produced in chemical industries as an intermediate in syntheses, such as of carboxymethyl cellulose, phenoxy acetate, thioglycolic acid, glycine, indigoid dyes, and drugs. The total annual world production is about 400,000 tons (BERARDI, et al., 1987; DARTCH, et al., 2000; KULLLING, et al., 1992; SAGHIR et al., 2001,). In industrial fields, MCA is used as an 80% solution, as flakes or as its sodium salt (KULLLING, et al., 1992).

MCA is a strong irritant to the skin, eyes and mucous membranes, and is highly corrosive to tissues in either its molten or solid form (BRYANT, et al., 1992; DARTCH, et al., 2000; KULLLING, et al., 1992; QUICK, et al., 1992;), and is known to be 25-40 times more toxic than acetic, dichloroacetic, or trichloroacetic acids (ROGERS, 1995).

Further, MCA is known to cause severe damage to the large energy-consuming tissues by interfering with the metabolism of fuel molecules of the tricarboxylic acid (TCA) cycle and gluconeogenesis (SAGHIR et al., 2001). It enters the TCA cycle as a two-carbon chloroacetate and forms chlorocitrate by reacting with oxaloacetic acid, inhibiting further oxidation in the TCA cycle. MCA also inhibits pyruvate carboxylase in the gluconeogenesis pathway, which further aggravates energy depletion (SAGHIR et al., 2001). The inhibition of pyruvate carboxylase by MCA has also

been reported in isolated rat hepatocytes (THOMAS and HALESTRAP, 1981). Recently, reflecting its increased use, accidental exposures to MCA are often reported. Kulling et al. reported a fatal case where, after their body surface being splashed on with MCA at an industrial plant, a worker died several hours after the accident (KULLLING, et al., 1992). MCA is rapidly absorbed through the skin and can produce death by systemic poisoning (BERARDI, et al., 1987; BHAT, et al., 1990; DARTCH, et al., 2000; KULLLING, et al., 1992). However, the mechanism by which death occurs from MCA exposure is still not completely understood, and various treatments have been tried without conclusive evidence of their effectiveness (KULLLING, et al., 1992; KUSCH, et al., 1990; SAGHIR et al., 2001).

This investigation aimed to clarify the lethal toxicity mechanism in acute MCA poisoning, and to propose effective safety measures by devising an adequate emergency treatment for accidental exposure to MCA. We reproduced experimentally the state induced by the acute lethal toxicity after MCA exposure and obtained laboratory data on the early phase situation.

Materials and Methods

MCA solution

MCA (purity>99%) was purchased from Wako Pure Chemical Industries; Ltd., and dissolved in saline (OTSUKA NORMAL SALINE: Na^+ 154mEq/L, Cl^- 154mEq/L, pH 6.4). The concentration of the MCA in solution was 243mg/ml, and pH was 3.0.

Animals

Ten-week-old male SPF Sprague-Dawley rats were acclimatized in our animal facility for 1 week before the experiment. The animal rooms were maintained at a constant temperature (22.0±1.0℃) and with a 12-hour light/dark cycle (lighting period: 8:00-20:00,dark period: 20:00-8:00). Five or four rats were housed in each polycarbonate cage with free access to stock feed (Funabashi Farm MM-3) and tap water. They were given only tap water and no diet for 18 hours before the experiment.

MCA administration and collection of blood

Eighteen rats weighing from 290 to 320g were divided into two groups; MCA treated and control. Before MCA treatment, animals were sedated by intraperitoneal injection of pentobarbital sodium (75mg/kg.) The lateral side of the left thigh was shaved trying not to damage the skin

The rats of the MCA group were injected subcutaneously with 0.2ml MCA using a plastic hypodermic syringe (1ml, Seijo-do Tokyo Japan) mounted with a 27G needle (TERMO Tokyo Japan). The dose of MCA was 162mg/kg, which is reported to be the dosage for LD90 after 24 hours in Male Sprague-Dawley rats (HAYES et al. 1973). To clarify the mechanism of death occurring several hours after MCA exposure, blood was collected by exsanguination in the carotid artery exactly 2 hours after MCA administration. The rats of the control group were subcutaneously injected with 0.2ml of physiological saline using a plastic syringe and blood was collected as for the MCA treated group.

Blood analysis

Blood glucose was measured by an enzymatic electrode assay (GA-1160, Arkray) Serum free fatty acids and ketone bodies (acetoacetate and 3-hydroxybutyric acid) were determined using an enzymatic assay kit (AU800, Olympus). Serum carnitine was measured by an enzymatic cycling assay (JCA-BM12, Nihondenshi). Plasma ammonia concentration was determined using ammonia gas BCG chromometry (Amino check meter, Kyoto Daiichi). Serum concentrations of ACTH, cortisol and glucagon were determined by immunoradiometric assay (ARC-950, Aloka). Serum concentrations of catecholamines and insulin were determined by high performance liquid chromatography (Hitachi L6200, Hitachi) and enzyme immunoassay (AIA1200i, Toso), respectively.

Stastical analysis

Differences between numerical data were evaluated by the unpaired Student's t-test if the variances were equal, and Welch's t-test if variances were unequal (F-test) ($p<0.05$). Statistical analysis was performed with SPSS® 10.0J software from SPSS INC. Results were expressed as mean±SD. A p value less than 0.05 was considered significant.

Results

As shown in Table 1, the blood glucose level 2 hours after the injection of MCA decreased by about 80% in the MCA-treated group compared to controls ($p<0.01$). Serum free fatty acid was increased about 40% ($p<0.01$), whereas ketone bodies, acetoacetate and 3-hydroxybutyric acid, were significantly reduced in the MCA-treated

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

	Controls (N=9)	MCA treated group (N=9)
blood		
Glucose(mg/dl)	72.6±10.4	16.0±3.7**
serum		
Free fatty acid (μEq/L)	829±110	1180±260**
Acetoacetate (μmol/L)	535±220	138.8±18.7**
3-Hydroxybutyric acid (μmol/L)	1023±339	53.2±11.4**
Total cartnitine (μmol/L)	72.8±8.6	42.0±7.7**
Free carnitine (μmol/L)	31.3±2.4	20.2±3.5**
Acylcarnitine (μmol/L)	41.4±6.8	21.7±4.4**
plasma		
Ammonia (μg/dl)	58.5±5.4	263±135**

mean±S.D, **p<0.01

group compared with controls (p<0.01). Total carnitine, free carnitine and acylcarnitine levels in the serum were significantly decreased in the MCA-treated group compared with controls (p<0.01). Two hours after injection with MCA, the concentration of plasma ammonia in the treated group was significantly elevated, being more than 5 times higher than that of the controls (p<0.01).

As shown in Table 2, the serum concentrations of the hyperglycemic hormones (adrenaline, noradrenaline, cortisol and glucagon) were significantly increased 2 hours after the injection with MCA compared with controls (p<0.01). Serum concentrations of dopamine and ACTH were also significantly increased in the MCA-treated group (p<0.05), while the serum concentration of insulin was within the reference value (<2.0 μ U/ml) in both groups.

Table 2 Concentrations of glucose regulatory hormones in serum in MCA-treated and control groups

	Controls (N=9)	MCA treated group (N=9)
ACTH(pg/ml)	124±117	278±137*
Adrenaline (pg/ml)	576±864	4347±2104**
Noradrenaline (pg/ml)	278±56	614±259**
Dopamine (pg/ml)	53.1±13.6	65.9±11.5*
Cortisol (μg/dl)	3.51±0.66	4.71±0.85**
Glucagon (pg/ml)	83.2±7.3	1632±536**
Insulin (μU/ml)	2.0>	2.0>

mean±S.D, *p<0.05, **p<0.01

Discussion

The dosage of MCA used here, 162 mg/kg s.c. is the amount reported for LD90 after 24 hours in Male Sprague-Dawley rats (HAYES, et al., 1973). Some rats died several hours after MCA exposure (KULLING, et al., 1992; ROGERS, 1995), but almost all rats died about 3 hours after exposure to the same dose of MCA in our previous study. We assumed that this dosage matches the amount inducing acute lethal toxicity.

Hypoglycemia, hypoketonemia, hyperammonemia and the increase of free fatty acid suggest that MCA has an inhibitory effect on the oxidation of fatty acid. Fig.1 shows the changes in concentrations of metabolites in fatty acid degradation after MCA administration. Fatty acids are the precursors of ketone bodies, and once activated long chain acyl-CoA combining to carnitine can be transported through the mitochondrial membrane. β-oxidation of fatty acids involves successive cleavage and release of acetyl-CoA, which is oxidized in the citric acid cycle, or enters the ketogenesis pathway. We suggest that hypoglycemia partly resulted from reduced gluconeogenesis owing to impaired oxidation of fatty acids, because gluconeogenesis is dependent upon fatty acid oxidation, and any impairment in fatty acid oxidation leads to hypoglycemia. We consider that hypoglycemia and hypoketonemia were partly the cause of death. Carnitine, synthesized from methionine and lysine in the liver and kidney, transports long-chain fatty acids into the mitochondrial matrix to provide cellular energy for β-oxidation. Carnitine deficiency results in impaired β-oxidation of fatty acid. (WANG et al. 2000). Total carnitine level in serum was markedly decreased in the MCA-treated group, while free- and acylcarnitine levels in serum were also reduced. From these results we suggest that MCA

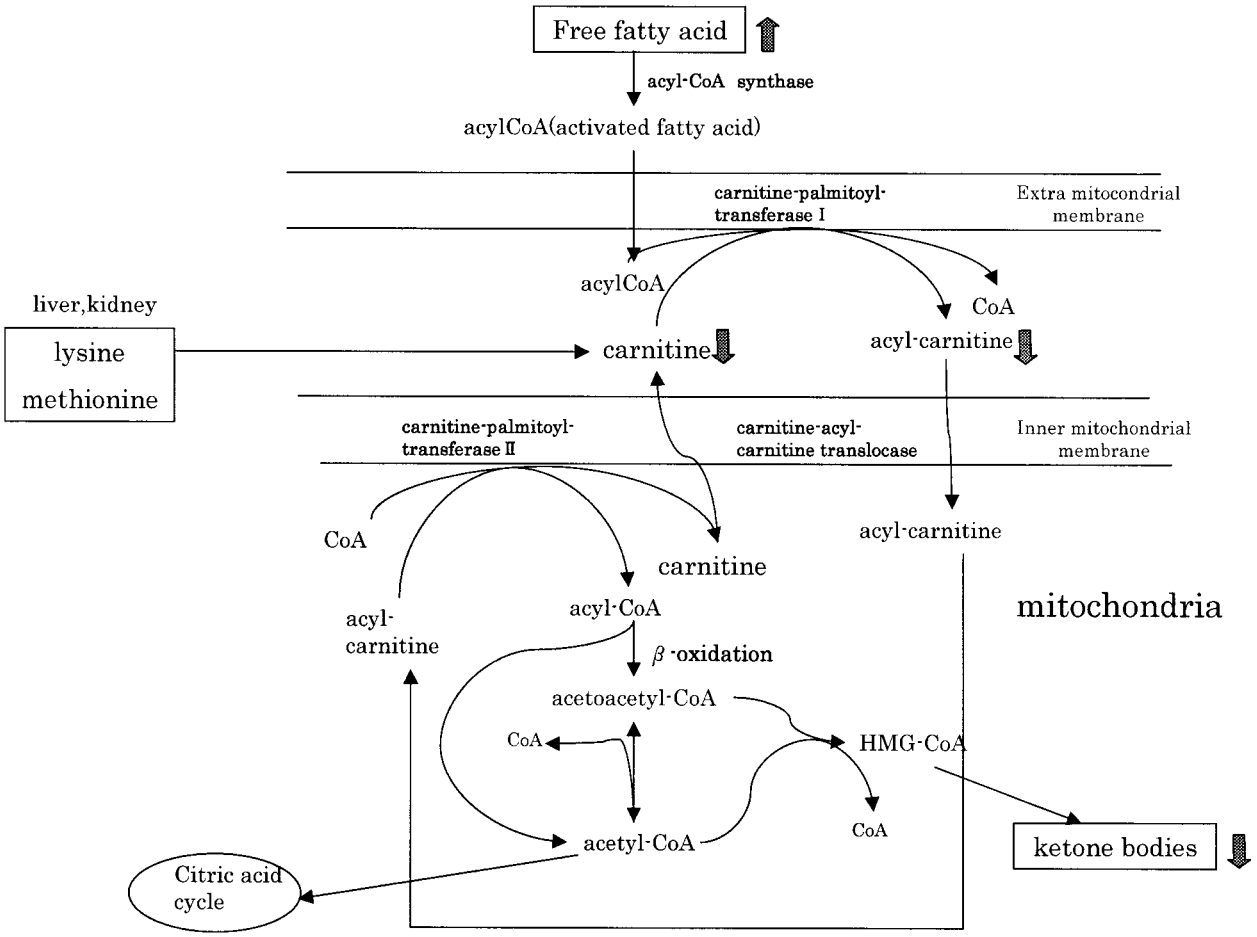

Fig. 1 Changes of materials in the pathway from free fatty acid to ketone bodies after MCA injection. '⬆' and '⬇' indicate increases and decreases in the corresponding compounds, respectively. HMGCoA is the abbreviation for 3-hydroxy-3-methylglutaryl CoA.

affects the synthesis of carnitine, and that the carnitine deficiency causes the impairment of the pathway from free fatty acid to ketone bodies. We suggest that hyperammonemia is also partly induced by the impaired β-oxidation. In cases of fatty acid oxidation inhibition, hyperammonemia can arise from low concentrations of acetyl-CoA, as suggested by the limited availability of N-acetylglutamate, an essential cofactor of the urea cycle enzyme carbamoylphosphate synthase (RÖSCHINGER, et al., 2000). Ammonia intoxication is life threatening, with even minute quantities of ammonia being toxic to the central nervous system (MATHIAS, 2001). Hyperammonemia may disrupt the blood brain barrier (BBB) (ZIYLAN, et al., 1993), and be a part of the lethal toxicity. Common clinical features of fatty acid oxidation disorders are hepatomegaly, heart failure, respiratory distress, acute encephalopathy (Reye's-like syndrome), fatty infiltration of parenchymal organs, acute dysfunction of fatty-acid dependent tissues (skeletal muscle, heart, and liver), hypoglycemia owing to reduced gluconeogenesis, hyperammonemia, impaired ketogenesis (hypoketonemia) in the presence of raised plasma free fatty acid, and metabolic acidosis (AL ODAIB, et al., 1998; KAMIJO, et al., 1997; MATERN, et al., 1999; TOKUNAGA, et al., 2000; ROSCHINGER, et al., 2000; YAMAGUCHI, et al., 2001; WANG, et al., 2000; PANDE, 1999). Numerous features of MCA poisoning have been reported and can be summarized as central nervous system features, severe myocardial depression with shock or nonspecific myocardial damage, and severe metabolic acidosis (DARTSCH, et al.; 2000; KULLING, et al., 1992;). As well, an autopsy has shown fatty infiltration of the liver in MCA poisoning (ROGERS, 1995). These features closely resemble those of fatty acid oxidation disorders, supporting the idea that MCA exposure affects the pathway from fatty acid to ketone bodies, including β-oxidation. We also investigated whether hypoglycemia could be induced by hormone abnormality. There are few reports concerning disturbances of glucose regulatory hormones after exposure to MCA. Increases in hyperglycemic hormones were seen in the MCA-treated group, but no change in insulin in either group suggested that glucose regulatory hormones did not cause hypoglycemia; rather that the increase of counter-regulatory hormones seemed to be induced by hypoglycemia or the stress of MCA injection. Therefore, MCA-induced hypoglycemia may not be due to hormonal disturbance. This study suggested the lethal toxicity induced by MCA exposure is, in part, made up of hypoglycemia, hypoketonemia and hyperammonemia. We found that MCA-treatment caused carnitine deficiency. Then, we suggest that carnitine deficiency may cause impairment of fatty acid oxidation, resulting in hypoglycemia, hypoketonemia and hyperammonemia.

In accidents involving MCA exposure, immediate monitoring and correction of the states of the serum glucose, serum ketone bodies and plasma ammonia may help to prevent death.

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Note. The Experiments described in this paper were performed with adherence to the guidelines of Japanese association for Laboratory Animal Science for the use of experimental animals.

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