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PATHOLOGICAL STUDIES ON THE CHRONIC TOXICITY OF METHAMPHETAMINE ADMINISTRATION

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ABSTRACT: *Toxic effects of chronic administration of methamphetamine (MA) to SD rats were studied in respect to histopathological changes induced in each organ. In experimental groups liver weights decreased and brain weights increased markedly compared with controls in the 12th month after subcutaneous injection of 0.5 mg and 5 mg/kg/BW MA. Serum alkaline phosphotase levels increased, but marked decreases of cholesterol, triglyceride, and BUN levels were checked depending on both the dose of MA and duration of treatment. Microscopic examination revealed severe hyperplasia of biliary epithelia, cellular degeneration, and mild fibrosis in livers; gliosis, activation of microglia, and vascular hypertrophy in brain; and edema and vascular hypertrophy in heart. These results indicate that long-term administration of MA has the potencial to induce hepatic, nervous, and cardiac toxicity. Also it may produce vascular hypertrophy in certain vessels which eventually lead to hypertension.*

Key words: *Methamphatamine (MA), hyperplasia of biliary epithelia, activation of microglia vascular, hypertrophy, hepartic, nervous, and cardiac toxicity.*

INTRODUCTION

Methamphetamine (MA) is one of the many amphetamine derivatives that were first synthesized in 1914 by Schmidt and massively produced by the phamaceutical industry from 1919 (Giannini,1989). Today, stimulent drugs such as the MA remain among the most widely used and abused of the many psychoactive compounds available in some coutries (Robinson,1986). MA use is also emerging as an important drug abuse problem from the mid 1960's in Korea, and the National Institute of Health reported that MA related crimes against psychotropic drug control law accounts for 85.8% among total drug abuse cases in 1988 (The Ministry of Health and social Affairs, 1990).

In spite of the nation-wide abuse of MA, the detailed morphological alteration

due to administration of MA and their possible damage mechanism have not been clarified. The purpose of this study was to identify the sites of the target damage and to define the pathological changes following long-term administration of MA.

MATERIALS AND METHODS

Animals

Male SD rats were obtained from KRICT, and the bred weighing 140 to 160g in our facilities were used. The animals were randomly distributed in polypropylene cage on sawdust bedding (Beta-Chip) with tap water and commercial rodent chow pellet(Sam Yong) available *ad libitum*.

Chemical Administration

MA was obtained from the Korean authorities and was prepared as a saline solution. In this report, consecutive subcutaneous injections were carried out for 12 months at two dose levels: 0.5 mg/kg and 5 mg/kg/BW, in a volume of 0.1 m*l*. The control group received saline doses of the same volume. The drugs were daily administered between 08:30 and 09:30 a.m.

Weights of Body and Organ

In month 6 and 12, animals were weighed and terminated by cervical dislocation. The organs were removed, trimmed of excess connective tissue, and weighed. Results were analysed by absolute weights and ratio to body weight.

Blood Samples and Data Analysis

A number of animals from each group were selected for retrospective study of the hematologic response to MA administration. These animals had a base-line hemogram within acceptable laboratory values and complete hemologic data for a 12-month period. Hematologic variables were examined once each scheduled month. For comparative purpose, base-line values and determinations at each scheduled month were also analyzed.

Histopathological Observation

All organs of the sacrificed animals were closely examined for gross lesions. Tissue blocks were selected from each animal organ, and were fixed in buffered formalin for hematoxylin-eosin(H-E) staining. Each tissue sample from the animals was prepared for electron microscopy (EM) by the conventional EM preparation methods.

RESULTS

General Observation

The behavior of the MA treated rats was characterized by frequent head shaking, bristling of hairs, salivation which occurred soon after the drug administration, and subsequent elevation of body temperature in most animals.

Changes in Body and Organs Weights

The gain of body weight of the long-term MA-treated group is shown in Table 1. Although body weights showed considerable variations among the individual rats throughout the duration, the mean body weights of 0.5 mg/kg and 5 mg/kg treated groups showed an increasing tendency throughout the duration as control group did. The final body weights of the control, 0.5 mg/kg, and 5 mg/kg treated groups in the 12th month were 352 gm, 353 gm, and 366 gm, respectively.

Upon dissection, organs were weighed at the time of sacrifice to determine if the MA affected the animal's organ weights. The liver weights of 0.5 mg/kg and 5mg/kg MA-treated animals showed a significant decreasing tendency measured in the 12th month, while the brain weights of 5 mg/kg MA-treated group revealed a significicant increase in the 6th and the 12th month. 0.5 mg/kg group also showed increased brain weights in the 12th month. The spleen weights of the 5 mg/kg group also increased significantly in the 12th month. The change in the ratio of each organ to body weight was almost the same as the change of absolute weight of each organ(Table 1).

Blood Changes

Serum alkaline phosphatase levels increased depending on both the dose of MA and the duration of treatment. Marked decrease of cholesterol,triglyceride, and BUN levels developed according to both the dose of MA and the duration of treatment. (Table 2)

Histopathological Changes

Since we were interested in studying chronic changes caused by long-term treat-

Table 1. Effects of Methamphetamine on Body Weights and Various Organ Weights.

EXP.Group	Body (gm)	Heart(%)[a] (mg)	Liver(%)[a] (mg)	R. Kidney(%)[a] (mg)	Brain(%)[a] (mg)	Spleen(%)[a] (mg)
6 Month						
Conrtol	275± 27	1.11± 0.02 (0.0040)	9.22± 0.35 (0.0335)	0.94± 0.03 (0.0034)	1.92± 0.05 (0.0069)	0.77± 0.02 (0.0028)
0.5 mg/kg MA	270± 43	1.17± 0.04 (0.0043)	8.29± 0.41 (0.0307)	0.97± 0.05 (0.0035)	1.88± 0.03 (0.0069)	0.85± 0.05 (0.0032)
5 mg/kg MA	278± 35	0.96± 0.04 (0.0035)	8.57± 0.36 (0.0308)	1.02± 0.04 (0.0037)	2.09± 0.06* (0.0075)	0.94± 0.07 (0.0034)
12 Month						
Control	352± 34	1.21± 0.04 (0.0034)	12.38± 0.48 (0.0352)	1.15± 0.05 (0.0034)	2.00± 0.09 (0.0057)	0.83± 0.03 (0.0024)
0.5 mg/kg MA	353± 27	1.12± 0.07 (0.0033)	10.15± 0.62* (0.0289)	1.11± 0.04 (0.0033)	2.37± 0.12* (0.0069)	0.92± 0.04 (0.0027)
5 mg/kg MA	366± 38	1.17± 0.05 (0.0032)	10.94± 0.73* (0.0309)	1.18± 0.06 (0.0034)	2.34± 0.14* (0.0068)	1.06± 0.07* (0.0031)

Rats were injected with MA for 360 concecutive days with daily dose of 0.5 mg/kg and 5 mg/kg body weights respectively. Values are represented as mean± SDa. expressed as percentage to body weight. *$p<0.05$, as compared to the control.

Table 2. Hematologic Variables of Rats following MA Administration.

Item	Baseline	Months of Treatment with 0.5 and 5 mg MA			
		6 (0.5 mg;5 mg)		12 (0.5 mg;5 mg)	
RBC($\times 10^6/\mu l$)	6.66± 1.51	6.94± 1.37	6.87± 1.64	6.80± 1.73	7.02± 1.85
WBC($\times 10^3/\mu l$)	9.6± 2.3	10.4± 2.5	11.2± 3.4	10.2± 3.1	10.9± 2.8
Platelet($\times 10^3/\mu l$)	843± 77.4	783± 56.6	826± 48.2	962± 56.7	819± 155.7
Hgb(g/dl)	13.3± 2.4	12.6± 1.8	13.6± 2.8	13.5± 1.8	13.8± 2.5
PCV(%)	36.4± 5.7	35.7± 4.3	37.3± 4.3	37.8± 4.9	39.2± 5.1
MCV(fl)	55.4± 8.2	51.3± 6.8	54.8± 3.9	55.8± 7.4	56.5± 7.7
MCH(pg)	19.8± 4.8	18.7± 3.9	19.4± 5.6	19.5± 5.6	19.9± 7.1
MCHC(g/dl)	36.4± 9.2	34.8± 5.2	36.1± 7.2	35.8± 9.7	35.3± 6.8
ALK phos(Iu/l)	85± 7.4	99± 5.8	131± 9.4	117± 11.4	174± 13.5
Total Bile(mg/dl)	0.2± 0.07	0.3± 0.04	0.2± 0.06	0.2± 0.04	0.2± 0.05
ALT(SGPT)(Iu/l)	74± 6.2	72± 5.4	75± 4.9	70± 4.2	81± 5.7
AST(SGOT)(Iu/l)	155± 15.3	158± 13.9	149± 7.7	146± 13.5	135± 6.5
T.Protein(g/dl)	8.2± 1.8	7.6± 2.3	8.3± 1.5	8.1± 1.7	7.8± 2.2
Albumin(g/dl)	5.1± 1.3	4.7± 1.6	5.2± 2.3	4.9± 1.9	4.8± 1.4
Cholesterol(mg/dl)	127± 11.6	108± 9.7	102± 6.4	92± 8.4	88± 5.3
Triglyceride(mg/dl)	303± 25.6	254± 19.6	216± 15.8	217± 22.7	145± 18.4
BUN(mg/dl)	29± 4.3	24± 4.9	20.5± 5.1	23± 5.3	16± 4.2
Creatinine(mg/dl)	0.7± 0.13	0.8± 0.21	0.6± 0.31	0.7± 0.21	0.6± 0.18
Glucose(mg/dl)	107± 11.6	98± 9.5	109± 13.2	103± 9.6	113± 8.8

Data are expressed as mean± SD. BUN; Blood urea nitrogen.

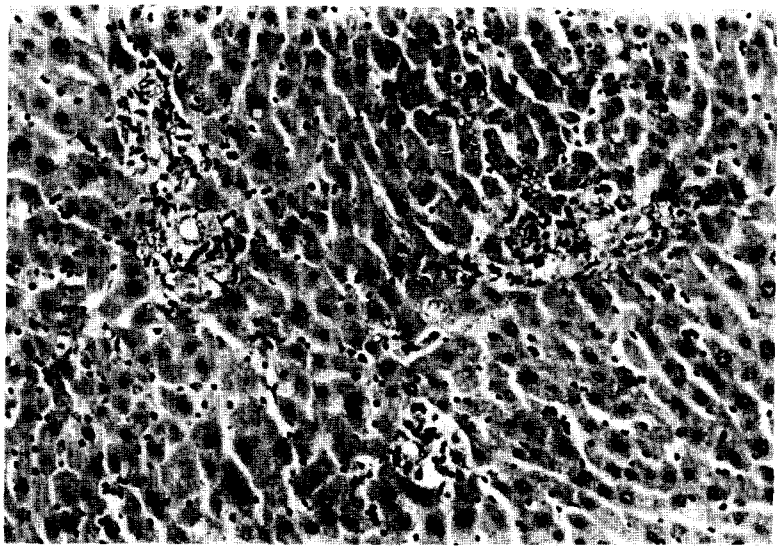

Figure 1. Light micrograph of a liver from a rat that received daily doses of 5 mg/kg MA for 12 months, demonstrating biliary proliferation, lymphocyte infiltration, and mild finrosis. H & E stain ×200.

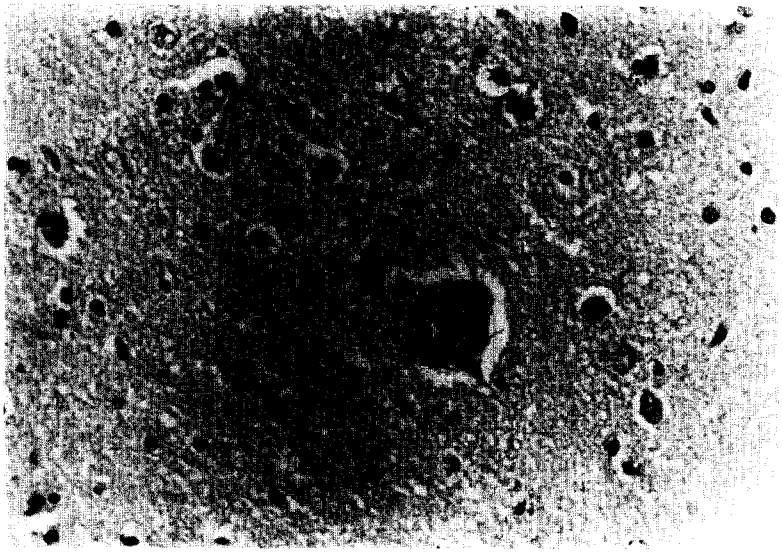

Figure 2. Light micrograph of cerebrum from a rat that sacrificed in 12 months after administration of daily dose of 5 mg/kg MA, showing moderate hypertrophy of small arteriole accompanying edema. H & E stain ×400.

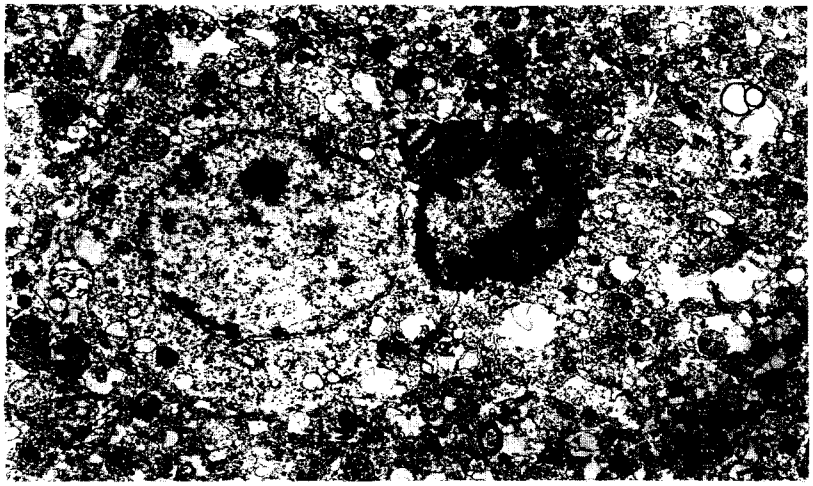

Figure 3. Electron micrograph of the brain from a rat that received daily doses of ×3,000.

ment of MA, each organ of every sacrificed animals was observed by light and electron microscopy. Hepatocytes of 5 mg/kg group showed moderate vacuolar and fatty degerneration accompanied by swelling of the mitochondria and rupture of SER membrane from the 6th month. Prominent hyperplasia of biliary epithelia throughout more tortuous bile ductules and slight interlobular fibrosis were noted in both experimental groups in the 12th month with moderate lymphocyte infiltation. (Figure 1) In the rats treatd with higher doses for 6 months and in both groups in after 12 months, the brains had increased neuroglia in edematous in rstitial tissue, and some anterioles showed moderate hypertrophy. (Figure 2) A number

Figure 4. Light micrograph of the myocardium from a rat that received daily dose of 5 mg/kg/MA for 6 months, demostrating, edema, and hypertrophy of smaller arteriole. H & E stain ×400.

of microglia were found invading degenerated neurons so called neronophagia. (Figure 3) In the hearts of both of the treated groups, blood vessels revealed moderate congestion, and diffuse interstitial edema was observed during the experimental period. Also some sarcolemmas were broken, and vesicular structures and swollen mitochondria were increased beneath sarcolemma. The arterial hypertrophy of heart appeared more apparently in higher dose groups in each period. (Figure 4) Spleens showed an increased cellularity in the red pulp, and RES cells took more hemosiderin.

DISCUSSION

Most of reports noted that MA and certain similar drugs had been used in the treatment of obesity because these drugs have anorective action. (Robinson, 1986; Goodman, 1991) But the anorexic effect of amphetamine is attenuated after chronic administration (Magour, 1974) even though the locomotor response to amphetamine is augmented following chronic treatment. (Rebec *et al.*, 1979; Robinson *et al.*, 1986) Meanwhile, this study demonstrates that the body weights of both lower and higher dose groups reveal an increasing tendency equivalent to that of the controls throughout the experimental period.

The cardiotoxicity of amphetamine had been suggested in some experimental studies. (Zalis *et al.*, 1967; Nogi *et al.*, 1988) A single intraperitoneal injection of methamphetamine hydrochloride (10 mg/kg) given to rats in a warm (30℃) and humid environment caused an uncommon form of cardiac lesion during the first few hours. Disseminated loss of myoglobin was demonstrated immunohistochemically in the ventricular myocardium, and sarcolemmal damage was noted by freeze-fracture electron microscopy. (Kaiho *et al.*, 1989) Also in autopsy cases

of fatal methamphetamine abuse, cardiac lesions along with pathological alteration in multiple organs have been found. It is noteworthy that we found no apparent severe cellular damages that led to cardiac dysfunction except that vascular hypertrophy and long standing congestion were seen in the long-termed MA administration.

Numerous recent studies indicate that when amphetamines are administered continuously or in high doses, they exert long-lasting toxic effects on dopamine neurons in the CNS, (Ricaurte *et al.*, 1982; Ricaute *et al.*, 1984) and such reports reveal the morphological evidences of nerve terminal degeneration. (Hotchkiss *et al.*, 1980) Also increases in number of sparse glia cells in the caudate and intense gliosis were reported. (Hess *et al.*, 1990).

Increased brain weights in MA treated groups were concomitant with edema, congestion, and vascular hypertrophy. In this connection, activation of microglia may be related to degeneration of the nervous system.

There is some evidence that long-termed administration of MA may produce vascular hypertrophy in various organs which eventually lead to hypertention. Elevated blood levels of alkaline phosphotase and marked decrease in blood cholesterol, triglyceride, and BUN levels of MA groups may be presumably due to hyperactivity and continuous intense stereotypy of the animals.

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