Effects of Methamphetamine on Cortisone Concentration, NK Cell Activity and Mitogen Response of T-lymphocytes in Female Cynomolgus Monkeys

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Abstract: As a model for studying methamphetamine (MAP) abuse, which has become a social problem in Japan, we investigated the changes in serum cortisone, NK cell activity and mitogenic response of T-lymphocytes after a single injection of MAP (3.0 mg/kg) in female cynomolgus monkeys. Serum cortisol concentration was significantly elevated to 2.66 times pre-injection levels at 6 h post-injection, and the effect was still observed 24 h later. NK cell activity was significantly elevated at 6 h after MAP injection, but at 24 h after injection had dropped markedly to 49.5% of baseline. Mitogen (PHA) response of lymphocytes was elevated when MAP was injected, and this increased level continued up to 24 h. We speculate that the transient increase in NK cell activity followed by a distinct drop, as well as the changes in T-lymphocytes, may be strongly related to the cortisone concentration.

Key words: cynomolgus monkey, immunosuppression, methamphetamine

Methamphetamine hydrochloride (MAP) has strong affinity to the central nervous system (CNS) and stimulates the sympathetic nervous system through its action on blood vessels and smooth muscle. The pharmacological action of MAP in CNS promotes the release of dopamine and noradrenaline from nerve endings. Furthermore, MAP suppresses the re-uptake of catecholamine discharged at synapses. When MAP is administered, drowsiness and fatigue disappear, resulting in transient elevation of mental and physical activity levels. In Japan, MAP has been illegally used as a stimulant in recent years to relieve fatigue, and its abuse has spread among the general public, young people and even housewives. There are reports of deaths in humans [4, 8], and animal experiments have investigated the causes of death from acute addiction [3, 5, 7, 14, 15]. In Japan, MAP is mostly used as a stimulant, whereas in western countries amphetamine (AMP) is the stimulant of choice. Martin [9] has edited many reports on animal experiments, investigating the emo-
tional behavior of AMP-administered rats as well as changes in metabolism, neurotransmitter, behavior, diet/water intake and endocrinology when AMP or MAP is administered. Although there are some studies that have used monkeys to examine behavioral pharmacology [1, 12, 13] and drug metabolism [2, 6, 10], there have been few reports on immunological function. In our study, cynomolgus monkeys were given a single injection of MAP, and changes in blood cell count, biochemistry values, serum cortisone level, natural killer (NK) cell activity and the mitogen response of T-lymphocytes were investigated.

Four female cynomolgus monkeys (Macaca fascicularis) obtained from the Department of Wild Animals, Nippon Veterinary and Life Science University, were used. The monkeys used in this study were 13 to 19 years old (weighing 4.1 to 5.6 kg). They were kept in an animal room at 18–25°C with 12-h lighting (7:00–19:00) and ventilation 12 times/h. MAP was injected to the monkeys and blood samples were collected in the same animal room. All procedures were in accordance with the NIH Guide for the Care and Use Committee of the Nippon Veterinary and Life Science University.

Methamphetamine hydrochloride (MAP: 1-phenyl-2-methylaminopropane hydrochloride from Dainippon Sumitomo Pharma, Osaka, Japan) was dissolved in sterilized saline solution (adjusted to 1.0 mg/ml) and a single injection of 3.0 mg/kg was given via the femoral vein.

Without anesthesia, blood (7.0 ml) was drawn from the femoral vein to obtain an accurate measurement of the cortisone concentration. Three blood samples were taken: before MAP injection, and at 6 h and 24 h after MAP injection. A blood sample with heparin was used to measure blood cell count, NK cell activity and the mitogenic response of T-lymphocytes. Serum was stored at –80°C for biochemical analysis and measurement of adrenocortical hormone.

Leukocyte and erythrocyte counts were performed with an automatic blood count device (Sysmex F-300, SYSMEX, Kobe, Japan). Biochemical measurement of sera was conducted with an automatic analyzer (Hitachi-736, HITACHI, Tokyo, Japan) for the following items: total protein (TP), albumin (ALB), total cholesterol (TC), triglyceride (TG), blood urea nitrogen (BUN), creatinine (CRE), calcium (Ca) and inorganic phosphorus (IP). Adrenocortical hormone (cortisone) was measured using a Spac-S Cortisone kit (Daiichi Radioisotope Laboratories, Tokyo, Japan).

NK cell activity was measured by the chromium-51 releasing assay. Briefly, effector cells were prepared from blood samples by separating lymphocytes using Ficoll-Conray solution (specific gravity: 1077, Lympho-Sepal Immuno-Biological Laboratories, Takasaki, Japan). Chromium-51 (Na₂⁵CrO₄; MP Biomedicals, CA, USA) labeled K562 cells were used as target cells. Effector cells (50 × 10⁴/0.1 ml) and target cells (1 × 10⁶/0.1 ml) were prepared with culture medium (fetal bovine serum 10%, L-glutamine, gentamicin, and streptomycin added to RPMI-1640 medium, SIGMA CHEMICAL, MO, USA) and cultured for 4 h (at 37°C, 5% CO₂) on 96-well plates (U96 microwell plate; NUNC, Denmark), and the radioactivity of free chromium-51 in the supernatant was calculated to evaluate NK cell activity as follows:

\[
\text{Activity} (\% \text{ cytotoxicity}) = \frac{(A-B)}{(C-B)} \times 100 (\%),
\]

where A=test cpm, B=spontaneous cpm, and C=maximum cpm.

Measurement of the mitogenic response of T-lymphocytes was performed by the following method. After separating lymphocytes from the blood samples, they were adjusted to 50 × 10⁴/0.1 ml with culture medium. Phytochaemagglutinin-P (5 µl/ml, PHA; DIFCO, MI, USA) and Concanavalin A (5 µl/ml, Con A; Pharmacia Fine Chemicals, Sweden) were used as mitogen for T-lymphocytes and were cultured on 96-well plates (F96 microwell plate; NUNC) with ³H-thymidine (methyl-³H; MP Biomedicals) of 37 kBq for 72 h at 37°C, 5% CO₂. ³H-thymidine uptake in lymphocytes was then measured.

Behavioral changes were seen in the cynomolgus monkeys immediately after MAP injection. We studied the changes in behavior of each monkey after i.v. injection of 3.0 mg/kg of MAP (Table 1). Although there were some individual differences, no clear increase in spontaneous exercise or stereotypic activity was seen. All monkeys showed hyperactive responses to sound.

The changes in leukocytes and erythrocytes are shown
in Table 2. There were individual differences in leukocyte count (ranging from 7,700–13,400 cells/µl) among the monkeys (No.1–4) before MAP injection. All animals showed a significant increase ($P<0.05$) in leukocytes 6 h post-injection, averaging 185% of baseline. All animals then showed a sharp drop at 24 h (to 83% of baseline). All monkeys showed a decrease in erythrocytes at 6 h post-injection, to 87% of baseline, and this trend continued up to 24 h; the changes were not statistically significant.

Changes in serum biochemical parameters (8 items) after MAP injection are shown in Table 3. TP, ALB, TC and TG showed a decreasing trend at 6 h and at 24 h post-injection. All animals showed an increase in BUN 6 h after the injection, averaging 142% of baseline; the differences among the animals were not significant. The BUN values then tended to return to baseline at 24 h. The average of CRE value at baseline was 0.522 ± 0.013 mg/dl, with differences among individual monkeys being relatively small. At 6 h after MAP injection, however, the average CRE level was significantly elevated (0.730 ± 0.064 mg/dl; 140% of baseline, $P<0.05$), only to swiftly return to baseline at 24 h. Ca showed a transient decline to 97% of baseline post-injection, but then rapidly recovered. IP was elevated in all monkeys after MAP injection, reaching 131% of baseline at 6 h, then declining slightly to 122% at 24 h, but these changes were not statistically significant.

Cortisone concentrations before and after MAP injection are shown in Fig. 1. Before MAP injection, cortisone concentration was within a range of 14.2–24.8 µg/dl, the average being 17.8 ± 2.0 µg/dl. It was markedly elevated in all monkeys 6 h after MAP injection, the average becoming 47.6 ± 7.3 µg/dl, 266% of baseline. The average of CRE value at baseline was 0.522 ± 0.013 mg/dl, with differences among individual monkeys being relatively small. At 6 h after MAP injection, however, the average CRE level was significantly elevated (0.730 ± 0.064 mg/dl; 140% of baseline, $P<0.05$), only to swiftly return to baseline at 24 h. Ca showed a transient decline to 97% of baseline post-injection, but then rapidly recovered. IP was elevated in all monkeys after MAP injection, reaching 131% of baseline at 6 h, then declining slightly to 122% at 24 h, but these changes were not statistically significant.

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baseline, a significant difference ($P<0.01$). At 24 h, the average level, although somewhat lower, was still significantly elevated ($37.3 \pm 4.0 \, \mu g/dl, P<0.01$).

NK cell activities before and after the administration of MAP are shown in Fig. 2. Individual differences were small throughout the experiment; the pre-injection range was 59.7–73.9%, an average of 64.2 ± 2.8%. The average was significantly elevated at 6 h ($78.3 \pm 2.3%; P<0.01$), and then dropped significantly at 24 h post-injection (49.5% ± 1.9%) to 77.1% of baseline, which was also statistically significant ($P<0.01$).

Mitogen responses of T-lymphocytes in monkeys injected with MAP are shown in Table 4. The uptake of $^3$H-thymidine was measured after adding PHA and ConA to lymphocytes before and after MAP injection. Before MAP injection, the count of PHA-added lymphocytes was 4,888 ± 901 cpm. It was then elevated at 6 h after MAP injection, reaching 8,460 ± 1,537 cpm, 173% of baseline. Even at 24 h, the average was still 7,682 ± 1,457 cpm (157%). The baseline of Con A was 13,995 ± 1,317 cpm, and Con A increased to an average of 17,381 ± 2,186 at 6 h post-injection, 124% of baseline. It then returned to close to the baseline value, becoming 14,918 ± 1,865 (about 107% of baseline) at 24 h. There was no significant change in the uptake of $^3$H-thymidine by T-lymphocytes before and after MAP injection. As for the Con A/PHA ratio, the pre-injection average was 3.029 ± 0.025, that at 6 h was 2.202 ± 0.155, and that at 24 h was 2.062 ± 0.227 ($P<0.05$), a significant decrease.

In this study, 3.0 mg/kg of MAP was injected intravenously to 4 cynomolgus monkeys. Although the level of excitation differed among the monkeys, the violent neck shaking, abnormal hair plucking, and apparently

| Table 4. Mitogen-induced proliferation of lymphocytes in female cynomolgus monkeys injected with MAP$^{\text{a,b}}$ |
|-----------------|-----------------|-----------------|
|                 | Baseline$^b$    | 6 h after injection | 24 h after injection |
| Mitogen (-)     | 567 ± 98$^c$   | 443 ± 90         | 520 ± 62           |
| PHA             | 4,888 ± 901     | 8,460 ± 1,537    | 7,682 ± 1,457      |
| Con A           | 13,995 ± 1,317  | 17,381 ± 2,186   | 14,918 ± 1,865     |
| Con A/PHA       | 3.029 ± 0.025$^d$ | 2.202 ± 0.155    | 2.062 ± 0.227*     |

$^a$, $^c$: The data are shown as in Table 2. $^b$: Each value represents mean ± S.E. (cpm, n=4). $^d$: Con A cpm/PHA cpm ratio (mean ± S.E.). Statistically significant differences from the baseline by Student’s $t$-test are indicated by* ($P<0.05$).
abnormal stereotypic behavior reported by Ridley et al. [13] were not observed. Only one monkey, No. 4, exhibited persistent interest in the metal part of a drink bottle, touching it for a number of hours.

As for blood cell numbers after MAP injection, leukocytes were elevated transiently, followed by a swift decrease, and erythrocytes showed a decreasing tendency. Some serum biochemical parameters (TP, ALB, TC, BUN, Ca) became lower at 6 and 24 h after MAP administration, while others (CRE, IP) were elevated; only CRE showed a statistically significant change. The pre-injection biochemical parameters of the monkeys were all within the reported normal range [18].

The cortisone concentration increased 2.0–2.6 times after MAP injection. It is a known fact that an increase in cortisone concentration causes a decrease in leukocyte cell numbers, suppressing the immune function. Scheiman et al. [16] suggested that nuclear factor kappa B, a regulator of the immune system and inflammation genes, might be a target for glucocorticoid-mediated immunosuppression. The transient increase in leukocytes and NK cell activity following MAP injection and the rapid drop thereafter is speculated to be strongly related to the change in cortisone concentration.

The mitogen response of T-lymphocytes, on the other hand, was clearly elevated against PHA after MAP injection. This suggests that lymphocyte activity may be elevated or lymphocyte numbers may be increased by MAP administration. Lectin, a plant-derived mitogen, triggers blastogenesis of lymphocytes regardless of its antigen specificity. PHA, a mitogen of T-cells, causes helper T-cells to differentiate, whereas Con A induces both helper T-cells and suppressor T-cells to differentiate [11, 17]. The Con A/PHA ratio, therefore, roughly reflects the suppressor-helper balance. The Con A/PHA ratio declined after MAP injection, suggesting that elevated helper T-cell activity and/or increase in the number of T-cells were induced.

Various effects on the immune system of cynomolgus monkeys were induced by a single injection of MAP. We speculate that multiple injections of MAP, as performed by human stimulant addicts, may inhibit immune functions.

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References