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STUDIES ON IMMUNOTOXIC POTENTIAL OF METHAMPHETAMINE (MA) IN Balb/C MICE

I. Changes of Lymphoid Organs and Inhibitory Effect of Lymphocyte Proliferation to Mitogen

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ABSTRACT *: The immune system is partially under the control of the sympathetic and parasymphathetic nervous systems through the regulatory feedback loop. Methamphetamine (MA) is a neurotoxic chemical which affects the neurotransmitter system. The objective of this study was to investigate the immunotoxic effect of MA on the major immune target organ and lymphocyte proliferation to the various mitogens. Female Balb/C mice, 15 to 20 g, were injected subcutaneously with 0, 0.5, or 5 mg MA/kg for 14 consecutive days. In MA treated mice, the body weight gain and relative spleen and thymus weight were decreased in dose-related manner. Histopathologically, there was a paucity of lymphoid follicles and germinal centers in the spleen, and thymic cortical atrophy with lymphophagocytosis was prominent. Apoptosis also occurred in germinal centers of spleen and thymic cortex. The threshold and peak of lymphocyte proliferation at various concentration of mitogens showed similar patterns. However, the response to lipopolysaccaride (LPS) and pokeweed mitogen (PWM) in the 5 mg MA/kg treated group showed threshold and peak proliferation at high concentration of mitogens (25 μg LPS/ml for MA vs 15 μg LPS/ml for control; 60 μg PWM/ml for MA vs 45 μg PWM/ml for control), which suggest that MA impairs T cell dependent-B cell function. This preliminary study indicated that MA affected the lymphoid organs and immune function.*

Key Words : *Methamphetamine, Lymphoid organ, Mitogen, Lymphocyte proliferation.*

I. INTRODUCTION

Methamphetamine (MA) is one of the many amphetamine derivatives (James *et al.*, 1989), which was dumped into civilian markets after World War II. MA use is emerging as an important drug abuse problem since the mid 1960s in Korea.

MA appears to affect the neurotransmitter by release of dopamine and norepinephrine from the presynaptic neuron and blocking the reuptake of catecholamines by presynaptic neurons (Azzaro *et al.*, 1974; Carlsson, 1970). This chemical is well known as a neurotoxic phenethylamine which produces long-term deficits in brain dopaminergic, serotominergic neuronal system, and morphological evidence of nerve terminal degeneration (Hotchkiss *et al.*, 1980; Ricaurte *et al.*, 1982; Wagner *et al.*, 1980).

Immunohistochemical studies from many investigators have shown the presence of autonomic nerve fibers in specific compartments of both primary and secondary lymphoid organs. In the lymphoid tissues, sympathetic nerve endings contact postganglionic sympathetic fibers that richly innervate lymphoid organs and lymphocyte at a distance which is even shorter than that in a synapse (Felten *et al.*, 1985, 1987). In the spleen, the neurotransmitter acts in the spleen as both a paracrine secretion, available to receptors on cells in the white pulp, and a localized neurotransmitter in nerve terminals that directly contact T lymphocyte in the periarteriolar lymphocyte sheath (PALS) (Felten *et al.*, 1986). Therefore, the communication from the nervous system to the immune system may occur via innervation of lymphoid tissues, with neurohormones and endocrine hormones influenced by neurohormones or neuropeptides. Despite the wide abuse of MA, there has been little research on the effect of this drug on the immune system. The need

for such studies has become more important because of the increased susceptibility to infection with various diseases, and the high prevalence of AIDS among abusers (Harold *et al.*, 1985; Nestor *et al.*, 1989). This study was designed to assess the effects of repeated exposure to MA on lymphoid organs and lymphocyte proliferation to the various mitogens.

II. MATERIALS AND METHODS

1. Animals

Female Balb/C mice weighed 15 to 20 g were obtained from KRICT. The animals were randomly distributed in polypropylene cages on sawdust bedding (β-chip) with tap water and commercial rodent chow pellet (Sam yang) available *ad libitum*.

2. MA Treatment

MA was obtained from Korean authorities, and dissolved in saline prior to use. Mice were injected subcutaneously for 14 consecutive day at 0.5 mg MA/kg or 5 mg MA/kg, while control mice were given saline as a vehicle.

3. Pathology

Animals were weighed every two other days. On day 14, blood was drawn from the retro-orbital plexus in order to perform a blood count. Animals were terminated by cervical dislocation. The weight of spleen and thymus and cellularity of spleen were measured as a part of the immunotoxic evaluation. Results were analyzed as absolute weights, and relative weight to body weight. The tissue samples from spleen and thymus were fixed 10% neutral buffered formalin, then processed routinely, sectioned at 4 microns, stained with hematoxylin and eosin (H & E), and examined by light microscopy.

4. Spleen Cell Suspension

Splenocyte suspensions were prepared by gently squeezing the organs in cold RPMI 1640 (Gibco) and passing the cells though a nylon filter with a pore size of 53 μm (Spectrum, USA). Following red blood cell lysis by hypotonic shock and wash, cells were resuspended in complete culture media. The culture medium was RPMI 1640 supplemented with gentamycin sulfate (100 mg/L), L-glutamine (2 mM), Hepes buffer (10 mM), and 10% fetal calf serum (Gibco). Cell numbers were determined with a hemocytometer and viability was measured by exclusion of trypan blue (Leslie *et al.*, 1989).

5. Mitogen-induced Lymphocyte Proliferation Assays

Proliferation assays were carried out in 96 well flat-bottomed microtiter culture plates (Falcon). 100 μl containing 4×10^6/ml of splenocytes suspension was added to wells with 50 μl of medium and 25 μl of various concentrations of concanavalin A (Con A; Sigma, Type IV), lipopolysaccharide (LPS; E. Coli 0111:B4), and pokeweed mitogen (PWM; Gibco). Every assay was performed in triplicate. The plates were incubated at 37°C for 42 hr in a humidified 5% CO_2 incubator. ^{3}H-thymidine incorporation into the cells was measured by adding 0.5 μCi (83 Ci/mM, Amersham, New England) 18 hr prior to harvesting the cultures onto fiber glass filter paper (Flow laboratories) using a multiple automatic cell harvester (Inotech, Swiss). The resulting filter paper spots were dried and placed in minivials to which 2 ml of scintillation cocktail solution (PPO 5 g, POPOP 100 mg, toluene 1 L) was added. Incorporated radioactivity into DNA of dividing lymphocytes was determined in a β-liquid scintillation counter (Pockard, USA) (Gin *et al.*, 1990).

6. Statistical Analysis

The data were analyzed using paired Student's t-test and considered significant at $p<0.05$.

III. RESULTS

1. General Observations

The observable behavioral effects of MA treated mice were rapid locomotion, frequent head shaking, bristling of hairs and salivation. These responses occurred soon after the drug treatment, and there was a subsequent elevation of body temperature was noted in most mice.

2. Pathology

The growth rate of treated groups was decreased in a dose dependent manner. Even with considerable variation among the individuals, the body weights of 5 mg MA/kg treated group dropped on day 4 and showed the greatest significant difference from the control. After that, the growth of this group tended to increase (Fig. 1). The number of peripheral leukocytes was affected only in the high dose treated group ($4.45 \pm 0.57 \times 10^3$ cell/mm^3 for MA vs $6.29 \pm 0.69 \times 10^3$ cell/mm^3 for MA). Meanwhile, the splenic cellularity was decreased in a dose dependent manner. The decrease in splenic cellularity was associated with the decreased spleen weight (Table 1).

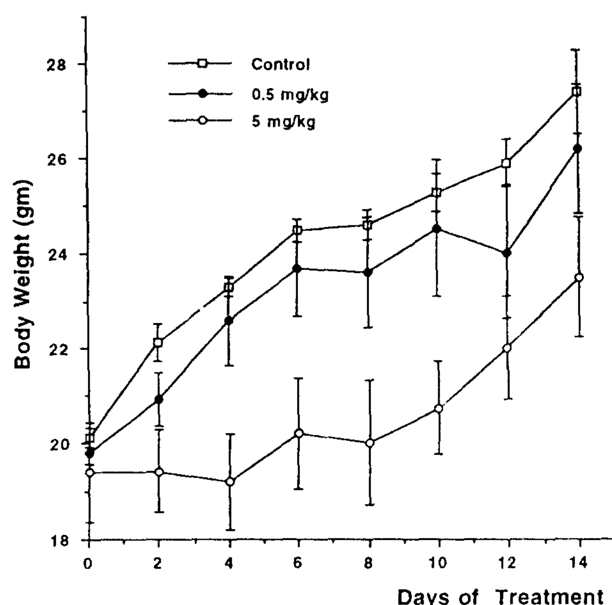

Fig. 1. Effect of methamphetamine in the Balb/C mice injected MA for 14 days. Body weights were measured every 2 other day. Data expressed as mean ± SD (n=4).

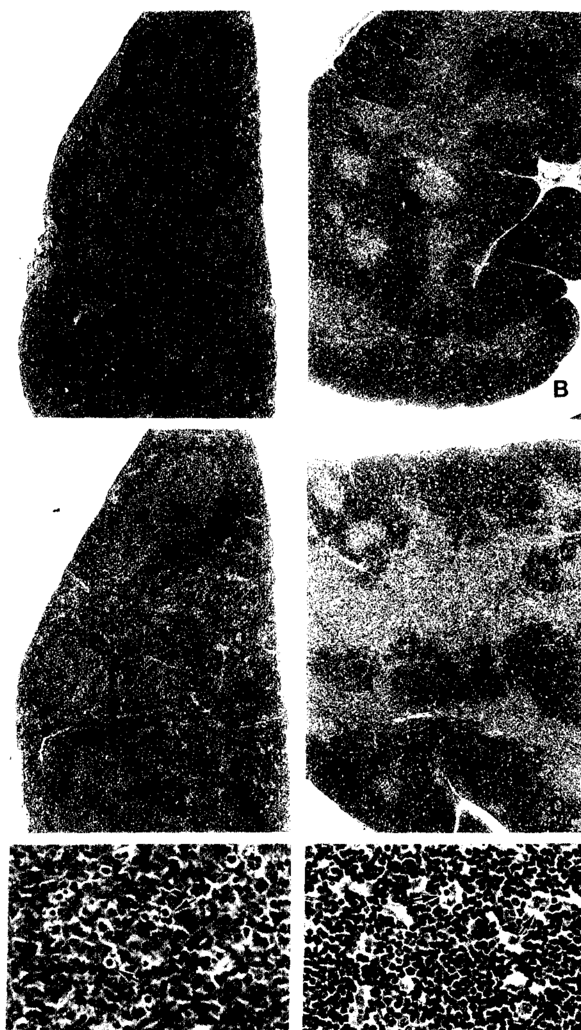

Fig. 2. Photomicrographs of the spleen and thymus from control Balb/C mice and methamphetamine treated mice at a dose of 5 mg/kg for 14 days. (A) Control spleen and (B) thymus; (C) MA treated spleen and (D) thymus. HE X 90. Note the decrease in the number of white pulps with germinal center in the spleen and moderate cortical atrophy and relatively increased medulla in the thymus. Apoptotic cells were characterized by capping nuclei and well defined halo within the germinal center (E), and phagocytized by neighboring macrophages in the thymic cortex (F) (Starry-sky apperance). HE X930.

Table 1. Final body weight, absolute and relative organ weight and celluarity of Balb/C mice injected MA for 14 days.

Group	gm Body Wt	mg Spleen Wt (%)[a]	mg Thymus Wt (%)[a]	Cellularity	
				Spleen X 10^7	WBC $(X10^3)$[b]
Control	24.54 ± 1.21	152 ± 17 (0.61)	112 ± 16 (0.41)	9.52 ± 0.60	6.29 ± 0.69
0.5 ng MA/kg	23.78 ± 0.93	140 ± 13 (0.58)*	109 ± 16 (0.46)	8.93 ± 0.54*	6.00 ± 0.82
5 mg MA/kg	21.24 ± 0.94*	104 ± 11 (0.49)**	84 ± 8 (0.39)*	7.39 ± 0.54**	4.45 ± 0.75*

On day 15, body and lymphoid organs were weighed (mean ± SD; n=4). Blood was collected from retro-orbital plexus.
[a]Expressed as percentage of body weight.
[b]white blood cell.
*, **Significantly different from control; *p<0.05, **p<0.01.

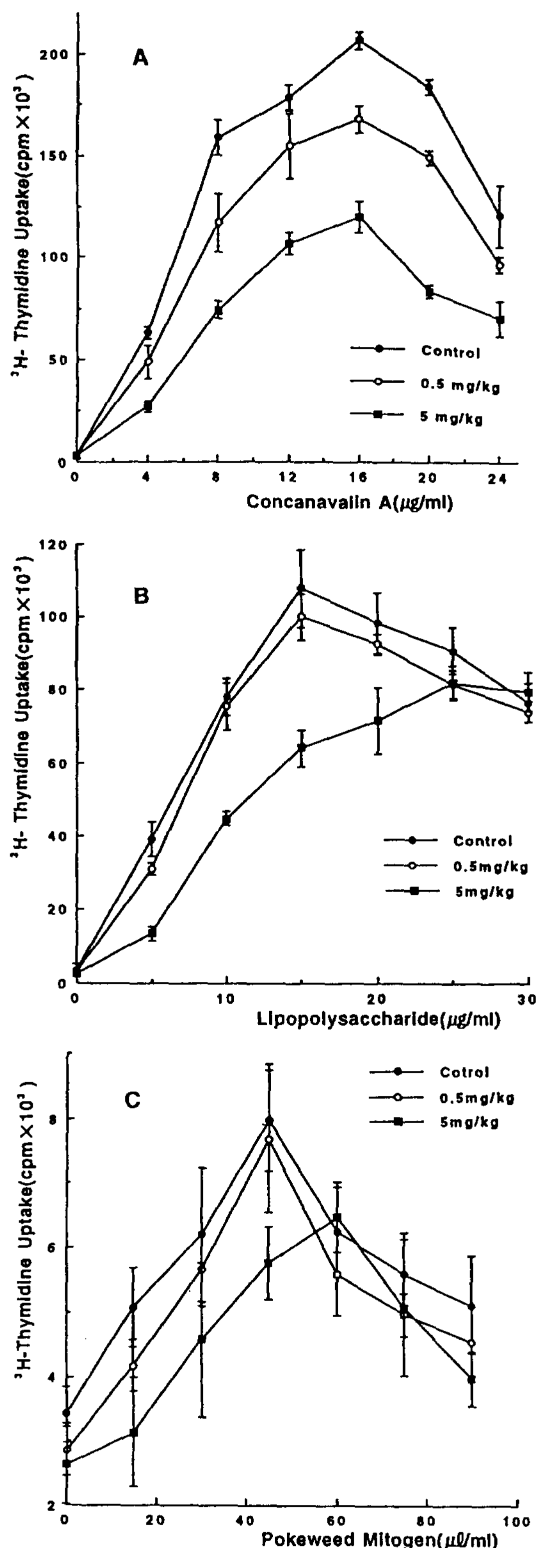

Fig. 3. Effect of methamphetamine on splenic lymphocitic proliferation at different concentration of Con A (A), LPS (B) and PWM (C) (mean±SD; n=3). Balb/C mice were injected with MA for 14 days. On the 15th day. Splenocytes (4×10^6/ml) were cultured in complete media contraining mitogen. Cells were harvested at 42 hr and assayed ^{3}H-thymidine uptake.

The relative splenic weights of the high dose treated group showed obvious atrophic change (0.49% for MA vs 0.57% for control). Changes in the thymus were similar, although not as marked.Histopathologically, lymphoid organs of control animals showed relatively normal architecture (Fig. 2A, 2B). However, the spleens of the treated group showed a paucity of white pulp including lymphoid follicles and germinal centers (Fig. 2C). Cell death in the germinal center occurred singly characterized by capping surrounded by a well defined halo (Fig. 2E). The thymus revealed moderate cortical atrophy with an accopmanying widening in medulla (Fig. 2D). Lymphophagocytosis (Starry-sky appearance) was observed (Fig. 2F). Apoptosis was characterized by single cell death without infiltration of inflammatory cell, which was phaogcytized by neigboring macrophages.

2. Mitogen-induced Lymphocyte Proliferation

The threshold and peak concentration for the proliferation response of the spleen cells to Con A, a T cell mitogen, were similar among the three groups. However, the magnitudes of the response were decreased in the 5 mg MA/kg treated group as compared with control (Fig. 3A). At 16 μg Con A/ml, the maximum stimulating concentration in the high dose group was nearly half of those of control. In the high dose group the threshold and peak proliferative response to LPS was shown at 25 μg LPS/ml, while those of control showed at 15 μg LPS/ml (Fig. 3B). Similarly, response to PWM was shown at a higher concentration of mitogen (60 μg PWM/ml for 5 mg MA/kg vs 45 μg PWM/ml for 0.5 mg MA/kg and control) (Fig. 3C).

IV. DISCUSSION

Within the past 10 years, research concerned with the relationships among brain, behavior, and immunity has expanded rapidly (Ader *et al.*, 1987). Although systemic research in psychoneuroimmunology is relatively new, the range of the phenomena that bear on brain and immune system interaction is quite broad (Ader *et al.*, 1990, Madden *et al.*, 1995).

Weight and morphology of lymphoid organs are

the first parameters studies in toxicity assessment. The data from this study showed that daily exposure to MA in Balb/C mice affected the lymphoid organs and altered lymphoblastic response. The body weight gain in MA treated mice was decreased and some studies supported this data by saying that the site of anorectic action of this drug is probably in the lateral hypothalamic feeding center. Alternation in diet and nutritional status could be one of possible indirect effects of MA in changing immune function (Chandra *et al.*, 1994).

In the thymus, absolute weight of MA treated mice was reduced, but the relative weight to body was not overtly changed. The cortical thymocyte, mainly CD^{4+8-3-}, CD^{4-8+3-} and $CD^{4+8+3-/lo}$, possessing scant cytoplasm, makes up about 85% of the total thymic lymphocyte population. Pathologic finding in the spleen included a defect in lymphoid follicles and germinal centers indicating B cell deficiency. Apoptosis was observed in thymic cortex with tingible body macrophage and splenic germinal center. Currently, apoptosis is emphasized in toxicologic field. This removal mode of dead cell is generally benifical to the organism and not biologically threatening because toxic contents of dying cells are phagocytized and internally digested (Corcoran *et al.*, 1994).

MA seemed to have a lymphocytolytic effect in mice associated with splenic cellularity as well as peripheral leukocytes. Considering most circulating mouse leukocytes are T cells (David *et al.*, 1990), their loss seemed to be related to substantial damage of the thymus, which may prevent or delay the migrating process of T cells into the peripheral blood. Decreases in WBC count of MA treated mice was thought to somewhat commensurate with results of Manuel F.G. *et al.* (1991) who reported the quantification of blood cellularity.

Mitogens are commonly used to assess lymphocyte function. The lymphocyte proliferation in MA treated mice was decreased with Con A, which stimulates the T cell via direct binding to the T cell Receptor (TCR) and TCR associated molecules. LPS and PWM either randomly insert into the lipid bilayer or attach to multiple surface receptors of large numbers of B cells. PWM predominantly reflects T cell-dependent B cell proliferation or the ability of T-helper cells to communicate with and stimulate B cell proliferation (Paul, 1989). The peak proliferation of the 5 mg MA/kg treated group was shifted to higher concentrations of LPS and PWM than that of control. Based on these results, it appears that MA impairs T cell dependent B cell function. This data might support the report provided by Johnson *et al.* (1981) that lymphocyte responsiveness to Con A and LPS was found to be suppressed following exposure of β_2 adrenergic receptor(AR) selective agonist, norepinephrine or epinephrine. However, this is contradictory to other study (Ader *et al.*, 1990).

The findings suggest that methamphetamine has a immunotoxic effect to lymphoid organs and inhibits the lymphoblastogenic response to various mitogens. More detailed immuno-toxicological assessments of MA will be further studied.

REFERENCE

Ader, R., Felten, D.L., and Cohen, N., (1990): Interactions between the brain and the immune system, *Ann. Rev. Pharmacol. Toxicol.*, **30**, 561-602.

Azzaro, A.J., Ziazce, R.J., and Rutledge, C.O. (1974): The importance of neuronal intake of amines for amphetamine induced release of ^{3}H-norepinephrine from isolated brain tissue. *J. Pharmacol. Exp. Ther.* **189**, 110-118.

Carlsson, A. (1970): Amphetamine and brain catecholamine in amphetamines and related compounds. (Raven, New York). 289-299.

Chandra, R.K., and Kumari, S. (1994): Nutrition and immunity: An overview. *J. Nutr.*, **124**, 1433S-1435S.

Corcoran, G.B., Fix, L., Jones, D.P., Moslen, M.T., Nocotera, P., Oberhammer, F.A., Buttyan, R. (1994): Contemporary issues in toxicology. Apoptosis: molecular control point in toxicology. *Toxicol. and Appl. Phamacol.*, **128**, 169-181.

David, N.K., Anthony, J.M., and Robert, E.F. (1990): Effects of stress on the immune system, *Immunol. Today*, **11**, 170-175.

Felten, D.L., Bellinger, D.L., Ackerman, K.D., and Felten, S.Y., (1986): Denervation of splenic sympathetic fibers in the young adult rat. *Soc. Neurosci. Abstract*, **12**, 1064.

Felten, D.L., Felten, S.Y., Bellinger, D.L., Carlson, S.L., Ackerman, K.D., Madden, K.S., Olschowki, J.A., and Livnat, S. (1987): Noradrenergic sympathetic neural interactions with the immune system: structure and function. *Immunol. Rev.*, **100**, 225-260.

Felten, D.L., Felten, S.Y., Carlson, S.L., Olschowka, J.A., and Livnat, S. (1985): Noradrenergic and peptidergic innervation of lymphoid tissue. *J. Immunol.*, **135**,

755-765.

Gin, C.H., Robert, D.R.P., Raghubir, P.S., and Brian, J. H. (1990): Subclinical effects of ground water contaminants. *Arch. Toxicol.*, **64**, 320-328.

Harold, M.G., Stanley, H.W., Mhairi, G.M., and Robert, L.H. (1985): HTLV-III exposure among drug users. *Cancer Res. (Suppl)*, **45**, 4605s-4608s.

Hotchkiss, A.J., and Gibb, J.W. (1980): Long-term effects of multiple doses of methamphetamine on trytophan hydeoxylase and tyrosine hydroxylase activity in rat brain. *J. Pharmacol. Exp. Ther.*, **214**, 257-262.

James, A.G., and Andrew E.S. (1989): Amphetamine and its derivatives in drugs of abuse. (Med. Economics Books, New Jersey), 15-82.

Johnson, D.L., Ashmore, R.C., Gordon, M.A. (1981): Effects of β-adrenergic agents on the murine lymphocyte response to mitogen stimulation. *J Immunopharmacol.*, **3**, 205-219.

Leslie, H., and Frank, C.H. (1989): Preparation of lymphocytes from solid lymphoid organs in practical immunology (3rd ed.), (Blackwell Sci. Pub., New York), 23-24.

Madden, K., and Felten, D.L. (1995): Experimental basis for neuralinnune interactions. *Physiological Reviews*, **75**, 77-106.

Manuel, F.G., Jose, L.B., Maria, J.N., Maria, T.C., Jose, B.L., Jose, C.F., and Angel, B. (1991): Effects of amphetamine on T cell immune response in mice, *Life Science*, **49**, PL107-PL112.

Nestor, T.A., Tamamoto, W.I., and Schults, T. (1989): Acute pulmonary oedema caused by crystalline methamphetamine. *Lancet*, **35**, 1277-1278.

Paul, W.E. (1989): T lymphocyte activation in *Fundamental Immunology*. (2nd ed.), (Raven Press, New York), 359-384.

Ricaurte, G.A., Seiden, L.S., and Schuster, C.R. (1982): Dopamine nerve terminal degeneration produced by high doses of methyamphetamine in the rat brain. *Brain Res.*, **235**, 93-103.

Wagner, G.C., Ricautre, G.A., Seiden, L.S., Schuster, C. R., Miller, R,J., and Westley, J. (1980): Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated admiuistoration of metham phetamine. Brain Res., **181**, 151-160.