Arch. Pharm. Res. Vol.18, No.4, pp. 267-270, 1995

Effects of Chronic Dextromethorphan Administration on the Cellular Immune Responses in Mice

Hyoung-Chun Kim, Wang-Kee Jhoo, Myung-Sang Kwan[1] and Jau-Shyong Hong[2]

College of Pharmacy, [1]College of Animal Agriculture, Kangwon National University, Chuncheon 200-701 and [2]Laboratory of Environmental Neuroscience, National Institute of Environmental Health Science, National Institute of Health, Research Triangle Park, North Carolina 27709, USA

(Received April 5, 1995)

We examined the chronic effect of dextromethorphan (DM) on the cellular immune responses in mice. T cell stimulator, phytohemagglutinin did not show significant effect on lymphocyte proliferation. Costimulator of T and B cell, pokeweed mitogen, and B cell stimulator, lipopolysaccharide exhibited DM-induced decreased lymphocyte proliferation. Significantly suppressed natural killer (NK) cell cytotoxicity was evidenced following 6 months DM exposure. These results suggest that chronic DM administration perturb B cell functioning and NK cell cytotoxicity. In addition, prenatal DM exposure did not potentiate the immunomodulation in postnatal effect induced by chronic DM.

Key words : Chronic dextromethorphan, Cellular immune responses

INTRODUCTION

Dextromethorphan (DM) is a synthetic derivative of morphine that is used as an antitussive. It has been reported to have no analgesic, sedative or respiratory depressant properties, which are ideal characteristics. Therefore, it has been widely used as an over-the-counter cough medicine. During the past decade, investigators have already documented that DM has a N-methyl-D-aspartate (NMDA) receptor antagonistic effect with neuroprotection (Kim *et al.*, 1995; Tortella *et al.*, 1989). However, to achieve the neuroprotective effect, the DM dosage needed is much higher than the cough suppressant dosage (Albers *et al.*, 1992). Conversely, several NMDA receptor antagonists in high doses induced neurotoxicity as shown by neuronal vacuolation in the rodent brain (Olney *et al.*, 1989). Clinically, high doses of DM can induce psychotropic effects/euphoria (Jasinski *et al.*, 1971). Futhermore, DM has been recognized as one of the goals for drug-seeking behavior by some teenagers in Korea (Jhoo and Kim, 1990), European countries and California (F. C. Tortella, Personal communication). We already shown that behavioral abnormalities were induced by the chronic oral administration of DM in the mouse model (Kim and Jhoo, 1994). It is well known that the central nervous system and behavior can influence immune function (Madden and Felten, 1995). The sympathetic nervous system has been implicated in the modulation of splenic natural killer (NK) cell activity (Hellstrand *et al.*, 1985). In some cases, psychoactive compounds suppressed splenic NK cell activity and mitogen-induced lymphocyte proliferative responses (Di Francesco *et al.*, 1993; House and Thomas, 1994). This study investigated the alterations in the immune system after chronic exposure to DM with or without prenatal DM exposure.

Correspondence to: Hyung-Chun Kim, College of Pharmacy, Kangwon National University, Chuncheon 200-701, Korea

MATERIALS AND METHODS

Animals and drugs

Eight-week-old male and nulliparous female BALB/c mice were used for this study. The animal room was maintained on a 12 hr light/dark cycle. One week after arrival, the mating period was initialized by placing each female into a male's cage. The females subsequently were inspected twice daily at 9 A.M. and 8 P.M., for the presence of a vaginal plug. The occurrence of the vaginal plug was designated pregnancy day 0. Gravid mice received DM (50 mg/kg, p.o.) daily through the entire gestation period. On postnatal day 21, the pups (G2) were weaned and examined for gender-distinguishing characteristics. The females were discarded. To get the age-matched condition corresponding to the adult (G1) group, the male pups (G2), which were prenatally exposed to DM, were housed separately until the experiment

commenced. DM (50 mg/kg, p.o.) or the same volume of saline was administered to both the first generation (G1) and second generation (G2) of 50-day-old male mice 5 times a week by gastric intubation throughout the 6 month period.

Lymphocyte proliferation assay

Spleen cells were cultured in flat-bottomed 96-well plates (Flow Laboratories, U.K.) at 5×10^5 cells/well in a final volume of 0.2 ml RPMI-1640 medium (Gibco, USA) containing 10% fetal calf serum (Gibco), 20 units/ml of penicillin G, 100 g/ml of streptomycin sulfate, and 3 g /ml of fungizone (Gibco). Various concentrations of mitogen were added and the cultures were kept in a humidified atmosphere containing 10 % CO_2 in air. After 48 hr of incubation at 37°C, 0.5 Ci of [^{3}H] thymidine (2 Ci/mM; Amersham, UK) was added to each well and incubation was continued for 6 hr. Cells were harvested with a Titertek multiharvester (Flow Laboratories) and radioactivity was counted in a packard liquid scintillation counter (IL, USA). Incubations were usually done in triplicates and DNA synthesis, as measured by [^{3}H] thymidine incorporation, was expressed as counts per min (cpm).

Natural killer (NK) cell cytotoxicity

NK cell cytotoxicity was measured by the method of Florentin *et al.* (1989) using YAC-1 as the target cell.

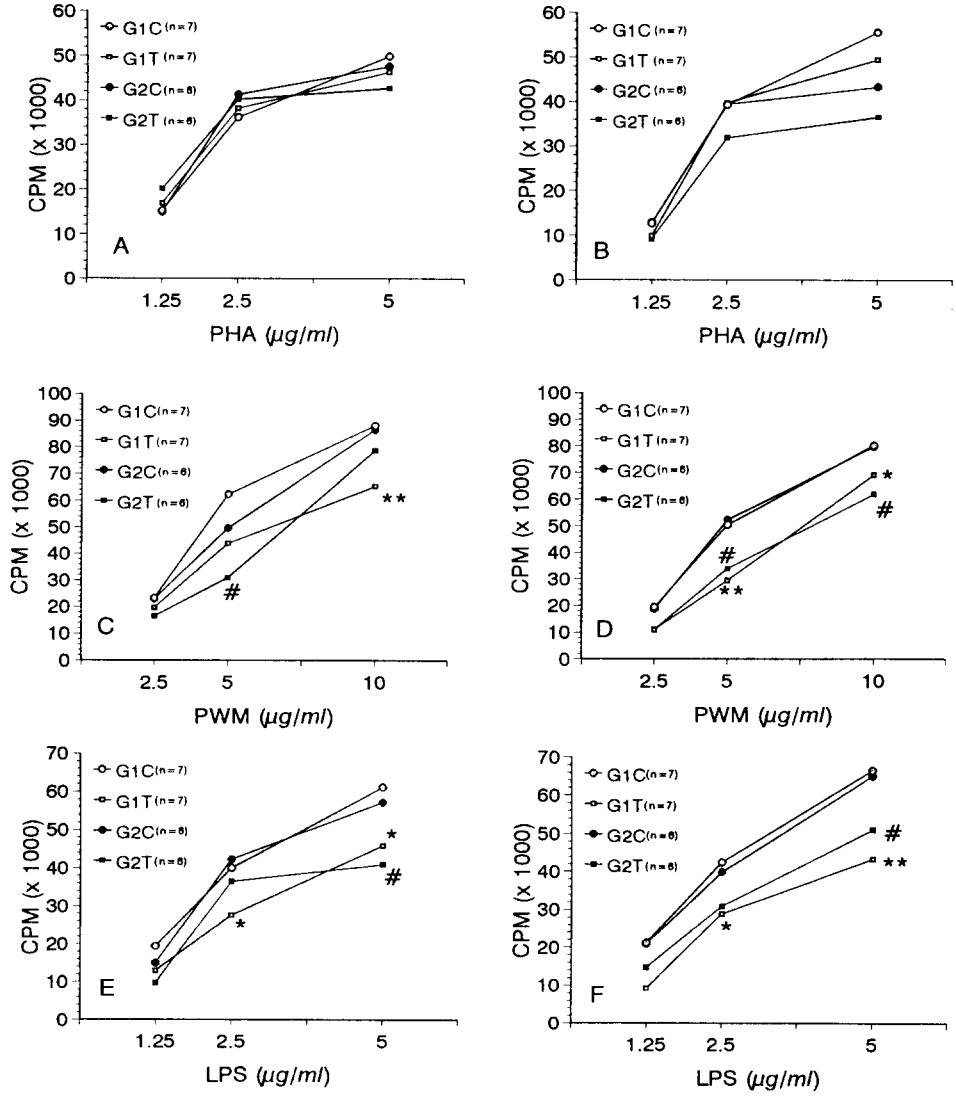

Fig. 1. Effects of DM on proliferative response to mitogens in mouse splenic lymphocyte. DM 50 mg/kg was orally administered to each group in the absence (G1) and/or in the presence (G2) of prenatal Dm exposure for 90 days (A, C, E) and 180 days (B, D, F). Each point indicates mean of 6 to 7 experiments. G1C: saline alone, G1T: DM alone, G2C: Saline treatment following initial exposure to DM through the entire gestation period, G2T: DM treatment following initial exposure to DM through the entire gestation period. *G1C v G1T: $P<0.05$, **G1C v G1T: $P<0.02$, # G2C v G2T: $p<0.05$

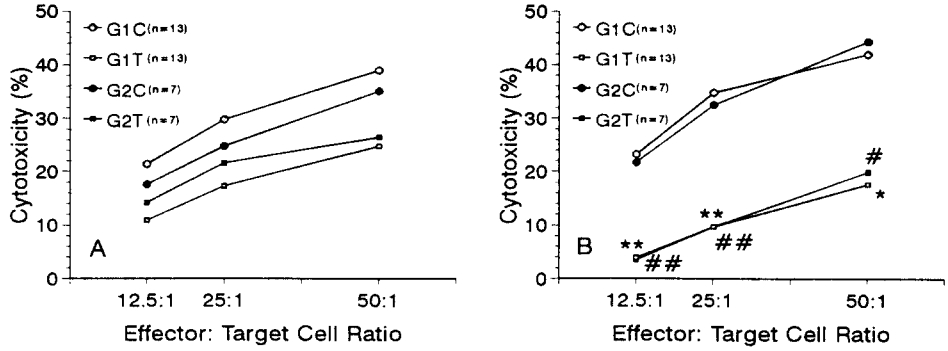

Fig. 2. Effects of DM on natural killer (NK) cell activity in mouse splenic lymphocyte. DM 50 mg/kg was administered orally to each group in the absence (G1) and/or in the presence (G2) of prenatal DM exposure for 90 days (A) and 180 days (B). Each point indicates the mean of 7 to 13 experiments. G1C: saline alone, G1T: DM treatment following initial expousure to DM through the entire gestation period. *G1C vs. G1T=P<0.02; **G1C vs. G1T=P<0.001; #G2C vs. G2T=p<0.02; ##G2C vs. G2T=P<0.01.

Briefly, effector cells were adjusted to varying concentrations and added to 1×10^4 ^{51}Cr-labelled YAC-1 cells in U-bottomed 96-well plates in a total volume of 160 μl. Following incubation for 4 hr at 37°C in a 5 % CO_2 incubator, the plates were centrifuged at 350× g for 10 min; 80 μl samples of supernatant were collected and their radioactivity was measured with a α-counter (LKB-Pharmacia, Sweden). All assays were performed in triplicate. The baseline ^{51}Cr release never exceeded 10 % of the total counts incorporated by target cells. Experimental results were expressed as cytotoxicity obtained at effector/target (E/T) ratios of 50 : 1, 25 : 1 and 12.5 : 1. The percentage of cytotoxicity was expressed as (E-S) / (T-S) × 100, where E=cpm of ^{51}Cr released in the presence of splenic effector cells ; S=cpm of ^{51}Cr released with unlabelled target cells in place of effector cells, and T=total cpm of ^{51}Cr incorporated into the target cells.

Statistics

The statistical significance of differences between the DM-treated group and the control group was determined with the Student's *t*-test.

RESULTS AND DISCUSSION

A careful review of the literature indicated that immunomodulation after exposure to DM had not been reported. The purpose of our study, therefore, was to examine possible immunomodulation after chronic exposure to DM. This paper provides new information about chronic DM-related impairment of B cell function and reduction of NK cell cytotoxicity. Unexpectedly, prenatal DM exposure (G2) did not potentiate the immunomodulation in postnatal effect induced by chronic DM administration. Splenic lymphocytes did not show any significant response to phytohemagglutinin (PHA), a T cell stimulator after prolonged DM administration, although the response tended to be reduced compared to the control animals (Fig. 1 A, B), suggesting that the administration of DM did not impair T cell function. The lumphocyte proliferative response in the presence of long-term DM exposure was suppressed by mitogen concentrations of of 5 μg/ml and 10 μg/ml for pokeweed mitogen (PWM), a costimulator of T cells and B cells, and 2.5 μg/ml and 5 μg /ml for lipopolysaccharide (LPS), a B cell stimulator, suggesting that in the spleen, the main target for DM-induced suppression resides in PWM and LPS-responsive populations, presumably, B lymphocytes (Fig. 1 C, D, E, F). It is possible that the impairment of immune surveillance by long-term DM exposure is a high-risk factor for aquiring diseases, in particular infectious diseases. As seen in Fig. 2 the NK cell activity of mouse splenic cells was significantly suppressed only at 180 days after prolonged DM administration. The mechanism of the reduction of NK cell activity by long-term exposure to DM remains uncharacterized. Finally, the present finding that long-term exposure to DM significantly suppressed B cell function and NK cell cytotoxicity is similar to immunosuppressive effects caused by drug abuse (Di Francesco *et al.*, 1993; House and Thomas, 1985). Therefore, chronic DM-induced immunosuppressive action may be a potential risk factor. The mechanism of DM action following chronic DM exposure is not clearly understood, and is currently under investigation.

ACKNOWLEDGEMENTS

H. C. Kim was supported by the Korea Science and Engineering Foundation (1992, Post-doctoral program). We would like to thank Dr. J. L. Maderdrut for his critical reading of this manuscript.

REFERENCES CITED

Albers, G. W., Saenz, R. E. and Moses, Jr., J. A., To-

lerability of oral dextromethorphan in patients with a history of brain ischemia. *Clin. Neuropharmacol.*, 15, 509-514 (1992).

Di Francesco, P., Gaziano, R., Pica, F., Palamara, A. T., Innocenti, F. and Favalli, C., In vivo phencyclidine-induced inhibition of lymphocyte proliferation: prevention by cell activation. *Immunopharmacol. Immunotoxicol.*, 15, 69-78 (1993).

Florentin, I., Maral, J., De Sousa, M., Berardet, M., Hertz, F. and Cloarec, A., Modulation of immune responses in mice by oral administration on nifulmic acid. *Int. J. Immunopharmac.*, 11, 173-183 (1989).

Hellstrand, K., Hermodsson, S. and Strannegard, ., Evidence for a β-adrenoreceptor- mediated regulation of human natural killer cells. *J. Immunol.*, 134, 4095-4099 (1985).

House, R. V. and Thomas, P. T., Immunological consequences of in vitro exposure to lysergic acid diethylamide (LSD). *Immunopharmacol. Immunotoxicol.*, 16, 23-40 (1994).

Jasinski, P. R., Martin, W. R. and Maski, P. A., Progress report on the assessment of the antagonist nalbuphine and GPA-2087 for abuse potential and studies of the effects of dextromethorphan in man. *National Academy of Science/National Research Council.*, 1, 143-178 (1971).

Jhoo, W. K. and Kim, H. C., Drug abuse among Korean adolescents. *The International Congress of Applied Psychology (Kyoto, Japan)*, 22, 330 (1990).

Kim, H. C. and Jhoo, W. K., Alterations in motor activity induced by high dose oral administration of dextromethorphan throughout two consecutive generations in mice. *Arch. Pharm. Res.*, 18, 146-152 (1995)

Kim, H. C., Pennypacker, K. R., Bing, G., Bronstein, D., McMillian, M. K. and Hong, J. S., The effects of dextromethorphan on kainic acid-induced seizures in the rat. *NeuroToxicol.* in press.

Madden, K. S. and Felten, D. L., Experimental basis for neural-immune interations. *Physiol. Rev.*, 75, 77-106 (1995).

Olney, J. W., Labruyere, J. and Price, M. T., Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science*, 244, 1360-1362 (1989).

Tortella, F. C., Pellicano, M. and Bowery, N. G. Dextromethorphan and neuromodulation: old drug coughs up new activities. *Trends Pharmacol. Sci.*, 10, 501-507 (1989).