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IMMUNOMODULATING PROPERTIES OF MDMA ALONE AND IN COMBINATION WITH ALCOHOL: A PILOT STUDY

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Abstract: Cell-mediated immune response after the administration of MDMA alone and in combination with alcohol was evaluated in a randomized, double-blind, double-dummy, cross-over pilot clinical trial conducted in four healthy MDMA consumers who received single oral doses of 75 mg MDMA (n = 2) or 100 mg MDMA (n = 2), alcohol (0.8 mg/kg), MDMA and alcohol, or placebo. Acute MDMA treatment produced a time-dependent immune dysfunction associated with MDMA plasma concentrations. Although total leukocyte count remained unchanged, there was a decrease in the CD4 T/CD8 T-cell ratio as well as in the percentage of mature T lymphocytes, probably because of a decrease in both the percentage and absolute number of T helper cells. The decrease in CD4 T-cell counts and in the functional responsiveness of lymphocytes to mitogenic stimulation was dose-dependent. The correlation between MDMA pharmacokinetics and the profile of MDMA-induced immune dysfunction suggests that alteration of the immune system may be mediated by the central nervous system. Alcohol consumption produced a decrease in T helper cells, B lymphocytes, and PHA-induced lymphocyte proliferation. Combined MDMA and alcohol produced the greatest suppressive effect on CD4 T-cell count and PHA-stimulated lymphoproliferation. Immune function was partially restored at 24 hours. These results provide the first evidence that recreational use of MDMA alone or in combination with alcohol alters the immunological status. © 1999 Elsevier Science Inc.

Key Words: MDMA, humans, immunity, alcohol

Introduction

Recreational use of 3,4- methylenedioxymethamphetamine (MDMA, "ecstasy") either alone or in combination with other drugs, such as alcohol and cannabis, has become increasingly popular among young people (1). MDMA ingestion can induce neurochemical, behavioral, and endocrine alterations similar to those produced by exposure to acute stress, suggesting its potential as a "chemical stressor". It has been shown that stress can produce a dysfunction of immune function and alteration of the distribution of immune cells. The effect of MDMA on immune function has

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been evaluated in a few number of *in vitro* studies (2). In a rat model, acute MDMA administration was followed by rapid and sustained suppression of total leukocyte count and mitogen-induced lymphocyte proliferation accompanied by an increase in plasma corticosterone concentrations (3). Furthermore, a dose-related differential alteration in T-helper cell function has been reported (4).

Although it is well known that drugs of abuse modify the immune status in humans (5,6), to date no clinical studies have assessed immune function in MDMA users. In this report, we present results obtained during a pilot study initially designed to obtain preliminary data on the pharmacological interactions between MDMA and alcohol. Total leukocyte counts, blood lymphocyte subsets, and lymphocyte proliferative response to mitogenic stimulation, as well as plasma drugs and cortisol concentrations were investigated after the administration of MDMA alone and in combination with alcohol.

Methods

Study Design. Four healthy male volunteers familiar with MDMA effects gave their written informed consent to participate in a randomized, double-blind, double-dummy, cross-over pilot clinical trial. The study was approved by the institutional review board and authorized by the Spanish health authorities (DGFPS No. 98/112). All subjects participated in four different experimental sessions randomly assigned in which they were given single doses of MDMA, alcohol (0.8 mg/kg), MDMA and alcohol, or placebo by the oral route. The doses of MDMA administered were 75 mg (n = 2) and 100 mg (n = 2) in form of capsules prepared by the Department of Hospital Pharmacy of Hospital del Mar. Sessions were separated by 1-week washout period.

Determination of plasma drugs concentration. Blood samples for determination of drug concentrations were drawn before treatment and at 15, 30, 45, 60, 75 and 90 min and 2, 3, 4, 6, 8, 10 and 24 hours after drugs administration. MDMA was measured in plasma by gas chromatography equipped with a nitrogen-phosphorous detector (7).

Determination of plasma cortisol. Plasma cortisol concentrations were determined by fluorescence polarization immunoassay (FPIA) (Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions. The intra-assay coefficients of variation (CV) were 2.9 and 2.6 for low (4.0 μg/dL) and high (40.0 μg/dL) controls, respectively. The assay sensitivity is reported to be 0.45 μg/dL.

Blood cell preparation for immunological tests. Blood samples for immunologic tests were drawn before treatment and at 1, 2, 6, and 24 hours after drug administration. Peripheral blood was collected in evacuated tubes containing ethylenediaminetetraacetic acid (0.47 *M*). Complete blood profile and count was obtained for each participant. Peripheral blood mononuclear cells (PBMC) were obtained by centrifuging whole blood on a Ficoll-Hipaque density gradient (Pharmacia Fine Chemicals, Uppsala, Sweden). PBMC were rinsed and suspended in RPMI 1640 medium containing penicillin (100 U/mL), streptomycin (100 mg/L), and 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY, USA). This suspension was used as the effector cells in the immunoassays.

Response to mitogen. PBMC collected as described were adjusted to a final concentration of $1x10^7$ cell/mL in culture medium, placed in 0.1 mL aliquots in microtiter platewells, and stimulated with phytohaemoagglutinin A (PHA, 1 μg/ml) diluted in culture medium. This dose was found to produce optimal stimulation of lymphocyte proliferation. All cultures were incubated at 37°C in 5% $C0_2$ for 24 h, pulsed with 1 μCi methyl ^3H thymidine, and incubated during 18 h. Cells were harvested in filter paper Skatra 7031 using a Skatron automatic cell harvester. Incorporation of ^3H-thymidine was determined by counting in 3 mL lipoluma scintillation fluid by a β-counter. All cultures were performed in triplicate. Radioactivity was measured in counts per minute (cpm). Results were expressed as Stimulation Index (SI), defined as the ratio of mean cpm in PHA-stimulated versus non-stimulated cultures.

Lymphocyte immunophenotyping. 100 μL of whole blood were stained using 20 μL of monoclonal antibody reagent. Becton-Dickinson FACS Lysing solution was used to lyse red cells after which stained cells were washed once with PBS and fixed with 1% paraformaldehyde. Stained and fixed lymphocytes were analyzed using a Ortho Cytoron Absolute 4 flow cytometer (Ortho Instruments). The LeucoGATE (CD45/CD14) fluorescent information, with forward and side scatter, was used to set an electronic gate around the lymphoid population. This gate included at least 95% lymphocytes and less than 5% non-lymphocytes (granulocytes, monocytes, and debris).
Dual-color immunophenotyping was performed using the following Becton-Dickinson matched murine monoclonal antibody reagents directly conjugate to phycoerythrine (PE) or fluorescein isothiocyanate (FITC): CD14 / PE - CD45 / FITC (leukogate reagent for electronic gating), CD8 / FITC - HLA-DR / PE (cytotoxic/suppressor cells), CD4 / FITC - HLA-DR / PE (helper/inducer cells), CD3 / PE - CD16 - CD56 / FITC ($CD3^+$:T mature lymphocytes and $CD3^-$-$CD16^+$-$CD56^+$:natural killer cells), CD3 / PE - CD19 / FITC (T mature lymphocytes and B lymphocytes). Absolute lymphocyte numbers were determined by multiplying the percentage of each subset of lymphocytes obtained from the flow cytometer by the absolute lymphocyte count.

Results

In all volunteers, immunologic parameters were within normal limits before drug treatment. In table I the mean (and relative standard deviation, RSD) of basal values of leukocyte subpopulations for each subject in each clinical trial treatment condition (n=4) are presented.

TABLE I
Basal leukocyte subpopulations in four healthy volunteers (mean and RSD of absolute values)

	Subject 1	Subject 2	Subject 3	Subject 4
T cells/μL				
CD3	1422.8 (2.2)	1416.8 (2.8)	1546.4 (6.7)	1426.0 (5.2)
CD4	1006.7 (2.9)	988.3 (3.2)	1135.6 (5.4)	972.3(11.7)
CD8	376.4 (6.3)	448.9 (4.8)	474.0 (24.1)	411.1(13.1)
CD4/CD8 ratio	2.7 (4.7)	2.2 (4.7)	2.5 (17.8)	2.3 (12.3)
B cells/μL				
CD19	253.9 (14.1)	77.7 (14.2)	170.4(13.1)	283.3 (5.5)
NK cells/μL	88.2 (22.5)	136.5 (17.7)	185.6 (26.6)	107.4 (32.1)
Stimulation index	72.8 (5.3)	86.2 (8.0)	101.4 (5.5)	66.5 (8.5)

Acute MDMA treatment produced an immune dysfunction (Fig. 1). Immune dysfunction was time-dependent and showed a parallelism with both MDMA plasma concentrations and MDMA-induced cortisol stimulation kinetics. All alterations regarding immune parameters tested peaked between 1 and 2 h from the start of the treatment (data not shown).

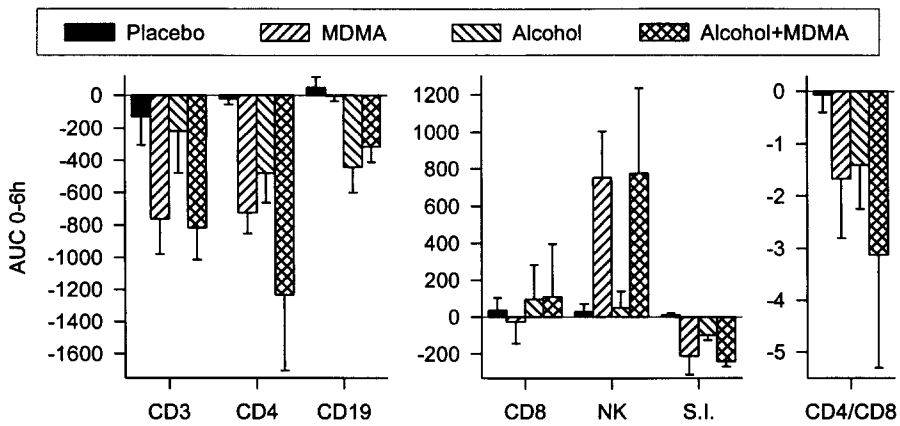

Fig 1.

Immunophenotyping and functionality of the immune system in the four treatment conditions. Results are expressed as the AUC0-6 hours (cells/μL*hour^{-1}, except for the ratio CD4/CD8 and SI) of experimental values (mean value and SD, n=4). Experimental values have been normalized by calculating the difference over basal values

Although total leukocyte count remained unchanged, there was a decrease in the ratio of CD4 to CD8 T-cells as well as in the percentage of mature T lymphocytes (CD3), probably because of the decreased proportion of circulating T helper cells (CD4). Lymphoproliferative response to PHA stimulation also was reduced. No differences were found in the percentage of cytotoxic/suppressor lymphocytes (CD8) and B lymphocytes (CD19). By contrast, there was a high increase in the percentage of NK cells. The decrease in CD4 T-cell counts and in the functional responsiveness of lymphocytes to mitogenic stimulation was dose-dependent. In the two subjects treated with 100 mg of MDMA, as early as 1 h after drug administration, CD4 T-cell count and PHA-induced lymphocyte proliferation decreased by an average of 24.5% and 55.5%, respectively, as compared with placebo. In the other two participants treated with 75 mg of MDMA, decreases of 13.5% and 36% were found.

Alcohol administration produced a decrease in the proportion of circulating T helper cells (CD4) and B lymphocytes (CD 19) and in the PHA-induced lymphocyte proliferation. Combined MDMA and alcohol produced the greatest suppressive effect on CD4 T-cell count and PHA-stimulated lymphoproliferation (Fig. 1). In fact, 1 h after receiving 100 mg MDMA and 0.8 mg/kg alcohol, the CD4 T-cell count and the lymphocyte proliferative response to PHA showed a mean decrease of 40% and 82%, respectively, as compared with placebo. The immune function was partially restored after 24 h for all treatment conditions.As shown in Fig. 2, cortisol plasma concentrations did not change in the placebo and alcohol treatment conditions.

MDMA produced a mean rise in cortisol concentrations at 2 h after drug administration of 21±7.5 μg/dL (17.5 μg/dL for MDMA 75 mg and 24.4 μg/dL for MDMA 100 mg). When alcohol was co-administered with MDMA cortisol plasma concentrations rise was partially blunted (mean value 13.7±7.5 μg/dL, 12.1 μg/dL for MDMA 75 mg and 15.4 μg/dL for MDMA 100 mg).

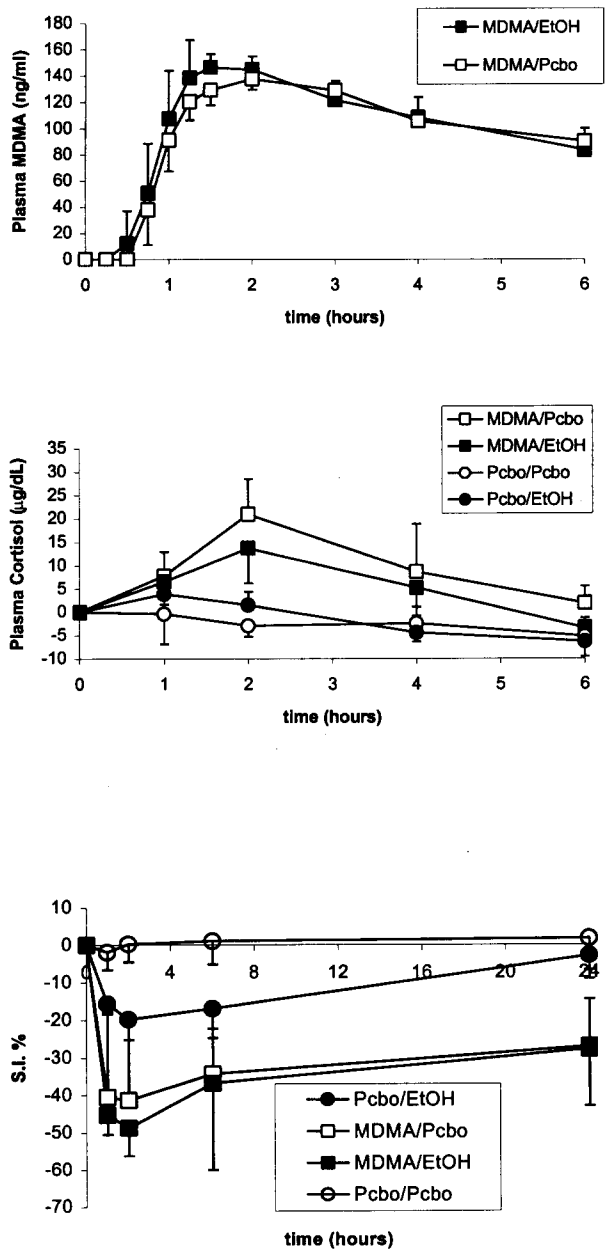

Fig. 2

Upper Trace: Plasma MDMA concentrations (mean and SD, n=4) after administration of MDMA alone or in combination with alcohol (EtOH). 'Pcbo' stands for placebo treatment. *Central Trace*: Plasma cortisol concentrations for the four treatment conditions (mean and SD, n=4) *Lower Trace*: Stimulation index (SI) (mean and SD, n=4). Values have been normalized by subtracting to experimental values the corresponding basal value of each treatment condition (central and lower trace)

Discussion

The results show that MDMA administered at doses compatible with its recreational use cause pronounced changes in certain neuroendocrine and immunologic parameters, and that these changes occur very rapidly. Indeed, 1 h after the start of treatment, there was a significant reduction of CD4 T-cell count, of proliferation of PHA-stimulated lymphocytes, and an increase in NK cell count. The few number of participants prevents any statistical treatment of data. However, variations observed can be attributed to treatment conditions because the cross-over design of the study and the small variations in the parameters tested in basal conditions at the beginning of each treatment sessions (randomly assigned) in four consecutive weeks (see Table 1).

A decrease in circulating CD4 helper T-cell and lymphocyte proliferation response to a mitogen and a simultaneous increase in circulating NK cells do not provide a certainty of immunosuppression or immunoenhancement. Otherwise, the reaction of the immune system to MDMA administration appears as an alteration of physiologic equilibrium or homeostasis. Interestingly, the same immune reactions were observed in the rapid response to several acute psychologic and physical stress in human volunteers (8). In fact, volunteers exposed to acute psychologic stress showed as early as 4 min after the start of challenge, a significant elevation in the percentage of NK cells and a fall in the CD4 cell percentages (8). These observations suggest that MDMA could be regarded as a "chemical stressor".

A rise of cortisol plasma concentrations similar to which was observed in the rat model (3) parallels alterations in immune function. These results would be complementary with observations made in human volunteers where MDMA stimulated cortisol and ACTH secretion (9,10). It should be noted that alterations in cortisol secretion and immune function seem to be dose-dependent. In view of cortisol results, it may be assumed that a consequence of the effects of MDMA on central monoaminergic systems is the release of corticotropin-releasing factor (CRF) from the median eminence of the hypothalamus and subsequent HPA-axis and SNS activation. Much evidence indicates that CRF is the coordinator of the response to stress (11). In fact, within minutes of acute stress, CRF induces the production of corticosteroids and catecholamines which represent two of the major classes of stress hormones (12). Different studies have indicated that corticosteroids inhibit many functions of lymphocytes and modify the production of many cytokines and inflammation mediators (13). In addition, elevation of norepinephrine and epinephrine levels, which accompanies stress, may produce changes in lymphocyte functions generally down regulating immune system function (14). From the data obtained in the present study, it cannot be defined the specific contribution of cortisol and serotonine and catecholamines involved in MDMA mechanism of action on the immune function.

In this study we also observed that MDMA-related immune dysfunction was dose-dependent. These results are in agreement with those found by Connor et al. (4), who observed in animal model a dose-dependent suppression of mitogen-stimulated lymphocyte proliferation and reduction in the number of circulating white blood cells. Indeed, this drug of abuse can modulate the peripheral blood lymphoid subsets changing the number of circulating regulatory lymphocytes. The decrease of lymphocyte proliferation response to a PHA mitogen presumably reflects the lower frequency of circulating CD4 helper T-cell. As previously described, alcohol consumption produced a decrease in the absolute number of T helper cells and B lymphocytes and in the PHA-induced lymphocyte proliferation (15).

As stated previously, alterations in immune function are more marked when combining MDMA and alcohol. In the combination treatment there is an additive phenomenon regarding the immune dysfunction induced by each drug. This observation is relevant as combination of both drugs, despite the current believe that non-alcoholic beverages are consumed with MDMA, occurs in as much as 76% of users of this drug (16). From the data obtained in four volunteers, we cannot affirm that there is an evident metabolic interaction between MDMA and alcohol. Studies in a larger number of subjects should be conducted to demonstrate definitively that alcohol does not affect MDMA metabolism/elimination.

We conclude that the recreational use of MDMA alone or combined with alcohol alters the immunologic status in humans. The correlation between MDMA pharmacokinetics and the profile of MDMA-induced immune dysfunction suggeststst that the alteration may be mediated by the CNS. Alternatively, MDMA may act directly on immune cells or inderectly by promoting the release in the periphery of serotonin from platelets stores. In the present study only acute effects are reported. Taking into account the particular consumption pattern of MDMA where abuse tends to be irregular (e.g., weekends), the impact of MDMA on immune function has to be assessed in longitudinal studies. The present findings, however, tentatively indicate that MDMA ingestion represents a potential serious health hazard for an increased risk of immune system-related diseases.

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