Immunomodulating Activity of MDMA

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ABSTRACT: MDMA (3,4-methylenedioxymethamphetamine) use can cause neurochemical, behavioral and endocrine alterations, similar to those produced by exposure to acute stress, suggesting its potential as a "chemical stressor." It is known that stressful stimuli can produce a depression of immune function and an alteration in immune cells distribution. *In vitro* exposure to MDMA resulted in a modulation of several immune functional parameters such as T-cell regulatory function, cytotoxic T-lymphocyte activity, natural killer cell activity and macrophage function.

Administration of MDMA in rats produced a rapid and sustained suppression of induced lymphocytes proliferation and a significant decrease in circulating lymphocytes. These alterations in rat immune function were accompanied by a significant rapid increase in plasma corticosterone concentrations. It was postulated that the result of altered induced proliferation response of lymphocytes could have been due to a combined effect of direct action of MDMA on lymphocytes and to the activation of the hypothalamic pituitary adrenal axis (HPA axis) and/or the sympathetic nervous system (SNS) via central mechanisms.

In humans, 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 $CD4^+$ T-cells and functional responsiveness of lymphocytes to mitogenic stimulation, while percentage of natural killer cells significantly increased. A rise of cortisol plasma concentrations similar to that observed in the rat model supported the hypothesis of MDMA-induced release of corticotrophin-releasing factor from the median eminence of the hypothalamus and subsequent HPA axis and SNS activation. The present findings indicate that MDMA ingestion may represent a potential health hazard for an increased risk of immune system-related diseases.

INTRODUCTION

MDMA (3,4-methylenedioxymethamphetamine, "ecstasy") is a widely abused psychomotor stimulant with behavioral effects related to amphetamines and hallucinogens.[1,2] Ingestion of MDMA can induce several undesirable side effects includ-

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ing, jaw clenching, bruxism, headache, nausea, sweating, muscle aches, fatigue, and insomnia. Acute medical complications include malignant hyperthermia, hypertension, arrythmias, seizures, cerebral hemorrhage, hepatitis, rhabdomyolisis, disseminated intravascular coagulation, and acute renal failure.[3] There are a number of reports concerning severe intoxication and death after MDMA abuse.[4] Studies in animal models (rats, monkeys) have shown neurotoxicity in the serotoninergic system after single and repeated doses of MDMA.[5] Even if not fully substantiated, neurotoxicity is seen as the main long-term side effect associated to MDMA consumption in humans.[6,7] Some effects observed in clinical trials with healthy volunteers after MDMA administration, like a dramatic rise in cortisol plasma concentrations, suggest that this drug could be considered as a chemical stressor.[8] It has been shown that stress can produce immune dysfunction and alteration of the distribution of immune cells.[9] To date, however, few reports have dealt with the effect of this recreational drug of abuse on the immune system.

The immune system is a highly regulated organ system and, as such, presents a variety of potential targets for modulation. This modulation may take the form of immunosuppression, possibly leading to an enhanced susceptibility to infection; conversely, it may take the form of immunostimulation, possibly resulting in hypersensitivity (allergy) or autoimmune disease.[10]

In the present paper, experimental data from *in vitro* and *in vivo* animal models concerning the interaction of MDMA and other natural and synthetic amphetamines on the immune system are reviewed. Data obtained in healthy volunteers administered with MDMA (single-dose studies) are presented.

IN VITRO STUDIES ON ANIMAL MODELS AND HUMANS

In vitro studies on the immunomodulatory effect of MDMA and other substituted amphetamines have been performed in murine splenocytes, peritoneal macrophages, and peripheral blood mononuclear leukocytes (PBML).[11,12] Several key immune function parameters were monitored: proliferation of B lymphocytes, T-cell regulatory function, evaluated as the production of cytokines by $CD4^+$ T-cells, cytotoxic T-lymphocyte (CTL) activity, natural killer (NK) cell activity, a nonspecific host resistance mechanism, and finally macrophage function, assessed as production of cytokine IL-6 and tumor necrosis factor (TNF).

In vitro exposure to MDMA in the concentration range of 0.0001–100 μM had no effect on B-cell proliferation. Concerning cytokines produced by $CD4^+$ T-cells, MDMA induced a suppression of IL-2 production at high doses (100 μM), but an enhancement at low doses (0.0001 μM), with intermediate doses (0.001–10 μM) failing to significantly alter this parameter. Conversely, IL-4 production was not affected by exposure to any concentration of MDMA, suggesting a differential alteration in T-helper cell function by this compound. CTL induction was significantly suppressed only at 100 μM MDMA. This observation was probably related to the modulation of IL-2 production. Moreover, exposure to MDMA resulted in an elevation of NK cell function at concentrations between 0.0001 and 1.0 μM when evaluated at an effector:target cell ratio of 100:1. This enhancement showed a bell-shaped dose response curve with a maximum at 0.01 μM MDMA. Finally, exposed mac-

rophages exhibited a trend toward lower TFN production with increasing MDMA concentrations.

To date, *in vitro* studies have not been performed in humans. Gagnon and coworkers presented some in vitro data performed in PBML, testing several 4-substituted amphetamine designer drugs, but not MDMA.[12] Both amphetamine and 4-substituted designer amphetamines inhibited cell proliferation in response to T-cell mitogen phytohemoagglutinin A (PHA). Designer drugs proved more potent than amphetamine in this experimental paradigm. In addition, they suppressed cell proliferation caused by the B-cell pokeweed mitogen. Also, it is noteworthy that amphetamine racemate and the *d*-enantiomer were active in the inhibition of cell proliferation, whereas the *l*-enantiomer was inactive. An increase in NK cell activity was observed with amphetamine at low concentrations (10^{-12} to 10^{-10} M range).

Although the data presented above showed for the first time the direct effect of MDMA on immune cells, *in vitro* studies suffer from some drawbacks. It is well established that alterations in the activity of the central nervous system (CNS) can affect immune function through neuroendocrine changes and the activity of the sympathetic nervous system (SNS).[13] Conversely, products of the immune system can modulate CNS function.[14] Specifically, MDMA acts on the central monoaminergic systems, releasing corticotrophin-releasing factor (CRF) from the median eminence of hypothalamus and hence activating the hypothalamic pituitary adrenal axis (HPA axis) and the SNS, factors known to modulate leukocyte distributions and function.[15,16] These relationships between the neuroendocrine, sympathetic, and immune systems cannot be easily evaluated with *in vitro* studies. Furthermore, they cannot account for the eventual effect of metabolites of MDMA on immune function. Indeed, 3,4-methylenedioxyamphetamine (MDA) and 3-hydroxy-4-methoxymetamphetamine (HMMA), the main MDMA metabolites, are 4-substituted amphetamines and hence their contribution on the overall MDMA effect over the immune system would not be unexpected.

IN VIVO STUDIES ON ANIMAL MODELS

Investigations in the mouse model with amphetamine appear to reproduce some of the results observed *in vitro*.[17,18] Amphetamine was shown to decrease T-cell populations and lymphoproliferation stimulated by the mitogen concavalin A (Con A). An apparent contradictory effect was a dose-related suppression of NK cell activity. Nevertheless, doses assayed in the animal model were much higher than concentrations tested in the *in vitro* model where at low concentrations activation was reported. Indeed, during the withdrawal of rats treated chronically with amphetamine, a significant increase of NK cell activity was observed.[19] Moreover, a report suggested that amphetamine reduction of lymphoproliferation appears as independent from the induced rise of corticosterone levels, as propranolol, a nonselective beta-blocker, was able to prevent immunedysfunction.[20] Finally, in a chronic *d*-amphetamine administration protocol, this drug was shown to facilitate immunosuppresion induced in rats by an aversive stimulus (foot shock stress).[21] A decrease in the percentage of T lymphocytes and a reduction in the delayed-type hypersensitivity reaction (DHT) were observed.

Regarding MDMA, investigations on female rat model were conducted by Connor and co-workers.[22,23] In the first report they examined the effect of single MDMA dose (20 mg/kg i.p.) on both circulating leukocyte number and the functional responsiveness of lymphocytes to mitogenic stimulation with Con A. Administration of MDMA produced a rapid (30 min) and sustained (6 h) suppression of induced lymphocyte proliferation and a significant decrease in total leukocyte count that persisted at least six hours after the treatment. These alterations in rat immune function were accompanied by a significant increase in plasma corticosterone concentrations by 30 min after drug administration which returned to baseline within six hours. It was postulated that the result of altered Con A-induced proliferation response of lymphocytes, in contrast with that from *in vitro* studies, could have been due to a combined effect of direct action of MDMA on lymphocytes and to activation of the HPA axis and/or SNS via central mechanisms. This hypothesis was supported by an MDMA-induced increase in corticosterone plasma concentrations, probably responsible for the reduction in total leukocyte count. However, it was not established whether MDMA-induced suppression in lymphocyte activity could be attributed to an increased SNS activity or simply to elevated plasma corticosterone concentrations. Furthermore, it was not clear what leukocyte subset was altered after MDMA administration.

In a subsequent report dealing with different doses of MDMA (1.25–40 mg/kg i.p.), it was shown that acute administration of MDMA caused a reduction in circulating lymphocytes without a significant alteration in the number of neutrophils or monocytes in peripheral blood. The decrease in circulating lymphocytes was observed at doses of MDMA (10 mg/kg), which induced an increase in plasma corticosterone concentrations, thus suggesting the intervention of MDMA-induced activation of the HPA axis. Conversely, MDMA produced a suppression of lymphocytes function even at doses that failed to increase plasma corticosterone concentrations, assuming that the reduced responsiveness of lymphocytes to mitogenic stimulation might be mediated by glucocorticoid independent mechanisms.

IN VIVO STUDIES IN HUMANS

Pilot Study

Preliminary results on cell-mediated immune response after the administration of MDMA were obtained in a randomized, double-blind, double-dummy, crossover pilot clinical trial conducted in four healthy male MDMA consumers who received single oral doses of 75 mg MDMA ($n = 2$) or 100 mg MDMA ($n = 2$), or placebo.[24]

Both pilot and definitive studies were approved by the local institutional review board and authorized by the Spanish health authorities (DGFPS No. 98/112). All subjects participated in two different randomly assigned experimental sessions in which they were given single oral doses of MDMA, or placebo by the oral route. Sessions were separated by a one-week washout period.

Blood samples to determine 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 h after drug administration. MDMA was measured in plasma by gas chromatography equipped with a nitrogen-phosphorous detector.[25] Plasma cortisol concentrations were determined by fluorescence polarization immunoassay (FPIA).

Blood samples for immunologic tests were drawn before treatment and at 1, 2, 6, and 24 h after drug administration. Peripheral blood was collected in evacuated tubes containing ethylenediaminetetraacetic acid (0.47 M). Complete blood profile and count were obtained for each participant. For lymphocyte immunophenotyping, 100 μl of whole blood were stained using 20 μl of monoclonal antibody reagent. 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% nonlymphocytes (granulocytes, monocytes, and debris). Each subset of lymphocytes was obtained as a percentage directly from the flow cytometer. Functional responsiveness of lymphocytes to mitogenic stimulation (PHA) was measured by incorporation of [^{3}H]thymidine test.

Although total leukocyte count remained unchanged, there was a decrease in the $CD4^+$ T /$CD8^+$ T-lymphocyte 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. A dose-dependent decrease in the functional responsiveness of lymphocytes to mitogenic stimulation was observed (FIG. 1). Indeed, 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. By contrast, there was a high increase in the percentage of NK cells.

The profile of MDMA-induced immune dysfunction parallels MDMA pharmacokinetics, suggesting that alteration of the immune system could be mediated by the CNS. Immune function was partially restored at 24 h. These results prompted the development of a final clinical trial on eight MDMA consumers treated with a dose commonly encountered in recreational use.

Definitive Clinical Trial

Eight healthy male volunteers familiar with MDMA participated in a randomized, double-blind, crossover clinical trial. All subjects participated in two different

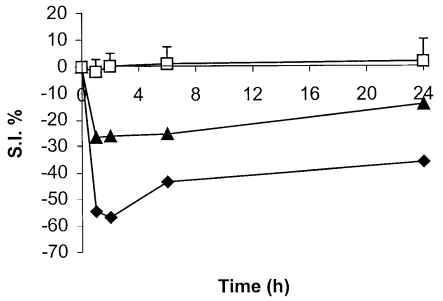

FIGURE 1. Lymphocyte mitogen induced proliferation over time in the MDMA 75 mg (▲, $n = 2$), MDMA 100 mg (◆, $n = 2$) and placebo (□, $n = 4$) treatment groups. Stimulation Index (SI %) is defined as the ratio of mean counts per minute in the incorporation of [^{3}H]thymidine test of PHA-stimulated versus nonstimulated cultures (mean and SD).

randomly assigned experimental sessions in which they were given single doses of 100 mg MDMA, or placebo by the oral route. Sessions were separated by a one-week washout period. Experimental conditions were the same as the pilot study. Blood samples for immunologic tests were drawn also at 0, 1, 1.5, 2, 6, and 24 h after drug administration.

Statistical analysis of data was conducted by a repeated measure analysis of variance, with treatment conditions and time as variables. The mean square error term from the dose condition vs. time interaction was used to calculate Tukey's honestly significant difference to make all possible pairwise comparisons at each postdrug observation. Tests were considered statistically significant when $p < 0.05$.

Acute MDMA treatment produced an immune dysfunction that was time-dependent and showed a parallelism with both MDMA plasma concentrations and MDMA-induced cortisol stimulation kinetics (FIG. 2). All alterations regarding immune parameters tested peaked between 1 and 1.5 h from the start of the treatment.

Likewise, in the pilot study, total leukocyte count remained unchanged. Nonetheless, a significantly significant decrease in $CD4^+$ T-cells was found between 1 and 24 h after MDMA administration. The decreased proportion of circulating $CD4^+$ T-cells negatively affected $CD4^+$ T/$CD8^+$ T-cell ratio as well as the percentage of mature T lymphocytes ($CD3^+$ T-cells). Administration of MDMA had no effect on the percentage of cytotoxic/suppressor lymphocytes ($CD8^+$) and B lymphocytes ($CD19^+$). By contrast, there was an increase in the percentage of NK cells, statisti-

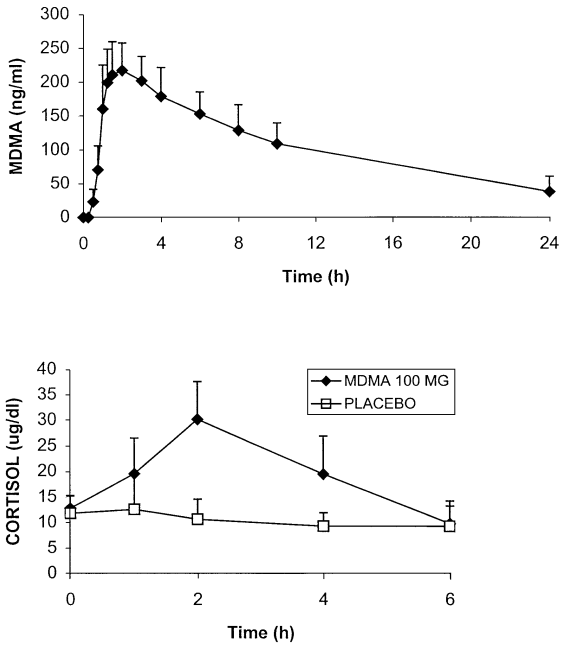

FIGURE 2. MDMA (*upper trace*) and cortisol (*lower trace*) plasma concentrations (mean and SD, $n = 8$ for each data point) over the time curves in the MDMA 100 mg (◆) and placebo (□) treatment groups.

cally significant between 1 and 24 h. Although the modified immune parameters remained statistically different between placebo and MDMA treatment, there was a trend toward basal condition values at 24 h.

As shown in FIGURE 2, cortisol plasma concentrations did not change in the placebo while MDMA produced a mean rise in cortisol concentrations at 2 h after drug administration of 20 mg/dl. The results confirmed 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, one hour after the start of treatment, a significant reduction of $CD4^+$ T-cell count, $CD4^+$ T/$CD8^+$ T-cell ratio, $CD3^+$ T-cells occurred and an increase in NK cell count (FIG. 3). However, these results 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 psychological and physical stress in human volunteers.[9] In fact, volunteers exposed to acute psychological 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 $CD4^+$ T-cell percentages.[9] These observations suggest that MDMA could be regarded as a "chemical stressor."

A rise of cortisol plasma concentrations similar to which was observed in a rat model, [22] parallels alterations in immune function. These results would be comple-

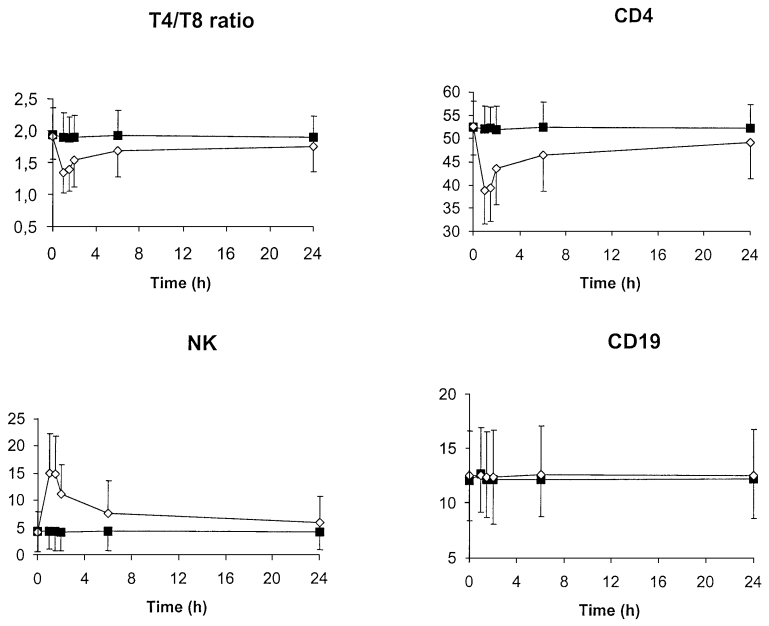

FIGURE 3. Immunophenotyping parameters over time (mean and SD, $n = 8$ for each data point) for the MDMA 100 mg (◆) and placebo (□) treatment groups. Values are expressed as percentage of leukocyte formula, except for the ratio T4/T8 ($CD4^+/CD8^+$).

mentary with observations made in human volunteers where MDMA stimulated cortisol and corticotrophin (ACTH) secretion.[8,26] It should be noted that alterations in cortisol secretion and immune function seem to be dose-dependent. Much evidence indicates that CRF is the coordinator of the response to stress.[27] In fact, within minutes of acute stress, CRF induces the production of corticosteroids and catecholamines that represent two of the major classes of stress hormones.[28] Different studies have indicated that corticosteroids inhibit many functions of lymphocytes and modify the production of many cytokines and inflammation mediators.[29] In addition, elevation of norepinephrine and epinephrine levels, which accompanies stress, may produce changes in lymphocyte functions generally down-regulating immune system function.[30] From the data obtained in the present study, the specific contribution of cortisol and serotonin and the catecholamines involved in the MDMA mechanism of action on immune function cannot be defined.

We conclude that the recreational use of MDMA alters the immunologic status in humans. Considering the results obtained *in vitro* and on animal models for amphetamine, MDMA seems to display a differential effect on the immune function. Apparently, MDMA has no effect *in vivo* on cytotoxic T-lymphocytes activity and B lymphocytes, in contrast with amphetamine. Nevertheless, 4-substituted amphetamines, structurally related to MDMA, look more potent than amphetamine over NK cells count and PHA-stimulated proliferation of T lymphocytes. The correlation between MDMA pharmacokinetics and the profile of MDMA-induced immune dysfunction suggests that the CNS may mediate the alteration. In the present study, only acute effects are reported. Taking into account the particular consumption pattern of MDMA where abuse tends to be irregular, the impact of MDMA on immune function has to be assessed in controlled MDMA repeated doses in clinical trials and in longitudinal studies in MDMA users. The present findings, however, tentatively indicate that MDMA ingestion represents a potential health hazard for an increased risk of immune system-related diseases.

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