Cell-Mediated Immune Response in MDMA Users After Repeated Dose Administration

Studies in Controlled versus Noncontrolled Settings


ABSTRACT: Acute administration of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) produces time-dependent immune dysfunction in humans. Recreational use of MDMA generally includes repeated drug consumption, often in association with other drugs, such as alcohol and cannabis. In the laboratory setting, repeated MDMA administration to healthy MDMA consumers produced a time-dependent immune dysfunction similar to that observed with the ingestion of a single dose, and the first of the two administrations paralleled the time-course of MDMA-induced cortisol stimulation kinetics and MDMA plasma concentrations. A significant decrease in CD4 T-helper cells with simultaneous increase in natural killer (NK) cell and a decrease in functional responsiveness of lymphocytes to mitogenic stimulation was observed. Response to the second dose was either long-lasting compared with the first dose or disproportionate and did not show any parallelism with cortisol and MDMA plasma concentrations. This circumstance extended the critical period during which immunocompetence is highly impaired as a result of MDMA use. Accumulation of MDMA in the body of a poor metabolizer induced higher immunomodulatory effects with statistically significant differences in NK cell function compared with extensive metabolizers. When basal values of lymphocyte subsets were examined in a population of recreational MDMA users participating in different clinical trials, alterations in several immunological parameters were observed. The absolute number of lymphocytes, in particular T lymphocytes and CD4 T-helper cell subsets, showed a trend toward reduced values, although cell counts were within normal limits. By contrast, NK cells in MDMA consumers were reduced to one-third of those from healthy persons. A statistically significant decrease in affected immune parameters was recorded during a 2-year observation period in a subgroup of recreational MDMA users. These permanent alterations in immunologic homeostasis may result in impairment of general health and subsequent increased susceptibility to infection and immune-related disorders.

KEYWORDS: 3,4-methylenedioxymethamphetamine (MDMA); immune dysfunction; lymphocytes; natural killer (NK) cells; ecstasy

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INTRODUCTION

Immunomodulating activity of MDMA (3,4-methylenedioxyamphetamine, "ecstasy") after single-dose administration has recently been assessed in various animal models and in humans. In particular, administration of MDMA in rats produces a rapid and sustained suppression of induced lymphocyte proliferation and a significant decrease in circulating lymphocytes. In humans, a single dose of 100 mg MDMA caused a decrease in CD4 T-helper cells, a simultaneous increase in natural killer (NK) cells, and a decrease in functional responsiveness of lymphocytes to mitogenic stimulation. The correlation of MDMA pharmacokinetics and MDMA-induced cortisol secretion kinetics with the profile of MDMA-induced immune dysregulation suggested a possible implication of the central nervous system in the impairment of immunological status. Subsequent investigations showed that acute MDMA produced a large increase of immunosuppressive cytokines and an imbalance toward antiinflammatory response. In any case, although a critical period during which immunocompetence was highly impaired was evidenced, the immune function showed a trend toward baseline levels 24 hours after a single MDMA administration.

Studies concerning patterns of MDMA use by consumers, however, show that recreational use of MDMA includes repeated drug administration (binge-ing) by "stacking" (i.e., taking several tablets at one time) or "boosting" (i.e., taking several tablets but at intervals over a period of time such as an evening or even several days). These circumstances could probably extend the period following MDMA administration during which immunocompetence is highly impaired. Furthermore, MDMA is consumed either alone or in combination with other drugs, such as ethanol, cannabis, and cocaine, which also are known to induce immune function alterations. Hence, such effects have to be combined with those elicited by MDMA. Thus, more pronounced and long-lasting immunological changes may result from the aforementioned MDMA consumption patterns with potentially enhanced susceptibility to infection and immune-related disorders.

In this paper, experimental data on cell-mediated immune response in volunteers administered repeated doses of MDMA at different time intervals are reviewed. The impact of MDMA metabolism by cytochrome P 450 2D6 (CYP2D6) isoenzyme (debrisoquine 4-hydroxylase) on MDMA immunomodulatory effects is discussed. Data regarding immune baseline parameters in MDMA recreational users participating in different clinical trials during a two-year interval were also reviewed as a preliminary approach to midterm effects of MDMA immunotoxicity.

STUDIES IN CONTROLLED SETTINGS: CLINICAL TRIALS OF REPEATED MDMA ADMINISTRATION

Cell-mediated immune response after the administration of two repeated doses of 100 mg MDMA at 4-hour and 24-hour intervals was evaluated in two randomized, double-blind and crossover clinical trials conducted in 18 healthy male MDMA consumers. Total leukocyte counts, blood lymphocyte subsets, and lymphocyte proliferative response to mitogenic stimulation, as well as cortisol and MDMA plasma concentrations, were investigated. Subjects were phenotyped for CYP2D6 activity
using dextromethorphan as a drug probe. The destromethorphan/dextrorphan ratio was used to classify subjects as poor or extensive metabolizers. All the subjects but one turned out to be extensive metabolizers.

MDMA administration produced a time-dependent decrease in the number of CD4 T-helper cells, a decrease in the functional responsiveness of lymphocytes to mitogenic stimulation, and a simultaneous increase in natural killer cells as already described in single-dose studies. In the case of two 100-mg MDMA doses given 4 hours apart to eight volunteers who were extensive metabolizers, immune alterations produced by the first dose were strengthened by the second one. In fact, the first 100-mg dose produced alterations in immune parameters, which peaked at 1.5 hours from the start of the treatment, when $C_{\text{max}}$ of MDMA was attained in plasma. A mean 30% decrease in CD4 proportion from baseline, a mean reduction of lymphoproliferative response to PHA stimulation of 68%, and a mean 103% peak increase of NK

![Time-course of CD4 T-helper cells (A) and NK cells (B) after the administration of two doses of 100 mg MDMA with a 24-hour interval in nine extensive metabolizers ( for MDMA administration and  for placebo) and one poor metabolizer ( for MDMA administration and  for placebo). Arrows below the abscissa indicate the administration of 100 mg MDMA. Values have been normalized by subtracting to experimental values the corresponding basal value of each treatment condition.](image-url)
cells from the initial value were observed. At 1.5 hours after the administration of the second MDMA dose (5.5 hours after the beginning of the experiment) CD4 T cells and lymphocyte proliferative response to PHA showed a mean 40% and 87% decrease from basal values, respectively, compared with placebo. In contrast, the increase in NK cells showed a 141% rise from baseline values. A mean 20% peak rise was observed in cortisol concentrations at 2 hours after both the first and second MDMA dose. At 24 hours after the first administration, statistically significant residual effects were observed for all the altered immune parameters, in contrast with recovered homeostasis of immune function observed after MDMA single dose.1

In the second clinical trial, two 100-mg doses of MDMA were given 24 hours apart to nine extensive-metabolizer volunteers and one poor-metabolizer volunteer. In extensive metabolizers, the first dose produced alterations in immune parameters of the same magnitude and time course as those observed in previous studies.1,4 All variables returned to baseline values within 24 hours. However, the second dose caused immunological changes significantly greater than those induced by the first pharmacological challenge (TABLE 1). The magnitude of alterations induced by MDMA when comparing those observed after the first administration and two consecutive doses may be exemplified by comparing area under the curve (AUC)_{24–48 h} versus AUC_{0–24 h} of CD4 T-helper and NK cell kinetics (TABLE 1 and Fig. 1). Average increases for the second dose were threefold for CD4 T-helper cells and three and a half times for NK cells. It can also be noted that MDMA AUC also increased, on average, 49%. Conversely, peak values of cortisol concentration at 1.5 hours after the first and the second MDMA dose were not different.

Regarding the poor-metabolizer volunteer, he showed MDMA AUCs higher than those from extensive metabolizers after both the first and second MDMA doses (60% greater in AUC for both time intervals compared with extensive metabolizers), with a statistically significant difference after the second drug administration (TABLE 1). Nevertheless, this effect apparently did not influence the rise in cortisol and the decrease in CD4 T-helper cells, which were similar to those from extensive metabolizers after both the first and second MDMA doses. In contrast, NK cell kinetics showed a statistically significant increase in comparison with those of extensive metabolizers after both the first and second MDMA administration (Fig. 1). Peak effects of immune response appeared to show a delayed onset (TABLE 1). Similarly to the other volunteers, the poor metabolizer presented a 41% increase in MDMA AUC after the second drug dose, whereas altered immune parameters changed more than twofold.

Results obtained in clinical trials of double MDMA administration confirm the findings postulated in single-dose protocols; that is, MDMA administration induces changes in peripheral blood lymphocyte redistribution.12 It is possible that some cell subsets (such as circulating CD4 T-helper cells) migrate to certain compartments to be protected from potential deleterious effects of MDMA. At the same time, other cell subpopulations (NK cells) may migrate from the immune compartment, like an adaptive compensatory response. This fact can be interpreted as an immunosuppressive action, since cells are removed from the primary site of action with a subsequent reduction in their cytotoxic effect.14 This hypothesis is supported by the decrease in the functional responsiveness of lymphocytes to mitogenic stimulation observed after single and repeated MDMA administration.1,12 Furthermore, in animal models,
MDMA induces the release of stress challenge-related neurotransmitters such as serotonin, norepinephrine, and dopamine. It is possible that an increase in stress hormones and neurotransmitters may be involved in switching immune cells from the blood and various immune tissues. Interestingly, the same immune reactions were
observed in the rapid response to several acute psychological and physical stressors in human volunteers. In fact, volunteers exposed to acute psychological stress showed a significant elevation in the percentage of NK cells and a fall in the CD4 cell percentages as early as 4 min after the start of challenge. In addition, a study showed a number of lymphocytes reduced in blood but increased in several immune tissues as a result of acute stress or acute administration of glucocorticoids.

Nevertheless, immune response to a second MDMA dose did not show a parallel course with cortisol or MDMA plasma concentrations. They were either longer lasting compared with the first dose and/or otherwise disproportionate.

These findings are consistent with those of Connor et al., who observed a reduction in functional activity of lymphocytes in rats after MDMA administration even in the absence of corticosterone secretion. Therefore, it can be postulated that both in animal models and in human beings, MDMA-induced immune dysfunction may be also mediated by a glucocorticoid-independent mechanism directly involving the sympathetic nervous system (SNS). Alternatively, the possibility that some kind of immune memory mechanism could also play a role cannot be discarded. Phenotypic analysis of blood-naive and activated/memory CD4 T cells after one and two administrations of MDMA could reveal important differences in the concentration of lymphocyte subsets, and it could justify the alteration of the mechanisms responsible for maintaining the systemic balance between functional subsets of peripheral lymphocytes.

The accumulation of MDMA in the body of the poor metabolizer seemed to induce immune alterations higher than those observed in the extensive metabolizers. In any case, the increase was marginal in the case of CD4 cells; whereas, for the NK cells of the poor metabolizer, both AUC0–24 h and AUC24–48 h were more than doubled compared to the other volunteers. The fact is that NK cells are the cells of the innate immune response, which provides the first line of defense against infectious agents, offering an immediate response to the aggression against immune homeostasis. Conversely, CD4 T helper cells are immunoregulatory cells, which in the case of host offense, migrate to lymphoid tissues to generate effector lymphokine responses. Thus, it may be postulated that the NK activation response is more affected by MDMA kinetics than the CD4 regulatory response, which seems to show a “ceiling effect.” By contrast, NK cells do not own memory subsets. For the same reason, even if CD4 cell response in a poor metabolizer is only minimally increased, it is possible that this fact could be attributed only to a single CD4 subset, which could be highly modified.

Repeated administration of single oral doses of 100 mg MDMA at 4-hour and 24-hour intervals extended the critical period in which immunocompetence is highly impaired. The greater immunomodulating effects observed after the second dose suggest that MDMA-induced changes in immune function may have significant consequences for the ability of the immune system to respond to potential or ongoing immune challenge, because it can occur that appropriate leukocytes may not be present at the right time in the right place. Furthermore, it can be postulated that repeated aggression against immune homeostasis with continuous peripheral blood lymphocyte recirculation could collapse the whole cell-mediated immune surveillance. These hypotheses prompted the revision of basal values of cell-mediated immune parameters in habitual MDMA users and a two-year follow-up of immune function in a selected number of those consumers.
STUDIES IN NONCONTROLLED SETTINGS: BASAL CELL-MEDIATED IMMUNE RESPONSE IN MDMA RECREATIONAL USERS

Basal values of lymphocyte subsets were examined in a population of recreational users of MDMA who participated in different clinical trials that have been carried out at the Pharmacology Unit of our center over a two-year period. Eligibility criteria required the recreational use of MDMA on at least five occasions. Exclusion criteria included consumption of more than 20 cigarettes per day and more than 50 g ethanol/day (6 units/day). A total of 30 volunteers were observed. The participants had a mean age of 24.0 years (range: 20–36 years), mean weight of 67.9 kg (range: 56.5–86.0 kg), and a mean height of 175.4 cm (range: 167.0–189.0 cm). All the subjects declared that they were MDMA consumers. A history of drug and alcohol abuse was recorded for each volunteer. All of them were habitual users of cannabis with previous experience with cocaine and/or methamphetamine consumption. None had a history of drug abuse or dependence according to DSM-IV criteria (except for nicotine dependence) nor showed any adverse medical or psychiatric reaction after MDMA consumption. Subjects were requested to abstain from consumption of any drug of abuse during the clinical trials, and urine drug testing was performed before each experimental session for opioids, cocaine, cannabis, and amphetamines.

At the first visit, each participant underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram. Furthermore, total leukocyte counts and blood lymphocyte subsets were investigated following a method described elsewhere.12 Cell-mediated immune response was checked during the clinical trial sessions, and none of the participants showed statistically significant changes or modifications (ANOVA for repeated measures) during the four sessions of the trial. Furthermore, the cell-mediated immune response of six MDMA consumers (range of consumption on 5 to 50 occasions) out of 30 participants in different trials could be checked three times during the two-year period and compared to that of a control group of eight healthy volunteers, matched for age and physical characteristics, who did not consume any kind of drug of abuse. The two populations were examined for immune parameters by the same laboratory using identical techniques.12

TABLE 2. A comparison of lymphocyte subpopulations in healthy Spanish blood donors and healthy recreational MDMA users participating in clinical trials

<table>
<thead>
<tr>
<th>Population</th>
<th>Blood donors (n = 24)</th>
<th>Recreational MDMA users (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD Median</td>
<td>Mean ± SD Median</td>
</tr>
<tr>
<td>Total lymphocytes (cell/µl)</td>
<td>2199.7 ± 918.4 2061.0</td>
<td>2022.9 ± 391.2 1864.0</td>
</tr>
<tr>
<td>T lymphocytes (cell/µl)</td>
<td>1694.4 ± 664.0 1525.5</td>
<td>1577.0 ± 311.0 1441.3</td>
</tr>
<tr>
<td>CD4 T cells (cell/µl)</td>
<td>1004.6 ± 443.8 936.5</td>
<td>977.4 ± 240.0 919.0</td>
</tr>
<tr>
<td>CD8 T cells (cell/µl)</td>
<td>559.6 ± 270.1 551.5</td>
<td>588.4 ± 146.6 570.4</td>
</tr>
<tr>
<td>B lymphocytes (cell/µl)</td>
<td>233.2 ± 134.9 191.0</td>
<td>241.4 ± 102.7 215.9</td>
</tr>
<tr>
<td>NK (cell/µl)</td>
<td>246.6 ± 180.3 217.0</td>
<td>89.4 ± 68.7** 70.7</td>
</tr>
</tbody>
</table>

**p < 0.001 in relation to NK from blood donors.
FIGURE 2A. Time-course of total number of lymphocytes, CD4 T-helper cells, and CD8 T-suppressor cells in six MDMA consumers and eight healthy volunteers during a two-year period. Data are expressed as mean and standard deviation (bars). Statistical significance was obtained using a one-way analysis of variance (ANOVA). If any significant change was found, post hoc multiple comparisons were performed using the Tukey’s test. Statistically significant differences between MDMA users and healthy controls are indicated with filled columns ($p < 0.05$), between MDMA users checked at different years with asterisks (*$p < 0.05$; **$p < 0.01$) in relation to users’ basals and with section mark ($§p < 0.05$) in relation to users’ first year.
Apparent alterations in several immunological parameters were observed in the 30 healthy recreational consumers of MDMA (Table 2). The absolute number of lymphocytes and absolute number of T cells and CD4 T-helper cells showed a decreasing trend if compared to mean values obtained in a Spanish population of blood donors having the same age range, although counts from recreational users fell within normal population ranges.23 Conversely, NK cells in drug abusers were reduced to one-third of those from healthy persons. In addition to that observation, a progressive impairment of cell-mediated immune response was observed in the subgroup of six MDMA users followed for two subsequent years (Fig. 2).

Indeed, if comparing the six users with the eight healthy controls, at first observation (users-basals), MDMA consumers presented the immunological parameters

**FIGURE 2B.** Time-course of CD-19 B lymphocytes and NK cells in six MDMA consumers and eight healthy volunteers during a two-year period. Data are expressed as mean and standard deviation (bars). Statistically significant differences between MDMA users and healthy controls are indicated with filled columns, between MDMA users checked at different years with asterisks (*p < 0.05; **p < 0.01) in relation to users (basals) and with section mark (§p < 0.05) in relation to users (first year).
tested, but for NK cells, similar to those from nonconsumers. One year after the first observation, the total number of lymphocytes decreased ($p < 0.05$), and at the end of second year the total number of lymphocytes, CD4 T-helper cells, CD19 (B lymphocytes), and NK cells all resulted in statistically significantly lower values. Furthermore, if considering only the time as a factor in the six MDMA users, a statistical impairment in the two following years was still observed, apart from NK cells, which were one-third of the values found in the eight controls and remained so during the two years of observation.

Results regarding basal cell-mediated immune response in MDMA recreational users seem to confirm the hypothesis that repeated challenge to immune homeostasis by MDMA consumption can affect cell-mediated immune response. The major impact is observed in case of NK cells. Evidently, as aforementioned, the high and temporary increase in NK cell number following acute MDMA administration could be an immune-compensatory response to alteration in the number and function of T helper cells. This adaptive or maladaptive response potentially could result in a net cost to the body when immune homeostasis is repeatedly challenged by use of MDMA. Therefore, it is not surprising that a large reduction of these cells, which in a normal population account for approximately 10–20% of peripheral blood lymphocytes, to less than 5% is observed in recreational users of MDMA under baseline conditions. Furthermore, a positive correlation with a history of abuse seems evident, although the small sample size does not allow definitive conclusions.

Several clinical studies show that persons with a reduced number and/or function of NK cells may experience a higher risk of bacterial and viral infections. In addition, recent experimental studies in mice selectively lacking NK cells demonstrated a critical role of these cells in defense against cytomegalovirus and herpes virus infections and resistance to tumor cells. In addition, other immune parameters, such as total number of lymphocytes and CD4 T-helper cells, already involved in acute response to MDMA administration, and also in number of CD19 B lymphocytes, which did not appear implicated in acute response, were affected by chronic MDMA consumption.

These observations, together with all the evidence regarding acute effects of MDMA administration (single and repeated doses) on cell-mediated immune response, allow us to develop a pharmacological hypothesis for MDMA action on the immune surveillance system. Recreational use of MDMA has been associated with elevated scores of self-reported measures of depression and former chronic ecstasy users reported higher levels of depression than matched nonconsumer controls. On the other hand, depressed mood has been associated with reduced NK cell activity, inversely correlated with the intensity of depression and reversible by means of serotonin-selective reuptake inhibitors (SSRIs). Indeed, serotonergic pathways in the central nervous system are related to psychiatric disorders such as depression. In vitro studies indicate that serotonin regulates T-cell and NK-cell function and that it may be absolutely required for T-cell blastogenesis through its action on 5-HT$_{1A}$ receptors. The same receptors are involved in stimulation of T cells and mitogen-activated B-cell proliferation. Hence, serotonin itself and SSRIs, such as paroxetine or fluoxetine, are able to stimulate NK-cell activity in depressed patients exhibiting low activity at baseline. Furthermore, the administration of fluoxetine was associated with an alteration of leukocyte trafficking in primates, and the 5-HT$_{1A}$
receptor agonist ipsapirone, which is given to depressed patients, led to a significant reduction of peripheral CD4 cells.32

The observations reported here are similar to those of our studies on acute and chronic effect of MDMA administration. In fact, MDMA consumers not under the direct effect of the drug present, at baseline levels present a situation of immunocompetence matching that of the depression mode. By contrast, acute effects of MDMA mimics the enhancing effects of serotonin and SSRI administration in depressed patients. It has to be said that even if direct determinations of neurotransmitters and/or their metabolites in biological fluids after MDMA administration had been never reported in humans, in animal models the release of stress neurotransmitters such as serotonin, norepinephrine, and dopamine was demonstrated after MDMA dose.15

The hypotheses illustrated here are supported by observations on in vitro cytokines release by SSRIs. Indeed, suppressed production of IL-2 and IFNγ by stimulated T lymphocytes and suppressed production of IL-1β and TNFα by stimulated monocytes by citalopram, fluoxetine, and sertraline33 is in agreement with what is reported in our previous study after MDMA administration in healthy consumers.5

The clinical impact of immunomodulatory effects of MDMA consumption is still difficult to evaluate, especially when considering the type of consumers and patterns of abuse. In general, this phenomenon involves young people, well educated—most being students and employees—that consume ecstasy mainly on a weekend basis.34 Hence, it can be acknowledged that risk of infection is certainly low, given the cohort considered. On the other hand, however, it has been reported that adolescents are the population at higher risk for acquiring transmitted diseases and that health problems, including depression and low self-esteem, may play an important role in the development and maintenance of high-risk sexual behavior.35 In addition to this, MDMA abuse has been recently associated with high-risk sexual behavior among men who have sex with homosexual and bisexual men.36 Therefore, the point is that MDMA-induced immune effects can enhance the susceptibility to infectious diseases, which can be a major health problem in case of association with high-risk sexual behavior. Nevertheless, a limitation in such observations is that they cannot be solely linked to MDMA consumption, because MDMA consumers included in the study are concurrent misusers of other substances. Neither can a definitive conclusion be drawn about the trend toward a progressive two-year impairment of the immune function in the MDMA consumers. Indeed, the observation was retrospective and included a small number of individuals, limiting the possibility of performing any kind of correlation between MDMA consumption and rate of impairment.

There are several reports on the impact of substances like nicotine, cannabinoids, or cocaine on the immune system, suggesting that under acute conditions they are able to induce profound alterations, whereas in chronic consumers results are more contradictory.37 In addition, the impact on the immune system of other dance-scene drugs (like γ-hydroxybutyric acid [GHB] or ketamine) frequently ingested by the same MDMA users is still unknown. Even in the case of the hypothesis of lack of direct cause and effect between MDMA and immunologic impairment, however, it can be said that MDMA consumption in eventual association with other drugs of abuse could lead to pronounced immunological changes with enhanced susceptibility to infectious diseases and immunocorrelated pathologic conditions. Indeed, recently some cases of meningococcal meningitis were correlated with MDMA
abuse,38 and cocaine, which displays a common pattern of immune function alteration with MDMA, was suggested to be linked with a higher risk of infectious diseases including AIDS.11

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