

Effects of repeated doses of MDMA (“Ecstasy”) on cell-mediated immune response in humans

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Abstract

Cell-mediated immune response after the administration of two repeated doses of 100 mg 3,4-methylenedioxymethamphetamine (MDMA) at 4-hour and 24-hour intervals was evaluated in two randomised, double-blind and cross-over clinical trials conducted in healthy male MDMA consumers. MDMA produced a time-dependent decrease in the CD4/CD8 T-cell ratio due to a 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. In case of two 100 mg MDMA doses given 4 hour apart, immune alterations produced by the first dose were strengthened by the second one. At 24 hours after treatment, statistically significant residual effects were observed for all the altered immune parameters after the administration of two MDMA doses if compared to single dose and placebo. In the second clinical trial, the second 100 mg MDMA dose given 24 hours after the first dose produced immunological changes significantly greater than those induced by the initial drug administration and which seemed to show a delayed onset. Significant residual effects were observed for all the immune parameters as late as 48 hours after the second dose. These results show that repeated administration of MDMA with both a short and a long time interval between doses extends the critical period following MDMA administration, already observed after a single dose, in which immunocompetence is severely compromised. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: MDMA; Humans; Immunity; Repeated administration

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Introduction

Recent studies have shown that acute administration of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) produced a time-dependent immune dysfunction in animals and humans [1–3]. A single dose of 100 mg MDMA caused a decrease in CD4 T-helper cells, simultaneous increase in natural killer (NK) cells, and a decrease in functional responsiveness of lymphocytes to mitogenic stimulation, whereas total leukocyte count remained unchanged. The correlation of MDMA pharmacokinetics and MDMA-induced cortisol stimulation kinetics with the profile of MDMA-induced immune dysregulation suggested an implication of the central nervous system in the impairment of immunological status. Subsequent investigations showed that acute MDMA produced a high increase of immunosuppressive cytokines (transforming growth factor-beta and interleukin-10) and a switch from Th1-type cytokines (interleukin-2 and interferon-gamma) to Th2-type cytokines (interleukin-4 and interleukin-10) [4]. Dysregulation in the production of pro- and anti-inflammatory cytokines with an imbalance towards anti-inflammatory response was also observed. However, the immune function showed a trend towards baseline levels at 24 h after MDMA administration [4]. It seems that following MDMA consumption there is a critical period in which immunocompetence is highly impaired. On the other hand, these studies were conducted in the laboratory setting, which is in contrast to the environment associated with the recreational use of MDMA. This includes repeated drug consumption and/or in association with ethanol, cannabis, cocaine, or tobacco; crowded conditions; stressed physical activity without control of food intake; etc. These circumstances could even lead to more pronounced immunological changes with potential enhanced susceptibility to infection and immune-related disorders [4–8].

The present investigation was designed to examine changes in cell-mediated immune function after repeated (double) administration of MDMA occurring in a relatively short (4 hours) and long (24 hours) time interval in recreational MDMA users. Time intervals chosen try to mimic the real consumption patterns of MDMA users during weekends. Total leukocyte counts, blood lymphocyte subsets and lymphocyte proliferative response to mitogenic stimulation as well as cortisol and MDMA plasma concentrations were investigated.

Methods

Study design

A total of 17 male volunteers familiar with MDMA use gave the written informed consent to participate in two randomised, double-blind, and cross-over clinical trials. Studies were conducted in accordance with the Declaration of Helsinki and approved by the institutional review board and the national health authorities (CEIC-IMAS). In the first trial (named *Study I*), eight subjects participated at random in three experimental sessions that included the following conditions: a single oral dose of 100 mg MDMA at time 0 and placebo at 4 hours; a single oral dose of 100 mg MDMA both at time 0 and at 4 hours; and placebo both at time 0 and at 4 hours. In the second trial (*Study II*), nine subjects participated at random in two experimental sessions in which they received a single oral dose of 100 mg MDMA or placebo both at time 0 and at 24 hours.

Blood cell preparation for immunological tests

Blood samples for immunological tests were drawn before treatment (baseline) and at 1.5, 4, 5.5, and 24 hours after drug administration at time 0 in *Study I*, and at baseline and at 1.5, 4, 6, 24, 25.5, 28, 30, 48, 54, and 72 hours in *Study II*. Peripheral blood was collected in evacuated tubes containing ethylenediaminetetraacetic acid (0.47 M). Complete blood profile and count was obtained for each participant. Functional responsiveness of lymphocytes to mitogenic stimulation was performed in *Study I*. Peripheral blood mononuclear cells were obtained by centrifuging whole blood on a Ficoll-Hipaque density gradient. Peripheral blood mononuclear cells were rinsed and suspended in tissue culture medium (RPMI-1640 medium) containing penicillin (100 U/mL), streptomycin (100 mg/L), and 10% foetal bovine serum (Gibco Laboratories, Grand Island, NY, USA).

Response to mitogen

PBMC samples were adjusted to a final concentration of 1×10^7 cells/mL, placed in 0.1 mL aliquots in microtiter platewells, and stimulated by phytohemagglutinin A (PHA, Sigma, St. Louis, MO, USA), 1 µg/mL diluted in culture medium. This dose was found to produce optimal stimulation of lymphocyte proliferation [2]. Unstimulated cultures were incubated with an equal volume of culture medium instead of PHA. All cultures were incubated at 37°C in 5% CO₂ 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 (S.I.), defined as the ratio of mean cpm in PHA-stimulated versus non stimulated cultures (expressed as percentage).

Lymphocyte immunophenotyping

Dual-color immunophenotyping was performed using 100 µL of whole blood stained using 20 µL of monoclonal antibody reagent. Becton-Dickinson (Becton-Dickinson Italia S.p.A., Milan, Italy) 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 analysed using a Ortho Cytoron Absolute 4 flow cytometer (Ortho Instruments, Ortho-Clinical Diagnostic, Milan, Italy). 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 conjugated to phycoerythrin (PE) or fluorescein isothiocyanate (FITC) were used: 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⁺:NK cells), CD3 / PE - CD19 / FITC (T mature lymphocytes and B lymphocytes).

Determination of plasma MDMA and cortisol

Blood samples for determination of MDMA plasma concentration were collected at the same time interval as for immunological tests. Blood samples for assessment of plasma cortisol concentration were drawn at baseline and at 1, 2, 4, 5, 6, 10 and 24 hours after administration of MDMA at time 0 in *Study I*, and at baseline and at 1.5, 4, 6, 24, 25.5, 28, 30, 48, 54, and 72 hours in *Study II*. Plasma MDMA was measured in plasma by gas chromatography equipped with a mass spectrometer detector [9]. Plasma cortisol concentrations were determined by fluorescence polarization immunoassay (FPIA) (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer’s instructions. The intra-assay coefficients of variation 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.

Statistical analysis

Values from lymphocyte subsets, S.I., and plasma cortisol concentrations were transformed to differences from baseline. Mean and standard deviation (SD) were used to express peak effects. In *Study II*, the 0–24 hour and 24–48 hour areas under the curve (AUC_{0–24h}, AUC_{24–48h}) of CD4 T-helper cells and NK cells values and MDMA plasma concentrations versus time were calculated by the trapezoidal rule. Data were analysed 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. Differences associated with *p* values lower than 0.05 were considered to be statistically significant.

Results

Data on immunological parameters at baseline are shown in Table 1. Repeated MDMA administration produced a time-dependent immune dysfunction that for the first acute administration paralleled time-course of MDMA-induced cortisol stimulation kinetics and MDMA plasma concentrations (Figures 1 and 2). Despite changes observed in lymphocyte subsets and in functional responsiveness of lymphocytes to mitogenic stimulation, total leukocyte count remained unchanged in all 17 subjects.

Study I (two doses of 100 mg MDMA at 4-hour intervals)

The single dose of 100 mg MDMA produced alterations in immune parameters, which peaked at 1.5 hours from the start of the treatment. In fact, there was a decrease in the CD4/

Table 1
Basal values (mean and SD) of lymphocyte subpopulations and stimulation index in 17 males MDMA consumers

	CD4 (cells/µL)	CD8 (cells/µL)	CD4/CD8 ratio	NK (cells/µL)	S.I. (%)
<i>Study I</i> (n = 8)	991.6 (188.9)	561.9 (163.9)	1.8 (0.4)	139.5 (118.4)	113.0 (18.2)
<i>Study II</i> (n = 9)	984.2 (155.5)	677.8 (183.1)	1.4 (0.2)	107.6 (61.9)	NA*

* NA: not available.

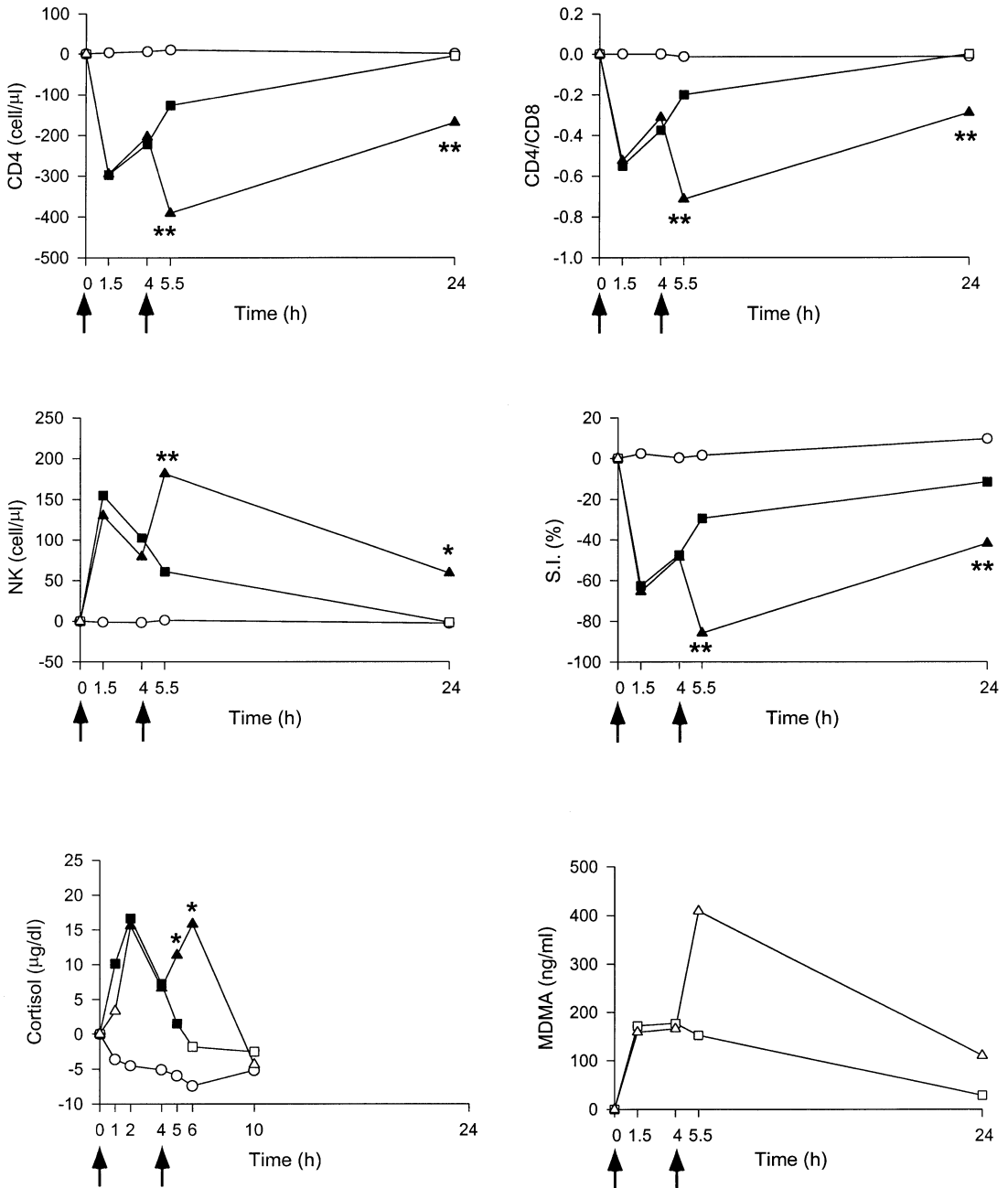


Fig. 1. Time-course of immunophenotyping parameters, stimulation index (S.I.), plasma MDMA concentration and cortisol kinetics ($n = 8$ for each data point) after the administration of a single 100 mg MDMA dose (□), two doses of 100 mg MDMA with a 4-hour interval (△) and placebo (○). Arrows below the abscissa indicate the administration of 100 mg MDMA. Statistically significant differences from placebo are indicated with filled symbols and between MDMA doses with asterisks (*= $p < 0.05$, **= $p < 0.01$). Values have been normalised by subtracting to experimental values the corresponding basal value of each treatment condition.

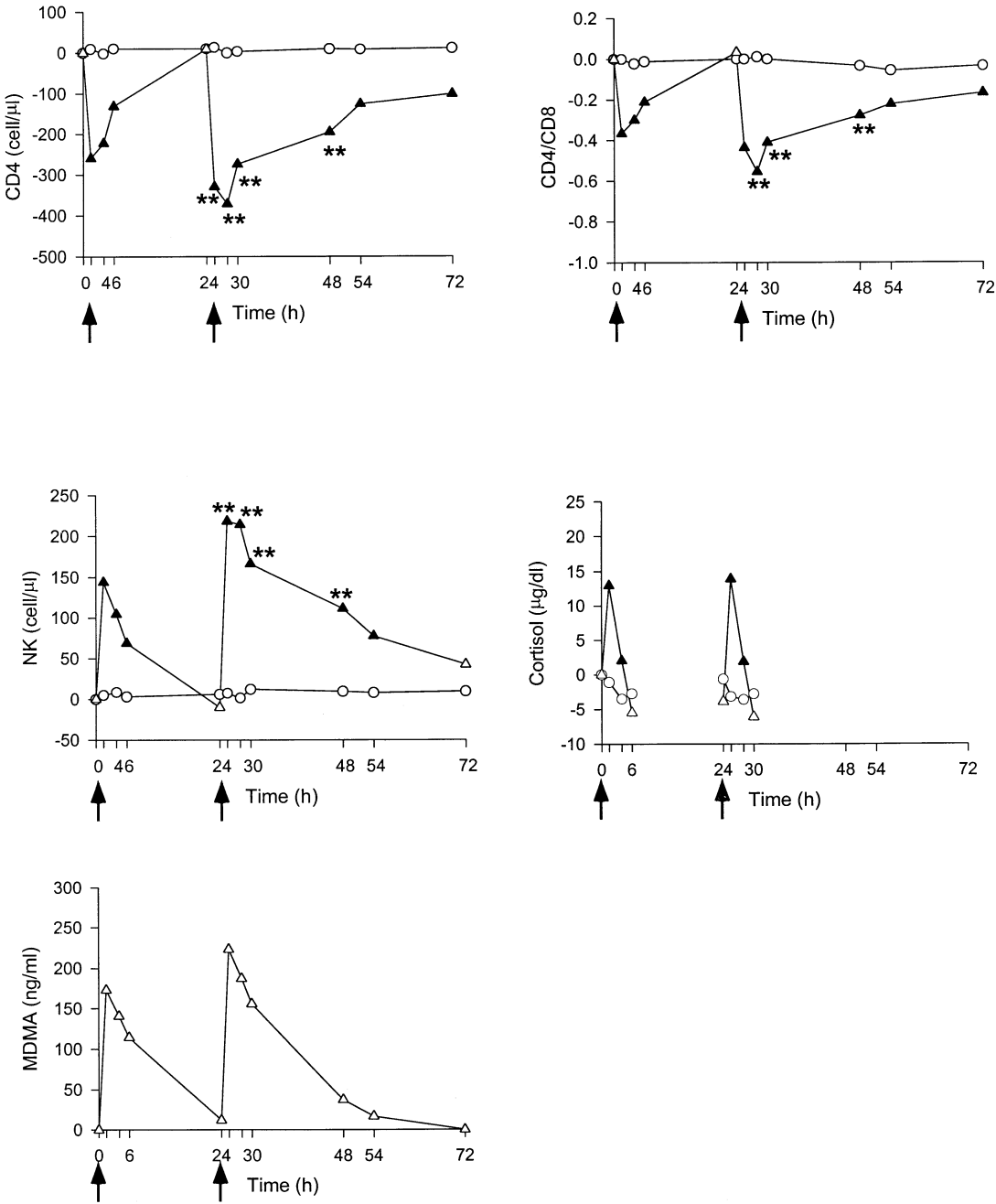


Fig. 2. Time-course of immunophenotyping parameters, stimulation index (S.I.), plasma MDMA concentration and cortisol kinetics (n = 9 for each data point) after the administration of two doses of 100 mg MDMA with a 4-hour interval with a 24-hour interval (△) and placebo (○). Arrows below the abscissa indicate the administration of 100 mg MDMA. Statistically significant differences from placebo are indicated with filled symbols and between MDMA doses with asterisks (** $p < 0.01$). Values have been normalised by subtracting to experimental values the corresponding basal value of each treatment condition.

CD8 T-cell ratio (mean and SD: -0.5 ± 0.1) because of decreased proportion of circulating T-helper cells (CD4), which showed a maximum mean (SD) difference of -300.2 (33.4) cell/ μL (equivalent to a reduction of 29% from baseline) between MDMA and placebo. Lymphoproliferative response to PHA stimulation was also reduced to a maximum (SD) of -64.8 (14.0)%. No differences were found in the amount of cytotoxic/suppressor lymphocytes (CD8). In contrast, there was a high increase in NK cells with a mean (SD) peak of 156.1 (113.4) cells/ μL (equivalent to a 137% increase from initial value). When two MDMA doses were administered, the first 100 mg dose produced immune alterations, which overlapped those of the single dose condition. Indeed a mean (SD) decrease in the CD4/CD8 T-cell ratio of -0.5 (0.1), a mean (SD) decreased CD4 proportion of -298.0 (54.0) cell/ μL (30% decrease from baseline), a mean (SD) reduction of lymphoproliferative response to PHA stimulation of -67.9 (6.2)%, and a mean peak (SD) increase of NK cells of 131.2 (100.0) cells/ μL (103% rise from initial value) were observed. The second drug dose enhanced the effects induced by the first dose. In fact, 1.5 hours after the second administration CD4 T-cells and lymphocyte proliferative response to PHA showed a mean (SD) decrease of -401.1 (76.9) cells/ μL (40% reduction from baseline) and -87.4 (10.7)% respectively, as compared with placebo. In contrast, the increase in NK cells showed a mean (SD) peak of 179.9 (152.8) cells/ μL (equivalent to 141% rise from initial value). At 24 hours, statistically significant residual effects for all altered immune parameters in subjects treated with two MDMA doses in comparison with the single dose group and placebo were found (Figure 1). A single MDMA dose produced a mean increase in cortisol concentration at 2 hours after drug administration of 16.7 ± 6.2 $\mu\text{g/dL}$. The administration of two doses of MDMA at 4-hour interval produced a mean rise in cortisol concentrations of 15.6 ± 5.4 $\mu\text{g/dL}$ at 2 hours after drug administration at time 0 which decreased to 6.6 ± 9.7 $\mu\text{g/dL}$ at 4 hours just before the administration of the second dose. A further plasma cortisol increase to 15.8 ± 7.2 $\mu\text{g/dL}$ was observed at 2 hours after the administration of the second MDMA dose.

Study II (two doses of 100 mg MDMA at 24-hour intervals)

The first dose of MDMA produced alterations in immune parameters of the same magnitude and time course to those observed for the first MDMA dose in *Study I*. The mean (SD) peak decrease in the CD4 T-cells was -276.4 (57.8) cells/ μL (equivalent to 28% reduction from baseline) after MDMA administration if compared to placebo. On the contrary, NK cells presented a mean (SD) maximum increase of 140.4 (66.9) cells/ μL (130 % rise from baseline) between MDMA treatment and placebo. All variables returned to baseline values within 24 hours. However, the second MDMA dose, produced immunological changes greater than those induced by the first administration and which seemed to show a delayed onset. Indeed, the suppression in the CD4 T-cells showed a mean (SD) at 4 hours of -392.9 (61.3) cells/ μL (equivalent to 40% decrease from initial condition) between MDMA treatment and placebo. This value was significantly higher than that from the first dose and with a delay of about 2.5 hours. Significant differences between the first and the second MDMA dose were also observed in case of NK cells increase, which reached a mean (SD) plateau between 25.5 and 28 hours (1.5 and 4 hours after the second administration) of 237.0 (86.3) cells/ μL (240% increase from baseline). At 24 hours after the second MDMA dose, residual effects were observed for all the immune parameters with statistically significant differences

from the first MDMA dose and placebo as late as 48 hours after the second MDMA dose. The magnitude of differences between doses can be exemplified by comparing AUC_{24-48h} versus AUC_{0-24h} of CD4 T-helper and NK cells kinetics (Figure 2). Average increases for the second dose were three fold and three and a half fold respectively. It can also be noted that MDMA AUC also increased in average by one half. There was a mean rise in cortisol concentrations of $15.6 \pm 6.1 \mu\text{g/dL}$ at 2 hours after the first dose of MDMA and of $20.9 \pm 5.1 \mu\text{g/dL}$ at 2 hours after the second dose. Peak values were not statistically different.

Discussion

Repeated administration of 100 mg of MDMA at either short (4-hour) or long (24-hour) intervals, compatible with the consumption patterns among users, was followed by rapid and sustained changes of certain immunological and neuroendocrine parameters. First dose administered induced an immune dysfunction, which was time-dependent and showed a parallelism with both MDMA plasma concentrations and MDMA-induced cortisol stimulation kinetics. These findings are consistent with our previous single dose studies, being at present extended to more than 20 subjects [1,2,4].

In both studies plasma MDMA concentrations after the second dose were about 30%–50% higher than expected (estimation based on AUC values). Nevertheless, relatively modest changes in MDMA kinetics cannot solely account for the immune dysfunction observed. Regarding cortisol concentrations, in both experiments after the second MDMA dose there was a dissociation between MDMA plasma concentrations and cortisol secretion. Cortisol plasma concentrations were rather constant while those of MDMA increased. These observations are in contrast with those concerning the immune parameters. Response to the second dose either was long-lasting as compared with the first dose and/or disproportionate, and did not follow any parallelism with cortisol and MDMA plasma concentrations.

In single doses protocols, it has been postulated that changes in immune function were linked to increased cortisol concentration, which is in turn a consequence of the effects of MDMA administration on central monoaminergic system with the release of corticotrophin-releasing factor and subsequent activation of the hypothalamic-pituitary-adrenal axis [10]. It might be possible that elevation of stress hormones may be involved in switching of immune cells from the blood and different immune tissues. In fact, a recent 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 [11]. The observed changes in lymphocyte subpopulation numbers and percentage could be the result of MDMA-induced changes in leukocyte turnover (production/destruction), or of MDMA-induced changes in leukocyte distribution. We hypothesise that MDMA induced changes in leukocyte redistribution rather than changes in leukocyte turnover. The following reasons support this hypothesis: we have demonstrated that although acute MDMA administration caused immediately significant changes in number and percentage of peripheral blood lymphocytes subpopulation, a relatively rapid return of lymphocytes subpopulation numbers and percentage to baseline levels was observed. It is unlikely that over 40 % of the circulating lymphocytes pool could be destroyed within 2 h following exposure to MDMA, and completely replaced within 24 h after the administration. Besides, a significant amount of evidence indicates that in human

glucocorticoid-induced changes in blood leukocyte numbers represent changes in leukocyte redistribution and they are not the result of leukocyte destruction [12]. It is possible that some subpopulation migrate to certain compartments to be protected from potential deleterious effects of MDMA. Alternatively, other subpopulation may migrate from immune compartment, like an adaptive response, to compensate. Redistribution of lymphocytes, following MDMA, administration, led to a decrease in circulating T-helper cells (CD4) with a consequent decline in the CD4/CD8 T-cell ratio and simultaneous increase in number of NK cells. According to Friedman and Irwin [13], this fact represents an immunosuppressive action since cells are removed from primary site of action with a reduction of cytotoxic action of these cells. In fact, what is observed after single and repeated doses MDMA administration is a decrease in the functional responsiveness of lymphocytes to mitogenic stimulation [2]. The increase in NK cells could be paradoxically considered potentially beneficial for subjects under MDMA. Nevertheless, as reported previously [4] and as it can be also observed in subjects participating in this study (see Table 1), basal values of NK in MDMA users not under acute drug effects show a trend towards low values as compared with reference population ones. This fact can be considered detrimental for subjects health.

The selectivity and reproducibility of the observed effects, and the greater immunomodulating effects observed after repeated doses, 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 are not present in the right place at the right time.

However, it should be noted that in case of double MDMA doses, a further increase in immune dysregulation produced by the second dose occurred without a similar increase in cortisol secretion. These findings are consistent with those of Connor et al. [14] who observed a reduction in functional activity of lymphocytes in rats after MDMA administration even in 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 involving directly sympathetic nervous system (SNS) [14]. Indeed, it has been shown indirectly that MDMA administration activates SNS through neuroendocrinological changes induced by the drug (i.e. cortisol, prolactin) [10,15]. Up to date there are no direct evidences of SNS activity through direct determinations of neurotransmitters and/or their metabolites in biological fluids in humans after MDMA administration. Recently it has been shown in animal models that MDMA induces the release not only of serotonin but also norepinephrine and dopamine [16]. The ability of MDMA in promoting norepinephrine release is of interest as an elevation of sympathetic tone induces a suppression of cell-mediated immunity [13].

Alternatively it cannot be discarded that some kind of immune memory mechanism could operate. Phenotypic analysis of blood naive and activated/memory CD4 T cells after one and two administration of MDMA could reveal important differences in the concentration of lymphocytes subsets and it could justify the alteration of mechanism responsible for maintaining the systemic equilibrium between functional subsets of peripheral lymphocytes [17,18].

We conclude that 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 as a result of MDMA use. Recovery of function at pre-exposure levels could require

energetic consumption whose cost on health is difficult to evaluate. This fact could lead to enhanced susceptibility to infection and immune-related disorders as observed in some cases of meningococcal meningitis correlated to MDMA abuse [19] and as suggested for cocaine, which displays a common pattern of immune function alteration with MDMA [20]. Longitudinal studies are required to assess long-term effects of MDMA consumption on the immune system.

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