The effect of 3,4-methylenedioxymethamphetamine (MDMA) on human genetic material: an in vitro study

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Abstract

Background: 3,4-methylenedioxymethamphetamine (MDMA), is a synthetic illicit psychostimulant drug that affects mood and social interactions. The aim of the present study is to investigate the in vitro effect of MDMA on human genetic material, by estimating sensitive cytogenetic indices.

Methods: MDMA solutions (A=15μg/ml, B=30μg/ml, C=45μg/ml, D=60μg/ml, E=75μg/ml) were added in cultures of peripheral blood lymphocytes of four healthy donors. After 72 hours of incubation, the cultured lymphocytes were collected, plated on glass slides, stained with the Fluorescence plus Giemsa method and SCEs, PRI and MI were measured with the optical microscope. Sister Chromatid Exchanges (SCEs) is a sensitive marker of genotoxicity, Proliferation Rate Index (PRI) is a reliable marker of cytostatic activity and Mitotic Index (MI) is a reliable indicator of cell ability to proliferate.

Results: Result analysis revealed: a) a statistically significant (p=0.001) reduction of SCEs on lower MDMA concentration and a significant induction (p=0.001) of SCEs after the effect of higher MDMA concentrations, b) PRI and MI reduction (p=0.001) after the effect of MDMA concentrations 45, 60 and 75μg/ml. Correlation was observed between a) SCE and PRI index variations, b) MI and SCE index variations and c) PRI and MI index variations.

Conclusions: MDMA exhibited an interesting cytogenetic activity in vitro. It seems to affect human T lymphocytes by epigenetic and DNA replication modifications. This may provide additional information about the mechanism of action of the drug. Further studies in other cell lines and in vivo experimental settings are needed in order to evaluate its potential effects on human genetic material.

Keywords: MDMA, Cytogenetic Activity, Sister Chromatid Exchanges, Proliferation Rate Index, Mitotic Index.

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Introduction

MDMA, known as ecstasy, is one of the most popular illicit psychostimulant drugs. It is a ring-substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. It was initially developed as an appetite suppressor in 1914. MDMA is widely used because of its effect in consciousness (Jeng et al., 2010). It causes heightening of the sensory perception, euphoria, increased energy, and heightened self-acceptance and empathy (Jeng et al., 2010). MDMA, acting at dopamine (DA) transporter (DAT) and serotonin (5-HT) transporter (SERT), stimulates non-exocytotic release of DA and 5-HT (Górska et al., 2018). These actions result from the interaction of MDMA with the membrane transporters involved in neurotransmitter reuptake and vesicular storage systems (de la Torre et al., 2004). Its abuse has several side effects, including anxiety, neurodegeneration (Górska et al., 2018), hepatotoxicity (Cerretani et al., 2011), cardiotoxicity (Koczor et al., 2015) and decreased immune function (Boyle et al., 2010). Studies in animal models and humans suggest its potential action in innate and adaptive immune system by enhancing serotonergic neurotransmission and increasing cortisol levels (Bigler et al., 2015). Moreover, MDMA seems to affect human lymphocytes after a single exposure causing a significant decrease in circulating lymphocytes (Pacifici et al., 2002). Mechanisms of neurotoxicity and immune system modulation are not yet clear (Górska et al., 2018). These toxic and inflammatory effects of MDMA are exacerbated by its co-administration with other psychoactive substances like cocaine and alcohol (Górska et al., 2018). The aim of the present study was to investigate the in vitro effect of MDMA on human cultured lymphocytes, by estimating sensitive cytogenetic indices, such as sister chromatid exchanges (SCEs), proliferation rate index (PRI) and mitotic index (MI). SCEs is the exchange of genetic material between two identical sister chromatids and they appear to be the result of DNA-replication mistakes on a damaged template, probably at the replication fork. SCEs has been identified as one of the most sensitive indices among sensitive biomarkers of genotoxicity, such as chromosomal aberrations, comet assay and micronuclei. PRI and MI have been used as sensitive indicators for the evaluation of the cytostatic activity of various environmental hazards or therapeutic agents (Mourelatos et al., 2016).

Materials and Methods

Healthy Blood Donors

Blood donors were four healthy young people (2 male and 2 female, mean age 19±1 years). They were non-smokers, not receiving any drugs, not consuming considerable quantities of alcohol, or not having suffered any kind of infection for the last 15 days. No pharmacological treatment was taken before blood sampling. Venous blood (5-7ml) was collected after informed consent from the donors and it was used immediately for the SCE, PRI and MI assays.
**In vitro** SCE, PRI and MI assays. Human lymphocyte cultures were prepared by adding in 5 ml chromosome medium (RPMI-1640, Biochrome, supplemented with 20% fetal calf serum, 0.63% L-glutamine, 0.63% penicillin/streptomycin and 2% phytohaemagglutinin) at the beginning of the culture life the following:

- 11-12 drops of human peripheral heparinized whole blood
- 0.1ml of 5-bromodeoxyuridine (BrdU) water solution (500 μg/ml), in addition, in experimental tubes:
  - 0.1 ml MDMA solution (A=0.05μg/ml or B=0.125μg/ml or C=0.25μg/ml or D=0.5μg/ml or E=1μg/ml final concentrations per corresponding culture) and
in the control tube:
  - 0.1 ml of MDMA solvent: double distilled water: ethanol, 9:1.

MDMA concentration of 0.25μg/ml (C) is found in the blood of a regular MDMA user. MDMA concentration of 0.5μg/ml (D) correlates with MDMA neurotoxicity. T lymphocyte cultures were incubated at 37° C for 72 hours in a dark incubator to minimize photolysis of 5-Bromodeoxyuridine. Colchicine was added 2h before the end of the incubation. T lymphocytes were then collected by centrifugation and exposed to 0.075M KCl (potassium chloride) for 12 minutes. The hypotonic solution spreads chromosomes and causes hemolysis of red blood cells. Pellet was fixed three times with methanol: acetic acid (3:1) solution. Drops of concentrated suspension of cells were placed on microslides and allowed to air dry. For SCE, PRI and MI analysis, the slides were stained by a modification of the fluorescence plus Giemsa procedure to obtain harlequin chromosomes.

**Statistical Analysis.** For SCE estimation, 30 suitably spread second division cells from each culture were blindly scored. For PRI calculation 100 cells in the first, second, third and higher divisions from each culture were blindly scored. PRI=M1+2M2+3M3/100, where M1, M2 and M3+ are the percent values of cells in the first, second, third and higher divisions, respectively. For MI analysis, all cell divisions that are present in an optical field of 1000 nuclei were scored. MI=number of cells in mitosis/total number of nuclei (1000). The statistical analysis was carried out using the SPSS, vs 22.0 statistical package. All values were expressed as mean ± standard error of the mean (SEM). Comparison of values between the different groups and subgroups was accomplished by the nonparametric Kruskal-Wallis test. It was also used for the evaluation of the dosage effect of MDMA on cytogenetic indices. The Spearman's rank correlation coefficient was applied for calculating the correlation between SCEs, PRI and MI

**Results**
Table I illustrates the effect of MDMA on SCE frequency. T lymphocyte SCE frequency is presented as a dose-dependent increase after the effect of MDMA solutions: C=0.25μg/ml, D=0.5μg/ml and E=1μg/ml. This observed increase is statistically
A significant (p=0.001) and the concentration of 1μg/ml duplicates the SCE rate in cultured T lymphocytes of all blood donors. A small but statistically significant (p=0.001) decrease of SCE rate is observed at the concentration 0.05μg/ml for all blood donors. Table II summarizes the effect of various MDMA concentrations on PRI index variations on T lymphocyte cultures.

A significant PRI reduction is observed for concentrations C, D and E (p=0.001). Table III summarizes the effect of various MDMA concentrations on MI index variations on T lymphocyte cultures. A significant MI reduction is observed for concentrations B, C, D and E (p=0.001). Table IV illustrates the values and statistical significance of correlation rates between SCEs, PRI and MI.

**Table 1:** Effect of MDMA on SCE frequency (Mean SCE/cell ±SE) in T lymphocyte cultures of healthy donors

<table>
<thead>
<tr>
<th>MDMA  (μg/ml)</th>
<th>Healthy donors (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>7.42±0.43</td>
</tr>
<tr>
<td>0.05</td>
<td>6.02±0.21*</td>
</tr>
<tr>
<td>0.125</td>
<td>8.20±0.59</td>
</tr>
<tr>
<td>0.25</td>
<td>11.66±0.12*</td>
</tr>
<tr>
<td>0.5</td>
<td>13.67±0.34*</td>
</tr>
<tr>
<td>1</td>
<td>15.53±0.11*</td>
</tr>
</tbody>
</table>

* statistically significant (p=0.001) alteration over the corresponding control (Kruskall Wallis test).
Table 2: Effect of venlafaxine on PRI frequency in T lymphocyte cultures of healthy donors

<table>
<thead>
<tr>
<th>MDMA (μg/ml)</th>
<th>Healthy donors (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>37±0.89</td>
</tr>
<tr>
<td>0.05</td>
<td>36±0.74</td>
</tr>
<tr>
<td>0.125</td>
<td>32±0.36***</td>
</tr>
<tr>
<td>0.25</td>
<td>27±0.12***</td>
</tr>
<tr>
<td>0.5</td>
<td>22±0.88***</td>
</tr>
<tr>
<td>1</td>
<td>19±1.23***</td>
</tr>
</tbody>
</table>

**statistically significant (p=0.001) decrease over the corresponding control (Kruskall Wallis test).

Table 3: Effect of venlafaxine on MI frequency in T lymphocyte cultures of healthy donors

<table>
<thead>
<tr>
<th>MDMA (μg/ml)</th>
<th>Healthy donors (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>2,35±0,10</td>
</tr>
<tr>
<td>0.05</td>
<td>2,37±0,07</td>
</tr>
<tr>
<td>0.125</td>
<td>2,02±0,11</td>
</tr>
<tr>
<td>0.25</td>
<td>1,88±0,09**</td>
</tr>
<tr>
<td>0.5</td>
<td>1,52±0,10**</td>
</tr>
<tr>
<td>1</td>
<td>1,31±0,05**</td>
</tr>
</tbody>
</table>

***statistically significant (p=0.001) decrease over the corresponding control (Kruskall Wallis test).
Discussion
MDMA has become increasingly popular in Europe and North America over the past 10 years as a recreational, “party” drug among young people. Its intake can produce several acute and chronic adverse events with a variety of possible mechanisms (Xin et al., 2017). MDMA neurotoxicity has been studied extensively and has revealed a lot of possible mechanisms of long term neuron damage as excitotoxicity, hyperthermia, oxidative stress, and apoptosis (Górska et al., 2018, Xin et al., 2017). The immune system seems to be affected as well. The possible mechanism could be related to DNA damage and/or epigenetic alterations (Connor et al., 2005, Wanner et al., 2019). Moreover, chronicity of exposure and increased dose may induce toxicity through different mechanisms (Xin et al., 2017, Patel et al., 2015). Animal studies in vivo showed that chronic exposure in low MDMA doses can cause neuroinflammation and worsen neurotoxicity (Xin et al., 2017). DNA damage has been studied in animal models with induced MDMA neurotoxicity and seems to be linked with oxidative stress and amphetamine-initiated neurodegeneration (Jeng et al., 2010, Górska et al., 2018). MDMA has clearly been demonstrated to be a potent immunomodulatory agent not only in cell lines in vitro, but also in animal models and humans in vivo (Connor, 2004). MDMA seems to suppress the number of circulating CD4+ T lymphocytes along with mitogen and antigen stimulated T lymphocyte cell proliferation capacity (Connor, 2004). It has also been observed that MDMA promotes the switch in a Th2 type cytokine profile lasting for at least 6 hours (Connor, 2004). Furthermore, a two year follow up of chronic MDMA users showed that B lymphocytes and natural killers (NK) cell numbers have also been reduced (Pacifici et al., 2002, Connor, 2004).

In the present study, the potential genotoxic and cytostatic effects of MDMA were evaluated using SCEs, PRI and MI assays in human cultured T lymphocytes in vitro. This method has the advantage of testing the indices mentioned above in human T lymphocyte populations (CD4+ and CD8+). These two T lymphocyte

Table 4: Correlation rates

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Value</th>
<th>p^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCEs -PRI</td>
<td>-0.937</td>
<td>0.01</td>
</tr>
<tr>
<td>SCEs -MI</td>
<td>-0.870</td>
<td>0.01</td>
</tr>
<tr>
<td>PRI -MI</td>
<td>0.964</td>
<td>0.01</td>
</tr>
</tbody>
</table>

^1Spearman’s rho
populations do not seem to be equally affected by MDMA.

The results of the present in vitro study come in agreement with other studies related with in vivo cytostatic effect of MDMA on T lymphocytes (Pacifici et al., 2002). MDMA on higher concentrations (C, D, E) seems to reduce PRI and MI indices significantly. This reduction for both indices negatively correlates with SCEs induction. This finding may indicate that DNA damage could lead T lymphocytes to apoptosis and it could be a possible mechanism of T lymphocyte cytostaticity (Demirtzoglou et al., 2016). This pattern of DNA damage has been observed with other drugs that affect serotonin system, such as escitalopram (Cobanoglu et al., 2018), probably because lymphocytes also express transporter sites for both serotonin and dopamine molecules (Boyle et al., 2010). In addition, MDMA and related amphetamines bind to trace amine receptors, which are also expressed on lymphocytes, so MDMA can interact directly with molecular targets expressed on lymphocytes (Boyle et al., 2010).

The mechanism of DNA damage reflected by SCE index induction is not yet clear but a possible mechanism is an insufficiency in DNA damage repairing by MDMA. Both DNA repair enzymes and epigenetic mechanisms that regulate DNA repair system may be involved. For instance, histone acetylation, one of histone post-translational modifications, plays an important role in DNA damage response system. Histone acetylation alterations may affect DNA integrity (Kim et al., 2019). Studies also indicate a possible role of DNA methylation in MDMA’s behavioral effect (Wanner et al., 2019). DNA methylation status can also alter DNA damage response (He et al., 2020). However, SCEs arise when damaged DNA induces the replicative bypass repair mechanisms during cell replication. When post-replication repair processes are involved, as in MDMA DNA damage induction hypothesis, chromosomal aberrations may be the possible mechanism (Sasaki, 1980, Azab et al., 2019).

MDMA studies showed that exposure of human and animal cell lines to different MDMA doses in vitro, as well as animal studies in vivo and human studies with chronic MDMA users in vivo, can lead to different effects concerning the immune system function (Connor, 2004). For instance, IL-2 production, a major Th1 cytokine, seems to be enhanced at lower MDMA doses whereas it seems to be reduced in higher MDMA doses (Boyle et al., 2010). In this study, low MDMA concentrations (A=0.05 μg/ml) reduced SCEs significantly without increasing PRI and MI. This indicates that acute exposure to low doses of MDMA may act as a genoprotective agent by protecting from chromosomal damage and enhancing the repair of damaged DNA. Another possible mechanism is that MDMA in low doses may reduce oxidative DNA damage. Several studies indicate the possible rapid antidepressant response of MDMA (Patel et al., 2015). As other fast acting antidepressants, like esketamine (Hashimoto, 2020), it may alter reactive oxygen species (ROS) production in low doses. PRI and MI
are not affected, probably due to differences between T lymphocytes subpopulations (CD4+/CD8+) that exist after phytohemagglutinin stimulation in cell cultures (van de Griend et al., 1980). MDMA cytogenetic activity in low doses needs further investigation in order to evaluate its possible genoprotective actions.

**Conclusions**

Taking into consideration the results of this study, MDMA shows a genotoxic and cytostatic cytogenetic behavior on higher concentrations *in vitro*. Low doses seem to be beneficial concerning DNA damage *in vitro*. In order to explicate these observations further studies are needed as well as cytogenetic studies in other cell lines (B lymphocytes, mononuclear cells etc.) and other cytogenetic indices (chromosomal aberrations) *in vitro*, and/or *in vivo* from chronic MDMA users and other illicit substances users.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**


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