

Research Article

3,4-methylenedioxypropylamphetamine (MDPV) Induces Cytotoxic Effects on Human Dopaminergic SH-SY5Y Cells

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Abstract *Background.* Synthetic cathinones are a rapidly growing group of psychostimulant drugs usually referred to as “bath salts” and have been used as an alternative to classic amphetamine-like drugs, with 3,4-methylenedioxypropylamphetamine (MDPV) being one of the most prevalent constituents. Consistent with the effects of other psychostimulants, MDPV may induce neurotoxicity by altering monoamine systems in the brain or by inducing neuronal apoptosis. *Purpose.* The aim of this study was to evaluate the effects of MDPV on the human dopaminergic cell line SH-SY5Y. *Experimental design.* After 24-hour exposure to MDPV (100 μ M to 2.5 mM), cytotoxicity, cellular proliferation, and apoptosis were evaluated, whereas reactive oxygen species (ROS) production was evaluated at 2 h, 4 h, 6 h, 22 h, and 24 h. *Results.* MDPV increased ROS production after 1 h, 4 h, and 6 h of exposure in all but the highest concentration; a moderate increase was observed at 22 h and 24 h. Only high concentrations of the drug decreased cellular proliferation and induced apoptosis and necrosis. *Conclusion.* MDPV induces dopaminergic toxicity by decreasing cellular proliferation and by increasing apoptosis and necrosis. The production of ROS may play a role in the early response to the drug.

Keywords 3,4-methylenedioxypropylamphetamine; apoptosis; reactive oxygen species; cytotoxicity; SH-SY5Y cells

1. Introduction

In recent years, the use of novel synthetic psychostimulant drugs has increased as a legal alternative to illicit drugs such as methamphetamine (METH), cocaine, and 3,4-methylenedioxymethamphetamine (MDMA) [1]. Among these types of drugs are the so-called “bath salts,” composed of a mixture of synthetic cathinones, with 3,4-methylenedioxypropylamphetamine (MDPV) being one of the most common constituents [2,3]. Apart from the energizing effects of this drug [4], MDPV induces hyperthermia, paranoia, hallucinations, panic attacks, excited delirium, and, in extreme cases, death [5,6,7]. Pharmacokinetic studies show that MDPV is rapidly absorbed into the blood and reaches the brain in about 5 min [8], probably due

to its high transport across the blood-brain barrier [9]. Once in the brain, MDPV induces alterations on the monoamine systems [10]. More specifically, MDPV binds with high affinity to the dopamine transporter (DAT) and norepinephrine transporter (NET), acting as an uptake inhibitor for these two neurotransmitters without promoting their release [9,10]. These effects of MDPV on the DA system resemble those of cocaine, since both drugs block the uptake of DA, however MDPV is the more potent of the two [11,12], whereas METH and MDMA induce the release of this neurotransmitter [9]. Apart from its effects on the monoamine systems, little is known about other effects of MDPV in the brain. Neurotoxic effects have been reported for different drugs of abuse that also alter the DA system. For instance, prenatal cocaine administration induces apoptosis in the fetal rat brain [13]; METH induces apoptosis on the rat midbrain [14], and MDMA has the same effect in the rat hippocampus [15]. In vitro, METH decreases cellular viability [16] and induces apoptosis through modulation of reactive oxygen species (ROS) and increases in caspase-3 activity in human neuroblastoma SH-SY5Y cells [17,18]. MDMA decreases cellular viability [19] and induces apoptosis [20] in PC12 cells, while a mixture of MDMA and its metabolites decrease cellular viability and induce cell death through activation of caspase-3 [21,22,23] in SH-SY5Y cells. Cocaine induces cell death [24] and apoptosis through caspase-3 [25] and -9 [26] in PC12 cells and through increase in oxidative stress in human neuronal progenitor cells [27]. There is only one study that reports neurotoxic effects of MDPV, which is reported to induce apoptosis in the neonatal mouse brain [28]. Therefore, the aim of this study was to analyze the toxic effects of MDPV on human neuroblastoma SH-SY5Y cells.

2. Materials and methods

2.1. Chemicals

Racemic MDPV was synthesized in Drug Design and Synthesis Section at NIDA by Kenner C. Rice [29]. The 5-bromo-2'-deoxyuridine (BrdU) labeling and detection kit III, cellular DNA fragmentation ELISA, and the cytotoxicity detection kit (LDH) were purchased from Roche Applied Sciences (Mannheim, Germany). Dulbecco Modified Eagle Medium (DMEM) and Ham's F-12 Medium were purchased from Cellgro (Manassas, VA, USA). All remaining media supplements and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Treatments

SH-SY5Y cells were cultured in DMEM/F12 medium with 10% fetal bovine serum, in a humidified incubator, under 5% CO₂. At 85% confluence cells were treated with MDPV (0, 10, 25, 50, 100, 250, 500, 1,000, and 2,500 μM) or 100 μM H₂O₂, as a positive control for cell death, for 24 h.

2.3. Cytotoxicity assay

Measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells was measured in the supernatant as an index of necrosis. Briefly, cells were seeded in 96-well plate at 85% confluence and treated with the drugs. After 24 h, 100 μL of the supernatant was collected and transferred to 96 well plates and then the reaction mixture was added and incubated in the dark for 30 min at room temperature. LDH activity was quantified by measuring the absorbance at 490 nm [30,31].

2.4. Cellular proliferation

Cell proliferation was determined using a commercial bromodeoxyuridine (BrdU) incorporation kit according to the manufacturer's instructions. Briefly, BrdU was added to the cells at 70% confluence with or without the drug treatments and incubated for 24 h. The BrdU-containing media was then removed and the cells were fixed for 30 min (ethanol 70%/HCl 25%, H₂O 5%). Nucleases were added for 30 min to digest the BrdU-labeled DNA and then the samples were incubated for 30 min with anti-BrdU-Peroxidase antibodies. Peroxidase substrate was then added and optical density measured at 405 nm (490 nm reference) to determine DNA synthesis and therefore proliferation.

2.5. Apoptosis

Apoptosis was determined by monitoring the production of DNA fragments using a commercial kit. Briefly, after a 24-hour treatment with MDPV, the cells were lysed and the lysates transferred to streptavidin-coated microplates to which anti-histone-biotin and anti-DNA-Peroxidase antibodies were added. The samples were incubated for 2 h and then peroxidase substrate was added. The optical

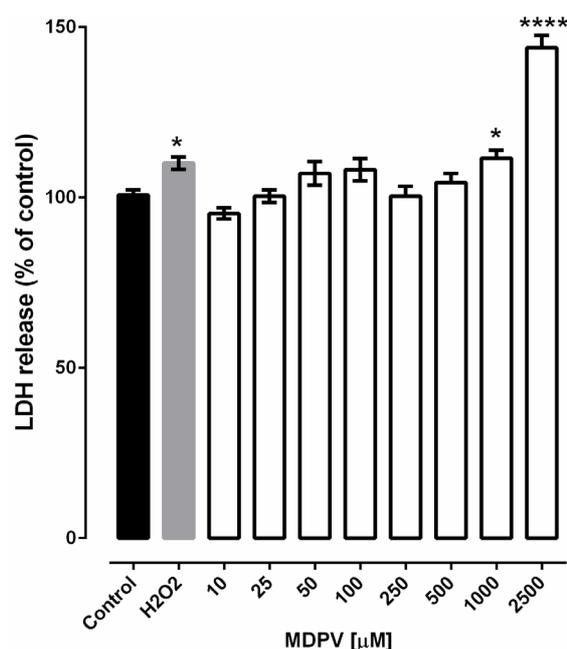


Figure 1: High doses of MDPV induce necrosis. SH-SY5Y cells were treated with increasing concentrations of MDPV (10 μM–2.5 mM) for 24 h. LDH release was used as an index of necrosis. Data represent the mean values ±SEMs from three independent experiments conducted with samples in triplicate. * $P < .05$ and **** $P < .0001$ versus control.

density was measured at 405 nm (490 nm reference) to determine DNA fragments, therefore apoptosis.

2.6. Reactive oxygen species production

ROS production was determined by dichloro-dihydro-fluorescein diacetate (DCFH-DA) oxidation [32]. Briefly DCFH-DA at a final concentration of 100 μM was added to the cells along with the various drug treatments. After 2 h, 4 h, 6 h, 22 h, and 24 h, fluorescence intensity was measured at 485 nm with a reference wavelength of 530 nm. This assay measured oxidized DCF, an indicator of ROS production.

2.7. Statistical analysis

Data were collected in triplicate from three independent experiments and analyzed using one-way ANOVA followed by Tukey analysis. The data are presented as means ±SEMs and the difference from control was considered significant if $P < .05$.

3. Results

3.1. MDPV induces necrosis only at high concentrations

After 24-hour exposure, only 1 mM and 2.5 mM MDPV increased LDH release by 11.5% and 43.9%, respectively, all of the other doses failed to induce necrosis on SH-SY5Y cells (Figure 1).

3.2. MDPV decreases cellular proliferation

The effects of MDPV on cellular proliferation were observed at concentrations lower than the ones that induced necrosis. MDPV decreased cellular proliferation by 12.6%, 16.8%, 21.1%, and 43.6% at 250 μM , 500 μM , 1 mM, and 2.5 mM, respectively (Figure 2).

3.3. MDPV induces apoptosis

In addition to decreased cellular proliferation, MDPV also induced apoptosis on SH-SY5Y cells. The same concentrations that decreased cellular proliferation, 250 μM , 500 μM , 1 mM, and 2.5 mM, increased DNA fragmentation by 23.4%, 38.4%, 69.8%, and 102.7%, respectively (Figure 3).

3.4. MDPV induced time- and concentration-dependent ROS production

The production of ROS was analyzed at different time points after initial exposure to MDPV. After 2-hour exposure, MDPV induced an increase in ROS production at all of the concentrations used (Figure 4(a)). Interestingly, the increase in ROS production induced by lower concentrations (10–250 μM) was higher than the production of ROS induced by higher concentrations, starting to gradually decrease from 500 μM up to 2.5 mM, but always being significantly higher than control (Figure 4(a)). A similar pattern was observed after 4 h (Figure 4(b)) and 6 h (Figure 4(c)) of exposure. Note that neither 1 mM nor 2.5 mM increased ROS production after 22 h (Figure 4(d)) and 24 h (Figure 4(e)) of exposure. It was also observed that the increase of ROS production was elevated shortly after initial exposure and gradually decreased as the exposure period increased (Figure 4(f)).

4. Discussion

The present study reports for the first time, the cytotoxic effects of MDPV on SH-SY5Y dopaminergic cells. MDPV induces toxicity by inhibiting cellular proliferation and promoting apoptosis and necrosis in a concentration-dependent manner. Previous reports indicate that drugs of abuse that also alter the DA system induce neurotoxic effects such as METH [16, 17, 18], MDMA [21, 22, 23], and cocaine [24, 25, 26] in different dopaminergic cell lines. Comparing these reports with the present study, MDPV seems to induce toxic effects at lower concentrations than the other drugs, as most of the studies showed neurotoxic effects starting at mM concentrations [16, 17, 18, 24, 33, 34] and MDPV induces toxic effects starting at μM concentrations. Even when MDPV induces in vitro toxicity at lower concentrations than other drugs of abuse, the doses used here are much higher than the ones found in the blood of drug users [7, 35]. However, several factors need to be considered when studying the toxic effects of MDPV. For instance, the fact that MDPV induces hyperthermia [5, 35, 36] is extremely important;

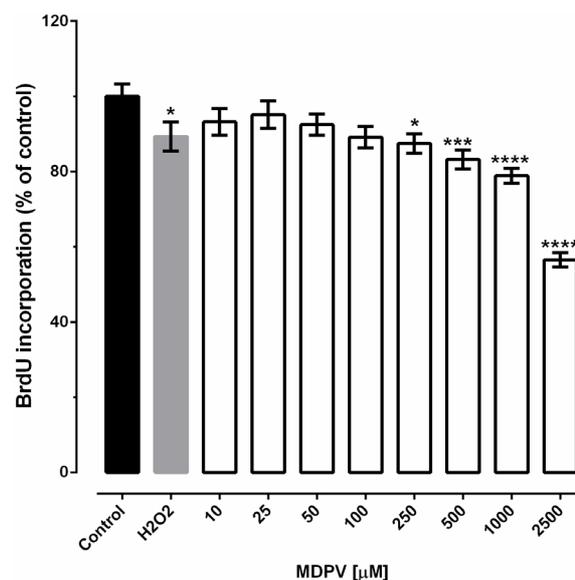


Figure 2: MDPV inhibits cellular proliferation. SH-SY5Y cells were treated with increasing concentrations of MDPV (10 μM –2.5 mM) for 24 h. BrdU incorporation was used as an index of cellular proliferation. Data represent the mean values \pm SEMs from three independent experiments conducted with samples in triplicate. * $P < .05$, *** $P < .001$, and **** $P < .0001$ versus control.

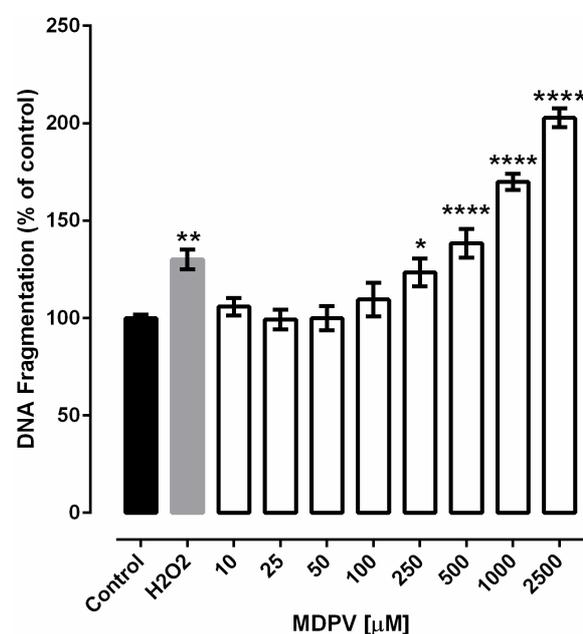


Figure 3: MDPV induces apoptosis. SH-SY5Y cells were treated with increasing concentrations of MDPV (10 μM –2.5 mM) for 24 h. DNA fragmentation was used as an index of apoptosis. Data represent the mean values \pm SEMs from three independent experiments conducted with samples in triplicate. * $P < .05$, ** $P < .01$, and **** $P < .0001$ versus control.

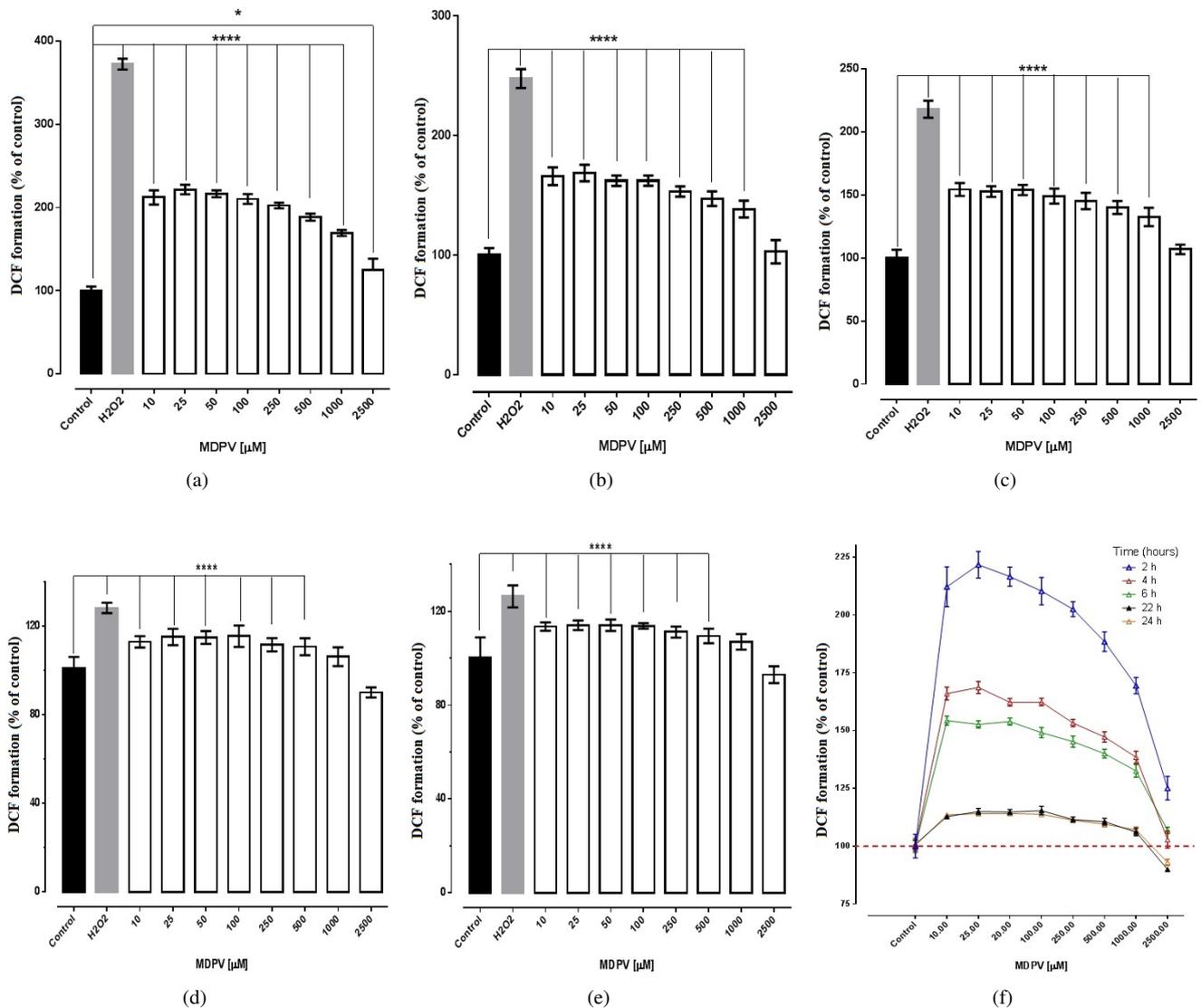


Figure 4: MDPV increases ROS production. SH-SY5Y cells were treated with increasing concentrations of MDPV (10 μM –2.5 mM) for 2 h (a), 4 h (b), 6 h (c), 22 h (d), and 24 h (e). DCFH-DA oxidation was used as an index of ROS production. Data represent the mean values \pm SEMs from three independent experiments conducted with samples in triplicate. * $P < .05$ and **** $P < .001$ versus control.

since it has been demonstrated for METH that hyperthermia is a key factor in the developing of neurotoxicity [37,38]. Additionally, there is only one study that addresses the effect of MDPV on hepatotoxicity in vitro [39]. Primary cultured rat hepatocytes exposed to different concentrations of MDPV showed an increase in toxicity at 40.5 $^{\circ}\text{C}$ compared to 37 $^{\circ}\text{C}$, indicating that MDPV-induced hyperthermia is a key factor in the hepatotoxic effects of this drug. It is also possible that, under hyperthermia, the toxic effects of MDPV in SH-SY5Y cells that we show here can be potentiated and observed at lower concentrations of the drug. In addition, metabolism of MDPV may also play a role in its toxic effects. There has been reported up to 10 MDPV

metabolites after human administration of the drug [40]; however, whether or not these metabolites also induce toxicity has not been studied. One of the reasons to believe that MDPV metabolites can be toxic is because toxicity of the MDMA metabolites has been reported [21,22,23]. Mixtures of MDMA and its metabolites induced neurotoxic effects on SH-SY5Y cells, interestingly the mixture of metabolites induced toxicity at very low concentrations compared to MDMA alone, inducing toxicity at in vivo relevant concentrations [21,22,23]. Due to the structural similarities between MDMA and MDPV, the possible toxic effects of MDPV metabolites cannot be ruled out and need to be evaluated.

One interesting finding on this study was the induction of ROS production by different concentrations of MDPV. ROS production is one of the mechanisms of toxicity induced by METH in SH-SY5Y cells [17,18,41]. However, METH induced increases of ROS production at high concentrations (1.68 mM and higher) and after longer exposure periods (12–48 h) [18,41]. Unlike the effects of METH, a higher increase in ROS production was observed at lower concentrations (starting from 10 μ M) and at shorter times (2–6 h). Despite the increase in ROS production, we believe that this effect is not involved in the toxic effects of MDPV, at least not in the apoptotic or necrotic effects, since higher ROS levels were induced by concentrations of MDPV that did not induce cell death. Even when there was an increase in its production by a concentration that also induced apoptosis, the levels of ROS were much lower than the ones induced by H₂O₂ and the percentage of cell death was higher. Apart from being mediator of cell death, ROS play different roles in cellular physiology, acting as signaling molecules [42] enhance neuronal cell survival [43] and even induce neurogenesis [44]. It is possible that ROS production induced by MDPV may play a role in cell survival at lower concentrations of the drug, rather than being involved in the toxic effects.

The results of this study indicate for the first time the toxic effects of MDPV on neuronal cells in culture, which seem to be independent of ROS production. Further studies are underway to elucidate the mechanisms of action of MDPV as well as to evaluate if hyperthermia and the presence of MDPV metabolites can potentiate the toxic effects as reported for other drugs of abuse that alter the DA system.

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Conflict of interest The authors declare that they have no conflict of interest.

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