

Role of Microglia in 3, 4 Methylenedioxymethamphetamine (MDMA) - Induced Neurotoxicity: A Mini Review

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MDMA (3, 4 Methylenedioxymethamphetamine) is a psychoactive drug under the amphetamine-type stimulant group. While the modulatory effects of MDMA on serotonin neurotransmission and its neurotoxicity in the central nervous system are well studied, MDMA's effects on modulating microglial neuroimmune functions have attracted considerable attention. Resident glial cells, including microglia in the brain, are implicated in contributing to MDMA-induced neurotoxicity. In their response to the disturbances around neurons, microglia can take on the role of the first line of defence against pathogens by the production of a variety of inflammatory mediators such as tumour necrosis factor-alpha (TNF- α), interleukin (IL) 1 β , IL-6, nitric oxide (NO), and reactive oxygen species (ROS). They also can act as anti-inflammatory mediators to initiate recovery from an insult. Hence, the current review illuminates MDMA-induced neurotoxicity by summarising studies reporting microglial activation after MDMA exposure *in vitro* and *in vivo*. A modulation between cytotoxic states to a neuroprotective state of microglia probably can make up an important strategy to reduce the negative impairments made by MDMA on neuronal cells by targeting microglial cells.

Keywords: MDMA; Neurotoxicity; Microglial Activation

I. INTRODUCTION

MDMA is a psychostimulant drug and widely abused illicit amphetamine derivatives. Statistics of drug abuse from the Malaysian National Anti-Drug Agency (NADA) 2020 reported that drug misuse related to amphetamine-type stimulants is more than 60% of all drug types, dominated by methamphetamine and followed by MDMA and amphetamine (National Anti-Drugs Agency, 2021). MDMA was considered to be a hallucinogen and has no recognised medicinal use. Hence, it was grouped into Class A drugs (the most harmful) in most countries (Advisory Council on the Misuse of Drugs, United Kingdom, 2009). Despite its harmful

effects, MDMA which is also known as Ecstasy has gained attention among teenagers for its recreational use due to its euphoric effects and enjoyable feelings. The excessive abuse of MDMA would result in brain damage and psychological disorders. Besides that, the increasing use of MDMA by pregnant women causes a public health concern because it is associated with health risks for mothers and their developing children (Barenys *et al.*, 2020).

Neurological insult is the most common form of 3, 4 Methylenedioxymethamphetamine (MDMA) neurotoxicity, which causes substantial damage to the brain by causing both apoptotic and necrotic cell death in the brain. Besides the overproduction of neurotransmitters, exposure to MDMA

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also causes the neuronal immune cell such as microglial cells in the brain to be activated in response to the danger-associated signals from the endangered neurons. The responses of microglia have been studied following MDMA administration to laboratory animals and *in vitro*, and the present objective is to summarise and evaluate MDMA-induced microglial responses that occur following MDMA exposure. Microglial response as a first-line defence has been implicated in MDMA toxicity in the sense that their activation is thought to contribute to neurotoxicity.

Here, we review the evidence indicating that the popular drug of abuse, methylenedioxymethamphetamine (MDMA; 'Ecstasy') has effects on microglia functioning and can result in increased disease susceptibility.

A. The Use of MDMA

MDMA was initially used in the 1970s to enhance psychotherapy, but later, it has grown in popularity as a recreational substance (Passie, 2018). Due to that, it was classified as a Schedule 1 narcotic in most countries including Malaysia, as stated in Act 234 Dangerous Drugs Act 1952, Laws of Malaysia along with raw opium, coca leaves, poppy-straw, cannabis heroin, ketamine, heroin, and morpheridine, indicating it had a high potential for abuse and no recognised therapeutic benefit (Pharmaceutical Services Programme, Ministry of Health Malaysia, 2022). Some researchers, however, are still interested in its efficacy in psychotherapy when administered to patients in carefully monitored settings. MDMA is currently being tested in clinical studies as a potential therapy for post-traumatic stress disorder (PTSD), anxiety, and other psychological distress related to life-threatening illnesses (Wolfson *et al.*, 2020; Mitchell *et al.*, 2021).

B. Microglial Cells

Microglia are a type of glial cell found in the central nervous system. Microglia has a role related to immune defence and phagocytosis of potentially harmful elements for neurons. The term "microglia" was coined in 1920 by Pío del Río Hortega, a pioneer student of neuroscience Santiago Ramón y Cajal (Tremblay, 2015). The immune function of these cells is known from the time of their discovery, although knowledge of their characteristics has advanced over the past

few decades. He was the first to demonstrate that mesoglia were composed of microglia, which are of mesodermal origin, and oligodendroglia, which, along with astroglia and neurons, are of neuroectodermal lineage. Río-Hortega framed a "modern conception of microglia" that remains relevant to this day (Rock *et al.*, 2004).

Microglial cells have several morphological features, which are amoeboid, ramified, and reactive microglia. The presence of microglial is crucial for brain homeostasis in both health and disease. Immune defence and CNS preservation or maintenance are the two main aspects of microglia function. As immune cells, they can rapidly respond to pathological insults, becoming activated to induce a range of effects that may contribute to both pathogenesis or confer neuronal protection (Wake *et al.*, 2011). Under the inflammatory conditions of the active immune response, dysregulated microglial activation and microglia-induced inflammation can exert direct effects on neurons, contributing to disease progression (Ginhoux *et al.*, 2013). Numerous endogenous and exogenous factors, such as invasive pathogens, neurodegeneration, ageing and toxic substances, can trigger microglial cells activation (Puzi & Vidyadaran, 2020).

The activation of microglial cells is indicated by several criteria. According to Hoogland *et al.* (2015), the activated microglial is characterised by its morphology, the number and size of the microglial cells, and the expression of microglial markers. As for the morphology, the activated microglial is shown by immunohistochemically staining in a brain tissue sample that marks several antibody markers, such as Ilb-1 and CD-68 proteins (Stankov *et al.*, 2015). However, *in vitro* study using BV2 cells demonstrated that the morphology can be distinguished without staining, in which the activated cells exhibit an amoeboid shape with reduced dendrites and processes (Dang *et al.*, 2014). The number and/or size of activated microglial cells also should be significantly increased as compared to the normal group. A morphometric study on the size of microglia cells in humans and mice found that the reactive microglia increased in cell body size and has fewer processes, almost 2-fold as compared to ramified microglia (Torres-Platas *et al.*, 2014). In the early phase of acute neuroinflammatory response, the number of microglia increases immediately due to the microglial activation program (Streit & Xue, 2009;

Matsudaira & Prinz, 2022). For instance, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment in mice showed a significant increase of microglial cells in the striatum and substantia nigra as compared to the control/untreated group (Członkowska *et al.*, 1996). As for the microglial markers, there should be a significant increase in the expression of a microglial marker in the activated microglial cells as compared to the control group, such as IL-1 β , TNF- α , ROS, and NO levels (Jiang *et al.*, 2020; Jurga *et al.*, 2020). If these criteria do not appear (no changes in their morphology, the number and size of the microglial cells do not increase, and no expression of microglial markers), then the microglia are inactive. However, if one or more criteria are present, microglia are activated. If there is any contradiction between these criteria, such as increased microglial marker expression but no change in morphology, then the microglia are judged as moderately activated (Hoogland *et al.*, 2015).

In the past few years, studies on microglia function are not limited to its immune function. There is data that appears to show a new and fundamental role for microglia in controlling the proliferation and differentiation of neurons as well as in the formation of synaptic connections (Hughes, 2012). Hence, microglia become an important target of therapy to improve the defence system and CNS maintenance caused by various neurological disorders and brain injuries.

C. Neurotoxicity of MDMA and Microglial Activation

Histopathological examinations and immunohistochemistry approaches showed that MDMA causes neuronal damage. It was indicated by the morphological changes, a decrease of intact neuronal cells, or several apoptotic markers (Schmued *et al.*, 2005; Meamar *et al.*, 2010; Soleimani *et al.*, 2013). Besides that, an *in vitro* study on human neuronal cell lines showed that MDMA could activate apoptotic processes increase expression levels of pro-apoptotic Bax and caspase 3 activity (Sogos *et al.*, 2021). Biochemical studies have also reported a reduction of serotonin transporter and the depletion of dopamine and serotonin (Li *et al.*, 2014). MDMA also induces a hyperthermic response, which appears to modulate the long-term neuronal damage caused by the drug (Goni-Allo *et al.*, 2008). Besides that, all of these effects are the outputs of the mechanism of actions of MDMA, starting

from its administration into the body until the pharmacological changes taking place. Then, the cascade signals will activate the intracellular response, which leads to a sequence of events that will lead to neuronal damage and apoptosis pathway.

Along with the events, microglial cell activation also plays a vital role in MDMA neurotoxicity (Thomas *et al.*, 2004; Herndon *et al.*, 2014; Costa *et al.*, 2021). As the immune cells, microglia act as protectors, detecting the first signs of invasion by MDMA or tissue damage. Activation of microglia leads to specific differentiation of microglial phenotypes. Similar to macrophages, activated microglia will undergo classical/pro-inflammatory (M1) or alternative/anti-inflammatory (M2) activated phenotypes. The classical activation (M1) state of microglia exhibits cytotoxic and harmful properties, whereas an alternative (M2) activation state demonstrates neuroprotective and reparative functions. While the M2 phenotypes promote tissue repair and phagocytosis of protein aggregates and cell debris, the M1 phenotypes are more likely to be detrimental to the brain by inducing neuronal toxicity through the secretion of proinflammatory cytokine and chemokine and production of reactive oxygen species (ROS) (Peng *et al.*, 2017). The activated cells will also proliferate and migrate to the site of injury, followed by the changes in the morphology and the inflammatory secretion profiles (Kettenman *et al.*, 2011; Garaschuk & Verkhratsky, 2019).

In vivo studies have shown that MDMA had caused microglial activation in many parts of the brain (Torres *et al.*, 2011; Frau *et al.*, 2013). This shifted the microglia to be in the M1 phenotype and release pro-inflammatory substances and neurotoxic factors such as cytokines tumour necrosis factor- α (TNF- α) (Frau *et al.*, 2016; Mohamad *et al.*, 2022), nuclear factor kappa B (kB), and interleukin-1 β (IL-1 β) (Orio *et al.*, 2010; Salem *et al.*, 2011; Torres *et al.*, 2011; Frau *et al.*, 2016). In the meantime, it is still unclear whether MDMA could modulate the release of pro-inflammatory and anti-inflammatory cytokines from the microglia cells. Even though there are no specific reports on the modulation between M1 and M2 phenotypes following the activation of microglia by MDMA, however, several studies reported on the modulation of both pro-inflammatory and anti-inflammatory cytokines after microglial activation induced by methamphetamine, in

which, 150 μ M methamphetamine exposure to BV2 cells increased the expression of M1 markers (iNOS) and down-regulated the expression of the anti-inflammatory markers arginase and SOCS3 (Chao *et al.*, 2017). In MDMA administration in mice, it was found that MDMA could induce over-expression of BDNF that affects serotonergic and dopaminergic transmission in the nucleus accumbens and leads to dependence and psychosis (Mouri *et al.*, 2017). Besides that, exposure of BV2 cells to MDMA also changed the resting state of microglia into a more rounded shape, with the increase of TNF- α levels (Mohamad *et al.*, 2022). Excessive abuse of MDMA would lead to over-activation of microglia and can be deleterious to the neurons in protecting the neuronal function instead of worsening the neuronal injury (Woodcock *et al.*, 2019; Downey & Loftis, 2021). It is important to understand that the neurotoxicity of MDMA is associated with the microglial activation through the fact that the cytokines released such as TNF- α by the activated microglia can activate glutamate neurotransmission, which promotes excitotoxicity (Zou & Crews, 2005).

However, previous studies have shown that stimulation with an anti-inflammatory compound such as a parthenolide, a sesquiterpene lactone extracted from the medical herb feverfew (*Tanacetum parthenium*),

candesartan (an angiotensin II type I receptors antagonist), rutin (a dietary flavonoid) and other phytochemicals could promote the M2 state of microglia, deactivates the pro-inflammatory (M1) cell phenotype, and induce the increased expression of glia-derived neurotrophic factors (Popiolek-Barczyk *et al.*, 2015; Saqib *et al.*, 2018; Qie *et al.*, 2020; Lang *et al.*, 2021). Thus, the ability to switch the microglial phenotype during microglial activation and the associated inflammatory responses would be an important strategy for the development of effective therapy against MDMA neurotoxicity. Although many studies had shown that MDMA had caused various neuroimmune activation markers, specific phenotypic M1 versus M2 markers have not been examined in the context of determining microglial phenotype involving MDMA neurotoxicity. It is useful to identify the microglial activation states to enable more understanding of the pathogenesis of MDMA.

The effects of MDMA on microglial activation were studied in various models either *in vivo* or *in vitro*. Most previous research investigated the effects of MDMA on microglial cell activation *in vivo* by using mice or rats. Even though there were studies conducted *in vitro*, however, a lack of study was reported in the literature. Table 1 below summarised several studies on the effects of MDMA on microglia.

Table 1. Previous studies on the effects of MDMA on microglia.

Parameters	Species	MDMA Doses	Effect of MDMA	Treatment	Authors
IL-1 α , IL-1RI.	Dark Agouti rats.	12.5 mg/kg, i.p.	IL-1 α expression levels rise whereas IL-1RI expression declines.	CB2 agonist JWH-015 prevented the MDMA-induced microglial activation.	Torres <i>et al.</i> , 2011
IL-1 β , Hyperthermia and 5-HT.	Dark Agouti rats.	12.5 mg/kg, i.p.	MDMA caused microglial activation; increased IL-1 β , and it was found that the release of IL-1 β was associated with hyperthermia and was not related to acute 5-HT depletion.	Fluoxetine prevented the MDMA-induced acute 5-HT depletion.	Orio <i>et al.</i> , 2004

Cannabinoid CB2 Receptor.	Dark Agouti.	12.5 mg/kg, i.p.	Increased CB2 receptor expression in microglia.	JWH-015 decreased MDMA-induced microglial activation and interleukin-1b release and slightly decreased MDMA-induced 5-HT neurotoxicity.	Torres <i>et al.</i> , 2010
Number of activated microglial cells and morphology.	Sprague Dawley rats.	20 mg/kg, s.c	Increased microglial occupancy at 12- and 72-h.	None.	Herndon <i>et al.</i> , 2014
Number of activated microglia (HRP-conjugated isolectin B4 (ILB4) staining) and glial fibrillary acidic protein (GFAP) response.	Female C57BL/6 mice.	20 mg/kg (four injections (i.p.) with a 2 h interval between injections	Increased the number of activated microglia and GFAP response.	None.	Thomas <i>et al.</i> , 2004
CD11b and GFAP immunoreactivity.	Male C57BL/6J mice.	4 x 20 mg/kg	Increased CD11b and GFAP immunoreactivity in the striatum and only CD11b was significantly higher than the vehicle in substantia nigra pars compacta (SNc).	Caffeine (10 mg/kg) increased the CD11b and GFAP in the striatum but not in the SNc of MDMA-treated mice.	Khairnar <i>et al.</i> , 2010
CD11b, GFAP, Body temperature.	Male old C57BL/6J mice.	4 x 20 mg/kg, i.p.	Increased CD11b immunoreactivity in the striatum, nucleus accumbens, motor cortex, and substantia nigra; the increase in GFAP immunoreactivity, and body temperature.	None.	Frau <i>et al.</i> , 2013

NFκB binding activity, IL-1β, [3H]-paroxetine binding.	Dark Agouti rats.	12.5 mg/kg; i.p.	Increased NFκB activation, IL-1β release, and microglial activation in the frontal cortex and the hypothalamus; and reduction in the density of 5-HT uptake sites after 7 days.	Minocycline, a semi-synthetic tetracycline antibiotic twice a day for 2 days (45 mg/kg on the first day and 90 mg/kg on the second day; 12-h apart, i.p.).	Orio <i>et al.</i> , 2010
GFAP, ionised calcium-binding adaptor molecule 1 (Iba-1).	Male and female Wistar rats.	Two daily injections of MDMA (10 mg·kg ⁻¹ , s.c.) at 4h intervals.	Increased Iba-1 in male rats but not in females; decreased the proportion of SERT-positive fibres.	- Δ9-tetrahydrocannabinol (THC) increased GFAP response in both sexes. - a combination of both drugs resulted in a 'normalisation' of Iba-1 to control values. - reduced immunostaining for CB1 receptors.	Lopez-Rodriguez <i>et al.</i> , 2014
GFAP, Complement receptor type, 3 (CD11b), tyrosine hydroxylase (TH).	Male C57BL/6J mice.	10 mg/kg twice daily, two times a week	No increase in astroglia and microglia in the SNc and striatum.	MPTP (20 mg/kg × 4) induced a higher microglial and astroglial response in both the striatum and the substantia nigra pars compacta (SNc).	Costa <i>et al.</i> , 2013
CD11b and GFAP immunoreactivity as markers of microglia and astroglia activation in the striatum.	CD1 mice.	three repeated doses of 20 mg/kg, i.p., at 2-h intervals)	MDMA induced microglial activation; hyperthermia; increased CD11b, and GFAP staining in the striatum.	Caffeine (10 mg/kg) chronically administered completely prevented MDMA-induced glial activation.	Ruiz-Medina <i>et al.</i> , 2013

CD11b and GFAP, to mark microglia and astroglia in the caudate-putamen.	C57BL/6J adolescent (4 weeks old) and adult (12 weeks old) mice.	MDMA (4 × 20 mg/kg, 2-hour intervals, intraperitoneally [i.p.]	Higher activation of astroglia in the caudate putamen (Cpu).	None.	Frau <i>et al.</i> , 2016
Dopaminergic degeneration by measuring tyrosine hydroxylase (TH), astrogliosis, and microgliosis by measuring glial fibrillary acidic protein (GFAP), pro-inflammatory interleukin (IL) IL-1β or the anti-inflammatory IL-10.	Male offspring from pregnant C57BL6/J dams .	MDMA, 4 × 20 mg/kg, 2 h apart, sacrificed 48 h later) administered in either adolescence or adulthood.	Dopaminergic damage and glial activation in the nigrostriatal tract.	None.	Costa <i>et al.</i> , 2021
BDNF mRNA expression, serotonin, dopamine, conditioned place preference test, and locomotor activity.	C57BL/6J mice.	A single dose of MDMA (10 mg/kg)	MDMA-induced BDNF expression affects serotonergic and dopaminergic transmission in the nucleus accumbens and leads to dependence and psychosis.	Dopamine receptor antagonists attenuated the effect of repeated MDMA administration on BDNF mRNA expression in the nucleus accumbens.	Mouri <i>et al.</i> , 2017
Morphological changes of microglia and TNF-α level.	BV2 cells.	500 µg/mL of MDMA, incubated for 24H.	Activated BV2 cells showed an amoeboid shape and were bigger in size in the MDMA group, and increased TNF-α level as compared to the control group.	None.	Mohamad <i>et al.</i> , 2021

II. CONCLUSION

In this review, we have presented evidence that the activation of microglial cells is involved in causing the neurodegenerative effects of MDMA in experimental animals. In summary, most studies pointed out the activation of microglia following the exposure of MDMA to rodents either through the numbers of activated microglia, microglial morphological changes, and the biomarkers of microglia activation. The parameters include the increase in IL-1 α and IL-1 β , the decrease in IL-1RI expression, increased CB2 receptor expression in microglia, increased CD11b expression, increased Iba-1 expression, increased TNF- μ level, and the increase in GFAP immunoreactivity. Moreover, the cytokines released by activated microglia can activate

glutamate neurotransmission, resulting in excitotoxicity. Nevertheless, different mechanisms and factors appear to be involved in the noxious effects of MDMA, as indicated by findings in experimental animals. Thus, investigation of the neurotoxic insults generated by MDMA *in vivo* and *in vitro* is required for a better understanding of this amphetamine-related drug.

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