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Review

Molecular mechanism of methamphetamine-induced neurotoxicity: Musing on the not-so-funny side

Xiaochun Zhang[1,2] and Jun Ren[2,*]

[1]Pathology & Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH 44195,
[2]Division of Pharmaceutical Sciences, University of Wyoming, Laramie, WY 82071, USA

ABSTRACT

Methamphetamine is one of the most widely abused drugs in the world and its abuse is frequently associated with serious health consequences such as Human immunodeficiency virus (HIV) infection. Ample evidence has indicated that both methamphetamine abuse and HIV infection can result in neuronal injury *en route* to the development of neurodegenerative diseases. The major neurotoxic effects triggered by methamphetamine include depletion of neurotransmitter dopamine and serotonin as well as the persistent damage to monoaminergic terminal. In this review, we try to summarize recent updates of the cellular and molecular mechanisms underneath methamphetamine-induced neurotoxicity including disturbance of dopamine metabolism, oxidative stress, excitotoxicity, neuroinflammation and interconnections among these pathways.

KEYWORDS: methamphetamine, neurotoxicity, neuroinflammation, oxidative stress, excitotoxicity, ER stress, apoptosis

***Correspondence should be addressed to: Dr. Jun Ren, Division of Pharmaceutical Sciences, Graduate Neuroscience Program & Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences Laramie, WY 82071, USA.
jren@uwyo.edu

INTRODUCTION

Methamphetamine (MA), a potent addictive stimulant illegally manufactured, distributed and abused in the United States, represents a major public concern due to its abuse frequency and the associated serious health complications [41]. It is usually referred to as “speed”, “crystal”, “crank”, “go”, and “ice”. It is estimated that a US population of 12.3 million use the drug during their life time, which is equivalent to 5.2% of the Americans of age 12 years and older. MA, a cationic lipophilic molecule derived from amphetamine, can provoke profound effects on the central nervous system and trigger neurotoxicity featured by long-lasting depletion of striatal dopamine (DA) and serotonin as well as damage to striatal dopaminergic and serotonergic axon terminals [9, 16, 33, 34, 42]. Observations from animal studies have demonstrated MA-induced death of nonmonoaminergic cell bodies in multiple brain areas such as the striatum, cortex and hippocampus [65]. In human drug users, systematic histological, immunohistochemical and imaging investigations have revealed significant alterations in the brain including hypometabolism, microglial activation, widely spread axonal damage, dopaminergic terminal marker deficits, and neuronal loss [5, 31, 34, 46]. Taken together, these findings suggested that MA-related neurotoxicity comprises a cascade of interacting cellular and molecular events which finally result in wide spread disturbances in the central nervous system. A thorough understanding of the

mechanisms responsible for MA-related neurotoxicity is needed to facilitate the development of new therapeutic or preventive approaches. In this mini-review, we will update the mechanisms underlying MA-induced neurotoxic effects as well as the interconnection among different pathways.

1. Dopamine signaling: Source of reactive oxidative species (ROS), victim and mediator in MA-induced neurotoxicity

It has been well known that dopaminergic signaling is a major target of MA-induced neurotoxicity. On the other hand, however, DA signaling itself acts as a crucial mediator in MA intoxication. In general, dopaminergic signaling is orchestrated by a delicate balance among synthesis, release, storage and re-uptake of DA by the presynaptic terminal and almost every link in this pathway is affected by MA and mediated MA-induced neurotoxicity to a certain degree [43].

DA is synthesized from tyrosine using a two-enzymatic step. Tyrosine hydroxylase (TH) catalyzes the first reaction and functions as the rate-limiting enzyme in the production of dopamine. A persistent reduction of TH has been found in human users [34]. Dopamine transporter (DAT) residing on presynaptic terminals removes DA from synapse after its release. It plays an important physiologic role in recycling of DA and protecting it from the enzymatic degradation by monoamine oxidase (MAO) [43]. Notably, DAT is capable to transport DA in either direction depending on the concentration gradient [48]. Under physiological conditions, DA is stored in vesicles in nerve endings. When nerves are depolarized following stimulation, the vesicles release their contents into the synaptic cleft in response to elevated intracellular Ca^{2+} [4]. To the contrary, MA elicits an increase in extracellular DA level via a Ca^{2+}-independent mechanism, a process being referred to as exchange diffusion [13]. MA may enter the nerve terminal through binding to DAT as well as diffusion. Within the neuron, it is thought that MA permeates synaptic vesicles in its base form, and becomes charged in the acidic interior of vesicles and retains as a cation. The resultant increase in pH collapses the synaptic vesicle proton gradient, leading to a displacement of dopamine from vesicles to the cytoplasm. As a result, intracellular DA concentrations are drastically increased [13]. The elevated intracellular DA is then metabolized by MAO accompanying production of H_2O_2. High intracellular DA levels are thought to be a major source of ROS and contribute to MA-induced neurotoxicity (Fig. 1) [16]. Moreover, high cytoplasmic levels of DA induced by MA can bind to the intracellular “facing” DAT, and are transported reversely to extracellular space, and cause increased stimulation of postsynaptic receptors [13]. In addition, MA may indirectly prevent the reuptake of the released DA into vesicles, thus causing them to remain in the synaptic cleft for a prolonged period of time. An *in vitro* study using synaptosomes prepared from the MA-treated rats showed that single or multiple high-dose administrations of MA decrease DA uptake within 1 hr, indicating a significant decrease in DAT activity in response to MA exposure [20]. *In vivo* administration of a single high dose of MA was reported to reversibly dampen DAT function, which may be due to phosphorylation and internalization of DAT [12, 45]. Distinct from observation using a single high dose, multiple high-dose injections of MA result in a persistent decrease in striatal DAT activity and protein abundance [25, 35]. The MA-induced reduction in DAT activity and/or cell surface localization may decrease DAT-mediated DA uptake, leading to the accumulation of extracellular DA and DA-mediated ROS. More intriguingly, a neurotoxic MA regimen was found to remarkably decrease the DAT monomer immunoreactivity accompanied by the formation of higher weight DAT complexes 12 to 48 hrs following treatment [3]. This finding provides direct evidence that MA is capable of eliciting structural alteration to DAT, although the significance of such change remains to be defined. Furthermore, observation from knockout models showed that the DAT homozygote knockout mice were refractory to MA-induced DA terminal toxicity. These results provide some convincing evidence that DAT and DA are major mediators of MA-induced dopaminergic terminal toxicity.

MA can also disturb DA homeostasis via affecting vesicular monoamine transporter 2 (VMAT-2).

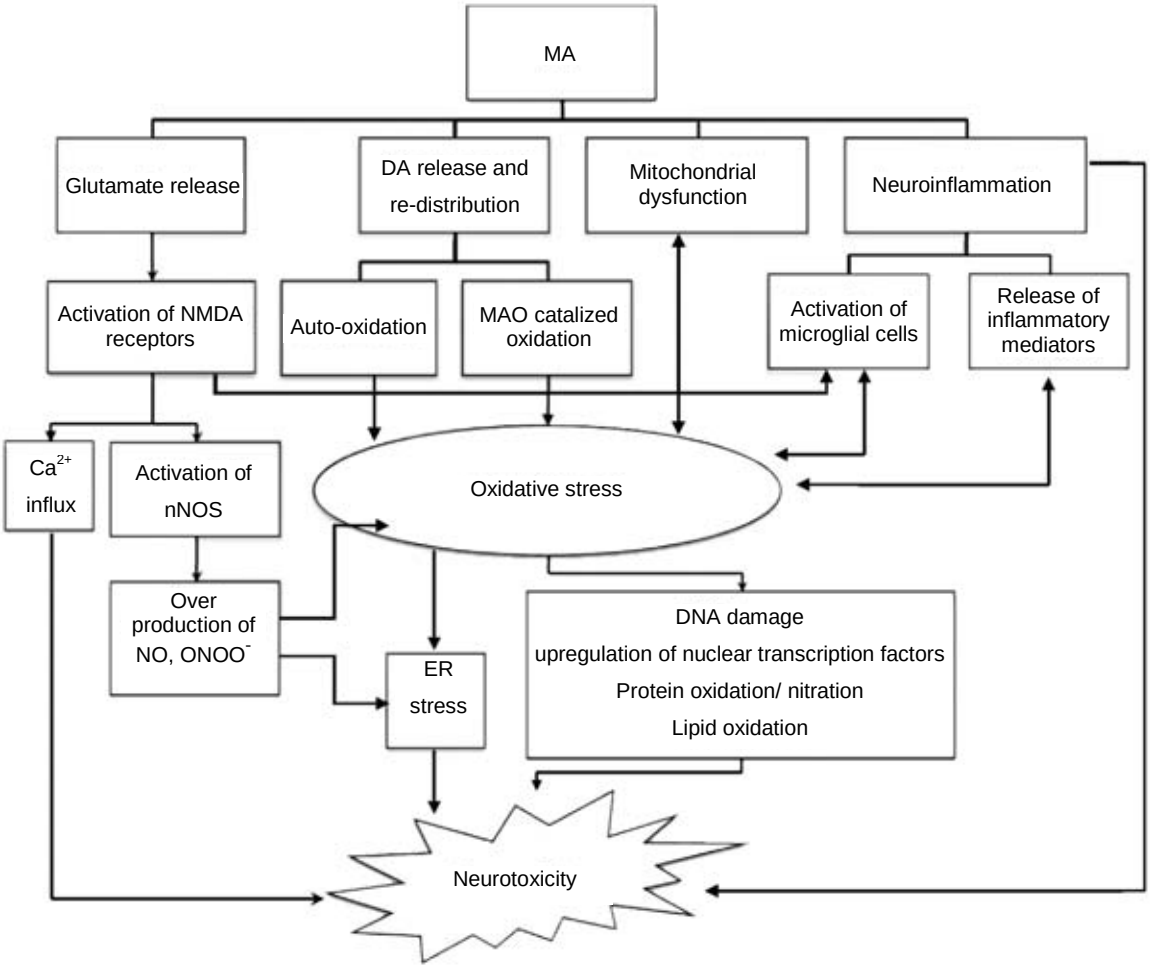

Fig. 1. Proposed mechanism for methamphetamine-induced neurotoxicity. A complex pathway network composed of dopamine oxidation, mitochondria dysfunction, neuroinflammatory response and excitotoxicity underlies MA-induced neurotoxic effects such as degeneration of monoaminergic terminals and death of nonmonoaminergic cell bodies. These pathways are intimately connected to each other with oxidative stress functions as a central link. All the four pathways can produce oxidative stress either directly or indirectly. Sequentially, oxidative stress leads to oxidative damage of proteins, lipids and DNA as well as apoptosis. Endoplasmic Reticulum (ER) Stress is another consequence of oxidative stress and activation of excitotoxic pathway. Increased ER stress can directly lead to cell apoptosis.

VMAT-2 is responsible for packaging cytoplasmic DA into vesicles for storage, thus playing an important role in regulating intracellular DA concentrations [43]. Several studies have shown that MA-treated animals develop long term reductions of VMAT-2 [38]. Riddle and fellow researchers also demonstrated that MA may alter the subcellular distribution of VMAT-2 [44]. Multiple high-dose administrations of MA were found to redistribute VMAT-2 protein in the nerve terminal within 1 hr, resulting in fewer VMAT-2 available to sequester DA into the terminal vesicles [44]. Using the heterozygous VMAT knockout mice (homozygotes are not viable after one week), Fumagalli and colleagues found greater toxicity in knockout mice [22]. Given that synaptosomal uptake of DA by the DAT was similar between wild-type and knockout animals, the exacerbated toxicity of MA may be mainly due to elevated intracellular DA [16]. It is thus plausible to speculate vesicular storage as an essential player to the defense against MA-induced neurotoxicity.

Although MA also induces serotonin release, a recent study [56] showed that endogenous serotonin does not play a role in MA-induced damage to DA nerve endings in the striatum. In summary, DA seems to play a rather important role in MA neurotoxicity. Disturbance of the delicate balance among cytoplasmic, vesicular and extracellular DA pools may be a key mechanism in the neurotoxic effect of MA. Nevertheless, the precise DA pool responsible for this toxicity warrants further scrutiny.

2. Formation of ROS and oxidative stress: A central link leading to DNA damage, protein oxidation and apoptosis

An increasing body of evidence suggests that MA-induced neurotoxicity is dependent upon production of reactive species and consequently oxidative stress. Oxidative stress in turn leads to oxidative damage of proteins/lipids/DNA, ER stress, mitochondria dysfunction and apoptosis.

The reactive species mediating MA toxicity include reactive oxygen species (ROS) and reactive nitrogen species (RNS). The formation of RNS is associated with glutamate/excitotoxicity pathway, which will be addressed in the next section. Three machineries may contribute to the overproduction of ROS (Fig. 1): (1) DA release and subsequent enzymatic oxidation, (2) DA auto-oxidation and (3) mitochondrial dysfunction [16]. DA can be metabolized in the brain by several enzymes with MAO serving as the major enzyme in the metabolic degradation of catecholamine. DA is metabolized mainly by MAO to dihydroxyphenylacetic acid (DOPAC) accompanied by H_2O_2 formation [49]. The redistribution of DA from synaptic vesicles to cytoplasmic compartments caused by MA leads to a significant elevation of oxidizable DA concentrations, therefore promoting hydroxyradial formation. DA can also be non-enzymatically oxidized by molecular O_2 to form 6-hydroxy (OH) DA and superoxide radicals referred to as auto-oxidation [16]. The excess DA caused by the DAT-mediated outward transportation is thought to encourage auto-oxidation of DA [33]. Moreover, 6-OHDA is extremely labile and H_2O_2 can be generated during the further reactions [33]. Mitochondria are a major source of ROS production, and MA has been known to cause mitochondria dysfunction, leading to overproduction of ROS [64]. In addition, MA may also enhance ROS formation through stimulating microglial cells. Kuhn and colleagues [53] found that MA administration led to microglial activation and activated microglia can produce reactive species [26].

ROS such as superoxide and hydroxyradicals produced from DA metabolism has been suggested to play an important role in the MA-induced neurotoxicity. Administration of antioxidants, such as ascorbic acid or vitamin E, were shown to attenuate MA toxicity [6], whereas, inhibition of superoxide dismutase (SOD) by diethyldithiocarbamate may increase the neurotoxicity [23]. Moreover, the specific involvement of superoxide radicals in the MA-induced neurotoxicity has been evaluated using transgenic mice overexpressing SOD. Cadet and coworkers found that chronic MA does not cause the loss of DA in striatum or cortex of these SOD overexpressing mice compared to wild-type mice [11].

3. Excitotoxicity/Nitric oxide (NO) pathway

MA can cause excess release of glutamate and sequentially lead to calcium influx, RNS formation, microglial activation and ER stress. This has been considered as an important route to MA-induced dopaminergic deficits (Fig. 1). For instance, parenteral administration of MA enhances striatal glutamate release [43]. Blockade of the N-methyl-D-aspartate (NMDA) type of glutamate receptor by dizocipline (MK-801) can also attenuate the MA-induced neurotoxicity [28]. Furthermore, inhibition of nNOS using 7-nitro-indazole (7-NI) was demonstrated to offset MA-induced DA deficits [16] while nNOS knockout mice are protected from the MA-induced neurotoxicity [33].

The increased glutamate release in striatum was thought secondary to the enhanced DA release via a cortico-striatothalamo-cortical negative feedback loop [33]. Glutamate further binds to NMDA-receptor and promotes Ca^{2+} influx. Excessive intracellular Ca^{2+} can lead to mitochondrial damage and cell death [43]. In addition, activation of NMDA receptor is related

to stimulation of the nNOS, which produce NO. NO mediates neurotoxicity by forming free radicals peroxynitrite ($ONOO^-$) via further reaction with ROS such as O_2^-. The interaction of NO and ROS can produce higher oxides of nitrogen, which are potent pathological mediators. Peroxynitrite is the most prevalent ROS among such oxides. Due to the radical nature, NO and O_2^- can combine rapidly to form $ONOO^-$. Actually, the rate of $ONOO^-$ formation is close to diffusion limited, and this reaction is about three-fold faster than the reduced reaction of O^{2-} catalyzed by SOD [16]. Peroxynitrite is a strong two-electron oxidant and is capable of nitrating molecules. Thus it can result in a number of toxic effects including DNA strand breaks, nucleic acid modification, lipid oxidation, protein oxidation and nitration, which ultimately lead to cell death [16]. Specially, peroxynitrite may directly inactivate TH. Imam and coworker performed a Protein Data Bank survey for the crystal structure of TH and noticed that several tyrosine moieties are clustered around the active center of the enzyme. Among those, tyrosine 371, 314, and 289 are within 10Å bond length of the active center of the enzyme. Furthermore, the oxygen atom of Tyr 371 has a bond length of 5.02Å from the iron atom of the active center. Nitration of the tyrosine 371 by peoxynitrite can lead to the reduction of bond length between the iron atom of the active center and the oxygen atom of the tyrosine residue 371 [28]. This reduction of the bond length can create a steric hindrance at the active center of the enzyme, and result in reversible or irreversible inactivation of the enzyme [28]. This hypothesis may therefore explain MA-induced TH dysfunction although direct experimental evidence is still lacking.

4. Mitochondria and energy balance: Direct link to oxidative stress, Endoplasmic reticulum (ER) stress and apoptosis

The current data also strongly favors that MA interrupts mitochondrial function thus directly leading to oxidative stress, ER stress and apoptosis. MA has been found to inhibit the electron transport chain (ETC) and many ETC inhibitors can promote formation of reactive species and result in dopaminergic neurotoxicity [43]. In contrast, administration of substrates for energy metabolism attenuates MA-induced toxicity [43].

MA disrupts mitochondrial function through both direct and indirect mechanisms (Fig. 1). First, due to its cationic lipophilic nature, MA may diffuse into the mitochondria and remain there. The accumulation of positively charged MA in the cristae of mitochondria further interrupts the electrochemical gradient established by ETC [16, 58]. The gradient is essential to preserve functional ATP synthase and integrity of mitochondrial membrane. Therefore, a failure in maintaining normal electrochemical gradient will endanger cell survival. Second, elevated semi-quinones, metabolites of DA, caused by MA can result in excess Ca^{2+} release in the mitochondria, which lead to activation of caspase-related apoptotic pathway [16, 39]. Third, increased ROS and RNS products induced by MA can result in mitochondria dysfunction. The mitochondrion is itself a source of reactive species through leakage from the ETC and is especially vulnerable to oxidative stress. The permeability transition pore (PTP) and the four complexes of the ETC are the major targets for oxidative attack. Attacks to the PTP can lead to opening of pores and Ca^{2+} release. Complexes I-III can be irreversibly modified by peroxynitrite through both oxidation and nitration and then lead to severe mitochondrial damage or even cellular necrosis [58, 59]. Complex IV contains a heme protein, cytochrome c oxidase. Cytochrome c can rapidly combine with NO to produce a ferrous nitrosyl complex. Although this combination is reversible, the bond between NO and iron is so strong that low levels of NO can significantly inhibit the function of complex IV [14, 16]. Since neurons have a very high-energy demand and are thus particularly sensitive to mitochondrial damage.

5. Pathways of MA-induced apoptosis

Toxic dose of MA can cause neural cell death in the striatum, cortex, and hippocampus of rats and mice [7, 18]. As evidenced by DNA strand breaks, chromatin condensation and nuclear fragmentation, MA-induced cell death was thought to have a mechanism similar to apoptosis [6, 17]. A number of signaling pathways have been suggested to mediate MA-induced apoptosis.

5.1. Activation of JNK/SAPK-c-Jun pathway

The JNK/SAPK (Jun N-terminal Kinases/Stress Activated Protein Kinase) plays an important role in stress responses, cell proliferation, and apoptosis. This pathway is part of the MAPK (Mitogen-Activated Protein Kinase) superfamily. The JNK kinase cascade has been established as follows: "Receptors --> Adaptors (Crk, CrkL, Cas) --> MAP4K (HPK, GCK) -->MAP3K (MEKK1, TAK1, MLK3) --> MAP2K (MKK4, MKK7) --> JNK(JNK1, JNK2 and JNK3) --> c-Jun [6].

cDNA array data has shown that MA causes very early induction of several transcription factors, including c-Src and c-Jun [6]. c-Jun activity is regulated via phosphorylation at serines 63 and 73, and this phosphorylation is mediated by JNK1, JNK2, and JNK3. MA administration causes intensive phosphorylation of c-Jun at ser73 at 4–16 h and phosphorylation at ser63 at 2-4 hrs after injection. JNK protein expression was later found to increase with time, and showed a pattern that was similar to c-Jun [7]. Phosphorylation of JNK at threonine 183 and tyrosine 185 was also observed after a toxic regimen of MA administration. The protein expression of some upstream members of the JNK pathway including c-Src, Cas, MKK4, and MKK7 were all increased after MA treatment [7]. Since JNK was found to be involved in several models of neuronal apoptosis, activation of this pathway might contribute to the MA-induced apoptotic events in the rodent brain. This hypothesis actually was supported by the c-jun knockout study where c-jun knockout mice were protected against MA-induced apoptosis [6].

5.2. Mitochondrial cell death pathway

Bcl-2 family is an important component of the mitochondrial apoptotic pathway. Proteins of the Bcl-2 family can be functionally divided into pro- or anti-apoptotic members. BAX, BAK, BAD, and BID are inducers of apoptosis whereas Bcl-2, Bclw, and Bcl-XL are known to promote cell survival [6]. The data from cDNA array analyses showed a significant up-regulation of proapoptotic genes of the Bcl-2 family several hours following MA injection [8]. Further studies using RT-PCR and Western blot confirmed that MA administration enhanced the expression of BAX, BAK, BAD and BID [6]. In contrast, both the mRNA and protein expression of Bcl-2 and Bcl-XL were decreased [7]. In addition, overexpression of Bcl-2 was found to protect cultured neural cells from MA-induced apoptosis [7]. These results suggest that administration of toxic doses of MA lead to a shift in the ratios of death promoters to death repressors that might result in neuronal apoptosis.

Besides the Bcl-2 family, some other components of the mitochondria death pathway are also suggested to play a role in MA-induced apoptosis. MA can induce cytochrome *c* release from mitochondria and activation of caspases 9 and 3 [17] although the precise mechanisms of action are still not fully understood. The anti-apoptotic properties of some members of the Bcl-2 family are thought to depend upon their blockade of cytochrome c release [67]. Meanwhile, the pro-apoptotic members of the family are found to enhance cytochrome c release [47].

5.3. ER-dependent stress pathway

ER is an important organelle which participates in the regulation of Ca^{2+} homeostasis and protein folding within the cell [7]. Oxidative stress can also cause ER dysfunction (Fig.1) and thus lead to activation of ER-associated apoptotic pathway [7]. Cadet and colleagues reported that MA at doses to turn on apoptosis can induce activation of calpain, which is a key mediator of ER-associated apoptosis [7]. In addition, expression of a number of proteins, such as caspase-12, GRP78/BiP (glucose-regulated protein/immunoglobulin heavy chain binding protein), and CHOP/GADD153 (C/EBP homology protein) was altered by administration of MA [6]. These proteins are involved in the ER-induced apoptosis and unfolded protein response (UPR). Therefore, these findings indicate that MA-induced apoptosis may be partly due to increased ER stress. Nevertheless, ER stress may be a secondary effect in the MA-mediated oxidative stress [6] due to the shift in BAX/Bcl-2 balance induced by the drug of abuse [29]. Enhanced expression of BAX and BAK by MA may play important roles in producing ER stress. Accumulation of these molecules has been observed in the ER and mitochondria followed by

early caspase-independent Bcl-2-sensitive release of Ca^{2+} from ER and subsequent Ca^{2+} accumulation in mitochondria [6]. Enhanced Ca^{2+} influx into the mitochondria may further lead to cell death via release of cytochrome *c* and activation of the caspase dependent apoptotic pathway. In summary, cross talk between the ER and mitochondria signaling might be a key factor in MA-induced apoptosis.

6. DNA damage and MA intoxication

Gene expression study using microarray showed that MA administration caused changes in the expression of certain genes involved in the DNA repair such as APEX, PolB and LIG1 [10]. DNA damage is evidenced by apoptosis following MA administration. Immunohistochemical results showed that MA increased 8-hydroxy-2'-deoxyguanosine (8-OH-dG) levels in multiple brain regions and provided direct evidence of MA-induced peroxidative DNA damage [60, 63]. In addition, MA has also been shown to promote deletion in brain mtDNA [64]. Oxidative damage to mitochondrial DNA (mtDNA) in the heart and brain is inversely related to maximum life span of mammals, suggesting that accumulation of mtDNA damage may be involved in MA related accelerating brain senescence and neurodegeneration [64].

7. Neuroinflammation and MA intoxication

Neuroinflammation has been evidenced in both MA-treated animals and human users [1, 37, 66]. It may contribute to the MA-induced neurotoxicity directly or by interacting with other pathways such as oxidative stress (Fig. 1). Studies have demonstrated that several drugs with anti-inflammatory effect were protective to MA-caused neural damage [24, 51, 61] .

7.1. Microglial activation and its role in MA-induced neurotoxicity

7.1.1. MA administration can cause significant microglial activation

Microglial activation following acute toxic regimen of MA (4 x 5 to 10 mg/kg, at 2 hrs internal) has been reported in both mouse and rat models. In striatum, the lowest dose tested that can induce significant microglial action is four injections of 2 mg/kg, and 5 mg/kg caused marked enhanced microglial activation when compared to the effects of 2 mg/kg. However a higher dose of 10 mg/kg caused similar microglial activation at a dose of 5 mg/kg [57]. Significant microglial activation was observed in the striatum as early as 1 day after the last injection of MA, and the peak activation appears on the second day [36, 57]. Microglial activation in the striatial tissue lasts about a week, and the number of activated microglia return to the control level after 7 days [57]. Focal microglial activation in response to acute MA treatment has also been found in parietal and piriform cortices, and in the ventromedial column of the periaqueductal gray. However it was not observed in hippocampus, substantia nigra, nucleus accumbens, or raphe nuclei [36]. Moreover, only neurotoxic amphetamines including D-methamphetamine, 3,4-methylenedioxy-methamphetamine, d-amphetamine, and p-chloro-amphetamine, are potent to induce microglial activation in the striatum, whereas the non-neurotoxic amphetamines L-methamphetamine, fenfluramine, and 2,5-dimethoxy-4-iodoamphetamine (DOI) do not possess such adverse effects [57]. Since neurotoxic amphetamines have been associated with DA and serotonin nerve terminal damage, these findings depict a possible link between microglial activation and neurotoxicity. Moreover, a number of non-neurotoxic chemicals which partially mimic the pharmacologic effect of MA on dopamine system have been tested, and none of them induce microglial activation [57]. These data reinforced the possible link between microglial activation and MA-induced dopaminergic terminal degeneration.

7.1.2. Does hyperthermia mediate MA-induced microglial activation?

Methamphetamine intoxication is known to cause hyperthermia which may be related to its neurotoxicity. To determine whether body temperature triggers the activation of microglial following MA administration, Thomas and colleagues kept the mouse core body temperature at either 38-40^{o}C for 6 hrs or 10-12°C for 8 hrs by changing ambient temperature prior to assessment of the effect of MA on microglial cells. Their findings suggested that neither high nor low

temperature can elicit microglial activation [57]. Administration of MA at reduced ambient temperature is known to reduce both MA-induced hyperthermia and neurotoxicity [57]. Further evidence on the impact of hypothermia on MA-induced microglial activation revealed that the number of activated microglial cell is significantly reduced when MA was administrated at 5^{o}C compared with room temperature [36]. However, the microglial response was still clearly above control levels [36]. These findings suggest that high or low body temperature per se does not cause activation of microglial cells, but low temperature is capable of attenuating MA-induced microglial response.

7.1.3. Role of microglial activation in MA-induced neurotoxicity

Activated microglia can release various cytokines, reactive oxygen species and reactive nitrogen species, which are suspected to contribute to MA-induced neurotoxicity. However, due to lack of method which can purely block microglial activation without other apparent effects, the role of microglial activation in MA-induced neurotoxicity remains unclear. LaVoie *et al.* compared the temporal changes of tyrosine-hydroxylase immunoreactivity (a stable marker for dopaminergic terminal) and microglial response in the striatum, and found that a robust microglial response temporally prior to the reduction of tyrosine hydroxylase [36]. These results indicate that rather than a secondary consequence of dopamine system damage, the microglial activation may be a cause of neuropathology [36]. In addition, the effect of MA on microglia was related to dose and showed an inverse relationship with striatal dopamine levels. The higher MA dose resulted in a stronger microglial response but lower striatal dopamine concentration [57]. This finding also supports the link between microglial activation and MA-induced neurotoxicity. The excitatory neuro-transmitter, glutamate has long been well accepted as a mediator of MA-related neurotoxicity by stimulating NMDA receptors. NMDA receptor blocker, MK-801 and dextromethorphan, were found to significantly attenuate MA induced microglial activation [55]. Since NMDA receptor blockers have neuroprotective effects against MA-induced neurotoxicity, the data may also indicate a causal link between microglial response and MA-induced neurotoxicity. However, the pharmacological effects of MK-801 and dextro-methorphan are very complex. For example, they are both non-competitive NMDA receptor antagonists. Thus their neuroprotective effects may result from the blockage of NMDA receptors rather than the inhibition of microglial activation. However, another chemical minocycline which was found to attenuate MA-mediated microglial activation failed to afford neuroprotection. Minocycline is a derivative from tetracycline and is capable of inhibiting inflammation and free radical formation. The lack of neuroprotection was shown to be due to the inability to abolish the induction of TNF-α signaling. These results oppose the proposal that microglial activation mediates MA-induced neurotoxicity, and instead emphasize the importance of inflammatory mediator TNF-α [50].

7.2. Expression of inflammatory mediators associated with MA abuse

A number of inflammatory mediators have been shown to play a pivotal role in the MA-induced neurotoxic outcomes. These inflammatory mediators include cyclooxygenase (COX), TNF-α, interleukin 1, and interleukin 6.

7.2.1. COX expression following acute toxic treatment of MA

COX is the rate-limiting enzyme in biosynthesis of prostaglandins which are a group of important inflammatory mediators and exert diverse roles in the inflammatory response. Two distinct isoforms of COX have been identified. COX-1 is constitutively expressed throughout the brain. COX-2, initially characterized as an inducible enzyme that is expressed in response to pathological stimuli, cytokines and mitogens, is now known to be also present in the normal CNS and kidney [27, 62]. Constitutive COX-2 in the brain has been associated with neurodevelopment and fundamental brain functions such as synaptic activity and memory consolidation [30, 62]. COX-2 induction is associated with neuro-degeneration, and there is an increased interest in using nonsteroidal anti-inflammatory drugs

(NSAIDs) which inhibit COX as potent therapeutic agents to slow the progression of neurodegeneration [15, 19, 21, 52]. However, owing to their complex function, COX and prostaglandins exert both pathologic and physiologic conditions in CNS, the role of COX and NSAIDs in neurodegenerative diseases is still highly controversial despite the intense research over the last decade. A number of studies also investigated possible implication of COX in dopamine terminal degeneration caused by MA intoxication [2, 33, 54].

Two independent research groups have assessed the effects of MA on COX expression in mouse model. Both studies showed a significant increase in COX-2 expression following a neurotoxic regimen of MA. However these two displayed discrepancy with regards to the time course of COX-2 induction. In the first study [32], a significant upregulation of striatal COX-2 was not observed until 72 hrs following MA administration. In the second study, COX-2 expression was found to be overtly elevated as early as 3 hrs after the last MA injection, and continued to rise up to 48 hrs with a peak elevation at 24 hrs following treatment [54]. Up-to-now, only one report examined the effect of MA on prostaglandin levels in mice. The results indicate that a neurotoxic regimen of MA failed to change striatal prostaglandin E_2 content. Besides mouse model, rats also have been used to study MA-induced neuroinflammatory response. Similarly, Zhang and colleagues [68] detected a significant upregulation of COX-2 protein in rat striatum 72 hrs following MA treatment (5 mg/kg, i.p.,). Interestingly, this study revealed a significant decrease of both COX-2 protein expression and number of COX-2 positive cells in rat striatum at 24 h after MA treatment. Meanwhile, the prostaglandin E_2 levels, IκB phosphorylation and translocation of NFκB, a key regulator of COX-2 expression, were measured in this study and the data supported their findings. Moreover, a regional difference in COX-2 induction has also been observed. Increase of COX-2 is significant in the striatum but not obvious in the hippocampus or cerebral cortex [32]. Most of the existing studies reported that MA administration has little effect on the expression of COX-1.

7.2.2. Effects of COX in MA-induced neurotoxicity

Both nonsteroidal anti-inflammatory drugs (NSAIDs) and knockout models have been used to explore the effect of COX in MA-induced neurotoxicity. COX-1 knockout mice showed similar striatal dopamine reduction as compared to the wild-type mice, whereas COX-2 knockout mice had significantly greater dopamine levels than the wild-type [54]. These results suggest COX-2 but not COX-1 may mediate MA-induced neurotoxicity.

Upon a revisit of the effect of NSAIDs on MA-induced neurotoxicity, eight different NSAIDs have been tested. These data are summarized in Table 1. In general, the result is somewhat surprising when compared with the larger role of the COX in mediating other forms of neuronal injury or degeneration. Most of the NSAIDs that have been studied, no matter it is a COX-1 selective, COX-2 selective, COX-3 selective or non-selective inhibitor, did not show protective effect against MA-induced neurotoxicity. Therefore, the current data does not support COX as a major mediator in MA caused neurotoxic effects. The beneficial effect of Ketoprofen, Indomethacin or Ibuprofen, if it exists, may depend on their other effects rather than the inhibition of COX enzymes. For instance, the protective effects of Ibuprofen against MA-induced neurotoxicity have been suggested to be based on its anti-peroxisome proliferator-activated receptor γ agonistic properties (PPAR γ; [61]. In addition, the disagreement in outcome between COX-2 knockout mice and COX-2 selective inhibitor with regards to MA-induced neurotoxicity could be attributed to the different inhibition of peroxidase activity of COX-2 [54].

7.2.3. TNF-α and MA-induced neurotoxicity

Although acute MA treatment at a dose of 2 mg/kg (2 mg/kg x 4, s.c., at 2 hrs interval) did not change TNF-α mRNA expression, significant elevated mRNA expression of TNF-α and its receptor TNFR was observed when animals were treated with either 20 mg/kg (acute) or chronically with 2 mg/kg for 5 days . Furthermore, pretreatment with exogenous TNF-α (4 μg, i.p.) was found to abolish MA (4 mg/kg, s.c.; four injections at 2 h interval)-induced DA depletion in frontal cortex

Table 1. Effects of NSAIDs in MA-induced Neurotoxicity.

NSAIDs treatment	Category of NSAIDs	Treatment regimen	Animal species	Effects of NSAIDs
[1]Aspirin	COX1/2 none selective	100 mg/kg x 4; s.c. 30 min prior to each MA injection.	mouse	No effect on MA-induced reduction of striatal dopamine transporter immunereactivity
[2]Aspirin	COX1/2 none selective	40 or 100 mg/kg x 4; i.p. 30 min prior to each MA injection.	mouse	No attenuating effects on effect on MA-inducted striatal DAT levels and microglial activation
[5] Ibuprofen	COX1/2 none selective	20 mg/kg x 4; s.c. 30 min prior to each MA injection.	mouse	Prevented reduction of PPARγ and attenuated MA-induced reduction of striatal DAT
[1]Indomethacin	COX1/2 none selective	5 mg/kg x 4; s.c 30 min prior to each MA injection.	mouse	Attenuate DAT signal and microglial activation
[6]Indomethacine	COX1/2 none selective	10 mg/kg; i.p. 30 min prior to a dose of 30 mg/kg MA	mouse	Prevented MA-induced gila activation in hippocampus; Has no effects on MA caused downregulation of syntaxin, synaptophysin and calbindin D28K protein in hippocampus.
[2]Ketoprofen 2 or 5 mg/kg x 4	COX1/2 none selective	2 or 5 mg/kg x 4; i.p. 30 min prior to each MA injection.	mouse	Dose dependently attenuate the reduction of striatal DAT levels and microglial activation induced by MA
[3]Ketoprofen 10 mg.kg	COX1/2 none selective	10 mg/kg; i.p. 1 h before the first MA injection and 1 h after the last MA injection.	mouse	No effect on MA-induced striatal DA depletion
[3]SC-560	COX1 selective	10 mg/kg; i.p. 1 h before the first MA injection and 1 h after the last MA injection.	mouse	No effect on MA-induced striatal DA depletion
[4]Celecoxib 7.5 mg/kg x 4	COX2 selective	7.5 mg/kg x 4; i.p. 10 min after each dose of MA	rat	No effect on MA-induced apoptosis or proliferation of glial cells. Exacerbated MA-induced striatal DA depletion.
[3]NS398 10 mg/kg	COX2 selective	10mg/kg; i.p. at 1 h before the first MA injection, 1 h after the last MA injection, and then twice daily for 2 days.	mouse	No effect on MA-induced striatal DA depletion

Table 1 continued..

[3]Rofecoxib	COX2 selective	25 mg/kg; gavage. Once per day for 5 days preceding MA treatment and 2 days after MA.	mouse	No effect on MA-induced striatal DA depletion
[3]Antipyrine	COX3 selective	100 mg/kg; i.p. 30 min prior to MA injection.	mouse	No effect on MA-induced striatal DA depletion

Note: 1: Asnuma *et al.*, 2004; 2: Asanuma *et al.*, 2003; 3: Thomas and Kuhn 2005; 4: Zhang *et al.*, 2007; 5: Tsuji *et al.*, 2010; 6: Goncalves *et al.*, 2009.
MA treatment regimen: Report 1 and 2: 4 mg/kg x 4, i.p. with 2 hrs interval; report 3 and 4: 5 mg/kg x 4, i.p. with 2 hrs interval. DAT: Dopamine transporter; DA: Dopamine.
s.c.: Subcutaneous injection. i.p.: Intraperitoneal injection.

and striatum [40]. In addition, TNF-α knock-out mice [TNF-α(-/-)] exhibited enhanced neurotoxicity when exposed to MA. These results suggest a neuroprotective role of TNF-α.

CONCLUSION

As summarized in Fig. 1, a complex pathway network composed of dopamine oxidation, mitochondria dysfunction, neuroinflammatory response and excitotoxicity underlies MA-induced neurotoxicity. These pathways are intimately connected and oxidative stress functions as a central link in the network. It should be noted that most of the current knowledge about mechanisms underlying MA-induced neurotoxicity are originated from animal models especially mice and rats. The MA animal models greatly facilitate our understanding of the molecular and cellular aspects of MA intoxication. However, there is apparent difference between human being and rodents at least in MA metabolism. The mechanism in human MA-intoxication may differ from that in animal models. In addition, the frequent combination of MA abuse with other exposures, such as alcohol and HIV infection add more complexity and severity to MA-induced toxicity.

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