Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans

Rafael de la Torre¹,² and Magí Farré¹,³

¹Institut Municipal d’Investigació Mèdica, Pharmacology Unit (IMIM), Dr. Aiguader 80, 08003 Barcelona, Spain
²Universitat Pompeu Fabra, Dr. Aiguader 80, 08003 Barcelona, Spain
³Universitat Autònoma de Barcelona, Dr. Aiguader 80, 08003 Barcelona, Spain

Several studies suggest that MDMA-induced acute toxicity and long-term neurotoxicity is dependent on the metabolic disposition of MDMA. Differences in MDMA metabolism among animal species might therefore account for different sensitivities to its neurotoxic effects. The kinetic parameters of enzymes that regulate the formation of neurotoxic metabolites of MDMA differ among species, as does the ability of MDMA to self-inhibit these enzymes and the degree of genetic polymorphisms exhibited by these enzymes. Such features limit allometric scaling across animal models.

The neurotoxic effects of 3,4-methylenedioxymethamphetamine [MDMA (also known as ecstasy)] are well documented in animal models [1]. By applying allometric scaling, researchers claim that results obtained in animal studies using doses that are close to those used recreationally by humans have direct clinical application [2]. On this basis, some clinical pharmacology studies in which MDMA has been administered have been criticized on ethical grounds [3]. The extrapolation of results from animal models to humans implies two assumptions: (i) the toxic effects of MDMA among species are similar; and (ii) the metabolic disposition of MDMA among species is comparable. In this article, we discuss why these assumptions cannot be applied and also describe the limitations to extrapolating from animals to humans.

MDMA metabolism

MDMA is cleared mainly by hepatic metabolism (Figure 1) [4]. MDMA is N-demethylated to form 3,4-methylenedioxymethamphetamine (MDA) and O-demethylelated to form 3,4-dihydroxymethamphetamine (HHMA). HHMA is highly unstable and conjugates with sulfate and glucuronic acid [5]. HHMA can also be oxidized rapidly to its corresponding quinone and form adducts with glutathione and other thiol-containing compounds [6]. HHMA is further O-methylated to form 4-hydroxy-3-methoxy-methamphetamine (HMMA). Following administration of MDMA to mice, MDMA is the main chemical species observed in both plasma and brain. By contrast, although MDMA is observed in rats and humans following its administration, HHMA and HMMA, which are produced at different rates in rats and humans, are also present in high concentrations in plasma. In fact, MDMA metabolism, except in mice, is qualitatively similar for its major metabolic reactions in most animal species and humans; nevertheless, there are relevant quantitative differences. In rats, the N-demethylation of MDMA leading to the formation of MDA is one of the main metabolic pathways ([MDA]/[MDMA] = 0.5) at low doses [7], whereas in humans the O-demethylation of MDMA to HHMA predominates at any dose tested [4]. In rats, the rate of N-demethylation is dose dependent and at doses higher than 10 mg kg⁻¹ there is saturation of hepatic clearance [7] (Figure 2). In monkeys given 10 mg kg⁻¹ of MDMA twice daily for four consecutive days, MDMA plasma concentrations increased by 30% and MDA plasma concentrations increased by 200%, compared with levels present following the first dose of MDMA; MDA concentrations were as high as 18% of the concentration of MDMA [8]. By contrast, MDA plasma concentrations are usually <5% of the concentration of MDMA in humans following administration of MDMA [4]. It is postulated that in mice, in which MDMA predominates in tissues following its administration, MDMA-induced neurotoxicity is mainly dopamine mediated: MDMA causes the release of dopamine, which when oxidized leads to the generation of reactive oxygen species (ROS), which in turn cause toxicity. In other animal species, including humans, it is postulated that hepatic metabolism is a key factor in the production of MDMA-induced neurotoxicity to 5-HT-containing neurons.

Which MDMA metabolite is active in the brain?

MDMA-induced neurotoxicity results from formation of ROS. Although in mice ROS formation might result from a direct action of MDMA on dopamine-containing neurons, in other animal species the metabolites of MDMA also contribute to ROS formation. In animal species other than mice, the neurotoxicants MDMA and its active metabolite MDA, which are neurotoxic to 5-HT-containing neurons when given systemically, are not neurotoxic when injected directly into the brain at concentrations that result following systemic administration [9]. This suggests that previous
metabolic activation is needed for the development of neurotoxicity [7]. The administration of the MDMA metabolites HHMA and HMA into the brain also fails to reproduce neurotoxicity in rats [10]. These observations prompted an investigation into the pharmacological–toxicological profile of minor MDMA-hydroxylated metabolites found in rats [10]. However, this study was unable to reproduce the typical neurodegeneration of 5-HT-containing neurons induced by MDMA. Thioether adducts with quinones that are formed from the autooxidation of the MDMA catechol-type metabolites HHMA and 3,4-dihydroxymethamphetamine (HHA) [6,11] have been postulated as the most likely chemical species to be involved in MDMA-induced neurotoxicity. When injected into the striatum or the cortex of rats they can reduce the concentration of 5-HT in the brain and cause long-term depletion of 5-HT in the CNS. Thioether adducts can easily enter into redox cycling and generate ROS, which underlies MDMA-induced neurotoxicity. It is postulated that neurotoxic metabolites are formed in the liver and cross the blood–brain barrier using glutathione specific transporters [12]. Their formation in vivo in rats has been reported recently [13]. Thus, if thioether conjugates are accepted as the most likely neurotoxic species, a proper understanding of the MDMA O-demethylation reaction that gives rise to their metabolic precursors is required.

**MDMA O-demethylation and CYP2D6 inactivation**

Most amphetamine-related substances interact with the polymorphic enzyme cytochrome P450 (CYP) involved in the N-demethylation and O-demethylation metabolic reactions in rats are highlighted in blue whereas those corresponding to enzymes in humans are shown in red [26,27]. The parent compound is N-demethylated to form 3,4-methylenedioxymethamphetamine (MDA) and O-demethylated to form 3,4-dihydroxymethamphetamine (HHMA). HHMA is further O-methylated to 4-hydroxy-3-methoxyamphetamine (HMAA). In rats, N-demethylation to MDA is one of the main metabolic pathways, whereas in humans O-demethylation to HHMA predominates. 3,4-Dihydroxymethamphetamine (HMA) and HHMA are the precursors of neurotoxic species. In mice, MDMA-induced neurotoxicity is mainly dopamine mediated because MDMA causes the release of dopamine, which leads to the generation of reactive oxygen species as a result of dopamine oxidation. In other animal species, including humans, hepatic metabolism is a key factor involved in the production of MDMA toxicity to 5-HT-containing neurons. Abbreviations: COMT, catechol-O-methyl transferase; HMA, 3-methoxy,4-hydroxyamphetamine; MAO, monoamine oxidase; SULT, sulfotransferase; UDPGT, glucuronosyltransferase.
The enzyme in humans has lower V<sub>max</sub> (CYP2D1/CYP2D6), there are no major differences among animal species functionally identical [18]. Interestingly, although homologous to human CYP2D6, the enzymes are not of MDMA is CYP2D1. Although this enzyme is which are involved in the generation and inactivation of

In rats, the enzyme involved in O-demethylation of MDMA is CYP2D1. Although this enzyme is homologous to human CYP2D6, the enzymes are not functionally identical [18]. Interestingly, although there are no major differences among animal species in the affinity of substrate probes for this enzyme (CYP2D1/CYP2D6), there is a tenfold difference in its V<sub>max</sub> [19]. The enzyme in humans has lower V<sub>max</sub> values than the enzyme in rats and monkeys. Although in humans a single dose of MDMA can fully inhibit CYP2D6, in non-human primates several doses of MDMA are required for full inhibition of the enzyme [8]. Thus, differences in both enzyme kinetics and the inhibition of CYP2D6 by MDMA through the formation of an enzyme–metabolite complex suggest that different rates of formation of metabolic neurotoxic species and different susceptibilities to the development of neurotoxicity exist among species. MDMA-mediated inhibition of CYP2D6 might confer to humans a certain degree of protection against the development of neurotoxicity. However, this hypothesis should be balanced with pharmacogenetic factors related to CYP2D6 and other enzymes involved in the disposition of MDMA metabolites.

**Pharmacogenetics and the disposition of MDMA metabolites**

CYP2D6 and catechol-O-methyl transferase (COMT), which are involved in the generation and inactivation of HHMA and HHA (precursors of neurotoxic species), respectively, are highly polymorphic in humans. Thus, the link between genetic polymorphisms and the development of neurotoxicity should be examined experimentally. It is postulated that the poor-metabolizer phenotype for CYP2D6 might confer a higher susceptibility to acute toxicity induced by MDMA but lower susceptibility to neurotoxicity induced by MDMA. By contrast, subjects who have multiple functional copies of the gene encoding CYP2D6 might exhibit a higher risk of neurotoxicity. Because animals do not display such genetic polymorphisms, such potential differences in susceptibility cannot be examined in animal models. Lack of such genetic polymorphisms is another reason why extrapolations of data from studies of animals to humans should be considered with caution [20]. In CYP2D-deficient female Dark Agouti rats, the animal model that resembles most closely the poor-metabolizer CYP2D6 phenotype in humans, a higher sensitivity to acute hyperthermia [21], but lower neurotoxic effects in terms of 5-HT depletion when compared with other rat strains [7], were observed following administration of MDMA. Thus, the use of transgenic animals that express human metabolic enzymes should be considered for future studies [22].

This approach will not only reproduce the enzymatic machinery responsible for MDMA bioactivation in humans, but might also reproduce extreme phenotypes when combined with selective enzyme inhibitors. Therefore, different susceptibilities to the development of neurotoxicity, depending on the different genotypes that might occur in humans (which are usually overlooked), might be able to be predicted in suitable animal models.

**MDMA dosing**

MDMA-induced neurotoxicity in animal models is dependent on the doses used and the dose regimen employed. High and frequent doses result in neurotoxicity, which occurs when endogenous free-radical-scavenging mechanisms become overwhelmed or exhausted [23].

There are reports that a single dose of MDMA can induce neurotoxicity in animal models. However, protocols that involve the administration of several doses during a short period of time [i.e. ‘binge-dosing’ (e.g. 4-day dosing regimen)] are preferred because more-pronounced effects are observed. In rhesus monkeys, using an equivalent overall drug exposure, the neurotoxic effects that are observed following ‘binge-dosing’ protocols are not observed by lengthening the intervals between doses of MDMA administered [24]. This suggests that pharmacokinetic factors and the achievement of a threshold concentration of toxic metabolites might contribute to the development of neurotoxicity. Epidemiological studies report that usually between two and three tablets are taken by humans during a single session but ~25% of users take four tablets or more. Approximately 70–80% of MDMA users are exposed to the drug less than once a week; however, ~10% of MDMA users consume the drug several times a week and are at higher risk for
neurotoxicity [25]. The magnitude and severity of the health problems posed by MDMA is dependent on whether these risky behaviour patterns will increase.

Longitudinal studies that evaluate MDMA-induced neurotoxicity in humans have limitations because of a poor estimation of both the exposure to the drug and the dose of MDMA per tablet. A further factor to consider is that MDMA users are polydrug users of ‘dance drugs’, which further compounds the ability to evaluate MDMA-induced neurotoxicity.

Concluding remarks

MDMA-induced acute toxicity and neurotoxicity is dependent on its metabolic disposition, which in turn depends on non-linear kinetics in the main metabolic pathway, the regulation of metabolic pathways by enzymes that are highly polymorphic in humans, and the formation of neurotoxic metabolic species. These factors are not present consistently among animal species and thus limit allometric scaling across animal models. Toxokinetically animal studies should be encouraged to accurately interpret toxicity results.

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