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ORGAN TOXICITY AND MECHANISMS

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Role of metabolites in MDMA (ecstasy)-induced nephrotoxicity: an in vitro study using rat and human renal proximal tubular cells

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Abstract The metabolism of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) has recently been implicated in the mechanisms underlying ecstasy-induced neurotoxicity and hepatotoxicity. However, its potential role in ecstasy-induced kidney toxicity has yet to be investigated. Thus, primary cultures of rat and human renal proximal tubular cells (PTCs) were used to investigate the cytotoxicity induced by MDMA and its metabolites methylenedioxyamphetamine (MDA), α-methyldopamine (α-MeDA), and the glutathione (GSH) conjugates 5-(glutathion-*S*-yl)-α-MeDA and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA. Cell viability was evaluated using the mitochondrial MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. MDMA and MDA were not found to be toxic to either rat or human PTCs at any concentration tested (100–800 μM). In contrast, 800 μM α-MeDA caused 60% and 40% cell death in rat and human PTCs, respectively. Conjugation of α-MeDA with GSH resulted in the formation of even more potent nephrotoxicants. Thus, exposure of rat and human PTC monolayers to 400 μM 5-(glutathion-*S*-yl)-α-MeDA caused approximately 80% and 70% cell death, respectively. 5-(Glutathion-*S*-yl)-α-MeDA (400 μM) was more toxic than 2,5-*bis*(glutathion-*S*-yl)-α-MeDA to rat renal PTCs but equally potent in human renal PTCs. Pre-incubation of rat PTCs with either acivicin, an inhibitor of γ-glutamyl transpeptidase (γ-GT), or bestatin, an inhibitor of aminopeptidase M, resulted in increased toxicity of 5-(glutathion-*S*-yl)-α-MeDA but had no effect on 2,5-*bis*(glutathion-*S*-yl)-α-MeDA-mediated cytotoxicity. The present data provide evidence that metabolism is required for the expression of MDMA-induced renal toxicity in vitro. In addition, metabolism of 5-(glutathion-*S*-yl)-α-MeDA by γ-GT and aminopeptidase M to the corresponding cystein-*S*-yl-glycine and/or cystein-*S*-yl conjugates is likely to be associated with detoxication of this compound. Thus, it appears that toxicity induced by thioether metabolites of ecstasy at the apical membrane of renal proximal tubular cells is the result of extracellular events, presumably redox cycling.

Keywords MDMA · Metabolites, Glutathione conjugate · Rat · Human · Renal proximal tubular cells

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA, or ecstasy) is a ring-substituted amphetamine derivative that rapidly achieved popularity as a recreational drug of abuse mainly among young people. Several cases of acute or chronic renal failure secondary to ecstasy consumption have recently been reported (Fahal et al. 1992; Henry et al. 1992; Woodrow et al. 1995; Bingham et al. 1998; Walubo and Seger 1999). However, despite these reports, the mechanisms of toxicity remain to be elucidated.

Several factors may contribute to the adverse renal effects of ecstasy, including disseminated intravascular coagulation (DIC), vasoconstriction, rhabdomyolysis, hyperthermia and metabolism. DIC and rhabdomyolysis

are frequent clinical consequences of ecstasy abuse (and of amphetamines in general), resulting in varying degrees of microvascular obstruction due to fibrin-platelet or myoglobin deposition, respectively (Fahal et al. 1992; Fineschi and Masti 1996; Cunningham 1997). This process may result in sudden renal ischaemia and acute tubular necrosis. Hyperthermia, a pro-oxidant aggressive condition, may also play a part (Dar and McBrien 1996). In addition, MDMA metabolism results in the formation of redox-active metabolites, which have recently been implicated in the mechanisms underlying ecstasy-induced neurotoxicity (Miller et al. 1995, 1997; Bai et al. 1999, 2001) and hepatotoxicity (Carvalho et al. 2001).

In the present work, the role of these putative metabolites in ecstasy-induced nephrotoxicity was investigated. MDMA is demethylenated to *N*-methyl-α-methyldopamine and *N*-demethylated to α-methyldopamine (α-MeDA) by cytochromes P450 2D, 2B and 3A (Kumagai et al. 1994; Kreth et al. 2000) (Fig. 1). These reactive metabolites are readily oxidized to the corresponding *o*-quinones. Quinones are highly redox-active molecules which can enter redox cycles with their semiquinone radicals, leading to formation of reactive oxygen species (ROS), including the superoxide anion, hydrogen peroxide, and ultimately the hydroxyl radical (Bolton et al. 2000). Alternatively, the reactive *o*-quinone intermediates are Michael acceptors, and cellular damage can occur through alkylation of crucial cellular proteins and/or DNA. In the presence of glutathione (GSH), the oxidation of α-MeDA to the corresponding *o*-quinone is followed by conjugation with GSH to form 5-(glutathion-*S*-yl)-α-MeDA (Hiramatsu et al. 1990). The GSH-conjugate remains redox active and is readily oxidized to the quinone-GSH conjugate followed by the addition of a second molecule of GSH to yield 2,5-*bis*(glutathion-*S*-yl)-α-MeDA (Miller et al. 1997).

The conjugation of GSH with electrophilic compounds usually results in their detoxication and subsequent elimination as mercapturic acids. Recently, however, several examples have been reported where conjugation of quinones with GSH fails to eliminate their biological or toxicological reactivity (for detailed reviews see Monks and Lau 1994, 1997; van Bladeren 2000; Dekant 2001), and evidence is accumulating that GSH conjugates of a variety of polyphenols are nephrotoxicants in animal models (Monks et al. 1985, 1988; Lau et al. 1988; Monks and Lau 1990; Fowler et al. 1991; Lau 1995). We report for the first time the differential toxicity of ecstasy and its metabolites methylenedioxyamphetamine (MDA), α-MeDA, and the GSH conjugates 5-(glutathion-*S*-yl)-α-MeDA and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA in primary cultures of rat and human renal proximal tubular cells (PTCs). Furthermore, the contribution of different metabolic pathways were evaluated using selective enzyme inhibitors, such as acivicin for γ-glutamyl transpeptidase (γ-GT) and bestatin for aminopeptidase M.

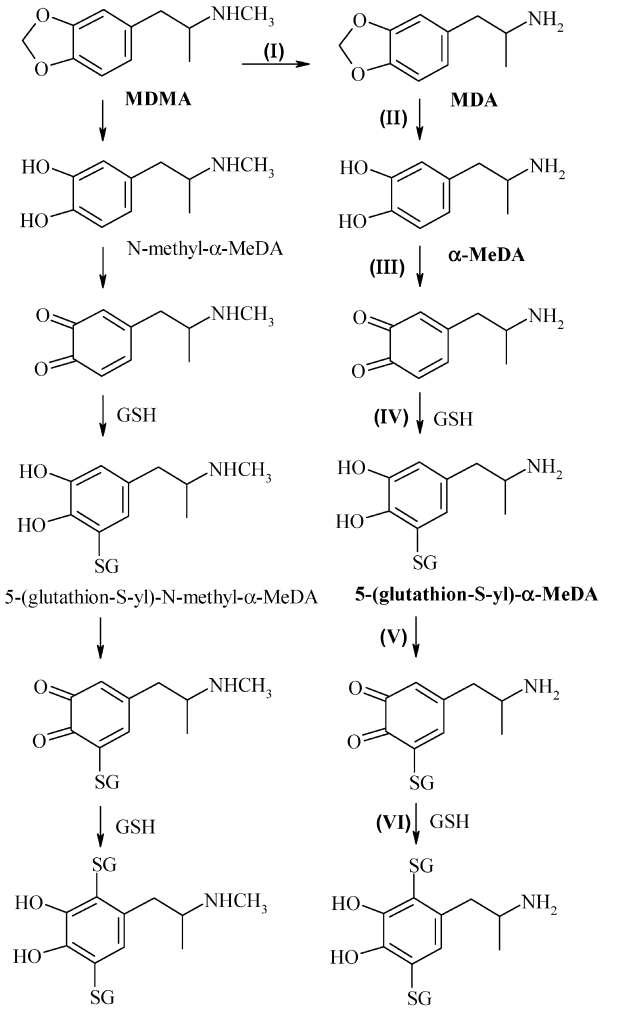


Fig. 1. Proposed pathway for 3,4-methylenedioxymethamphetamine (MDMA) metabolism to nephrotoxic metabolites. MDMA can undergo *N*-demethylation to methylenedioxyamphetamine (MDA) (*I*). Cytochrome P450-mediates demethylenation of MDMA and MDA to *N*-methyl-α-methyldopamine and α-methyldopamine (α-MeDA), respectively (*II*). The catechols are readily oxidized to the corresponding *o*-quinones (*III*), which may react readily with glutathione (GSH) to form the corresponding GSH conjugates (*IV*). Quinol-linked GSH conjugates are also susceptible to oxidation to the quinone-thioethers (*V*), which undergo a second GSH addition to form the *bis*-GSH substituted catechol (*VI*)

Materials and methods

Chemicals

Collagenase (clostridiopeptidase A; EC 3.4.24.3), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], glutathione (GSH), acivicin and bestatin were purchased from Sigma Chemical Company (St. Louis, Mo., USA). All other reagents were of the highest grade commercially available. MDMA (HCl salt), MDA (HCl salt) and α-MeDA (HBr salt) were synthesized in the Organic Chemistry Department, Porto University, Portugal. 5-(Glutathion-*S*-yl)-α-MeDA and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA were synthesized in the Center for Molecular and Cellular Toxicology, College of Pharmacy, University of Texas, Austin, USA.

Biological material

Rat renal tissue

Male Wistar rats (200–250 g) were used for all experiments, and were housed in the Biological Services Unit, Foresterhill, University of Aberdeen, UK. The animals had free access to a commercial diet (CRM, Special Diet Services, Witham, UK) and tap water, and were kept on a 12-h/12-h day/night cycle. Animals were killed by cervical dislocation and the kidneys were excised and placed on ice.

Human renal tissue

Human kidney samples were obtained from surgical nephrectomies at Aberdeen Royal Infirmary (UK). Kidneys were transferred on ice to the Pathology Department and tissue samples were collected from areas defined as normal by a consultant pathologist. Renal cell isolation took place immediately after sample collection.

Cell isolation and culture

Rat and human proximal tubular cells were isolated following the procedure of Hawksworth (Rodilla and Hawksworth 1996), consisting essentially of a collagenase enzymatic digestion of renal cortex tissue followed by filtration and isopycnic Percoll density centrifugation. The cell yield was $20–30\times10^6$ cells/rat and $10–15\times10^6$ cells/g of human cortical tissue. Viability was routinely higher than 85%. PTCs (8×10^4 cells/cm^2) were cultured on 96-well microtitre plates at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was Dulbecco's modified Eagle's medium nutrient mixture F12 Ham (Sigma) supplemented with 10% fetal bovine serum, penicillin-streptomycin (50 U/ 50 μg/ml), ofloxacin (5 μg/ml) and amphotericin B (1.5 μg/ml) (all from Gibco BRL, Paisley, UK). Culture medium was changed every 24 h.

Cytotoxicity assay

As judged by light microscopy, rat and human PTC monolayers were confluent 4 days after plating. At that time, monolayers were incubated for 24 h with 100, 200, 400, or 800 μM MDMA, MDA, α-MeDA, 5-(glutathion-*S*-yl)-α-MeDA or 2,5-*bis*(glutathion-*S*-yl)-α-MeDA. When indicated, monolayers were pre-treated with 250 μM acivicin or 10 μM bestatin for 20 min. Cell viability was measured using the mitochondrial MTT assay. MTT is a yellow water-soluble dye that is reduced in viable cells to an insoluble purple coloured product, MTT-formazan (3-[4,5-dimethylthiazol-2-yl]-3,5-diphenylformazan) by the mitochondrial enzyme succinate dehydrogenase. After exposure to the compounds, the medium was removed and the cells were washed twice with fresh culture medium. Subsequently the cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 4 h with 100 μl MTT (0.5 mg/ml). MTT solution was then removed from the cells and intracellular MTT-formazan was solubilized in 100 μl dimethyl sulfoxide and the absorbance measured at 540 nm in a microplate reader.

Statistical analysis

Data are expressed as mean ± SEM of measurements from the indicated number of separate cell isolations. Statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Scheffé's test. Differences were considered significant at $P < 0.05$.

Results

Apical exposure of rat (Fig. 2) or human (Fig. 3) PTC monolayers to MDMA or MDA for 24 hours had no adverse effects at any concentration tested (100 to 800 μM) as determined by changes in mitochondrial function. In contrast, exposure of rat and human renal PTCs to α-MeDA at the highest concentration tested (800 μM) induced a significant decrease in cell viability, measured by decreases in mitochondrial function (Figs. 2 and 3), causing 60% and 40% cell death in rat and human PTCs respectively.

GSH conjugates of α-MeDA were clearly more potent nephrotoxicants than the parent catechol. Thus, exposure of rat and human PTC monolayers to 400 μM 5-(glutathion-*S*-yl)-α-MeDA caused approximately 80% and 70% cell death, respectively (Figs. 2 and 3). 5-(Glutathion-*S*-yl)-α-MeDA (400 μM) was more toxic than 2,5-*bis*(glutathion-*S*-yl)-α-MeDA to rat renal PTCs. 5-(Glutathion-*S*-yl)-α-MeDA (400 μM) caused 80% cell death, whereas 800 μM 2,5-*bis*(glutathion-*S*-yl)-α-MeDA was required to cause similar increases in this parameter (Fig. 2). In contrast, the two conjugates were equally potent in human renal PTCs (Fig. 3). Thus, human PTCs were more sensitive to 2,5-*bis*(glutathion-*S*-yl)-α-MeDA than rat PTCs.

To determine the role of γ-GT and aminopeptidase M in the cytotoxicity mediated by 5-(glutathion-*S*-yl)-α-MeDA and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA, rat

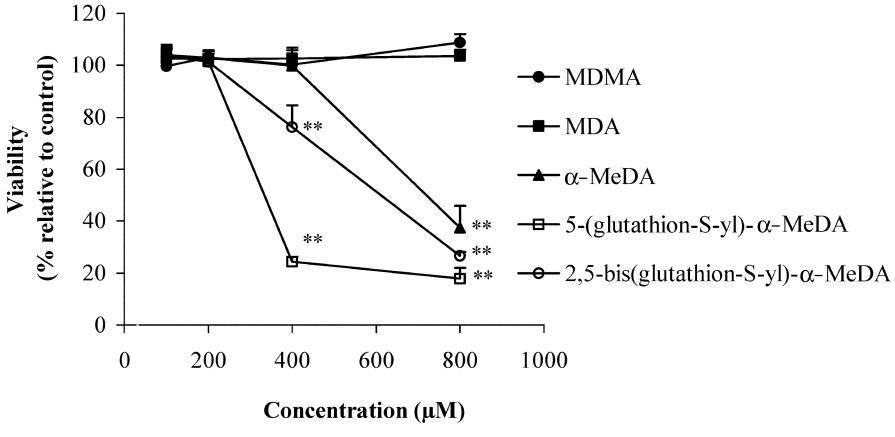


Fig. 2. Cellular viability determined by MTT assay 24 h after incubation of rat proximal tubular cell monolayers with 100, 200, 400 and 800 μM 3,4-methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA) and α-methyldopamine (α-MeDA) ($n = 7$ cultures, four replicates), 5-(glutathion-*S*-yl)-α-MeDA ($n = 4$ cultures, four replicates) and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA ($n = 3$ cultures, four replicates). Data represent means + SEM. $^{**}P < 0.01$, compared with control

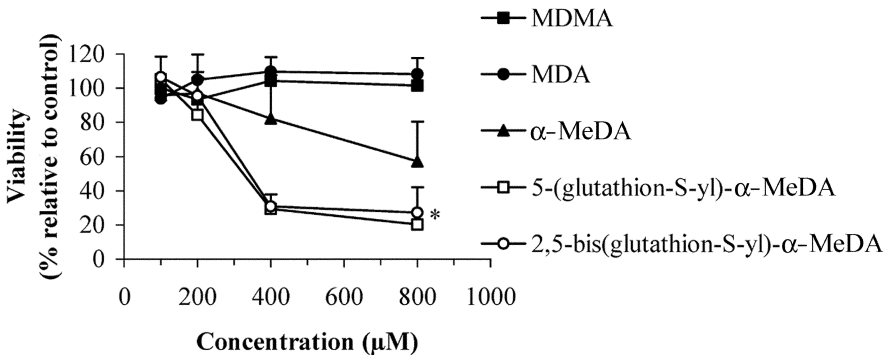


Fig. 3. Cellular viability measured by MTT assay 24 h after incubation of human proximal tubular cell monolayers with 100, 200, 400 and 800 μM 3,4-methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA) and α-methyldopamine (α-MeDA) ($n = 3$ cultures, four replicates), 5-(glutathion-*S*-yl)-α-MeDA ($n = 2$ cultures, four replicates) and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA ($n = 1$ culture, four replicates). Data represent means + SEM. *$P < 0.05$, compared with control

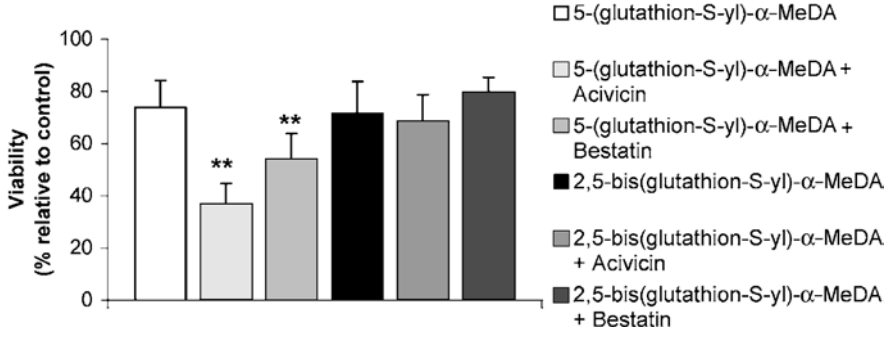
PTCs were pre-treated with acivicin, an inhibitor of γ-GT, or bestatin, an inhibitor of aminopeptidase M, prior to exposure of the monolayers to these conjugates. Because of the requirement for concentrations at which potentiation of 5-(glutathion-*S*-yl)-α-MeDA and 2,5-*bis*(glutathion-*S*-yl)-α-MeDA toxicity could be easily observed, we selected concentrations of 300 μM and 400 μM, respectively. Interestingly, inhibition of either γ-GT or aminopeptidase M potentiated 5-(glutathion-*S*-yl)-α-MeDA-mediated cytotoxicity but had no effect on 2,5-*bis*(glutathion-*S*-yl)-α-MeDA-mediated cytotoxicity. Although 5-(glutathion-*S*-yl)-α-MeDA (300 μM) or 2,5-*bis*(glutathion-*S*-yl)-α-MeDA (400 μM) decreased cell viability (Fig. 4) at these concentrations, the decreases were not statistically significant. However, following pretreatment with acivicin, the same concentrations of 5-(glutathion-*S*-yl)-α-MeDA significantly decreased cell viability by 65% compared with controls. Pretreatment with bestatin caused a more modest, but significant, decrease (approximately 45%) in cell viability. Acivicin or bestatin treatment alone had no statistically significant effect on cell viability.

Fig. 4. Effect of acivicin and bestatin on 5-(glutathion-*S*-yl)-α-methyldopamine- and 2,5-*bis*(glutathion-*S*-yl)-α-methyldopamine-induced toxicity. Rat proximal tubular cell monolayers were pre-treated for 20 min with 250 μM acivicin or 10 μM bestatin. After this period, monolayers were treated with 300 μM 5-(glutathion-*S*-yl)-α-MeDA ($n = 5$ cultures, four replicates) or 400 μM 2,5-*bis*(glutathion-*S*-yl)-α-MeDA ($n = 2$ cultures, four replicates). Viability was measured by MTT assay and data are expressed as means + SEM relative to controls. **$P < 0.01$, compared with control

Discussion

We have shown that neither MDMA nor MDA are directly toxic to either rat or human PTCs. In contrast, several known and putative metabolites of these amphetamines produced cytotoxicity in primary PTC cultures of both human and rat origin. These findings indicate that metabolism is a prerequisite for the renal toxicity of MDMA and MDA, at least in vitro. Mammalian kidneys are capable of metabolizing xenobiotics via a broad variety of pathways, including cytochrome P450-dependent oxidation, and conjugations with GSH, glucuronic acid and sulfate (Boogaard et al. 1990). However, whether metabolites of MDMA can be formed directly within kidney cells in sufficient quantities to produce toxicity is not known and requires further investigation. Activities of the cytochrome P450 isoenzymes decrease considerably in primary cultures of PTCs (Schaaf et al. 2001), the greatest loss in activity occurring within 24 h of culture. Such effects may contribute to the lack of toxicity of MDMA and MDA in rat and human PTCs. Alternatively, metabolites of MDMA and MDA formed in the liver may reach the kidney via the circulation.

MDMA and MDA are demethylenated to α-MeDA by cytochromes P450 2D, 2B and 3A isoenzymes (Kreth et al. 2000). The reactivity of this redox-active metabolite probably contributes to the observed decrease in rat



and human PTC viability. α-MeDA is a catechol, with the capacity to redox cycle with the concomitant generation of ROS, initially the superoxide anion, but subsequently hydrogen peroxide, and ultimately the hydroxyl radical (Bolton et al. 2000). Thus, superoxide anion radical ($O_2^{\cdot-}$) undergoes either spontaneous or enzyme-catalysed dismutation to form hydrogen peroxide. $O_2^{\cdot-}$ also reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), which is a powerful cytotoxic species (Halliwell et al. 1999). In addition, $O_2^{\cdot-}$ reduces Fe^{3+} to Fe^{2+}, and hydrogen peroxide then reacts with Fe^{2+} to generate the hydroxyl radical (see Fig. 5), which is probably the reactive species responsible for damage to essential macromolecules (Bolton et al. 2000). Furthermore, α-MeDA may undergo further oxidative coupling to give rise to dimeric and polymeric products. The presence of insoluble black pigments within PTC monolayers exposed to α-MeDA is indicative of such polymerization reactions that ultimately give rise to melanin-like pigments. Persistence of insoluble pigments may cause tubular obstruction and irritation, which could contribute to the chronic toxicity of ecstasy.

In contrast to the generally accepted role of GSH conjugation serving as a detoxication mechanism, it is now known that conjugation of GSH with electrophiles may result in preservation or enhancement of biological activity (Monks and Lau 1992). In particular, although quinones formed by oxidation of α-MeDA are readily conjugated with GSH to form 5-(glutathion-*S*-yl)-α-MeDA (Hiramatsu et al. 1990), the conjugate itself is readily oxidized to the quinone-thioether (Miller et al. 1997), followed by the reductive addition of a second molecule of GSH to form 2,5-*bis*(glutathion-*S*-yl)-α-MeDA. Thus, like other GSH conjugates of a variety of polyphenols that are potent nephrotoxicants (Lau 1995), the thioether metabolites of ecstasy retain the ability to redox cycle with the corresponding generation of ROS.

The first step in the metabolism of GSH conjugates involves either hydrolysis or transamination by γ-GT, a membrane-bound enzyme, which has its active site oriented on the outer surface of the cell membrane. The kidney possesses the highest levels of γ-GT activity and the outer surface of the microvillus membrane (brush border) in the proximal tubule is the main site of γ-GT activity (Horiuchi et al. 1978). The product of the γ-GT catalysed reaction is a S-substituted cysteinyl-glycine dipeptide, which, in turn, is subject to hydrolytic cleavage of the glycine residue. The latter reaction is catalysed by the membrane enzymes aminopeptidase M and dipeptidase. Aminopeptidase M is more abundant than the dipeptidase and rat kidney possesses the highest levels of aminopeptidase M activity (Commandeur et al. 1995). The transfer of an acetyl group to the S-substituted cysteine conjugate catalysed by cysteine-thioether *N*-acetyltransferase completes the detoxication function of mercapturic acid biosynthesis (Monks and Lau 1987). However, in contrast to the detoxication function of mercapturic acid synthesis, GSH S-conjugates biosynthesized from several quinones, hydroquinones and aminophenols are nephrotoxic in animal models (Lau 1995; Monks and Lau 1998). A major factor in the nephrotoxicity of these polyphenolic GSH-conjugates seems to be the ease with which their metabolites oxidize to the corresponding quinones. For example, the half-wave oxidation potentials of 2-Br-(*bis*-cystein-*S*-yl)hydroquinone and 2,3,5-(*tris*-cystein-*S*-yl)hydroquinone are lower than those of 2-Br-(*bis*-glutathion-*S*-yl)hydroquinone and 2,3,5-(*tris*-glutathion-*S*-yl)hydroquinone (Monks et al. 1994). In this manner, γ-GT and dipeptidases serve to deliver a more readily oxidizable, and thus more reactive, metabolite to PTCs.

However, the cytoxicity of 5-(glutathion-*S*-yl)-α-MeDA in rat PTCs is potentiated by inhibition of renal γ-GT and aminopeptidase M, indicating that metabolism of the conjugate to the corresponding cystein-*S*-yl-glycine and/or cystein-*S*-yl conjugate is a detoxication pathway. In addition, pre-incubation of rat PTCs with either acivicin or bestatin had no effect on

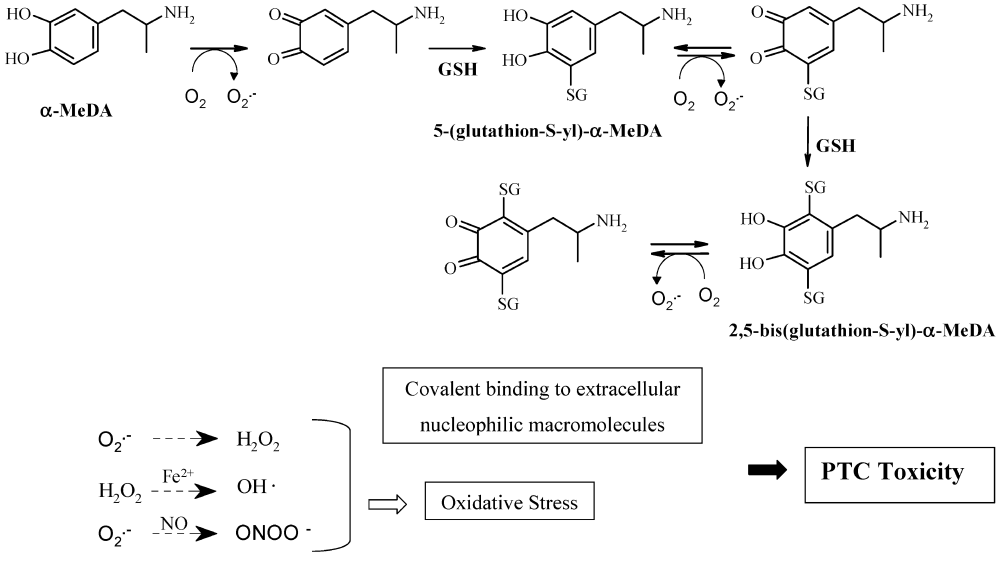


Fig. 5. Formation of reactive oxygen species by redox-cycling of glutathione (GSH) conjugates of quinones formed from α-methyldopamine (α-MeDA) (*PTC* renal proximal tubular cell)

2,5-*bis*(glutathion-*S*-yl)-α-MeDA-mediated cytotoxicity. Thus, it seems that increasing GSH substitution results in a decreased affinity for γ-GT of the GSH-conjugate. Since γ-GT-mediated cleavage of the GSH portion of quinones is a prerequisite for cellular uptake of the conjugates, the observed results are suggestive of an extracellular mechanism of toxicity. The mechanism of quinone-GSH conjugate-mediated toxicity is unclear, but based upon the known properties of quinones, is likely to be initiated either via the arylation of cellular macromolecules that are critical for cell structure and function, or via the generation of ROS during quinone redox cycling. Potentiation of toxicity by acivicin pretreatment has also been observed with GSH conjugates of chlorohydroquinones (Mertens et al. 1991), *p*-aminophenol (Anthony et al. 1993) and menadione (Haenen et al. 1994). These findings suggest that for a number of GSH-conjugates degradation by γ-GT may be a detoxication mechanism. To explain this effect, it was hypothesized that inhibition of γ-GT by acivicin prevents the formation of a benzothiazolyl-like compound via cyclization of the cysteinylglycine and/or cysteine conjugate (Monks 1995). γ-GT-mediated hydrolysis of 5-(glutathion-*S*-yl)-α-MeDA and subsequent aminopeptidase M-mediated glycine cleavage may thus lead to oxidative cyclization of the cysteine conjugate (Fig. 6). In this reaction, the cysteinyl amino group condenses with the quinone carbonyl to give a benzothiazolyl-like compound. Since this reaction eliminates the reactive quinone function from the molecule, it effectively prevents redox cycling of the thioether. The intramolecular cyclization reaction can therefore be considered an intramolecular detoxication reaction. Inhibiting the metabolism of these conjugates results in a high extracellular concentration of GSH-conjugates. In addition, quinone-thioethers are known to inhibit a variety of enzymes that utilize GSH as either a substrate or a cosubstrate, including γ-GT (Monks and Lau 1998). Such an effect of the α-MeDA–GSH conjugate on γ-GT at PTC membranes may subsequently also increase extracellular concentrations of nephrotoxic metabolites of MDMA. Since these conjugates retain the ability to redox cycle, with the concomitant generation of ROS, it is possible that they induce lipid peroxidation of cell membranes. In addition, GSH-linked *o*-quinones interact with thiol groups of extracellularly located functional proteins, which play a critical role in cell function.

Inhibition of γ-GT by acivicin enhances MDMA-induced toxicity to serotoninergic neurons (Bai et al. 2001). γ-GT is also present in high concentrations in the brain. Such enhancement may involve increased uptake of thioether metabolites of MDMA into the brain due to the increased pool of thioether conjugates available for uptake via the intact GSH transporter. At present, a similar GSH transporter capable of transferring GSH conjugates into the cell has not been identified in the apical membrane of renal PTCs.

The GSH conjugate 5-(glutathion-*S*-yl)-α-MeDA was more toxic to rat PTC monolayers than 2,5-*bis*(glutathion-*S*-yl)-α-MeDA. The greater potency of the former mono-substituted GSH conjugate may be related to its ability to maintain redox activity, since *bis*-, and *tris*-substituted thiol conjugates may be more predisposed to dimerization or polymerization reactions.

By contrast, the two conjugates were equally potent in human renal PTCs. Thus, human PTCs were more sensitive to 2,5-*bis*(glutathion-*S*-yl)-α-MeDA than rat PTCs. The reasons for such differences in susceptibility to the *bis*-substituted GSH conjugate are unclear at present. Species differences in the specific activity of renal γ-GT have been reported (Lau 1995). The species variations of γ-GT result not only from differences in concentrations of γ-GT but also from differences in the γ-GT -protein. The different forms of γ-GT may have different specificity for γ-glutamyl acceptor substrates (Commandeur et al. 1995). However, factors other than γ-GT appear to be involved since metabolism mediated by this enzyme had no effect on renal toxicity induced by 2,5-*bis*(glutathion-*S*-yl)-α-MeDA. Thus, it is more likely that variability in antioxidant defences contributes, at least in part, to the differential toxic effects of quinone thioethers.

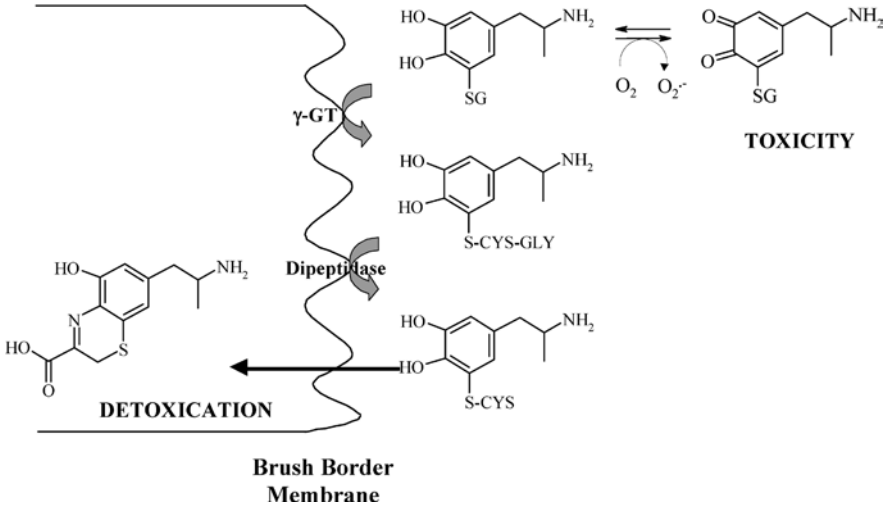


Fig. 6. Proposed mechanism resulting in detoxication of 5-(glutathion-*S*-yl)-α-methyldopamine in renal proximal tubular epithelial cells (γ-*GT* γ-glutamyl transpeptidase)

In summary, the results of the present work indicate that metabolism of MDMA and MDA plays an important role in the development of nephrotoxicity in vitro. In addition, conjugation of the putative metabolite, α-MeDA, with GSH results in compounds that are even more potent nephrotoxicants. Furthermore, the ability of acivicin and bestatin to potentiate the nephrotoxicity of 5-(glutathion-*S*-yl)-α-MeDA suggests that metabolism of this conjugate by γ-GT and aminopeptidase M constitutes a detoxication reaction. Thus, an extracellular mode of toxicity rather than uptake of cysteine-*S*-yl conjugates seems to account for the toxic effects of the thioether metabolites of ecstasy. Further work is therefore clearly required to delineate the extracellular mechanism(s) by which these compounds exert toxicity in renal cells.

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