

Original Article

Induction of P450 1A by 3-methylcholanthrene protects mice from aristolochic acid-I-induced acute renal injury

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Abstract

Background. Cytochrome P450 1A, an enzyme known to metabolize polycyclic aromatic hydrocarbons (PAHs), participates in the metabolism of aristolochic acid I (AAI) in liver and kidney microsomes isolated from humans and rodents. This study was designed to investigate whether P450 1A plays a role in AAI-induced renal injury in C57BL/6 mice.

Methods. Separate groups of mice were given AAI (10 mg/kg and 20 mg/kg) or pretreatment with 3-methylcholanthrene (3-MC, an agent known to induce P450 1A expression in many species including rodents) at 60 mg/kg given at 24 h before AAI injection. Renal function and histopathology were determined at the 3rd day following the high dose of AAI and at the 14th day following the low dose of AAI treatment. For both doses, we determined *in vivo* AAI clearances and pharmacokinetic parameters. We also determined *in vitro* P450 1A1/2 activity and the ability of liver microsomes from 3-MC-treated and vehicle-treated mice to metabolize AAI. Finally, the effect of 3-MC on protein levels of P450 1A1/2 in both liver and kidney was measured by western blotting.

Results. Pretreatment with 3-MC greatly protected mice against renal failure induced by AAI. *In vivo* AAI clearance was more rapid in 3-MC-pretreated mice than in the vehicle-pretreated mice. In addition, the P450 1A1/2 activity and the ability to metabolize AAI in hepatic microsomes isolated from 3-MC-treated mice were much greater than in vehicle-treated mice. Western blotting showed that protein levels of hepatic P450 1A1/2 were greatly increased in 3-MC-treated mice than in vehicle-treated mice.

Conclusion. These results demonstrated that the induction of hepatic P450 1A1/2 protected against AAI-induced kidney injury through faster *in vivo* clearance of AAI and suggested an important role for hepatic P450s in the detoxification of AAI-induced renal injury.

Keywords: aristolochic acid nephropathy; P450 1A; 3-methylcholanthrene

Introduction

Aristolochic acid-associated nephropathy (AAN) is characterized by hypocellular interstitial fibrosis, intimal thickening of interlobular and afferent arterioles, with glomerular sparing or mild sclerosis [1,2]. Since the first reported case in Belgium, many instances of AAN in adults have been reported throughout the world [3,4,5]. In all cases of AAN, patients had taken Chinese herbs containing aristolochic acid (AA) [6]. The well-known nephrotoxicity of AA had been established in animal models treated with AA or AA-containing herbs [7]. Balkan endemic nephropathy (BEN) appears similar to AAN on both morphological and clinical grounds [2], and a recent report provided direct evidence that AA may be a significant risk factor for BEN [8,9].

AA is a mixture of derivatives of 3,4-methylenedioxy-10-nitro-1-phenanthrenecarboxylic acid and has been used as an anti-inflammatory agent in Germany [10]. There are at least six components in AA, among which aristolochic acid I (AAI) is the main ingredient and the most cytotoxic [11]. Metabolic studies examining AA in different species including humans showed that aristolactams (AL) are the major metabolites found in excreta after nitro reduction [12]. In both rat and human hepatic microsomes, the *in vitro* activation of AA was mostly attributed to cytochrome P450 1A1/2 [13], and the major metabolite was AAIa under aerobic conditions in rat liver 9000 g supernatant [14]. Although the actions of P450s and other enzymes to form AA-DNA adducts have been widely studied in AA carcinogenesis experiments [15,16], little research has focused on the *in vivo* mechanisms of AA-induced kidney toxicity, especially at the level of enzymes and their *in vivo* metabolic effects. Thus, the role that P450s play in AA-induced kidney toxicity and whether *in vivo* induction of P450 1A, an important P450 isoform involved in the metabolism of AA, will affect AA-induced kidney toxicity are largely unknown.

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It has been reported that 3-methylcholanthrene (3-MC), a potent inducer of aryl hydrocarbon hydroxylase activity [17], induces P450 1A expression at the level of both mRNA and protein in many species, including mice [18–20]. Thus, the aim of this study was to investigate the effect of P450 1A induction by 3-MC on AAI-induced renal injury in C57BL/6 mice. We found that pretreatment of the mice with 3-MC greatly reduced the kidney toxicity induced by AAI. These findings suggest an important role for hepatic P450s in the detoxification of AAI-induced renal toxicity.

Subjects and methods

Reagents

Aristolochic acid I (AAI), 3-methylcholanthrene (3-MC), 7-ethoxyresorufin and 7-methoxyresorufin were purchased from Sigma Chemical Co. (St Louis, MO, USA). The standard for aristolochic acid Ia (AAIa) was a generous gift from Dr Minghua Xu. The L-Type Creatinine F kit and L-Type UN kit were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) Rabbit monoclonal antibodies to GAPDH were from Cell Signaling Technology, Inc. (Danvers, MA, USA), mouse monoclonal antibodies to cytochrome P450 1A1/2 and rabbit polyclonal antibodies to cytochrome P450 reductase (CPR) were from Abcam (Cambridge, UK), and sheep polyclonal antibodies to P450 1A2 were from Chemicon (Temecula, CA, USA). All other reagents were from Sigma and were of analytical grade.

Animal protocols

Animal-use protocols were approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica (Shanghai, China). Two-month-old male C57BL/6 mice were used in the studies. Animals were maintained at 22°C with a 12-h on and 12-h off light cycle. 3-MC (60 mg/kg, in corn oil) pretreatment was given 24 h before AAI injection, whereas animals in the control groups received vehicle. For AAI clearance experiments, the animals were given a single intraperitoneal (i.p.) injection of AAI (10 mg/kg and 20 mg/kg, in warm saline), and blood samples were collected by tail-bleeding at various times after the injection. For serum blood urea nitrogen (BUN) and creatinine determinations as well as for histological examinations, blood and tissue samples were collected at 14 days or 3 days after a single i.p. injection of AAI (10 mg/kg or 20 mg/kg, respectively).

Determination of serum BUN and creatinine levels

Serum creatinine and BUN levels were determined by the L-Type Creatinine F kit and the L-Type UN kit according to vendor instructions using a Hitachi-7080 biochemical analyzer.

Histopathological examination

The mice were killed at various times after AAI injection. Blood samples were drawn by cardiac puncture, and

livers and kidneys were then promptly collected and fixed in 10% formalin for 24 h. The tissues were sectioned at 2–3 mm and fixed in 10% formalin for an additional 24 h. After dehydration through a series of alcohols, tissues were cleared in xylene and impregnated with paraffin wax prior to embedding in blocks of paraffin wax. Sections were then cut at 3–4 µm and floated onto glass slides, baked at 56°C for 30 min and stained with haematoxylin-eosin (HE) and Masson's trichrome staining.

Determination of blood AAI and its major metabolite levels

Blood samples (15 µl each) were collected in heparin-coated capillary tubes and were mixed with an equal volume of saline. The samples were spun at 4000 g for 5 min at 4°C. The supernatant fractions were mixed with one-half volume of methanol and spun again at 14 000 g for 5 min to remove the precipitates. Aliquots of the final supernatant were analysed for AAI concentration on a high-performance liquid chromatograph according to the method described by Krumbiegel [12]; typical HPLC chromatograms are shown as supplementary data. Pharmacokinetic parameters were calculated using the WinNonLin software, version 3.1.

Isolation of microsomes

Hepatic tissues from three mice were homogenized in three volumes of 0.15 M KCl and centrifuged at 9000 g for 20 min. Microsomes were obtained by centrifugation of the supernatant at 100 000 g for 1 h and resuspension of the resulting pellets in a buffer containing 0.25M sucrose and 0.05M Tris buffer at pH 7.5. All steps were performed at 4°C. Protein concentrations were determined using a BCATM protein assay kit (Pierce, IL, USA).

Measurement of ethoxyresorufin and methoxyresorufin O-dealkylase activity

O-dealkylase activity was determined by the formation of 7-ethoxyresorufin O-deethylation (EROD) and 7-methoxyresorufin O-demethylation (MROD) as described by Burke [21]. Liver microsomes (2 mg/ml protein) were preincubated with a NADPH generating system (0.3 mM NADP⁺, 0.4 mM glucose 6-phosphate, 1.2 mM MgCl₂ and 0.4 U/ml glucose-6-phosphate dehydrogenase) in 0.1M phosphate buffer, pH 7.4 (total volume 0.25 ml) at 37°C for 5 min. Reactions were initiated with 7-ethoxy- or 7-methoxyresorufin (10 µM) and terminated at 120 min by adding 125 µl ice-cold acetonitrile. Samples were allowed to precipitate on ice and centrifuged for 10 min at 14 000 g. Supernatants were analysed spectrofluorometrically for product (resorufin) at an excitation of 545 nm and emission of 590 nm. Experimental values were extrapolated using a standard curve in the linear range.

Microsome metabolism

Incubations were carried out at 37°C for 2 h in tubes containing 20 µg/ml AAI dissolved in 25 µl DMSO, 2 mg/ml

microsomal protein and an NADPH generating system (5 mM glucose-6-phosphate, 3 mM MgCl₂, 1 mM NADP⁺ and 1.5 units of glucose-6-phosphate dehydrogenase) in 100 mM sodium phosphate, pH 7.4, in a total volume of 250 μ l. The reaction was initiated by an addition of the NADPH generating system and stopped by the addition of 125 μ l ice-cold acetonitrile. The precipitate was removed by centrifugation at 14 000 *g* for 10 min. AAI concentration in the supernatant was detected on a high-performance liquid chromatograph according to the method as described [12].

Western blotting

Proteins were separated by electrophoreses on a SDS-10% polyacrylamide gel and then transferred to the PVDF membrane (Amersham). The membranes were blocked in 5% skim milk powder in PBS before overnight incubation with a monoclonal rabbit anti-mouse GAPDH (1:1000) or anti-mouse cytochrome P450 1A1/2 (1:500) or polyclonal rabbit anti-mouse CPR (1:1000) or sheep anti-rat P450 1A2 (1:1000) antibody. Proteins were visualized using an ECL detection system (Amersham, NJ, USA) and a Kodak Image Station (Kodak, CT, USA).

Statistical analysis

Data are expressed as means \pm SD. One-way ANOVA and Student's *t*-test were used for statistical analysis, with *P* < 0.05 accepted as significant difference.

Results

AAI-induced renal fibrosis in C57BL/6 mice

To establish the animal model for AAN in our laboratory, we first performed acute toxicity experiments to determine appropriate doses and sacrifice times. We found that the median lethal dose (LD₅₀) was \sim 37 mg/kg for male C57BL/6 mice (data not shown), and that AAI induced a rapid progressive tubulointerstitial fibrosis in mice kidneys at 14 days after single i.p. injections at doses of 10 and 20 mg/kg. Figure 1 shows the characteristics of mice kidneys at 14 days after 10 mg/kg AAI that included hypocellular interstitial fibrosis, tubular atrophy, limited interstitial infiltrates and relatively mild glomerular changes, which are characteristics of this form of nephropathy [22]. These results suggest that a single 10 mg/kg AAI i.p. injection can induce AAN in an animal model of C57BL/6 mice.

3-MC-pretreatment prevented renal injury induced by AAI

To assess the effect of 3-MC on renal injury induced by AAI, we first examined BUN, serum creatinine and kidney histopathology. As shown in Figure 2, single injections of AAI at 10 or 20 mg/kg greatly elevated both BUN and creatinine levels. Pretreatment with 3-MC blocked these elevations induced by 10 mg/kg AAI at the 14th day post-injection (Figure 2A and B). At the 3rd day following injection of 20 mg/kg AAI, both the 3-MC-pretreated and vehicle-pretreated groups had higher BUN and creati-

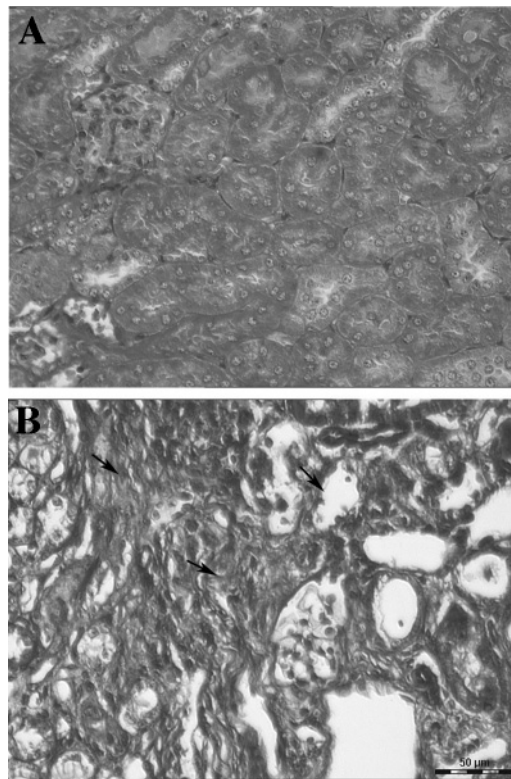


Fig. 1. Representative photomicrographs of renal cortex. (A) No abnormalities were found in control mice. (B) Severe tubular atrophy and interstitial fibrosis (arrows) were observed in mice at 14 days following AAI (10 mg/kg) (Masson's trichrome staining. Original magnifications \times 400).

nine levels than controls; however, these elevations were mild in the 3-MC-pretreated group (Figure 2C and D). Histopathological examination of the kidneys showed a normal appearance in control mice (Figure 3A and D). At 14 days following 10 mg/kg AAI, vehicle-pretreated mice had severe tubulointerstitial lesions, hyperplasia and fibrosis (Figure 3B), which contrasted with a loss of tubular epithelia with slight interstitial hyperplasia and fibrosis in 3-MC-pretreated mice (Figure 3C). At the 3rd day after 20 mg/kg AAI, vehicle-pretreated mice exhibited a large area of tubular lesions, loss of tubular brush border, necrotic cells and cellular fragments in the lumen of proximal tubules (Figure 3E), while 3-MC-pretreated mice showed the same tubular lesions but within a much smaller area (Figure 3F). We graded lesion severity in these groups of mice and found that the severity was greater in vehicle-pretreated mice than in 3-MC-pretreated mice at both doses of AAI (Table 1). These results suggest that 3-MC pretreatment may reduce the renal injury induced by AAI.

AAI clearance in 3-MC-pretreated mice was more rapid than in vehicle-pretreated mice

We next examined whether there are differences in AAI metabolism between vehicle-pretreated mice and 3-MC-pretreated mice. As shown in Figure 4, blood levels of AAI at 5–60 min after injection were markedly reduced in 3-MC-pretreated mice compared with levels in the

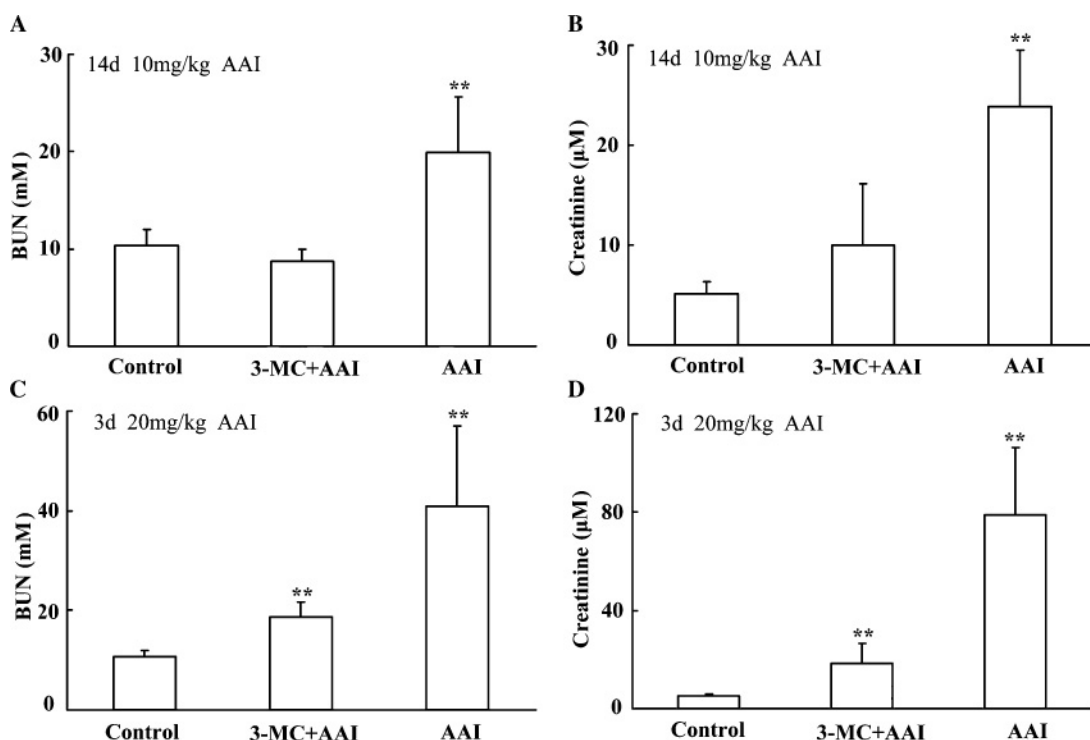


Fig. 2. Effect of 3-MC on blood urea nitrogen (BUN) and serum creatinine. (A and B) Mice treated with 10 mg/kg AAI and assayed at 14 days after the treatment. (C and D) Mice treated with 20 mg/kg AAI and assayed at 3 days after the treatment. Values are means \pm SD. $n = 5$ for each group. ** $P < 0.01$, compared with control group.

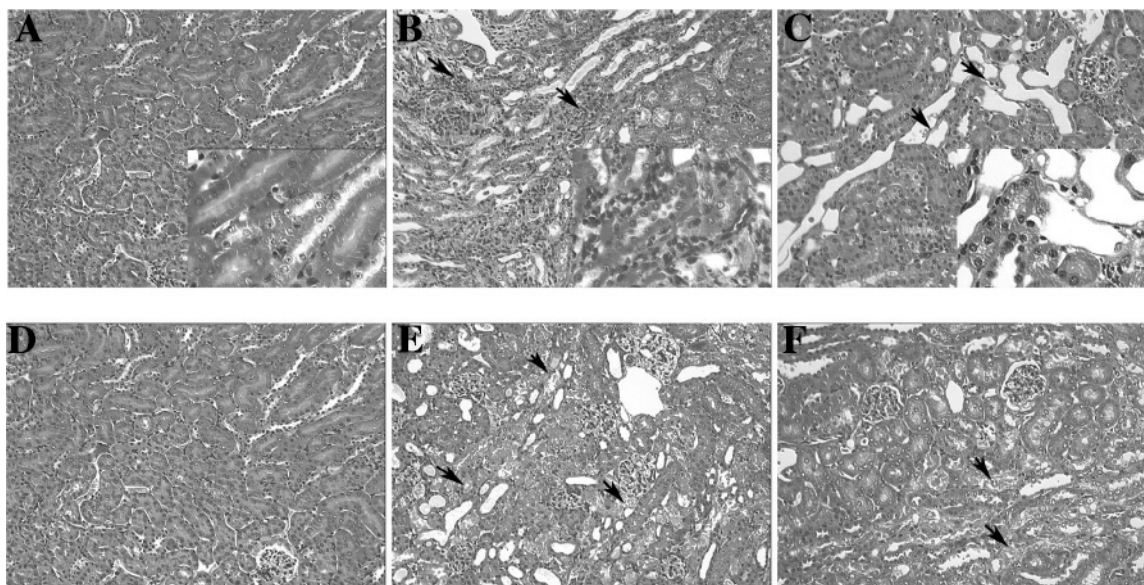


Fig. 3. Histological examination of mice kidneys. (A) Control mice, (B) 10 mg/kg AAI-treated mice and (C) 3-MC pretreated 10 mg/kg AAI-treated mice, all at the 14th day following AAI treatment; (D) control mice, (E) 20 mg/kg AAI-treated mice and (F) 3-MC pretreated 20 mg/kg AAI-treated mice, all at the 3rd day following AAI. Arrows indicate the main area of injury. (H&E stain, original magnifications $\times 200$, $\times 400$.)

vehicle-pretreated mice ($P < 0.01$). Blood AAI level began to noticeably decrease in 3-MC-pretreated mice at 5 min after AAI injection, while in vehicle-treated mice it peaked at 15 min after AAI injection. The pharmacokinetic parameters, shown in Table 2, demonstrated that T_{peak} , C_{max} , AUC and CL/F_s in 3-MC-pretreated mice were significantly dif-

ferent than the values in vehicle-pretreated mice. We also examined major metabolites of AAI in serum (Figure 4C and D), of which only AAIa was detectable. Serum AAIa levels were lower in 3-MC-pretreated mice than in vehicle-pretreated mice, and the time to initial decrease was earlier in 3-MC-pretreated mice than in vehicle-pretreated mice.

These results suggest that 3-MC pretreatment accelerated the *in vivo* clearance of AAI, and that the major metabolite generated *in vivo* is AA1a.

Enzyme activities demonstrated by EROD and MROD assays

Since P450s play a critical role in metabolizing AAI [14], we tested the effects of 3-MC on hepatic microsomal P450 activities. EROD and MROD assays were used to determine P450 1A1 and 1A2 activities. We found that 1A1 (Figure 5A) and 1A2 (Figure 5B) activities in hepatic microsomes isolated from 3-MC-pretreated mice were 70 times

and 8 times, respectively, higher than in vehicle-pretreated mice. These results suggest that pretreatment with 3-MC increased the enzyme activity of P450 1A in the liver of the mice.

In vitro metabolism of AAI by microsomes

To further determine whether AAI metabolism was affected by 3-MC, we examined the metabolic capacity of hepatic microsomes isolated from vehicle- or 3-MC-pretreated mice to metabolize AAI. After incubation for 2 h, AAI concentrations in microsomes isolated from vehicle-treated mice remained ~70% of the initial concentration; whereas it was ~50% of the initial concentration in 3-MC-pretreated mice (Figure 6A). We also examined the major metabolite AA1a generated during incubation, which was quantified from the peak area of the AA1a peak in the HPLC chromatogram (Figure 6B). We found that AA1a levels were significantly greater in microsomes from 3-MC-pretreated mice than that from vehicle-pretreated mice. These results suggest that 3-MC treatment increased the capacity to metabolize AAI in the liver of the mice.

Table 1. Extent of kidney injury in vehicle-pretreated and MC-pretreated mice

Time	AAI dose	Group	Number of mice in each grade		
			+	++	+++
3 days	20 mg/kg	AA 10 mg/kg	0	1	4
		MC+AA 10 mg/kg	1	4	0
14 days	10 mg/kg	AA 20 mg/kg	0	0	5
		MC+AA 20 mg/kg	1	4	0

Two-month-old male mice were treated with a single injection of AAI at 10 or 20 mg/kg. Kidney histopathology was examined at the 3rd or 14th day after AAI treatment. The severity of lesions in the tissues was graded as follows: +, mild; ++, moderate; +++, severe.

P450 1A1/2 and CPR expression in liver and kidney of vehicle- and 3-MC-pretreated mice

To determine whether 3-MC induces P450 1A1/2 expression in liver to then enhance the metabolism of AAI, we examined protein levels of these enzymes in the mice

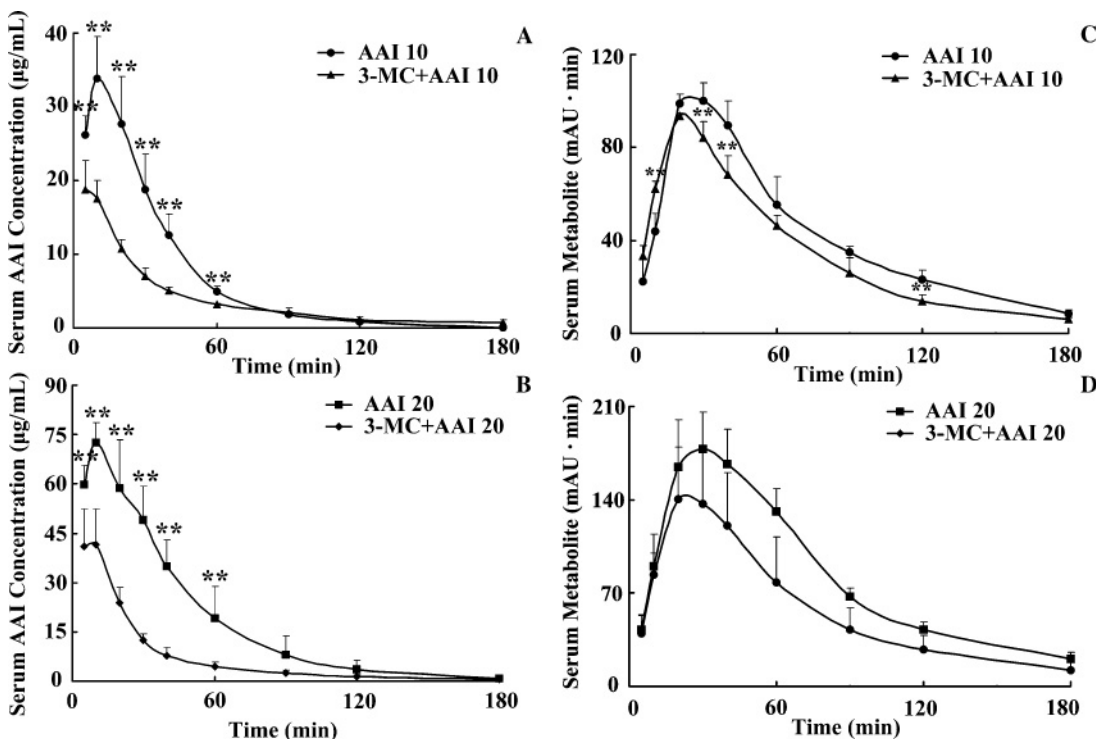


Fig. 4. Serum clearance of AAI, its major metabolite (AA1a) in C57BL/6 mice. Vehicle-pretreated and 3-MC-pretreated mice were given a single i.p. injection of 10 mg/kg (A, C) or 20 mg/kg (B, D) AAI, and blood samples were collected after injection for the determination of serum AAI concentration and for the quantity of the major metabolite. AA1a is expressed as the peak area (mAU min) of the AA1a peak in the HPLC chromatogram. Values are means \pm SD. $n = 5$ for each group. $**P < 0.01$, compared with 3-MC-pretreated group.

Table 2. Pharmacokinetic parameters of AAI in vehicle-pretreated and 3-MC-pretreated mice

Parameter	$T_{1/2}$ (min)	$T_{(peak)}$ (min)	$C_{(max)}$ ($\mu\text{g/ml}$)	AUC [$(\mu\text{g/ml}) \times \text{min}$]	CL/ $F_{(s)}$ [$\text{mg/kg/min}/(\mu\text{g/ml})$]
AA 10 mg/kg	15.49 \pm 1.71	10.17 \pm 1.48	32.39 \pm 4.52	1208.18 \pm 217.89	0.00450 \pm 0.00042
MC+AA 10 mg/kg	15.93 \pm 1.55	6.14 \pm 1.03*	20.90 \pm 2.55*	570.49 \pm 23.31*	0.01755 \pm 0.00069*
AA 20 mg/kg	20.55 \pm 1.72	11.18 \pm 1.25	69.08 \pm 4.02	2990.41 \pm 272.91	0.00337 \pm 0.00029
MC+AA 20 mg/kg	11.28 \pm 2.08*	7.01 \pm 0.88*	47.28 \pm 8.28*	1174.76 \pm 42.13*	0.01704 \pm 0.00061*

All mice were treated with a single injection of AAI at 10 or 20 mg/kg and pharmacokinetic parameters were determined as described in the Materials and methods section. * $P < 0.05$, 3-MC-pretreated mice versus vehicle-pretreated mice.

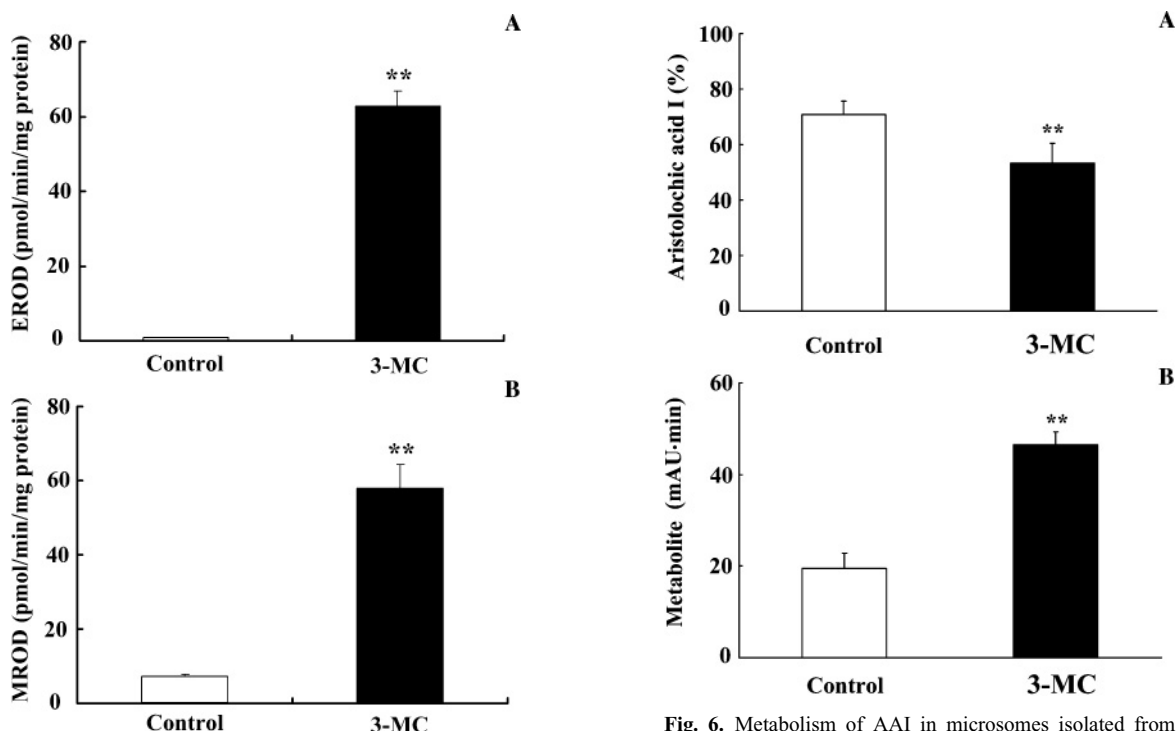


Fig. 5. Effect of 3-MC on hepatic *O*-dealkylations of ethoxyresorufin (EROD) and methoxyresorufin (MROD) in vehicle- and 3-MC-pretreated mice. (A) EROD assay for P450 1A1 activity. (B) MROD assay for P450 1A2 activity. Values are means \pm SD. $n = 5$ for each group. ** $P < 0.01$, compared with vehicle-pretreated mice.

Fig. 6. Metabolism of AAI in microsomes isolated from the liver of vehicle- and 3-MC-pretreated mice. (A) Relative concentration of AAI after the incubation of microsomes. (B) Quantity of the major metabolite generated in the incubation. AAIa is expressed as the peak area (mAU min) of the AAIa peak in the HPLC chromatogram. Values are means \pm SD. $n = 5$ for each group. ** $P < 0.01$, compared with control group.

(Figure 7). Western blot analysis revealed that protein levels of P450 1A1/2, and especially P450 1A2, were greatly increased in 3-MC-pretreated mice compared with the levels in control mice. CPR, the obligate redox partner for all microsomal P450s, was slightly increased in the liver of 3-MC-pretreated mice. These results support the notion that 3-MC induces hepatic P450 1A1/2 expression to then facilitate AAI clearance from the body. It has been reported that P450s in kidney also can metabolize AAI. Because of this, we also examined whether 3-MC treatment affected the expression of P450 in kidney. We found that the expressions of P450 1A2 and CPR were almost unchanged in 3-MC treated mice compared with controls. These results suggest that 3-MC treatment increased the expression of P450 1A1/2 in liver but not in kidney, which may have contributed to the faster clearance of AAI and the reduced renal toxicity in these mice.

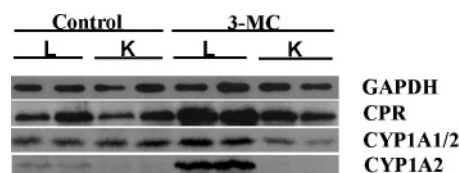


Fig. 7. Western blot analysis of cytoplasmic preparations from liver and kidney of vehicle- and 3-MC-pretreated mice. L indicates liver; K indicates kidney. Each lane contained a tissue pool from two mice.

Discussion

AA intake has been associated with the development of AAN and Balkan endemic nephropathy (BEN) [8]. The mechanisms of AA-induced diseases have been studied both *in vitro* and *in vivo* [23,24]. In spite of many *in vitro* studies examining AA metabolism and mechanisms of its carcinogenesis [13,16], there has been little *in vivo*

investigation on the mechanism by which AA induces renal toxicity. Furthermore, it remains unknown which organs participate in the *in vivo* activation of AA and whether AA or its metabolite contributes to the renal toxicity. In the present study, we report a protective effect of 3-MC, a potent inducer of P450 1A, against kidney injury induced by AAI, the major component of AA. Our results provide a possible explanation for the *in vivo* mechanism by which AAI induces renal toxicity and suggest that hepatic P450 1A1/2 contributes to the clearance of AAI, probably by a detoxification process acting against AAI-induced kidney injury.

Here we showed a protective effect of 3-MC, as demonstrated by the renal function analysis and histological examination, and then studied possible mechanisms. While examining the *in vitro* metabolism of AAI, we found that liver microsomes isolated from 3-MC-pretreated mice had greater activity than liver microsomes isolated from vehicle-pretreated mice. This occurred in parallel with 3-MC-induced elevated activities of hepatic P450 1A1 and 1A2, which was probably a result of the increased expression at the protein level. As a result, the *in vivo* clearance of AAI was faster in 3-MC-pretreated mice than in vehicle-pretreated mice, as demonstrated by serum AAI concentrations and pharmacokinetic parameters. In addition, we found that the major metabolite generated *in vivo* is AAIA, which is in agreement with previous *in vitro* studies using rat homogenate and which was authenticated by the AAIA standard. AAIA levels were lower in 3-MC-pretreated mice, and this was possibly because of lower AAI bioavailability. AAIA can be transformed into aristolactam Ia (ALIA) [25], which explains why the principal metabolite of AAI found in urine and faeces in rats was ALIA [12]. Both AAIA and ALIA are much less cytotoxic and mutagenic than AAI [11,14], and it is clear that the metabolites generated in liver were less toxic than AAI. Thus, the *in vivo* toxicity of AAI was correlated with its serum concentration, and a more rapid clearance of AAI may lead to less accumulation, resulting in less kidney toxicity.

In vitro metabolic studies using inhibitors of various enzymes and recombinant enzymes that detected and quantified specific AA-DNA adducts showed that prostaglandin H synthase (PHS) also contributes to the activation of AAI in the kidney [26]. Although a possible role for PHS in the kidney activation of AAI could not be ruled out, the finding that protein levels of P450 1A1/2 remained unchanged in kidney suggested that the kidneys may play a less important role in metabolizing AAI. Moreover, there are no reports that 3-MC affects the expression and activity of PHS or other related enzymes. Although 3-MC also induces P450 1A in intestine and seminal vesicles in rat, the increase is very mild [27]. Therefore, it is unlikely that 3-MC will influence the metabolism of AAI in the kidney or other organs, and if it does this would be to a much smaller extent.

The *in vivo* expression and *in vitro* metabolism findings for P450 1A in the current experiment are consistent with a previous *in vitro* study showing that P450 1A1/2, and especially 1A2, is responsible for most of AA metabolism [13]. High levels of hepatic P450 1A1/2 proteins lead to rapid metabolism of AA in the liver, which subsequently result in less toxicity in the kidney. There is a variability

of P450 1A in human beings [28], such that people with low expression of P450 1A face a high risk of AAN and BEN, which may explain why not all people exposed to AA subsequently develop AAN or BEN. An assay for variability of P450 1A in populations that have potential for exposure to AA may provide a means to predict and prevent the risk of AAN and BEN.

AA is activated and subsequently reacts with cellular proteins and DNA, producing DNA adducts in both kidney and liver [29]. Although the liver is the major organ for biotransformation of xenobiotics, AA induces tumours only in the kidney. In addition, a case report showing *Aristolochia*-related urinary tract cancer without significant renal failure suggested a dissociation between carcinogenicity and nephrotoxicity of AA [30]. In the present study we focused on the *in vivo* mechanisms of nephrotoxicity of AA by investigating the *in vivo* effects of major enzymes responsible for AA metabolism on AA-induced kidney toxicity. Understanding the underlying mechanisms of tissue-specific carcinogenicity of AA will require further study.

In conclusion, we have shown that inducement of P450 1A by 3-MC can increase the *in vivo* clearance of AAI by inducing enzymes involved in AAI metabolism, resulting in the reduced AAI toxicity in the kidney. These results suggest that hepatic P450s play a detoxification role in AAI-induced renal injury. A mouse model in which hepatic P450s are specifically suppressed will be useful to provide further evidence that hepatic P450s play a key role in AA-induced kidney toxicity. Future studies will also be needed to identify AAI metabolites generated in the liver and kidney of mice.

Supplementary data

Supplementary data is available online at <http://ndt.oxfordjournals.org>

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Conflict of interest statement. None declared.

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