Inhibition of Renal NQO1 Activity by Dicoumarol Suppresses Nitroreduction of Aristolochic Acid I and Attenuates its Nephrotoxicity

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Aristolochic acid I (AAI) is the major toxic component of aristolochic acid that causes aristolochic acid nephropathy and Balkan endemic nephropathy. Nitroreduction is an essential metabolic process for AAI rapid clearance in different species including humans. However, which enzyme participates in AAI nitroreduction in vivo and whether this metabolic process contributes to AAI nephrotoxicity are unclear. Here, we showed that NAD(P)H:quinone oxidoreductase 1 (NQO1) was highly expressed in mouse renal tubular epithelial cells. Inhibition of NQO1 activity by dicoumarol pretreatment significantly decreased renal aristolactam I (ALI) levels, a major reductive metabolite of AAI, whereas it increased renal AAI and its major oxidative metabolite 8-hydroxy-aristolochic acid I (AAIa) levels in male C57BL/6 mice. Similar changes in renal ALI, AAI, and AAIa levels were also observed in mice pretreated with another NQO1 inhibitor, phenindione. Consistent with higher levels of renal AAI and AAIa found in dicoumarol-pretreated mice, their serum clearance was much slower compared with vehicle-pretreated mice. The survival rate of mice pretreated with dicoumarol was markedly increased when higher doses of AAI were given. Similarly, pretreatment of mice with phenindione also attenuated AAI-induced nephrotoxicity. These results indicate that NQO1 plays an important role in renal AAI nitroreduction and may thus contribute to AAI-induced nephrotoxicity.

Key Words: aristolochic acid nephropathy; NQO1; nitroreduction; aristolactam I; dicoumarol.

The metabolism of AAI has been widely studied in different species and it has been reported that the 8-hydroxy-aristolactam I (ALI) is the major metabolite found free or as conjugates in urine and feces (Krumbiegel et al., 1987). ALI might be produced by either nitroreduction of AAI to aristolactam I (ALI) that is additionally demethylated or demethylation of AAI to 8-hydroxy-aristolochic acid I (AAIa) that is further reduced (Fig. 1) (Chan et al., 2006; Chan et al., 2007; Krumbiegel et al., 1987; Schmeiser et al., 1986). The average proportion of ALI, AAIa, and ALIa found in urine and feces of rodents was 3.4, 3.2, and 86.2%, respectively (Krumbiegel et al., 1987), indicating that production of ALIa by reductive metabolism is an essential process for AAI rapid clearance in vivo.

Recently, the role of AAI oxidative metabolism in its nephrotoxicity has been investigated. Results showed that hepatic CYP1A, especially CYP1A2 demethylated AAI (Levova et al., 2011; Rosenquist et al., 2010; Sistkova et al., 2008; Xiao et al., 2009; Xue et al., 2008) to form less toxic AAIs (Shibutani et al., 2010) and attenuated AAI-induced nephrotoxicity. However, the role of AAI reductive metabolism in its nephrotoxicity remains unclear. AAI was expected to produce an ALI-nitrenium intermediate by nitroreduction (Fig. 1), which can react with DNA resulting in the formation of AAI-DNA adducts (Pfla et al., 1990a; Pfla et al., 1990b; Schmeiser et al., 1988). In vitro, the enzymes involved in the formation of AAI-DNA adducts included NAD(P)H:quinone oxidoreductase 1 (NQO1) (Levova et al., 2011; Stiborova et al., 2002; Stiborova et al., 2003; Stiborova et al., 2011), xanthine oxidase (Schmeiser et al., 1988; Stiborova et al., 2002; Stiborova et al., 2003), CYP1A1/2 (Levova et al., 2011; Stiborova et al., 2001b; Stiborova et al., 2005), NADPH:CYP reductase (Stiborova et al., 2001c; Stiborova et al., 2005), and prostaglandin H synthase (Stiborova et al., 2001a; Stiborova et al., 2005). Given the different reaction conditions in vivo and in vitro, which enzyme...
actually participates in AAI nitroreduction in vivo remains unclear (Stiborova et al., 2008b).

NQO1 is a flavoprotein that catalyzes two electron reductions of quinones and nitrogen oxides (Riley and Workman, 1992; Ross et al., 1993). It has been demonstrated that the activity of NQO1 was high in the kidney, a target organ of AAI-induced toxicity in humans and mice (Radjendirane et al., 1998; Schlager and Powis, 1990). In the present study, we examined whether dicoumarol, the most potent inhibitor of NQO1 (Chen et al., 1999; Hosoda et al., 1974), and phenindione, another NQO1 inhibitor (Chen et al., 1999), affected AAI nitroreduction and nephotoxicity in mice. We found that inhibition of renal NQO1 activity suppressed AAI nitroreduction and attenuated its nephrotoxicity.

MATERIALS AND METHODS

Drug and chemicals. AAI, dicoumarol, phenindione, 2,6-dichloroindophenol, rabbit polyclonal antibody to NQO1, and mouse monoclonal antibody to β-actin were purchased from Sigma Chemical Co. (St Louis, MO). The standard for AAla and ALI were a generous gift from Dr Minghua Xu (Shanghai Institute of Materia Medica).

Animal treatment. All animal experiments were proved 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 12-h on and 12-h off light cycle. Dicoumarol, as a suspension in corn oil, was administered orally to mice twice at either doses of 15, 30, and 60 mg/kg, once in the afternoon and then again the following morning. For examination of the effect of dicoumarol on renal NQO1 and hepatic CYP1A2 activity, the animals were killed 2.5 h after the second dose and kidney samples were taken for immediate enzyme assay. In the study on the effect of dicoumarol on AAI nitroreduction and nephrotoxicity, the AAI dissolved in 1% NaHCO3, at a dose of 10 or 20 mg/kg, was administered by a single ip injection 2.5 h after the second dose of dicoumarol. Experiments with phenindione, another inhibitor of NQO1, were similar to dicoumarol. In all experiments, animals in the control groups received the vehicle only.

Renal NQO1 activity and Western blot analysis, hepatic CYP1A2 activity analysis. Kidney and liver from vehicle- and dicoumarol-pretreated mice were homogenized in 50mM Tris (pH 7.4) containing 0.25mM sucrose, and centrifuged at 105,000 × g for 1 h to obtain cytosolic and microsomal fractions. Dicoumarol-sensitive NQO1 activity in cytosolic fractions was measured by a method reported previously (Benson et al., 1980). The reaction mixtures contained 25mM Tris-HCl (pH 7.4), 0.2 mg/ml bovine serum albumin at pH 7.4, 5µM FAD, 0.01% Tween 20, 0.2mM NADPH, 0 or 10µM dicoumarol, an appropriate amount of cytosolic proteins, and the 40µM 2,6-dichloroindophenol was added to initiate the reaction. The reaction rate was monitored by measuring the decrease in absorbance at 600 nm due to the reduction of 2,6-dichloroindophenol. Hepatic CYP1A2 activity in microsomal fractions was assayed as described by Burke et al. (1985). The reaction mixtures, containing 0.1M phosphate buffer (pH 7.6), 1 mg/ml microsomal proteins, 0.2mM NADPH, and 10µM 7-methoxyresorufin were incubated at 37°C for 2 h. The various cytosolic fractions were also analyzed for NQO1 expression by Western blot analysis. Proteins were separated by electrophoreses on an SDS-10% polyacrylamide gel and then transferred to the polyvinylidene fluoride membrane (Amersham, NJ). The membranes were blocked in 5% skim milk powder in Tris-buffered saline before overnight incubation with a polyclonal rabbit anti-mouse NQO1 (1:2000) or monoclonal anti-mouse β-actin (1:2000) antibody. Proteins were visualized using an ECL detection system (Amersham) and a Kodak Image Station (Kodak, CT).

Determination of serum BUN and creatinine levels. Blood urea nitrogen (BUN) and creatinine levels were determined by an automatic Hitachi Clinical Analyzer Model 7080 (Hitachi High-Technologies Corporation, Tokyo, Japan).

Histopathological and immunohistochemical examination. The mice were killed at various times after AAI injection. Kidneys were collected and processed by a standard pathology procedure. Sections of 3–4 µm were stained with hematoxylin and eosin. For demonstration of NQO1 expression in normal mice, a commercially available kit Polink-2 plus (catalog number PV-9001, GBI) was used. The deparaffinized tissue sections were rehydrated and incubated in endogenous peroxidase activity was blocked using 3% hydrogen peroxide solution (for 10 min at room temperature). Subsequently, the sections were incubated with 10% bovine serum substituted with the primary antibody. The reaction product was visualized (brown staining) by the reaction product was visualized (brown staining) by the chromogen DAB. Sections were then counterstained using hematoxylin.

Determination of the levels of AAI and its metabolites in blood and kidney. For the determination of AAI concentrations, blood samples were collected by tail bleeding at various time points after the injection. Blood samples (20 µl each) were collected in heparin-coated capillaries and were mixed with an equal volume of saline. The samples were spun at 4000 × g for 5 min at 4°C. Pharmacokinetic parameters were calculated using the WinNonLin software.
Tissue samples were homogenized in saline (1 ml saline/g tissue), then the homogenate was spun at 14,000 × g for 10 min, and then the supernatants were mixed with an equal volume of acetonitrile and spun again at 14,000 × g for 5 min to remove precipitated proteins. Aliquots of the final supernatants were analyzed and quantified for the levels of AAI and the metabolites AAla and ALI by high-performance liquid chromatography (HPLC). The recovery of AAI, AAla, and ALI in kidney was 88.32, 85.27, and 82.01%, respectively.

**HPLC analysis of AAI and its metabolites.** The quantification of AAI and its metabolites in the samples was performed on an e2695-2998 HPLC system (Waters Corp., Milford, MA). HPLC analysis was carried out using a Welch Hybrid XB-C18 column (5 μm, 46.6 × 250 mm; Welch Materials, MD) at a flow rate of 0.8 ml/min. An isocratic mobile phase of methanol, 0.1% acetic acid in H2O (7:3) was used for separation. The photodiode array detector was set at 250 nm. In addition, liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis were conducted on an Agilent 6300 LC/MSD Trap XCT Ultra (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) to confirm and characterize AAI and its metabolites. The testing conditions refer to Xiao et al. (2008).

**Statistical analysis.** Data are presented as mean ± SD and their statistical differences were determined by Student’s t-test and one-way ANOVA followed by a Tukey post hoc test. Differences were considered significant at p < 0.05.

**RESULTS**

**Specific Expression of NQO1 in Mouse Renal Tubular Epithelial Cells**

We first examined the expression of NQO1 in the kidney of male C57BL/6 mice by immunohistochemistry. As shown in Figure 2B, NQO1 was highly expressed in renal cortex but not in medulla compared with negative control (Fig. 2A). Morphologically, the expression of NQO1 extended radially from inner to outer cortex. Further observation found that NQO1 was mainly located in proximal tubular epithelial cells but not in distal convoluted tubule, glomeruli, and vasculature (Fig. 2C). As the tubular epithelial cells are the first to meet AAI coming from the blood, our results provide the anatomic base for NQO1 to metabolize AAI in the kidney.

**Pretreatment of Mice with Dicoumarol Inhibited Renal NQO1 Activity**

We then examined whether dicoumarol could affect renal NQO1 activity. As shown in Figure 3A, administration of dicoumarol to the mice decreased renal NQO1 activity in a dose-dependent manner with the peak inhibition at 60 mg/kg. The maximal inhibition was up to 62.5% compared with control vehicle (Fig. 3A). However, dicoumarol had no or little effect on renal NQO1 expression (Fig. 3B) and hepatic CYP1A2 activity (Fig. 3C), an enzyme mainly responsive for AAI demethylation (Levova et al., 2011; Rosenquist et al., 2010; Sistkova et al., 2008; Xiao et al., 2008).

**Pretreatment of Mice with Dicoumarol Inhibited Renal AAI Nitroreduction and Slowed AAI Serum Clearance**

The levels of ALI, a major reductive metabolite of AAI detected at 30 min after a single intraperitoneal injection of AAI at 20 mg/kg, were the highest in kidney among different tissues in male C57BL/6 mice (Fig. 4), indicating that kidney is an organ with the strongest reductive capability to AAI. To examine the role of NQO1 in AAI nitroreduction, using authentic standard and LC-MS/MS analysis, we determined the levels of renal AAI and its major metabolites in dicoumarol-pretreated mice at 30 min after injection of AAI at 10 or 20 mg/kg. Representative HPLC chromatograms from the kidney of vehicle- and dicoumarol-pretreated mice given 20 mg/kg AAI are shown in Figure 5A. The levels of ALI in the kidney of dicoumarol-pretreated mice were significantly lower than those in vehicle-pretreated mice at both AAI doses injected (Fig. 5B). However, renal AAI and its major oxidative metabolite AAla levels were much higher (Figs. 5D and C). Similar results were also obtained in mice pretreated with phenindione, another NQO1 inhibitor.
inhibitor (Supplementary fig. 1). Linear regression analysis showed that renal ALI levels positively correlated to NQO1 activity (Fig. 6).

To further examine the effect of NQO1 on AAI reductive metabolism, we determined the levels of AAI and its major metabolite in the blood of mice pretreated with vehicle and 60 mg/kg dicoumarol at different time points. The serum levels of AAI and AAIa in dicoumarol-pretreated mice were significantly higher than those in vehicle-pretreated mice at time points ranging from 30 to 180 min after a single ip injection of AAI at 10 mg/kg (Figs. 5E and G) or 20 mg/kg (Figs. 5F and H). The pharmacokinetic parameters, shown in Table 1, demonstrated that \( t_{1/2} \) and area under the curve in dicoumarol-pretreated mice were markedly higher than the values in vehicle-pretreated mice. In our testing conditions, serum levels of ALI in all mice were not detected, indicating that the ALI found in kidney was produced in the local but not from the blood. Collectively, our results suggested that NQO1 participated in renal AAI nitroreduction, and inhibition of its nitroreduction significantly slowed AAI serum clearance in mice.

**Pretreatment of Mice with Dicoumarol Attenuated AAI-induced Nephrotoxicity**

The AAI-induced nephrotoxicity was then examined in vehicle- and dicoumarol-pretreated mice with AAI at 10 or 20 mg/kg. In 10 mg/kg AAI-treated groups, serum levels of BUN and creatinine in vehicle- and 15 mg/kg dicoumarol-pretreated mice markedly increased compared with the control group, whereas their levels were normal in 30 or 60 mg/kg dicoumarol-pretreated mice (Figs. 7A and B). In 20 mg/kg AAI-treated groups, serum levels of BUN and creatinine were normal only in 60 mg/kg dicoumarol-pretreated mice (Figs. 7A and B). Serum levels of BUN and creatinine in mice, which were measured at 8–10 days after AAI injection, were correlated with renal levels of ALI in mice detected at 30 min after AAI injection (Figs. 8A and B). Histologically, at 10 days following 10 mg/kg AAI injection, vehicle-pretreated mice had severe tubulointerstitial lesions, hyperplasia, and fibrosis (Fig. 7F) compared with the control mice (Fig. 7E), whereas the extent of renal lesions was attenuated in 30 mg/kg dicoumarol-pretreated mice (Fig. 7G), and no renal lesions were found in mice pretreated with 60 mg/kg dicoumarol (Fig. 7H). The severity of renal lesions was graded among vehicle- and dicoumarol-pretreated mice (Table 2). Similarly, pretreatment of mice with another NQO1 inhibitor, phenindione, also attenuated AAI-induced nephrotoxicity (Supplementary fig. 2).

The survival rate and body weight were determined in vehicle-pretreated and 60 mg/kg dicoumarol-pretreated mice within 14 days after AAI treatment to further find the difference in the susceptibility to AAI toxicity. After a single ip injection
of AAI at 20 mg/kg, no death was observed in dicoumarol-pretreated mice, whereas all of the vehicle-pretreated mice were dead after 10 days (Fig. 7C). Similarly, the body weight of vehicle-pretreated mice started to decline on the second day after the injection, whereas no changes in dicoumarol-pretreated mice were found (Fig. 7D).

**FIG. 5.** The levels of AAI and its major metabolites in the kidney and blood of vehicle- and dicoumarol-pretreated mice. The tissues were collected from male C57BL/6 mice at 30 min after a single ip injection of AAI at 10 or 20 mg/kg. Tissues were processed for the determination of ALI, AAla, and AAI levels as described in “Materials and Methods” section. (A) Typical HPLC chromatograms were obtained from the samples of mice, given the 20 mg/kg AAI. Renal levels of ALI (B), AAla (C), and AAI (D) in mice. Quantity of AAla was expressed as the peak area (μV·S) of its peak in the HPLC chromatogram. Values are presented as mean ± SD (n = 6). For detection of serum levels of AAI and its metabolites, blood samples were collected at different time points after AAI injection and were processed for the determination of AAla and AAI levels as described in “Materials and Methods” section. Serum levels of AAI (E, F) and AAla (G, H) in mice. Quantity of serum AAla was expressed as the peak area (μV·S) of its peak in the HPLC chromatogram. Values are presented as mean ± SD (n = 5). *p < 0.05, **p < 0.01 versus vehicle-pretreated mice.

**DISCUSSION**

*In vitro* studies have established that enzymes involved in AAI reductive activation under anaerobic conditions included NQO1, CYP1A, XO, PHS, and CPR (Stiborova et al., 2008a). However, *in vivo* which of them actually participates in AAI
Nitroreduction is still unknown. We reported here, for the first time, that NQO1 plays a critical role in renal AAI nitroreduction. Moreover, it appeared that this metabolic process may contribute to AAI-induced nephrotoxicity.

Nitroreduction is an essential metabolic process for AAI rapid clearance in vivo (Fig. 1). Kidney is an organ with the strongest reductive capability to AAI in male C57BL/6 mice (Fig. 4) as observed in rodents earlier (Dong et al., 2006; Shibutani et al., 2007). The levels of ALI in mouse tissues were not fully consistent with that of AAI-DNA adducts, which might be due to the differences in species, route of AAI administration, and sampling time (Dong et al., 2006; Shibutani et al., 2007). Because we did not find the ALI, a major reductive metabolite of AAI, in mouse blood, its detection in kidney should be produced in the local. Pretreatment of mice with dicoumarol or phenindione, two inhibitors of NQO1, significantly decreased renal levels of ALI (Fig. 5B; Supplementary fig. 1A), indicating that NQO1 actually participated in AAI nitroreduction in vivo as observed in vitro previously (Levova et al., 2011; Stiborova et al., 2002; Stiborova et al., 2003; Stiborova et al., 2011). The renal levels of AAI did not change significantly in dicoumarol-pretreated mice with the increase of dicoumarol dosage (Fig. 5D), which might be due to the balance of AAI oxidation (AAIa increase) and reduction (ALI decrease) (Figs. 5B and C) as proposed by previous reports (Rosenquist et al., 2010; Stiborova et al., 2008a; Stiborova et al., 2008b). In addition to nitroreduction, the oxidation of AAI catalyzed by hepatic CYP1A2 is also an important metabolic pathway for its rapid clearance in vivo as shown in Figure 1. As dicoumarol had no effect on hepatic CYP1A2 activity (Fig. 3C), the slower serum clearance of AAI found in dicoumarol-pretreated mice might be due to the inhibition of AAI nitroreduction.

Previous studies have shown that hepatic CYP1A demethylated AAI (Levova et al., 2011; Rosenquist et al., 2010; Stiborova et al., 2008; Xiao et al., 2008; Xiao et al., 2009; Xue et al., 2008) to form less toxic AAIa (Shibutani et al., 2010) and attenuated AAI-induced nephrotoxicity in mice. Due to a decrease of both AAI and ALI levels in the kidney of mice pretreated with β-naphthoflavone (Xiao et al., 2009) or 3-methylcholanthrene (Xiao et al., 2008), the question whether AAI itself or a reductive intermediate is responsive for AAI-induced nephrotoxicity remains to be answered as proposed recently by Rosenquist et al. (2010). In the present study, we found that pretreatment of mice with dicoumarol or phenindione decreased renal ALI but increased AAI levels (Figs. 5B and D; Supplementary fig. 1A and C), and these mice were protected from AAI-induced nephrotoxicity (Fig. 7; Supplementary fig. 2). These results indicated that the nephrotoxicity induced by AAI was correlated with the amounts of renal ALI but not AAI as shown in Figures 8A and 8B. Because ALI has been shown to be nontoxic to mice (Sato et al., 2004), the ALI-nitrenium intermediate produced by AAI nitroreduction (Pfau et al., 1990a; Pfau et al., 1990b; Schmeiser et al., 1988) might contribute to AAI-induced nephrotoxicity (Fig. 1). Similarly, the kidney, a target organ of AAI-induced toxicity, had the highest levels of ALI among different tissues in male C57BL/6 mice (Fig. 4). The location of NQO1 expression (Fig. 2) was highest levels of ALI among different tissues in male C57BL/6 mice (Fig. 4). The location of NQO1 expression (Fig. 2) was consistent with that of renal lesion induced by AAI (Cosyns et al., 1994; Depierreux et al., 1994), and renal ALI levels positively correlated with NQO1 activity (Fig. 6), suggesting that NQO1 may play a key role in AAI-induced nephrotoxicity.

Although dicoumarol has been widely used as an inhibitor of NQO1, whether the observed modulation of AAI

### TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>t$_{1/2}$ (min)</th>
<th>T$_{max}$ (min)</th>
<th>C$_{max}$ (µg/ml)</th>
<th>AUC ([µg/ml] × min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAI 10 mg/kg</td>
<td>27.40 ± 3.50</td>
<td>10 ± 0</td>
<td>26.39 ± 1.43</td>
<td>957.48 ± 30.11</td>
</tr>
<tr>
<td>Dic 60 + AAI 10 mg/kg</td>
<td>31.95 ± 1.25*</td>
<td>15 ± 5.77</td>
<td>26.26 ± 3.43</td>
<td>1513 ± 267.41**</td>
</tr>
<tr>
<td>AAI 20 mg/kg</td>
<td>27.58 ± 3.48</td>
<td>10 ± 0</td>
<td>61.59 ± 8.46</td>
<td>2714.76 ± 316.06</td>
</tr>
<tr>
<td>Dic 60 + AAI 20 mg/kg</td>
<td>33.79 ± 1.81**</td>
<td>18 ± 4.47**</td>
<td>62.74 ± 4.17</td>
<td>3675.18 ± 390.11**</td>
</tr>
</tbody>
</table>

*Note. 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 “Material and Methods” section.

*p < 0.05, **p < 0.01, dicoumarol-pretreated mice versus vehicle-pretreated mice.*
Nitroreduction and nephrotoxicity is due solely to effects upon NQO1 activity cannot be stated with certainty. The NQO1-null mice model has been successfully set up (Radjendirane et al., 1998), and it is needed to further confirm its contribution to AAI nitroreduction and nephrotoxicity using this model.

The proof of NQO1 involved in AAI nitroreduction and nephrotoxicity is of great importance because the activity of NQO1 in humans may differ significantly among individuals. It is influenced by several factors including drugs, environmental chemicals, and genetic polymorphisms (Nioi and Hayes, 2004).

**FIG. 7.** Effect of dicoumarol on AAI-induced nephrotoxicity. Serum levels of BUN (A) and creatinine (B) in vehicle- and dicoumarol-pretreated mice at the 10th or 8th day after a single injection of AAI at 10 or 20 mg/kg. Values are presented as mean ± SD (n = 6). **p < 0.01 versus vehicle-pretreated mice; ***p < 0.01 versus 15 mg/kg dicoumarol-pretreated mice; $$$p < 0.01 versus 30 mg/kg dicoumarol-pretreated mice. Survival rate (C) and body weight (D) of 60 mg/kg dicoumarol- and vehicle-pretreated mice within 14 days after a single injection of AAI at 20 mg/kg, n = 8 in each group. **p < 0.01 versus control mice. Histological examination of the kidney in negative control mice (E) and mice pretreated with vehicle (F), 30 (G) and 60 mg/kg dicoumarol (H) at the 10th day after a single injection of AAI at 10 mg/kg (hematoxylin and eosin stain, original magnifications ×200).
Humans with higher activity of renal NQO1 may have a higher risk for AAN and BEN.

In conclusion, NQO1 plays an important role in renal AAI nitroreduction in mice, and the metabolic process may contribute to AAI-induced nephrotoxicity. Our findings provide new insights into the mechanism of AAI-induced nephrotoxicity and possible new approaches to the intervention/prevention of AAN and BEN.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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REFERENCES


FIG. 8. Serum levels of BUN and creatinine in mice treated with AAI were correlated with renal levels of ALI. Serum levels of BUN and creatinine were determined in vehicle- and dicoumarol-pretreated mice at the 10th or 8th day after a single injection of AAI at 10 or 20 mg/kg. Renal levels of ALI were determined in vehicle- and dicoumarol-pretreated mice at 30 min after a single injection of AAI at 10 or 20 mg/kg.

TABLE 2

Extent of Kidney Injury in the Mice Pretreated with Vehicle, 15, 30, and 60 mg/kg Dicoumarol (Dic)

<table>
<thead>
<tr>
<th>Time</th>
<th>AAI dose</th>
<th>Group</th>
<th>−</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>10 mg/kg</td>
<td>AAI 10 mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dic 15 + AAI 10 mg/kg</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dic 30 + AAI 10 mg/kg</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dic 60 + AAI 10 mg/kg</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8 days</td>
<td>20 mg/kg</td>
<td>AAI 20 mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dic 15 + AAI 20 mg/kg</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dic 30 + AAI 20 mg/kg</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dic 60 + AAI 20 mg/kg</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. 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 10th or 8th day after AAI treatment. The severity of lesions in the tissues was graded as follows: −, no lesions; +, mild; ++, moderate; ++++, severe.


