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NAD(P)H:quinone oxidoreductase expression in *Cyp1a*-knockout and *CYP1A*-humanized mouse lines and its effect on bioactivation of the carcinogen aristolochic acid I

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ABSTRACT

Aristolochic acid causes a specific nephropathy (AAN), Balkan endemic nephropathy, and urothelial malignancies. Using Western blotting suitable to determine protein expression, we investigated in several transgenic mouse lines expression of NAD(P)H:quinone oxidoreductase (NQO1)—the most efficient cytosolic enzyme that reductively activates aristolochic acid I (AAI). The mouse tissues used were from previous studies [Arlt et al., *Chem. Res. Toxicol.* 24 (2011) 1710; Stiborova et al., *Toxicol. Sci.* 125 (2012) 345], in which the role of microsomal cytochrome P450 (CYP) enzymes in AAI metabolism in vivo had been determined. We found that NQO1 levels in liver, kidney and lung of *Cyp1a1(−/−)*, *Cyp1a2(−/−)* and *Cyp1a1/1a2(−/−)* knockout mouse lines, as well as in two *CYP1A*-humanized mouse lines harboring functional human *CYP1A1* and *CYP1A2* and lacking the mouse *Cyp1a1/1a2* orthologs, differed from NQO1 levels in wild-type mice. NQO1 protein and enzymic activity were induced in hepatic and renal cytosolic fractions isolated from AAI-pretreated mice, compared with those in untreated mice. Furthermore, this increase in hepatic NQO1 enzyme activity was associated with bioactivation of AAI and elevated AAI-DNA adduct levels in ex vivo incubations of cytosolic fractions with DNA and AAI. In conclusion, AAI appears to increase its own metabolic activation by inducing NQO1, thereby enhancing its own genotoxic potential.

Introduction

The herbal drug aristolochic acid (AA) derived from *Aristolochia* species has been shown to be the cause of so-called Chinese herb nephropathy, now termed aristolochic acid nephropathy (AAN) (Debelle et al., 2008; Schmeiser et al., 2009). The plant extract AA is a mixture of structurally related nitrophenanthrene carboxylic acids, the major components being aristolochic acid I (AAI) and aristolochic acid II (AAII).

AAN is a rapidly progressive renal fibrosis that was initially observed in a group of Belgian women who had ingested weight loss pills containing *Aristolochia fangchi* (Nortier et al., 2000; Vanherweghem et al., 1993). Within a few years of taking the pills, AAN patients also showed a high risk (~50%) of upper urothelial tract carcinoma and, subsequently, bladder urothelial carcinoma. In the meantime, similar cases have been reported elsewhere in Europe and Asia (Schmeiser et al., 2009). Dietary exposure to AA has also been linked to Balkan endemic nephropathy (BEN) and its associated urothelial cancer (Arlt et al., 2007; Grollman et al., 2007; Moriya et al., 2011); this nephropathy is endemic in certain rural areas of Serbia, Bosnia, Croatia, Bulgaria and Romania.

Exposure to AA was demonstrated by identification of specific AA-DNA adducts in urothelial tissue of AAN and BEN patients (Arlt et al., 2002; Grollman et al., 2007; Jelakovic et al., 2012; Nortier et al., 2000; Schmeiser et al., 1996; Yun et al., 2012). The most abundant DNA adduct detected in patients is 7-(deoxyadenosin-N^6-yl)

Abbreviations: AHR, aryl hydrocarbon receptor; AA, aristolochic acid; AAI, aristolochic acid I; AAIa, aristolochic acid Ia; AAN, aristolochic acid nephropathy; ARE, antioxidant response element (also known as EpRE, electrophile response element); BEN, Balkan endemic nephropathy; CYP, cytochrome P450; dA-AAI, 7-(deoxyadenosine-N^6-yl)aristolactam I; dA-AAII, 7-(deoxyadenosine-N^6-yl)aristolactam II; dG-AAI, 7-(deoxyguanosin-N^2-yl) aristolactam I; HRN, Hepatic P450 Reductase Null; KEAP1, Kelch-like ECH-associating protein 1; NATs, *N,O*-acetyltransferases; NQO1, NAD(P)H:quinone oxidoreductase; NRF2, nuclear factor-erythroid-related factor 2; POR, P450 oxidoreductase; RAL, relative adduct labeling; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SULTs, sulfotransferases; TLC, thin-layer chromatography; UUC, upper urinary tract urothelial carcinoma; WT, wild-type; XRE, xenobiotic response element (also known as AHRE, AHR response element).

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aristolactam I (dA-AAI) (Fig. 1), which causes characteristic AT→TA transversions. Such AT→TA mutations have been observed in the *TP53* tumor suppressor gene in tumors from AAN and BEN patients (Grollman et al., 2007; Lord et al., 2004; Moriya et al., 2011), indicating a probable molecular mechanism associated with AA-induced carcinogenesis (Arlt et al., 2007; Kucab et al., 2010). More recently, AA exposure was discovered to contribute to the high incidence of upper urinary tract urothelial carcinoma (UUC) in Taiwan, where medicinal use of *Aristolochia* plants is widespread (Chen et al., 2012); again, the *TP53* mutational signature in patients with UUC was predominant among otherwise rare AT→TA transversions (Olivier et al., 2012). AA has been classified as a Group I carcinogen in humans by the International Agency for Research on Cancer (Grosse et al., 2009).

The activation pathway for AAI is nitroreduction, catalyzed by both cytosolic and microsomal enzymes; in this process NAD(P)H:quinone oxidoreductase (NQO1) is the most efficient cytosolic nitroreductase (Chen et al., 2011; Stiborova et al., 2003, 2008a, 2008b) (Fig. 1). In contrast to NQO1, conjugation enzymes such as human sulfotransferases (SULTs) or *N,O*-acetyltransferases (NATs) did not significantly activate AAI (Martinek et al., 2011; Stiborova et al., 2011). In human hepatic microsomes, AAI is activated by cytochrome P450 1A2 (CYP1A2) and, to a lesser extent, by CYP1A1; P450 oxidoreductase (POR) also plays a minor role (Stiborova et al., 2001a, 2005). Human and rodent CYP1A1 and 1A2 are also the principal enzymes involved in oxidative detoxication of AAI to 8-hydroxyaristolochic acid I (aristolochic acid Ia, AAIa, Fig. 1) (Shibutani et al., 2010; Sistkova et al., 2008).

The role of cytochrome P450 enzymes, particularly CYP1A1 and CYP1A2, both in the reductive activation and oxidative detoxication of AAI, was demonstrated in several animal studies. Two studies used the *H*epatic P450 *R*eductase *N*ull (HRN) mice – in which the *Por* gene is deleted specifically in hepatocytes – resulting in the absence of CYP activity (Levova et al., 2011; Xiao et al., 2008), two others used *Cyp1a1(−/−)*, *Cyp1a2(−/−)* and/or *Cyp1a1/1a2(−/−)* mouse lines (Arlt et al., 2011a; Rosenquist et al., 2010).

We also evaluated AAI metabolism mediated by human CYP1A1 and 1A2, employing two *CYP1A*-humanized mouse lines, both carrying functional human *CYP1A1* and *CYP1A2* genes in place of the orthologous mouse genes; one line carries the high-affinity aryl hydrocarbon receptor (AHR) [*hCYP1A1_1A2_Cyp1a1/1a2(−/−)_Ahr^{b1}*], whereas the other line carries the poor-affinity AHR [*hCYP1A1_1A2_Cyp1a1/1a2(−/−)_Ahr^{d}*] (Stiborova et al., 2012). The latter line is believed to be more relevant to human risk assessment vis-à-vis human CYP1A1 and CYP1A2 substrates, because poor-affinity, rather than high-affinity, AHR is known to predominate by far in human populations (Nebert et al., 2004). Overall, AAI-DNA adduct levels were higher in *CYP1A*-humanized mice than in wild-type (WT) mice, suggesting strongly that human CYP1A1 and 1A2 cause higher AAI bioactivation than mouse CYP1A1 and CYP1A2 (Stiborova et al., 2012).

Moreover, an exclusive role of human CYP1A1 and 1A2 in AAI oxidation to AAIa was observed in human liver microsomes under aerobic (i.e. oxidative) conditions (Stiborova et al., 2012). Our results suggest that, in addition to CYP1A1 and 1A2 expression levels, pO_2 levels in specific organs or even cells might affect the balance between AAI nitroreduction (bioactivation) and demethylation (detoxication) which, in turn, would influence tissue-specific toxicity or carcinogenicity. However, reductive activation of AAI in these mouse lines may not only be dictated by CYP1A1/1A2 (Arlt et al., 2011a; Stiborova et al., 2012) but also by NQO1 expression. Indeed, higher AAI-DNA adduct levels in HRN than in WT mice are not only the result of lack of hepatic AAI demethylation by the CYP-dependent

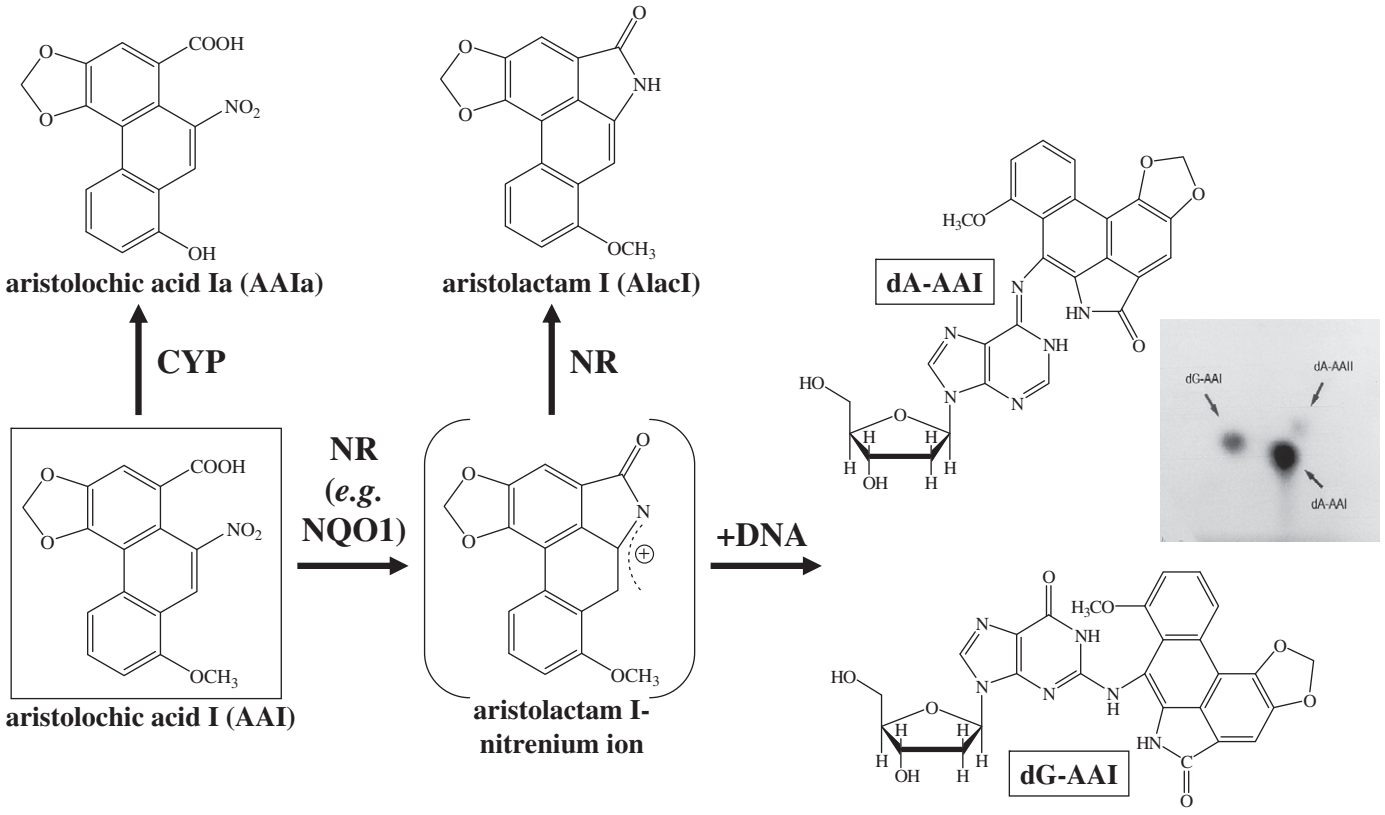

Fig. 1. Pathways of biotransformation and DNA adduct formation of AAI. dA-AAI, 7-(deoxyadenosin-N^6-yl)aristolactam I; dG-AAI, 7-(deoxyguanosin-N^2-yl)aristolactam I; NQO1, NAD(P)H:quinone oxidoreductase; NR, nitro-reductase. Inset: Autoradiographic profile of AAI-DNA adducts formed by incubation of AAI with hepatic cytosol from WT mice, using the nuclease P1 enrichment version of the ^{32}P-postlabeling assay. The adduct profile shown is representative of those obtained in hepatic cytosolic fractions of other mouse lines.