Biotransformation enzymes in development of renal injury and urothelial cancer caused by aristolochic acid

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Ingestion of aristolochic acid (AA) is associated with the development of AA-nephropathy and Balkan endemic nephropathy, which are characterized by chronic renal failure, tubulointerstitial fibrosis, and urothelial cancer. Understanding which enzymes are involved in AA activation and/or detoxification is important in assessing susceptibility to AA. Xiao et al. demonstrate that hepatic cytochrome P450s in mice detoxicate AA and thereby protect kidney from injury. The relative contribution of enzymes activating AA to induce urothelial cancer in humans remains to be resolved.


The paper by Xiao et al. (this issue) addresses the interesting and still unset-tled question of whether the metabolism of aristolochic acid (AA) determines its pathophysiological effects, and, if so, which enzymes participating in this process are responsible. AA, a naturally occurring nephrotoxin and carcinogen, is associated with urothelial cancer development in patients suffering from Chinese herb nephropathy, now termed aristolochic acid nephropathy (AAN), and may also be a cause of the development of a similar type of kidney fibrosis with malignant transformation of the urothelium, Balkan endemic nephropathy (BEN),²,³ The molecular mechanisms for AA-mediated renal injury, and whether it is an early stage of the urothelial-specific tumor development, are still matters of debate and need further investigations. In this context, it is noteworthy that a case of AA-induced tumor development without renal injury⁴ suggests dissociation between AA-mediated nephrotoxicity and carcinogenicity. AA seems to directly cause renal injury by activating mitochondrial permeability transition, which was found recently in human renal tubular epithelial cells,⁵ and metabolic activation of AA to species forming DNA adducts is an important step for AA-induced malignant transformation.⁶ Indeed, the molecular mechanism of AA-induced carcinogenesis demonstrates a strong association between DNA adduct formation, mutation pattern, and tumor development.³

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both these metabolites were found to be excreted. However, even though aristolactam I is not a direct DNA-binding species, low amounts of the dA-AAI adduct, with the highest levels in one of the target tissues, the renal pelvis, were generated in rats treated with aristolactam I.9 This result is consistent with the finding of formation of the dA-AAI adduct by aristolactam I after its in vitro activation with different peroxidases, of which several, such as COX-1 and/or COX-2, are expressed at high levels in renal tissue. It is still questionable whether enzymes capable of conjugating the proximate carcinogenic metabolite of AAI, N-hydroxyaristolactam I, are involved in AAI activation. Meinl et al.10 demonstrated that expression of some human sulfotransferases (SULTs), particularly SULT1A1, in bacterial and mammalian target cells enhances the mutagenic activity of AAI. Moreover, an increase in AAI-induced mutagenicity was correlated with higher AAI-DNA adduct levels in V79 cells transfected with human SULT1A1.6 However, an increase in AAI-DNA adduct levels was found neither in human hepatic and renal cytosols, to which the SULT cofactor was added, nor in an in vitro system consisting of human NQO1 and SULT1A1.6 Although the enzymes catalyzing the reductive activation of AAI in vitro have already been identified, the question of which of them actually participates in this process in vivo remains to be answered. Additional factors such as route of administration, absorption, renal clearance, and tissue-specific enzyme expression make it difficult to extrapolate from data found in vitro to the in vivo situation.

In contrast to the enzymes activating AAI in vitro, those participating in AAI detoxication both in vitro and in vivo had not been investigated until the study of Xiao et al.1 Hepatic cytochrome P450 reductase-null (HRN) mice, which had been shown previously to be a suitable model to determine hepatic xenobiotic metabolism in vivo and had suggested for use to elucidate AA metabolism,6 were successfully used in their interesting study.1 The authors’ novel results clearly indicate that hepatic CYPs detoxify AAI by its demethylation to aristolochic acid Ia (AAIa) and thereby protect the kidney from AAI-induced injury.1 AAIa or its conjugates the O-glucuronide, O-acetate, and O-sulfate esters were excreted in urine. AAIa is also reduced to N-hydroxyaristolactam Ia, forming aristolactam Ia, which together with its conjugates, the N- and O-glucuronides, is excreted.6 Enzymatic reactions leading to aristolactam Ia and its metabolites seem to be purely a detoxication pathway, as DNA adducts containing aristolactam Ia structure have as yet not been found. The observations of Xiao et al.,1 combined with results found previously, support strongly the former hypothesis6 that a key point determining the carcinogenic and nephrotoxic effects of AAI lies in the balance of activities of reductases such as NQO1, catalyzing the AAI-DNA adduct formation, and enzymes such as CYPs, which detoxicate AAI to AAIa.

![Figure 1 | Proposed pathway for metabolic activation and detoxication of aristolochic acid I (AAI), leading to renal injury and urothelial cancer. AAN, aristolochic acid nephropathy; BEN, Balkan endemic nephropathy; COX, cyclooxygenase; CPR, NADPH:CYP reductase; CYP, cytochrome P450; dA-AAI, 7-(deoxyadenosin-N6-yl)aristolactam I; NQO1, NAD(P)H:quinone oxidoreductase; SULT, sulfotransferase.](image-url)
The question of which of the CYP enzymes are responsible for formation of AAla remains still to be investigated. The in vitro experiments of Xiao et al. indicate that CYP1A generates AAla. However, the model used to evaluate CYP1A participation in formation of AAla in vivo, mice treated with an inducer of CYP1A 3-methylcholanthrene (MC), did not bring unambiguous results. Namely, MC also induces other enzymes besides CYP1A. Although treatment of mice with MC leads to a decrease in AAI concentrations in the liver and kidney, an increase in AAla concentrations was found not in the liver but only in the kidney of mice treated with the higher dose of AAI (20 mg/kg). An increase in excretion of AAla due to its conjugation with glucuronide, caused by induction of UDP-glucuronosyltransferase with MC, could occur. Nevertheless, because CYP1A enzymes also activate AAI to species forming DNA adducts, the decrease of AAI in liver and kidney might also result from this reaction. Moreover, NQO1, which is also efficiently induced by MC, could contribute to decreased AAI levels in MC-treated mice.

Taking into account all data known at the present time, we propose that the pathways of AAI metabolism are dictated by the binding affinity of AAI to CYP1A or NQO1, and their enzymatic turnover, as well as by the balance of the efficiency of CYP1A at oxidizing versus reducing AAI. In order to confirm this assumption and to complement the work of Xiao et al., we have started a study investigating formation of AAI-DNA adducts in the HRN mouse model and in models in which CYP1A genes are deleted.

Although the impact of individual enzymes that metabolize AAI on its nephrotoxicity and carcinogenicity in vivo is still not entirely resolved, one question was unambiguously answered by Xiao et al.: hepatic CYP enzymes detoxicate AAI in mice, thus decreasing its renal toxicity. The evaluation of interindividual variations in the human enzymes playing a major role in AAI activation and detoxication, including their genetic polymorphisms, remains a major challenge to explain an individual’s susceptibility to AAI and to predict cancer risk among AAN and BEN patients.

REFERENCES

Plasma exchange for myeloma kidney: cast(s) away?

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Leung et al. (this issue) present a retrospective study of 40 patients. Observations in 14/40 led to the suggestion of restitution of plasma exchange for light-chain responsive, biopsy-proven myeloma kidney until a better randomized control trial (RCT) is constructed. A careful analysis of their study and a recent RCT suggest little difference in outcome between plasma exchange and control groups. The analysis supports restitution of a better RCT of plasma exchange for myeloma kidney rather than off-label use.

Acute kidney injury in the setting of multiple myeloma has a strong impact on patient morbidity, mortality, health-care utilization, and cost.1–4 A unique and important cause of acute kidney injury in multiple myeloma is cast nephropathy, in which renal inflammation results from an excess of filtered monoclonal light chains that are transported to the interstitium of the kidney via specific receptors in the proximal tubule. The receptors become overloaded by the light chains, which then combine with Tamm–Horsfall protein, forming obstructive casts in the renal tubules.5 Plasma exchange has been shown to remove light chains transiently and may have an adjunctive effect when combined with effective chemotherapy in the treatment of cast nephropathy.1,2,5

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