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DNA adduct formation and mutation induction by aristolochic acid in rat kidney and liver

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Abstract

Aristolochic acid (AA) is a potent nephrotoxin and carcinogen and is the causative factor for Chinese herb nephropathy. AA has been associated with the development of urothelial cancer in humans, and kidney and forestomach tumors in rodents. To investigate the molecular mechanisms responsible for the tumorigenicity of AA, we determined the DNA adduct formation and mutagenicity of AA in the liver (nontarget tissue) and kidney (target tissue) of Big Blue rats. Groups of six male rats were gavaged with 0, 0.1, 1.0 and 10.0 mg AA/kg body weight five times/week for 3 months. The rats were sacrificed 1 day after the final treatment, and the livers and kidneys were isolated. DNA adduct formation was analyzed by ^{32}P-postlabeling and mutant frequency (MF) was determined using the λ Select-*cII* Mutation Detection System. Three major adducts (7-[deoxyadenosin-N^6-yl]-aristolactam I, 7-[deoxyadenosin-N^6-yl]-aristolactam II and 7-[deoxyguanosin-N^2-yl]-aristolactam I) were identified. There were strong linear dose-responses for AA-induced DNA adducts in treated rats, ranging from 25 to 1967 adducts/10^8 nucleotides in liver and 95–4598 adducts/10^8 nucleotides in kidney. A similar trend of dose-responses for mutation induction also was found, the MFs ranging from 37 to 666×10^{-6} in liver compared with the MFs of 78–1319×10^{-6} that we previously reported for the kidneys of AA-treated rats. Overall, kidneys had at least two-fold higher levels of DNA adducts and MF than livers. Sequence analysis of the *cII* mutants revealed that there was a statistically significant difference between the mutation spectra in both kidney and liver of AA-treated and control rats, but there was no significant difference between the mutation spectra in AA-treated livers and kidneys. A:T → T:A transversion was the predominant mutation in AA-treated rats; whereas G:C → A:T transition was the main type of mutation in control rats. These results indicate that the AA treatment that eventually results in kidney tumors in rats also results in significant increases in DNA adduct formation and *cII* MF in kidney. Although the same treatment does not produce tumors in rat liver, it does induce DNA adducts and mutations in this tissue, albeit at lower levels than in kidney.

Keywords: Aristolochic acid; DNA adduct; Mutagenicity; Transgenic rat

1. Introduction

Herbal drugs derived from *Aristolochia* species have been used for medicinal purposes since antiquity. Aristolochic acid (AA) is a family of structurally related nitrophenanthrene carboxylic acids, mainly consisting of aristolochic acid I (AAI) and aristolochic acid II (AAII), and is the active component of the extracts used in these herbal medicines. Following the observation that AA is mutagenic and carcinogenic, the sale of pharmaceuticals containing AA was banned in many European (e.g. Germany, UK) and Asian countries (e.g. Japan), Australia, and Canada.

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Long-term oral treatment of mice and rats with AA results in the time- and dose-dependent induction of tumors in multiple tissues. When AA was administered orally to rats for 3 months in doses ranging from 0.1 to 10.0 mg/kg, the animals developed squamous cell carcinomas in the forestomach and malignant tumors in the kidney and urinary tract [1]. In mice, AA treatment results in squamous cell carcinoma of the forestomach, adenocarcinoma of the glandular stomach, kidney adenomas, lung carcinomas, and uterine haemangiomas [2]. AA is also mutagenic in bacterial [3,4] and mammalian [5,6] short-term tests.

In the early 1990s, a rapidly progressive interstitial nephropathy, originally called Chinese herb nephropathy (CHN) and now known as aristolochic acid nephropathy (AAN [7]), was reported in Belgian patients who used Chinese herbs containing AA as part of a weight-loss program [8,9]. Similar cases subsequently were observed world-wide [10,11]. Soon thereafter, AA-associated urothelial cancer was reported in CHN/AAN patients [12–14], and specific AA-derived DNA adducts, identified as 7-(deoxyadenosin-N^6-yl)-aristolactam I (dA–AAI), were found in the kidney, ureter, bladder, liver, lung, and spleen of the patients [15–17]. These findings provide strong evidence linking the use of herbal products containing AA with cancer development. Due to the potential for serious public health risk, the U.S. Food and Drug Administration issued a Consumer Advisory in April 2001 warning consumers against using dietary supplements and other botanical products containing AA and requesting a recall of these products [18].

It is hypothesized that AA is bioactivated by cytochrome P450s and subsequently reacts with cellular proteins and DNA, leading to multiple forms of toxicity, including gene mutation and tumor induction. Previously we found that the mutant frequency (MF) in the kidneys of Big Blue rats treated with different doses of AA was positively correlated with the tumor incidence previously reported for the same treatments [19]. The available data indicate that AA is activated in the liver, kidney, and other tissues, but only targets the kidney and forestomach for tumor induction in rodents. In the present study, we used transgenic Big Blue rats to compare DNA adduct and mutation induction in kidney (target tissue) and liver (nontarget tissue) to determine their relationships with tumorigenicity.

2. Materials and methods

2.1. Chemical and animals

Aristolochic acid (AA) was purchased from Sigma (St. Louis, MO). The AA content of the test agent was 96% (40% AAI and 56% AAII). Male Big Blue transgenic rats were obtained from Taconic Laboratories (Germantown, NY) through purchase from Stratagene (La Jolla, CA). All animal procedures followed the recommendations of the NCTR Institutional Animal Care and Use Committee for the handling, maintenance, treatment, and sacrifice of the rats.

2.2. Treatments

The treatment schedule was based on the previous carcinogenesis study [1]. Six-week-old Big Blue rats were treated with AA as its sodium salt at concentrations of 0.1, 1.0, and 10.0 mg/kg body weight by gavage five times/week for 12 weeks. Vehicle control rats were gavaged with 0.9% sodium chloride using the same schedule as for the AA-treated rats. Six rats from each treatment group were sacrificed 1 day after the last treatment. The livers and kidneys were isolated, frozen quickly in liquid nitrogen, and stored at −80 °C.

2.3. DNA adduct analysis by ^{32}P-postlabeling

Liver and kidney DNAs were isolated by a standard phenol extraction method. DNA adducts were determined by the ^{32}P-postlabeling procedure described recently [20], with minor modifications. DNA samples (4 μg) were digested with micrococcal nuclease (240 mU; Sigma, Poole, UK) and calf spleen phosphodiesterase (60 mU; Calbiochem, Nottingham, UK) in digestion buffer containing 20 mM sodium succinate and 10 mM calcium chloride (pH 6.0) for 3 h at 37 °C in a total volume of 10 μl. For nuclease P1 enrichment, the digests were incubated with 4 μg nuclease P1 (MP Biomedicals, London, UK) in 3 μl of a buffer containing 0.8 M sodium acetate (pH 5.0) and 2 mM zinc chloride for 30 min at 37 °C. The reaction was terminated by the addition of 3 μl Tris base (427 mM). DNA digests were then ^{32}P-labeled by adding 4 μl of a mixture consisting of 400 mM bicine (pH 9.5), 200 mM magnesium chloride, 300 mM DTT, 10 mM spermidine, 50 μCi [γ-^{32}P]ATP (approximately 7000 Ci/mmol; MP Biomedicals), and 6 U T4 polynucleotide kinase (USB, Cleveland, OH), and incubated for 30 min at 37 °C. Resolution of ^{32}P-labeled adducts was by thin layer chromatography (TLC) on polyethyleneimine-cellulose (PEI-cellulose) sheets (10 cm × 20 cm; Macherey-Nagel, Düren, Germany). TLC sheets were scanned using a Packard Instant Imager (Dowers Grove, IL), and DNA adduct levels were calculated from the adduct cpm, the specific activity of [γ-^{32}P]ATP, and the amount of DNA (pmol of DNA-P) used. Results are expressed as DNA adducts per 10^8 normal nucleotides. Enzymatic preparation of AA–DNA adduct reference compounds was performed as described previously [21].

2.4. cII mutation assay

High-molecular-weight genomic DNA was extracted from rat livers and kidneys using the RecoverEase DNA Isolation Kit (Stratagene) and stored at 4 °C until DNA packaging was performed. The packaging of the phage, plating the pack-