Detection of DNA Adducts Formed by Aristolochic Acid in Renal Tissue from Patients with Chinese Herbs Nephropathy

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Abstract

A unique type of rapidly progressive renal fibrosis, designated Chinese herbs nephropathy (CHN), has been described in young Belgian women who had followed a slimming regimen including recently introduced Chinese herbs (Stephania tetrandra and Magnolia officinalis). Aristolochic acid (AA), a known nephrotoxin and carcinogen, was suspected as its causal factor. To substantiate this hypothesis, renal tissue from five patients with CHN and six patients with other renal diseases was analyzed for the presence of AA-derived DNA adducts, a described biomarker of AA exposure associated with its carcinogenic and mutagenic activity. Using the \(^{32}\)P-postlabeling method, a major distinct DNA adduct spot was found in all five cases of CHN and identified by cochromatographic analyses with authentic markers as the deoxyadenosine adduct of AA-1 [7-(deoxyadenosin-\(\beta\)-yl)-aristolactam I], the major component of the plant extract AA. This DNA adduct was absent in the six control cases. The 7-(deoxyadenosin-\(\beta\)-yl)-aristolactam I adduct levels in CHN ranged from 0.7 to 5.3/10\(^7\) nucleotides. Our data demonstrate that AA is implicated in CHN. They suggest a mechanism for the urothelial atypia and cancers observed in this disease and raise the possibility that a DNA mutation is responsible for the kidney-destructive fibrotic process.

Introduction

CHN\(^2\) nephropathy is a recently described, rapidly progressive interstitial nephritis associated with the intake of Chinese herbs during a slimming cure (1). The nature of the postulated toxic agent, however, remained in doubt. The observation that one of the prescribed Chinese herbs (Stephania tetrandra) had probably been replaced by another one (Aristolochia fangchi; Ref. 2) led to the hypothesis that AA might be the causative agent, despite the fact that it could not be identified in the original pills (1). The active principle of herbal drugs derived from Aristolochia species is AA. AA is a mixture of structurally related nitrophenanthrene carboxylic acids, with AA-1 [8-methoxy-6-nitrophenanthro(3,4-d)-1,3-dioxolo-5-carboxylic acid] being the major component.

In 1964, AA was shown to be nephrotoxic in humans (3), and in 1982, it was shown to be a strong carcinogen in rodents (4). Recently, we demonstrated that its carcinogenic and mutagenic effects were associated with the formation of AA DNA adducts (5, 6). We have characterized the major adducts and used the \(^{32}\)P-postlabeling method to detect and identify them (7, 8). In the present study, we have taken advantage of this method and demonstrate the presence of AA DNA adducts in the kidney tissue of five patients with CHN. These results indicate that AA is implicated in CHN and provide an explanation for the urothelial atypias and transitional cell carcinomas reported in patients with CHN (9, 10, 11).

Materials and Methods

Nephroureterectomy Specimens. Seven native kidneys were removed at the time of transplantation or subsequently in five patients with documented CHN. Samples were analyzed by light and immunofluorescent microscopy as described previously (9). In addition, corticomedullary (cases 1 and 2) and/or cortical tissue samples (cases 1–5) were frozen at −20°C for DNA extraction. Similarly, corticomedullary samples were obtained from six end-stage kidneys removed from six patients at the time of transplantation. Our diagnosis in this control group included lupus nephritis, membranoproliferative glomerulonephritis type I, chronic interstitial nephritis, reflux nephropathy, cystic nephropathy, and nephrosclerosis (one case each).

Identification of DNA Adducts. The method has been described elsewhere (12). Briefly, DNA was isolated from kidney tissue by the phenol extraction method. DNA samples (12.5 µg) were digested enzymatically to nucleoside 3’-phosphates and enriched for adducts by nuclease P1 digestion (13). The digests were then analyzed by \(^{32}\)P-postlabeling. Nuclease P1-resistant adducts were 5’ labeled with \(^{32}\)P-orthophosphate, separated by PEI-cellulose TLC, and detected by autoradiography; autoradiography was performed at 23°C for 6 h. Adducts were measured by assay of their \(^{32}\)P content (12). Their tissue levels were determined in at least three separate experiments and expressed as relative adduct labeling values, as described previously (13, 14).

The comparison of different chromatographic mobilities on PEI-cellulose or on reversed-phase HPLC of single \(^{32}\)P-postlabeled adducts obtained in vivo with those of in vitro-synthesized reference compounds was used for identification purposes. Individual spots detected by the \(^{32}\)P-postlabeling assay were excised from thin-layer plates and extracted as described (12).

Results

Patients. The characteristics of patients with CHN are described in Table 1. All were female, aged 27–42 years. Medical histories prior to attending the slimming clinic were unremarkable. They had taken the Chinese herbs-containing pills (formula 2 regimen as described in the original publication) for 13–23 months. Renal transplantation was performed after an average of 5 (range, 2–10) months of hemodialysis in four patients (three cadavers and one living donor graft) and prior to hemodialysis in one patient (living donor graft). The delay between the end of the slimming cure and renal transplantation ranged from 9 to 27 months. Pathological features have been reported extensively (9) for the first three patients. The kidneys of the two last patients exhibited the same characteristics, including extensive hypocellular interstitial fibrosis, tubular atrophy, global glomerular sclerosis with a corticomedullary gradient, and fibrosis of mainly interlobular arteries. Extensive atypia and atypical hyperplasia of the epithelial lining of the collecting ducts, calyces, pelvis, and ureter were also present. Additional findings included: in case 4, diffuse, intense granular mesangial deposits of IgA; and in case 5, numerous corticomedullary cysts ranging from 1 to 15 mm in diameter, typical of early polycystic disease.

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Table 1  Relevant clinical data and relative adduct labeling (RAL) values of dA-AA-I in kidney tissue of patients with CHN

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)/Sex</th>
<th>Duration of formula 2 regimen (months)</th>
<th>PreTP HD* (months)</th>
<th>Interval from regimen end to kidney removal (months)</th>
<th>RAL in renal tissue (mean ± SD/10^7 nucleotides)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32/F</td>
<td>19</td>
<td>0</td>
<td>Right, 9</td>
<td>1.7 ± 0.9, 3.5 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>28/F</td>
<td>13</td>
<td>4</td>
<td>Left, 17</td>
<td>2.3 ± 1.2, 2.6 ± 2.0</td>
</tr>
<tr>
<td>3</td>
<td>27/F</td>
<td>20</td>
<td>10</td>
<td>Right, 20</td>
<td>5.3 ± 3.2, 3.4 ± 2.1</td>
</tr>
<tr>
<td>4</td>
<td>42/F</td>
<td>21</td>
<td>2</td>
<td>Right, 18</td>
<td>4.1 ± 2.7, n.d.</td>
</tr>
<tr>
<td>5</td>
<td>42/F</td>
<td>23</td>
<td>2</td>
<td>Right, 11</td>
<td>3.0 ± 1.8, n.d.</td>
</tr>
</tbody>
</table>

* PreTP HD, pretransplantation hemodialysis duration.
* Mean of at least three determinations in separate experiments.
* n.d., not determined.
* TCC, transitional cell carcinoma.

Table 2  Control subjects

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age (yr)/Sex</th>
<th>PreTP HD* (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupus nephritis</td>
<td>14/F</td>
<td>51</td>
</tr>
<tr>
<td>MPGN I</td>
<td>12/F</td>
<td>0</td>
</tr>
<tr>
<td>CIN*</td>
<td>27/F</td>
<td>7</td>
</tr>
<tr>
<td>PI</td>
<td>41/F</td>
<td>11</td>
</tr>
<tr>
<td>Cystic nephropathy</td>
<td>10/F</td>
<td>7</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>38/M</td>
<td>12</td>
</tr>
</tbody>
</table>

* PreTP HD, pretransplantation hemodialysis duration.
* MPGN I, membranoproliferative glomerulonephritis type I.
* CIN, chronic idiopathic interstitial nephritis.
* PI, pyelonephritis due to lithiasis.

The characteristics of the control patients are given in Table 2. They include four females and one male aged 10–41 years. Renal transplantation was performed after an average of 18 (range, 7–51) months of hemodialysis in five patients and prior to hemodialysis in one patient.

DNA Adduct Detection and Identification. Using the nuclease P1-enhanced variation of the ^32P-postlabeling assay, one major (spot 1) and several minor spots of DNA adducts were observed in all renal samples obtained from the five patients with CHN (Fig. 1). To obtain reference compounds for the identification of AA DNA adducts by cochromatography, the deoxyadenosine (dA-AA-I) and deoxyguanosine adducts [7-(deoxyguanosin-β-yl)-aristolactam 1] of AA-I were prepared by in vitro incubation, as described previously (12). By cochromatographic analysis on PEI-cellulose TLC plates (data not shown) and reversed-phase HPLC (Fig. 2) with those reference compounds, we found that the spots in Fig. 1 were chromatographically indistinguishable from dA-AA-I and thus assigned as 3',5'-bisphospho-7-(deoxyadenosin-β-yl)-aristolactam 1.

It is clear from Fig. 2 that spot 1 from Fig. 1a eluted with a retention time of 24 min (Fig. 2b), identical to the dA-AA-I standard (Fig. 2a). The identity of the two spots was also confirmed by cochromatographic HPLC analysis, shown in Fig. 2c. Equal amounts of radioactivity obtained from spot 1 (Fig. 1a) and the dA-AA-I standard were mixed prior to analysis and found to elute as a single peak.

The levels of this adduct in DNA samples ranged from 0.7 to 5.3/10^7 nucleotides and were similar in cortical and corticomedullary samples (Table 1).

By contrast, DNA isolated from the control kidneys was virtually free of adducts in the area where AA-derived spots were located (Fig. 1f).

Discussion

These results conclusively demonstrate for the first time that CHN is associated with the intake of AA. Furthermore, they provide a physiopathological clue as to the cause of the urothelial atypias observed in several patients and of the transitional cell carcinoma of the bladder observed in patient 3.

Fig. 1. Autoradiographs of two-dimensional chromatograms of ^32P-labeled DNA adducts from human kidneys. (a–e), patients with CHN: a, case 1; b, case 2; c, case 3; d, case 4; e, case 5; f, control patient with lupus nephritis.
The role of AA in the genesis of CHN is still under debate (15). Although the evidence that the prescribed herb, *S. tetrandra*, was replaced by another is compelling, because the *Stephania* alkaloid tetrandrine was not present in the pills used (1), AA was not initially identified in the Chinese herbs contained in the pills. Subsequent analysis of the batches of Chinese herbs imported in Belgium between July 1990 and August 1992 under the name of *S. tetrandra* disclosed variable amounts of AA. The failure to detect AA in the pills was then ascribed to an inadequate extraction procedure (2). The discovery of AA adducts in the kidneys of five patients with CHN conclusively demonstrates that AA had indeed been ingested in amounts sufficient to alter cellular DNA.

The natural extract of aristolochia plants consists of structurally related nitrophenanthrene carboxylic acids, AA-I and -II, being the major components. In rats given AA-I or the natural extract AA, it persists apparently throughout the animals' lives (16), a feature that probably explains why dA-AA-I was still detectable in the kidneys of patients with CHN 9–27 months after the interruption of formula 2 pills. The persistence of such specific adducts suggests nonrepairable lesions in a variety of tissues exposed to carcinogens (17).

The specific adduct levels of 0.7–5.3/10^7 nucleotides found in this study are approximately 10 times higher than the levels of bulky DNA adducts in humans reported by others (18, 19). They are similar to those reported in DNA from forestomachs of rats treated with AA at a dose of 10 mg/kg body weight twice weekly for 2 weeks (12). Both interindividual variability in the metabolism of AA and batch-to-batch differences of AA contents in the capsules are major factors influencing adduct levels. Hence, a correlation between adduct levels and the duration of capsule intake is unlikely and was indeed not found.

Specific DNA adducts within the genomic DNA have an important biological significance as premutagenic lesions. This has been verified by us for dA-AA-I adducts; Ha-ras proto-oncogenes are activated with high frequency by a A→T transversion mutation in codon 61 from CAA to CTA in the DNA of AA-I-induced forestomach carcinomas in rats (5).

The role of the dA-AA-I adducts in AA-induced mutagenesis and carcinogenesis appears relevant and might be responsible for the atypia observed in the urothelia of patients with CHN (9) as well as for the multifocal transitional cell carcinomas observed in two of them (10, 11).

Finally, the possibility that the AA DNA adduct also initiated the renal fibrotic process that destroyed the kidney remains an intriguing possibility. The fact that the fibrotic process was not confined to the kidney but also extended to the pelvis and ureter in one patient (9) and contributed perhaps to the occurrence of aortic valvular lesions in 40% of the patients with CHN supports a generalized effect of the ingested toxic compound. The amount of ingested AA calculated on the basis of the batch analyses (2) is minimal when compared with that given experimentally to rats (12) and in the range prescribed without untoward effects in traditional Chinese medicine (20). Still, the fact that such quantities are capable of altering DNA to a significant extent raises the possibility that AA has a toxic effect synergistic with some of the other compounds included in the slimming cure (15).

Altogether, we demonstrate the presence of AA metabolites as DNA adducts in patients with CHN, thus establishing that CHN is indeed associated with the presence of AA. This observation further suggests that dA-AA-I DNA adducts are responsible for the urothelial cancers observed in CHN. Therefore, patients with CHN should undergo regular follow-ups for urothelial cancer.

References


3 Unpublished observations.
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