**Aristolochic acid associated nephropathy:**

*Pathology*

Extrarenal manifestations:
- Aortic insufficiency
- Peritoneal fibrosis
- Periureteral fibrosis

Michel Depierreux et al - 1994
The clinical and pathological manifestations of aristolochic acid nephropathy: Report of 58 cases

- Acute tubular necrosis
- Tubular dysfunction – degeneration atrophy – R.T.A., Fanconi syndrome
- Chronic aristolochic acid nephropathy


Aristolochic acid associated nephropathy: Pharmacology

Structure activity relationships of AA analogues: toxicity in cultured epithelial cells – nitro- and methoxygroup critical

Studies on pharmacodynamic characteristics of AA I in rats
- oral administration
- highest concentration in liver and kidney
- partial metabolism in the liver and accumulation in the kidney
**Aristolochic acid associated nephropathy: Mechanism (1)**

Aristolochic acids induce chronic renal failure with interstitial fibrosis in salt-depleted rats


**Rabbit model:**

Cosyns JP et al:
Chronic aristolochic acid toxicity in rabbits: A model of Chinese herbs nephropathy?
Kidney Int 59: 2164-2173, 2001

**Aristolochic acid associated nephropathy: Mechanism (2)**

The renin-angiotensin system blockade does not prevent renal interstitial fibrosis induced by aristolochic acids


Dexfenfluramine does not enhance aristolochic acid nephrotoxicity in rats

### Aristolochic acid nephropathy: A worldwide problem

**Most relevant studies investigating the renal effects of aristolochic acid in animal models**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosage</th>
<th>Duration</th>
<th>AA components</th>
<th>Renal findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/Mice</td>
<td>38-88 mg kg⁻¹ day⁻¹ orally or 130-300 mg kg⁻¹ orally and i.v., depending on species</td>
<td>One shot</td>
<td>AA (0.7%)/AA (21%)</td>
<td>Several PT cells necrosis, C3H ranging from 56 to 203 mg kg⁻¹</td>
<td>Debelle FD et al, Kidney Int 74: 158-169, 2008</td>
</tr>
<tr>
<td>Rat</td>
<td>6.2 / 16.5, or 25 mg kg⁻¹ / day orally or parenterally</td>
<td>26 days</td>
<td>AA (0.7%)/AA (21%)</td>
<td>Proteinuria and granulocytes, PT cells apoptosis and mild necrosis at highest dose, renal interstitial inflammatory cells infiltration, AT 100 mg kg⁻¹</td>
<td>Lebeau C et al: Kidney Int 60: 1332, 2001</td>
</tr>
<tr>
<td>Rat</td>
<td>16, 50, or 100 mg kg⁻¹ orally or parenterally</td>
<td>1-40 mg kg⁻¹ per day</td>
<td>AA (0.7%)/AA (21%)</td>
<td>1a, 2a, and 3a, and necrosis of PT (granulocytes)</td>
<td></td>
</tr>
<tr>
<td>MDW</td>
<td>6.1 mg kg⁻¹ day⁻¹, 17-31</td>
<td>AA (44%)/AA (56%)</td>
<td>1a, 2a, and 3a, and necrosis of PT (granulocytes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>1 or 10 mg kg⁻¹ day⁻¹, s.c.</td>
<td>35 days</td>
<td>AA (40%)/AA (60%)</td>
<td>Renal tubular interstitial fibrosis.</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>15 mg kg⁻¹ day⁻¹, i.p.</td>
<td>14 days</td>
<td>AA (44%)/AA (56%)</td>
<td>During the regeneration phase (day 29), circulating transferrin-receptor positive cells reduced, AA-induced renal fibrosis partially through a decrease in TIMP-1 and TGF-β activity.</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>15 mg kg⁻¹ day⁻¹, s.c.</td>
<td>35 days</td>
<td>AA (40%)/AA (60%)</td>
<td>AA inulin clearance reduced ED-1 + macrophage infiltration but not interstitial fibrosis induced by AA.</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>25 mg kg⁻¹ day⁻¹, 24 h</td>
<td>14 days</td>
<td>AA (0.7%)/AA (40%)</td>
<td>AA or AA/AA or Aristolochic acid</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>10 mg kg⁻¹ day⁻¹, s.c.</td>
<td>35 days</td>
<td>AA (40%)/AA (60%)</td>
<td>AA/AA or AA or Aristolochic acid. AA inulin clearance reduced ED-1 + macrophage infiltration but not interstitial fibrosis induced by AA.</td>
<td></td>
</tr>
</tbody>
</table>

**Control**

10 µmol AA
20 µmol AA
20 µmol AA + 24 h recovery

**Reference**


---

### Aristolochic acid associated nephropathy: Physiopathology (1)

Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells


---

**Control**

15 µM CdCl₂
0.1% DMSO
20 µM AA

**Results**

10 µmol AA
20 µmol AA
20 µmol AA + 24 h recovery
Aristolochic acid associated nephropathy: Physiopathology (2)

Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells


Expression of megalin and exposure to aristolochic acid in OK cells: immunoblot analysis

Aristolochic acid associated nephropathy: Physiopathology (3)

Synergetic effect of monocyte-chemotactic protein-1 and AA on transdifferentiation of human tubular epithelial cells in vitro


AA-induced apoptosis in LLC-PK1 cells and amelioration of the apoptotic damage by calcium antagonists


Patients with AA nephropathy: mast cell infiltration – proliferation of myofibroblast

Aristolochic acid associated nephropathy: Treatment

- Cortisone treatment – unsuccessful–successful  

- Effects of prostaglandin E1 in the progression of aristolochic acid nephropathy: slowing of the progression (6 months)  

- Chemoprotective effect of five drugs on renal interstitial fibrosis induced by an aristolochic acid-containing Chinese herb in rats  

- Simvastatin: shows a protective effect on development of renal fibrosis induced by Mu Tong (an aristolochic acid-containing Chinese herb)

- Transgene-derived hepatocyte growth factor attenuates reactive fibrosis in aristolochic acid nephrotoxicity  

possible therapeutic effect of low-dose HGF

Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi)

"The prevalence of urothelial carcinoma among patients with end-stage Chinese herb nephropathy (caused by aristolochia species) is high"

<table>
<thead>
<tr>
<th>Mean duration of use and total doses of components of weight-reducing pills and levels of aristolochic acid–related DNA adducts</th>
<th>UROTHELIAL CARCINOMA PRESENT (N=10)</th>
<th>UROTHELIAL CARCINOMA ABSENT (N=21)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ±SD</td>
<td>mean ±SD</td>
<td></td>
</tr>
<tr>
<td>Compound labeled as containing Stephania aurea*</td>
<td>226±23</td>
<td>167±17</td>
<td>0.038</td>
</tr>
<tr>
<td>Total dose (g)</td>
<td>15±1.4</td>
<td>12±1.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Duration of use (mo)</td>
<td>93.1±10.1</td>
<td>66±7.6</td>
<td>0.036</td>
</tr>
<tr>
<td>Magnolia officinalis (g)</td>
<td>47.3±6.1</td>
<td>24.8±5.5</td>
<td>0.013</td>
</tr>
<tr>
<td>Atractylodes (g)</td>
<td>17.8±2.6</td>
<td>12.5±2.1</td>
<td>0.13</td>
</tr>
<tr>
<td>Testicular (g)</td>
<td>14.0±2.2</td>
<td>12.2±1.9</td>
<td>0.20</td>
</tr>
<tr>
<td>Norepinephrine (g)</td>
<td>29.0±9.3</td>
<td>31.2±7.8</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*The compound actually contained Aristolochia fangchi.

Aristolochic acid associated nephropathy: Urothelium

| Mild urothelial atypia | Moderate urothelial atypia | Severe urothelial atypia |

Aristolochic acid associated nephropathy: Aristolochic acid associated nephropathy: Urothelium


Aristolochic acid associated nephropathy: Urothelial carcinoma in situ

Overexpression of p53 protein

Chinese Herbs: Stromal Invasion


Chinese Herbs: Papillary Transitional Cell Carcinoma

Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi)

Autoradiograms of DNA adducts in renal tissue from two patients with Chinese-herb nephropathy


Aristolochic acid and the etiology of endemic (Balkan) nephropathy

HPLC, MS and Quant. P32 postlabeling DNA adducts found in renal cortex of patients with BEN

“signature” mutation

p53 mutational spectra in transitional cell carcinomas

AA adducts and mechanism of toxicity ???

Activation of p53 Promotes Renal Injury in Acute Aristolochic Acid (AA) Nephropathy

Li Zhou,*† Ping Fu,* Xiao R. Huang,†‡ Fei Liu,*† Kar Neng Lai,† and Hui Y. Lan†‡

- AA induced dephosphorylation of STAT3 and the subsequent activation of p53 and TEC apoptosis.
- In contrast, overexpression of STAT3, p53 inhibition, or p53 knockdown with small interfering RNA all attenuated AA-induced TEC apoptosis.
- These results suggest that AA induces TEC death via apoptosis by dephosphorylation of STAT3 and posttranslational activation of p53, supporting the hypothesis that:
- p53 promotes renal injury in acute AAN.

Li Zhou et al JASN 21: 31 – 41 ; 2010
Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi)

Risks factors for urothelial carcinoma in patients with end-stage Chinese herb nephropathy

<table>
<thead>
<tr>
<th></th>
<th>ALL PATIENTS</th>
<th>UROTHelial CARCINOMA PRESENT (N = 18)</th>
<th>UROTHelial CARCINOMA ABSENT (N = 21)</th>
<th>P VALUE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of compound labeled as containing Stephania tetrandra</td>
<td>39 (100)</td>
<td>18 (100)</td>
<td>21 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>Concomitant use of Magnolia officinalis</td>
<td>38 (97)</td>
<td>18 (100)</td>
<td>21 (100)</td>
<td>0.99</td>
</tr>
<tr>
<td>Concomitant use of acetazolamide</td>
<td>27 (69)</td>
<td>14 (78)</td>
<td>13 (62)</td>
<td>0.32</td>
</tr>
<tr>
<td>Concomitant use of desferrioxamine</td>
<td>16 (41)</td>
<td>5 (28)</td>
<td>11 (52)</td>
<td>0.19</td>
</tr>
<tr>
<td>Concomitant use of phenytoin</td>
<td>7 (18)</td>
<td>3 (11)</td>
<td>5 (24)</td>
<td>0.43</td>
</tr>
<tr>
<td>Mesotherapy</td>
<td>31 (79)</td>
<td>15 (82)</td>
<td>16 (76)</td>
<td>0.70</td>
</tr>
<tr>
<td>Concomitant regular use of analgesics‡</td>
<td>8 (21)</td>
<td>3 (17)</td>
<td>5 (24)</td>
<td>0.70</td>
</tr>
<tr>
<td>Concomitant regular use of nonsteroidal antiinflammatory drugs‡</td>
<td>7 (18)</td>
<td>4 (24)</td>
<td>3 (14)</td>
<td>0.68</td>
</tr>
<tr>
<td>Concomitant smoking</td>
<td>12 (31)</td>
<td>7 (39)</td>
<td>5 (24)</td>
<td>0.49</td>
</tr>
</tbody>
</table>


Aristolochic acid nephropathy: A worldwide problem


Chinese herb/AAN and Balkan endemic nephropathy: the two faces of Janus?
Aristolochic acid induces proximal tubule apoptosis and epithelial to mesenchymal transformation

Representative photomicrographs and time course of the semiquantitative score of N-cadherin and Jones membrane staining

Historical Henna

- Bangladesh 12th C. AD
- Knossos 1600 BC
- Persia 15th C. AD
- Morocco

Catherine Cartwright Jones
Takaout: Hair Dye

Natural Takaout El Beldia
(Tamaris Orientalis) (Morocco).

- Natural plant
- Non toxic
- Rare
- Black dye hair

Takaout Roumia
(Paraphenylene diamine) (Soudan).

- C6H4(NH2)2
- Mineral
- Highly toxic
- Free selling
- Black dye hair
- Cosmetic agent
- Films

Poisoning with hair-dye containing paraphenylene diamine

Renal failure

- Acute:
  - Ingestion
  - Suicidal attempt

- Chronic:
  - Skin contact
  - Focal glomerulosclerosis

Paraphenylene diamine intoxication

Glomerular (A) and tubular (B-D) lesions. H&E staining.

A. Glomerular swelling and hypercellularity. B. Inflammatory cells are present around eosinophilic intraluminal material (arrow). Some tubular epithelial cytoplasm is attenuated. C. Tubular basement membrane is distinct in some areas. D. Intraluminal inflammatory cells are seen with a small proteinaceous cast. The tubular basement membrane is indistinct.

Nephrotoxicity due to Chronic Paraphenylene Diamine (Hair Dye)

Hamdouck M Abdelraheem et al
Aslam Kidney Center
Khartoum, Sudan

- 72 hairdressers, Henna tattoo designers (mean age 40.3 years) with chronic contact with PPD
- Duration of exposure varied from 1 to 21 years.
- Albuminuria 26.4%, haematuria 41.1%, anaemia 28.8%, hypertension 29.4%.
- In eleven of the participants to this study renal function impairment was detected. 1.6-26 mg/dl
- Chronic PPD exposure may cause renal damage related to duration and type of PPD used.
- Renal lesion is characterized by prominent interstitial fibrosis and tubular atrophy.

Different features of Chinese Herb Nephropathy and Balkan Endemic Nephritis

<table>
<thead>
<tr>
<th></th>
<th>Chinese Herb Nephropathy</th>
<th>Balkan Endemic Nephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of progression</td>
<td>rapid</td>
<td>very slow</td>
</tr>
<tr>
<td>Kidney survival</td>
<td>2 years (17%)</td>
<td>over 20 years</td>
</tr>
<tr>
<td>Extrarenal manifestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic insufficiency</td>
<td>documented in 40%</td>
<td>not described</td>
</tr>
<tr>
<td>Peritoneal fibrosis</td>
<td>described</td>
<td>not found</td>
</tr>
<tr>
<td>Perireteral fibrosis</td>
<td>described</td>
<td>not found</td>
</tr>
<tr>
<td>Urinary deposit</td>
<td>aseptic leukocyturia, 40%</td>
<td>scarce deposit</td>
</tr>
<tr>
<td>Gross morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney atrophy</td>
<td>asymmetric, 54%</td>
<td>symmetric</td>
</tr>
<tr>
<td>Outline</td>
<td>irregular, 31%</td>
<td>smooth</td>
</tr>
<tr>
<td>Kidney morphology</td>
<td>extensive interstitial fibrosis and loss of tubular structures</td>
<td>interstitial fibrosis</td>
</tr>
</tbody>
</table>
Kidney syndromes from alternative medicines (1):• Acute tubular necrosis
  - Traditional African medicine: toxic plants
    (Securidaca longipedunculata, Euphorbia matabalensis, Crotalaria laburnifolia, Heliotropium, Symphytum, Senecio plants, Callilepsis laureola, Atractylis gummifera, Cape aloes) or adulteration by dichromates
  - Sri Lankan traditional Ayurvedic pharmacopoeia: toxic plants
    (Crotalaria species, Cassia auriculata, Hollarrhena antidysenterica)
- Asian rural areas: raw carp bile
- Saudi Arabia: raw sheep bile
- China: Taxus celebrica
- Hydrazine sulfate
- Mentha spicata
- Chinese herbs contaminated with arsenic (Niu Huang Chieh tu Pien, …)
- Morocco: Takaout roumia (paraphenylenediamine, PPD)
- Sudan: henna adulterated with PPD
- Cantharidin
- Chelation therapy with EDTA

Kidney syndromes from alternative medicines (2):• Proximal tubulopathy (Fanconi’s syndrome)
  - Glycyrrhiza species (herbal cough mixtures, (Chinese) herbal teas, gancao, bougiougiougi--toutou
  - Chinese herbs contaminated with cadmium
• Distal tubular toxicity
  - Germanium
• Acute interstitial nephritis
  - Traditional African medicine: toxic plants (Cape Aloes)
  - Peruvian medicine (Uno degatta)
  - China: Taxus celebrica
  - Chinese herbs adulterated with non steroidal anti-inflammatory drugs: (Tung Shueh pills)
  - Hypericum, ledum
• Analgesic nephropathy, Papillary necrosis
  - Willow bark (Salix species)
  - Chinese herbs adulterated with non steroidal anti-inflammatory drugs (indomethacine, diclofenac, mefenamic acid, phenylbutazone): Chuilfong Tuokuwan, Tung Shueh pills, other
• Hypertension
  - Glycyrrhiza species (herbal cough mixtures, (Chinese) herbal teas, gancao, Bougiougiougi--toutou
**Kidney stones**
- Ephedra containing herbal preparations (*Ma huang*, dietary supplements containing ephedra alkaloids)
- Cranberry juice (oxalate)
- With cadmium contaminated Chinese herbs

**Urinary retention**
- Niger: *Datura species* (Sobi-lobi) [56]
- Chinese herbs: *Rhodondendron molle*, *Rehmania glutinosa*, *Carthamus tinctorius*, *Atropa belladona*, *Hyoscyamus niger*, *Datura species*

**Acute interstitial nephritis**
- Traditional African medicine: toxic plants (*Cape Aloes*)
- Peruvian medicine (*Uno degatta*)
- China: *Taxus celebica*
- Chinese herbs adulterated with non steroidal anti-inflammatory drugs: (*Tung Shueh pills*)
- *Hypericum, ledum*

**Chronic tubulo-interstitial nephritis with fibrosis**
- *Chinese herbs containing Aristolochia sp.: Belgian slimming regimen, Mokutsu, Boui*
- China: *Jia Wey Guo Sao pills (herbal mixture, no aristolochia)*

**Chronic kidney disease of unknown etiology in Sri Lanka**
- Slow, progressive, asymptomatic development
- Age less than 20 years at start
- Men working in agriculture
- ESRF 40-60 yrs
- Anaemia
- Bilateral small kidney, loss of cortico-medullary differentiation. Renal biopsy, tub atrophy, fibrosis.
- Hypertension is not prevalent
- Ochratoxin excluded, cadmium; other environm toxin???
Chronic kidney disease of unknown etiology in Sri Lanka

Teaching hospital Anuradhapura - ↑ of deaths to CKD


Mortality due to renal causes:
- Vanumija first
- Anuradhapura 3th
- Polonnaruwa 4th
- Jaffna 6th

Organigram

National task force
Secretary Ministry of Health, Country Representative WHO and others

Technical advisory committee
Steering committee
Lead investigators of main studies
Central database management (Imperial College/WHO HQ)

International advisory board

Project field officer

Collection of samples
- renal biopsy
- post mortem tissues

Environmental studies

- epidemiological survey
- case control studies
- diet studies