Renal Excretion of Aristolochic Acid I in the IPK

Mariana Babayeva MD, PhD
Touro College of Pharmacy,
New York, NY, USA
Aristolochic Acid I

- Compound extracted from plant *Aristolochia*
- Nephrotoxin and carcinogen
Adverse Effects of AA-I

- AA-I is an organic anion eliminated by the kidney
- Produces nephrotoxic effect to S3 segment of renal proximal tubule

- **Chinese herbs nephropathy (CHN)**
- **Belgian nephropathy**
- **Balkan endemic nephropathy (BEN)**
- **Herbal remedies**
- Slimming pills: *Aristolochia fangchi*
- Bread contaminated by *Aristolochia clematitis*
Renal Excretion

- Glomerular Filtration
  - GFR: glomerular filtration rate

- Tubular Secretion
  - Active transport
    - Secretion transporters: OATs, OCTs, etc.

- Tubular Reabsorption
  - Active transport
    - Reabsorptive transporters
  - Passive transport

- Glomerular Filtration (1)
- Tubular Secretion (2)
- Tubular Reabsorption (3)
- Renal Excretion (4)
Renal Organic Anion Transport (OAT)

- OAT system plays an important role in tubular secretion and reabsorption of compounds (organic anions)
Objectives

- The overall goal of the research was to assess transport mechanism of renal excretion of AA-I.

- Further identify potential strategies to mitigate drug toxicity by reducing renal uptake
Methods

Step 1: HPLC Method

Step 2: Protein Binding Studies

Step 3: IPK Studies
Isolated Perfused Kidney (IPK) Model

- Assessment of renal drug excretion mechanism
  - Dose-linearity
  - Inhibition studies
- Drug interaction screening
- Model for nephrotoxicity
- Probing renal drug metabolism
- Gender differences in renal function and drug excretion
- Correlation between drug excretion and membrane transporter expression
- Model for aging
- Studies in mutant strains (genetic “knockout” animals)
IPK Apparatus
Perfusate Composition

- Krebs-Henseleit buffer (KHS buffer)
- Bovine serum albumin (BSA)
- Dextran
- Glucose
- Inulin
- Amino acids
  - Mixture of 20 amino acids
# IPK Study Groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Compound(s) (Concentration)</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Perfusion</td>
<td>None</td>
<td>Establish viability of preparation and allow for evaluating of drug effects on kidney function</td>
</tr>
<tr>
<td>AA-I Excretion</td>
<td>Aristolochic acid I (20uM)</td>
<td>Obtain baseline parameter values of renal excretion and of AA-I</td>
</tr>
<tr>
<td>AA-I Transport Inhibition</td>
<td>Aristolochic acid I (20uM) + Probenecid (1mM)</td>
<td>Study mechanisms of AA-I renal transport</td>
</tr>
</tbody>
</table>
IPK Experimental Design

- **Stabilization Period**
  - Time = 0 min (Initiation of Perfusion)
  - Time = 10 min (Vehicle or Probenecid Added) (Urine Collection 1)

- **Distribution Period**
  - Time = 15 min Perfusate Sample 1
  - Time = 20 min (AA-I Added) (Urine Collection 2)

- **Distribution Period**
  - Time = 25 min Perfusate Sample 2
  - Time = 30 min (Urine Collection 3)

- **Drug Excretion Periods** (total = nine)

  - 10-minute urine collection intervals (40 min (collection 4), 50 min (collection 5) etc.)
  - Perfusate sampled at midpoint of interval (35 min (sample 3), 45 min (sample 4), etc.)
## IPK Viability Criteria

<table>
<thead>
<tr>
<th>Viability Parameters</th>
<th>Minimum Acceptable Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR</td>
<td>&gt; 0.5 ml/min</td>
</tr>
<tr>
<td>Glucose Reabsorption (FR$_{\text{Glu}}$)</td>
<td>&gt; 90%</td>
</tr>
<tr>
<td>Sodium Reabsorption (FR$_{\text{Na}}$)</td>
<td>&gt; 85%</td>
</tr>
<tr>
<td>Urine Flow Rate</td>
<td>&gt; 0.03 ml/min</td>
</tr>
</tbody>
</table>
Renal Excretion Parameters

\[ Cl_r = \frac{dX_u}{dt/C_p} \]

- \( dX_u/dt \) = urinary drug excretion rate
- \( C_p \) = perfusate drug concentration

\[ XR = \frac{Cl_r}{fu \times GFR} \]

- \( XR \) = excretion ratio
- \( CLr \) = clearance
- \( fu \) = fraction unbound
- \( GFR \) = glomerular filtration rate

\( XR > 1 \) = net secretion process
\( XR < 1 \) = net reabsorption process
Results

- Protein Binding of AA-I
- IPK Viability Parameters
- AA-I Renal Excretion Parameters
Protein Binding Studies

Ultrafiltration technique was used for the protein binding studies

Table 1. Summary of AAI Protein Binding Studies in Perfusate

<table>
<thead>
<tr>
<th>AA-I Concentration (uM)</th>
<th>Unbond Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3.16 ± 0.1373</td>
</tr>
<tr>
<td>10</td>
<td>2.63 ± 0.2622</td>
</tr>
<tr>
<td>5</td>
<td>2.71 ± 0.4946</td>
</tr>
</tbody>
</table>

Data reported as mean ± SD

There were no significant differences in protein binding among the different concentrations of AA-I (ANOVA, p >0.05)
Protein Binding Studies

Table 2. Effect of Probenecid on AAI Protein Binding in Perfusate

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Unbond Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-I (20 uM)</td>
<td>2.980 ± 0.3456</td>
</tr>
<tr>
<td>AA-I (20 uM) &amp; PBC (1 mM)</td>
<td>3.250 ± 0.4931</td>
</tr>
</tbody>
</table>

Data reported as mean ± SD

There was no significant difference in protein binding between the two study groups (ANOVA, p >0.05)
Kidney function was well maintained across all study groups.
The IPK technique has been successfully applied.
### Renal Excretion Studies

<table>
<thead>
<tr>
<th>Renal Excretion Parameter</th>
<th>AA-I (20 μM)</th>
<th>AA-I (20 μM) Probenecid (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>0.90 ± 0.24</td>
<td>0.80 ± 0.28</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>0.0020 ± 0.0008</td>
<td>0.0035 ± 0.0013</td>
</tr>
<tr>
<td>XR</td>
<td>0.081 ± 0.042</td>
<td>0.165 ± 0.097</td>
</tr>
<tr>
<td>Perfusate Recovery (% Dose)</td>
<td>80.99 ± 8.78</td>
<td>93.02 ± 5.81</td>
</tr>
<tr>
<td>Urinary Recovery (% Dose)</td>
<td>0.23 ± 0.086</td>
<td>0.46 ± 0.157</td>
</tr>
<tr>
<td>% Dose Unaccounted</td>
<td>18.78 ± 8.81</td>
<td>6.51 ± 5.67</td>
</tr>
</tbody>
</table>

*Data presented as mean ± SD of data representing IPK drug excretion periods.

The renal excretion parameters ~ 2-fold higher in studies with PBC
Effect of Probenecid on AA-I Excretion in the IPK.

Plot of Cumulative Amount of AA-I Excreted in Urine vs Time.
Results

- XR of AA-I < 1 (0.08 and 0.17) for both study groups
  - The results suggest net reabsorption

- XR of AA-I was more than 2-fold higher in the presence of probenecid
  - Probenecid inhibited the tubular reabsorption of AA-I most probably by interaction with renal organic anion transport system

- The amount of unrecovered AA-I in Phase I studies was 3-fold higher than in Phase II
  - Probenecid decreased accumulation of AA-I in the kidney cells
Discussion

Bidirectional Transport: Oat2 / Oat5
The S3 segment of proximal tubule is the most vulnerable part to AA-I toxicity.

Rat Oat2 and Oat5 are localized to the apical membrane of S3 segment of proximal tubules.

AA-I has a high affinity to Oat2.

Previous findings:

Oat2 and Oat5 may take part in active renal reabsorption of AA-I.

Reabsorption of AA-I by Oat2 and Oat5 may cause tubular injury of S3 segments of proximal tubules.

Inhibition of reabsorption can decrease nephrotoxicity of AA I.

Assumption:
Conclusion

- The present study described the transport mechanism of Aristolochic acid I renal excretion.

- The observation suggested that the renal apical transporters (Oat2 and Oat5) may function as reabsorptive pathway during renal elimination of AA-I.

- Inhibition of AA-I reabsorption can decrease nephrotoxicity of AA-I. This assumption requires further investigation.
Thank You!