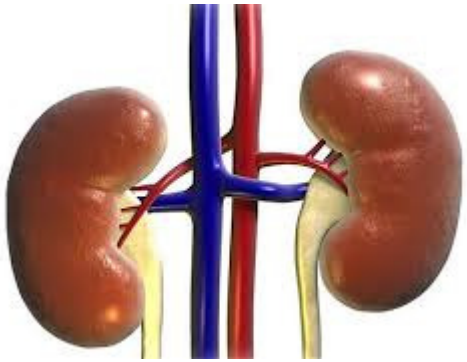
Renal Excretion of

Aristolochic Acid I in the IPK



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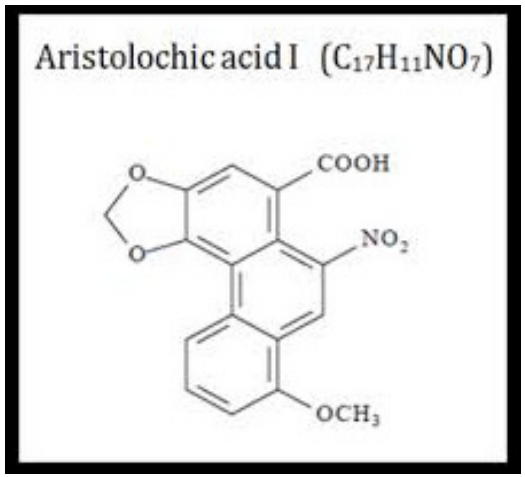
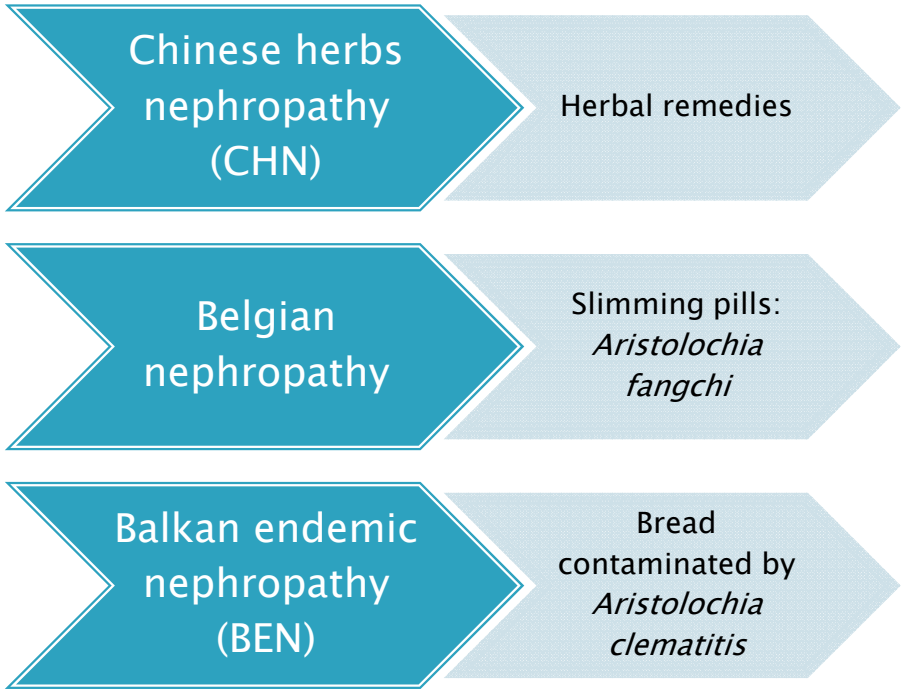
Aristolochic Acid I

- Compound extracted from plant *Aristolochia*
- Nephrotoxin and carcinogen



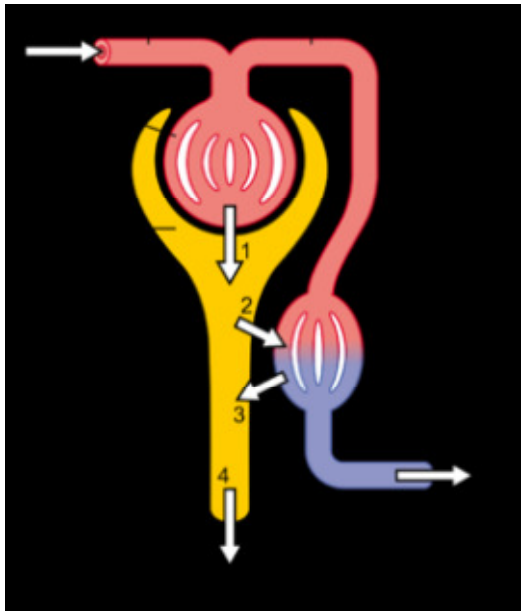
Adverse Effects of AA-I

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



Renal Excretion

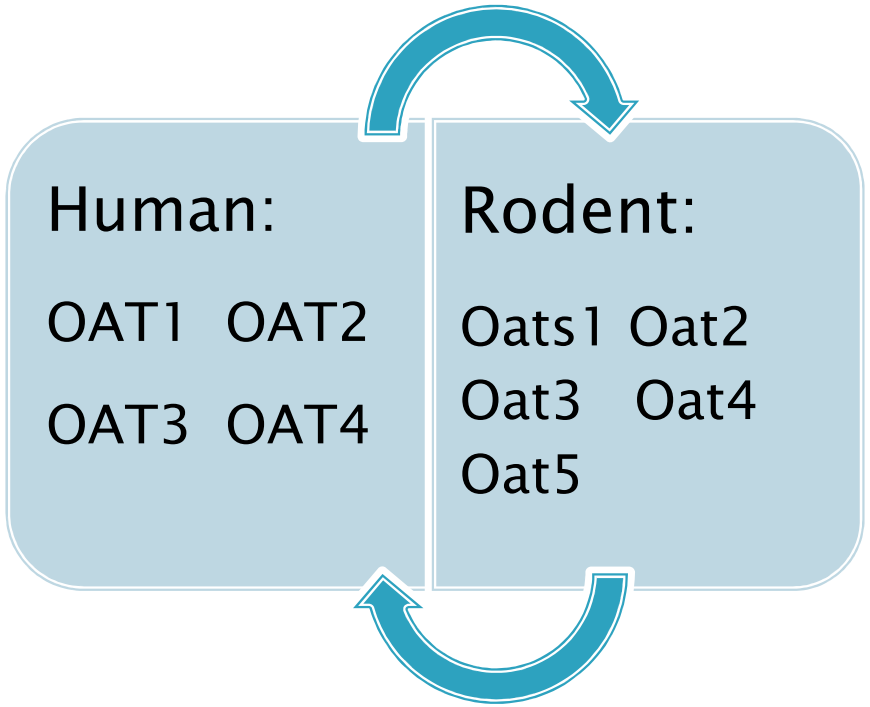
- ❑ Glomerular Filtration
- GFR: glomerular filtration rate
- ❑ Tubular Secretion
- Active transport
  - Secretion transporters: OATs, OCTs, etc.
- ❑ Tubular Reabsorption
- Active transport
  - Reabsorptive transporters
- Passive transport



- ❖ 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)

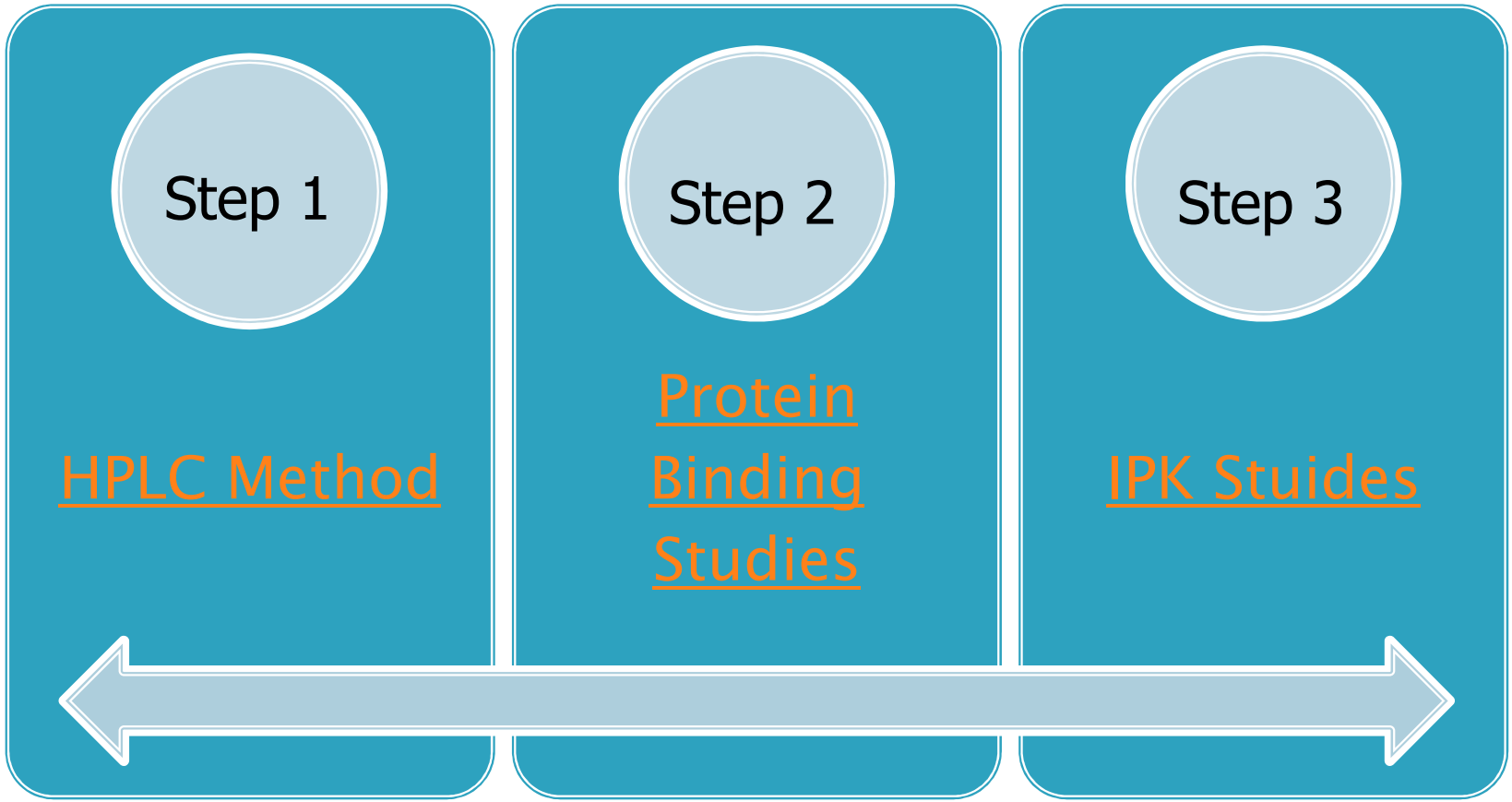


Objectives

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

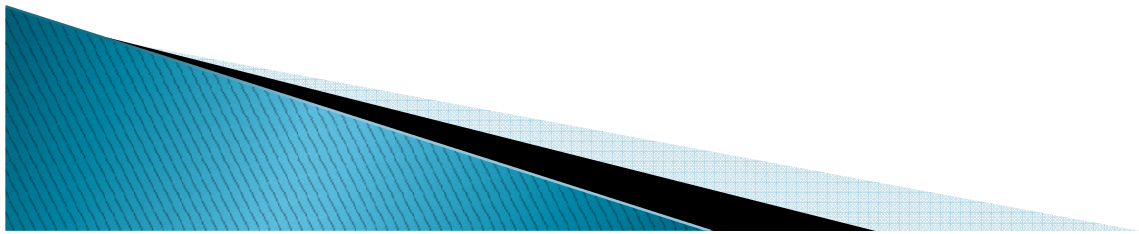
- Further identify potential strategies to mitigate drug toxicity by reducing renal uptake

Methods

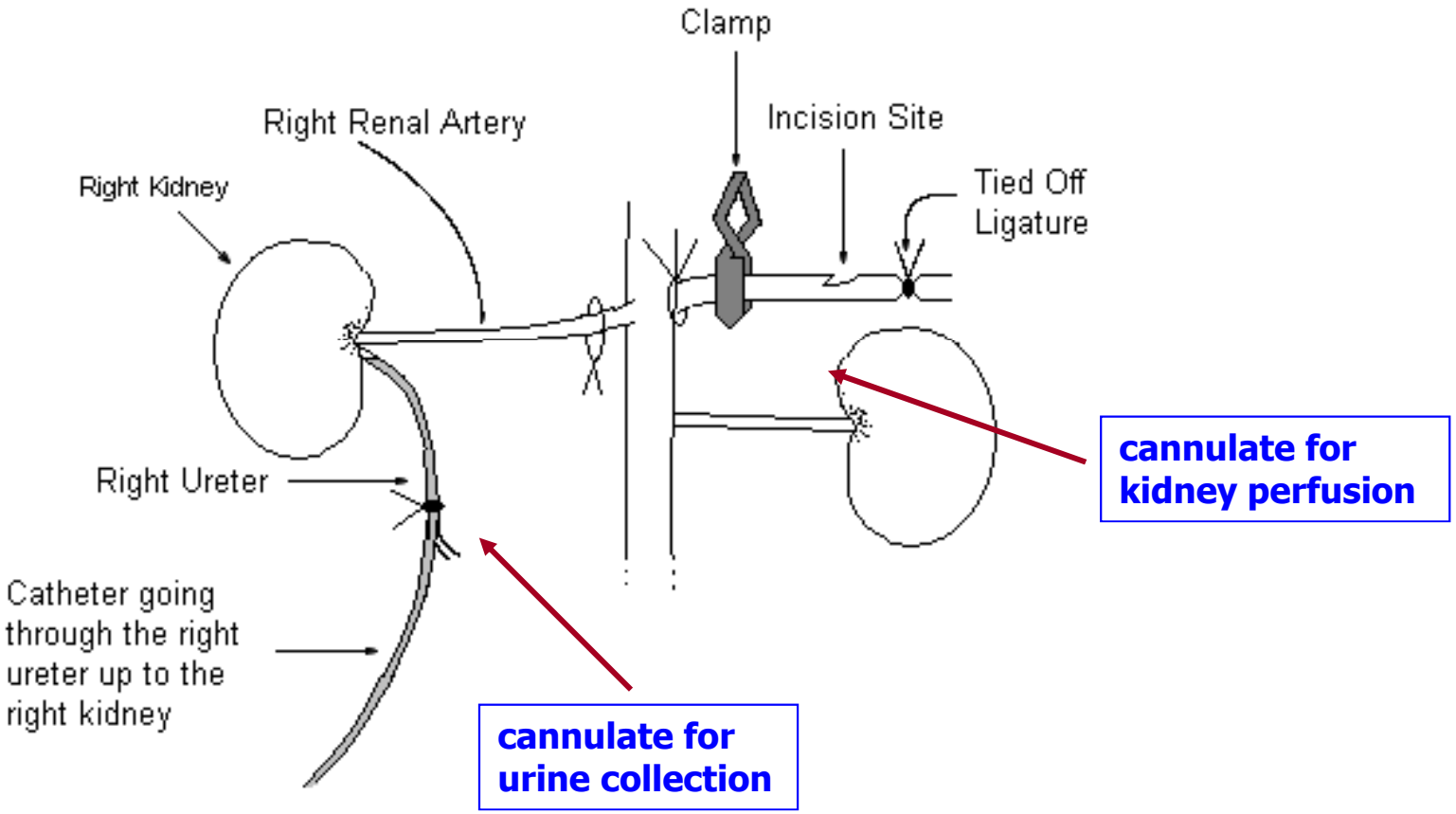

Step 1
HPLC Method
Step 2
Protein Binding Studies
Step 3
IPK Stuides

Isolated Perfused Kidney (IPK) Model

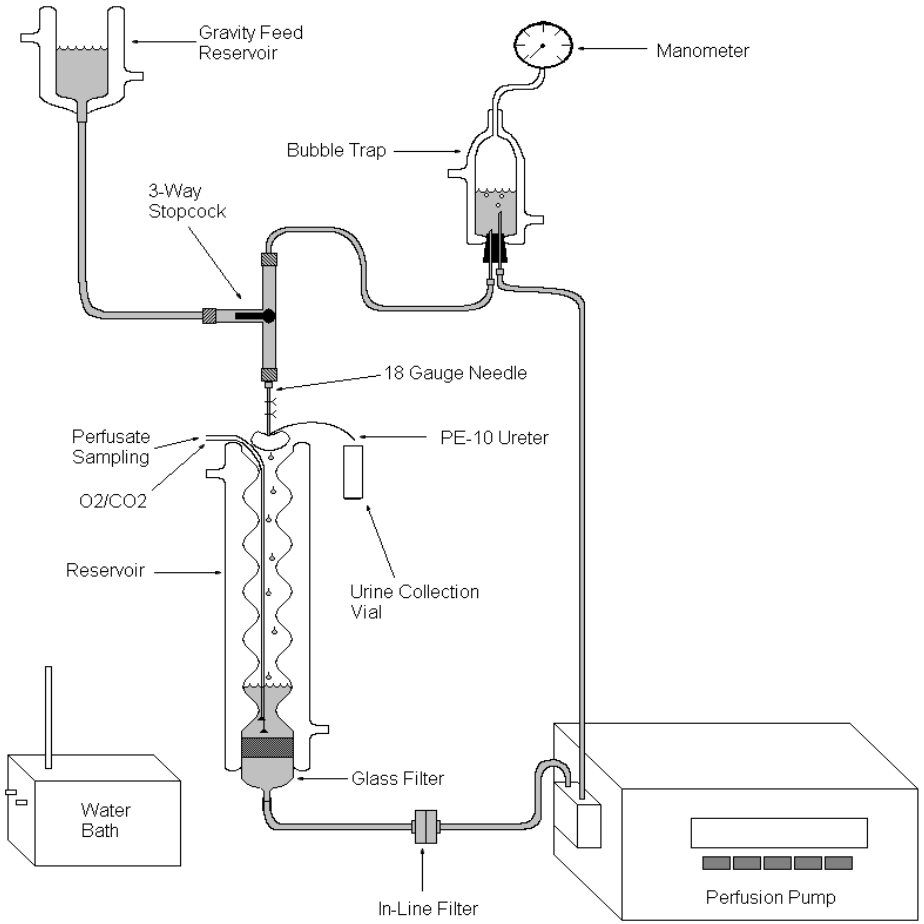
- Assessment of renal drug excretion mechanism
  - Dose-linearity
  - Inhibition studies
- Drug interaction screening
- Model for nephrotoxicity
- Probing renal drug metabolism
- Gender differences in renal function and drug excretion
- Correlation between drug excretion and membrane transporter expression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



IPK Surgical Procedure

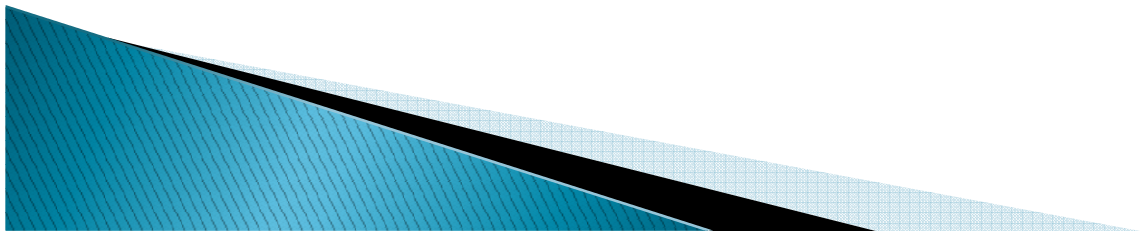


IPK Apparatus



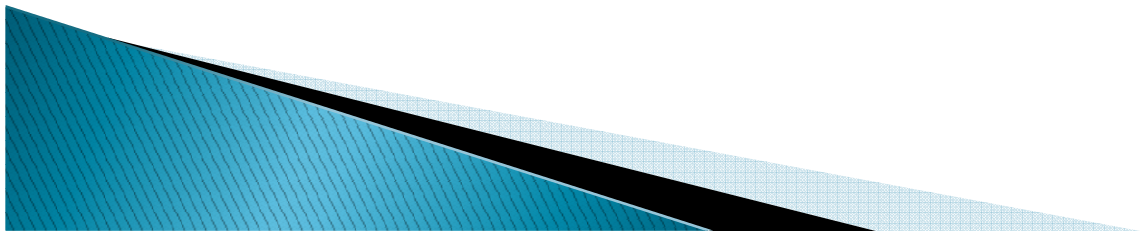
Perfusate Composition

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

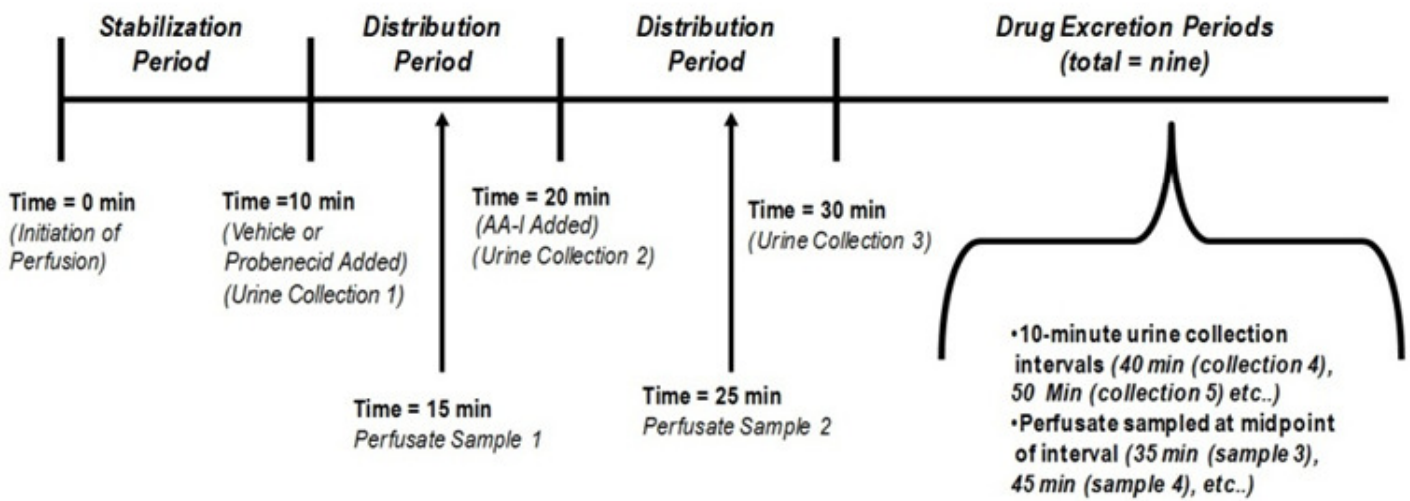


IPK Study Groups

Treatment Group	Compound(s) (Concentration)	Justification
Control Perfusion	None	Establish viability of preparation and allow for evaluating of drug effects on kidney function
AA-I Excretion	Aristolochic acid I (20uM)	Obtain baseline parameter values of renal excretion and of AA-I
AA-I Transport Inhibition	Aristolochic acid I (20uM) + Probenecid (1mM)	Study mechanisms of AA-I renal transport

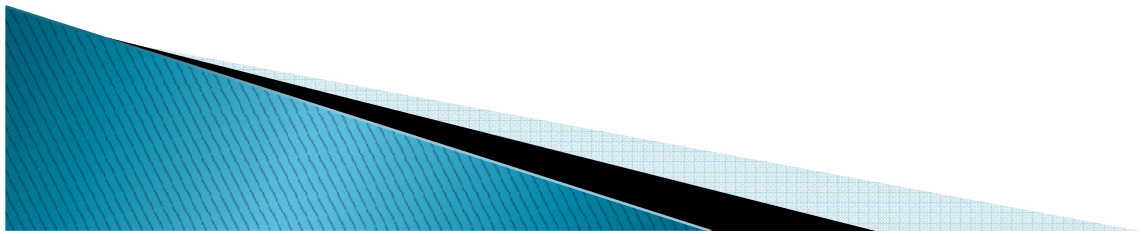


IPK Experimental Design



IPK Viability Criteria

Viability Parameters	Minimum Acceptable Value
GFR	> 0.5 ml/min
Glucose Reabsorption (FR_{Glu})	> 90%
Sodium Reabsorption (FR_{Na})	> 85%
Urine Flow Rate	> 0.03 ml/min



Renal Excretion Parameters

$$Cl_r = \frac{dX_U / dt}{C_p}$$

- dXU/dt = urinary drug excretion rate
- Cp = perfusate drug concentration

$$XR = \frac{Cl_r}{f_u \times GFR}$$

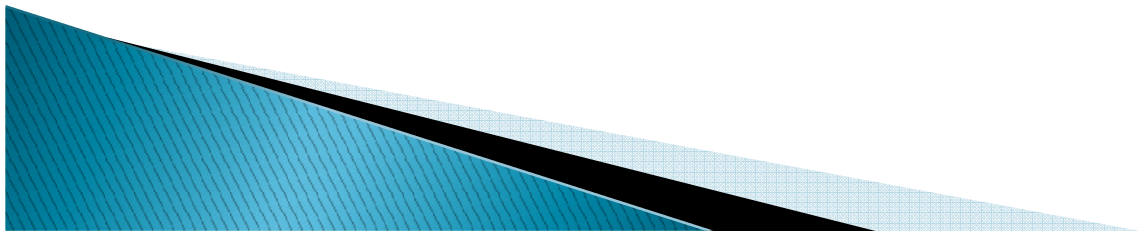
- XR = excretion ratio
- CLr = clearance
- fu = fraction unbound
- GFR = glomerular filtration rate

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

AA-I Concentration(uM)	Unbond Percent (%)
20	3.16 ± 0.1373
10	2.63 ± 0.2622
5	2.71 ± 0.4946

[a] 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

Experimental Group	Unbond Percent (%)
AA-I (20 uM)	2.980 ± 0.3456
AA-I (20 uM) & PBC (1 mM)	3.250 ± 0.4931

[a] Data reported as mean ± SD

There was no significant difference in protein binding between the two study groups (ANOVA, p >0.05)

Viability of the Perfused Kidney

Table 4. Summary of IPK Viability Parameters [a]

IPK Viability Parameter	Control	AA-I (20uM)	Transport Studies AA-I (20uM) & PBC (1 mM)
Urine Flow Rate (ml/min)	0.11 ± 0.03	0.13 ± 0.03	0.14 ± 0.01
Urine pH	7.16 ± 0.27	7.05 ± 0.55	6.91 ± 0.26
GFR (ml/min)	0.63 ± 0.11	0.9 ± 0.24	0.8 ± 0.28
$FR_{glucose}$	93.75 ± 2.04	93.7 ± 2.42	92.75 ± 5.12
FR_{Sodium}	90.84 ± 2.50	92.22 ± 4.58	87.01 ± 5.37

[a] Data presented as mean ±SD of data representing IPK drug excretion periods

[b] Abbreviation for transport inhibitor: PBC, probenecid

Kidney function was well maintained across all study groups
The IPK technique has been successfully applied

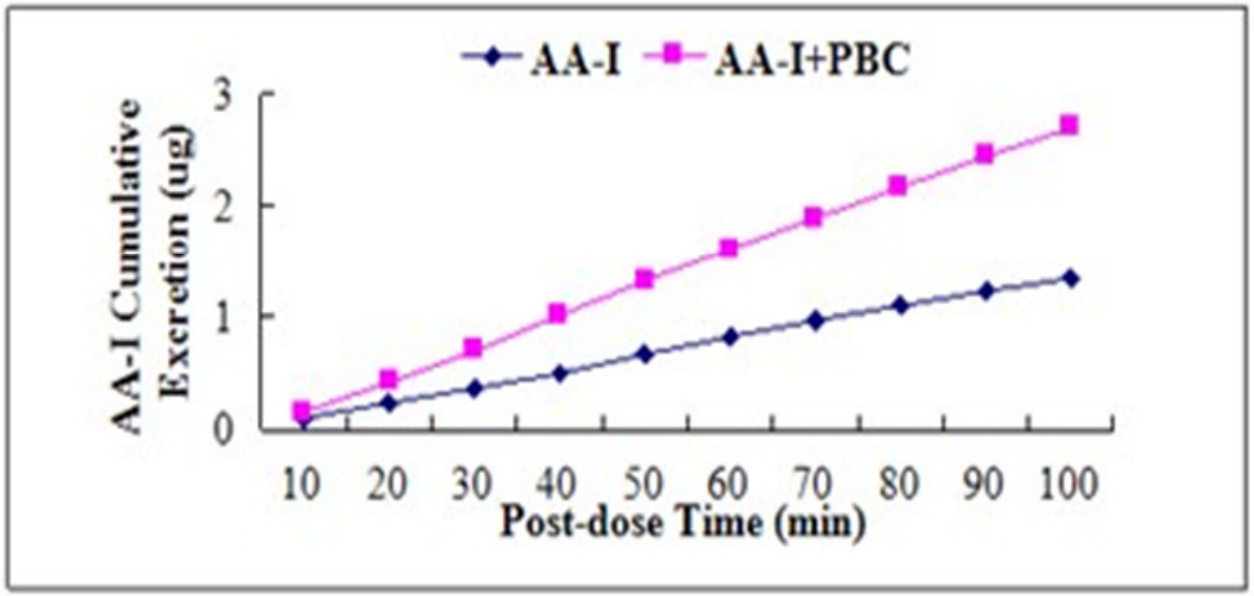
Renal Excretion Studies

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

[a]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.



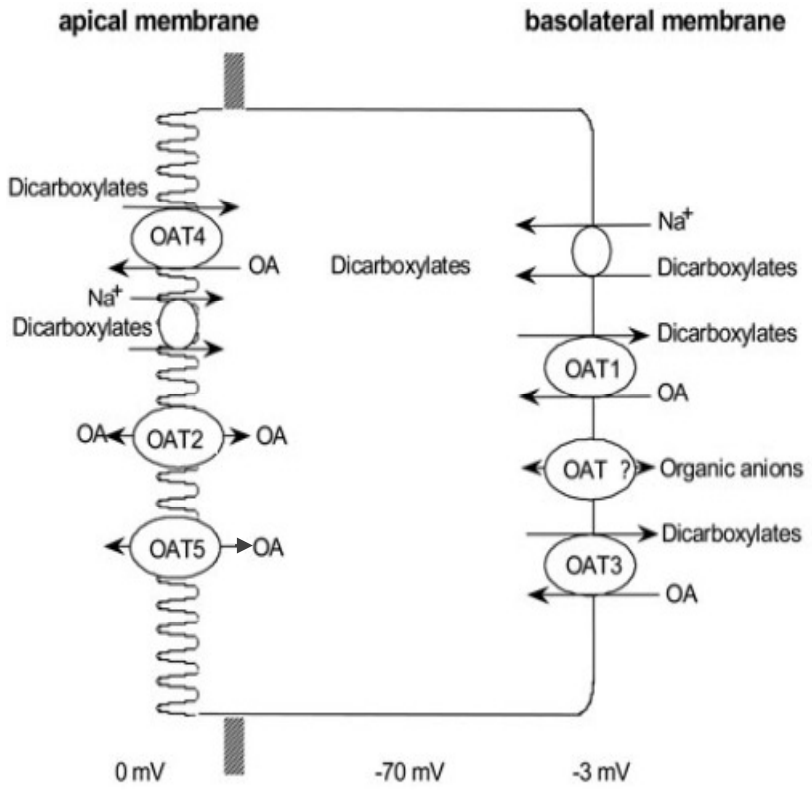
Plot of Cumulative Amount of AA-I Excreted in Urine vs Time.

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



Discussion

- 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 Oat5may 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!