SUSTIVA: UNCOVERING THE MECHANISM FOR THE METABOLISM-DEPENDENT, SPECIES-SELECTIVE NEPHROTOXICITY

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SUSTIVA®
EFAVIRENZ

- CURRENTLY THE PREFERRED NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR FOR THE COMBINATION THERAPY OF HIV-AIDS
- EFFECTIVE AT 600 MG ONCE DAILY
- INDUCES CYP 2B, 3A AND UGT ENZYMES IN RODENTS AND PRIMATES INCLUDING HUMANS
- IS A SUBSTRATE FOR CYP 2B6 (8’-HYDROXYLATION)
YOU KNOW YOU’RE HAVING A BAD DAY WHEN...

CONTROL (20X)  EFAVIRENZ-TREATED (20X)

EFAVIRENZ PRODUCED PROXIMAL CONVOLUTED TUBULAR EPITHELIAL CELL NECROSIS IN RATS (BUT NOT MONKEYS) IN A DOSE-DEPENDENT MANNER
THE LACK OF NEPHROTOXICITY IN MONKEYS IS NOT THE RESULT OF POOR EXPOSURE

**Graphs showing Cmax and AUC for different treatments in rats, monkeys, and humans.**

- Cmax: Rat, Monkey, Human
  - 250 mg/kg bid (rat)
  - 75 mg/kg bid (monkey)
  - 600 mg (human)

- AUC: Rat, Monkey, Human
  - 250 mg/kg bid (rat)
  - 75 mg/kg bid (monkey)
  - 600 mg (human)
DRUG-RELATED CRYSTALS PRESENT IN RAT URINE AFTER NEPHROTOXIC ORAL DOSES OF EFAVIRENZ
EFAVIRENZ PRODUCES PROXIMAL TUBULAR EPITHELIAL CELL NECROSIS IN RATS

• WHAT WAS KNOWN
  – NEPHROTOXICITY WAS FOUND ONLY IN RATS, NOT MONKEYS
  – METABOLITE PROFILE IN ANY SPECIES WAS UNDEFINED
  – A CRYSTALLINE SEDIMENT WAS PRESENT IN RAT URINE

HYPOTHESES

HIGH CONCENTRATIONS OF A METABOLITE WERE PRECIPITATING OUT IN THE TUBULES LEADING TO RENAL DAMAGE AND CRYSTALURIA (CHEMICAL PATHWAY)

OR

A SPECIES-SPECIFIC REACTIVE METABOLITE WAS FORMED OR PROCESSED IN RATS (BIOCHEMICAL PATHWAY)
ISOLATION AND IDENTIFICATION OF THE URINARY CRYSTALS AS THE 8-HYDROXYGLUCURONIDE

- CRYSTALS WERE ISOLATED FROM THE URINE OF RATS DOSED ORALLY WITH 500 MG/KG/DAY, DRIED AND DISSOLVED IN 2H METHANOL

- STRUCTURE WAS DETERMINED BY MS AND NMR AND ULTIMATELY CONFIRMED BY SYNTHESIS OF AUTHENTIC METABOLITE

- pH DEPENDENT SOLUBILITY WAS DETERMINED BY PHARMACY
  - pH 5.3 0.5 mg/mL  
  - pH 6.4 8.7 mg/mL

- TOXICOKINETIC STUDIES WITH ISOLATED AND SYNTHETIC 8-OH GLUCURONIDE AND 8-OH EFAVIRENZ WERE CONDUCTED WITH RATS

- CONCENTRATIONS OF BOTH METABOLITES WERE MEASURED IN PATIENT PLASMA AND URINE
HYPOTHESIS B: RATS FORM OR PROCESS A SPECIES-SELECTIVE NEPHROTOXIC METABOLITE

KEY QUESTIONS

WHAT ARE THE METABOLITES FORMED AND EXCRETED BY EACH SPECIES, INCLUDING HUMANS?

IF THERE IS A RAT-SELECTIVE METABOLITE, HOW IS IT FORMED AND HOW CAN THE FORMATION AND PROCESSING BE RELATED TO THE NEPHROTOXICITY OBSERVED?
SPECIES-DEPENDENT METABOLISM OF EFAVIRENZ

R=rats
M=monkeys
H=humans

M1 (R, M, H)
M2 (R, M, H)
M6 (M, H)

M4 (R, M, H)

M3 (R, M)

M11 (R, M)

M14 (M, H)

M7 (M, H)

M5 (M, H)

M8 (M)

M10 (R)

M9 (R)

M1 (R, M, H)
M2 (R, M, H)
M6 (M, H)

M11 (R, M)

M14 (M, H)

M7 (M, H)

M5 (M, H)

M8 (M)

M9 (R)

M10 (R)
Rat-Specific Formation of Efavirenz Glutathione Conjugate

Metabolite Formed By:
Human, Monkey, Rat
Monkey, Rat
Rat
SELECTIVE DEUTERATION INHIBITS EFAVIRENZ OXIDATION AND ULTIMATE GLUTATHIONE CONJUGATION
STUDY PROTOCOL FOR DEUTERATED EFAVIRENZ ADMINISTRATION

Day 1 | Day 2 | Day 3 | Day 4 | Day 5
---|---|---|---|---
EFA 30 mg/kg bid po

Collect 24-Hour Urine

EFA 700 mg/kg po
or
$^{2}$H-EFA 700 mg/kg po

NECROPSY
DEUTERATED EFAVIRENZE DECREASES THE URINARY EXCRETION OF THE CYS-GLY BUT NOT THE 8-OH GLUCURONIDE METABOLITE OF EFAVIRENZE

* p < 0.05
DEUTERATED EFAVIRENZ DECREASES THE SEVERITY AND INCIDENCE OF NEPHROTOXICITY

**EFA 700 mg/kg po**

**2H-EFA 700 mg/kg po**
INHIBITION OF EFAVIRENZ GLUTATHIONE CONJUGATE CATABOLISM BY ACIVICIN

Pretreatment with Acivicin Inhibits GGT Activity
STUDY PROTOCOL FOR THE INHIBITION OF GGT ACTIVITY
BY THE PRETREATMENT WITH THE SELECTIVE INHIBITOR
ACIVICIN

Day 1 | Day 2 | Day 3
---|---|---
EFA 30 mg/kg bid po

Day 4 | Day 5
---|---
ACIVICIN 10 mg/kg iv
EFA 700 mg/kg po

Collect 24-Hour Urine
1 HR

NECROPSY
ACIVICIN PRETREATMENT DECREASES THE URINARY EXCRETION OF THE CYS-GLY BUT NOT THE 8-OH GLUCURONIDE METABOLITE AFTER EFAVIRENZ.

* p < 0.05
ACIVICIN PRETREATMENT DECREASES THE INCIDENCE AND SEVERITY OF NEPHROTOXICITY

**Graph:**

- **EFA 700 mg/kg po**
  - Number of affected rats: 1, 2, 3, 4
  - Histo-logy severity score: 0, 1, 2, 3, 4

- **ACIVICIN + EFA 700 mg/kg po**
  - Number of affected rats: 3, 4
  - Histo-logy severity score: 0, 1, 2, 3,
RENAL PROCESSING OF GLUTATHIONE CONJUGATES

Glutathione-S-Transferase (GST)

R + Glu-Cys-Gly $\rightarrow$ Glu-Cys-Gly

$\gamma$ - Glutamyltranspeptidase (GGT)

R-Cys-Gly

Dipeptidase

R-Cys

N-Acetyltransferase

R-Cys-Ac

$\beta$-lyase

R-SH

Potentially Toxic Intermediates
FORMATION OF POTENTIAL NEPHROTOXIC REACTIVE METABOLITES

Pathway a

Cysteinylglycine conjugate, M10

Pathway b

Interaction with cellular nucleophiles

Sulfoxide intermediate

β-lyase

Formation of thioketene

Interaction with cellular nucleophiles

R = cysteine or cysteinylglycine

TOXICITY

Cysteine conjugate

Dipeptidase

CYP450

Formation of thioketene

Pathway a

Pathway b
CONCLUSIONS

• SELECTIVE DEUTERATION DIMINISHED THE CYP MEDIATED OXIDATION AND FORMATION OF THE CYCLOPROPANOL INTERMEDIATE, AN OBLIGATE SPECIES IN THE FORMATION OF THE ULTIMATE NEPHROTOXICANT

• CONJUGATION WITH GLUTATHIONE AND SUBSEQUENT RENAL PROCESSING OF THIS CONJUGATE ULTIMATELY LEADS TO THE RAT SELECTIVE NEPHROTOXICITY

• MECHANISMS AND RELEVANCE OF REACTIVE INTERMEDIATE FORMATION AND DISPOSITION CAN ONLY BE DETERMINED BY THE APPLICATION OF BIOANALYTICAL CHEMISTRY, DRUG METABOLISM, KINETICS AND BIOCHEMICAL TOXICOLOGY