Deciphering the Mechanism of Cinchophen-Induced Liver Injury

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Introduction

Drug induced liver injury (DILI) is a leading cause of drug attrition due to preclinical toxicity. The drug cinchophen (discovered in 1887) and introduced to patients in 1908 as a treatment for gout and subsequently for treatment of pain, was one of the first examples of causing DILI. With a high fatality rate of ~50%, it resulted in the death of hundreds of patients over a 30 year period.

Its mechanism of DILI remains unclear. Toxic cirrhosis with jaundice were a common account of clinical cases and post-mortem examination of liver tissue from fatal cases revealed severe liver damage characterised by widespread necrosis, cell infiltration and marked tissue atrophy.

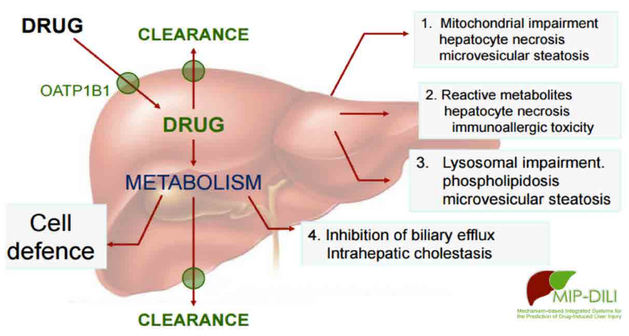


Figure 1: Chemical Insults and liver injury

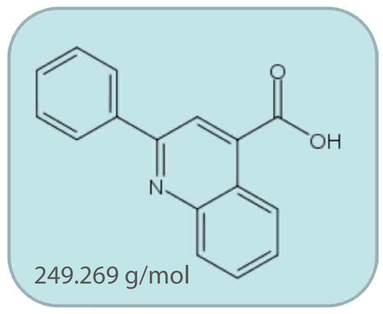


Figure 2: Cinchophen molecular structure

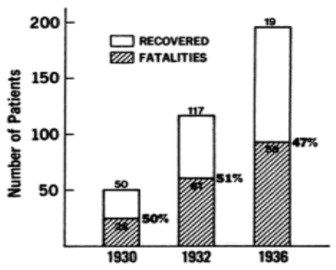


Figure 3: Case- fatality rates for cincophen jaundice in three different reviews, each published in the year shown below their respective bar. Note that the rate remained constant[3].

Cinchophen

- First prepared by Doebner & Gieskel in 1887
- Introduced to the medical world in 1908 by Nicolaier & Dohrn, as a treatment for gout.
- Its use became widespread owing to its remarkable pain-relieving properties in arthritic and neuritic conditions
- One of the first drugs to introduce the phenomenon of drug-induced liver injury (DILI) to the medical world: First signs of toxicity were reported by Worster-Drought in the British Medical Journal in 1923[1].
- Fatality rate of 50% based on 3 reviews: From Weir and Comfort review: an account of 117 cases of toxic cirrhosis due to Cinchophen; nineteen of the cases were seen at the Mayo Clinic, and ninety-eight were collected from the literature on this subject. Of the 117, sixty-one were fatal[2].

Bile Salt & Bilirubin Disposition

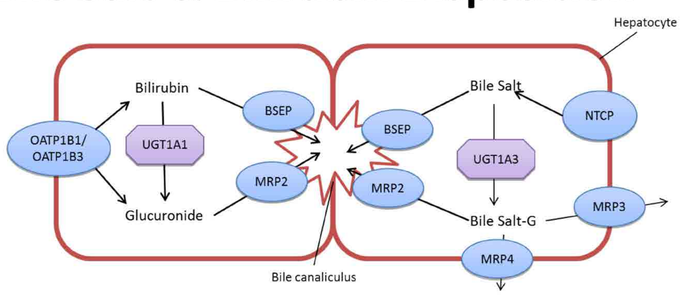


Figure 4: Transporters and enzymes involved in bilirubin and bile salt disposition.

Cinchophen ADME Properties

- Hydrophilic acid
- High aqueous solubility
- Mid-range passive permeability
- High apparent permeability, with involvement of transporters
- High plasma protein binding
- Low/medium turnover in liver microsomes and cryopreserved hepatocytes

$LogD_{7.4}$	Solubility (µM)	PAMPA P_e (10^{-6} cm/s)
-0.23	>100	2.2

Caco-2 P_{app} (10^{-6} cm/s)		Efflux Ratio
Basal to Apical	Apical to Basal	
14	16	0.9

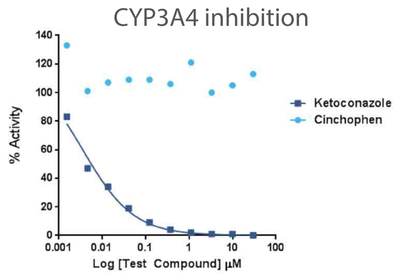
Species	Liver Microsomes Clint (µL/min/mg protein) + NADPH	Plasma Protein Binding (% bound)	Hepatocyte Clint µL/min/10^6 cells	Hepatocyte Half-life (min)	Pred CL_b mL/min/kg (%Q)	Scaled CL mL/min/kg
Mouse	25	97	21	65	5	5
Rat	11	>99	36	39	2	1
Human	9	98	16	231	4	1

- Comparably low turnover in cultured hepatocytes

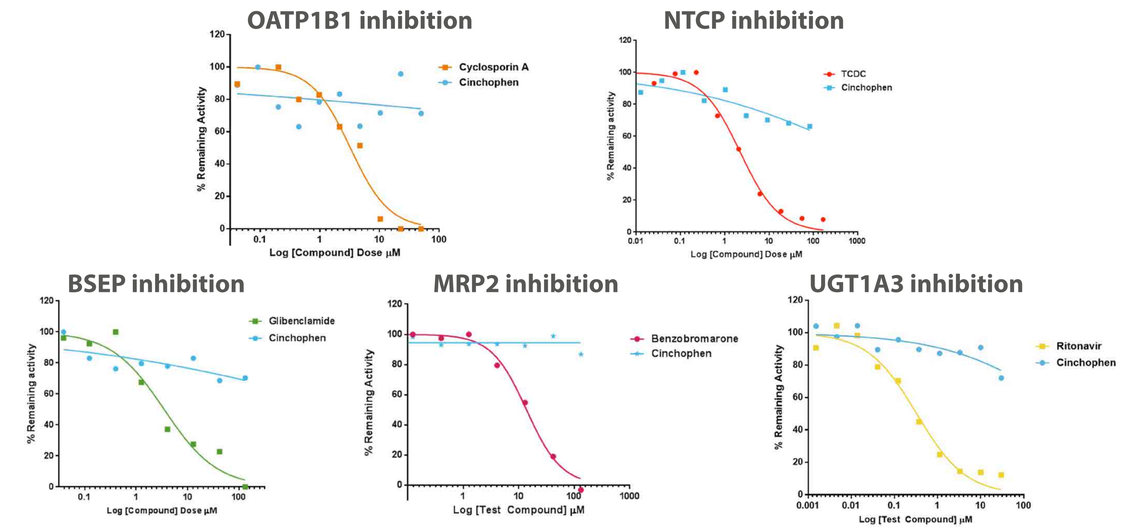
Species	Cultured Hepatocyte Clint (µL/min/10^6 cells)	Cultured Hepatocyte half/life (h/min)
Human	3	3.5h/210min

- No CYP3A4 inhibition

CYP3A4 inhibition

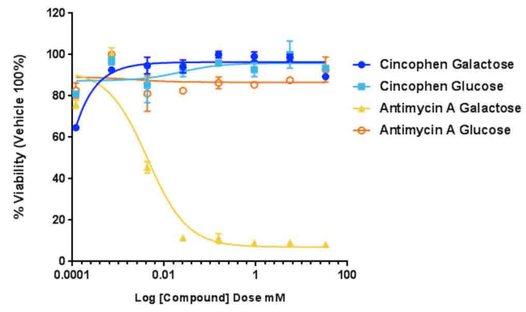


Cinchophen affinity for liver transporters and enzymes involved in bilirubin and bile salt disposition



Cinchophen cell and mitochondrial toxicity

Cell and mitochondrial toxicity in HepG2 cells



- No affinity for hepatic uptake transporters OATP1B1 and NTCP
- No affinity for hepatic efflux transporters MRP2 and BSEP
- No UGT1A3 inhibition
- No cell and mitochondrial toxicity

Cinchophen *In Vivo* pharmacokinetics and elimination routes

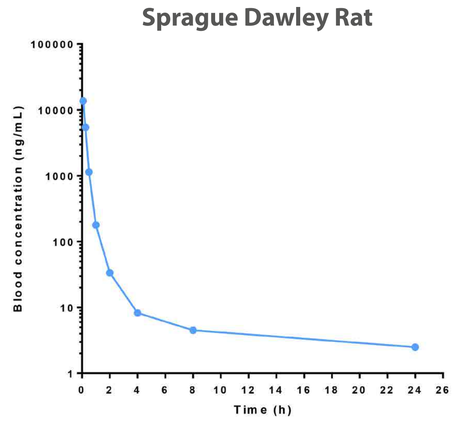
Blood
- t½= ~13hrs
- Vdss= 1.9L/kg
- CL= 37.2ml/min/kg
- AUC = 4430ng/ml*hr

Bile
- Biliary excretion= ~3.5%

Urine
- Renal excretion= ~1.6%

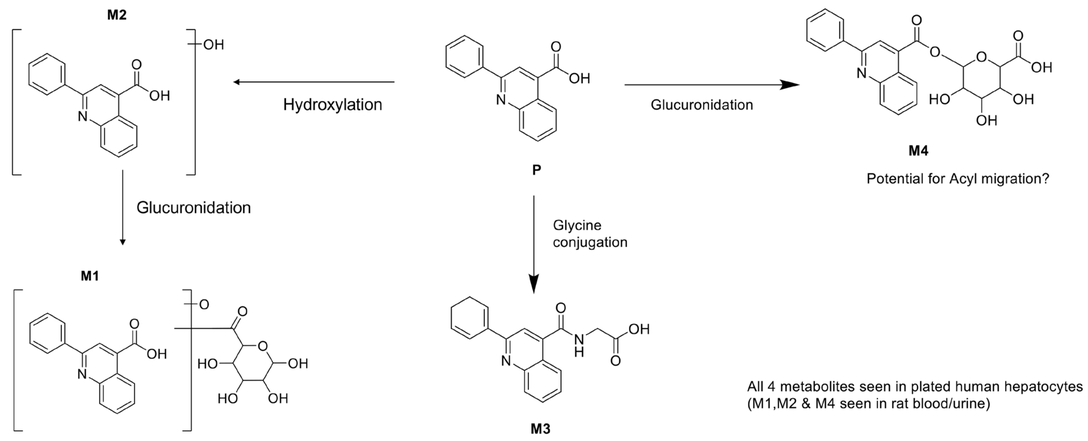
Liver
- Negligible [Cinchophen] after 24 hours

Cinchophen 10mg/Kg IV bolus
Sprague Dawley Rat



- Passive biliary/renal excretion
- Negligible [cinchophen] in liver after 24hrs
- Likely metabolites? (~90% cinchophen unaccounted for in bile/urine)

Cinchophen metabolites



All 4 metabolites seen in plated human hepatocytes (M1,M2 & M4 seen in rat blood/urine)

Solving the riddle…

The causes of DILI are often difficult to predict and identify during pre-clinical development despite modern tools, models and techniques.

- We have studied cinchophen ADME properties and characterised its affinity to hepatic transporters and enzymes involved in bilirubin and bile salt disposition. None of the results so far would have predicted DILI.
- Other mechanisms: UGTs? Phospholipidosis? Renal drug transporters (OAT1 and OAT2)? – to be investigated.
- Metabolites are likely to be involved in the causes of DILI:
  - Significant acyl migration has been observed.
  - Acyl glucuronidation may lead to widespread damage to hepatic proteins and/or hapten may lead to immunogenic ADR.

References

[1] Worster-Drought, C (1923) Brit. J. Med., 1:148 [2] Weir, J.F. and Comfort, M.W. (1933) Arch Int Med, Iii
[3] Zimmerman, H.J. (1999) pp 517-547 Lippincott Williams&Wilkins, USA.

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