Assessment of the *in vivo* disposition of Cinchophen in rat and potential mechanisms of drug-induced liver injury

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Introduction

Drug induced liver injury (DILI) is a leading cause of drug attrition due to preclinical toxicity, and the uricosuric drug cinchophen was one of the first medical examples of DILI, with it being introduced to patients in 1908 for the treatment of gout and subsequently withdrawn from the market in the 1930s due to serious hepatotoxic adverse drug reactions (ADRs). Today the mechanism of toxicity for this drug remains unclear, despite many historical reports of toxic cirrhosis with jaundice, severe liver damage and significant necrosis and tissue atrophy.

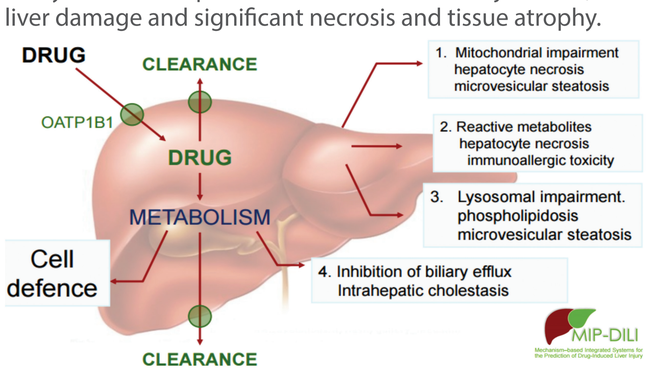
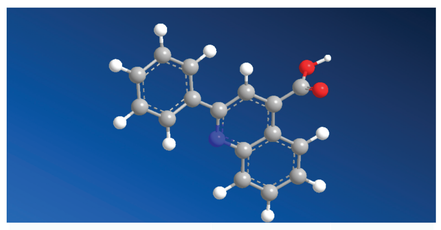


Figure 1: Chemical Insults and liver injury



Chemical Property	Ideal Criteria	Cinchophen
No. of H Bond donors	<5	1
No. of H Bond acceptors	<5	2
Molecular weight	<500	249.269
Log P	<5	3.801

Figure 2: Cinchophen molecular structure

History of Cinchophen

- First synthesised by Doebner & Gieskel in 1887.
- Introduced as a treatment for gout in 1908 by Nicolaier & Dohrn, and later used to treat arthritic and neuritic conditions due to its remarkable pain-relieving properties.
- By 1913, mild ADRs such as peptic ulcers and skin rashes were associated with cinchophen.
- In the British Medical Journal in 1923[1], the first signs of liver toxicity were reported by Worster-Drought, including a 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].
- Cinchophen toxicity was one of the first examples of drug-induced liver injury (DILI) to be introduced to the medical world.

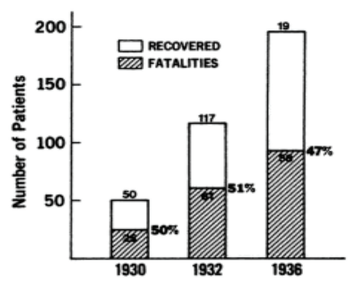


Figure 3: Case- fatality rates for cincophen jaundice in three different reviews, each published in the year shown below their respective bar. Note that the rate remained constant[3].

Cinchophen 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

Species	Plasma Protein Binding (% bound)
Mouse	97
Rat	>99
Human	98

Cinchophen *in vivo* pharmacokinetics and elimination routes

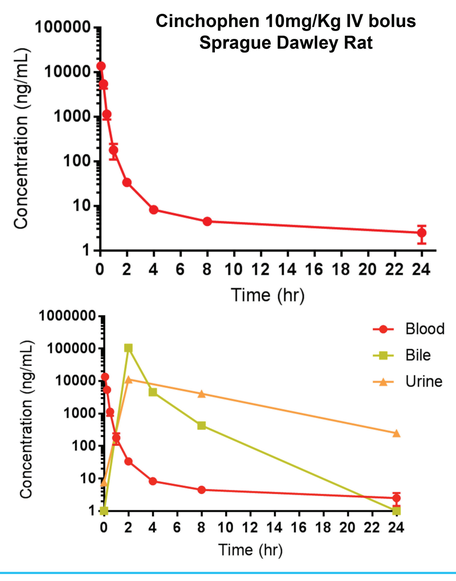
Blood
- $t_{1/2}$= ~13hrs
- Vd_{ss}= 1.9L/kg
- CL= 37.2ml/min/kg
- AUC = 4430ng/ml*hr

Bile
- Biliary excretion= ~7.5%

Urine
- Renal excretion= ~1.6%

Liver
- Negligible [cinchophen] after 24 hours

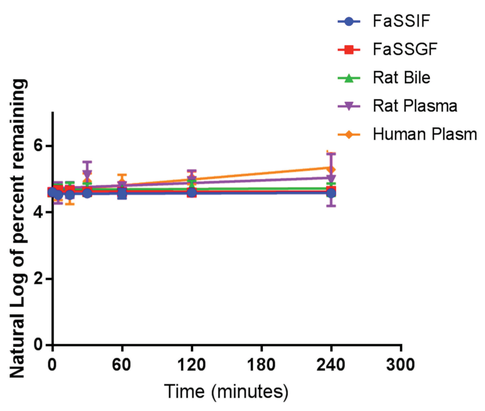
- Passive biliary/renal excretion.
- Negligible [cinchophen] in liver after 24hrs.
- ~90% cinchophen unaccounted for in bile/urine.
- Likely to be highly distributed in tissues or a significant amount of metabolites formed.



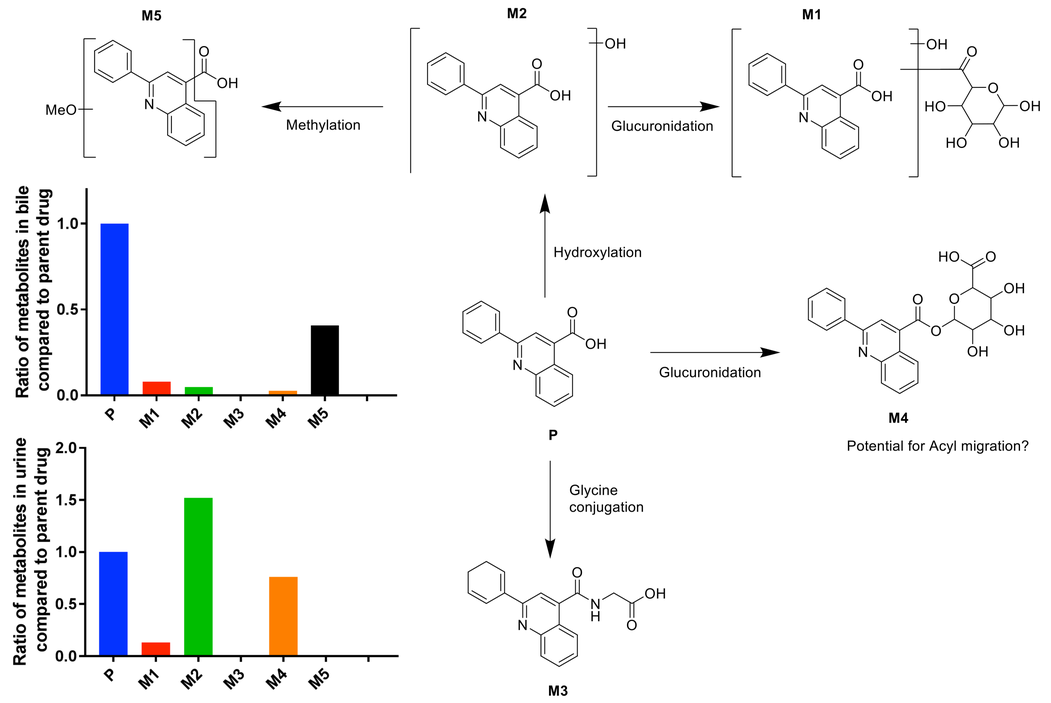
Chemical stability

Historically the quinoline nucleus of cinchophen and the potential for the drug to degrade endogenously have been suggested to be the cause of the hepatotoxic effects[4], despite the lack of in vivo or in vitro evidence for this. Additionally, in silico toxicity prediction software highlights this aspect of the molecule as a risk for hepatotoxicity.

Chemical stability assays performed in several biologically relevant matrices (plasma, bile and simulated stomach and intestinal fluid), show cinchophen to be chemically stable ($t_{1/2}$>24 hrs). Subsequently, it is unlikely to break down into a toxic quinoline or reactive molecule.



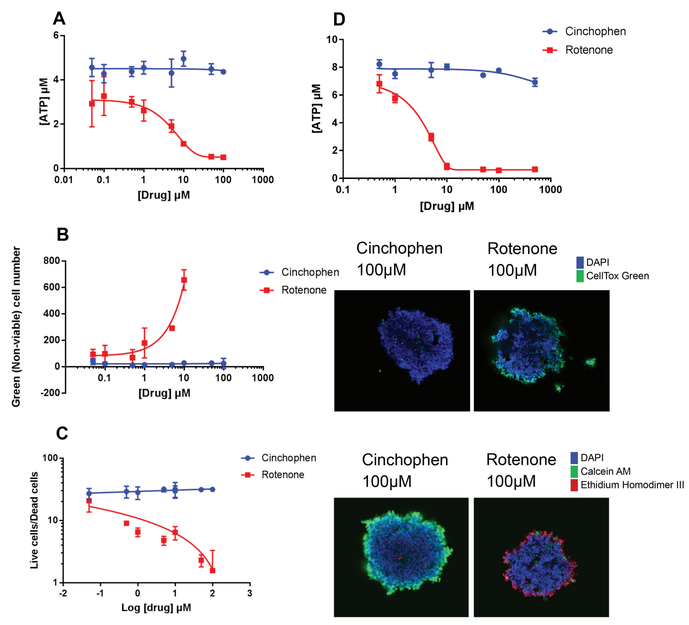
Cinchophen metabolite identification



A total of 5 metabolites were identified using LC-MS-TOF, 4 of the metabolites were found in rat tissues (blood, bile and urine), and 4 were found in human plated hepatocytes exposed to 1µM cinchophen for 24 hours. M3, the glycine conjugate was unique to human and M5 was only found in rat bile following purification by solid phase extraction. Although glucuronide conjugates, hydroxycinchophen, and methoxycinchophen have previously been suggested in literature[4], this is the first study to identify these metabolites *in vivo* using LC-MS techniques. Additionally, the glycine conjugate has not previously been described in the literature. Subsequent *in vitro* assays using liver microsomes suggest cinchophen is hydroxylated by CYP1A2 and CYP2C9, in order to form the M2 hydroxycinchophen metabolite.
The acyl glucuronide conjugate (M4), shows evidence of significant acyl migration, a property that has been linked to reactive and toxic drug metabolites that may form protein adducts. This could be a potential cause of cinchophen-induced drug liver injury and would share similarities with a suggested mechanism of hepatotoxicity for other NSAIDs such as diclofenac.

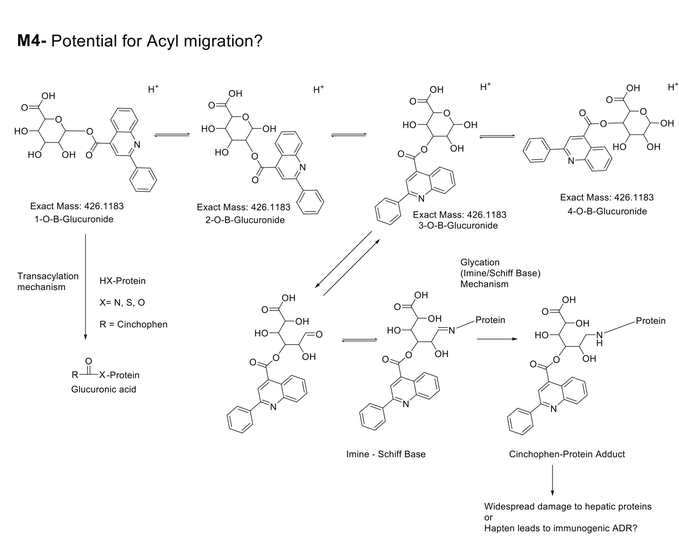
3D HepG2 cell viability

A 3D HepG2 model was used to investigate cinchophen-induced hepatotoxicity. 3D cell culture with HepG2 has been suggested to have improved CYP450 and UGT mediated xenobiotic metabolism in comparison to 2D models.
ATP quantification(A), plasma membrane integrity (B & C) and esterase function (C) were used as measures of cell viability following 24hr exposure to 0.05-100µM cinchophen. No significant cytotoxicity was observed in these assays (n=3). An additional ATP quantification assay (D) was performed with a 48hr exposure of 500µM cinchophen. However cinchophen does not appear to be significantly toxic to HepG2 at biologically relevant concentrations.



Potential mechanisms of cinchophen-induced liver injury

DILI remains one of the leading causes of preclinical drug attrition, primarily due to the difficulty to predict these ADRs using current models and techniques.
- With regards to cinchophen, we have performed multiple DMPK and molecular biology assays, and yet none of the current results highlight a definitive mechanism of DILI.
- Acyl glucuronidation may be a potential mechanism of DILI. Reactive products may cause damage by binding to hepatic proteins and/or hapten formation may lead to immunogenic ADRs.
- Other potential mechanisms may relate to drug transporters or phospholipidosis.



Future work

Future work may seek to focus on chronic toxicology models, additional DMPK studies such as chemical reactivity assays or the use of NMR on the acyl glucuronide. Further optimisation of a suitable 3D cell culture model may also prove useful in elucidating the mechanism of DILI using *in vitro* techniques.

References

1 Worster-Drought, C (1923) Brit. J. Med., 1:148. **2** Weir, J.F. and Comfort, M.W. (1933) Arch Int Med, lii. **3** Zimmerman, H.J. (1999) pp 517-547 Lippincott Williams&Wilkins, USA. **4** Hueper, W.C. (1948) pp 43-103 Medicine 27:1.