Detection and Characterisation of the Biliary Metabolism of the anti-cancer agent Ifosfamide using in vivo and analytical 31P MRS and Mass Spectrometry

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Synopsis. Biliary excretion is a significant component in the metabolism of many drugs. Using ^{31}P 3d-CSI it is shown that Ifosfamide and its metabolites can be detected in the gall bladder in a guinea pig model. Analysis of extracted bile using high-resolution MRS and mass spectrometry identifies peaks to include Ifosfamide, carboxyifosfamide, and a major contribution from a previously unreported glutathione conjugate of Ifosfamide. These results may help to identify causes of oxazophosphorine-related cholecystitis reported in patients.

Introduction

The biliary compartment is pharmacologically important given the many drugs that undergo excretion via that route. The biliary system therefore has significant influence on the pharmacokinetics and toxicology of many drugs and/or metabolites. Studies of biliary drug excretion in vivo however remain highly invasive requiring either direct ductal cannulation or the use of peroral endoscopic techniques. In this study we demonstrate in an animal model using *in vivo* ^{31}P MRS the excretion of ^{31}P-containing metabolites of Ifosfamide (IF), an alkylating agent used in the treatment of a wide range of cancers. We also describe a hitherto unknown biliary metabolite of IF that dominates high resolution ^{31}P-MR spectra of extracted bile. The unknown metabolite is putatively assigned to GSH-ifosfamide on the basis of analytical ^{31}P-MRS and MS

In vivo studies

Ten male Dunkin-Hartley guinea pigs (900 ± 20 g) were studied. Venous access was secured via jugular vein cannulation and the animals were anaesthetised using intramuscular and intravenous fentanyl/fluanisone (0.5 mg/kg) and midazolam (0.5 mg/kg) Animals were placed prone over a 5cm diameter ^{1}H/^{31}P dual-resonance coil system (1). Studies used a 1.5T Siemens Vision MR system, with a triggered SMIS spectroscopy console to provide the double resonance capabilities. "Unlocalised" ^{31}P data were acquired using a 1.28ms tanh adiabatic 90^{o} RF pulse, while 3d CSI measurements used the same RF pulse and a voxel size of 2cm. Unlocalised spectra acquired 20 minutes after commencing administration of 500mg/kg IF show signals from the normal ^{31}P-containing metabolites of muscle and liver, with an additional peak at about 18ppm downfield from PCr at the expected frequency of non-metabolised IF (Fig 1). The 3d-CSI data set (fig 2) shows that IF signal arises from liver. There is also a very large and previously unexpected contribution from the gall bladder. The presence of prominent biliary metabolites raises the possibility of their being implicated in previously described oxazophosphorine related cholecystitis (2-4)

High Field ^{31}P MRS Studies of Bile

High-resolution ^{31}P MRS spectra were acquired at 11.74 T from 600 µl samples of bile taken about 2.5 hours after IF dosing (Fig 3). By spiking the sample, Peak 5 is identified as Ifosfamide, and is set to 15.85 ppm relative to H_3PO_4 (5). Published data (5) also suggest Peak 2 is carboxy IF, while peak 3 is either 2-dechloroethyl IF or 2,3-dechloroethyl IF. The large peak at 16.02 ppm (Peak 4) has not been previously reported. Identification using Mass Spectrometry is described below. Peak 1 is not identified. Quantification of peak areas is summarised in the Table 1. Assuming an average gall bladder volume of 4cc this indicates that approximately 1.9 % of the injected IF is present as IF and its metabolites in the bile.

Identification of 16.02 ppm peak using Mass Spectrometry

Bile samples were analysed by liquid chromatography mass spectrometry (LCMS). LC eluant was subjected to electrospray ionisation to produce protonated (MH^+) ions of analytes and the masses of these ions ascertained using an ion trap mass spectrometer. Results showed that the most intense peak detected that was not present in control bile had a molecular weight of 531 (MH^+=532) which is consistent with formation of a conjugate of IF where one chlorine atom has been replaced with glutathione. Comparative MSMS fragmentation of glutathione (MH^+=308), IF (MH^+=261) and the putative GS-IF conjugate showed similar patterns of fragment ions consistent with the proposed product.

Conclusions

This study has demonstrated that ^{31}P MRS signals *in vivo* from IF and its metabolites arise predominantly from liver and gall bladder. Biliary excretion of IF or its metabolites has not previously been reported. High-resolution ^{31}P MRS and mass spectrometry show the main metabolite present to be GSH conjugate of IF. Further work is required to assess potential implications of these metabolites for observed toxicity in patients.

Acknowledgements.

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References

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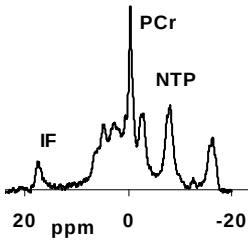

Fig 1. Unlocalised ^{31}P MRS *in vivo* after 500 mg/kg IF

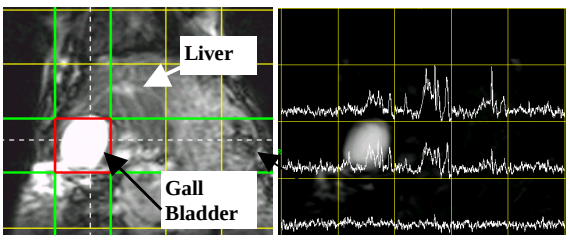

Fig 2. CSI-localised ^{1}H-decoupled ^{31}P MR Spectra from guinea pig following administration of 500 mg/kg IF

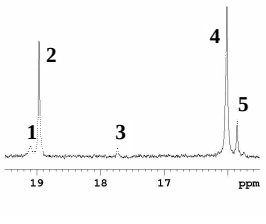

Fig 3. ^{31}P MR spectrum from bile at 11.74 T

Peak	ppm	Conc (mM)
1	19.09	0.49 ± 0.25
2	18.96	2.04 ± 1.04
3	17.74	0.16 ± 0.07
4	16.02	4.05 ± 2.38
5	15.86	1.19 ± 1.47

Table 1. Metabolites in bile (N=10; mean ± sd)