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 31P 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 has a wide range of cancers. We also describe a hitherto unknown biliary metabolite of IF that dominates high resolution 31P-MR spectra of extracted bile. The unknown metabolite is putatively assigned to GSH-ifosfamide on the basis of analytical 31P-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 1H/31P dual-resonance coil system (1). Studies used a 1.5T Siemens Vision MR system, with a triggered SMIS spectroscopy console to anaesthetised using intramuscular and intravenous fentanyl/fluanisone (0.5 mg/kg) and midazolam (0.5 mg/kg) Animals were placed prone over a

High Field 31P MRS Studies of Bile

High-resolution 31P 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 H3PO4 (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 31P 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 31P 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.

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References


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<tr>
<th>Peak</th>
<th>ppm</th>
<th>Conc (mM)</th>
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<tbody>
<tr>
<td>1</td>
<td>19.09</td>
<td>0.49 ± 0.25</td>
</tr>
<tr>
<td>2</td>
<td>18.96</td>
<td>2.04 ± 1.04</td>
</tr>
<tr>
<td>3</td>
<td>17.74</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>4</td>
<td>16.02</td>
<td>4.05 ± 2.38</td>
</tr>
<tr>
<td>5</td>
<td>15.86</td>
<td>1.19 ± 1.47</td>
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Table 1. Metabolites in bile (N=10; mean ± sd)