Acetaminophen (APAP) overdose is the most frequent cause of acute liver failure in the U.S. The mechanism of APAP hepatotoxicity in rodents involves formation of a reactive metabolite, glutathione (GSH) depletion, mitochondrial dysfunction, and necrosis. To investigate these mechanisms in a human system, a metabolically competent cell line is needed. In this study, we tested the value of a human hepatoma-derived cell line (HepaRG) for APAP toxicity studies. Cells were treated with 20 mM APAP and the time course of cell dysfunction and injury was evaluated. APAP caused a decline in cellular GSH levels to 60% of control at 6 h and 30% at 24 h. The mitochondrial membrane potential (JC-1) was unaffected at 6 h but was reduced to 20% of control at 12 h and 40% at 24 h. Lactate dehydrogenase (LDH) release was not observed until 24 h (30%) and 48 h (64%) indicating cell necrosis. In addition, a clear dose response (5–20 mM) was observed with each of these parameters. Experiments with HepG2 cells, which are not metabolically competent, showed no GSH depletion, mitochondrial dysfunction or cell injury. Conclusion: APAP toxicity in HepaRG cells mimics closely the sequence of events observed in rodents. Thus, HepaRG cells may be a useful model for mechanistic studies of drug hepatotoxicity.

I. Time course of APAP-induced injury in HepaRG cells

![Figure 1. GSH depletion in HepaRG cells after APAP treatment.](image)

![Figure 2. Mitochondrial dysfunction in HepaRG cells after APAP treatment.](image)

![Figure 3. LDH release from HepaRG cells after APAP treatment.](image)

II. Dose response of HepaRG cells to APAP

![Figure 4. GSH depletion in HepaRG cells after APAP treatment.](image)

![Figure 5. Mitochondrial dysfunction in HepaRG cells after APAP treatment.](image)

![Figure 6. LDH release from HepaRG cells after APAP treatment.](image)

III. Oxidant Stress in HepaRG cells after APAP treatment

![Figure 7. Oxidant stress in HepaRG cells after APAP treatment.](image)

IV. Comparison to HepG2 cells

![Figure 8. LDH release from HepG2 cells after APAP treatment.](image)

![Figure 9. Mitochondrial dysfunction in HepG2 cells after APAP treatment.](image)

![Figure 10. GSH depletion in HepG2 cells after APAP treatment.](image)

Table I. Lack of APAP toxicity in HepG2 cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (mmol/Mg)</th>
<th>JC-1 (Red/Green)</th>
<th>LDH Released (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1 ± 0.02</td>
<td>4.38 ± 0.1</td>
<td>10 ± 0.3</td>
</tr>
<tr>
<td>20 mM APAP</td>
<td>0.01 ± 0.01</td>
<td>4.43 ± 0.1</td>
<td>10 ± 0.3</td>
</tr>
</tbody>
</table>

Summary & Conclusions

- The mechanism of APAP toxicity in HepaRG cells closely resembled what has been observed in rodents: GSH depletion, followed by mitochondrial dysfunction and cell injury.
- The time course of APAP-induced injury in HepaRG cells resembled what has been reported in humans.
- APAP treatment caused oxidant stress.
- HepaRG cells are a useful model for drug toxicity studies.

Materials & Methods

HepaRG cells were obtained from Biopredic Int. (Rennes, France) (Gripon P, 2002). Cells were cultured according to instructions. GSH was measured in cell lysate and is reported as % of total activity released into the medium. All data expressed as mean ± SEM, n = 4. * p < 0.05 vs. control.

References


Abstract

Acetaminophen (APAP) overdose is the most frequent cause of acute liver failure in the U.S. The mechanism of APAP hepatotoxicity in rodents involves formation of a reactive metabolite, glutathione (GSH) depletion, mitochondrial dysfunction, and necrosis. To investigate these mechanisms in a human system, a metabolically competent cell line is needed. In this study, we tested the value of a human hepatoma-derived cell line (HepaRG) for APAP toxicity studies. Cells were treated with 20 mM APAP and the time course of cell dysfunction and injury was evaluated. APAP caused a decline in cellular GSH levels to 60% of control at 6 h and 30% at 24 h. The mitochondrial membrane potential (JC-1) was unaffected at 6 h but was reduced to 20% of control at 12 h and 40% at 24 h. Lactate dehydrogenase (LDH) release was not observed until 24 h (30%) and 48 h (64%) indicating cell necrosis. In addition, a clear dose response (5–20 mM) was observed with each of these parameters. Experiments with HepG2 cells, which are not metabolically competent, showed no GSH depletion, mitochondrial dysfunction or cell injury. Conclusion: APAP toxicity in HepaRG cells mimics closely the sequence of events observed in rodents. Thus, HepaRG cells may be a useful model for mechanistic studies of drug hepatotoxicity.