Acetaminophen-induced injury in HepaRG cells: a novel human cell line for studies of drug hepatotoxicity

Mitchell R. McGill, Huimin Yan, Anup Ramachandran, and Hartmut Jaeschke

Department of Pharmacology, Toxicology, & Therapeutics, University of Kansas Medical Center, Kansas City, KS

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 oncotic 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 50% 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 human model for mechanistic studies of drug hepatotoxicity.

Introduction

Acetaminophen (APAP) is a popular analgesic and antipyretic, found in many over the counter and prescription formulations. At therapeutic levels, most is glucuronidated or sulfated in the liver and subsequently excreted. Remaining parent compound is converted to the reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which can be conjugated to glutathione (GSH) and safely removed from the body. However, at supratherapeutic doses, APAP becomes hepatotoxic. In fact, overdose of APAP is the foremost cause of acute liver failure in the U.S. and the U.K.

Most of our current knowledge of the hepatotoxicity of APAP is based on rodent studies. In these models, toxicity begins with saturation of Phase II metabolic processes, resulting in overabundance of NAPQI. Excess NAPQI depletes GSH stores and the remainder is then free to bind to cellular proteins. It has been well established that binding of NAPQI to proteins in mitochondria can lead to mitochondrial dysfunction and cell injury. The end result is oxidant stress leading to massive centrilobular hepatocyte necrosis (Jaeschke & Bajt, 2006; Jaeschke et al., 2003; Knight et al., 2002; Knight et al., 2001). Limited clinical data suggest that the early mechanisms of cell injury are similar in humans (NAPQI formation and GSH depletion), but detailed studies of later toxic events have not been completed. Also, important differences in the progression of injury exist. In particular, the time course of enzyme release in patients appears to be delayed and elongated when compared with mice (Singer et al., 1995; Rumack and Matthew, 1975). Clearly, further investigation into the mechanism of APAP hepatotoxicity in humans is needed in order to explain these differences.

To study the mechanism of APAP hepatotoxicity in humans, a reliable human *in vitro* system is needed. Unfortunately, the cost of primary human hepatocytes can be prohibitive and results with these cells are not always consistent. Furthermore, most available liver parenchymal-like cell lines do not express the necessary cytochrome P450 (CYP450) enzymes to perform biotransformation of APAP (Aninat et al., 2006; Jennen et al., 2010). Recently, the HepaRG cell line has been shown to express CYP450s at levels similar to primary human hepatocytes. The aim of this study was to test the usefulness of HepaRG cells for the study of APAP hepatotoxicity.

Results

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

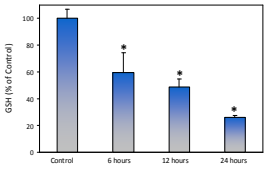

Figure 1. GSH depletion in HepaRG cells after APAP treatment. Cells were treated with 20 mM APAP for various times. GSH was measured in cell lysate. Results are expressed as % of untreated control. Data are expressed as mean ± SEM, n = 6. * p < 0.05 vs. control.

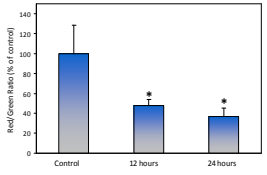

Figure 2. Mitochondrial dysfunction in HepaRG cells after APAP treatment. Cells were treated with 20 mM APAP for various times. Mitochondrial function was determined using the JC-1 assay. Data are expressed as red/green ratio % of control, mean ± SEM, n = 6. * p < 0.05 vs. control.

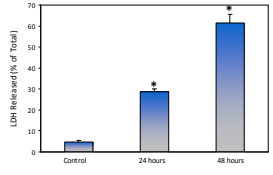

Figure 3. LDH release from HepaRG cells after APAP treatment. Cells were treated with 20 mM APAP for various times. LDH activity was measured in both medium and in cell lysate. Results are expressed as % of total activity found in the medium, mean ± SEM, n = 6. * p < 0.005 vs. control.

II. Dose response of HepaRG cells to APAP

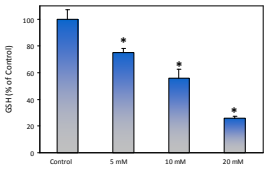

Figure 4. GSH depletion in HepaRG cells after APAP treatment. Cells were treated with various concentrations of APAP for 24 hours. GSH was measured in cell lysate. Results are expressed as % of untreated control, mean ± SEM, n = 6. * p <0.05 vs. control.

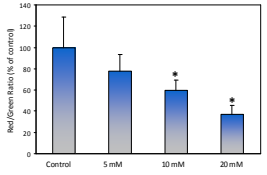

Figure 5. Mitochondrial dysfunction in HepaRG cells after APAP treatment. Cells were treated with various concentrations of APAP for 24 hours. Mitochondrial function was determined using the JC-1 assay. Data are expressed as red/green ratio % of control, mean ± SEM, n = 6. * p < 0.05 vs. control.

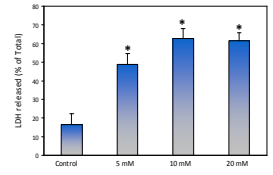

Figure 6. LDH release from HepaRG cells after APAP treatment. Cells were treated with various concentrations of APAP for 48 hours. LDH activity was measured in both medium and in cell lysate. Results are expressed as % of total activity found in the medium, mean ± SEM, n = 6. * p < 0.005 vs. control.

III. Oxidant Stress in HepaRG cells after APAP

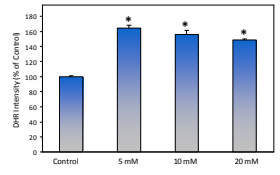

Figure 7. Oxidant stress in HepaRG cells following APAP treatment. HepaRG cells were treated with 20 mM APAP for 6 hours, then incubated with the oxidant stress-sensitive fluorescent probe DHR. After incubation, fluorescence intensity in the cells was measured. Data are expressed as DHR intensity % of control, mean ± SEM, n = 2-4. * p < 0.005.

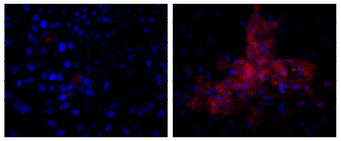

Figure 8. DHR staining of HepaRG cells. Representative images of HepaRG cells incubated with DHR with (a) and without (b) 20 mM APAP treatment.

IV. Comparison to HepG2 cells

Treatment	GSH (nmol/mg)	JC-1 (Red/Green)	LDH Released (% total)
Control	98.5 ± 12	4.38 ± 1	5.9 ± 2
20 mM APAP	90.8 ± 11	4.43 ± 0.1	10.3 ± 3

Table I. Lack of APAP toxicity in HepG2 cells. HepG2 cells were grown to 70-90% confluence and treated with 20 mM APAP for 24 hours. GSH was measured in cell lysate. JC-1 was measured in intact cells. LDH activity was measured in growth medium and in cell lysate and is reported as % of total activity released into the medium. All data expressed as mean ± SEM, n = 4.

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 as previously described (Bajt et al., 2004). For the JC-1 assay, untreated controls and APAP-treated cells were washed with PBS and incubated in the presence of the JC-1 dye at 37° C for 20 minutes. Following incubation, cells were washed and transferred to 96-well plates for fluorescence measurement. DHR experiments were conducted similarly. LDH activity was monitored by following the disappearance of NADH at 340 nm over time in a potassium phosphate buffer.

References

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