Mass balance phenotyping of primary human hepatocytes in 2D cultures treated with acetaminophen

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INTRODUCTION: This study applies a new MRS phenotyping methodology to determine acetaminophen (paracetamol, APAP) toxicity in two dimensional cultures of primary human hepatocytes. APAP, an analgesic and anti-pyretic, has been studied as a model hepatotoxin in humans and animals.¹ A recent clinical trial has shown that one-third of the people receiving the maximum recommended daily dose of 4 g per day of APAP experience moderate elevations in serum alanine aminotransferase (ALT) levels.² This ALT increase is indicative of liver injury. While there have been other studies demonstrating APAP toxicity in animals and humans, these studies usually examine hepatotoxic events due to APAP overdose, APAP toxicity potentiated by malnutrition. This study examines the sub-lethal events produced by extreme APAP toxicity.

METHODS: Human hepatocytes were obtained on collagen-coated 6-well plates with a well diameter of 35 mm and a cell density of 10⁶ cells per well. All hepatocytes were extracted 24 hours after arrival with a modified cold methanol/H₂O (65:35, stored on dry ice) extraction procedure.³ This procedure is used to extract the polar intracellular metabolites. Two hours prior to extraction, the media in all of the plates was changed to a 100% DMSO control, 1 mM APAP, and 10 mM APAP. The metabolites are grouped according to the metabolic function. Examination of the intracellular metabolites shown in Figure 3 show an approximately 2-fold decrease in acetate, formate, and NADP+ in the high and low APAP treated hepatocytes when compared to the controls. A 2-fold decrease in intracellular fumarate can be seen in the high APAP treated hepatocytes.

DISCUSSION: The results in Figure 2 show a decrease in sulfation at the higher APAP treatment conditions shown in Figure 3. The ALT increase is indicative of liver injury. While there have been other studies demonstrating APAP toxicity in animals and humans, these studies usually examine hepatotoxic events due to APAP overdose, APAP toxicity potentiated by malnutrition. This study examines the sub-lethal events produced by extreme APAP toxicity.

RESULTS: The aromatic region of a representative NMR spectrum of the hepatocyte extract can be seen in Figure 1. Peaks from the two APAP phase II conjugates: APAP-glucuronide and APAP-sulfate can be seen in this region. In addition, peaks from the unmetabolized parent APAP can be seen in addition to a few endogenous metabolites containing aromatic rings. The relative ratios of the APAP compounds can be seen in Figure 2 at the two APAP concentrations, 1 mM and 10 mM. One can see that the majority of the intracellular APAP is in the form of the glucuronide conjugate. At the lower concentration, there is more of the sulfate conjugate than parent APAP and the opposite is true at the higher dose. Figure 3 shows the endogenous metabolite profile of the hepatocytes at the 4 conditions: control, vehicle control, 1 mM APAP, and 10 mM APAP. The metabolites are grouped according to the metabolic function. Examination of the intracellular metabolites shown in Figure 3 show an approximately 2-fold decrease in acetate, formate, and NADP+ in the high and low APAP treated hepatocytes when compared to the controls. A 2-fold decrease in intracellular fumarate can be seen in the high APAP treated hepatocytes.