Molecular mechanisms of paracetamol induced liver damage

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Paracetamol (acetaminophen) is the most commonly used analgesic and antipyretic drug that is available over the counter in many countries, including Australia. At the same time, paracetamol overdose is the most common cause of acute liver failure and the leading cause of liver failure requiring liver transplantation in developed countries (Chun *et al.*, 2009). Paracetamol overdose causes a multitude of interrelated biochemical reactions in liver cells producing multitude of outcomes. Among those are covalent modification and inhibition of enzyme activity, protein oxidation, lipid peroxidation, DNA fragmentation, and deregulation of Ca^{2+} homeostasis, each contributing to paracetamol-induced liver damage (Jaeschke & Bajt, 2006). It has been known for a long time that paracetamol overdose causes a Ca^{2+} raise in hepatocytes; however, the importance of Ca^{2+} in paracetamol-induced liver toxicity is not well understood, primarily due to lack of knowledge about the source of Ca^{2+} rise (Thomas, 1993).

In this work we investigated the molecular pathways of Ca^{2+} entry activated by paracetamol in hepatocytes. Primary rat hepatocytes were isolated by liver perfusion with collagenase under general anaesthesia (intraperitoneal injection of pentobarbitone 50 mg/kg body mass). The experiments were conducted on cells maintained in culture for 24-48h. Cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) was measured using Fura-2 with the aid of a Nikon TE-300 inverted fluorescence microscope. Measurements of ion currents were conducted by standard patch clamping in whole cell mode using a computer-based EPC-9 patch-clamp amplifier and PULSE software.

Fura-2 experiments showed that application of 5 mM acetaminophen to the bath caused slow increase in $[Ca^{2+}]_{cyt}$ reaching levels above 1 μM in 45 min. Application of paracetamol in the absence of Ca^{2+} in the bath solution did not cause any changes in $[Ca^{2+}]_{cyt}$ suggesting that it activates Ca^{2+} entry across plasma membrane through Ca^{2+} permeable channels. In patch clamping experiments incubation of hepatocytes with 5-10 mM paracetamol for 30-60 min resulted in activation of a large linear non-selective cation current, which was inhibited by 20 μM clotrimazole, 100 μM chlorpromazin, 100 μM 2-APB, 5 μM N-(p-amylcinnamoyl)anthranilic acid (ACA), and 100 μM La^{3+}. Similar current was activated by treatment of hepatocytes with 100-500 μM of H_2O_2 for 15-30 min. The selectivity and pharmacological profile of the channels activated by paracetamol and H_2O_2 were consistent with those of the Transient Receptor Potential Melanostatin (TRPM) 2 channel. TRPM2 is a non-selective cation channel expressed in many tissues and is activated by ADP ribose and H_2O_2. We confirmed the expression of TRPM2 in primary rat hepatocytes by western blotting and RT-PCR. To ascertain the molecular identity of the channel mediating non-selective cation current activated by paracetamol, the expression of TRPM2 protein was suppressed by a specific siRNA. SiRNA-mediated knockdown of TRPM2 in rat hepatocytes resulted in almost complete elimination of the current activated by either paracetamol or H_2O_2. These results suggest that TRPM2 may play a significant role in paracetamol toxicity and oxidative damage in the liver.

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