The role of TRPM2 channels in paracetamol overdose-induced Ca^{2+} entry in hepatocytes

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TRPM2 (Transient Receptor Potential Melastatin 2) channels are non-selective Ca^{2+} permeable cation channels localized to both plasma membrane and lysosomes (Eisfeld & Luckhoff, 2007). They are activated by extracellular application of oxidants, such as hydrogen peroxide, or intracellular application of ADP-Ribose (Eisfeld & Luckhoff, 2007). TRPM2 are expressed in a range of tissues and have been shown to contribute to oxidative stress-mediated cellular damage in brain and pancreas (Fonfria *et al.*, 2004). Opening of TRPM2 channels on the plasma membrane results in a sustained increase of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), which leads the activation of Ca^{2+} dependent cell destructive enzymes and cell death. In the liver, there are several known pathologies associated with Ca^{2+} overload and hepatocellular death. One of them is paracetamol toxicity. It has been shown previously that paracetamol overdose causes accumulation of reactive oxygen species in the liver leading to cytoplasmic Ca^{2+} rise and cell damage (Bajt *et al.*, 2011). The source of this Ca^{2+} rise is currently not known. We hypothesize that the reactive oxygen species and ADPR generated in paracetamol overdose activate TRPM2 channels on the cell membrane, leading to Ca^{2+} overload and hepatocellular death.

TRPM2 expression was detected in rat hepatocytes using Immunofluorescence (IF), Western Blot analysis and RT-PCR. $[Ca^{2+}]_{cyt}$ was measured using Fura2-AM, and TRPM2 current was measured by whole cell patch clamping. Production of Poly-ADPR in rat hepatocytes treated with paracetamol and H_2O_2 was investigated using IF and poly-ADPR-specific antibodies. To evaluate the contribution of TRPM2 to H_2O_2 and paracetamol activated Ca^{2+} entry hepatocytes were treated with siRNA against TRPM2 for 48h. The effects of TRPM2 blockers on paracetamol- and H_2O_2-induced hepatocellular damage were assessed using Trypan-Blue. The *in vivo* study of paracetamol overdose was conducted using TRPM2 Knock-Out and Wild-Type mice.

The RT-PCR and Western blot analysis showed the presence of the long subtype of TRPM2 containing the NUDT9 (Homology with Nudix Box) located at the C-terminal segment in rat hepatocytes. It was identified that treatment of hepatocytes with H_2O_2 and paracetamol resulted in a rise of $[Ca^{2+}]_{cyt}$, which was prevented by application of clotrimazole and ACA ((N-(p-amylcinnamoyl) anthranilic acid). In patch clamping, the cation current activated by paracetamol and H_2O_2 was also inhibited by these blockers. siRNA-mediated knock down of TRPM2 in rat hepatocytes resulted in a significant reduction of the current activated by either paracetamol or H_2O_2. At the same time, treatment of hepatocytes with paracetamol or H_2O_2 produced a significant rise in intracellular concentration of Poly-ADPR. Inhibition of TRPM2 by clotrimazole and ACA in cultured rat hepatocytes diminished the paracetamol and H_2O_2-induced cell death. It was detected that TRMP2 knock-out mice were significantly less susceptible to paracetamol toxicity than wild type and TRPM2 Heterozygote mice.

In conclusion, we have shown that TRPM2 channels are expressed in hepatocytes and are activated by paracetamol and H_2O_2. Importantly, TRPM2 blockers significantly reduced paracetamol-induced hepatocellular death, potentially suggesting a new treatment for paracetamol overdose.

Bajt, M.L., Ramchandran, A., Yan, H.M., Lebofsky, M., Farhood, A., Lemasters, J.J. & Jaeschke, H. (2011) Apoptosis-inducing factor modulates mitochondrial oxidant stress in acetaminophen hepatotoxicity. *Toxicological Sciences*,**122,** 598-605.

Eisfeld J, Luckhoff A. (2007) TRPM2. *Handbook of Experimental Pharmacology*,**179**, 237-252.

Fonfria, E., Marshal, I.C., Benham, C.D., Boyfield, I., Brown, J.D., Hill, K., Hughes, J.P., Skaper, S.D. & Mcnulty, S. (2004) TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase. *British Journal of Pharmacology*, **143,** 186-192.