## Curcumin and chlorpromazine are novel inhibitors of TRPM2 channel

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Transient Receptor Potential Melastatin 2 (TRPM2) channel is a non-selective cation channel activated by intracellular ADP-Ribose generated in response to oxidative stress (Eisfeld & Luckhoff, 2007). Previous research suggests that TRPM2-mediated sustained rise in cytosolic $Ca^{2+}$ concentration can lead to cell death through apoptosis and necrosis (Fonfria *et al.*, 2004). Recently we have shown that activation of TRPM2 channels by reactive oxygen species generated in the liver plays a major role in hepatocellular death caused by paracetamol overdose, and inhibition of TRPM2 channels protects hepatocytes from oxidative stress-induced damage (Kheradpezhouh *et al.*, 2014). Involvement of TRPM2 in cell damage makes TRPM2 channels a potential therapeutic target for treatment of a range of oxidative stress-related diseases. However, none of the currently known inhibitors of TRPM2 channel can be used clinically due to their low affinity and poor specificity.

In this study, using whole-cell patch clamping and $Ca^{2+}$ imaging of primary rat hepatocytes and TRPM2-transfected HEK293T cells, we investigated whether curcumin and chlorpromazine inhibit TRPM2 channel activity. The rationale for choosing these two agents was that both have been shown to protect liver from paracetamol-induced oxidative damage *in vivo* (Ray *et al.*, 2001; Kheradpezhouh *et al.*, 2010). The results showed that curcumin and chlorpromazine inhibited TRPM2 channels, although through different mechanisms. Chlorpromazine blocked $H_2O_2$- and paracetamol-induced $Ca^{2+}$ entry and ADPR-activated TRPM2 current in hepatocytes and HEK293T cells. The complete block could be achieved within 20-30 s after application 50 μM chlorpromazine to the bath, and could be reversed by washing. Similarly, curcumin (2 μM) inhibited paracetamol- and $H_2O_2$-induced $Ca^{2+}$ entry, and the development of TRPM2 current in response to intracellular application of ADPR. However, to be effective, curcumin had to be present in the medium during the treatment of cells with paracetamol or $H_2O_2$, or applied to the bath prior to patch clamping with a pipette solution containing ADPR. The $IC_{50}$ for curcumin inhibition of ADPR-induced TRPM2 current was ~50 nM, and the effect of curcumin could not be washed out prior to losing the patch. If applied to the bath after full development of TRPM2 current, curcumin had no effect of the current amplitude. This suggests that curcumin affects the mechanism of TRPM2 activation, but does not block the open pore of the channel.

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