The role of TRPM2 channels in the liver ischemia-reperfusion injury

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Ischemia-reperfusion injury (IRI) of the liver after liver surgery or transplantation is a common clinical problem. One of the hallmarks of the pathogenesis of liver IRI is intracellular Ca^{2+} accumulation in hepatocytes. The identity of Ca^{2+} channels activated by ischemia-reperfusion (I-R) in hepatocytes is currently not known. Recent evidence suggests that the Transient Receptor Potential Melastatin 2 (TRPM2) channel, which is activated in oxidative stress, could play a major role in Ca^{2+} overload in the liver (Kheradpezhou *et al.*, 2014). In order to examine whether TRPM2 channels mediate intracellular Ca^{2+} rise during I-R in hepatocytes, and whether inhibition of TRPM2 channels can reduce liver IRI, we used an *in vitro* model of I-R in WT and TRPM2-KO isolated mouse hepatocytes and *in vivo* model of liver IRI in rat, respectively.

WT and TRPM2-KO mouse hepatocytes were isolated by liver perfusion with collagenase as previously described (Kheradpezhou *et al.*, 2014). Prior to the procedure each animal was anaesthetized by IP injection of ketamine (100 mg/kg) and xylazine (8 mg/kg). Isolated primary hepatocytes were cultured 24-48 h on coverslips and then subjected to ischemia in an airtight chamber under anaerobic condition using AnaeroGen^{TM} 2.5L for 1-2 h followed by reperfusion in DMEM for 1-3 h in 37^{o}C incubator. At the end of the reperfusion phase hepatocytes were loaded with FFP-18 (AM) Ca^{2+} indicator and transferred to BX51 Olympus microscope stage for intracellular Ca^{2+} measurements. To assess the effects of TRPM2 inhibitors on liver injury we used segmental liver I-R with bile flow measurements in rat as described (Schiesser *et al.*, 2009). Rats anaesthetized with 1.75-2.5% isoflurane inhalation were subjected to 45 min of segmental liver ischemia and 1 h reperfusion with no recovery. Each animal randomly received one of the TRPM2 inhibitors: chlorpromazine; N-(p-amylcinnamoyl)anthranilic acid (ACA) or curcumin as an IP injection 30 min prior to the surgery. The control groups only received the respective vehicles. Bile flow recovery during reperfusion phase was used as a measure of the final liver damage.

The results of the experiments on isolated WT mouse hepatocytes demonstrated that ischemia followed by 2-3 h reperfusion caused a significant rise in free cytoplasmic $[Ca^{2+}]_{cyt}$, compared to the untreated WT cells. In contrast, similar I-R treatment of isolated TRPM2-KO hepatocytes did not induce any change in $[Ca^{2+}]_{cyt}$. Interestingly, a shorter reperfusion, for 60 min or less, was insufficient to induce a noticeable $[Ca^{2+}]_{cyt}$ rise in WT hepatocytes after 1-2 h of ischemia. These data suggest that I-R can cause activation of TRPM2 channels in hepatocytes, however, the level of activation depends on the duration of the reperfusion, possibly due to gradual accumulation of ADP-ribose in the cells, which is the main TRPM2 ligand. Furthermore, bile flow measurements in rats subjected to segmental liver I-R demonstrated that none of the TRPM2 inhibitors employed in this study had a positive effect on bile flow recovery 1 h after ischemia. Neither ACA, nor curcumin had any effect on bile flow recovery, while chlorpromazine reduced bile flow recovery compared to the vehicle treated group. These results suggested that TRPM2 channels may not play a role in the initial liver damage during the period of ischemia and early reperfusion, which is consistent with the $[Ca^{2+}]_{cyt}$ measurements data in isolated hepatocytes.

Kheradpezhouh E, Ma L, Morphett A, Barritt GJ, Rychkov GY. (2014). TRPM2 channels mediate acetaminophen-induced liver damage. *Proc Natl Acad Sci USA* **111,** 3176-3181.

Schiesser M, Wittert A, Vincent B, Nieuwenhuijs VB, Morphett A, Padbury RTA, Barritt GJ. (2009) Intermittent ischemia but not ischemic preconditioning is effective in restoring bile flow after ischemia reperfusion injury in the livers of aged rats. *J Surg Res* **152,** 61–68.