TRPM2 channels contribute to liver ischemia and reperfusion injury

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Liver ischemia and reperfusion injury (IRI) as result of 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. It is well established that hepatocyte cytoplasmic and mitochondrial Ca^{2+} concentrations increase immediately following the onset of reperfusion. The identity of Ca^{2+} channels activated by ischemia-reperfusion (I-R) in hepatocytes, however, 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 (Kheradpezhouh *et al.*, 2014).

To assess the contribution of TRPM2 channels in liver IRI we used segmental liver I-R in WT and TRPM2-KO mice, as previously described (Abe *et al.*, 2009). Mice were subcutaneously injected with buprenorphine (0.1 mg/kg) prior to the surgery. While under 1.75-2.5% isoflurane inhalation anaesthesia, each animal was subjected to 45 min of segmental liver ischemia by clamping blood flow to the lateral and the medial lobes, followed by clamp removal and reperfusion. After suturing, the incision area was treated locally with bupivacaine (0.5-1 mg/kg). All animals were subcutaneously injected with amoxycillin (20 mg/kg) before returning to a cage for recovery. Mice in the sham group were subjected to a similar surgery procedure but without blood vessel clamping. After 24 h or 72 h reperfusion, each animal was anaesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (8 mg/kg), followed by the collection of blood plasma and liver tissue. Plasma samples were used to determine the levels of alanine transaminase (ALT) and aspartate transaminase (AST) in the blood, whereas liver tissues fixed in 10% formalin were used for histological analysis.

As expected, ALT and AST enzymes levels were significantly elevated in WT mice 24 h after liver I-R, compared to sham group. This was consistent with the presence of necrosis accompanied by vacuolization and pyknotic nuclei in the regions of the ischemic liver lobes. The liver damage assessed by quantitative histological analysis was found slightly reduced in TRPM2-KO mice, compared to WT animals 24 h after liver reperfusion. Similarly, there was some reduction in ALT and AST enzyme levels in TRPM2-KO, compared to WT. The difference between I-R induced liver injury in TRPM2-KO and WT mice was even more evident 72 h after reperfusion started, where the mean injury area in the ischemic liver lobes was 13±4.97% in TRPM2-KO, compared to injury area of 43±4.64% in WT mice. Furthermore, while ALT and AST levels were dramatically lower than those at 24 h, at 72 h ALT levels in WT mice were significantly higher than in TRPM2-KO mice. Taken together, our data suggest that TRPM2 channels play a detrimental role in IRI in the liver by promoting intracellular Ca^{2+} accumulation and subsequent cell death.

Abe Y, Hines IN, Zibari G, Pavlick K, Gray L, Kitagawa Y, Grisham MB. (2009). Mouse model of liver ischemia and reperfusion injury: Method to study reactive oxygen and nitrogen metabolites *in vivo*. *Free Radic Biol Med* **46(1):** 1–7.

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