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TNF- α Induced Apoptosis During Hepatic Ischemia Reperfusion Injury?

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In a recent study in *SHOCK* on the role of heme oxygenase-1 (HO-1) in hepatic ischemia-reperfusion injury, Kim *et al.* concluded that “the cytoprotective effects of HO-1 are mediated by suppression of TNF/TNFR1-mediated apoptotic signaling,” (1). Due to the word limitation, we will focus only on one fundamental concern with this study: the assumed role of apoptotic cell death in this rat model.

The evidence for apoptosis provided by the authors is based on an increase of caspase-3 and -8 activities, cytochrome c release and TUNEL-positive cells (1). A major contradiction is already quite obvious from the authors’ own data: The authors report a 2-fold increase in caspase activity compared to a massive increase in TUNEL-positive cells (60% of all hepatocytes) (1). In untreated controls the number of TUNEL-positive cells is generally <0.3% (2), which amounts to a more than 200-fold increase over baseline after ischemia-reperfusion (1). It is well recognized that the TUNEL assay detects DNA strand breaks independent of the mode of cell death (3) and in the case of hepatic ischemia-reperfusion injury, TUNEL-positive cells are almost exclusively necrotic (2,4). This is consistent with morphological assessment by professional pathologists who were unable to detect any relevant increases in the number of cells that showed cell shrinkage, chromatin condensation and apoptotic body formation during hepatic ischemia and up to 24h of reperfusion in a rat model (2). In addition, other features like mitochondrial cytochrome c release can also be found during necrotic cell death either after bax translocation or mitochondrial swelling after the membrane permeability transition pore opening (5). Thus, none of these parameters provides any support for apoptotic cell death being important in the pathophysiology. The only reliable parameter for apoptotic signaling is activation of caspases. However, quantitative changes need to be considered. A modest 2-fold increase in caspase activation is insufficient to trigger relevant apoptotic cell death (6). In a true model of TNF-mediated apoptosis (galactosamine/endotoxin), generally increases in caspase-3 activity of 20–50-fold of baseline can be observed when 20–30% of all hepatocytes undergoing apoptosis (7). Thus, if the massive cell death (60% of hepatocytes) during hepatic ischemia-reperfusion would include a relevant contribution of apoptosis, the caspase activities would have to be 2 orders of magnitude higher than reported here (1). Most importantly, a pancaspase inhibitor, even when administered after TNF formation, completely prevents apoptotic cell death and any related liver injury (7). Caspase inhibitors generally do not protect against ischemia-reperfusion injury (2,4) and even if there is some protection, it is likely due to an off-target effect on other proteases (8).

Why should we care if the mode of cell death is apoptosis or oncotic necrosis? First, in contrast to apoptosis, necrosis causes much more inflammation that aggravates the injury (9) but is also necessary for the repair (10). Second, for apoptosis we already have highly effective therapeutics (pancaspase inhibitors). However, because apoptosis is not a relevant process during hepatic ischemia-reperfusion injury, it is still necessary to evaluate therapeutic strategies such as HO-1 induction.

References

1. Kim SJ, Eum HA, Billiar TR, Lee SM. Role of heme oxygenase 1 in TNF/TNF receptor-mediated apoptosis after hepatic ischemia/reperfusion in rats. *Shock*. 2013; 39:380–388. [PubMed: 23423194]
2. Gujral JS, Bucci TJ, Farhood A, Jaeschke H. Mechanisms of cell death during warm hepatic ischemia-reperfusion in rats: apoptosis or necrosis? *Hepatology*. 2001; 33:397–405. [PubMed: 11172341]
3. Grasl-Kraupp B, Ruttkay-Nedecky B, Koudelka H, Bukowska K, Bursch W, Schulte-Hermann R. In situ detection of fragmented DNA (TUNEL Assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death: a cautionary note. *Hepatology*. 1995; 21:1465–1468. [PubMed: 7737654]
4. Jaeschke H, Lemasters JJ. Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. *Gastroenterology*. 2003; 125:1246–1257. [PubMed: 14517806]
5. Bajt ML, Farhood A, Lemasters JJ, Jaeschke H. Mitochondrial bax translocation accelerates DNA fragmentation and cell necrosis in a murine model of acetaminophen hepatotoxicity. *J Pharmacol Exp Ther*. 2008; 324:8–14. [PubMed: 17906064]
6. Williams CD, Koerner MR, Lampe JN, Farhood A, Jaeschke H. Mouse strain-dependent caspase activation during acetaminophen hepatotoxicity does not result in apoptosis or modulation of inflammation. *Toxicol Appl Pharmacol*. 2011; 257:449–458. [PubMed: 22023962]
7. Gujral JS, Hinson JA, Farhood A, Jaeschke H. NADPH oxidase-derived oxidant stress is critical for neutrophil cytotoxicity during endotoxemia. *Am J Physiol Gastrointest Liver Physiol*. 2004; 287:G243–252. [PubMed: 15044177]
8. Schotte P, Declercq W, Van Huffel S, Vandenabeele P, Beyaert R. Non-specific effects of methyl ketone peptide inhibitors of caspases. *FEBS Lett*. 1999; 442:117–121. [PubMed: 9923616]
9. Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol*. 2003; 284:G15–26. [PubMed: 12488232]
10. Van Sweringen HL, Sakai N, Tevar AD, Burns JM, Edwards MJ, Lentsch AB. CXC chemokine signaling in the liver: impact on repair and regeneration. *Hepatology*. 2011; 54:1445–1453. [PubMed: 21626524]