432p In-Vitro Model of Hepatic Ischemia/Reperfusion Injury

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Ischemia–reperfusion (I/R) injury is the main cause of primary dysfunction or nonfunction after liver transplantation. Steatotic (i.e. fatty) livers comprise up to 25% of the liver donor pool. Steatotic livers are more susceptible to cold or warm ischemia, suffer greater hepatocellular damage during reperfusion, and have impaired regeneration. These factors contribute to the increased postoperative complications and poor survival rates after transplantation or after resection surgery in experimental animals and humans with fatty livers. For example, patients receiving livers with mild-to-moderate steatosis, which are usually considered "marginally acceptable," have a 77% survival 2 years posttransplantation compared with a 91% survival in patients receiving nonsteatotic livers. The mechanisms underlying the high sensitivity of fatty livers to I/R injury are poorly understood. The motivation of this work is to develop an in vitro model of hepatic I/R so that the many potential factors involved can be decoupled. We cultured rat hepatocytes in the collagen sandwich configuration and made them fatty by exposure to fatty acid-supplemented culture medium. Cultures were exposed to defined periods of ischemia followed by normoxia and cell viability was determined. In some experiments, we co-cultured hepatocytes with nonparenchymal cells (NPCs) to determine the impact of these cells on the response. Results with cultured hepatocytes were consistent with in vivo studies. Fatty hepatocytes were more sensitive to I/R injury, as indicated by higher lactate dehydrogenase release when compared to lean hepatocytes. Hepatocyte damage increased as a function of duration of the ischemic period, and was highest during the reperfusion phase. Co-culture with Kupffer cells, which are known to be activated during I/R in vivo, exacerbated the damaging effect of I/R. This cell culture model will be useful to elucidate the mechanisms of I/R in the liver at the cellular level. Furthermore, this system may be a useful platform for high-throughput screening of treatments which could enhance the resistance of lean and fatty liver cells to I/R.