Stress in the liver: stereotypic genomic responses *in vitro* and *in vivo* involve inflammation and loss of metabolic functions

Agata Widera

Abstract:

Hepatocyte *in vitro* systems represent a well-accepted tool in many fields of research. Despite their widespread use, research with primary hepatocytes remains challenging. To obtain a comprehensive overview of the expression alterations caused by various culture conditions and after radically different interventions, a time-resolved gene array analysis of mouse hepatocytes in sandwich and monolayer cultures was carried out. The results were compared to livers *in vivo* after treatment with carbon tetrachloride (CCl_4), lipopolysaccharide (LPS) or partial hepatectomy (PHx). Global gene expression profiling exposed profound alterations within the first 24 hours that orchestrate the cellular response of primary mouse hepatocytes *in vitro*. All cultivation systems - sandwich, monolayer confluent and subconfluent - expressed similar pattern of up-regulation for lipocalin-2 (Lcn2), metallothionein-2 (Mt2) and serum amyloid A3 (Saa3) and down-regulation of the bile salt export pump (Bsep), multidrug resistance-associated protein 2 (Mrp2) and cholesterol 7 alpha-hydroxylase (Cyp7a1). The sandwich system offers clear advantages over monolayers by maintaining a more stable profile of expressional changes and preserving more of the *in vivo*-like features. Bio-statistical analysis identified a stereotypic gene expression response, which was similar for all the different types of stress tested: isolation by collagenase perfusion, intoxication with CCl_4 or lipopolysaccharide as well as after partial hepatectomy, namely an upregulation of inflammation and proliferation as well as a downregulation of metabolism-associated genes. This illustrates that the broadly applied hepatocyte in vitro systems do not represent healthy but rather critically inflamed livers. Luminex screening showed rapid and strong activation of stress-associated signaling kinases during isolation of hepatocytes suggesting a new time frame for possible interventions. An inhibitor screening demonstrated a prominent role of c-Jun N-terminal kinases which when inhibited during liver perfusion or subsequent cultivation resulted in a strongly repressed inflammation response. In contrast, none of the tested inhibitors was able to rescue the profound repression of metabolism-associated genes, indicating that yet undiscovered pathways control this response. In conclusion, this work identifies remarkable similarities between inflamed livers *in vivo* and cultivated hepatocytes, which open new paths for mechanistic studies on liver inflammation and a more accurate use of hepatocyte *in vitro* systems.