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Journal of Hepatology 40 (2004) 1027–1029

Journal of
Hepatology

www.elsevier.com/locate/jhep

Editorial

The cirrhotic hepatocyte: navigating between Scylla and Charybdis

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In the chronically injured and cholestatic liver, the various hepatic cell types are exposed to a vicious cocktail of toxic and potentially lethal ingredients, such as cytokines, reactive oxygen species (ROS), bile acids, endotoxin and Fas-Ligand expressed on immune cells. Some liver cells thrive in this hostile environment, e.g. stellate cells, which start to proliferate and produce excessive amounts of extracellular matrix and inflammatory and immune cells, which infiltrate the liver and produce cytokines. Others, e.g. hepatocytes, perish in this environment. It is still a matter of debate what the predominant mechanism of hepatocyte cell death is in chronic liver injury: necrosis or apoptosis. This debate is not trivial, because any intervention aimed to protect hepatocytes in the injured liver must be based on a correct understanding of the mechanism of cell death, e.g. apoptosis is a highly regulated process involving numerous steps and components with ample opportunity to intervene and inhibit apoptosis. Prevention of necrosis requires a fundamentally different approach aimed at reducing the necrosis-causing agent. In the chronically injured liver, liver enzymes in serum are elevated. Although increased AST and ALT levels are considered as markers of necrotic cell death, it may result from necrosis secondary to apoptosis (necro-apoptosis) [1]. With the introduction of methods to detect apoptosis, e.g. the TUNEL assay, apoptosis was proposed as the major mechanism of cell death in chronic cholestatic liver injury [2]. However, the TUNEL assay appeared to be prone to artifacts. Therefore, more specific methods have been applied, e.g. active caspase-3 staining and morphological criteria. Based on these latter methods, apoptosis as the predominant mode of cell death was questioned and necrosis has been proposed as the major mode of cell death in the cirrhotic liver [3–5].

Bile acids, Fas-Ligand, reactive oxygen species (ROS) and cytokines like TNF α are all potent inducers of apoptotic cell death in normal primary cultures of hepatocytes. This

raises the question why in vivo in the chronically injured or cirrhotic liver, apoptosis is not the prevailing mode of hepatocyte cell death. It appears that the hepatocyte in the chronically injured liver is somehow resistant against apoptotic cell death. Several mechanisms have been proposed which contribute to this resistant phenotype [4,6–9]. First of all, the expression of the bile acid importer NTCP (Na⁺-dependent taurocholate cotransporting polypeptide) is downregulated in chronic liver diseases, in particular cholestatic liver diseases [6]. Since bile acid-induced apoptosis is absolutely dependent on bile acid uptake [7], reduced NTCP expression contributes to the protection against bile acid induced apoptosis. Furthermore, in cholestasis hepatocytes are exposed to a mixture of many different bile acids, including pro-, anti- and non-apoptotic bile acids. The actual apoptotic potential of this bile acid mixture remains to be determined. Recently, another mechanism of increased resistance against apoptosis in the cholestatic liver has been described: activation of the transcription factor NF- κ B [4,9]. NF- κ B regulates the transcription of numerous inflammation-associated genes, including many anti-apoptotic genes [10]. Indeed, inhibition of NF- κ B increases hepatocyte apoptosis in chronic cholestasis [9]. Activation of NF- κ B prior to exposure to bile acids protects against bile acid-induced apoptosis, although bile acids themselves do not activate NF- κ B [4,7]. In vivo, NF- κ B in hepatocytes is activated by cytokines derived from inflammatory cells including Kupffer cells and increased expression of the NF- κ B-activating cytokines TNF α and interleukin-1 has been reported in chronic cholestasis [4,11]. Bile acids activate additional survival pathways, such as PI-3/Akt-kinase and the p38 and ERK MAP kinases, in various liver cell types, including hepatocytes, cholangiocytes and stellate cells [7,12–15]. Bile acid-mediated activation of the EGF-receptor and subsequent activation of ERK appears to be a common mechanism in these cell types [12–14].

In this issue of the Journal, Black and colleagues deepen

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our understanding of the resistance phenotype of hepatocytes in the chronically injured and cirrhotic liver [16]. Black et al. observed increased resistance against TGF- β -induced apoptosis in hepatocytes isolated from cirrhotic livers. Interestingly, TGF- β -induced apoptosis in normal hepatocytes is mediated via a ROS-dependent mechanism: inhibition of ROS exposure prevented TGF- β -induced apoptosis in *normal* hepatocytes. In contrast, basal ROS production was increased in hepatocytes isolated from cirrhotic livers and inhibition of ROS exposure in *cirrhotic* hepatocytes increased TGF- β -induced apoptosis. Subsequent investigations demonstrated that the initiation of the caspase-cascade via TGF- β -induced caspase-8 activation was less effective in cirrhotic hepatocytes due to decreased pro-caspase-8 protein levels in cirrhotic hepatocytes. This is a seminal study since it describes a completely novel mechanism of resistance against apoptosis and because it provides directions how to exploit this knowledge in future therapeutic applications. TGF- β is a pro-apoptotic cytokine for hepatocytes and is produced by non-parenchymal cells, in particular activated stellate cells, in chronic liver diseases. Therefore, hepatocyte exposure to increased concentrations of TGF- β is common in most chronic liver diseases, regardless of their etiology. The most intriguing observation in the study of Black is the “conditioning” of hepatocytes, converting increased ROS exposure from a pro-apoptotic factor into an anti-apoptotic factor. Considering that bile acids increase intracellular ROS generation in hepatocytes, “conditioning” of hepatocytes could also occur via chronic exposure to bile acids [17,18]. The findings of Black et al. are also highly relevant for any attempt to treat chronic liver diseases with anti-oxidants. Indeed, the disappointing results of anti-oxidants in the treatment of chronic liver diseases could be explained by the fact that any beneficial effects of anti-oxidants, e.g. lowering the exposure to inflammatory cell derived extracellular ROS and lipid peroxidation, are offset by the collapse of the anti-apoptotic phenotype in hepatocytes. Obviously, we need to know the mechanism(s) of the beneficial effect of increased basal ROS production in cirrhotic hepatocytes. Although Black and colleagues do not address this issue, there are some clues. First of all is the involvement of the JNK pathway. Reactive oxygen species activate JNK and activation of JNK in hepatocytes is considered to be pro-apoptotic event [19–21]. In cirrhotic hepatocytes chronically exposed to ROS-generating factors continuous JNK-activation could be prevented via some kind of de-sensitization mechanism, contributing to an anti-apoptotic phenotype. It remains to be explained why inhibition of ROS exposure then leads to a sudden increase in apoptosis. Another explanation could be that chronic ROS exposure leads to induction of the gene heme-oxygenase-1 (HO-1). HO-1 is an oxidative stress-induced gene and it is well known that expression of HO-1 protects against oxidative stress-induced cell death [22]. Indeed, HO-1 expression contributes to the phenomenon of protective pre-conditioning in organ transplantation [22].

Both hypotheses can be tested experimentally and additional possibilities may exist.

Another important observation in the study of Black et al. is the reduced content of pro-caspase-8 protein in cirrhotic hepatocytes. Since pro-caspase-8 mRNA levels were not investigated, we do not know whether this regulation is at the transcriptional or post-transcriptional level. Not much is known about the regulation of the caspase-8 promoter and mRNA expression, although gene-silencing and down-regulation of caspase-8 mRNA has been observed in several tumors [23–25]. This is extremely relevant for the present study since one of the hypotheses of Black et al. is that the increased resistance of cirrhotic hepatocytes to TGF- β -induced apoptosis facilitates inappropriate hepatocyte proliferation and predisposes to hepatocellular carcinoma. Reduction of caspase-8 activity to preserve hepatocytes in chronic liver injury could then turn out to be a wolf in sheep’s clothing because it may facilitate aberrant hepatocyte proliferation and tumor development and it may be futile since necrotic rather than apoptotic hepatocyte death prevails in chronic liver injury. In contrast, transient reduction of caspase-8 function may be a promising therapy for acute liver injury characterised by massive apoptosis of hepatocytes as recently reported by Zender et al. [26].

One potential pitfall of the study of Black et al. is the preservation of the anti-apoptotic phenotype of cirrhotic hepatocytes after isolation and in culture. Indeed, although there is strong evidence that NF- κ B is activated in cholestatic liver injury in vivo, Black et al. did not demonstrate any involvement of NF- κ B in the resistance against TGF- β induced apoptosis in cultured cirrhotic hepatocytes. The preservation of anti-apoptotic mechanisms in cultured cirrhotic hepatocytes, in particular NF- κ B activation, but also other mechanisms involving activity of kinases, remains to be addressed and is of considerable importance to obtain a full comprehension of the anti-apoptotic phenotype of cirrhotic hepatocytes. Finally, we should not forget that in chronic liver injury hepatocytes die and eventually liver failure will develop. Strengthening the anti-apoptotic phenotype of already apoptosis-resistant hepatocytes may be a futile approach or even undesirable because of the risk for cancer development. Much more benefit can be expected from preventing necrotic cell death. In fact, the anti-apoptotic phenotype of cirrhotic hepatocytes may be the cause of necrotic cell death, since inhibition of apoptosis often shifts the balance to necrotic cell death [27–30].

All in all, there are many mechanisms which protect cholestatic or cirrhotic hepatocytes against apoptosis: MAP kinases like ERK and p38, PI-3/Akt kinase, transcriptional events such as downregulation of bile acid importers and NF- κ B-mediated induction of anti-apoptotic genes. Black et al. have now reported a novel mechanism: reduction of caspase-8 function. Involvement of other pathways like JNK and HO-1 remain to be investigated. All these pathways suggest numerous novel intervention targets aimed at modulating apoptosis in the chronically injured or cirrhotic

liver. The question is: should we inhibit or promote apoptosis? Inhibition of apoptosis will strengthen the resistant phenotype and may promote proliferation of malignant hepatocytes and development of hepatocellular carcinoma. On the other hand, reverting the resistant phenotype may lead to excess hepatocyte apoptosis and liver failure. The true challenge will be to avoid both Scylla and Charybdis. In the end, reducing necrotic cell death, e.g. by reducing inflammation, may be the answer to this challenge [31].

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