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Utility of M30, an apoptotic serum marker, in liver diseases

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Abstract: The aim of this paper is to evaluate the role of apoptosis in some common liver diseases, and the utility of M30, an apoptotic serum marker, in the diagnosis of the severity of underlying hepatic injury. As is widely known, apoptosis is programmed cell death, and its deregulation results in an uncontrolled inflammatory process leading to upregulation of liver fibrogenesis. Both extrinsic and intrinsic pathways are crucial in apoptosis, and caspase cleavage of cytokeratin proteins occurs in both. Therefore, the measurement of caspase-cleaved cytokeratin fragments could be a novel method to assess the intensity of apoptotic cell numbers in epithelial tissue damage. M30 levels were found to increase not only in acute liver disorders, but also in some chronic liver injuries. We tried to summarize the recent studies focused on the role of apoptotic processes in liver diseases, mainly those that investigated the use of M30 in determining the severity of, or in predicting, ongoing liver injury.

Key words: Apoptosis, M30 level, liver injury

1. Introduction

Apoptosis, also known as programmed cell death, is a routine feature of healthy organs and tissues. However, apoptosis has become an emerging topic of interest in some acute and chronic liver diseases in the recent years (1). Apoptosis has been found to be crucial in viral hepatitis, alcoholic and nonalcoholic steatohepatitis, cholestatic processes, and Wilson disease (2). It appears that dysregulated apoptosis has an important role in inflammation, and also in fibrogenesis (3). While the number of apoptotic cells should be equal to the number of newly formed ones, upregulation of apoptosis alters this healthy homeostasis. The most important part of apoptosis is that physiological apoptosis does not induce proinflammatory cytokine secretion, whereas upregulation does (4). With an increasing apoptosis rate, there may also be a regeneration stimulus that could also contribute to a high cancer risk (5).

2. The link from apoptosis to hepatic fibrosis

Apoptotic bodies in the liver can be phagocytosed by both Kupffer cells and hepatic stellate cells (6,7). After engulfment of apoptotic bodies by Kupffer cells, the Kupffer cells express death ligands (tumor necrosis factor alpha, TNF-α; tumor necrosis factor-related apoptosis-inducing ligand, TRAIL; and Fas ligand, FasL), leading to a proinflammatory status (7). These death ligands are closely related with their receptors to hepatocyte-induced death receptor-mediated apoptosis. In massive liver injury, the apoptotic cell phagocytosis capacity of Kupffer cells is overwhelmed by the increase in apoptotic cell engulfment by hepatic stellate cells or myofibroblasts derived from hepatic stellate cells (8). Myofibroblasts engulf the apoptotic cells and subsequently release some profibrogenic cytokines (like tumor growth factor beta, TGF-β) and type I collagen, promoting the development of fibrosis and cirrhosis (7).

There are 2 known pathways leading to apoptosis: the first is a death receptor-mediated or extrinsic pathway, and the second is an intrinsic pathway. The engaging of one of the ligands (FasL, TRAIL, or TNF-α) to the death receptors (Fas/CD95, TRAIL 1-2, or TNF R1), causes some conformational changes, leading initially to the activation of caspases 8 or 10. Subsequently, a proteolytic cascade starts to activate caspases 3, 6, and 7 (the effector caspases) via direct cleavage, or through the activation of Bcl-2 family protein Bid makes mitochondrial dysfunction to release cytochrome-c (caspase-activating factor) (1,9).

There is a growing interest in caspase cleavage of cytokeratin proteins during apoptosis in all epithelial tissues. Measurement of the cytokeratin fragments cleaved by caspases (the most important cleavage caused

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by caspases 3 and 6) during apoptosis was found to be a marker of ongoing apoptosis (10), and it is possible to detect these cleavage cytokeratin fragments by sandwich ELISA (11). M30- and M65-based ELISA detects such cytokeratin fragments and all of the cytokeratin proteins (both full-length and fragmented ones), respectively. M30-based ELISA gives the amount of ongoing apoptosis, while M65 shows the total cell death.

3. Importance of apoptosis in some selected liver diseases

3.1. Acute liver failure

Acute liver failure (ALF) is associated with ongoing massive hepatocyte death (1,4). While both apoptosis and hepatic stellate cell activation were found to be upregulated during the early phase of acute liver injury, Dechene et al. investigated the amount of apoptosis and the degree of hepatic stellate cell activation by using M30 and tissue inhibitor of metalloproteinases (TIMP1 and 2) and hyaluronic acid ELISA, and by immunohistochemical studies defining caspase 3 and alpha smooth muscle (alpha SMA) as markers of apoptosis and hepatic stellate cell activation in acute liver failure patients (12). They found that both apoptotic cell and hepatic stellate cell activation (fibrogenesis) markers increase in ALF. By considering M30 to be a good marker of underlying apoptotic process, Rutherford et al. described a new index for predicting the survival rate of patients presenting with ALF, and also showed that this new index, combining clinical parameters and M30 level, had better efficacy in predicting outcome compared with the King's College criteria and the Model for End Stage Liver Disease (MELD) (13). On the other hand, an M65-based modified MELD score had already been determined as a better scoring system in predicting the lethal outcome in ALF patients than the M30 (14). In contrast, Craig et al. suggested that none of the cell death markers (M30 and M65) improved the qualification of the existing scoring systems, particularly in paracetamol-related ALF cases (15). Jochum et al. showed a significant drop in M30 and M65 values at the end of 1 week of therapy (16).

3.2. Nonalcoholic liver diseases

In contrast to ALF, the relation between chronic liver injury and ongoing apoptosis has been known for years (17). The presence of inflammation in the liver, with or without fibrosis, is crucial in separating nonalcoholic steatohepatitis (NASH), which indicates the possibility of progressive liver injury, from simple hepatosteatosis. Feldstein et al. showed increased TUNEL-positive cells in the livers (apoptotic cells) of NASH patients compared with a control group (2). Activated caspases 3 and 7, which have important roles in the late phase of apoptosis, were also detected in NASH patients. Moreover, the amount of apoptotic cells was found to be correlated with the severity of liver fibrosis (higher apoptosis in stage 3–4 fibrosis than 1–2), and Fas staining scores were higher in NASH patients than in the control, as expected. M30 level was determined as an independent predictor of NASH in the context of simple steatosis (18,19). However, Shen et al. found that both M30 and M65 values in serum correlated with histological progression in NASH patients, and both increased in simple steatosis compared with healthy controls, but they had a moderate impact on discriminating NASH from simple steatosis (20).

3.3. Alcoholic liver diseases

While there are limited therapeutic options in severe acute alcohol related liver disease (ASH), the use of early liver transplantation has begun to be investigated (17). Natori et al. revealed a higher amount of apoptosis by both TUNEL and immunohistochemical detection of activated caspase 3 (21). Higher levels of serum bilirubin (>3 g/dL) and AST (>75 U/L) were found to be correlated with a higher amount of apoptotic cells, but not correlated with the survival of ASH patients. Hepatic apoptosis was prominent in grade 4 steatohepatitis rather than grade 1 or 2 in ASH patients. TNF-α expression was also noted in ASH patients, but not in controls. In contrast to the NASH study mentioned above, the expression of TNF-R1 was not upregulated in ASH patients or controls; even TNF-α and FasR, which are both death receptors, triggered apoptosis. FasL expression was found to be high in alcohol-induced liver injury in rats (22). Moreover, plasma TNF-α levels were higher in ASH patients than controls (23). Gonzalez-Quintela et al. showed that tissue polypeptide specific antigen, also a keratin 18 fragment used as a tumor marker, has a positive correlation with apoptotic score in ASH patients (24).

3.4. Chronic viral hepatitis

In active hepatitis C virus (HCV) infection, death receptor-related apoptosis has already been shown to be a main part of immune-mediated tissue damage, which presents with viral antigens (9). The magnitude of apoptosis has been shown to be related to fibrosis progression of chronic HCV infection (25). Yılmaz et al. and Valva et al. showed that there is a significant correlation between M30 level and histological steatosis score in HCV patients (11,26). More than half of the chronic HCV patients with normal aminotransferases presented with higher M30 levels in serum; although the importance of this is unknown, it may perhaps be due to the underlying HCV-related steatosis in the liver (25). On the other hand, Jazwinski et al. were not able to determine any correlation between M30 levels and the degree of steatosis in the liver of chronic HCV patients. However, they were able to identify a positive correlation between M30 level and the fibrosis stage (27). A human trial with pan-caspase inhibitor IDN 6556 was shown to be effective in reducing ALT levels in HCV-infected patients and AST levels in NASH patients (28).

Serum M30 levels were found to be significantly higher in chronic hepatitis B virus (HBV) patients than inactive HBV carriers, and Eren et al. determined similar M30 levels in serum in both inactive HBV carriers and healthy controls (29,30). The usefulness of measuring serum M30 level in order to predict the grade of fibrosis or histological activity index is still unknown. Joka et al. gave a cut-off value for M30 sera level (240 U/L) to predict the presence of chronic hepatitis B, and Papatheodorios et al. gave one to establish the severity of underlying fibrosis (157.5 U/L) (29,31).

3.5. Hepatocellular carcinoma

Dysregulated apoptosis (defective apoptosis, increased cell proliferation) has an important role in hepatocarcinogenesis (1). Not only hepatocellular carcinoma (HCC) but also other epithelial-originated cancers (breast cancer and head and neck cancer) and their risk of recurrence have been shown to be correlated with the apoptosis index (32). Most of the antitumoral treatments are targeted to a tumor suppressor gene, p53. However, p53 is resistant against some cancer drugs and can be overwhelmed by TRAIL induction leading to TRAIL-mediated apoptosis (33). Reduced Fas receptor expression is negatively correlated with the survival of HCC patients (34). It has also been shown that the M30 level is significantly higher in HCC patients than control groups (35).

4. Antiapoptotic treatment strategy

It is uncertain as to whether improving the apoptosis rate in the liver enhances ongoing inflammation and fibrosis. The main caspase targeted for blocking the ongoing apoptosis leading to liver injury is caspase 8 (9). Genetic absence of caspase 8 attenuated liver injury in a mouse experimental model (36). Wedemeyer et al. determined that adiponectin, a hormone secreted from adipose tissue reduced free fatty acid, induced CD95/FasR-mediated apoptosis (37). Thus, an increase in the level of adiponectin seems to be beneficial in patients with steatohepatitis.

Due its strong relation to TNF-α–mediated apoptosis in alcoholic hepatitis, the anti-TNF-α agent infliximab was already used in treatment (38). However, a discouraging result arose. The rate of death (according to infection, hemorrhage, and renal insufficiency) in the treatment group was higher than in the placebo group and the study had to be stopped.

It has been detected that p53, a tumor suppressor gene, has some relations with apoptotic mechanisms, and upregulates some death receptors (Fas, TRAIL-R1) and their ligands (39). On the other hand, p53 has the ability to engage BH-3-only proteins (proapoptotic proteins) and Puma, Noxa, Bid, and Bax (BCL-2 homologues), leading to the escape of damaged cells from apoptotic clearance.

A controversial point is the possible side effect of antiapoptotic therapy resulting in cancer. Masuoka et al. discussed this issue in a review article and concluded that there is no carcinogenetic process induced by blocking apoptosis by pan-caspases inhibitors as there are 2 pathways leading to apoptosis and with this therapy only the death receptor-related apoptosis is prevented (9).

5. Conclusion

This article focused on the apoptosis of liver cells, not the apoptosis of cancer cells or hepatic stellate cells. The latter processes have quite different results. Cancer therapies are mostly directly targeted at the apoptosis of cancer cells, whereas the apoptosis of hepatic stellate cells is the main issue to overcome in the fibrotic process. Recent studies noted the importance of apoptosis in liver injury and the possible ways to block the underlying dysregulated apoptosis. However, it is still too early to suggest using antiapoptotic agents in acute or chronic liver diseases routinely. More experimental and clinical results are needed to investigate the promising topic of apoptosis in liver diseases.

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