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REVIEW
Apoptosis and fibrosis in non-alcoholic fatty liver disease

Non-alkolik yağlı karaciğer hastalığında apoptoz ve fibrozis

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Nonalcoholic fatty liver disease is becoming an increasingly common medical problem in the developed countries which, unfortunately, still is associated with the lack of any effective treatment. However, recent data favor a model in which a pathologically increased rate of hepatocytic apoptosis and the subsequent induction and upregulation of inflammation and fibrosis in the liver provide both a rationale for the pathogenesis of nonalcoholic fatty liver disease, as well as a clue for designing first effective therapeutic strategies. In order to illuminate this context, this article focuses on the pathogenesis and possible new therapeutic options in nonalcoholic fatty liver disease.

Key words: Nonalcoholic fatty liver disease, Non-alcoholic steatohepatitis, apoptosis, apoptotic bodies, inflammation, fibrosis

Özellikle gelişmiş ülkelerde giderek artan bir biçimde görülmekte olan alkole bağlı olmayan yağlı karaciğer hastalığı (NAFLD, Nonalcoholic fatty liver disease), etkin bir tedavisi bulunmayan ciddi bir tıbbi sorun olarak günümüzde karşımıza çıkmaktadır. Ancak, son dönemlerde yürütülen çalışmalar ile, hepatositlerde programlı hücre olumunun yani apoptozun patolojik bir biçimde arttığı gösterilmiş ve bunun sonucu olarak da, karaciğerde inflamasyonun indüklenerek fibrozun geliştiği ortaya konmuştur. Geliştirilen bu hastalık modeli alkole bağlı olmayan yağlı karaciğer hastalığı'nın hem patogenezini açıklığa kavuşturacak, hem de etkin tedavi stratejilerinin geliştirilmesine öncülük edecek niteliktedir. Bu derleme, sözkonusu bilgilerin ışığında, alkole bağlı olmayan yağlı karaciğer hastalığı'nın patogenezini irdelemekte ve olası tedavi opsiyonlarını gözden geçirmektedir.

Anahtar kelimeler: Alkole bağlı olmayan yağlı karaciğer hastalığı, alkole bağlı olmayan steatohepatoz, apoptoz, apoptotik cisim, inflamasyon, fibroz

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), as a clinicopathologic syndrome, encompasses a spectrum of liver injuries, ranging from steatosis to steatohepatitis, advanced fibrosis, and cirrhosis (1, 2). NAFLD is increasingly recognized as the most common liver disease in developed countries (3). Nonalcoholic steatohepatitis (NASH) represents an advanced sub-entity of NAFLD which comprises steatosis, inflammation, and/or fibrosis in varying degrees (3).

It is generally believed that simple steatosis is benign with only a minimal risk of progression, whereas NASH is progressive and can lead to end-stage liver disease (2). The frequency with which NASH progresses to cirrhosis is uncertain, with recent studies reporting rates up to 15% (4). Currently, data specifically relating to NAFLD and liver transplantation is not available. However, two studies suggest that 50% of cryptogenic cirrhosis may in fact have arisen from NASH (5, 6). Commonly associated risk factors for NAFLD include presence of obesity, hyperlipidemia, diabetes mellitus, other metabolic diseases, HIV infection, as well as usage of drugs and narcotics (5, 7). Although these risk factors appear to play a pivotal ro-

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le in the development of NAFLD, the pathogenesis of and progression to NASH remain poorly understood.

Numerous studies have explored various treatment strategies for NASH, but none of these approaches has shown a convincing benefit. Indeed, no effective therapies have become available for treating this disease entity since the first description of NASH by Ludwig *et al.* (8). Therefore, advanced insight into the pathomechanism(s) underlying the progression to steatohepatitis might help in the design of new therapeutic options.

A growing body of evidence suggests that pathologically increased hepatocyte apoptosis is an important, if not critical, mechanism contributing to inflammation and fibrogenesis of the liver (9-12). Therefore, since both inflammation and fibrosis are the prominent features of NASH, one may reasonably hypothesize that derailed hepatocyte apoptosis plays a key role in the progression of NAFLD to NASH (13). Indeed, recent findings as described below may help to identify the missing links in the pathogenesis of NAFLD/NASH, thereby possibly paving the way for novel therapies for patients suffering from this chronic liver disease.

Programmed Cell Death (Apoptosis)

Apoptosis is nature's pre-programmed form of cell death which is characterized by organized cellular fragmentation; remnants resulting from this structured decay, termed apoptotic bodies, are then cleared by phagocytosis (14, 15). Apoptotic cell death can be triggered by two alternative pathways, i.e. (i) the extrinsic death receptor-mediated pathway, or (ii) the intrinsic intracellular organelle-based pathway (16).

While hepatocytes can undergo programmed cell death via the extrinsic as well as the intrinsic pathways, both of which are operational in the liver, extrinsic signals clearly predominate (17). Death receptors (DRs) specifically expressed in the liver include Fas/CD95, tumor necrosis factor receptor-1 (TNFR-1/CD120a), and tumor necrosis factor-associated apoptosis-inducing ligand receptors-1 and -2 (TRAIL-R1/DR4 and TRAIL-R2/DR5) (17, 18). Intriguingly, besides inducing cellular demise, DRs also initiate separate signaling cascades, most prominently those stimulating the synthesis of proinflammatory mediators (18).

Mitochondria play a general role in the final stage of apoptosis, and their metabolism serves as the great unifier for integrating both extrinsic and intrinsic signals into a common final pathway. Thereupon, a near-universal apoptotic signaling event, the mitochondrial release of cytochrome-c, triggers a final caspase-dependent cascade culminating in cellular fragmentation (19).

Nevertheless, the pleiotropic action of DRs appears to encapsulate an important message: when functioning in an ordered manner, the close integration of apoptotic death and the immediate removal of the resulting debris is an entirely physiologic process.

However, recent results favor an understanding in which the hyper-activation of programmed cell death in some diseases must be considered as a derailed non-selective process. If uninhibited, this process may progressively exacerbate over prolonged periods of time, thus entailing a number of serious consequences.

In human and animal models of liver injury, it has been demonstrated repeatedly that an increased rate of intrahepatic apoptosis is very likely to be the first cellular response to a broad spectrum of noxious events (20-22). Still, in a healthy context, both regionally and episodically increased apoptosis may be considered as an integral element of the liver.

Apoptosis, Inflammation and Fibrosis

Upregulated apoptosis of hepatocytes is increasingly viewed as a nexus between liver injury and fibrosis (23). As previously mentioned, death receptor-mediated apoptosis is particularly prominent in the liver. Among these receptors, Fas/CD95 is upregulated in hepatocytes in the course of disease processes (20, 21). Indeed, the Fas ligand itself exerts a proinflammatory activity (24).

In addition to pathologic rates of apoptosis, virtually all liver diseases are associated with an enhanced inflammatory response (10). According to recent concepts, these events must be regarded in concert; current data indicate that pathologically upregulated apoptosis in the liver both directly and indirectly promotes inflammation and fibrosis (11, 25-28).

As demonstrated experimentally in a murine model, upregulated intrahepatic apoptosis can lead to severe liver damage by fulminant hepatic failure with massive inflammation and necrosis (29). Specifically, when the extent of apoptosis overrides the phagocytic clearance, the apoptotic bodies can undergo spontaneous disruption, thus releasing

their contents and causing tissue damage and inflammation (30). This concept was supported in a recent study by Takehara et al., in which hepatocyte-specific disruption of Bcl-X_L was shown to lead to continuous hepatocyte apoptosis and liver fibrosis (12).

Engulfment of apoptotic bodies by macrophages or Kupffer cells has also been reported to induce the expression of death ligands, with Fas ligand as their most prominent proinflammatory representative as mentioned before (24, 31-33). Such findings suggest that non-physiological deposition of apoptotic bodies in the liver leads to inflammation.

Recent data implicate that, in addition to causing apoptosis, deleterious DR-mediated signaling may directly contribute to liver inflammation (10, 23, 25, 26, 34). As an example, agonists of Fas/CD95 stimulate hepatic chemokine expression, neutrophil infiltration, and inflammation (27). Another example is TNFR-1/CD120a which, when ligated by TNF-α, affects down-stream activation of nuclear factor κB (NF-κB); this transcription factor in turn activates the expression of many proinflammatory cytokines (18). Mechanisms by which DR-mediated apoptosis promotes inflammation may further include the (over-)expression of inflammatory CXC chemokines (11, 27). In addition, Jaeschke and collaborators demonstrated that hepatocyte apoptosis is a potent stimulus for neutrophil infiltration and an increased susceptibility for endotoxin-induced liver injury (10, 26, 27, 34). These findings are in line with the fact that in both human and experimental alcoholic hepatitis, apoptotic hepatocytes co-localize with neutrophils, which correlates strongly with the severity of the tissue damage (10, 35, 36). Consistent with this data, inhibition of hepatocyte apoptosis blocks neutrophil transmigration into the liver during injurious conditions (10, 36).

One late-stage consequence of hepatic inflammation is that activated hepatic stellate cells (HSCs) assume a myofibroblastoid phenotype that continuously expresses and deposits collagen within the perisinusoidal spaces (37). Activated HSCs have also been shown to participate in, and mediate, the inflammatory response by expressing cytokines and adhesion molecules (38, 39). However, most recent evidence suggests that HSCs might participate in an even earlier stage of disease. It is an established fact that in both the pre-inflammatory and inflammatory stages of liver damage, hepatocytes, Kupffer cells and activated HSCs secrete considerable amounts of insulin-like growth factor I (IGF-I), which is thought to play an important role in the course of liver fibrogenesis (40). Thus, as to the role of apoptosis, HSCs might be much more important players than previously assumed.

Taken together, considerable evidence supports the pathophysiologic concept that excessive apoptosis in the liver acts as a proinflammatory and profibrogenic trigger.

NAFLD and Apoptosis

Despite the pervasive link between elevated hepatocyte apoptosis on the one hand and liver damage and fibrosis on the other (23), the concrete role of apoptosis in NAFLD/NASH has not yet been fully explored.

Current studies have shown that in NASH patients, hepatocyte apoptosis and Fas expression are even more markedly enhanced than in patients with alcoholic hepatitis (13, 41). This may explain the fact that higher rates of NASH patients develop liver cirrhosis when compared to patients with alcoholic hepatitis (13). In-vitro studies employing the HepG2 liver cancer cell line have revealed that free fatty acids promote upregulation of the Fas/CD95 receptor (42). Fas-induced hepatocyte apoptosis is mediated by caspase-8-dependent cleavage of Bid, a proapoptotic member of the Bcl-2 family that translocates into the mitochondria and, in concert with other proapoptotic proteins, induces dysfunction of this organelle (22). The successive mitochondrial release of cytochrome-c then activates effector caspases-3 and -7, and thus the apoptosis machinery. When considering the entire organ, liver samples from NASH patients reveal enhanced activation of caspases-3 and -7, which in turn positively correlates with the severity of disease and fibrosis (13, 41). In addition, Bantel et al. have demonstrated that in chronic hepatitis C virus (HCV) infection, hepatocyte apoptosis correlates with liver fibrosis (43).

Nevertheless, in mice receiving a high-carbohydrate diet, Fas receptor is also upregulated significantly (42). Accordingly, obese mice treated with Fas agonist (Jo2) show increased liver injury as evidenced by elevated levels of ALT, apoptosis and inflammation (42, 44). This vulnerability is partly due to DR-mediated apoptosis. In line with these experimental results, we have documented that obese patients are much more susceptible to acute liver failure when subjected to known risk factors (45).

Anti-Apoptotic Strategies as a Therapeutic Option in NAFLD

Taken together, it appears that increased hepatocyte apoptosis indeed plays a dominant role in the development and progression of NAFLD.

Although still preliminary, one may make a convincing case for the potentially high therapeutic benefit of inhibiting hepatocyte apoptosis in patients with steatohepatitis. Such a strategy obviously might prevent liver inflammation, fibrosis, and their sequelae. To this end, caspase inhibitors are currently being developed for clinical use. In the bile duct-ligated mouse model of cholestasis, the pan-caspase inhibitor, IDN-6556, has already proven beneficial as an antifibrotic agent (23). Likewise, treatment of HCV-positive patients with this inhibitor significantly reduces ALT values and, therefore, liver injury (46). However, perhaps the most promising approach to date has just been presented by Eichhorst *et al.*, who applied the already FDA-approved drug, suramin, to inhibit apoptosis in a mouse model of liver damage (47, 48). Their results appear highly encouraging, but caution is warranted because the therapeutic long-term employment of anti-apoptotic drugs might potentially promote excessive cell growth. Although much work remains to be done, the recent findings briefly outlined herein foreshadow exciting new treatment options for designing new therapeutic strategies for NAFLD.

Conclusion and Perspective

The data currently available favors a model for the pathogenesis of nonalcoholic fatty liver disease which is based on an apparent sequential relationship of intrahepatic apoptosis, inflammation and fibrogenesis. Based on both hepatic and peripheral insulin resistance, the hepatocellular accumulation of triglycerides, termed as steatosis, initially leads to an altered metabolism of glucose and free fatty acids in the liver (Figure 1). In response to these metabolic alterations, expression of death receptors in simple steatosis is increased, giving rise to enhanced hepatocyte susceptibility for pro-apoptotic stimuli, which in turn elicits excessive apoptosis and inflammation in the liver. Evidence indicates that these processes, if prolonged, activate both hepatic stellate and Kupffer cells, to further contribute to the vicious circle in which apoptosis, inflammation, cellular activation, and collagen deposition have already been upregulated (Figure 2).

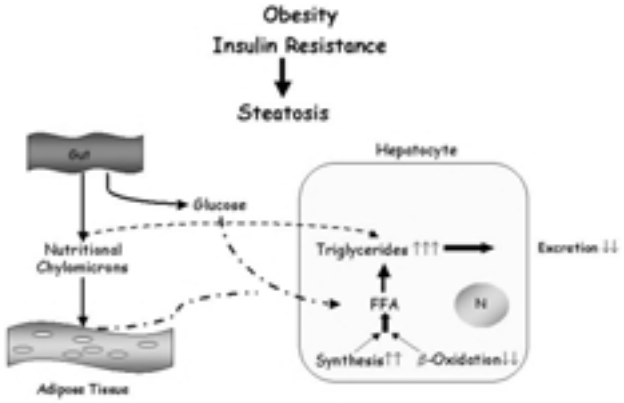

Figure 1. Hepatocellular accumulation of free fatty acids (FFAs) The accumulation of triglycerides (steatosis) in hepatocytes is thought to occur initially and primarily through hepatic and peripheral insulin resistance, which leads to altered glucose and FFA metabolism

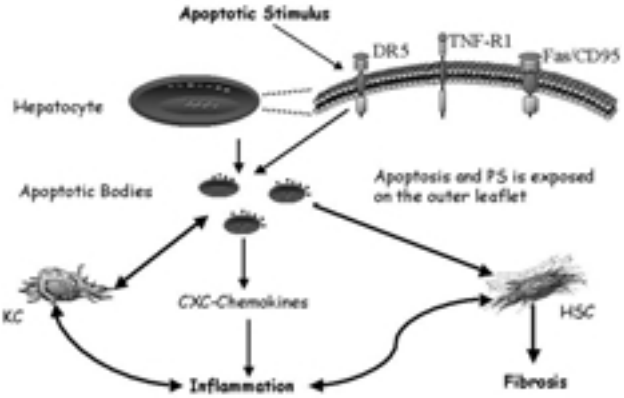

Figure 2. Enhanced death receptor expression in hepatocytes Enhanced expression of death receptors (Fas, DR5, TNF-R1) in simple steatosis enhances the susceptibility for pro-apoptotic stimuli, which induce hepatocyte apoptosis and pro-inflammatory response. In apoptosis, phosphatidylserine (PS) is exposed on the outer leaflet of the plasma membrane. Apoptosis in the long term activates stellate cells (HSC) and Kupffer cells (KC) which further promote hepatocyte apoptosis, culminating in hepatic inflammation, with generation of CXC chemokines and further HSC activation with collagen deposition

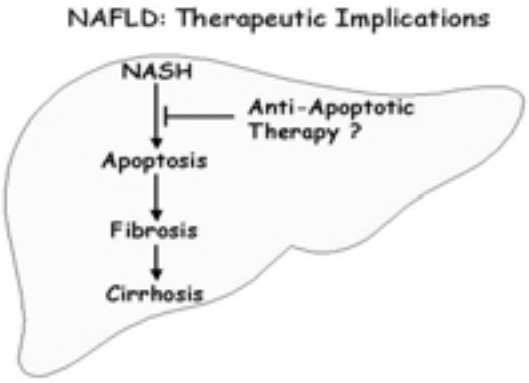

Figure 3. Therapeutic strategies in NAFLD/NASH

In conclusion, in nonalcoholic fatty liver disease, the crucial biological mechanism of apoptosis is turned into a key pathogenic event. The fight to be employed against this enhanced apoptosis may be the basis for novel therapeutic modalities developed in the future to slow down the progression of this disease (Figure 3).

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