Apoptotic Signaling Pathways as a Target for the Treatment of Liver Diseases

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Abstract: Dysregulation of apoptosis is a major contributor to the initiation and aggravation of liver injury. Agents that modulate apoptosis may be of therapeutic benefit in a number of liver diseases, and research related to cell type-specific activation or inhibition of apoptotic signaling pathways will provide new strategies for treatment.

Key Words: Liver, hepatitis, alcohol, hepatocarcinoma, cholestasis, fulminant hepatic failure, steatohepatitis, fibrosis.

INTRODUCTION

The importance of apoptosis is evident in the liver during development and homeostasis of the biliary tree, and apoptotic cell death is also increased in the aging liver [1]. However, in the context of liver disease, over activation involving mediators such as Fas, tumour necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and members of the Bcl-2 family, can lead to significant acute injuries, such as fulminant hepatic failure or even chronic sustained hepatocellular damage, as occurs with toxic liver injury, viral hepatitis, alcoholic and non alcoholic liver disease or cholestatic disorders. On the contrary, inhibition of apoptosis can promote the proliferation and transformation of cells and possibly hepatocellular carcinoma, protecting malignant hepatocytes from cellular suicide and avoiding removal of cells carrying mutated genes. This review gives an overview of the importance of apoptotic signaling pathways as a target for the treatment of liver diseases.

1. APOPTOTIC SIGNALING PATHWAYS IN THE LIVER

Two molecular pathways are mainly responsible for apoptotic signaling within the cells: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. Apoptotic events in the hepatocytes can be regulated by different stimuli that bind to death receptors in the cell membranes, such as Fas ligand (FasL), TNF-α or TNF-related apoptosis-induced ligand (TRAIL), which activate the extrinsic pathway. Other factors, particularly TGF-β, do not bind the death receptors, but its intracellular signals couple to the apoptotic machinery through activation of the intrinsic pathway. Binding of FasL or TNF-α to their corresponding death receptors induces the recruitment of procaspase 8-10 to form the death-inducing signaling complex, leading to cell death. TRAIL, which is regulated by two death receptors (TRAIL-R1 and TRAIL-R2), induces apoptosis in transformed cell lines but not in normal tissue (Fig. (**1**)). The intrinsic pathway is triggered by different intracellular or extracellular signals that induce hepatocytic mitochondrial dysfunction, resulting in the cytsolic release of mitochondrial proteins such as cytochrome c, SMAC/DIABLO, apoptosis-inducing factors or endonuclease G among others. Several intracellular proteins, in particular de Bcl-2 family are important regulators of the intrinsic pathway, intregrating death and survival signals [3]. This family includes both pro-apoptotic (t-Bid or Bax) and anti-apoptotic (Bcl-2 or Bcl-XL) proteins (Fig. (**1**)).

There is less information on the apoptotic signaling pathways in other liver cells. Cholangiocytes constitutively express Fas, but the apoptotic potential role of TNF-α or TRAIL is less clear [4]. Activated hepatic stellate cells become sensitive to Fas-mediated apoptosis, with increased TRAIL-R protein expression [3]. Kuppfer cells appear to regulate the apoptotic machinery with potential relevance for immunoregulation. Finally, endothelial cells express Fas, and Fas activation can induce apoptosis of endothelial cells from liver sinusoids [5].

2. APOPTOSIS IN LIVER DISEASES

2.1. Cholestatic Liver Disease

Cholestasis is characterized by an accumulation in the liver of hydrophobic bile acids which are thought to play a key role in liver injury. Although some studies have suggested that the predominant mode of cell death during obstructive cholestasis is oncotic necrosis [6], they have focused on cell death occurring in bile infarcts, and this is a late occurrence in which identification of cell killing mechanism by morphologic criteria may be difficult [7]. Moreover, pan-caspase inhibitors prevent bile infarcts in bile duct-ligated mice [8], although mechanisms other than suppression of apoptosis, such as blocking of cytokine signaling, might be involved [9]. In any case, widespread necrosis is not a prominent feature in most cholestatic liver diseases, and a higher rate of apoptosis compared with healthy controls occurs in patients with primary biliary cirrhosis [10].

It is well-known that hepatic levels of toxic bile acids correlate with the degree of liver damage [11], and hepatic retention of hydrophobic bile acids has long been implicated as a major cause of liver damage. The mechanisms of apop-

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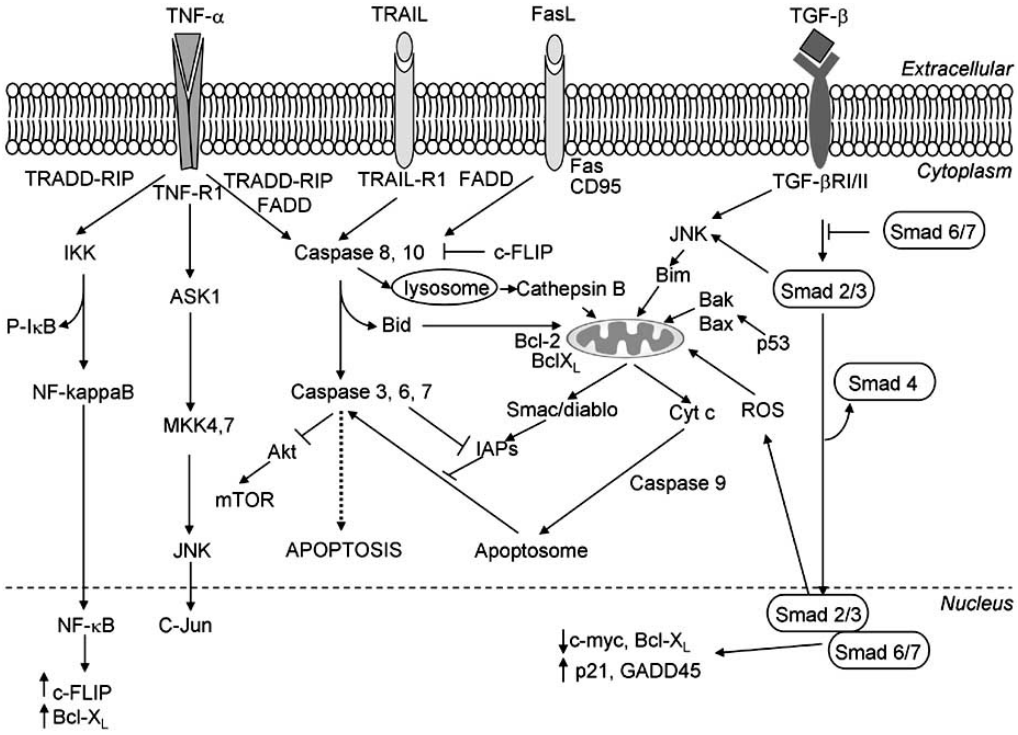

Fig. (1). Apoptotic pathways in hepatocytes.

tosis by bile acids have been elucidated in recent years. Although there is evidence of a Fas-dependent apoptotic signaling [12], bile acids may also induce apoptosis in Fas deficient lpr mice through a mechanism that involves transcriptional induction and oligomerization of TRAIL-R2, with recruitment of FADD and cleavage of caspase 8 [13]. In this model of apoptosis, signals converge to produce mitochondrial permeabilization, release of cytochrome c and activation of downstream caspases and cathepsin B [14]. This cysteine protease also participate as an important effector mechanism, because apoptosis and subsequent fibrosis are absent in mice genetically deficient in cathepsin B after bile duct ligation [15]. Mitochondrial dysfunction also appears to contribute to hepatocyte apoptosis in cholestatic liver disease. *De novo* expression of Bcl-2 has been observed in hepatocytes of bile duct-ligated rats, suggesting an adaptative mechanism to resist apoptosis by toxic bile acids. In fact, cholangiocytes, which are in direct contact with bile continuously, express Bcl-2 constitutively [11]. Induction and translocation of Bax to mitochondria, probably mediated by t-Bid, have been reported after bile duct ligation in lpr mice, and inhibition of Bid expression using an antisense technology appears to be a promising approach for the amelioration of cholestatic liver injury [13].

Ursodeoxycholate (3,7/3-dihydroxy-5/3-cholanoic acid) (UDC) is identical in structure to the primary hydrophobic bile acid chenodeoxycholic acid, except that the hydroxyl group at C-7 is in a β rather than an α configuration (Fig. (**2**)). This chemical structure renders UDC a non toxic hydrophilic bile acid which reduces apoptosis induced by toxic bile acids through a mechanism that seems to be associated to direct prevention of the mitochondrial permeability transition [16]. It has been demonstrated that the taurine conjugate of UDC protects against bile acid-induced apoptosis *via* the activation of survival pathways such as p38, extracellular signal-regulated kinases (ERK), and phosphatidylinositol 3-kinase (PI3K) pathways [17]. More recently, a cross-talk of UDC with nuclear steroids receptors has been suggested to contribute to the modulation of hepatocyte apoptosis [18]. Activation of kinase signals by bile acids also contributes to hepatocyte apoptosis and may be a target for new therapeutical strategies in cholestasis. Thus, it has been demonstrated that eupatilin (5,7-dihydroxy-3,4,6-trimethoxyflavone), a pharmacological active ingredient in *Artemisia asiatica*, attenuates caspase 8 cleavage through inhibition of bile acid-induced Jun terminal kinase (JNK) activation [19].

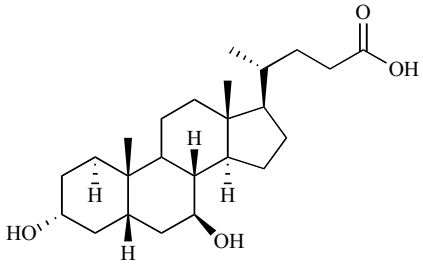

Fig. (2). The structure of the bile acid ursodeoxycholate.

2.2. Fulminant Hepatic Failure

Fulminant hepatic failure (FHF) may be induced by drugs, toxins or viral hepatitis, and is characterized by a severe hepatocellular dysfunction with a massive death of hepatocytes, in which apoptosis may play a role in addition to necrosis [20]. It has been indicated that serum cytochrome c is a possible new marker for acute liver failure in humans

and correlates to serum levels of AST and ALT [21]. Because FHF is associated with a high risk of lethality results and orthotopic liver transplantation is not always available in a timely fashion, the intervention on apoptotic pathways could be molecular targets for potential therapeutical approaches to FHF, contributing to temporary support while awaiting a liver transplant.

Some therapeutical approaches to FHF have tried to block the apoptotic cascade. Expression of antiapoptotic molecules such as Bcl-2, Bcl-X_L or a dominant negative FADD/MORT1 molecule, as well as treatment with the caspase inhibitor Ac-VAD-CMK (Ac-Val-Ala-Asp-chloro-methyl ketone) have been reported to rescue mice from anti-CD95 or TNF-induced failure [22]. Pretreatment with colchicine (N-((7S)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxo-benzo(a)heptalen-7-yl) acetamide) protects mice from a lethal dose of anti-CD95 antibody Jo2, probably by down-regulating the surface expression of CD95 on hepatocytes [23]. It has been described that CD95 and TNF-induced liver damage may also be prevented by the neurokinin 1-receptor (NK-1R) antagonists and the polysulfonated derivative of urea suramin. Anti-apoptotic effect of NK-1R antagonists is related to its ability to reduce the CD95- and TNF-R1-mediated caspase 3 activation, and suramin attenuates caspase 8-10 activation through the inhibition of the death-inducing signaling complex (DISC) [24].

The rabbit hemorrhagic disease (RHD) has been shown to fulfil many of the requirements of an animal model of FHF, being useful to improve our insight into the metabolic and physiological derangements of FHF [25]. In this model, apoptotic cell death is induced *via* both the intrinsic and the extrinsic signaling pathways, and the antioxidant N-acetylcysteine exerts an antiapoptotic effect related to the modulation of Bcl-2 and Bax genes. The protective effect provided by N-acetylcysteine suggests that oxidative stress is a primary pathway for apoptosis in this model of FHF [26]. In this same line, intraperitoneal injection of an antioxidant lignan compound from *Schisandra fructus*, has been reported to reduce DNA fragmentation and to attenuate the elevation of serum TNF-α and activation of caspase 3 in D-galactosamine and lipopolysaccharide-induced liver failure [27].

Suppressor of cytokine signaling-1 (SOCS1), which is a negative-feedback molecule for cytokine signaling, has been demonstrated to be rapidly induced during liver injury. Very recently it has been shown, using liver-specific SOCS1-conditional-knockout mice, that SOCS1 deletion in hepatocytes enhanced concanavalin A-induced hepatitis, and increased proapoptotic signals, including signal transducers and activators of transcription 1 (STAT1) and JNK activation, and higher expression of Fas antigen. These findings indicate that SOCS1 plays important negative roles in FHF and his forced expression could be of therapeutical use [28].

RNA interference (siRNA) is a relatively new and powerful approach with encouraging success in experimental models of apoptotic liver damage. Studies in mice have demonstrated the silencing effect of siRNA duplexes targeting the gene Fas in models of autoimmune hepatitis induced by concanavalin A [29]. Injection of caspase 8 siRNA during Ad-FasL- and adenovirus wild type-mediated liver failure prevents apoptosis and significantly attenuates acute liver damage [30].

2.3. Viral Hepatitis

Deregulated apoptosis appears both in acute and chronic viral hepatitis. Apoptotic bodies, previously recognized as Councilmen bodies, are present in the liver of patients with viral hepatitis [2]. The degree of hepatic inflammation in hepatitis C infection correlates with the degree of caspase activation [31], and patients with active HCV have elevated levels of caspase-generated cytokeratin 18 cleavage fragments compared with healthy controls [32]. Some data suggest that the apoptotic events may be mediated by the host immune system. Studies on human liver biopsy material have shown increased number of TUNEL-positive liver cells expressing Fas receptor closely associated with Fas ligand expressing cytotoxic T lymphocytes [33]. The activation of immune-mediated apoptotic pathways is corroborated by the fact that the transcripts for perforin/granzyme B are elevated in patients with HCV-related cirrhosis compared to normal liver or other no inflammatory causes of liver cirrhosis [34].

Hepatitis B virus (HBV) is one of the leading causes of chronic liver disease and is often associates with hepatocarcinogenesis. The virus consists of a nucleocapsid and an outer envelope composed mainly of three surface antigens. The nucleocapsid contains the core antigen, the viral genome and cellular proteins. The X protein of HBV (HBx) is a potent transactivator essential for virus replication and shows oncogenic properties in animal models [35]. HBx forms complexes with p53 in the cytoplasm and retains p53 from entering the nucleus [36]. HBx may also directly inhibit caspase 3 and activate PKB survival pathway [24]. Induction of NF-kappaB or JNK pathway may also contribute to the protection of HBx against anti-Fas-mediated apoptosis in human liver cells [37]. However, HBx may also sensitize cells to apoptotic killing by TNF-α, without up-regulation of TNF-R1 expression, through activation of MAPK kinase 1 and n-Myc [38], and also by abrogating the apoptosis inhibitory function of c-FLIP [39]. HBV viral variants containing two core promoter mutations associated with fulminant hepatitis have been reported to induce apoptosis in primary Tupaia hepatocytes. The core mutations resulted in two amino acid changes of the HBx protein, which suggests that HBx may be a potential candidate mediating this effect [40].

Hepatitis C virus (HCV) may frequently result in liver cirrhosis and hepatocellular carcinoma. HCV belongs to the flaviviridae and is a RNA virus whose RNA encodes for a polyprotein that is cleaved into different structural (E1, E2, core, p7) and non-structural (NS2, NS3·, NS4A, NS4B, NS5A, NS5B) proteins. Six different genotypes which differ genetically from one another by at least 30% have been identified. This genetic heterogeneity makes difficult to compare apoptotic pathways [41]. Multiple HCV proteins expressed in transgenic mice have been shown to inhibit Fas-mediated apoptosis by preventing the release of cytochrome c from mitochondria and activation of caspase 9, 3 and 7 [42]. The HCV non-structural NS2 protein has been reported to bind and protect from cell death-inducing DFF45-like effector (CIDE). Interactions blocks cytochrome c release from mitochondria and cell death triggering, and double staining of

NS2 and CIDE reveals partial overlapping signals in the perinuclear region [43]. NS5A protein has sequence homologies to Bcl-2 and inhibits apoptosis [44]. In addition, NS5A may sequester p53 in the cytoplasm and thereby inhibit apoptosis, activate the P13-kinase-Akt/PKB survival pathway [45], or activate STAT3 with enhanced expression of Bcl-X_L and p21 [46]. By contrast, a direct inhibition by proapoptotic Bim1, a tumour suppressor protein with a SH3 domain has been shown in hepatoma cells [47], and there are reports of a direct NS5A-induced apoptosis [48]. It has been shown that core protein inhibits c-myc mediated apoptosis in Chinese hamster ovarian cells and ciplastin-mediated apoptosis in human cervical epithelial cells [49]. Direct binding of core protein to the downstream death domain of FADD and c-FLIP has been reported to result in anti-apoptotic effects [50]. Moreover, it has been demonstrated that core proteins interact with the DNA-binding domain of Smad3, inhibiting the TGF-β receptor I/II apoptotic pathway [41]. However, over-expression of HCV core protein protects against chemotherapeutic drug-induced apoptosis, but not against CD95-induced apoptosis in hepatoma cells [51]. Core protein can bind to the cytoplasmic domain of TNF-R1 and this interaction promotes apoptosis in mouse and human cell lines [2]. Other studies have shown core-induced apoptosis through mitochondrial cytochrome c release and indirect activation of Bax and TRAIL-induced apoptosis in hepatoma cells [52]. Moreover, HCV triggers production of reactive oxygen species and lowering of the mitochondrial transmembrane potential, leading to consecutive caspase-independent cell death [53]. Envelope proteins E1 and E2 inhibit CD95L-mediated apoptosis in a transgenic mouse model expressing HCV proteins. Inhibition of TRAIL-induced apoptosis in hepatoma cells by E2 has been demonstrated, with no effect of E1. By contrast, E2 induces mitochondria-related and caspase-dependent apoptosis in the same hepatoma cell lines [41].

Thus, virus proteins may sensitize hepatocytes to apoptosis or inhibit apoptosis, and their role requires to be investigated in more detail before therapeutical goals can be achieved. The now available infectious tissue culture systems as well as future *in vivo* systems may give answer to these questions, may better reflect the *in vivo* situation and may help to clarify the role of the apoptotic pathways in the pathogenesis of HCV infection [41]. In spite of those limitations, some cell death-targeted approaches to viral elimination have been reported. Bid engineered to contain a specific cleavage site for the NS3/NS4A protease and delivered into mice with chimeric human liver is specifically activated and causes a considerably decline in HCV serum titres [54]. Effective reduction of liver damage and improvement of survival in mice injected with small interfering RNA (siRNA) against caspase 8 has been shown in models of acute hepatitis, and the same approach prevents the development of fibrosis in a model of chronic hepatitis [42]. Differential sensitivity of HBV-infected hepatocytes to TRAIL might be therapeutically useful for removing hepatocytes bearing CCC DNA which permit HBV to resume replication even during or after antiviral therapy [2]. In murine models of autoimmune hepatitis induced by injection of concavalin A or a Fas agonistic antibody, a nitric oxide derivative of ursodeoxycholic acid (NCX-1000; 2-(acetyloxy) benzoic acid 3-(nitrooxymethyl) phenyl ester) inhibits caspase activity [55]. Although longer studies are merited, the potent inhibitor of caspases IDN-6556 (3-{2-[*tert*-butyl-phenilaminooxalyl)-amino]-peopio-nylamino}-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxyl)-pentanoic acid) has been recently shown, in a multicenter, double-blind, placebo-controlled study with a 14-day dosing period, to significantly lower aminotransferase activity in HCV patients [56].

2.4. Non-Alcoholic Steatohepatitis and Alcoholic Liver Disease

Non alcoholic steatohepatitis (NASH) is characterized by an accumulation of fat in the liver along with inflammation in patients with no history of alcohol consumption or drug abuse. Many aspects of the pathophysiology of NASH are still unclear, but it is known that hepatocyte apoptosis is increased in patients with NASH and correlates with the disease severity and stage of fibrosis. Death receptor expression, especially Fas and TNF-R1, is also significantly enhanced [42]. Induction of the prooxidant cytochrome P450 2E1 (CYP2E1) is a general finding, and over-expression of CYP2E1 causes in hepatocytes activation of ERK1/2 and sensitizes cells to TNF-induced apoptosis through activation of JNK [57]. The fact that antioxidants reduce both MAPK activation and TNF-induced cell death in CYP2E1 expressing hepatocytes suggests a contribution of reactive oxygen species to increased apoptosis and hepatocellular injury in this disease [37]. Liver injury is associated with subclinical insulin resistance and diabetes mellitus in patients with NASH and evidence has been provided of a possible crosslink between metabolic and inflammatory pathways that leads to increased activation of apoptotic pathways. Thus, JNK activation causes insulin resistance *in vitro* and *in vivo*, and application of adiponectin, whose levels are decreased in patients with non-alcoholic fatty liver disease, counteracts TNF-induced liver injury in mice [58].

Liver disease related to alcohol consumption has a high mortality rate. Although the pathogenesis of alcoholic liver disease is still poorly understood, different studies have shown the importance of apoptosis in this type of liver damage. The severity of the disease can be correlated with the amount of apoptotic cells in liver biopsies from patients [11]. Increased expression of cytochrome CYP2E1, reactive oxygen species and lipoperoxides may contribute to hepatocyte apoptosis [59]. Production of reactive oxygen species, which is driven by increased availability of the reduced form of nicotinamide adenine dinucleotide as a consequence of acetaldehyde metabolism, may cause mitochondrial dysfunction and release of cytochrome c into the cytosol [42]. Antioxidants reduce acute hepatocellular injury in rats receiving ethanol through prevention of the mitochondrial release of apoptotic factors [37].

High FasL expression has been found in hepatocytes of patients with alcoholic liver disease. This may be induced by reactive oxygen species or by TNF-α-induced activation of NF-kappa B, which may upregulate the transcription of Fas and Fas ligand genes [42]. In fact, TNF-α levels are also increased during alcoholic hepatitis, and chronic ethanol administration has been reported to increase TNF-R1 expression on hepatocytes [11]. The important role of TNF-α in the

pathophysiology of alcoholic liver disease is reinforced by the demonstration that reduced TNF expression with antibodies anti-TNF, adiponectin therapy or impaired TNF signaling through TNF-R1 have reduce fatty acid infiltration and inflammation in experimental models of alcoholic liver disease [60].

TGF-β signaling appears to be also important in alcoholic steatohepatitis. TGF-β has been reported to be produced by hepatic stellate cells in alcoholic liver disease and might have a double impact on the progression of the disease by promoting fibrogenesis and killing hepatocytes by apoptosis [11]. Moreover, studies on HepG2 cells indicate that potentiation of TGF-β -induced cell death by CYP2E1 may contribute to mechanisms of alcohol-induced liver disease [61]. T-cells and NK T-cells have been implicated in the development of alcoholic liver disease by the increased numbers found in human liver following ethanol injury. Cytotoxic T cells can be generated in response to acetaldehyde-modified cells, and it has been reported that aldehyde-modified proteins at high levels can cause cell death and apoptosis [62]. Therefore, the build-up of these adducts in the liver could potentially increase the level of apoptosis [63].

2.5. Hepatocellular Carcinoma

Development and progression of tumours of the liver and the biliary tree has been associated with insufficient apoptosis [42]. Hepatocellular carcinoma (HCC) is the most common primary malignant hepatic tumour, with a vast incidence through the world. The available evidence indicates the disruption of apoptosis in several steps of HCC development, especially in its promotion stage. Fas is partially or completely loss in HCC [64] and Fas expression negatively correlates to the degree of HCC differentiation and patient survival [65]. On the contrary, p53-mediated up-regulation of Fas expression and increased apoptosis have been reported in human hepatoma cells following treatment with different chemotherapeutic agents [66]. Nevertheless, down-regulation of Fas alone may not be sufficient to escape the immune response and many HCC also over express Bcl-X_L, which confers resistance to mitochondrial-induced apoptosis. As the death receptor-mediated pathway of apoptosis is linked to the mitochondrial pathway in hepatocytes, over-expression of Bcl-X_L may contribute to Fas resistance in HCC [67]. Several chemotherapeutic agents, such as etopoxide, camptothecin or norcantharidin, have been shown to up-regulate Fas expression and increase sensitivity to Fas mediated apoptosis in hepatoma cells [42].

Another important factor in HCC is disruption of TGF-β signaling. In HCC a significant reduction in the mRNAs for both TGF-β type 1 (TβR-I) and TGFG-β type 2 (TβRII) receptor has been reported and it has also been shown that type 2 receptor, although expressed in tumoural hepatocytes, is detected in the cytoplasm but not in the plasma membrane [68]. However, changes at the receptor levels do not appear to be as important as they are in other types of gastrointestinal tumours [69], and other factors disrupting TGF-β signaling may exist. Dysregulation of the EGF pathway has been observed in HCC [70] and EGF receptor ligands might protect tumoural cells from TGFG-β -induced apoptosis, perhaps through over expression of TAC/ADAM17 [3].

It is also worth mentioning that mutations in the p53 gene are common alterations in HCC. A dysfunctional p53 allows the tumour cells to escape apoptosis and results in cancer development [42]. Moreover, because several chemotherapeutic drugs induce apoptosis of tumour cells by activation of p53, tumours with disrupt p53 are generally resistant to chemotherapy and associated with an unfavorable prognosis [71]. It has been reported that decreased p53 function contributes to the loss of CD95 expression ad reduces sensitivity of HCC cells towards this apoptosis pathway [66]. Microinjections of wild-type p53 and treatment with bleomycin restore sensitivity towards CD95-induced apoptosis [66]. Nevertheless, delivery of p53 recombinant DNA into rodent models of HCC does not suppress tumour growth [72] and it has been hypothesized that the effect of p53 loss is probably associated to the presence of intact or dysfunctional telomeres [73]. Only preliminary and non conclusive results are available to date from p53 gene therapy trials for HCC.

Apoptosis inhibition in HCC also requires inhibitors of apoptosis proteins (IAPS), which inhibit caspase activation. One member of the IAP family, survivin, may play an important role in progression of HCC by promoting cell proliferation, and is positively correlated with high risk of disease recurrence and poor prognosis in HCC. Its expression may therefore serve as a prognostic factor for patients with HCC after hepatectomy [74]. Transfection of liver tumour cells HepG2 with antisense oligonucleotide (ASO) against survivin results in significant cells growth inhibition and reduction expression of survivin. Furthermore, systemic treatment with ASO significantly inhibits tumour growth in an orthotopic transplant model of HCC in nude mice, suggesting that ASO could potentially be a promising gene therapy approach to treatment of HCC [75].

Levels of c-FLIP are increased in HCC, contributing to apoptosis induced by CD95, TRAIL-R1 and TRAIL-R2. Down-regulation of c-FLIP by pre-treatment with cycloheximide and actinomycin D results in partial resensitization of tumour cells to apoptotic stimuli [23]. Increased expression of TRAIL-R1 may be a mechanism of cisplatin-induced sensitization to TRAIL-induced apoptosis in some HCC cells [76]. Different studies have also shown that various agents, such as selective COX-2 inhibitors, interferon-gamma and PK111195, a mitochondrial benzodiazepine receptor antagonist, increase the sensitivity of HCC to chemotherapeutic agents and radiation therapy. Enhancement of tumour sensitivity to apoptotic signals may improve the prognosis of patients with HCC [31].

New approaches to HCC treatment could benefit from RNA interference. Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic member of the Bcl-2 protein family which interferes with mitochondrial activation. This molecule is highly expressed in tissues of human HCC, and its specific down-regulation by RNA interference sensitizes HCC cells to different chemotherapeutic agents [77]. Inhibition of glioma-associated antigen-1 (Gli-1) mRNA in Huh7 cells through Gli-1 siRNA also induces apoptosis through down-regulation of Bcl-2 [78]. To avoid the deleterious effects of potential therapies from injury to healthy liver tissue tools allowing type-specific targeting may be important. Thus, adenoviral, tumour cell-specific delivery of TRAIL or cas-

pase 8 has been reported to induce increased rates of apoptosis in human HCC cells [79,80]. However, some conflicting results, suggesting that intrarterial injection of an adenovirus vector with ioidized oil esters can result in cancer-selective but not effective gene therapy for HCC, have been reported [81].

2.6. Apoptosis and Fibrosis

Acute and chronic liver diseases may induce repair mechanisms that cause excessive disposition of scar matrix (liver fibrosis) leading to liver cirrhosis. Although the relationship between apoptosis and liver fibrosis has not been fully explored, it is generally assumed that pro-apoptotic stimuli induce hepatocyte apoptosis. Engulfment of the hepatocyte apoptotic bodies by Kupffer and stellate cells enhances their expression of profibrogenic genes and death ligands, and persistent activation of these cells promotes further hepatocyte apoptosis, which culminates in generation of chemokines and sustained stellate cell activation [8]. Activation of stellate cells plays an important role in this process by transformation into myofibroblastic cells that synthesize scar tissue, and causes most of the pathological changes in cirrhosis. The activation of stellate cells involves the transdifferentiation from a quiescent state into myofibroblast-like cells, which are distinguished by accelerated proliferation and enhanced production of extracellular matrix components [82].

It therefore appears that both inhibition of apoptosis and selective targeting of hepatic stellate cells with apoptotic stimuli could be adequate antifibrotic strategies. From the first perspective it has been reported that Fas-specific siRNA attenuates hepatic fibrosis following repeated concavalin A administration in mice [29]. More recently it has been reported that cytosolic caspase 3 activity and cytosolic fractions of Bax, Bcl-2, cytochrome c, and calpain-mu protein expressions are decreased in rats receiving carbon tetrachloride plus S*alvia miltiorrhiza* extract, with no change in caspase 8 which suggests that the antiapoptotic effect is related to the antioxidant properties of *S. miltiorrhiza* [83].

Prevention of fibrogenesis by apoptosis of activated stellate cells has been demonstrated in different studies [84]. Thus, the fungal metabolite epipolythiodioxopiperazine gliotoxin reduces hepatic fibrosis, an effect accompanied by reduction by apoptosis of the numbers of activated hepatic stellate cells in the liver [85]. Gliotoxin is characterized by a heterobicyclic core containing a polysulfide bridge which plays a crucial role in its toxic effect (Fig. (**3**)). Because the use of apoptosis-inducing drugs may be limited due to lack of cell specificity, with a risk of severe adverse effects, very recently gliotoxin has been coupled to mannose-6-phosphate-modified human serum albumin (M6P-HSA), which selectively accumulates in liver fibrogenic cells. Administration of gliotoxin-M6P-HSA to bile duct-ligated revealed a significant decrease in alpha-smooth muscle actin mRNA levels and a reduced staining for this marker of hepatic stellate cells in fibrotic livers [86].

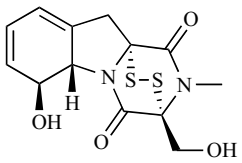

Fig. (3). The structure of the fungal metabolite gliotoxin.

Proteasome inhibitors induce apoptosis in transformed cells, especially those cells dependent upon NF-kappaB activation. Stimulated stellate cells also trigger NF-kappaB activation, and it has been reported that when the immortalized human stellate cell line, LX-2 or primary rat stellate cells are treated with the proteasome inhibitors bortezomib ([(1*R*)-3-methyl-1-[[(2*S*)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino] propyl]amino]butyl] boronic acid) and MG132 (N-[(phenylmethoxy)carbonyl]-L-leucyl-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide), there is an apoptotic response that is not related to proteasome inhibition-induced alterations in TRAIL, death receptor 5, and Bim, but to a blocked activation of NF-kappaB dependent upon the NF-kappaB target gene A1 [87]. Other studies have also demonstrated the possibility of acting on additional apoptotic mediators. Thus, the type 2 statin atorvastatin (calcium (3*R*,5*R*)-7-(3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoate) (Fig. (**4**)) induces apoptosis in activated rat stellate cells through an ERK-dependent cleavage of Bid and a highly increased protease activity of caspase 9 and caspase 3 [88]. This proapoptotic effect can be related to the lipophilic properties of atorvastatin, having been previously demonstrated that lipophilic statins are able to decrease the viability of cultured human hepatocytes, whereas hydrophilic statins have no influence on cell viability [89].

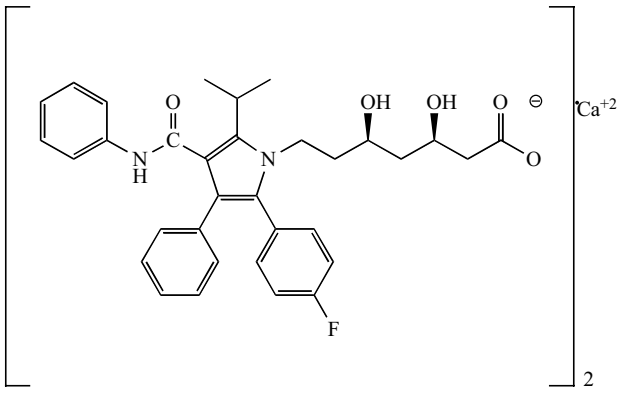

Fig. (4). The structure of the type 2 statin atorvastatin.

CONCLUSIONS

Apoptosis is an essential featuring contributing to liver injury in a wide range of acute and chronic liver diseases. Cell death by apoptosis can increase in toxic, viral, alcoholic and non alcoholic liver injury. A reduction of apoptosis could be beneficial in these diseases and the goal for the development of new drugs. Since multiple apoptotic pathways are activated in any of these conditions, combination therapies targeting different pathways may be useful. On the contrary, hepatocellular carcinomas seem to escape immune surveillance and apoptosis. Induction of apoptosis might therefore be a new option for the prevention and treatment of

hepatocarcinoma. Finally, liver fibrosis may require targeted approaches aimed to limit apoptosis to parenchymal cells while accelerating apoptosis of hepatic stellate cells.

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