Review

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Cyclophilin inhibition as potential therapy for liver diseases

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Summary

The cyclophilins are a group of proteins with peptidyl-prolyl isomerase enzymatic activity, localised in different cellular compartments and involved in a variety of functions related to cell metabolism and energy homeostasis, having enhanced expression in inflammation or malignancy. Cyclophilin A (CypA), the most abundantly expressed cyclophilin, is present mainly in the cytoplasm and is a host factor involved in the life cycle of multiple viruses. The extracellular fractions of CypA and CypB are potent pro-inflammatory mediators. CypD, located in mitochondria, is a key regulator of mitochondrial permeability transition pores, and is critical for necrotic cell death. Cyclosporines are the prototype cyclophilin inhibitors. Cyclic peptides, which bind and inhibit cyclophilins without having immunosuppressive properties, have been generated by chemical modifications of cyclosporin A. In addition, cyclophilin inhibitors that are structurally different from cyclosporines have been synthesized. The involvement of cyclophilins in the pathogenesis of different liver diseases has been established using both *in vitro* and *in vivo* investigations, thus indicating that cyclophilin inhibition may be of therapeutic benefit. This review summarises the evidence for potential therapeutic applications of non-immunosuppressive cyclophilin inhibitors, alone or in combination with other agents, in virus-induced liver diseases like hepatitis C, B or Delta, liver inflammation and fibrosis, acetaminophen-induced liver toxicity and hepatocellular carcinoma.

Introduction

Cyclophilins are a group of cellular proteins (collectively known as immunophilins), which display the enzymatic activity of a peptidyl-prolyl isomerase (PPIase) [1,2]. This enzyme catalyses the *cis* to *trans* conversion of proline-containing peptides and facilitates protein folding. Cyclophilins are ubiquitously expressed in all prokaryotic and eukaryotic cells. The first cyclophilin was identified in 1984 as a specific cytosolic protein that binds cyclosporin A (CsA) [3]. However it was not until 5 years later, in 1989, that it was demonstrated that the 18 kDa protein with PPIase activity and cyclophilin (CypA) were in fact the same protein [4,5].

Overall, 17 cyclophilins have been identified in the human genome, however the function of most of these is unknown and only 7 have been characterised for isomerase activity or binding to CsA [1,2]. Cyclophilins share a common domain of approximately 109 amino acids, the cyclophilin-like domain, which is surrounded by domains unique to each member of the family and associated with their subcellular compartmentalization and functional specialization. The subcellular localization of some cyclophilins has been defined, for example – CypA (Cyp18a, where 18 denotes its molecular mass of 18 kDa) and Cyp40 (40 kDa) are present in the cytosol; CypB (22 kDa) and CypC reside in the lumen of the endoplasmic reticulum; CypD – in mitochondria; CypE and CypA are found in the nucleus [1]. CypNK, a 150 kDa molecule, was identified on the surface of human natural killer cells [6].

During the last 20 years considerable knowledge has been accumulated for CypA, CypB, and CypD – concerning their involvement in specific cell functions and disease pathogenesis, while the functional characterization of other members of the cyclophilin family and potential roles in diseases have not been elucidated. CypA is one of the most abundant proteins in the cytoplasm (approximately 0.1% of total cytosolic proteins) and is involved in a range of cellular functions including protein folding, trafficking, immunomodulation and cell signalling [7]. The development of CypA knockout mice and CypA knockdown cell lines has demonstrated that CypA is not essential for cell growth and survival [8,9]. Importantly, CypA is secreted from cells spontaneously and in response to inflammatory stimuli or oxidative stress (reviewed in [10]), and the extracellular fraction of CypA acts as a potent pro-inflammatory mediator, which stimulates inflammatory responses and exerts chemotactic activity for neutrophils and monocytes via the CD147 cell receptor (Table 1). Increased CypA expression has a major role in various pathological conditions such as inflammatory reactions and cartilage destruction in rheumatoid arthritis [18–20]; progression of inflammatory diseases in the lung [24]; exacerbation of oxidative stress and inflammation [21,22]. CypA is also overexpressed in cancer cells and promotes metastasis [27,28].

CypB was the second cyclophilin identified [29]. It differs from CypA mainly by the presence of a cleavable N-terminal sequence

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Table 1. Key functions of cyclophilin A.

Functions	Target	Biological/pathological effects	[Ref.]
Intracellular			
Protein folding	Collagen	Folding of pro-collagen 1	11
	Transferrin	Folding of transferrin	12
Protein trafficking	Heterogenous nuclear RNP A2	CXCR4-mediated export of hnRNPA2	13
	CD147	Transport to plasma membrane	14
	Asialoglycoprotein receptor (ASGPR)	ASGPR transport between plasma membrane and endosomal pool	15
T-cell activation, cell signaling	Interleukin-2 tyrosine kinase (Itk)	Regulation of T-helper cell profile and cytokines	8,16
	VCAM-1, E-selectin	Proliferation and migration of vascular smooth muscle cells	17
Extracellular			
Stimulates proinflammatory signals	MMP-2, MMP-9 Interleukin-8	Promotes joint inflammation in rheumatoid arthritis, degradation of joint cartilage	18,19,20
Exacerbate oxidative stress	Vascular smooth muscle cells	Oxidative stress	21,22
Chemotaxis of inflammatory cells	CD147/EMMPRIN	Potent leukocyte chemoattractant for human monocytes, neutrophils, eosinophils, and T cells	14,23,24
		Stimulates inflammatory responses when injected *in vivo*	23
		Stimulates expression of adhesion molecules	25,26

hnRNPA2, heterologous nuclear ribonucleoprotein A2; VCAM-1, vascular cell adhesion molecule 1; MMP, metalloproteinase; NF-KB, nuclear factor kappa B; EMMPRIN, extracellular matrix metalloproteinase inducer, or CD147.

that directs the protein to the endoplasmic reticulum. CsA, and other cyclophilin inhibitors, specifically mobilize CypB from the endoplasmic reticulum and promote its secretion from cells, with the extracellular CypB fraction lacking the N-terminal signal sequence [30]. Unlike CypB, CypA is not secreted upon administration of cyclophilin inhibitors. The extracellular fractions of CypA and CypB are involved in cell-cell communications and inflammatory signalling, however on its own, CypB seems unable to induce proinflammatory cytokines [10].

CypD is another key cyclophilin, which is located in the mitochondria and has a central role in regulating the mitochondrial permeability transition pore (MPTP), hence it has been considered as potential therapeutic target for different diseases where mitochondrial dysfunction is central to the disease pathogenesis [31,32]. Mitochondria can be considered as a "firewall" that controls the Ca^{2+} concentration in different cell compartments. Across the outer mitochondrial membrane, the Ca^{2+} transport is mediated mainly by the poorly selective voltage-dependent anion channel (VDAC). Across the inner membrane, the uptake of Ca^{2+} occurs through the mitochondrial Ca^{2+} uniporter and/or the rapid uptake mode, while MPTP has a major role for Ca^{2+} efflux from mitochondria to the cytosol [33]. When opened in a flickering mode, the MPTP acts as a fast Ca^{2+} release channel, generating waves of cytosolic Ca^{2+} that propagate signals to other cell regions or are taken up by surrounding mitochondria. CypD is localized in the matrix of mitochondria and regulates MPTP opening and consequently the Ca^{2+} exchange between the mitochondria and the cytosol (Fig. 1). Several lines of evidence from experiments using hepatocytes, neurons or cardiomyocytes have established that CypD, is the key regulator of MPTP opening [32,33]. A persistent MPTP opening induces necrotic cell death, which is different from necroptosis – a regulated cell necrosis

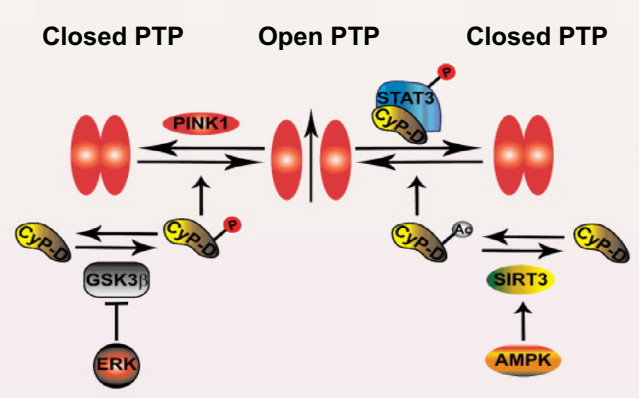

Fig. 1. Cyclophilin D (CyP-D) regulates opening of the mitochondrial permeability transition pore (PTP). (i) CyP-D phosphorylation by GSK3 facilitates PTP opening; (ii) phosphorylated STAT3 binds to CyP-D and inhibits PTP opening; (iii) CyP-D acetylation sensitizes the PTP to opening and is prevented by AMPK activation of the SIRT3 de-acetylase. Reprinted from [33] © 2014, with permission from Elsevier.

that is dependent on the receptor-interacting protein kinase 3, RIPK3 [34]. Recently, it has been shown that cell necrosis, as a result of mitochondrial permeability transition or necroptosis, occurs via two co-existing but separate pathways, and the combined blockade of both pathways, – such as CypD inhibition with cyclosporine or a non-immunosuppressive Cyp inhibitor (sanglifehrin A) together with necroptosis inhibition with the RIPK1 inhibitor necrostatin-1, has resulted in strong and additive protection from ischemia-reperfusion injury [34,35]. CypD plays a

fundamental role in the overall energy homeostasis in cells. The definitive proof that CypD is critical for necrotic signalling was provided in 2005 using the genetic deletion of *Ppif* (the CypD gene) [36]. These studies found that *Ppif*-null cells are highly resistant to cell death induced by cytosolic Ca^{2+} overload and hydrogen peroxide-mediated ROS stress.

The involvement of cyclophilins in the pathogenesis of different liver diseases has been established in both *in vitro* and *in vivo* investigations, thus, indicating that cyclophilin inhibition may be of therapeutic benefit. This review summarises the evidence for potential therapeutic applications of non-immunosuppressive cyclophilin inhibitors in liver diseases.

Key Points

- Cyclophilins are ubiquitous cellular proteins with peptidyl-prolyl isomerase enzymatic activity, which are involved in a variety of functions related to cell metabolism, energy homeostasis, and have enhanced expression in inflammation or malignancy
- Small molecules that bind and inhibit cyclophilins have been generated either as non-immunosuppressive derivatives of cyclosporin A, or as molecules that are structurally distinct from cyclosporines
- Blocking cyclophilin A has been shown to result in a potent antiviral effect against a range of viruses, with the largest *in vitro* and clinical database available for hepatitis C virus. In addition, blocking cyclophilin A has an anti-inflammatory effect and reduces its role in oxidative stress and chemotaxis of inflammatory cells
- Blocking cyclophilin D is effective in correcting pathological conditions that involve mitochondrial dysfunction, oxidative stress or cell necrosis
- Cyclophilin inhibitors alone or in combination with other agents, could be beneficial in the treatment of chronic hepatitis C, chronic hepatitis B, acetaminophen-induced liver toxicity; they may reduce liver inflammation and fibrosis in non-alcoholic steatohepatitis, possibly augment activity of chemotherapy against hepatocellular carcinoma and decrease the metastatic spread

Cyclophilin inhibitors: Structure and functional relationships

Cyclosporin A

The story of cyclophilin inhibitors began in 1970 when cyclosporin A (CsA) and cyclosporin C were isolated from the fungus *Tolypocladium inflatum* and found to suppress T-lymphocyte reactivity. CsA is a cyclic peptide comprising of 11 amino acids and its immunosuppressive action is exerted by forming a ternary complex between CsA, CypA and calcineurin [37]. CsA-induced immunosuppression is not a result of its binding to CypA, but from the interaction between the CypA-CsA complex with calcineurin, as the latter is required for T cell activation [1,9,38]. Formation of this ternary complex leads to inhibition of the intrinsic phosphatase activity of calcineurin, which abolishes the nuclear translocation of the nuclear factor of activated T cells (NFAT). This ultimately contributes to the failure of T cells to respond to antigenic stimuli [1,38].

Non-immunosuppressive cyclophilin inhibitors

Cyclophilin inhibitors with non-immunosuppressive properties have been generated by chemical modifications of the CsA molecule to produce alisporivir (DEB025), NIM811 and SCY-635 [9,38]. Alisporivir differs from the parent molecule CsA by purposely substituting two amino acids - sarcosine with D-alanine at position 3, and N-methyl-leucine with N-ethyl-valine at position 4 [39]. Although CypA and alisporivir form a complex with nanomolar affinity, the resulting binary complex is unable to bind calcineurin. Molecular modelling of a NMR-derived CypA-alisporivir complex, and further analyses of the CypA-alisporivir-calcineurin complex, have enabled to define, at a molecular level, the non-immunosuppressive properties of alisporivir. Whereas the calcineurin pocket is optimally occupied by the leucine-4 side chain, the valine-4 side chain of alisporivir cannot enter this pocket and impedes the formation of a stable ternary complex [40]. These findings established that the N-ethyl-valine at position 4 in the cyclic peptide of alisporivir is responsible for the inability to bind calcineurin, and consequently for the lack of immunosuppressive activity. The chemical structure of NIM811 differs from CsA by having methyl-isoleucine instead of methyl-leucine at position 4 [41]; for SCY-635 – the differences from CsA include a dimethylamino-ethylthio substituent at position 3, plus a hydroxyl group at position 4 [42]. Despite relatively small variations in the structure of CsA and its non-immunosuppressive derivatives, these molecules differ in their activity against HIV or HCV with marked differences in their EC_{50} [43,44].

Recently, cyclophilin inhibitors were developed that are not derived from CsA [9,45]. The family of sanglifehrins includes sanglifehrin A, B, C, D - a group of naturally occurring cyclophilin-binding polyketides that are structurally distinct from cyclosporines and are produced by soil *Streptomyces* bacteria. The natural sanglifehrins have immunosuppressive activity with a mechanism of action, which does not involve calcineurin, i.e., different from that of CsA, and non-immunosuppressive analogues of sanglifehrin A have been synthesized [46,47]. In addition, small (100–150 Da) non-peptide cyclophilin inhibitors have been identified using a fragment-based drug design approach by means of nuclear magnetic resonance (NMR) and X-ray crystallography. Two compounds (F680 and F684) showed significant inhibition of CypA, CypB, and CypD enzymatic activities. It is notable that these two compounds also exerted anti-HCV G1b activities and were devoid of cytotoxicity [9].

Chronic hepatitis C

The most extensive evidence at present concerns the role of cellular cyclophilins in the replication of hepatitis C virus (HCV). Experiments using siRNA, proteomic assays, and mRNA profiling have established that several cyclophilins are instrumental for HCV replication [48].

The effect of cyclophilin inhibition in suppressing HCV replication was first demonstrated by Shimotohno and colleagues using CsA in cultured hepatoma cells [49]. The antiviral activity was initially thought to be mediated by blocking CypB. Further studies extended this finding by demonstrating that the

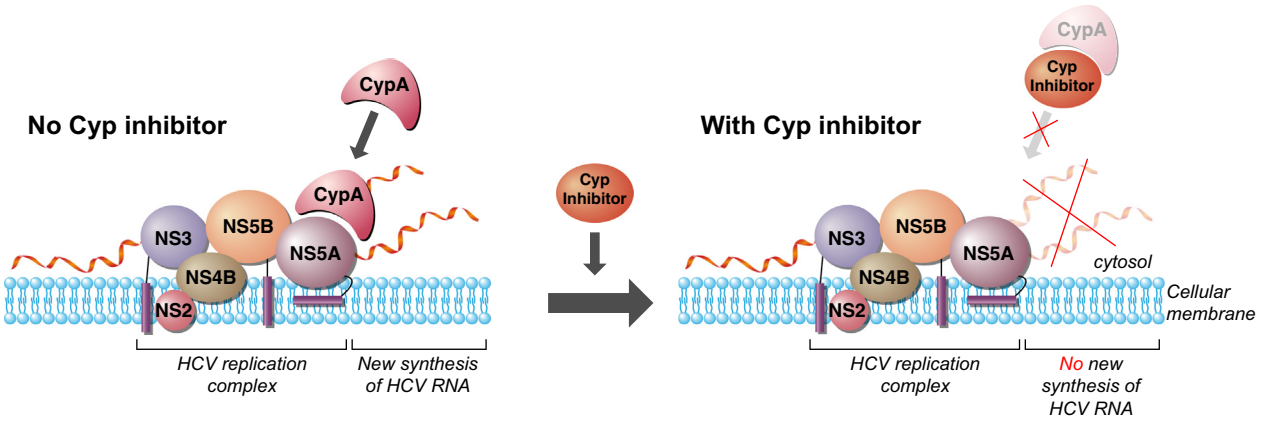

Fig. 2. Mechanism of action of cyclophilin (Cyp) inhibitors in blocking HCV replication. Cyclophilin A (CypA) binds to its ligand (domain II of the NS5A HCV protein) and interacts with the HCV replication complex on the membranous web. By blocking the interaction between CypA and NS5A, the cyclophilin inhibitors (alisporivir, NIM811, SCY-635 or sanglifehrins) abrogate HCV replication. Based on [44,45,55,57,59].

non-immunosuppressive CsA derivatives are even more effective in blocking HCV replication. Although several cyclophilins appear to be implicated in HCV replication [48–51], the accumulating evidence has convincingly demonstrated that CypA is the principal cyclophilin that is essential for viral replication, and its blockade underlines the anti-HCV activity of cyclophilin inhibitors [52–55]. This is further supported by *in vivo* data from a recently developed mouse model supporting the entire HCV life cycle – the HCV replication was drastically reduced in *Ppia* knockout mice (*Ppia*$^{-/-}$), confirming that CypA is an essential cellular cofactor [56]. The specific mechanisms how cyclophilin inhibition abrogates HCV replication have been elucidated by several key findings. First, the peptidyl-prolyl isomerase activity of CypA was shown to be critical for HCV replication [52,53]. Unlike wild type CypA, isomerase-deficient CypA variants with laboratory-generated mutations in their hydrophobic pocket are unable to support HCV replication. The second key finding was that CypA binds directly to the HCV nonstructural 5A (NS5A) protein, suggesting that the HCV NS5A protein is the viral ligand for the host CypA protein [55,57,58]. Importantly, the CypA-NS5A interaction is conserved among all HCV genotypes, which explains the pan-genotypic antiviral effect of cyclophilin inhibitors as demonstrated in clinical studies. The third key finding is that cyclophilin inhibitors prevent the formation of and disrupt CypA-NS5A complexes. All classes of cyclophilin inhibitors - CsA, non-immunosuppressive CsA derivatives (e.g., alisporivir or SCY-635), sanglifehrins and sanglifehrin derivatives – block CypA-NS5A interactions in a dose-dependent manner [44,45,55,57,59]. Therefore, CypA-NS5A interactions are critical for HCV replication, and the most direct impact of CypA inhibitors is the prevention of CypA-NS5A contacts, thus abrogating HCV replication (Fig. 2). In a recent *in vitro* testing of anti-HCV activity of alisporivir or sanglifehrin B in combination with different direct acting antivirals, we found the strongest synergy in the combination of a cyclophilin inhibitor with a NS5A inhibitor [60]. In addition, Ciesek *et al.* reported that the replication of a JFH1 full-length replicon (encoding non-structural NS2-NS5B proteins) was far more sensitive to CypA depletion and inhibition than subgenomic replicons (encoding non-structural NS3-NS5B proteins) [61]. The role of NS2 in enhancing HCV sensitivity to cyclophilin inhibitors was recently shown to be indirect, a consequence of reduced replication competence most likely as a result of NS2-mediated polyprotein cleavage, while NS2 is not a direct target for CypA inhibitors [62].

The clinical experience with non-immunosuppressive cyclophilin inhibitors, as a treatment option for chronic hepatitis C patients, varies considerably: – NIM811 and SCY-635 have been administered in proof-of-concept and small exploratory trials (<50 patients) involving up to 4-weeks treatment with these compounds; alisporivir has been given to more than 2000 HCV patients, while there is no clinical data with sanglifehrins at present [63–66]. Thus, full treatment results and sustained virologic response rates are currently available only for alisporivir given in combination with peg-interferon/ribavirin (Fig. 3), or alisporivir plus ribavirin as interferon-free treatment for HCV G2 and G3 patients [67,68].

Based on *in vitro* data, the cyclophilin inhibitors, provided as host-targeting antiviral agents, seem promising in combination with direct-acting antivirals (DAAs) in interferon-free treatment regimens for hepatitis C. Aside from the NS5B polymerase inhibitors, cyclophilin inhibitors are the only class that can provide a backbone in combination with direct antivirals (protease, nonnucleoside polymerase or NS5A inhibitors), which rapidly select resistant strains. The following attributes of cyclophilin inhibitors can be particularly useful in this context: (1) high barrier to viral resistance [44]; (2) broad genotype coverage; (3) demonstrated additive or synergistic antiviral effect and blocking the emergence of resistance to DAAs [60]; (4) fully active against all HCV mutants that confer resistance to protease, NS5A and NS5B polymerase inhibitors [58].

Apart from the antiviral effect in suppressing HCV replication, as a result of CypA inhibition, cyclophilin inhibitors may provide additional benefits in improving HCV-infected livers through the concomitant inhibition of CypD and correcting the HCV-induced mitochondrial dysfunction. HCV proteins produced in infected hepatocytes target mitochondria, leading to functional consequences and contributing to HCV-induced liver damage [69]. The HCV core protein was shown to increase the formation of reactive oxygen species (ROS) and affect calcium uptake and release. The *in vitro* treatment of cells transfected with HCV with

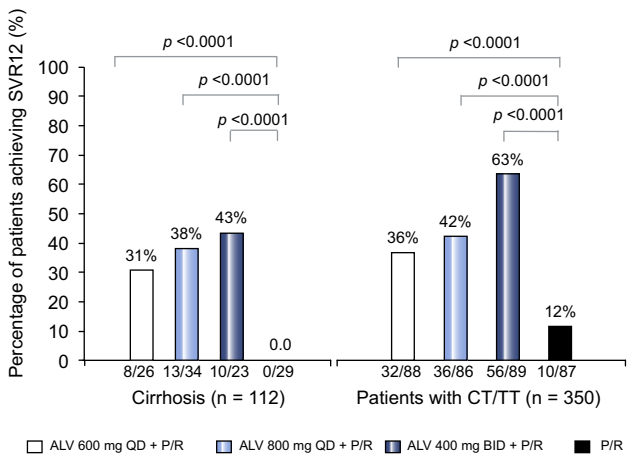

Fig. 3. Sustained Virologic Response (SVR12) in HCV genotype 1 previous treatment-failure patients for particular difficult to treat patient subgroups (with cirrhosis or *IL28B* genotype CT/TT) who received treatment with alisporivir (ALV) once daily (QD) or twice daily (BID) in combination with peg-interferon/ribavirin (P/R). ITT results from the FUNDAMENTAL study 2210 [67].

alisporivir was able to correct/prevent HCV protein-mediated mitochondrial dysfunction by blocking CypD, and obtaining an associated effect on MPTP [70].

Chronic hepatitis B

The accumulating evidence indicates that cyclophilins are involved in the life cycle of the hepatitis B virus (HBV) in hepatocytes. Early investigations using an HBx protein-dependent model for HBV replication in HepG2 cells, have suggested that changes in mitochondrial calcium flow and blocking cytosolic calcium signalling can impact HBV replication [71]. Cyclophilin inhibition (with either CsA or its non-immunosuppressive derivative NIM811) was shown to interfere with HBV replication by acting on Ca^{2+} cytosol levels and specifically on the mitochondrial permeability transition pores [71]. More recently, the role of cyclophilins in liver cells, and the effect of cyclophilin inhibitors (NIM811, alisporivir or the sangamide-based cyclophilin inhibitor - NVP018) on HBV replication and HBsAg production were investigated in greater details [72–74]. The involvement of individual cyclophilins was investigated after selective knockdown of CypA, CypC or CypD with specific siRNA [73]. Cyclophilin expression in HuH7 cells was markedly reduced (>80%) after transfection with the corresponding siRNA, which was associated with a significant lowering of HBV-DNA and HBsAg levels. The experiments indicated that CypA is the principal cyclophilin involved in HBsAg production and secretion; incubation with either alisporivir or NIM811 reduced HBV replication and HBsAg levels [72,73]. The effects of cyclophilin inhibition on HBV life cycle were further supported by *in vitro* and *in vivo* animal data using a sangamide-based cyclophilin inhibitor - NVP018 [74]. Two-week administration of NPV018 in a mouse HBV model resulted in a 1.2–1.5 log reduction of serum HBV DNA levels along with a decrease in HBsAg titres. HBsAg and CypA are closely associated during secretion from liver cells [75], thus disrupting the CypA/HBsAg complex by cyclophilin inhibitors will be one possible mechanism for reducing the envelope protein secretion.

Collectively, the available data indicate that cyclophilin inhibition with either non-immunosuppressive analogues of CsA or with a sanglifehrin-based inhibitor could be useful in the treatment of patients with chronic hepatitis B, potentially also for hepatitis Delta, as they interfere at multiple sites of the HBV life cycle and HBsAg: first, within the hepatocytes by blocking CypA and CypD, cyclophilin inhibitors will reduce HBV DNA replication as well as HBsAg production/secretion; secondly and independent of the cyclophilin inhibition, by blocking the sodium-taurocholate co-transporter polypeptide (NTCP) on the hepatocyte membrane, recently shown to be a specific receptor for HBV and hepatitis Delta virus [76–78], cyclophilin inhibitors would prevent the entry of these viruses into cells, and infection of new hepatocytes. The potential benefit of combining a cyclophilin inhibitor with some of the well-established HBV antiviral agents will need to be tested in clinical studies to assess whether it will accelerate and/or enhance HBsAg clearance.

Mitochondrial dysfunction in liver diseases and cyclophilin inhibition

The term "mitochondrial medicine" was introduced recently and refers to treatment approaches that are directed at preventing and/or treating mitochondrial dysfunction and its consequences [79]. Mitochondria play a key role in the normal function and the integrity of liver cells and a wide range of liver diseases are associated with mitochondrial dysfunction. As outlined in the introduction, CypD regulates the opening of MPTP and calcium exchange between the mitochondrial space and the cytosol and has a key role in cell necrosis or necroptosis [33,34]. Thus, CypD inhibition might be beneficial for the liver in preventing a cascade of events, triggered by mitochondrial dysfunction, and leading to cell death.

Liver regeneration

Mitochondrial calcium was recently shown to play a role in liver regeneration after partial hepatectomy [80]. Mitochondrial permeability transition underlies the dysfunction of small-for-size livers. Transplantation of quarter-size livers has been associated with liver cell necrosis, increase in ALT and bilirubin levels. In an experimental model, these were reduced by 70% using a cyclophilin inhibitor (NIM811), which decreased graft injury and stimulated liver regeneration by inhibiting CypD and MPTP [81]. An additional benefit of cyclophilin inhibition in small-for-size liver transplantation is the marked reduction of associated pulmonary injury [82]. NIM811 treatment profoundly reduced the expression of inflammatory cytokines and adhesion molecules (TNF-alpha, IL-1β, and ICAM-1), and as a result decreased lung inflammation and injury associated with small-for-size liver transplants [82].

Non-alcoholic steatohepatitis (NASH)

Hepatic mitochondrial dysfunction appears to contribute to liver damage in NASH. Patients with NASH have an impaired ability to synthesize ATP and there is a decrease in hepatic mitochondrial DNA. The mechanism is unknown but it may involve TNF-alpha, which has been found to increase the permeability of mitochondrial membranes (reviewed in [83]). Clinical evidence that cyclophilin inhibition reduces liver cell necrosis was obtained in the

first-in-human study of NIM811 when given as monotherapy in HCV-infected patients. Two-week treatment with ascending doses of NIM811 given alone had no effect on HCV RNA levels, however even at low doses it caused rapid normalization of serum aminotransferases despite lack of the antiviral effect [63].

Liver inflammation and fibrosis

Apart from impacting liver cell death via CypD inhibition and correction of mitochondrial dysfunction, cyclophilin inhibitors exert significant anti-inflammatory activity by interacting with extracellular cyclophilins. In response to inflammatory stimuli CypA, CypB, and CypC are secreted in the extracellular space and these extracellular cyclophilins interact with CD147 or EMMPRIN (extracellular matrix metalloproteinase inducer) on leukocytes [10]. Inhibition of extracellular cyclophilins has shown a strong anti-inflammatory effect in various *in vivo* models, indicating that extracellular cyclophilins may represent an initial trigger for the release of pro-inflammatory cytokines and leukocyte recruitment [10,84]. This notion is strengthened by findings using an experimental compound - a non-immunosuppressive cell-impermeable cyclophilin inhibitor, which demonstrated, in several inflammatory models, a strong anti-inflammatory effect as a result of inhibition of extracellular cyclophilins and their interaction with CD147 [84]. The marked effect of NIM811 in reducing lung inflammation and injury, in parallel with decreasing hepatic TNF-alpha and IL-1β expression, after small-for-size liver transplantation is an example of such an anti-inflammatory effect with a non-immunosuppressive cyclophilin inhibitor [82]. Similarly, blocking extracellular CypA and CD147 interaction significantly reduced myocardial inflammation and macrophage recruitment, and in addition a pronounced reduction of myocardial fibrosis was observed [85]. So far, there is very little information whether cyclophilin inhibitors might impact liver fibrosis – either by interacting directly with liver stellate cells, or as a consequence of their anti-inflammatory effects. In a rat model with carbon tetrachloride-induced liver fibrosis, NIM811 was found to reduce the expression of the tissue inhibitor of metalloproteinase-1, transforming growth factor-β along with significantly reducing the enhanced CypB expression [86]. The first clinical results that provide a preliminary indication that cyclophilin inhibition may have an antifibrotic effect come from a prospective, randomized study comparing CsA and tacrolimus for liver fibrosis development in 356 liver transplant recipients [87]. Among steroid-free patients in the study, liver fibrosis progression (score >2) was significantly less frequent ($p = 0.029$) with CsA, compared with tacrolimus at one year post-transplant. Additional investigations, involving more specific measurements of fibrosis, are needed to further clarify whether cyclophilin inhibitors have antifibrotic effects that would be of clinical benefit.

Acetaminophen liver toxicity

The toxicity of paracetamol/acetaminophen (APAP) is initiated by the excess of its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which binds cellular glutathione and leads to profound mitochondrial glutathione depletion, while the remaining NAPQI is available to covalently bind to cellular proteins especially in mitochondria [88]. The resulting mitochondrial oxidative stress leads to mitochondrial DNA damage and opening of the mitochondrial PTP, followed by nuclear DNA fragmentation and ultimately necrotic cell death with release of damage-associated molecular patterns (DAMP) that trigger an inflammatory response. Cyclophilins appear to be involved in APAP-induced liver injury via two mechanisms – first, mitochondrial toxicity and secondly, immune-mediated inflammatory damage. Several studies have provided experimental data that mitochondrial PTPs are involved in APAP-induced liver toxicity and that CsA can protect mouse hepatocytes from APAP toxicity [89–91]. In cultured human hepatocytes, acetaminophen toxicity was markedly decreased with CsA or its non-immunosuppressive derivative NIM811 through CypD and correction of the mitochondrial dysfunction, however this cytoprotective effect was lost after 16 h [90]. In contrast to these findings, a recent investigation of the role of CypD inhibition in a mouse model of APAP liver toxicity produced partially controversial results [92]. APAP (600 mg/kg) induced injury in the mouse liver was not prevented by genetic ablation of CypD in *Ppif*$^{-/-}$ mice, or by application of DEB025 (alisporivir) 10 mg/kg injected intraperitoneally 1.5 h after APAP administration, indicating the contribution of a parallel pathway, a CypD-independent mode of APAP-induced liver injury, which involves peroxynitrite-mediated cell damage. The results from this investigation differ from a similar study where hepatotoxicity in mice was induced by APAP 200 mg/kg and CypD-deficient *Ppif*$^{-/-}$ mice were completely protected against APAP-induced liver injury and DNA fragmentation [93]. Oxidant stress and peroxynitrite formation were blunted but not eliminated in CypD-deficient mice.

Another major cyclophilin, which is key to inflammatory response and immune-mediated APAP-induced injury, is CypA. Recent studies have demonstrated that mice lacking CypA were resistant to acetaminophen toxicity [94]. When wild type mice were injected with necrotic liver cells from mice lacking CypA, the inflammatory responses that caused necrotic liver damage were reduced. Conversely, the host inflammatory response was increased when CypA was injected together with necrotic liver cells. Antagonism of the extracellular receptor for CypA can also reduce acetaminophen-induced liver injury [94]. In patients with paracetamol induced liver damage, urinary CypA levels are significantly increased. Overall, the current evidence indicates the involvement of two cyclophilins – CypD (as a key determinant of mitochondrial functions) and CypA (as mediator of pro-inflammatory reactions) as major contributors for acetaminophen liver toxicity, thus indicating that pharmacological blockade with non-immunosuppressive cyclophilin inhibitors might be beneficial in reducing APAP-induced liver injury.

Hepatocellular carcinoma

Enhanced expression of cyclophilins has been observed in different types of cancer, in particular CypA was found to be upregulated in hepatocellular carcinoma (HCC) [95,96]. CypA overexpression induces resistance to hypoxia and to chemotherapeutic agents such as cisplatin in cancer cells. Cyclophilin inhibition with CsA or sanglifehrin A was shown to provide a synergistic effect with cisplatin and to increase cell death in HCC cells [97]. Preliminary data have indicated that cyclophilins may play a role in the metastatic potential of liver cancer cells, as well as in responsiveness to chemotherapy. CypB was shown to protect tumour cells – also in p53-defective HCC cells – against

hypoxia and apoptosis induced by chemotherapeutic agents such as cisplatin. CypB was found to be overexpressed in 78% and 91% of human HCC and colon cancer tissues, respectively. These results suggest that hypoxia-induced CypB expression stimulates the survival of HCC via a positive feedback loop with HIF-1α [98]. The level of CypA expression in two HCC-derived cell lines was shown to correlate with their metastatic capability, while ectopic CypA expression was found to promote cell adhesion, chemotaxis and lung metastasis *in vivo*, without affecting cell proliferation. Microarray studies have identified 21 different genes related to metastasis having altered expression profile in relation to CypA overexpression. The enhanced CypA expression in HCC cells was suggested to promote HCC metastasis through upregulation of the matrix metalloproteinases MMP3 and MMP9 [99]. Cyclophilin inhibitors could therefore constitute new agents that have the potential to augment the activity of chemotherapeutic agents against HCC and also to decrease the metastatic spread of cancer cells [96].

Perspective

Cyclophilins are ubiquitously expressed cellular proteins, which appear to be involved in the pathogenesis of a variety of liver diseases. Cyclophilin A is established as a host cofactor essential for the HCV replication complex in hepatocytes, and it also appears to be involved in the replication of HBV and in HBsAg production. Cyclophilin D is a key regulator of the mitochondrial permeability pores, thus regulating the calcium exchange between mitochondria and cytosol with implications for cell survival or death. Several cyclophilin inhibitors have been developed, which allow to investigate the potential benefit of cyclophilin inhibition as a therapeutic approach in liver diseases.

Conflict of interest

N. Naoumov is employee of Novartis Pharma AG and Honorary Professor of Hepatology, University College London, UK.

References

[1] Wang P, Heitman J. The cyclophilins. Genome Biol 2005;6:226.
[2] Davis TL, Walker JR, Campagna-Slater V, Finerty PJ, Paramanathan R, Bernstein G, et al. Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. PLoS Biol 2010;8:e1000439.
[3] Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science 1984;226:544–547.
[4] Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX. Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. Nature 1989;337:476–478.
[5] Takahashi N, Hayano T, Suzuki M. Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. Nature 1989;337:473–475.
[6] Anderson SK, Gallinger S, Roder J, Frey J, Young HA, Ortaldo JR. A cyclophilin-related protein involved in the function of natural killer cells. Proc Natl Acad Sci U S A 1993;90:542–546.
[7] Nigro P, Pompilio G, Capogrossi MC. Cyclophilin A: a key player for human disease. Cell Death Dis 2013;4:e888.
[8] Colgan J, Asmal M, Yu B, Luban J. Cyclophilin A-deficient mice are resistant to immunosuppression by cyclosporine. J Immunol 2005;174:6030–6038.
[9] Gallay PA. Cyclophilin inhibitors: a novel class of promising host-targeting anti-HCV agents. Immunol Res 2012;52:200–210.
[10] Hoffmann H, Schiene-Fischer C. Functional aspects of extracellular cyclophilins. Biol Chem 2014;395:721–735. http://dx.doi.org/10.1515/hsz-2014-0125.
[11] Steinmann B, Bruckner P, Superti-Furga A. Cyclosporin A slows collagen triple-helix formation in vivo: indirect evidence for a physiologic role of peptidyl-prolyl cis-trans-isomerase. J Biol Chem 1991;266:1299–1303.
[12] Lodish HF, Kong N. Cyclosporin A inhibits an initial step in folding of transferrin within the endoplasmic reticulum. J Biol Chem 1991;266:14835–14838.
[13] Pan H, Luo C, Li R, Qiao A, Zhang L, Mines M, et al. Cyclophilin A is required for CXCR4-mediated nuclear export of heterogeneous nuclear ribonucleoprotein A2, activation and nuclear translocation of ERK1/2, and chemotactic cell migration. J Biol Chem 2008;283:623–637.
[14] Yurchenko V, Pushkarsky T, Li JH, Dai WW, Sherry B, Bukrinsky M. Regulation of CD147 cell surface expression: involvement of the proline residue in the CD147 transmembrane domain. J Biol Chem 2005;280:17013–17019.
[15] Huang T, Deng H, Wolkoff AW, Stockert RJ. Phosphorylation-dependent interaction of the asialoglycoprotein receptor with molecular chaperones. J Biol Chem 2002;277:37798–37803.
[16] Brazin KN, Mallis RJ, Fulton DB, Andreotti AH. Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A. Proc Natl Acad Sci U S A 2002;99:1899–1904.
[17] Satoh K, Matoba T, Suzuki J, O'Dell MR, Nigro P, Cui Z, et al. Cyclophilin A mediates vascular remodeling by promoting inflammation and vascular smooth muscle cell proliferation. Circulation 2008;117:3088–3098.
[18] Kim H, Kim WJ, Jeon ST, Koh EM, Cha HS, Ahn KS, et al. Cyclophilin A may contribute to the inflammatory processes in rheumatoid arthritis through induction of matrix degrading enzymes and inflammatory cytokines from macrophages. Clin Immunol 2005;116:217–224.
[19] Yang Y, Lu N, Zhou J, Chen ZN, Zhu P. Cyclophilin A up-regulates MMP-9 expression and adhesion of monocytes/macrophages via CD147 signalling pathway in rheumatoid arthritis. Rheumatology (Oxford) 2008;47:1299–1310.
[20] Wang L, Wang CH, Jia JF, Ma XK, Li Y, Zhu HB, et al. Contribution of cyclophilin A to the regulation of inflammatory processes in rheumatoid arthritis. J Clin Immunol 2010;30:24–33.
[21] Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, et al. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nat Med 2009;15:649–656.
[22] Jin ZG, Melaragno MG, Liao DF, Yan C, Haendeler J, Suh YA, et al. Cyclophilin A is a secreted growth factor induced by oxidative stress. Circ Res 2000;87:789–796.
[23] Sherry B, Yarlett N, Strupp A, Cerami A. Identification of cyclophilin as a proinflammatory secretory product of lipopolysaccharide-activated macrophages. Proc Natl Acad Sci U S A 1992;89:3511–3515.
[24] Arora K, Gwinn WM, Bower MA, Watson A, Okwumabua I, MacDonald HR, et al. Extracellular cyclophilins contribute to the regulation of inflammatory responses. J Immunol 2005;175:517–522.
[25] Jin ZG, Lungu AO, Xie L, Wang M, Wong C, Berk BC. Cyclophilin A is a proinflammatory cytokine that activates endothelial cells. Arterioscler Thromb Vasc Biol 2004;24:1186–1191.
[26] Nigro P, Satoh K, O'Dell MR, Soe NN, Cui Z, Mohan A, et al. Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. J Exp Med 2011;208:53–66.
[27] Yang H, Chen J, Yang J, Qiao S, Zhao S, Yu L, et al. Cyclophilin A is upregulated in small cell lung cancer and activates ERK1/2 signal. Biochem Biophys Res Commun 2007;361:763–767.
[28] Howard BA, Furumai R, Campa MJ, Rabbani ZN, Vujaskovic Z, Wang XF, et al. Stable RNA interference-mediated suppression of cyclophilin A diminishes non-small-cell lung tumor growth in vivo. Cancer Res 2005;65:8853–8860.
[29] Price ER, Zydowsky LD, Jin MJ, Baker CH, McKeon FD, Walsh CT. Human cyclophilin B: a second cyclophilin gene encodes a peptidyl-prolyl isomerase with a signal sequence. Proc Natl Acad Sci U S A 1991;88:1903–1907.
[30] Price ER, Jin M, Lim D, Pati S, Walsh CT, McKeon FD. Cyclophilin B trafficking through the secretory pathway is altered by binding of cyclosporin A. Proc Natl Acad Sci U S A 1994;91:3931–3935.
[31] Waldmeier PC, Zimmermann K, Qian T, Tintelnot-Blomley M, Lemasters JJ. Cyclophilin D as a drug target. Curr Med Chem 2003;10:1485–1506.
[32] Elrod JW, Molkentin JD. Physiologic functions of cyclophilin D and the mitochondrial permeability transition pore. Circ J 2013;77:1111–1122.
[33] Rasola A, Bernardi P. Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis. Cell Calcium 2011;50:222–233.
[34] Linkermann A, Green DR. Necroptosis. N Engl J Med 2014;370:455–465.

[35] Linkermann A, Bräsen JH, Darding M, Jin MK, Sanz AB, Heller JO, et al. Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. Proc Natl Acad Sci U S A 2013;110:12024–12029.

[36] Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, et al. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature 2005;434:652–658.

[37] Borel JF. History of the discovery of cyclosporin and of its early pharmacological development. Wien Klin Wochenschr 2002;114:433–437.

[38] Gallay PA. Cyclophilin inhibitors. Clin Liver Dis 2009;13:403–417.

[39] Flisiak R, Jaroszewicz J, Flisiak I, Łapiński T. Update on alisporivir in treatment of viral hepatitis C. Expert Opin Investig Drugs 2012;21:375–382.

[40] Landrieu I, Hanoulle X, Bonachera F, Hamel A, Sibille N, Yin Y, et al. Structural basis for the non-immunosuppressive character of the cyclosporin A analogue Debio 025. Biochemistry 2010;49:4679–4686.

[41] Lin K, Gallay P. Curing a viral infection by targeting the host: the example of cyclophilin inhibitors. Antiviral Res 2013;99:68–77.

[42] Hopkins S, Scorneaux B, Huang Z, Murray MG, Wring S, Smitley C, et al. SCY-635, a novel nonimmunosuppressive analog of cyclosporine that exhibits potent inhibition of hepatitis C virus RNA replication in vitro. Antimicrob Agents Chemother 2010;54:660–672.

[43] Peel M, Scribner A. Cyclophilin inhibitors as antiviral agents. Bioorg Med Chem Lett 2013;23:4485–4492.

[44] Garcia-Rivera JA, Bobardt M, Chatterji U, Hopkins S, Gregory MA, Wilkinson B, et al. Multiple mutations in hepatitis C virus NS5A domain II are required to confer a significant level of resistance to alisporivir. Antimicrob Agents Chemother 2012;56:5113–5121.

[45] Gregory MA, Bobardt M, Obeid S, Chatterji U, Coates NJ, Foster T, et al. Preclinical characterization of naturally occurring polyketide cyclophilin inhibitors from the sanglifehrin family. Antimicrob Agents Chemother 2011;55:1975–1981.

[46] Zenke G, Strittmatter U, Fuchs S, Quesniaux VF, Brinkmann V, Schuler W, et al. Sanglifehrin A, a novel cyclophilin-binding compound showing immunosuppressive activity with a new mechanism of action. J Immunol 2001;166:7165–7171.

[47] Sedrani R, Kallen J, Martin Cabrejas LM, Papageorgiou CD, Senia F, Rohrbach S, et al. Sanglifehrin-cyclophilin interaction: degradation work, synthetic macrocyclic analogues, X-ray crystal structure, and binding data. J Am Chem Soc 2003;125:3849–3859.

[48] Gaither LA, Borawski J, Anderson LJ, Balabanis KA, Devay P, Joberty G, et al. Multiple cyclophilins involved in different cellular pathways mediate HCV replication. Virology 2010;397:43–55.

[49] Watashi K, Ishii N, Hijikata M, Inoue D, Murata T, Miyanari Y, et al. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol Cell 2005;19:111–122.

[50] Coelmont L, Kaptein S, Paeshuyse J, Vliegen I, Dumont JM, Vuagniaux G, et al. Debio 025, a cyclophilin binding molecule, is highly efficient in clearing hepatitis C virus (HCV) replicon-containing cells when used alone or in combination with specifically targeted antiviral therapy for HCV (STAT-C) inhibitors. Antimicrob Agents Chemother 2009;53:967–976.

[51] Hanoulle X, Badillo A, Wieruszeski JM, Verdegem D, Landrieu I, Bartenschlager R, et al. Hepatitis C virus NS5A protein is a substrate for the peptidyl-prolyl cis/trans isomerase activity of cyclophilins A and B. J Biol Chem 2009;284:13589–13601.

[52] Liu Z, Yang F, Robotham JM, Tang H. A critical role of cyclophilin A and its prolyl-peptidyl isomerase activity in the structure and function of the HCV replication complex. J Virol 2009;83:6554–6565.

[53] Chatterji U, Bobardt M, Selvarajah S, Yang F, Tang H, Sakamoto N, et al. The isomerase active site of cyclophilin A is critical for hepatitis C virus replication. J Biol Chem 2009;284:16998–17005.

[54] Kaul A, Stauffer S, Berger C, Pertel T, Schmitt J, Kallis S, et al. Essential role of cyclophilin A for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics. PLoS Pathog 2009;5:e1000546.

[55] Coelmont L, Hanoulle X, Chatterji U, Berger C, Snoeck J, Bobardt M, et al. DEB025 (Alisporivir) inhibits hepatitis C virus replication by preventing a cyclophilin A induced cis-trans isomerisation in domain II of NS5A. PLoS One 2010;5:e13687.

[56] Dorner M, Horwitz JA, Donovan BM, Labitt RN, Budell WC, Friling T, et al. Completion of the entire hepatitis C virus life cycle in genetically humanized mice. Nature 2013;501:237–241.

[57] Chatterji U, Lim P, Bobardt MD, Wieland S, Cordek DG, Vuagniaux G, et al. HCV resistance to cyclosporin A does not correlate with a resistance of the NS5A-cyclophilin A interaction to cyclophilin inhibitors. J Hepatol 2010;53:50–56.

[58] Gallay PA, Lin K. Profile of alisporivir and its potential in the treatment of hepatitis C. Drug Des Dev Ther 2013;7:105–115.

[59] Hopkins S, Bobardt M, Chatterji U, Garcia-Rivera JA, Lim P, Gallay PA. The cyclophilin inhibitor SCY-635 disrupts hepatitis C virus NS5A-cyclophilin A complexes. Antimicrob Agents Chemother 2012;56:3888–3897.

[60] Chatterji U, Garcia-Rivera JA, Baugh J, Gawlik K, Wong KA, Zhong W, et al. The combination of alisporivir plus an NS5A inhibitor provides additive to synergistic anti-hepatitis C virus activity without detectable cross-resistance. Antimicrob Agents Chemother 2014;58:3327–3334.

[61] Ciesek S, Steinmann E, Wedemeyer H, Manns MP, Neyts J, Tautz N, et al. Cyclosporine A inhibits hepatitis C virus nonstructural protein 2 through cyclophilin A. Hepatology 2009;50:1638–1645.

[62] Madan V, Paul D, Lohmann V, Bartenschlager R. Inhibition of HCV replication by cyclophilin antagonists is linked to replication fitness and occurs by inhibition of membranous web formation. Gastroenterology 2014;146:1361–1372.

[63] Lawitz E, Godofsky E, Rouzier R, Marbury T, Nguyen T, Ke J, et al. Safety, pharmacokinetics, and antiviral activity of the cyclophilin inhibitor NIM811 alone or in combination with pegylated interferon in HCV-infected patients receiving 14 days of therapy. Antiviral Res 2011;89:238–245.

[64] Hopkins S, DiMassimo B, Rusnak P, Heuman D, Lalezari J, Sluder A, et al. The cyclophilin inhibitor SCY-635 suppresses viral replication and induces endogenous interferons in patients with chronic HCV genotype 1 infection. J Hepatol 2012;57:47–54.

[65] Muir AJ, Rodriguez-Torres M, Borroto-Esoda K, Peel M, Hopkins S, Ribell Y, et al. Short duration treatment with SCY-635 restores sensitivity to Peg-IFN/RBV in difficult to treat, IL28B TT/CT, HCV Genotype 1 patients. Hepatology 2012;56:234A.

[66] Griffel L, Bao W, Orsenigo R, Guo V, Wu M, Loeffler J, et al. Interferon (IFN)-free alisporivir (ALV) has a better overall safety profile compared to IFN-containing treatment: a pooled analysis of the ALV development program. J Hepatol 2013;58:S336–S337.

[67] Buti M, Flisiak R, Rasenack J, Davis G, Alberti A, Goeser T, et al. Alisporivir (ALV) plus Peg-Interferon/Ribavirin (PR) achieves high SVR12 rates among null responders, IL28BCT/TT and cirrhotic HCVG1 patients (FUNDAMENTAL study). J Hepatol 2013;58:S572.

[68] Pawlotsky JM, Sarin SK, Foster GR, Peng CY, Rasenack J, Flisiak R, et al. Alisporivir plus ribavirin achieves high rates of sustained HCV clearance (SVR24) as interferon (FN)-free or IFN-add-on regimen in treatment-naïve patients with GT2 or GT3: final results of the VITAL-1 study. Hepatology 2012;56:309A, [abstr. 233].

[69] Piccoli C, Scrima R, Quarato G, D'Aprile A, Ripoli M, Lecce L, et al. Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. Hepatology 2007;46:58–65.

[70] Quarato G, D'Aprile A, Gavillet B, Vuagniaux G, Moradpour D, Capitanio N, et al. The cyclophilin inhibitor alisporivir prevents hepatitis C virus-mediated mitochondrial dysfunction. Hepatology 2012;55:1333–1343.

[71] Bouchard MJ, Puro RJ, Wang L, Schneider RJ. Activation and inhibition of cellular calcium and tyrosine kinase signaling pathways identify targets of the HBx protein involved in hepatitis B virus replication. J Virol 2003;77:7713–7719.

[72] Chokshi S, Phillips S, Riva A, Naoumov NV. Characterisation of antiviral activities of DEB025 (Alisporivir) and NIM811 on Hepatitis B virus (HBV) replication and HBsAg secretion in vitro. J Hepatol 2011;54:S437–S438.

[73] Phillips S, Chokshi S, Riva A, Naoumov NV. Alisporivir-induced inhibition of cellular cyclophilins disrupts hepatitis B virus (HBV) replication in vitro and is synergistic in combination with direct antiviral targeted HBV-DNA polymerase. J Hepatol 2012;56:S199–S200.

[74] Nilsson J, Moss SJ, Coates N, Bobardt M, Gallay P, Gregory M. NVP018, A cyclophilin inhibitor for treatment of chronic HBV infection. J Hepatol 2014;60:S423.

[75] Tian X, Zhao C, Zhu H, She W, Zhang J, Liu J, et al. Hepatitis B virus (HBV) surface antigen interacts with and promotes cyclophilin a secretion: possible link to pathogenesis of HBV infection. J Virol 2010;84:3373–3381.

[76] Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife 2012;1:e00049. http://dx.doi.org/10.7554/eLife. 00049.

[77] Nkongolo S, Ni Y, Lempp FA, Kaufman C, Lindner T, Esser-Nobis K, et al. Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. J Hepatol 2014;60:723–731.

[78] Watashi K, Sluder A, Daito T, Matsunaga S, Ryo A, Nagamori S, et al. Cyclosporin A and its analogs inhibit hepatitis B virus entry into cultured hepatocytes through targeting a membrane transporter, sodium taurocholate cotransporting polypeptide (NTCP). Hepatology 2014;59:1726–1737.

[79] Koopman WJ, Willems PH, Smeitink JA. Monogenic mitochondrial disorders. N Engl J Med 2012;366:1132–1141.
[80] Guerra MT, Fonseca EA, Melo FM, Andrade VA, Aguiar CJ, Andrade LM, et al. Mitochondrial calcium regulates rat liver regeneration through the modulation of apoptosis. Hepatology 2011;54:296–306.
[81] Zhong Z, Theruvath TP, Currin RT, Waldmeier PC, Lemasters JJ. NIM811, a mitochondrial permeability transition inhibitor, prevents mitochondrial depolarization in small-for-size rat liver grafts. Am J Transplant 2007;7:1103–1111.
[82] Liu Q, Rehman H, Harley RA, Lemasters JJ, Zhong Z. Small-for-size liver transplantation increases pulmonary injury in rats: prevention by NIM811. HPB Surg 2012;2012:13 pages. http://dx.doi.org/10.1155/2012/270372. [Article ID 270372].
[83] Pessayre D, Fromenty B. NASH: a mitochondrial disease. J Hepatol 2005;42:928–940.
[84] Malesevic M, Gutknecht D, Prell E, Klein C, Schumann M, Nowak RA, et al. Anti-inflammatory effects of extracellular cyclosporines are exclusively mediated by CD147. J Med Chem 2013;56:7302–7311.
[85] Seizer P, Klingel K, Sauter M, Westermann D, Ochmann C, Schönberger T, et al. Cyclophilin A affects inflammation, virus elimination and myocardial fibrosis in coxsackievirus B3-induced myocarditis. J Mol Cell Cardiol 2012;53:6–14.
[86] Wang H, Zhang Y, Wang T, You H, Jia J. N-methyl-4-isoleucine cyclosporine attenuates CCl_4-induced liver fibrosis in rats by interacting with cyclophilin B and D. J Gastroenterol Hepatol 2011;26:558–567.
[87] Levy G, Villamil FG, Nevens F, Metselaar HJ, Clavien PA, Klintmalm G, et al. REFINE: a randomized trial comparing cyclosporine A and tacrolimus on fibrosis after liver transplantation for hepatitis C. Am J Transplant 2014;14:635–646. http://dx.doi.org/10.1111/ajt.12620.
[88] Jaeschke H, McGill MR, Williams CD, Ramachandran A. Current issues with acetaminophen hepatotoxicity – A clinically relevant model to test the efficacy of natural products. Life Sci 2011;88:737–745.
[89] Haouzi D, Cohen I, Vieira HL, Poncet D, Boya P, Castedo M, et al. Mitochondrial permeability transition as a novel principle of hepatorenal toxicity in vivo. Apoptosis 2002;7:395–405.
[90] Kon K, Kim JS, Jaessche H, Lemesters JJ. Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. Hepatology 2004;40:1170–1179.
[91] Masubuchi Y, Suda C, Horie T. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. J Hepatol 2005;42:110–116.
[92] LoGuidice A, Boelsterli UA. Acetaminophen overdose-induced liver injury in mice is mediated by peroxynitrite independently of the cyclophilin D-regulated permeability transition. Hepatology 2011;54:969–978.
[93] Ramachandran A, Lebofsky M, Baines CP, Lemasters JJ, Jaeschke H. Cyclophilin D deficiency protects against acetaminophen-induced oxidant stress and liver injury. Free Radic Res 2011;45:156–164.
[94] Dear JW, Simpson KJ, Nicolai MP, Catterson JH, Street J, Huizinga T, et al. Cyclophilin A is a damage-associated molecular pattern molecule that mediates acetaminophen-induced liver injury. J Immunol 2011;187:3347–3352.
[95] Lim SO, Park SJ, Kim W, Park SG, Kim HJ, Kim YI, et al. Proteome analysis of hepatocellular carcinoma. Biochem Biophys Res Commun 2002;291:1031–1037.
[96] Lee J. Cyclophilin A as a new therapeutic target for hepatitis C virus-induced hepatocellular carcinoma. Korean J Physiol Pharmacol 2013;17:375–383.
[97] Lee J. Novel combinational treatment of cisplatin with cyclophilin A inhibitors in human hepatocellular carcinomas. Arch Pharm Res 2010;33:1401–1409.
[98] Kim Y, Jang M, Lim S, Won H, Yoon KS, Park JH, et al. Role of cyclophilin B in tumorigenesis and cisplatin resistance in hepatocellular carcinoma in humans. Hepatology 2011;54:1661–1678.
[99] Zhang M, Dai C, Zhu H, Chen S, Wu Y, Li Q, et al. Cyclophilin A promotes human hepatocellular carcinoma cell metastasis via regulation of MMP3 and MMP9. Mol Cell Biochem 2011;357:387–395.