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Connexin and pannexin (hemi)channels: emerging targets in the treatment of liver disease

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Abstract

Connexin proteins are the building blocks of hemichannels, which dock further between adjacent cells to form gap junctions. Gap junctions control the intercellular exchange of critical homeostasis regulators. By doing so, gap junctions control virtually all aspects of the hepatic life cycle. In the last decade, it has become clear that connexin hemichannels also provide a pathway for cellular communication on their own independent of their role as structural precursors of gap junctions, namely between the cytosol of an individual cell and its extracellular environment. In contrast to gap junctions, connexin hemichannels become particularly active in liver disease by facilitating inflammation and cell death. This equally holds true for cellular channels composed of pannexins, being connexin-like proteins recently identified in the liver that gather in structures reminiscent of hemichannels. This paper gives an overview of the involvement of connexin-based and pannexin-based channels in non-cancerous liver disease.

Key words: connexin, pannexin, hemichannel, gap junction.

Structure, function and regulation of connexin and pannexin (hemi)channels

Liver homeostasis is controlled by extracellular, intracellular and intercellular communication mechanisms. The latter is mediated gap junctions.[1-3] The liver was among the first organs in which gap junctions were studied. In 1974, Goodenough isolated 2 gap junction proteins from mouse liver that were designated connexins.[4] Today, more than 20 different connexin (Cx) species have been identified, of which 5 are expressed in liver (Table 1). They are all named based upon their molecular weight and are expressed in a cell type-specific way.[5] Nevertheless, they all share a similar structure consisting of 4 transmembrane domains, 2 extracellular loops, 1 cytosolic loop, 1 cytosolic carboxyterminal tail and 1 cytosolic aminotail (Figure 1). Following their synthesis, 6 connexins form a hemichannel at the plasma membrane surface, which then docks with another hemichannel from a neighboring cell to generate a gap junction.[1-3] Gap junctions mediate the passive intercellular diffusion of small and hydrophilic molecules, such as glucose, glutathione, adenosine triphosphate, cyclic adenosine monophosphate and inositol triphosphate, as well as ions, including calcium, sodium and potassium.[6] Numerous physiological processes are regulated by substances that are intercellularly exchanged *via* gap junctions and hence gap junctional intercellular communication is considered as a key mechanism in the control of tissue homeostasis.[1-3] In this respect, gap junctions are known to be indispensable for liver cell proliferation and differentiation as well as for the maintenance of liver-specific functionality, including albumin secretion, ammonia detoxification and xenobiotic biotransformation.[1,2] It has now become well accepted that connexin hemichannels, in addition to acting as the building blocks of gap junctions, also provide a pathway for communication, albeit between the cytosol and the extracellular environment.[7] The messengers that permeate connexin hemichannels show great overlap with those involved in gap junctional intercellular communication. Unlike gap junctions, connexin hemichannels become primarily active during disease. Furthermore, a

novel class of connexin-like proteins was discovered in 2000, namely the pannexin (Panx) family, which gather in a configuration reminiscent of connexin hemichannels, but that do not form gap junctions (Figure 1).[8] Pannexin channels facilitate paracrine communication, mainly by controlling the extracellular exchange of adenosine triphosphate, cyclic adenosine monophosphate, inositol triphosphate and calcium. Only 3 pannexins have yet been identified, named based on the order of their discovery, of which Panx1 is found in liver (Table 1).[9,10]

Pathophysiology of connexin and pannexin (hemi)channels

Acute liver failure

Hepatic gap junction functionality deteriorates in mice upon intoxication with acetaminophen, which is accompanied by a switch in connexin production from Cx32 and Cx26 to Cx43 (Table 2). The upregulation of Cx43 expression is due, at least in part, to *de novo* production by hepatocytes.[11] Upon administration of acetaminophen and other prototypical liver toxicants to Cx32-lacking rodents, decreased aminotransferase levels and less liver damage are observed.[12-14] Similarly, hepatocytes isolated from Cx32-deficient mice show reduced cell death when treated with acetaminophen *in vitro*.[15] This suggest a role for Cx32-based signalling either in spreading noxious messengers or in the removal of dead cells in order to restore the homeostatic balance. In contrast, protective effects of Cx32 in acetaminophen-triggered liver toxicity have been described, which might be linked to the trafficking of glutathione between hepatocytes *via* gap junctions.[16] This can be reconciled with the well-known decay of Cx32 production and concomitant reduced channel activity upon exposure of hepatocytes to liver toxicants.[1] Nevertheless, Cx32$^{-/-}$ and wild-type mice display no differences in inflammation, cell death and oxidative stress upon acetaminophen intoxication.[17] Hepatocellular gap junctions persist in the early phases of centrilobular necrotic cell death induced by thioacetamide in rat, yet they fade away during the subsequent proliferative response. In a later stage, gap junctions initially

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emerge in perinecrotic areas and ultimately in all zones.[18] Interestingly, in liver of rats overdosed with acetaminophen, Cx43 becomes detectable in hepatocytes and co-localizes with caspase 3, suggesting a role for Cx43 in cell death.[13] This is substantiated by reduced cell death -and hepatocellular injury observed in Cx43-lacking mice treated with carbon tetrachloride.[19] $Cx43^{+/-}$ mice tend to show increased liver cell death, inflammation and oxidative stress, suggesting that hepatic Cx43-based signalling may protect against acetaminophen-induced liver toxicity.[11] Furthermore, high Cx43 immunoreactivity is seen around inflamed and necrotic areas in acute-on-chronic liver failure in rat.[20] Interestingly, specific Cx32 and/or Cx43 hemichannel inhibition reduces levels of pro-inflammatory cytokines and alanine aminotransaminase after acetaminophen overdosing (Table 3).[21] Recently, it has been shown that levels of hepatic Panx1 increase in acetaminophen-overdosed mice (Table 2). Furthermore, $Panx1^{-/-}$ mice are less prone to oxidative stress and liver damage induced by acetaminophen.[22] This is in line with the observation that pharmacological suppression of Panx1 channels reduces levels of alanine and aspartate aminotransferase, counteracts recruitment of neutrophils to the liver and alters the hepatic oxidative status (Table 3).[23]

Hepatitis and cholestasis

Hepatitis patients present reduced amounts of Cx32 in the liver[24,25], a feature that can be experimentally reproduced in rodents when treated with lipopolysaccharide (Table 2).[26-28] Deterioration of Cx32 expression hereby results from mRNA degradation.[29] Downregulation of Cx32 production by pro-inflammatory cytokines is controlled by nuclear factor kappa beta signalling and mitogen-activated protein kinase, and is accompanied by abrogation of gap junction activity.[30] Hepatic Cx26, however, is positively affected by pro-inflammatory stimuli.[31] Likewise, Cx43 expression along with gap junction functionality becomes enhanced in cultures of hepatic stellate cells and Kupffer cells in inflammatory conditions.[32] Cx43 hereby

moves from the cytosol to the membrane surface in order to assemble into functional gap junctions. Upregulated Cx43 production also occurs during liver inflammation *in vivo*.[29] This is thought to reflect the activation of Kupffer cells, which assists in the removal of debris and apoptosis of damaged hepatocytes following inflammation.[32] Administration of lipopolysaccharide[33] as well as ischemia/reperfusion injury[34] elevate hepatic Panx1 levels in mice. Upon cholestasis induced by bile duct ligation, gap junction quantities and Cx32 amounts decrease in rodent liver.[29] While Cx26 levels also drop, Cx43 production rather increases in cholestatic animals (Table 2).[20] Interestingly, bile duct ligation in rat leads to dysfunction of cortical connexin hemichannels, mediated by ammonia, which could underlie the hepatic encephalopathy frequently occurring in chronic liver disease.[35] Recently, bile duct ligated Panx1$^{-/-}$ mice were found to display increased hepatocellular injury and anti-oxidant enzyme activity with a predominant immune response.[36]

Liver fibrosis and cirrhosis

Cx32 steady-state protein levels are reduced in cirrhosis patients (Table 2), a process that coincides with its relocalization to the cytoplasm of hepatocytes.[37] Furthermore, upregulated Cx43 production has been observed in human cirrhotic liver tissue (Table 2).[38] These findings are identical to those in rodents following chronic administration of thioacetamide or carbon tetrachloride.[39] The latter induces translocation of both Cx26 and Cx43 from the plasma membrane to the cytoplasm and nuclei of liver sinusoidal endothelial cells, a scenario that is equally seen for Cx32 in hepatocytes.[40] This could underlie the establishment of communication between stellate cells and hepatocytes under these conditions[41], whilst gap junctional signalling in cultured hepatocytes is suppressed by carbon tetrachloride.[42] Cx32$^{-/-}$ mice show more pronounced liver damage and enhanced collagen deposition in experimentally induced liver fibrosis.[43] Recently, inhibition of Cx43-based gap junctions and hemichannels

was found to lower the degree of thioacetamide-triggered liver fibrosis accompanied by superoxide dismutase overactivation and reduced production of inflammatory proteins in mice (Table 3).[44] Using the same mouse model, tenovir, a widely applied antiviral agent, was found to inhibit Panx1-mediated release of adenosine triphosphate and to counteract liver fibrosis.[45] This complies with a study showing reduced collagen content, hepatic stellate cell activation, inflammation and regeneration in Panx1^{-/-} mice upon repeated treatment with carbon tetrachloride.[36]

Non-alcoholic steatohepatitis

Non-alcoholic steatohepatitis has been linked to the activation of inflammasomes in numerous studies.[46,47] Besides driving the inflammasome, Panx1 contributes to pathophysiological adenosine triphosphate release in lipo-apoptosis induced by saturated fatty acids. In turn, this is capable of stimulating migration of human monocytes *in vitro* and may participate in the recruitment of monocytes in acute and/or chronic liver injury.[48] Thus, Panx1-based channels may play a role in hepatic inflammation by mediating an increase in extracellular adenosine triphosphate levels in lipotoxic liver injury. Furthermore, Panx1 channel inhibition attenuates inflammasome activation and hepatic injury after ischemia/reperfusion injury.[34] Panx1^{-/-} mice present less liver damage, oxidative stress and inflammation in diet-induced non-alcoholic steatohepatitis in mice.[22] Panx1 expression has been found to be elevated in liver samples of clinical patients suffering from non-alcoholic steatohepatitis.[49] In experimental non-alcoholic steatohepatitis, Cx32 exerts a protective effect as evidenced by increased levels of inflammatory cytokines and more pronounced oxidative stress in Cx32 dominant negative transgenic rats.[50] In accordance with this finding, more liver damage, inflammation and oxidative stress were observed in Cx32^{-/-} mice with non-alcoholic steatohepatitis, with no differences in insulin and glucose tolerance measurements or liver regeneration.[51] Inhibition of

hemichannels consisting of Cx32 or Cx43 in mice suffering from non-alcoholic steatohepatitis lowers levels of liver lipids and inflammatory markers (Table 3).[52]

Therapeutic relevance of connexin and pannexin (hemi)channels

While gap junctions are considered as the "good guys" that support normal hepatic functionality, connexin hemichannels are typically seen as the "bad guys", which become active in case of liver toxicity and disease. In fact, connexin hemichannels, like pannexin channels, play central roles in the induction and propagation of cell death and inflammation, being hallmarks of a plethora of liver diseases.[9,10] A major impediment in clinically exploring this strategy is the lack of specific inhibitors of connexin hemichannels and pannexin channels, which do not affect other channel types. An exception in this regard are a group of peptides that simulate amino acid modules in specific regions of connexins critical for hemichannel opening. Although promising, such peptides may cope with stability issues.[53] Current efforts are focused on chemically stabilizing these connexin mimetic peptides in order to make them applicable for clinical use. In parallel, the search for small molecules as potential new connexin hemichannel and pannexin channel inhibitors has been initiated. Further exploration of this area in the upcoming years is anticipated to open novel therapeutic avenues in the hepatology field.

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Legends to Figures and Tables

Figure 1

A. *Architecture of connexin and pannexin channels*. Gap junctions are formed by the interaction between 2 hemichannels of adjacent cells and mediate intercellular communication. Connexin hemichannels and pannexin channels are built up by 6 connexin proteins (light grey) and 6 pannexin proteins (dark grey), respectively, and support paracrine communication.

B. *Topology of connexin and pannexin proteins*. Connexins (light grey) and pannexins (dark grey) all consist of 4 transmembrane domains (TM), 2 extracellular loops (EL), 1 cytosolic loop (CL), 1 carboxyterminal (CT) and 1 aminoterminal (NT) tail.

Table 1

Expression of connexins and pannexins in liver.

Table 2

Effects of liver disease on connexins and pannexins (H, human; M, mouse; R, rat).

Table 3

Effects of peptide-based inhibition of connexin hemichannels and pannexin channels in experimental mouse models of liver disease (reduced cell damage (*) and no effect on inflammation (**) are observed upon simultaneous inhibition of Cx32 and Cx43 hemichannels).