Review

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Gap junctions in liver disease: Implications for pathogenesis and therapy

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Summary

In the normal liver, cells interact closely through gap junctions. By providing a pathway for the trafficking of low molecular mass molecules, these channels contribute to tissue homeostasis and maintenance of hepatic function. Thus, dysfunction of gap junctions affects a wide variety of liver processes, such as differentiation, cell death, inflammation and fibrosis. In fact, dysfunctional gap junctions have been implicated, for more than a decade, in cholestatic disease, hepatic cancer and cirrhosis. Additionally, in recent years there is an increasing body of evidence that these channels are also involved in other relevant and prevalent liver pathological processes, such as non-alcoholic fatty liver disease, acute liver injury and portal hypertension. In parallel to these new clinical implications the available data include controversial observations. Thus, a comprehensive overview is required to better understand the functional complexity of these pores. This paper will review the most recent knowledge concerning gap junction dysfunction, with a special focus on the role of these channels in the pathogenesis of relevant clinical entities and on potential therapeutic targets that are amenable to modification by drugs.

Keywords: Connexins; Gap junctions; Hemichannels; Intercellular communication; Cirrhosis; Liver.

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Gap junctions, hemichannels and connexins: Molecular characteristics and function

Cell-to-cell communication is of extreme importance in tissue homeostasis, which is maintained by transmission of regulatory signals[1] (Fig. 1). Intercellular communication via gap junctions (GJs) represents one of the most important routes of rapid signalling between cells. GJ channels span 2 plasma membranes and consist of 2 hemichannels (connexons), one belonging to each cell. Each hemichannel is formed by 6 connexin (Cx) subunits and is permeable to small molecules up to 1–1.5kD.[1] They serve to provide electrical and chemical conductance as well as metabolic assistance.[2,3] GJ communication is modulated by many factors such as cytokines, growth factors and nitric oxide (NO), making them susceptible to change during cell stress and injury.[4,5]

Cxs consist of 4 transmembrane helices (M1-M4). The N- and C-terminal ends are intracellular. The primary sequence of the intracellular loop is not well conserved, while the C-terminal sequence varies a lot between Cxs with Cx26 being ~20 amino acids and Cx43 being 150 amino acids long.[6] More than 20 Cxs have been identified with different molecular weights and their expression patterns vary between cell types and tissues. Many different Cxs have been observed in the liver. Endothelial cells, Kupffer cells and stellate cells mainly express Cx43, hepatocytes express Cx32 and to a lesser extent Cx26, while liver vascular cells express Cx37 and Cx40 (Fig. 2).[2,7–9]

Across Cxs isoforms, there is a wide variation in conductance (most hemichannels have a fixed negative charge in the pore making them cation selective) and permeability characteristics that have likely evolved according to the requirements of the tissue in which they are expressed. Moreover, their plasticity allows them to compensate for the loss or downregulation of other Cxs as revealed by several knockout models.[1] Furthermore, in these models, disturbed cell development has been observed, suggesting that GJs and hemichannels play an important role in processes such as migration, differentiation and proliferation.[3]

Cxs can also exist as functional hemichannels allowing the exchange of ions between the intra and the extracellular milieu.[2,4] Under normal physiological conditions, hemichannels are either closed[10] or in a flickering state.[11] Maintaining controlled gating to allow entry or exit of molecules from the cell is very important to preserve normal cellular integrity and function. Therefore, hemichannels are constantly under the control of factors such as membrane potential, pH, post-translational modification (phosphorylation, ubiquitination, S-nitrosylation), mechanical stimulation and intracellular/extracellular calcium.[3,12,13] Facilitated opening of hemichannels has been shown to correlate with cell death in cerebral ischaemia, resulting in the loss of osmoregulation, excitotoxicity and the spread of inflammation.[14] Although hemichannels and pannexins (structurally similar to Cx proteins) are also of great interest in liver disease, their role in liver disease

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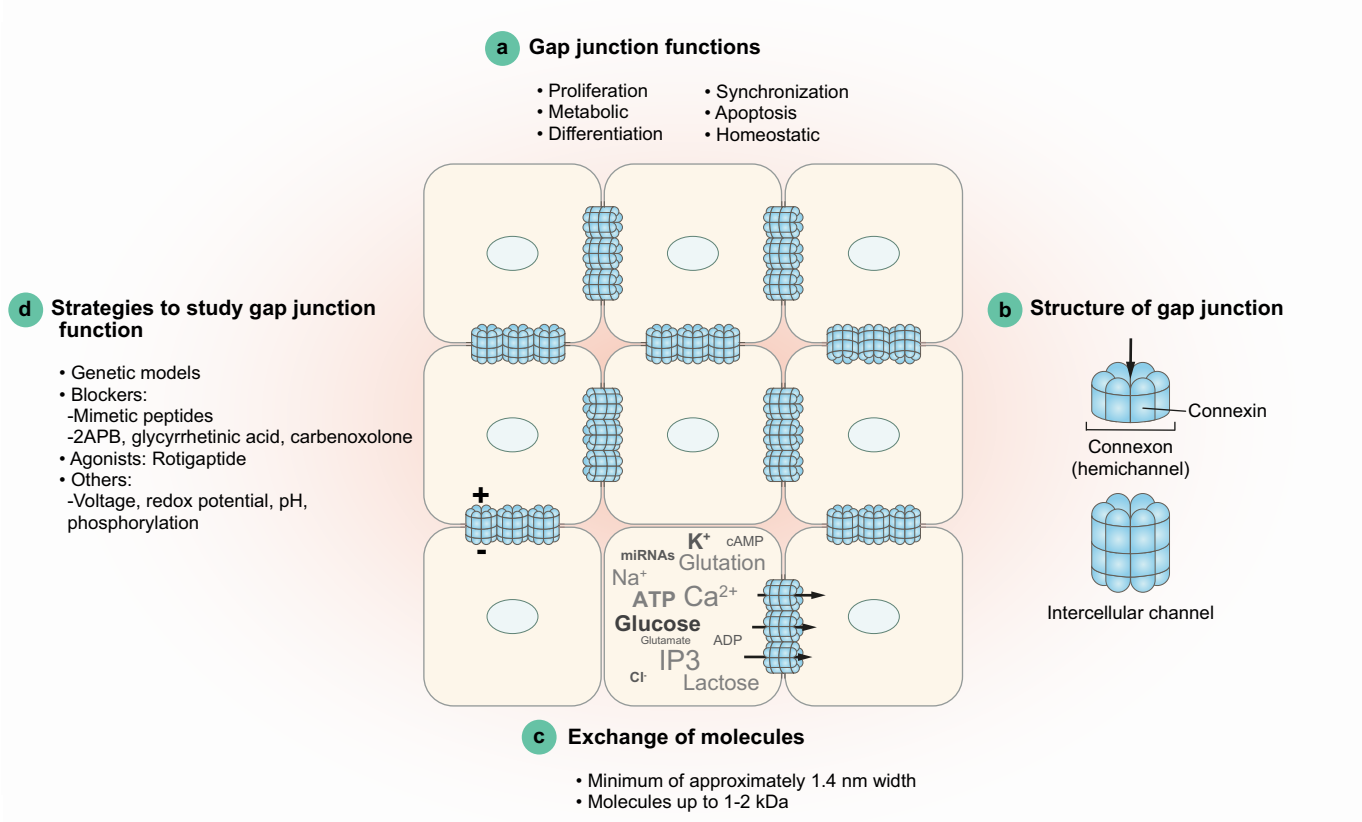


Fig. 1. Representation of hepatocytes and gap junction functions, structure, trafficking messengers and strategies to evaluate gap junction functions. a) Gap junctions participate in different functions. b) Individual connexins assemble intracellularly into hexamers, called connexons (hemichannels), which dock with other connexons in adjacent cells, assembling an axial channel spanning 2 plasma membranes and a narrow extracellular gap. c) Different molecules pass through the gap junctions. d) With different approaches the function of different connexins has been evaluated. 2-APB, 2-aminoethoxydiphenyl-borate.

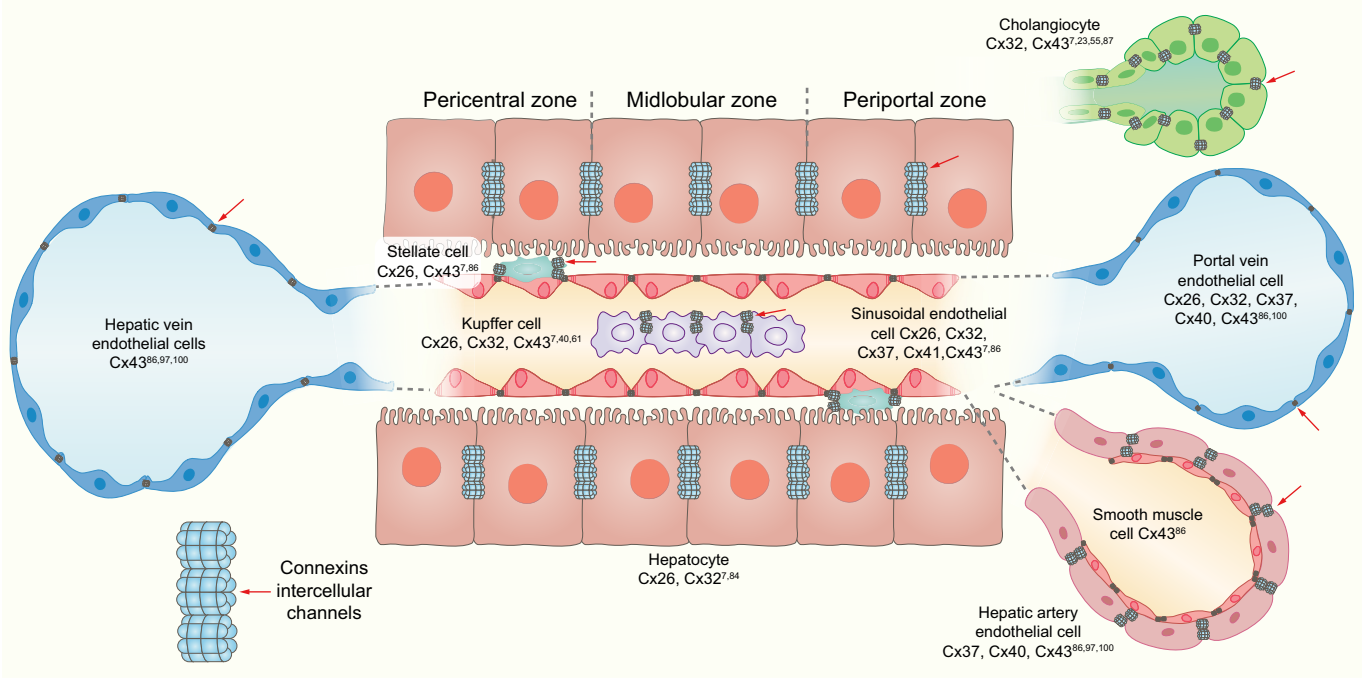


Fig. 2. Communication between liver cells through gap junctions. Diagram showing how different liver cells express Cxs. Cx, connexin. (See above-mentioned references for further information.)

will not be discussed in any detail in this review (for an extended review on liver pannexins see.[15]).

Connexin and gap junction alterations in disease

Cx protein mutations are associated with various diseases such as hearing loss, which is linked to Cx26 and Cx30, and atrial fibrillation which is associated with a Cx40 mutation.[16,17] Additionally, under other pathological conditions, such as focal ischaemia, opening of GJs serves a protective role, enabling cells to save their compromised neighbours by providing essential molecules to areas of high demand.[18] However, maintaining GJ communication in severely injured or diseased tissue areas allows the spread of toxic substances, propagating and worsening cell injury.[19] The diseases associated with congenital or acquired Cx involvement are shown (Fig. 3). It is notable that none of the mutations described affect the liver.

During cardiac ischaemia, a decrease in GJ coupling is observed, which results in slower conduction of electrical impulses and a higher risk of arrhythmias.[13,20] In Huntington's disease an increase in the expression of 5 Cxs was observed in the astrocytes in the brain, suggesting an adaptive protective response.[21] Cerebral ischaemia results in uncoupling of astrocytes due to a decrease in GJ function, which prevents astrocytes from being able to redistribute ions and neurotransmitters resulting in "cell swelling".[22] In cirrhosis and acute-on-chronic liver failure (ACLF),[23] studies indicated that increased expression of hepatic Cx43 was related to the severity of inflammation. This was suggested to be an adaptive response of the liver for protection through better intercellular communication.

Results on the role of Cx and GJ alterations during various pathological states are controversial (reviewed by[2]). However, the ability of GJ proteins to participate in different physiological and pathological states makes them attractive therapeutic targets in different diseases.[24] Therefore, this paper will review recent knowledge concerning the role of GJs in the pathogenesis of liver diseases.

Modulators of GJ function and targeting in diseases outside the liver

Opposing approaches aimed at increasing or decreasing GJ function have been explored to treat different diseases. To improve GJ function in heart diseases, GJ openers, such as the synthetic peptides rotigaptide[25] and danegaptide,[26] were tested and shown to reduce the burden of arrhythmias and myocardial infarct size. However, a recently published phase II study did not confirm the early results.[27] Other enhancers of GJ function such as ACT1, a peptide that mimics the carboxyl terminus of Cx43, have been evaluated in cutaneous ulcers[28] and arrhythmias, where they led to wound re-epithelialization and reduced inducible arrhythmias following ventricular injury, respectively.[29] Conversely, strategies that target specific Cxs with antisense oligonucleotide and mimetic peptides can be used if the goal is to block intercellular communication.[24] These strategies have been shown to reduce inflammation and improve neuronal survival after cerebral[30] and retinal ischaemia.[31] They have also been shown to promote wound healing.[32,33]

In addition to the direct beneficial effects of simply potentiating or blocking the channel, the Cx targeting drugs may be used as adjuvants potentiating the effects of other known therapeutic agents. This is of particular interest in hepatocellular carcinoma (HCC) where GJs may favour the delivery of cytotoxic drugs to tumour cells. In this regard, studies have shown that GJ mimetics facilitate the spread of cisplatin, conferring a better therapeutic effect.[34] Quinolone, a GJ opener was recently shown to enhance cisplatin-induced cytotoxicity,[35] supporting the rationale for combination therapies that include GJ openers in the treatment of various cancers such as colon,[36] prostate[37] and breast.[38] In addition, inhibition of GJ may reduce the toxic effects of drugs by preventing the propagation of inflammatory or death stimuli to neighbouring cells.[39] Given the potentially opposing effects of modulating GJ function, clinical application in a given disease needs to be carefully considered.

Key point

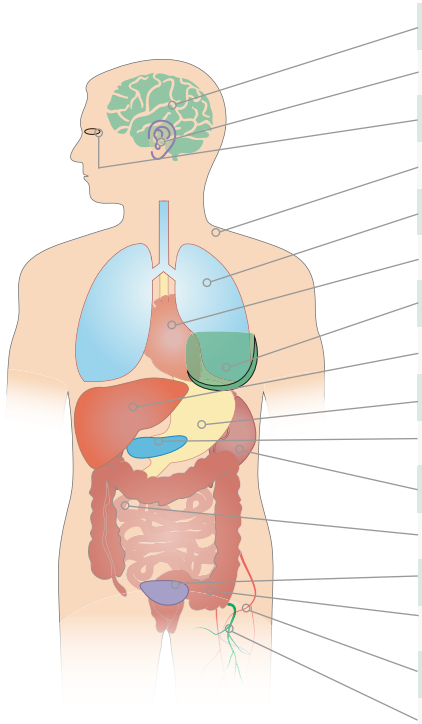
Gap junctions and hemichannels participate in a variety of liver diseases.

Acute liver injury and inflammation

GJs and Cxs are involved in settings where homeostatic regulation is crucial, such as during inflammation and cell death. Available data indicate that Cx26, Cx32 and Cx43 can contribute to acute liver injury and inflammation related to drugs, lipopolysaccharide (LPS) and ischaemia-reperfusion injury. Given that several immune cells including monocytes, macrophages and Kupffer cells express Cx43[40] and are known to be involved in autoimmune liver diseases, the role of GJs in specific autoimmune liver diseases should be explored.[41]

Acute liver injury

To better understand the role of GJs in drug-induced liver injury, studies in cells and animal models have been conducted wherein Cx expression was modified by gene therapy or drugs (Table 1). The observation that HeLa cells transfected with herpes simplex virus induced the killing of a neighbouring cell through the diffusion of toxic phosphorylated ganciclovir molecules after enhancement of GJs,[42] provided the rationale to explore the role of GJs in acute liver injury. Acute administration of carbon tetrachloride and dimethynitrosamine, which induce acute liver injury, resulted in reduced expression of Cx32[43] due to transcriptional downregulation.[44] Addition-



Central nervous system Cx30, Cx32, Cx43, Cx47[161,180]	Loss of memory, leukodystrophy
Cochlea Cx26, Cx30, Cx31, Cx43[154,157,160,162,172,173,174]	Deafness, oculodentodigital dysplasia, keratoderma-hypotrichosis-leukonychia totalis syndrome
Eye Cx46, Cx50[170,171,179,182]	Cataract
Skin Cx26, Cx30, Cx31, Cx43[152,153,158,159]	Palmoplantar keratoderma, psoriasis, erythrokeratodermia variabilis et progressiva
Lung Cx37, Cx40, Cx43[165,169,176]	Asthma, pulmonary arterial hypertension, idiopathic pulmonary fibrosis
Heart Cx37, Cx40[62,166,167,175]	Atrial fibrillation, infarct
Breast Cx26, Cx43[156,178]	Breast cancer
Liver Cx26, Cx32, Cx40, Cx43	DILI, portal hypertension, hepatocellular carcinoma, cholestasis, NASH, ischemia-reperfusion, acute liver failure, cirrhosis
Stomach Cx30, Cx32, Cx43[183]	Gastric cancer, gastric erosions
Pancreas Cx36[164]	Diabetes
Kidney Cx37, Cx40, Cs43, Cx45[168]	Hypertension
Duodenum Cx37[184]	Celiac disease
Prostate Cx43[37]	Prostate cancer metastasis
Colon Cx26, Cx32, Cx43[36,177,180]	Dysentery, diverticulosis, colon cancer, Crohn 's disease, Hirschsprung's disease
Vessels Cx47[181]	Lymphedema
Peripheral nervous system Cx32[163]	Charcot-Marie-Tooth disease

Fig. 3. Connexin types implicated in different organ diseases. Cxs are expressed in cells of almost every organ where dysfunction provokes different diseases. Cx, connexin. (See above-mentioned references for further information.)

Table 1. Experimental studies describing the role of connexins in acute liver injury.

Studied Cx	Animal model	Type and dose of toxic	Effects	Reference
Cx32	Sprague-Dawley male transgenic rat	Single intraperitoneal injection of D-galactosamine (300 mg/kg body wt) and carbon tetrachloride (0.5 ml/kg body wt)	Less evident necrosis and ballooning findings in Cx32 deficient rats	Asamoto *et al.*[45]
Cx32, Cx43	Sprague-Dawley male transgenic rat	Single intraperitoneal injection of acetaminophen (250, 500, 1000 mg/kg body wt)	Less inflammation after insult in rats lacking Cx32, and induction of Cx43 expression	Naiki-Ito *et al.*[46]
Cx32	Sprague-Dawley male rats	Single intraperitoneal injection of carbon tetrachloride (1.0 mL/kg body wt) and dimethylnitrosamine (6.3–25 mg/kg body wt)	Decrease in hepatic Cx32 expression after injury and inverse correlation with the increase in plasmic alanine-aminotransferase activity	Miyashita T *et al.*[43]
Cx32	Ceramide synthase (CerS2) null mice (altered gap junction function)	Intraperitoneal injection of acetaminophen (300 mg/kg body wt), D-galactosamine (800 mg/kg body wt), carbon tetrachloride (2 ml/kg body wt) and thioacetamide (200 mg/kg body wt)	Less acetaminophen-induced hepatotoxicity after ablation of Cx32	Park WJ *et al.*[47]
Cx26, Cx32	C57BL/6 Knock-out mice and wildtype mice treated with 2-aminoethoxydiphenyl-borate (Cx blocker)	Intraperitoneal injection of thioacetamide (200, 500 or 1000 mg/kg body wt) and acetaminophen (500 or 750 mg/kg body wt)	Mice deficient in Cx32 and wildtype mice (cotreated with 2APB) showed reduced inflammation and oxidative stress	Patel SJ *et al.*[50]
Cx32	C57BL/6 mice treated with 2-aminoethoxydiphenyl-borate	Intraperitoneal injection of acetaminophen (400 mg/kg body wt)	Protection by attenuation of c-jun-N-terminal kinase but not related to a specific role for Cx32	Du K *et al.*[150]
Cx32	C57BL/6 Knock-out mice	Intraperitoneal injection of acetaminophen (300 mg/kg body wt)	No influence of Cx32 deletion	Maes M *et al.*[151]
Cx32	C57BL/6 Knock-out mice	Intraperitoneal injection of acetaminophen (100, 200, or 300 mg/kg body wt)	More susceptible to liver damage 24 hours after the insult in Cx32 deficient mice	Igarashi I *et al.*[53]
Cx43	C57BL/6 Knock-out mice	Intraperitoneal injection of acetaminophen (300 mg/kg body wt)	Cx43-deficient animals tended to show increased liver cell death, inflammation and oxidative stress in comparison with wild type counterparts	Maes M *et al.*[44]

Cx, connexin; wt, weight.

ally, Cxs were mislocalized from the cell surface to the cytoplasm. Cx32 depleted animals exhibited less severe liver injury after acute administration of D-galactosamine, carbon tetrachloride, thioacetamide and acetaminophen.[45,46] The severity of liver injury increased to that in wild-type animals following restoration of Cx32 by gene transfection.[47] The potential role of GJs in contributing to cell death is further supported by studies in cultured hepatocytes, where suppression of Cx26 and Cx32 reduced the synchronization of cell death after administration of acetaminophen.[48] Taken together, these data suggest that the reduction in Cx32 during acute liver injury is likely to be an adaptive response aimed at protecting healthy cells from the propagation of toxins or messengers associated with cell death. These data have been translated into potential novel therapeutics targeting blockade of Cx26 and Cx32 using 2-aminoethoxydiphenyl-borate. Administration of 2-aminoethoxydiphenyl-borate[49] before, concurrently or after inducing acute liver injury was protective.[50]

In contrast to the decreased expression of Cx26 and Cx32 during acute liver injury, Cx43 expression increases.[44,46] This unexpected increase suggests that Cx43 may play a role in propagating death signals.[51] Accordingly, in liver cell cultures, a progressive increase in Cx43 mRNA and protein expression was observed during apoptosis.[52] It is possible that Cx43 mediates propagation of cell death through caspase-3, a relevant factor in the apoptotic cascade, as they co-localize when apoptosis is induced. In support of this hypothesis, inhibition of Cx43 resulted in downregulation of caspase-3.[46] Overall, these data suggest that blockade of Cx26 and Cx32, and counteracting Cx43 overexpression may represent potential therapeutic targets to reduce acute toxic liver injury. Despite this, there are data contradicting the protective role of Cx26 and Cx32 and the deleterious role of Cx43 in acute liver injury, suggesting that the situation may be more complex than first thought. Complete deletion of Cx32 was shown to worsen acute liver injury[53] and another recent study has suggested that the increase in Cx43 may well be an adaptive response, as knocking out Cx43 was actually associated with worse liver injury.[44] It is possible that these radically different observations may be due to differences in the animal species used, type of blocker/deletion and the route and dose of administration of toxins (Table 1).

Altogether, by means of targeted disruption of Cx genes or drug manipulation, these results argue for a crucial role of Cxs in the propagation of acute liver injury, irrespective of the type of hepatotoxin. However, the exact contribution of Cx remains unknown, as GJs may provoke a positive or negative effect on the severity of injury. The complexity lies in the fact that the cell death or survival response mediated by GJs may be determined by the transfer of molecules that can pass through them.[18]

Lipopolysaccharide-induced liver injury

There is compelling evidence in experimental animal models that administration of LPS, which induces an inflammatory response, results in decreased expression of Cx26 and Cx32[54–58] (Table 2). This reduction in levels of Cx26 and Cx32 protein expression in hepatocytes was related to inflammation.[55,59] However, a downregulation of these Cxs at the level of gene expression by a post-transcriptional mechanism has also been postulated.[56] In the setting of experimental cirrhosis, the administration of LPS resulted in a further reduction in both Cx26 and Cx32.[23] This argues in favour of a protective role of these Cxs, which shut down intercellular communication and propagation of inflammation.

Meanwhile, increased expression of Cx43 has been shown in stellate cells, macrophages, endothelial cells and also in leukocytes in response to LPS.[7,60] This increase in Cx43 expression was also associated with increased activity of Cx43, indicated by a higher dye coupling, suggesting that Cx43 may play a role in liver inflammation.[61] Interestingly, inhibiting Cx43 in rats treated with LPS using mimetic peptides was associated with increased hepatocellular necrosis, suggesting that the increased hepatic Cx43 expression is most likely an adaptive protective response.[23]

Key point

Connexins form gap junctions and hemichannels, which have different expression patterns depending of the type of liver disease.

Liver ischaemia and reperfusion

GJ channels and Cxs have a role in ischaemia-reperfusion injury of the heart,[62] brain[19] and vascular tissues.[63–66] The proposed mechanism is the initiation of an injury-signalling cascade that is propagated through GJs, affecting cellular metabolism.[67] In addition to the exchange of signals, ions and messengers between adjacent cells, functions independent of intercellular communication and related to the presence of Cx in the mitochondria have also been shown. In this case, Cx43 has important functions including modulation of mitochondrial respiration and production of reactive oxygen species.[68]

Hepatic ischaemia-reperfusion injury is commonly observed during partial hepatectomy and liver transplantation, and Cxs have been studied in this setting. In animal models of hepatic ischaemia-reperfusion, an early decrease in Cx26 and Cx32 mRNA and protein expression was observed.[69,70] Partial prevention of this effect was obtained with actinomycin D, which prevents the degradation of Cx32 mRNA, although protein expression of Cxs remained low, suggesting that its regulation occurs by different post-transcriptional and post-translational mechanisms.[71] This alteration is likely to represent an adaptive response aimed at restricting the spread of noxious signals to healthy areas. In keeping with this hypothesis, *in vitro* experiments using

Table 2. Experimental studies describing the role of connexins in inflammation induced by LPS.

Studied Cx	Animal model	Type and dose of toxic	Effects	Reference
Cx32	C57BL/6 Knock-out mice	Intravenous injection of LPS	Hypoglycemia was slightly prolonged, and cholestasis was much worse in Cx32-deficient mice	Correa PR *et al.*[54]
Cx26, Cx32, Cx43	Sprague-Dawley male rats	Intravenous injection of LPS 2 mg/kg body wt	Cx26 and Cx32 were reduced after LPS whereas Cx43 increased associated with prominent inflammation	Gonzalez HE *et al.*[55]
Cx32	Sprague-Dawley male rats	Intravenous injection of LPS 1 mg/kg body wt	A decrease in the level of Cx32 mRNA in rat liver occurred at the posttranscriptional level	Gingalewski C *et al.*[56]
Cx26,d Cx32	Sprague-Dawley male rats	Intravenous injection of LPS 1 mg/kg body wt	Decreased communication was observed associated to Cx mislocalization and decreased Cx32 mRNA	De MA *et al.*[57]
Cx43	Cell culture	LPS in culture medium	Cx43 is tyrosine phosphorylated showing intercellular resistance following exposure to LPS	Lidington D *et al.*[58]
Cx26, Cx32, Cx43	Sprague-Dawley male rats induced to bile-duct ligation	Intraperitoneal injection of LPS 1 mg/kg body wt	Cx26/32 expression inversely correlates with Cx43 expression after LPS. However, inhibiting Cx43 produced hepatocellular necrosis	Balasubramaniyan V *et al.*[23]
Cx26, Cx32, Cx43	Cell culture from Wistar male rats	LPS 1 μg/ml in culture medium	LPS up-regulate Cx43 protein and messenger RNA expression, and enhance intercellular communication in hepatic stellate cells	Fischer R *et al.*[7]
Cx43	Sprague-Dawley male rats	Intraperitoneal injection of LPS 6 mg/kg body wt	Kuppfer cells exposed to LPS showed Cx43 at cell-cell contacts associated with higher dye coupling	Eugenin EA *et al.*[61]

Cx, connexin; LPS, lipopolysaccharide; wt, weight.

cell cultures, targeting the Cx32 gene increased cell survival, which was associated with decreased molecular permeability of GJs.[72] An alternative explanation is that a reduction of cell-to-cell communication prevents disruption of cellular metabolism.[73]

Ischaemic preconditioning, which attenuates and protects against ischaemia-reperfusion damage, is NO dependent.[74,75] In this condition, cell-to-cell coupling appears to be necessary for the protective effect of preconditioning, as uncoupling by chemical inhibitors significantly reduced the protection provided by hypoxic preconditioning. In addition, preconditioning led to an increase in Cx43 expression, which was associated with increased GJ permeability.[76] Clearly, more research is needed to understand the pathophysiological alterations in ischaemia-reperfusion injury that are related to Cxs. This research may identify potential therapeutic approaches to reverse the effects of ischaemia-reperfusion injury.

Key point

In general, connexins aim to protect the liver from injury in response to several insults.

Role of connexins in hepatic fibrosis

Fibrosis is a consequence of pro-inflammatory cytokine release, oxidative stress and necrosis/apoptosis. Fibrosis is associated with the involvement of stellate cells, which transform from a quiescent state into a proliferative and contractile myofibroblast-like phenotype. Other neighbouring non-parenchymal cells including cholangiocytes, Kupffer cells and infiltrating monocytes interact and contribute to further activation of stellate cells.

In the normal liver, fenestrated liver sinusoidal endothelial cells induce senescence of hepatic stellate cells. Capillarization of sinusoids reduces the ability of endothelial cells to suppress stellate cell activity.[77,78] Although GJs may provide a direct pathway of intercellular communication, functional communication between endothelial cells and stellate cells has yet to be consistently identified.[7,79] However, as previously discussed, Cxs may contribute to intercellular transfer of angiocrine signals after injury[80] or may be incorporated into microvesicles involved in promoting fibrogenesis.[81] In this regard, Cxs, in particular Cx43, have been shown to contribute to the composition of membrane vesicles, making this an important target for future research.[82]

The role of GJs in liver fibrogenesis was studied recently.[83] Studies showed a significant decrease in liver fibrosis in Cx32 knockout mice compared to wild-type mice. Although the mechanism underlying this protective effect of Cx32 deletion is not clear, reduced oxidative stress was suggested as a possible explanation. In experimental models of cirrhosis induced by carbon tetrachloride, a downregulation of Cx32 was observed.[84] In humans, reduced expression, as well as a relocalization from the membrane to the cytoplasm were also observed.[71] This evidence argues for a protective role of Cx32.

In models of fibrosis such as after Schistosoma mansoni inoculation[85] or common bile duct ligation,[23,55,86] Cx43 expression was increased while the expression of Cx26 and Cx32 decreased.[23,55,87,88] By contrast, others have observed decreased expression,[84,86] or aberrant Cx43 positioned within the cytoplasm of cells,[89] after chronic carbon tetrachloride administration. Phenobarbital, which itself decreases GJs,[90] is usually co-administrated to promote fibrosis,[91] possibly explaining the observed discrepancy.

Current evidence points towards a role of Cx43 in collagen matrix deposition. Administration of chronic carbon tetrachloride to Cx43 deficient mice resulted in a similar grade of fibrosis as

observed in wild-type animals. Nevertheless, an intensification of collagen deposition and nodule formation with retraction of the liver capsule was more evident in Cx43 deficient animals.[89] In apparent contradiction, another study evaluating the role of Cx43 in fibrosis, aimed at discriminating between GJs and hemichannels. In both cases when Cx43 was inhibited, mice treated chronically with thioacetamide exhibited less fibrosis. Additionally, the authors concluded that hemichannel blockade mediated reduced stellate cell activation and reduced deposition of collagen.[92] To add more complexity, pannexins are involved in the transport of ATP to the extracellular space where it is converted to adenosine, which acts on its receptors to stimulate fibrosis. In another recent study, tenofovir, acting as a pannexin hemichannel blocker had a direct antifibrotic effect.[93]

Cirrhosis and its complications

Portal hypertension

In liver disease, increased intrahepatic vascular resistance contributes to the severity of portal hypertension.[94] In addition to the structural component of portal hypertension caused by fibrosis, a more dynamic component is also present.[95] In cirrhosis, intrahepatic vascular tone is increased due to dysfunction of sinusoidal cells and decreased NO, resulting in impaired vasorelaxation in response to acetylcholine.[96] GJs connect endothelial cells and allow for propagation of vasodilation.[97,98] Indeed, binding of acetylcholine stimulates calcium-activated potassium channels in the plasma membrane evoking hyperpolarization, which is conducted from cell to cell through GJs.

Cx37, Cx40, Cx43 and Cx45 regulate vascular tone.[99] Cx40 and Cx43 are involved in regulation of hepatic blood flow and are expressed in sinusoidal and endothelial cells of hepatic arteries and portal veins[7,86,100] (Fig. 4). This is consistent with the observation that Cx43 expression is likely to be absent during the resting state but induced during endothelial dysfunction.[101] It is noteworthy that the expression of Cx43 in stellate cells is increased in parallel with its activation, while its blockade inhibits propagated contraction in response to calcium.[7,102] Experiments conducted in our laboratory showed that blocking GJs increases portal perfusion pressure and reduces vasodilatory response to acetylcholine.[86] The mechanism underlying this observation is not clear but the data suggests this may be modulated by Cx-mediated NO release.[103]

Decreased endothelial NO synthase activity in the liver may also be due to upregulation of caveolin-1.[104] Interestingly, a strong association of Cx40 and Cx43 with caveolin-1 has been identified in endothelial and epidermal cells.[105] It is possible that Cx43 expression is implicated in caveolin-1 overexpression in cirrhotic livers. Shear stress is a potent inducer of NO production and its relationship with GJs has been evaluated. Shear stress promoted Cx43 expression in endothelial cells.[106] Although increased expression of Cx43 seems not to be limited to the sinusoidal liver cells, it is possible that the induction of Cx43 expression seen during cirrhosis is a compensatory mechanism to favour the transfer of molecules in response to shear stress. By contrast, changes in the Cx37 expression pattern caused by shear stress are less clear.[107] Interestingly, Kruppel-like factor 2 (KLF2), which is activated after induction of shear stress and upregulates eNOS, has been suggested to regulate Cx37 expression. Indeed, shear stress induced Cx37 expression was abrogated following KLF2 suppression, suggesting that KLF2 acts as a transcription factor for Cx37. Here again, the association of a relevant NO promoter such as KLF2 with Cxs suggests a role for GJs in the regulation of vascular tone.

In cirrhosis, following an increase in intrahepatic resistance, a progressive cascade of events leads to splanchnic and peripheral vasodilation. Sodium retention and volume expansion increases cardiac output that in turn contributes to the development of ascites, circulatory dysfunction and renal failure.[108] In opposition to the hepatic circulation, systemic NO is elevated. In addition to NO, other factors have also been hypothesized to participate in arterial vasodilation, such as the endothelium-derived hyperpolarizing factors[109] and more specifically epoxyeicosatrienoic acids.[110] GJs have been described as being fundamental in conducting hyperpolarization directly from the endothelium to vascular smooth muscle cells in the arteries.[110,111] In small resistance mesenteric arteries of cirrhotic rats, inhibiting epoxyeicosatrienoic acids promoted vasoconstriction, an effect that was shown to be independent of NO and prostaglandin, as it was still observed after their inhibition.[112] However, the effect of epoxyeicosatrienoic acids was blunted following pretreatment with a GJ blocker, suggesting that epoxyeicosatrienoic acids may initiate a hyperpolarizing response that is conducted to vascular smooth muscle cells by myoendothelial GJs with consequent vasorelaxation. NO is also responsible for improving Cx43 communication between endothelial and myoendothelial cells. This is because of its ability to nitrosylate proteins, thus modifying protein function.[113] Indeed, NO has been shown to s-nitrosylate Cx43 channels.[114]

Hepatic encephalopathy

Hepatic encephalopathy (HE) is an important neuropsychiatric complication that is associated with end-stage liver disease and has a multifactorial pathogenesis. Work from our laboratory recently demonstrated that Cx-hemichannel functionality, and consequently lactate transport, was impaired in the cerebral cortices of bile duct ligated rats with mild HE.[115] While the expression of the main

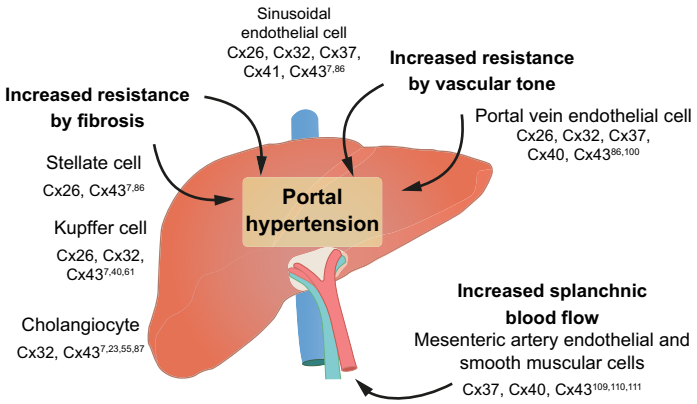


Fig. 4. Role of connexins in portal hypertension in cirrhosis. Different cells in the liver participate in fibrosis and vascular tone, contributing to increased intrahepatic resistance. Cxs participate in arterial vasodilation by conducting hyperpolarization directly from endothelium to vascular smooth muscular cell in the arteries. Cx, connexin. (See above-mentioned references for further information.)

Key point

The functions of gap junctions and hemichannels are amenable to modification with drugs, making them attractive therapeutic targets.

astrocytic and neuronal Cxs was unaffected, the results of this study suggest that HE is associated with impairment of hemichannel functionality in the central nervous system, with ammonia playing a key role. The data supporting Cx-hemichannel dysfunction provide evidence of a possible mechanism underlying the pathogenesis of HE, involving a potential neuronal energy deficit due to impaired hemichannel-mediated lactate transport between astrocytes and neurons.

Cholestatic disease

GJs are involved in bile secretion and regulation of bile flow,[116–119] and any alteration in intercellular transmission of secondary messengers might be expected to result in cholestasis. After bile duct ligation, GJ expression was decreased.[120,121] This was associated with a marked reduction in protein levels of Cx26 and Cx32,[23,55,87,88] which seems to be related to the associated inflammatory response.[55] In addition, an increase in cholestatic bile acids such as taurolithocholate, taurolithocholate-sulfate and taurochenodeoxycholate promotes the closed state of GJs and worsens intercellular communication, making cholestasis worse.[122]

However, the expression of Cx43 increases following bile duct ligation[55,87] and after the development of cirrhosis.[23,86] The protein expression of Cx43 was further increased following LPS challenge and reduced following treatment with anti-TNF drugs.[23] These data suggest that the activation and infiltration of macrophages contribute to this adaptive response, which involves the in the synthesis and recycling of Cx43.[55]

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease comprises a complex disease spectrum, including hepatic steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis and eventually HCC. Intracellular signalling cascades favour the deposition of fat in hepatocytes and induce inflammation.[123] Since GJs can modulate the transfer of molecules, Cxs potentially have an important role in NASH. Cx32 knockout rats with diet induced non-alcoholic fatty liver disease developed more pronounced oxidative stress, inflammation and liver injury than wild-type controls,[124,125] suggesting that GJ plays a protective role by maintaining homeostasis through cell-to-cell communication. However, using specific peptides to block Cx hemichannels decreased triglycerides, cholesterol, and inflammatory markers compared to controls, in animals fed a high-fat diet.[126] This apparently paradoxical observation may be explained by the fact that hemichannels are constitutively closed and open after a pathological stimulus contrary to Cx forming GJs. During injury different deleterious molecules are exchanged between the extracellular and intracellular environment of cells, so blocking hemichannels may be responsible for the beneficial phenotype reported in these studies. In keeping with this study, genetically modified obese rats treated with carbenoxolone, a non-specific blocker of Cx, had decreased liver steatosis, along with a significantly decreased body fat percentage, hypertriglyceridemia, hypercholesterolemia and insulin resistance.[127]

Pannexins, which form channels connecting cells with the extracellular environment, have also been studied in the setting of NASH.[128] When open these channels participate in inflammatory processes.[129] A decrease in lobular inflammation and oxidative stress was observed in mice with pannexin deletion. However, in this study a different gene expression profile was observed in pannexin deficient animals, particularly affecting lipid metabolism and genes involved in the inflammatory and oxidative stress response, suggesting that more experiments focussed on specifically blocking pannexins without modifying gene expression need to be performed. Interestingly, at

a cellular level, pannexins contribute to ATP release, which functions as a pro-inflammatory signal for recruitment and activation of inflammatory cells in lipoapoptosis.[130] Overall, these studies suggest that improving GJ permeability, or blocking hemichannels either constituted by Cxs or pannexins may represent relevant therapeutic targets.

Hepatocellular carcinoma

The ability of GJs to regulate cellular proliferation[131,132] supports the idea that these channels could be involved in cancer pathophysiology. In addition, there is evidence based on experimental studies suggesting a possible role of GJs in liver carcinogenesis. Targeted disruption of the Cx32 gene was associated with an increase in hepatic tumours, possibly because of a reduction in the propagation of apoptotic signals to adjacent cells.[133–135] In keeping with this and further supporting the idea that Cxs may show tumour suppressive properties, both Cx26 and Cx32 expression are decreased in HCC while a mislocalization (and dysfunction) of the Cxs from the cell membrane to the cytoplasm has also been observed.[136–140] In HCC tissues, a reduction in the expression of Cx32 was associated with larger more aggressive tumours, vascular invasion, and poorer survival. Thus, experiments exploring the potential benefit of GJ opening drugs should be explored. Concerning this observation, doxorubicin resistant HCC cell lines showed reduced expression of Cx32. By contrast, overexpression of Cx32 resulted in increased sensitivity of HCC cells to the chemotherapy drug, supporting the hypothesis that Cx32 could be also an important target for counteracting drug resistance of HCC.[141,142] More recently, sorafenib, an oral multikinase inhibitor approved for advanced HCC, was shown to be more efficacious after increasing GJ intercellular communication with all-trans retinoic acid. This effect was abolished after co-incubation with GJ inhibitor 18-alpha glycyrrhetinic acid and oleamide.[42,142]

In an apparent contradiction, the observation that Cx43 expression is increased in HCC cells suggests that Cx43 may possibly have oncogenic properties instead of suppressing tumorigenesis.[143–146] In fact, the magnitude of expression of Cx43 and its localization correlated with the malignant potential,[147] migration, invasive capacity and metastatic ability of HCC.[148] However, an alternative explanation could be that the increased expression of Cx43 is a compensatory response to mislocalization of Cx43 as has been postulated to occur in breast cancer.[149] Additional studies are needed to elucidate the exact role of Cx43 in hepatocarcinogenesis.

Conclusions

In conclusion, there is accumulating evidence that GJs have important functions related to cell-to-cell communication and that they contribute to tissue homeostasis. These functions have relevant consequences for the liver's tolerance to acute injury as well as chronic insult, such as that observed in cirrhosis. It is clear that Cxs are expressed in multiple cell types and have distinct or even opposing roles depending on the liver cell type studied and type of constituted channel. Different Cx subtypes are both downregulated and upregulated in many liver disease conditions. This may form the basis for new therapeutic strategies focussed on specifically limiting or improving the traffic of messengers. However, more research is needed to elucidate the exact molecular mechanisms involved in order to exploit this pathway for the treatment of liver diseases.

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Conflict of interest

Rajiv Jalan has research collaborations with Takeda, Ocera, and Yaqrit, and consults with Yaqrit. Rajiv Jalan is the founder of Yaqrit Limited, which is developing UCL inventions for treatment of patients with cirrhosis. Rajiv Jalan is an inventor of ornithine phenylacetate, which was licensed by UCL to Mallinkrodt Pharma. He is also the inventor of Yaq-001, DIALIVE and Yaq-005, the patents for which have been licensed by UCL into a spinout company, Yaqrit Ltd. The other authors declare that they have no conflict of interest. Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

MHG and RJ contributed in the concept and design of the manuscript; MHG and AH written the article.

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Supplementary data

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Author names in bold designate shared co-first authorship

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