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Over-activated hemichannels: A possible therapeutic target for human diseases

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ABSTRACT

In our body, all the cells are constantly sharing chemical and electrical information with other cells. This intercellular communication allows them to respond in a concerted way to changes in the extracellular milieu. Connexins are transmembrane proteins that have the particularity of forming two types of channels; hemichannels and gap junction channels. Under normal conditions, hemichannels allow the controlled release of signaling molecules to the extracellular milieu. However, under certain pathological conditions, over-activated hemichannels can induce and/or exacerbate symptoms. In the last decade, great efforts have been put into developing new tools that can modulate these over-activated hemichannels. Small molecules, antibodies and mimetic peptides have shown a potential for the treatment of human diseases. In this review, we summarize recent findings in the field of hemichannel modulation via specific tools, and how these tools could improve patient outcome in certain pathological conditions.

1. Introduction

Cells in our body are in constant chemical and electrical communication with neighboring cells [1]. This intercellular communication allows them to respond in a concerted way to changes in the extracellular milieu. A key player in the exchange of information between cells is a family of proteins known as connexins. There are 20 genes coding for different types of connexins in humans [2], and are named according to their molecular weight (e.g. connexin 43 is 43 kDa). Connexins are transmembrane proteins located mainly at the plasma membrane [3], although they have also been found in organelles [4] such as mitochondria [5,6], as well as in the cell nucleus, where they can modulate gene expression [7,8] (Fig. 1). Connexins have the particularity of forming two types of channels, hemichannels and gap junction channels. Notably, the cells use these two types of channels in different ways; while hemichannels allow the release of signaling molecules to the extracellular milieu, such as ATP [9] and glutamate [10], gap junction channels allow the exchange of ions and second messengers between cells in direct physical contact [11]. Interestingly, an old paradigm claiming that connexin-based channels participate only in short-range cell communication is changing, as recent data suggests that connexins (particularly connexin 43 and connexin 46) are involved in long distance vesicle-dependent "transfer of information" [12,13]. The following sections will address the role of hemichannels in physiological processes; the consequences of hemichannels with altered open probability (hereafter referred as "over-activated hemichannels") on cell survival; and how hemichannel modulation could be beneficial for the treatment of some human diseases.

2. Connexin hemichannels

Hemichannels are formed by the oligomerization of six connexin subunits [14,15] in the endoplasmic reticulum and the trans Golgi network [16–18]. Once hemichannels are formed, they are transported in vesicles from the Golgi apparatus to the plasma membrane, via an actin-dependent pathway [19]. Hemichannels show a large central pore, whose diameter can be different depending on the connexin that forms them. For example connexin 31.1 hemichannels have a diameter of ~8 Å which was determined by Cryo-EM [14], while connexin 26 hemichannels have a diameter of ~10 Å estimated by molecular dynamics [20] and ~14 Å by crystallography [21]. This pore size is large enough to allow the release of signaling molecules such as ATP [9], glutamate

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[10], prostaglandin E2 [22], NAD^+ [23] and reduced glutathione (GSH) [24] as well as the uptake of glucose [25], Ca^{2+} [26,27] and Na^+ [28,29].

2.1. Regulation of connexin hemichannels

Because ions and molecules that can pass through hemichannels are critical for metabolic and intercellular signaling processes, the opening of these channels is tightly regulated by a number of different mechanisms. For example, it is well known that negative membrane potentials and extracellular divalent cations like Ca^{2+} and Mg^{2+} (in mM range) allosterically stabilize connexin 46 hemichannels in its close state [30]. Similarly, posttranslational modifications such as phosphorylation [31,32], carbonylation [33], and S-nitrosylation [34–38] also have shown an effect on hemichannel open probability. In this regard, our group has suggested that the final effect of a given posttranslational modification induced by redox potential changes (i.e. carbonylation and S-nitrosylation) could be mediated by different cysteine residues of the connexin protein [39,40]. In addition to these mechanisms, protein-protein interactions can also modulate hemichannel opening/closing. For example, an increase in intracellular free Ca^{2+} concentration activates Ca^{2+}-calmodulin (CaM) [41], which in turn, can interact with connexin 32 [42], connexin 43 [43], connexin 46 [44] and connexin 50 [45] hemichannels, leading to an increase in their open probability. Additionally, other mechanisms for hemichannel regulation, such as interactions with CO_2 [46,47] and changes in intracellular and extracellular pH [26,48], have been proposed. In summary, hemichannels under physiological conditions are controlled by a myriad of molecular mechanisms, which keep hemichannels mostly in a close state. Despite the low open probability of hemichannels, they have several roles in many physiological processes, some of which are presented in the next section.

2.2. Physiological functions of hemichannels

The presence of hemichannels at the plasma membrane in different cell types has been demonstrated by the use of antibodies [49–51], electrophysiological recordings [52,53], cell surface biotinylation [34,54], fluorescent microscopy [55], atomic force microscopy [15,56,57], dye uptake [33,58–60], and mimetic peptides [61]. As mentioned earlier, hemichannels are permeable to large molecules and ions, which is why, for several years, it was believed that hemichannels under physiological conditions should be in a constant close state to prevent cell death due to overload. However, in the nineties, it was found that connexin 43 hemichannels in Novikoff cells allowed the uptake of 5(6)-carboxyfluorescein when the extracellular Ca^{2+} concentration was reduced [62,63]. In addition, in HeLa cells, hemichannels were shown to open in response to membrane depolarizations [52] and by dephosphorylation when they were present in proteoliposomes [64]. This evidence indicates that the hemichannel opening can be controlled by several factors, many of which can be found under physiological conditions. For example, in rat cancer-derived epithelial cells (BICR–M1Rk), connexin 43 hemichannels participate in their cell volume regulation associated with small changes in extracellular Ca^{2+} concentration [65]. This could be relevant in processes such as cell growth and metabolism [65].

Evidence for a role of hemichannels under physiological conditions is not limited to in vitro studies. In fact, in vivo and ex vivo studies have demonstrated that opening of connexin 43 hemichannels present in astrocytes located in the basolateral amygdala are fundamental for memory consolidation [66], likely through the release of gliotransmitters [10,66] into synapses. Similarly, astroglial connexin 43 hemichannels have been shown to increase their activity after acute and chronic restraint stress in mice, associated to an increase in glutamate and ATP release [67]. Moreover, astroglial connexin 43 hemichannels

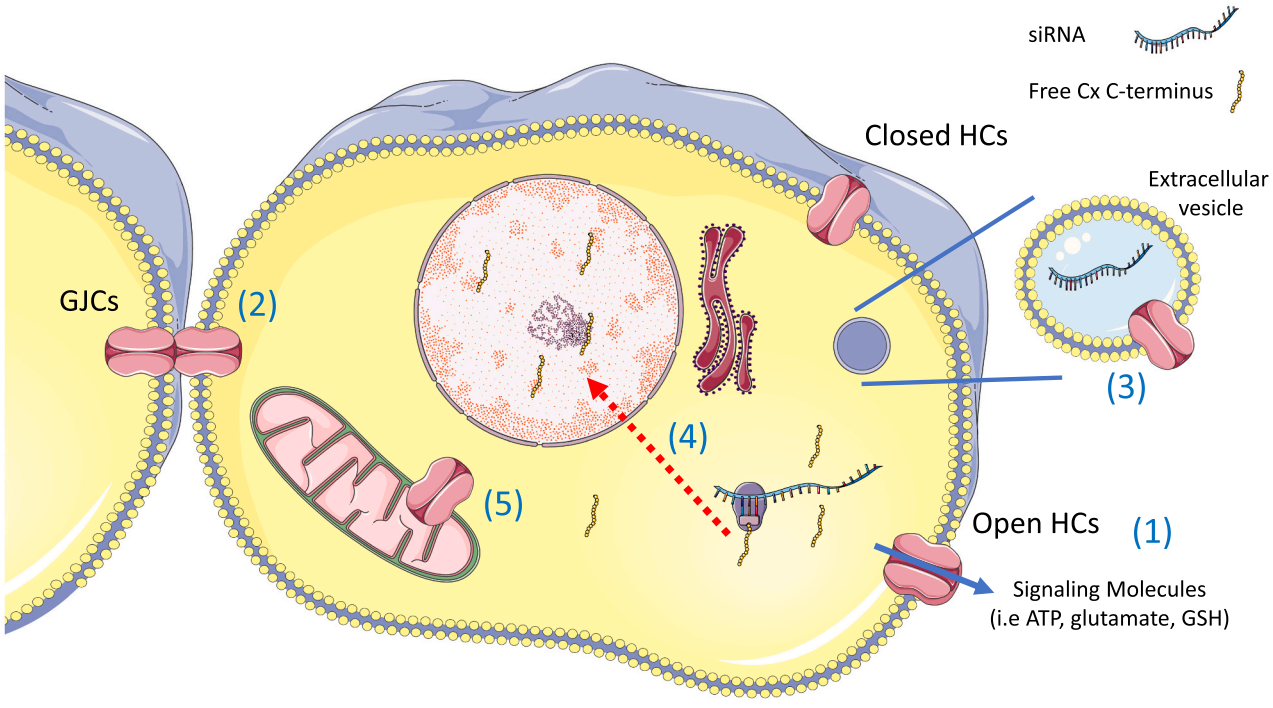

Fig. 1. - Role of connexins in cellular function and intercellular communication. Once connexins are formed, they oligomerize in hexamers known as hemichannels located at the plasma membrane, and when opened, they allow the release of signaling molecules to the extracellular milieu, where they can act as auto/paracrine molecules (1). Some hemichannels are located in areas of the membrane where two cells are in contact and dock to form a gap junction channel (2). These channels allow the exchange of metabolites, ions and second messengers between the cytoplasm of those two cells in contact. Additionally, it has been suggested that hemichannels could also be presents in the membrane of exosomes (3), where, they could modulate the siRNAs content and the interaction between the exosome and receptor cell. Hemichannels have also been found in the mitochondria (4), where they control K^+ movement. Finally, the C-terminal of some connexins can be translated separately from the rest of the protein, and this peptide can interact with other proteins both in the cytoplasm and in the cell nucleus (5).

have also been shown to regulate NMDA receptor-dependent synaptic plasticity in the prefrontal cortex [68], while in the hippocampus, augmented neuronal activity increases astroglial connexin 30 expression and hemichannel opening [69]. Other connexins have also been studied. For example, connexin 26 hemichannels present in horizontal cells in the retina participate in light processing, modulating glutamate release from cones [70,71], while astrocytes in the brainstem participate in the control of breathing by modulating the astroglial release of ATP [72]. Connexin hemichannels have been reported to participate in many other physiological processes, including control of fibroblast proliferation through a CD38/cADPR-mediated mechanism [23,73,74], Na^+ influx in the lens cells through connexin 46 hemichannels [28] and transport of glucose and GSH through connexin 50 hemichannels, which may contribute to maintaining metabolic and antioxidative functions in the outer cortical lens cells [75].

Additionally, our group has suggested that connexin 43 hemichannels could have greater open probability in vivo than in vitro because the in vivo partial oxygen pressure is lower (~10%) compared to that observed in vitro (~21%), and connexin 43 hemichannels seem to increase their opening state in "less oxidative" environments [55]. As mentioned, connexin 43 hemichannels also open in response to oxidative stress in both astrocytes [34] and skeletal muscle cells [76], which seem to be correlated with changes in the phosphorylation level of this connexin [38,55]. In summary, all these data suggest that hemichannels under physiological conditions show a controlled opening, which allows them to participate in important cellular processes.

2.3. Diseases related to overactivation of hemichannels

In 1991, Paul and co-workers found that 16 h after the injection of rat connexin 46 mRNA into *Xenopus laevis* oocytes, they showed large outward currents, a prominent membrane depolarization, and an evident increase in Lucifer yellow uptake. After 24 h, all those frog eggs were dead [77]. Similarly, the overexpression of bovine connexin 44 (which is very similar to rat connexin 46) on *Xenopus* oocytes induced membrane depolarization and cell lysis [78]. These initial findings suggested that an excessive hemichannel activity at the plasma membrane could lead to the cell malfunctioning and even cell death (Fig. 2). Currently, in many different human diseases, an increased hemichannel opening has been observed [79]. Probably one of the first studies connecting a "pathological condition" with an enhanced hemichannel opening was conducted by John et al., in 1999 [80]. In this work, HEK293 cells that were under metabolic inhibition with FCCP plus iodoacetic acid, showed a significant increase in connexin 43 hemichannel opening. Similar results were observed in cardiomyocytes subjected to simulated ischemia [81]. Connexin 43 hemichannel opening induced by metabolic inhibition generated an excessive overload of intracellular Ca^{2+} and Na^+ [80,82], which led to increased cell death.

Later, it was demonstrated that the connexin 43 hemichannel opening in astrocytes under metabolic stress is mediated by a combination of hemichannel dephosphorylation and S-nitrosylation [34]. Several other studies have demonstrated that connexin hemichannel posttranslational modifications can regulate hemichannel opening. For example, in connexin 46 hemichannels, S-nitrosylation induces hemichannel opening [36,83], while carbonylation can induce their closure [33]. It must be noted however, that posttranslational modifications of connexin, such as phosphorylation in specific residues in the connexin protein, may not only change their opening probability, but may also change their selectivity by changing the pore size. Studies using proteoliposomes have shown that connexin 43 hemichannel permeability can be affected by phosphorylation of specific residues of the connexin protein by PKC [64,84], or by S-nitrosylation [35].

Inflammatory processes can also induce hemichannel opening. For example, an increase in FGF-1 levels induced by a spinal cord trauma enhances connexin 43 hemichannel opening in astrocytes, a phenomenon associated to a rise in ATP release and the subsequent activation of surrounding astrocytes [85]. Similarly, in cultured mouse astrocytes exposed to conditioned media from activated microglia with LPS or exposed to IL1-β plus TNF-α, an increase in their ethidium uptake and in the number of ~220 pS events was observed, both phenomena being congruent with the opening of connexin 43 hemichannels [25]. Interestingly, the same work also reported a decrease in gap junction

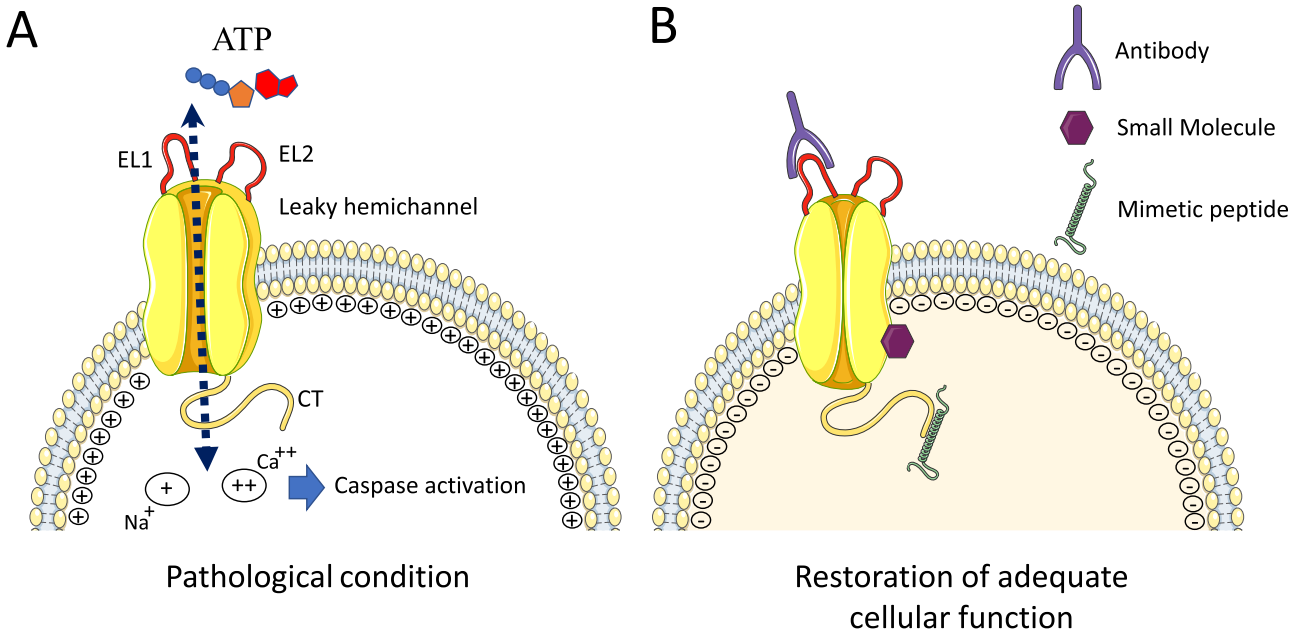

Fig. 2. - Effect of specific hemichannel inhibitors on cell function. (A) In pathological conditions where the over-activation of hemichannels occurs, a massive release of metabolites such as ATP, and the uncontrolled entry of Na^+ and Ca^{2+} to the cytoplasm can occur. The latter can cause depolarization of the plasma membrane as well as the activation of caspases. Altogether they can lead to cell malfunction and in extreme cases, their death. (B) The use of specific inhibitors such as antibodies, small molecules and mimetic peptides, could recduce the activity of the over-activated hemichannels, with the consequent reduction in symptoms of some pathologies.

channel-mediated communication between astrocytes. In another study, after 3 days of *Staphylococcus aureus* infection in the mice striatum, decreased gap junction-mediated communication and increased hemichannel activity in astrocytes near the primary inflammatory site were observed [86]. In another study, the exposure of pregnant mice to LPS rendered adult offspring with astrocytes showing higher connexin 43 hemichannel and Pannexin1 channel activity. This enhanced hemichannel activity was associated to a microglia-dependent production of IL-1β/TNF-α and the activation of p38 MAP kinase/iNOS/$[Ca^{2+}]_i$-dependent pathways [87]. The authors of this work suggested that their results could help to explain why human mothers exposed to infectious diseases during pregnancy give birth to children with a greater predisposition to generate neurological problems, such as schizophrenia, autism and cerebral palsy [87,88].

The immune system is so diverse and complex that it is difficult to identify the specific molecular mechanisms behind the possible relationship between hemichannel opening and inflammation. However several efforts have been made to understand such relationship [89–92]. For example, in the CNS, IL-1 increases hemichannel opening, possibly via a MyD88 (myeloid differentiation primary response gene 88) and TLR2 (Toll-like receptor 2) dependent pathways [93], while IL1-β plus TNF-α increase astroglial hemichannel opening via a NO and p38 MAPK dependent pathway [25], and potentially, via a rise in intracellular Ca^{2+} [94]. The same mix of cytokines in endothelial cells also induces cell permeabilization through hemichannels, but via a p38 MAPK, iNOS, COX2, PGE2 receptor, and P2X7/P2Y1 dependent pathways [95]. FGF-1 also induces connexin 43 hemichannel opening in spinal astrocytes, via a Ca^{2+} and phospholipase C dependent pathway [85]. Finally, Angiotensin II enhances hemichannel opening in mesangial cells, via a RhoA/ROCK dependent pathway [96].

3. Diseases associated to over-activated hemichannels

Not only metabolic changes or inflammatory conditions can induce hemichannel opening. It is very well known that some genetic alterations in connexin genes are responsible for over-activated

Table 1
Table showing a compendium of studies using different specific tools against channels formed by connexins and their effect on human disease models.

Tool	Name	Tissue/pathology	Directed against	Model	Possible clinical use	Ref
Antibody	MAbE2Cx43	Brain/glioma	Cx43	Rats	Coadjuvant	[110]
	MAbE2Cx43	Brain/glioma	Cx43	Rats	Coadjuvant	[111]
	Cx43(E2)	Brain/glioma	Cx43	Rats	Cancer cell labelling	[109]
	Cx43(E2)	Breast cancer metastasis	Cx43	Cell Culture	Decrease metastasis	[112]
	abEC1.1	Skin/KID syndrome	Cx26	Organotipic culture	Decrease symptoms	[115]
	abEC1.1	Skin/Clouston syndrome	Cx30	Mice	Decrease symptoms	[117]
Small molecules	Amphiphilic aminoglucosides	–	Cx26/Cx43/Cx46	*E. coli*	Skin and inner ear disorders and cancer	[134]
Mimetic peptides	Gap19	Heart/ischemia-reperfusion	Cx43	Mice	Decrease tissue damage	[153]
	Gap26	Heart/ischemia-reperfusion	Cx43	Cell culture	Decrease tissue damage	[151]
	Gap26	Heart/ischemia-reperfusion	Cx43	Ex vivo/Rats	Decrease tissue damage	[154]
	Gap27	Heart/heart failure	Cx43	Rats	Restore heart function/decrease arrythmias	[162]
	TAT-Gap19	Heart/heart failure	Cx43	Mice	Restore heart function/decrease arrythmias	[163]
	Gap19 and Gap26	Heart/heart failure	Cx43	Mice	Restore heart function/decrease arrythmias	[164]
	Peptide 5	Kidney/tubulointerstitial fibrosis	Cx43	Cell culture	Treatment for kidney dysfunction	[168]
	40GAP27	Lung/acute respiratory distress	Cx40	Mice	Restore lung vascular barrier failure	[170]
	Peptide 5	Lung/acute respiratory distress	Cx43	Mice	Decrease lung inflammation	[169]
	Gap19	Brain/food intake disorders	Cx43	Mice	Increase appetite control	[173]
	Gap19	Brain/ischemia-reperfusion	Cx43	Mice	Decrease tissue damage	[178]
	Peptide 5	Brain/ischemia-reperfusion	Cx43	Sheep	Decrease tissue damage	[174]
	Peptide 5	Brain/ischemia-reperfusion	Cx43	Sheep	Decrease tissue damage	[175]
	Peptide 5	Brain/ischemia-reperfusion	Cx43	Rats	Decrease tissue damage	[177]
	Gap26	Brain/ischemia-reperfusion	Cx43	Rats	Decrease tissue damage	[176]
	TAT-Gap19	Brain/ischemia-reperfusion	Cx44	Mie	Decrease tissue damage	[179]
	Gap19	Brain/hemorrhage	Cx43	Mice	Decrease neurological symptoms	[180]
	Gap19	Brain/epilepsy	Cx43	Mice	Anticonvulsant	[183]
	Peptide 5	Brain/epilepsy	Cx43	Ex vivo/Rats	Decrease neuronal loss	[182]
	Peptide 5	CNS/spinal cord injury	Cx43	Rats	Restore locomotor function	[186]
	Peptide 5	CNS/spinal cord injury	Cx43	Rats	Restore locomotor function	[187]
	Peptide 5	CNS/spinal cord injury	Cx43	Mice	Decrease chronic pain	[188]
	Gap26	PNS/orofacial pain	Cx43	Rats	Decrease chronic pain	[189]
	Peptide 5	Eye/retinal injury	Cx43	Rats	Retina protection from light-induced injury	[193]
	Peptide 5	Eye/retinal injury	Cx43	Rats	Retina protection from ischemia-induced injury	[194]
	Peptide 5	Eye/retinal injury	Cx43	Rats	Retina protection from ischemia-induced injury	[195]
	ACT1	Skin/wound	Cx43	Mice and Pig	Improve wound healing	[203]
	ACT1	Skin/wound	Cx43	Human	Improve diabetic wound healing	[206]
	Gap27	Skin/wound	Cx43	Cell culture	Improve wound healing	[204]
	Gap27	Skin/wound	Cx43	Ex vivo/human	Improve wound healing	[205]
	ACT1	Cornea/wound	Cx43	Rat	Improve diabetic wound healing	[210]
	Gap27	Cornea/wound	Cx43	Ex vivo/human	Improve diabetic wound healing	[209]
	ACT1	Silicon implants	Cx43	Rat	Improve postoperative healing after implants	[211]

hemichannels (in the case, the term of leaky hemichannels also has been used) that show high basal activity in conditions in which they should keep a closed state. These over-activated hemichannels are associated to the initiation and/or progression of some human diseases. For example, the mutation F235C in connexin 32 induces the appearance of over-activated hemichannels in Schwann cells, and their leaky activity is associated with symptoms of the severe X-linked Charcot-Marie-Tooth disease [97]. Similarly, the mutation G45E in connexin 26, which is associated to deafness, produces over-activated hemichannels and cell death when transfected in HEK cells [98]. In the eye, connexin 46G143R and connexin 50V44A mutations are associated to over-activated hemichannels and cataract development [99,100]. There are many other studies demonstrating that several human diseases are associated to over-activated hemichannel activity [79]. No matter whether they are congenital or acquired diseases, the inhibition of over-activated hemichannels could be an important step to reduce ailments in patients suffering from diseases associated to over-activated hemichannels.

4. Modulation of over-activated hemichannels, a promising new way to treat human diseases

In the last decade, the scientific community has recognized that hemichannels with a higher activity than normal, could become therapeutic targets to help reduce the symptoms of certain diseases, such as those mentioned in the previous chapters. The following strategies have been developed to selectively decrease hemichannel activity for specific connexins (Table 1).

4.1. Antibodies

For decades antibodies against connexins were used to study their localization (immunofluorescence, immunohistochemistry, electron microscopy, etc.) and its quantitative expression (Western blots). It is well accepted that connexin extracellular loops participate in gap junction channel formation [101–103]. Therefore, it was not a surprise when the use of antibodies directed against the first or second extracellular loops of connexin 32 or connexin 43, reduced in ~90% the dye transfer between Novikoff cells [104]. This result opened an opportunity to develop antibodies directed against Connexin- extracellular loops and use them as specific tools to inhibit gap junction channel-mediated intercellular communication; however, it was rapidly realized that these antibodies also blocked hemichannels. Thus, antibodies against connexin 43 extracellular loop one and two, reduced Lucifer yellow (LY) uptake in a cell line derived from astrocytes [49] and reduced Ca^{2+} release from the endoplasmatic reticulum [105]. In 2009, the connexin 43(E2) antibody, which is directed against amino acids 173–208 of the connexin 43 protein, was developed, and it was demonstrated to block connexin 43 hemichannels in C6 cells [106], which was later shown not to affect gap junction channel-mediated dye transfer in MLO-Y4 cells after a 4 h incubation period [107]. However, the possibility that it affects gap junction channel-mediated cell communication after longer periods of incubation cannot be readily ruled out. In 2013, it was reported that osteocyte-like cells (MLO-Y4) placed in simulated microgravity opened their connexin 43 hemichannels and released prostaglandin E2 (PGE2), which was decreased by the connexin 43(E2) antibody. Additionally, they showed that the connexin 43(E2) antibody exacerbated cell death of MLO-Y4 cells exposed to H_2O_2 [108]. This was unexpected since it was well accepted that the uncontrolled opening of hemichannels is correlated with cell death [79]. On the other hand, this result, despite being surprising, could suggest that in some cases, hemichannel opening could be a way to release toxic molecules, and therefore, their closure could concentrate these toxic molecules (i.e. ROS, RNS) in the cytoplasm, leading to cell death. A similar effect was observed in the rat lens, where the lipid peroxide 4-hydroxynonenal decreased connexin 46 hemichannel opening, leading to cataract formation [33]. This is relevant, as it implies that connexin hemichannel activity can be modulated to either increase or decrease, depending on the cell type and the subjacent pathology.

In an interesting turn of events, Baklaushev and colleagues injected intravenously the connexin 43(E2) antibody conjugated with Alexa fluor into rats with glioblastoma. After 72 h post injection, the antibody was located at the edge of a brain tumor, suggesting that invasive-like glioma cells have in their surface connexin 43 hemichannels [109]. Because it seemed that this antibody was binding to malignant cells, it was used in combination with temozolomide, or with fractionated γ-irradiation. The use of radiotherapy plus the connexin 43(E2) antibody (known later as MAbE2Cx43) resulted in the prolongation of life expectancy to almost double, as compared to treatments without the antibody in animals with glioblastoma [110]. Conversely, the combination of MAbE2Cx43 and temozolomide resulted in a reduction of life expectancy.

Taking advantage of the opportunity that connexin 43(E2) antibodies offer by "tagging" glioma cells, a cisplatin-loaded nanogel conjugated with monoclonal antibodies against connexin 43 was recently created, and induced an increase of the life expectancy to almost double that of the controls [111]. The original connexin 43(E2) antibody was directed against residues 173–208 of the connexin 43 protein, but overtime, the targeted sequence has been reduced to aminoacids 186–206. The antibody connexin 43(E2) targeting the shorter version of the rabbit connexin 43(E2) reduced cellular interactions between normal cells and MCF-7 cells in a non-adhesive agarose gel [112]. Similarly, connexin 43 hemichannel opening in MLO-Y4 cells induced ATP release, which inhibited the metastasis of breast cancer cells (MDA-MB-23) into the bone. The antibody connexin 43(E2) (directed against amino acids 185–206) reduced the degree of inhibition, thus enhancing the MDA-MB-23 aggressive characteristics [113]. The above data suggests that the use of antibodies should be taken carefully, as depending on the model, they may be considered antimetastatic or pro metastatic agents. It appears that the effects of modulating connexin 43 hemichannel activity depends, first, on the balance between gap junction channels and hemichannels, as they may have completely different (and even opposing roles), and second, on whether the main role of connexin hemichannels in a particular cell type under diseased conditions is to allow the release of molecules (e.g. ATP or toxic metabolites), or to increase the influx of molecules into the cell (e.g. calcium or sodium).

There are several connexin 26 and connexin 30 gene mutations that cause skin and inner ear disorders in humans, and some of them are associated to over-activated hemichannel activity [79,114]. For example, there are several mutations in the connexin 26 gene that produce Keratitis-ichthyosis-deafness (KID) syndrome in humans [114]. In 2017, an antibody against residues 41–56 of connexin 26- first extracellular loop known as abEC1.1, was designed. This antibody was shown to decrease the activity of hemichannels formed by both wild type and the mutated connexin 26 that produces KID syndrome, in HeLa cells, in a human skin keratinocyte cell line (HaCaT cells) and in a mouse organotypic cochlear model [115]. This antibody also inhibited connexin 30 hemichannel activity [51]. A congenic defect in the connexin 30 gene is responsible for the Clouston syndrome, which is a rare disease characterized for abnormalities in the skin, hair and nails. The connexin 30 mutation that causes this syndrome (A88V/A88V) produces connexin 30 over-activated hemichannels [116]. The systemic injection of abEC1.1 drastically inhibited connexin 30 hemichannel activity in vitro [117], and decreased cell hyperproliferation and the size of sebaceous glands to control levels in a mice model of Clouston syndrome [117]. We would like to note that, even though these results suggest that antibodies directed against extracellular loops of connexin 26 and connexin 30 can exert their effects through the inhibition of hemichannels, an effect on gap junction channels activity cannot be ruled out, because these antibodies could potentially interfere with the formation of gap junction channels, and thus decrease the coupling between cells.

4.1.1. Difficulties to solve

Antibodies against connexin extracellular loops can inhibit over-

activated hemichannel activity, but they can also affect gap junction channel-mediated communication. As far as we know, in vivo studies using connexin antibodies have not reported side effects attributable to gap junction channel blockade, but this issue must be methodically studied to corroborate the safety of the use of these antibodies in animal models and later in humans. Although, although the antibodies in question were designed against connexins, there is still very limited literature on their mode of action [118]. Furthermore, it is necessary to characterize the role of other connexins in various diseases, and to develop antibodies directed against other connexins different from connexin 43 [118]. In summary, the use connexin-derived antibodies with the potential to decrease the activity of the hemichannels could be promising for the treatment of human diseases. However, the issues of hemichannel vs gap junction channels specificity, mechanism of action and diversity should be addressed.

4.2. Small molecules

For a long time, the pharmacology of connexin-based channels (whether gap junction channels or hemichannels) has been catalogued as poor and unspecific [119]. Connexin blockers of common use in the scientific community include octanol [120,121], heptanol [10], lanthanum [80,122], gadolinium [121,123], chloride channel blockers (i.e. niflumic acid, flufenamic acid and diphenyl-2-carboxylate) [121], IP3 receptor blockers (2-aminoethoxydiphenyl borate) [124], carbenoxolone [10], 18α-glycyrrhetinic acid [10,125], aminosulfonate buffers [126], and quinine derived molecules [127–130]. As can be seen in this short list, none of these blockers is specific and -therefore-, they are not useful to treat a connexin-based disease and least, specifically hemichannel-associated pathologies. Recently, some groups of researchers have tried to develop small molecules with both a certain degree of specificity, and inhibition in a concentration range between nM and few μM. Among these, there are small molecules derived from antibiotic/aminoglycosides [131]. For a long time, it has been known that the use of aminoglycosides against bacterial infections can lead to deafness. In 2014, our group demonstrated that 200 μM gentamycin inhibited connexin 26 hemichannels in HeLa cells [132]. Later in 2016, we observed that gentamycin does not inhibit connexin 26 hemichannels formed by the mutant L10P [133], suggesting that gentamycin can bind directly to connexin 26, in a specific aminoacidic sequence which could be affected by the mutation at that position. Then, it was demonstrated that different aminoglycosides (kanamycin A, kanamycin B, geneticin, neomycin, and paromomycin) inhibit hemichannels formed by several connexins, including connexin 26, connexin 43 or connexin 46, when purified and expressed in *E. coli* [134]. As aminoglycosides cause mammalian cell damage, later on, amphiphilic aminoglycosides without cell toxicity were developed, retaining their ability to block connexin hemichannels [135,136].

Given the importance of finding specific modulators for connexin-based channels, in recent years several new techniques have been developed for high-throughput screening, including an assay based on *E. coli*, expressing purified connexins [137]. This technique is based on *E. coli* designed to be deficient in potassium uptake, and therefore they cannot proliferate. So, if hemichannels are present, *E. coli* can grow because hemichannels are permeable to potassium, and if a connexin inhibitor is present, the bacterial growth stops. Another technique published recently, verifies whether a molecule is an inhibitor of gap junction channels or not. This assay is based on two types of cells, one expressing the Gs protein-coupled adenosine A_{2A} receptor, and the other one acting as a biosensor, expressing a cAMP-sensitive GloSensor luciferase [138]. Therefore, the cAMP produced by the A_{2A} receptor expressing cells can be passed to biosensor cells through gap junction channels. If these channels are closed, no signal will be produced by the luciferase. A third assay measures the effect of small molecules on gap junction channel -mediated communication. In this technique, the diffusion of calcein from a donor to an acceptor cell is used as a measure of gap junction channel-mediated communication. The authors claimed that this assay was efficient enough to find a group of potential connexin 43 gap junction channel inhibitors within a library of 1280 Food and Drug Administration- and European Medicine Agency-approved drugs [139].

4.2.1. Difficulties to solve

Some groups recognize the importance of finding small molecules capable of modulating the activity of channels formed by connexins (both gap junction channels and hemichannels) but designing a molecule with such characteristics, which could be used in patients to treat pathology, is still out of our reach. One important problem in the use small molecules targeting over-activated hemichannels is their selectivity. Small molecules used to reduce hemichannel over-activation should be able to selectively diminish hemichannel activity without affecting the activity or formation of gap junction channels, as blocking of gap junction channels most likely will induce unwanted side effects. Another important point that should be addressed is the selectivity of the small molecules in terms of their capacity to discriminate between hemichannels formed by different connexins. Developing a wide range of small molecules with different selectivity profiles could be useful for evaluating their effects in particular cell types and models of specific diseases. Perhaps the search for small molecules using high throughput screening could help identifying a larger number of small molecules with clinical potential.

4.3. Peptides

In addition to the development of antibodies and small molecules against connexins, mimetic peptides have become a very popular and useful tool to study connexin hemichannels [140]. Originally, these peptides were conceived to mimic certain regions of connexin extracellular loops, with which they supposedly interact [141], and inhibit hemichannel opening [140]. Recently, mimetic peptides against intracellular regions have also been developed, some of which are specific for gap junction channels and others, for hemichannels [142]. There are several reviews discussing the importance of mimetic peptides in the connexin field of research [140,143–146]. We shall focus this section on studies in which peptides were used in models of human's diseases, but for detailed explanations of the peptides, other reviews are recommended [140,143–149].

4.3.1. Heart diseases

Heart failure is caused by several conditions, including coronary artery blockage, hypertension, defective heart valves, cardiomyopathy, and cardiac arrhythmias, among others. Connexin 43 is abundantly expressed in cardiomyocytes, where they mostly form gap junction channels and allow the propagation of action potentials across the myocardium that is essential for cardiac muscle contractions. In recent years, it has been also demonstrated that connexin 43 is expressed inside of the mitochondria in cardiomyocytes, where it seems to have an important role in ischemic preconditioning [150].

When a sudden heart attack induced by a coronary artery occlusion occurs, a massive but transient connexin 43 hemichannel opening has been observed in a model of ischemia in vitro [151], which has been associated to the massive influx of Ca^{2+} and Na^{+} into cardiomyocytes, which can lead to their malfunctioning or even their death [81]. Notably, in myocardial ischemia and reperfusion, oxidative stress induces the closure of connexin 43 gap junctions, while increasing hemichannel activity (reviewed in [152]). Interestingly, both the increased hemichannel activity and the Ca^{2+} intracellular overload induced by the hemichannel opening, can be prevented by Gap26 mimetic peptide (derived from the first extracellular loop of connexin 43, which blocks both connexin 43 hemichannels and gap junction channels) [151]. Similarly, the use of Gap19 (derived from the cytoplasmic loop of connexin 43, which blocks only connexin 43 hemichannels) drastically

reduced cardiomyocyte swelling, cell death in vitro, and infarct size after myocardial ischemia/reperfusion in mice in vivo [153]. Almost identical results were obtained using Gap26 on Langendorff-perfused intact rat hearts after occlusion of the left anterior descendent coronary [154]. In a similar model, the use of a Pannexin 1 mimetic peptide, did not reduce the size of necrotic tissue as Gap26 did [155].

One of the side effects of heart ischemia is the development of arrythmias, which are partially mediated by a decrease of gap junction channel communication between cardiomyocytes [156]. Several peptides that have anti-arrhythmogenic effects, induce an increase in connexin 43 gap junction channel-mediated communication [156]. For example, the peptide AAP10, which increases connexin 43 gap-junctional intercellular coupling, restored the normal spread of action potentials across the myocardium in a model of regional ischemia [157]. Interestingly, AAP10 increases gap junction channel-mediated electrical coupling in HeLa cells transfected with connexin 43 in a PKC dependent manner [158]. All these results strongly support the idea that connexin-mimetic peptides could be used to decrease the damage induced by a heart attack [159].

In heart failure associated with complications, such as hypertension, the membrane hemichannel pool may be increased in cardiomyocytes, producing current leakage and therefore, decrease the efficiency of the propagation of action potentials between cardiomyocytes [160,161]. In this case, mimetic peptides also demonstrated to have a great potential for the treatment of heart failure. For example, in a model of high-output heart failure in adult male rats, the administration via osmotic mini-pumps of the Gap27 peptide, which mimics a sequence of the second extracellular loop of connexin 43 and blocks both connexin 43 gap junction channels and hemichannels, induced better cardiac function, less cardiac arrhythmogenesis and less cardiac hypertrophy [162]. In a recent study, connexin 43 hemichannels were colocalized with RyR2 at microdomains in the intercalated discs between cardiomyocytes in both mice and pigs. Interestingly, they were activated by increases of intracellular calcium concentration ($[Ca^{+2}]_i$) during diastole or by adrenergic activation. The opening of these hemichannels induced the influx of Ca^{2+} to these microdomains, which in turn enhanced even more the $[Ca^{+2}]_i$. In the same study, the opening of connexin 43 hemichannels in cardiomyocytes from human hearts with heart failure, was associated to an electrical instability of these cells. Many of the effects associated to the opening of connexin 43 hemichannels in cardiomyocytes, were decreased by the use of the connexin 43 hemichannel mimetic peptide TAT-Gap19 [163]. These studies strongly suggest that the opening of connexin 43 hemichannels contributes significantly to arrhythmia in patients with heart failure, and therefore, could be a promising therapeutic target [162,163].

Interestingly, in a Duchenne mice model in which isoproterenol administration induces arrhythmias and eventual death of mice after 24 h, the use of Gap19 and Gap26 inhibited both connexin 43 lateralization and development of arrhythmias, and reduced lethality [164]. All these results suggest that connexin 43 lateralization is strongly associated with cardiac arrhythmias and that the inhibition of connexin 43 hemichannels could reduce them, helping to maintain overall cardiac function during heart failure and cardiac arrhythmias.

4.3.2. Nephropathies

Kidney diseases are often associated with sustained inflammation and fibrosis. In human diabetic nephropathy, there is an increase in connexin 26 and connexin 43 expression, which has been associated with in vitro evidence of increased TGFβ1 secretion from tubular cells under high glucose conditions [165]. In an in vitro model of proximal tubular epithelial cell damage, their exposure to TGFβ1 induced an increase of connexin 43 hemichannel activity that was correlated with a rise in ATP release and the activation of the integrin α2β1/integrin-like kinase signaling pathway [166,167]. Application of the connexin 43 hemichannel blocker peptide 5 (mimicking a sequence of the first extracellular loop of connexin 43 [168]) significantly reduced all the changes induced by TGFβ1. Although current evidence is limited to in vitro models, the above studies suggest that connexin 43 mimetic peptides could be used for the treatment of some types of kidney disease.

4.3.3. Lung injury

Acute respiratory distress compromises lung function and is characterized by inflammation and damage to the alveolar epithelial-endothelial barrier. In an in vivo model of acute lung injury, mice under anesthesia were instilled with intratracheal LPS, and an increase in connexin 43 hemichannel opening in alveolar cells was observed. A rise in high mobility group box protein 1 concentration in bronchoalveolar lavage fluid was also observed [169]. All these changes were decreased when peptide 5 was administered. Also the overall survival of animals with respiratory injury increased significantly [169]. Additionally, connexin 40 seems to be involved in the lung vascular barrier failure in LPS- and HCl-induced lung injury [170]. Interestingly, the use of connexin 40-inhibiting mimetic peptide ($^{40}GAP_{27}$) decreased the loss of thrombin-induced trans-endothelial electrical resistance, via a ROCK1 dependent pathway [170].

4.3.4. Central nervous system

4.3.4.1. Food intake disorders. Metabolic syndrome is a major public health problem. This syndrome involves a deregulation of food intake resulting in obesity and diabetes. It is well known that astrocytes modulate synapses in the central nervous system through the release of gliotransmitters [171,172]. Food intake is regulated by several neuronal circuits, being the most important, those located in the hypothalamus. In a recent work, it was demonstrated that in normal animals, astrocytes located in the arcuate nucleus of the hypothalamus show a high basal connexin 43 hemichannel activity and when Gap19 is administered intracerebro-ventricularly, mice decreased their food intake, without changes in glycaemia, energy expenditure or locomotor activity [173]. Therefore, connexin 43 hemichannels could be a target for the development of drugs to treat food intake disorders, such as metabolic syndrome.

4.3.4.2. Stroke. As in the heart, stopping the blood supply to the brain leads to ischemia of the affected brain region and sometimes, to the reperfusion of the same zone. In in vitro models of brain ischemia/reperfusion, it has been observed that connexin 43 hemichannels in astrocytes become permanently open, which can lead to cell death [34,122]. In models of cerebral ischemia induced by carotid [174] or umbilical cord occlusion [175] in near-term fetal sheep, a decrease in the number of dead neurons after the use of the peptide 5 was observed. In a similar model of cerebral ischemia but using neonatal rats, an increase in extracellular glutamate concentration measured by microdialysis was observed, condition that was reduced by the use of Gap26 [176], while in the same model, the use of peptide 5, also increased oligodendroglial survival in white matter and increased brain weight [177]. In a mouse model of brain stroke (induced by middle cerebral artery occlusion), it was observed that Gap19 was more efficient than Gap26 in preventing cell death. This effect was associated to a decrease of activated caspase-3 and Bax, and an increased level of Bcl-2 [178]. In the same model, an interesting work demonstrated that mice with a mutation in four serine residues (all changed to alanine) of connexin 43, which are usually phosphorylated by MAPK (MK4), drastically reduced both the size of the infarct volume and the number of reactive microglia. All these cellular changes were associated to an improvement in behavioral performance [179]. Interestingly, astrocytes from MK4 animals showed decreased connexin 43 hemichannel activity, suggesting that the opening of hemichannels is important for the deleterious effects of stroke. Accordingly, the use of TAT-Gap19 reproduced the effect of MK4 [179]. Therefore, the selective connexin 43 hemichannel blocker Gap19 could be acting as an antiapoptotic factor in brain stroke.

4.3.4.3. Intracerebral hemorrhage. After a head trauma, an intracerebral hemorrhage may occur. A mice model for intracerebral hemorrhage can be performed by microinjecting collagenase IV into the striatum. After this procedure, an increase in connexin 43 expression was observed, which was associated to an increase of astroglial connexin 43 hemichannel activity, measured by dye uptake [180]. Also, a clear hematoma was observed in the microinjection area, which was associated to neurological deficits observed in the mNSS test, balance beam, forelimb strength, and foot fault. All these deficits induced by the intra-striatum collagenase IV injection were greatly diminished by the intracranial injection of Gap19 [180]. Interestingly, Gap19 under normal conditions did not induce any changes in mice behavior [180]. These results support the notion that connexin 43 mimetic peptides could potentially be used for the treatment of brain strokes.

4.3.4.4. Epilepsy. As mentioned before, connexin 43 hemichannels present in astrocytes can modulate neuronal activity and current evidence suggests that connexin 43 function could be involved in the generation of epileptiform discharges. In an astrocyte specific connexin 30 and connexin 43 knockout mice, a reduction of epileptic activity was observed [181]. In an ex vivo model for epilepsy associated to neuronal damage by exposing hippocampal slices to $GABA_A$ receptor antagonist bicuculline for 48 h, the use of low doses of the mimetic peptide 5 reduced neuronal loss, suggesting a role for connexin 43 hemichannels [182]. Interestingly, incubation with the peptide 5 at a concentration high enough to block gap junction channels increased the damage induced by bicuculline [182]. Similarly, the use of Gap19 has also shown an anticonvulsant effect in a mice model of epilepsy [183]. In this work, pilocarpine-induced seizures triggered an increase in connexin 43 hemichannel activity and D-serine release, both of which were inhibited by Gap19 [183]. This indicates that connexin 43 hemichannels are involved in epileptic seizures and suggest that connexin 43 hemichannels could be targeted for the treatment of epilepsy in humans [184].

4.3.4.5. Spinal cord injury. Like in the brain, astrocytes in the spinal cord respond to injuries by becoming reactive, which can lead to neuronal damage or even neuronal death, process in which connexin 43 hemichannels participate [85,185]. In a recent study, it was shown that rats subjected to a spinal cord weight drop using an impactor, presented problems in their hindlimb locomotor function and evident mechanical allodynia three-six weeks after the injury [186]. All these symptoms were significantly reduced by intraperitoneal injection of peptide 5 [186]. Additionally, the number of activated astrocytes and microglia was reduced, neuronal survival was increased [186] and the levels of TNF-α and IL-1β were decreased [187]. Constriction of the sciatic nerve also induces spinal cord changes that are associated to chronic pain. In a mouse model of peripheral nerve damage, the sciatic nerve was constricted for 10 days, after which, animals developed neuropathic pain, and both spinal astrocytes and microglia became reactive [188]. The intrathecal injection of peptide 5 caused a significant improvement in mechanical pain hypersensitivity, and decreased astrogliosis [187,188]. Therefore, the present evidence suggests that the use of connexin 43 mimetic peptides could be beneficial in human injuries / accidents where the spinal cord is compromised.

4.3.5. Peripheral nervous system

4.3.5.1. Chronic pain. Pulpitis often causes ectopic orofacial pain associated with tooth-pulp inflammation. This phenomenon is associated with the activation of connexin 43 hemichannels in satellite glial cells (SGCs) of the trigeminal ganglion [189]. Daily administration of Gap26 into the trigeminal ganglion reduced pain-associated behavior and astroglial activation in SGCs [189]. The activity of connexin 43 hemichannels in the SCG may contribute to an exacerbated acidity in sensory neurons, which could be decoded in the brain as a permanent and/or enhanced pain sensation [190]. This opens the possibility of treating peripheral pain with connexin 43 hemichannel blockers.

4.3.6. Retinopathies

Intense light can induce irreversible cell damage to the retina [191]. In an in vivo model of light induced damage, rats were exposed to a light source with a luminance of 2700 lx for 24 h. This procedure induced damage to rods and cones measured in an electroretinogram, which was associated to a thinning of the retina [192]. This method also induced an increase if GFAP expression, which is an indicator of activation of Muller cells and astrocytes leading to gliosis [192]. All these changes were reduced by the intravitreal injection of peptide 5 alone, or with nanoparticles [193]. In addition, connexin 43 mimetic peptides have also been used for retinal injury caused by ischemia. In this case, peptide 5 was encapsulated into hyaluronic acid coated albumin nanoparticles [194] or was modified adding lipo-aminoacids [195] and injected into the vitreous body in rat models of retinal ischemia. Peptide 5 reduced inflammation and prevented retinal ganglion cell death [194,195].

4.3.7. Wound healing

After a wound, tissue repair is critical. This process involves several steps, such as cell differentiation and migration. The expression of connexins changes during these processes [196–198]. Furthermore, the expression and functionality of connexin hemichannels and gap junction channels are fundamental for these processes to occur correctly [199,200]. For example, the single application of a gel with an antisense against connexin 43 on top of an adult rat skin wound, decreased inflammation and enhanced the speed of healing [201], suggesting that the downregulation of connexin 43 expression and/or a reduction in hemichannel or gap junction channel activity, is beneficial for skin wound healing. A later study using Gap27 and a siRNA against connexin 43 demonstrated that the effect of connexin 43 on skin wound healing depends more on intracellular pathways activated or inhibited by connexin 43 (probably by C-terminal-mediated protein-protein interactions [202,203]), than via hemichannel or gap junction channel activity [204]. Interestingly, the authors observed that Gap27 enhanced cell migration of adult keratinocytes and juvenile foreskin fibroblasts but had no effect on human neonatal fibroblasts and adult dermal tissue [204]. However, in all those cell types, the siRNA against connexin 43 had a great impact in cell migration speed, which suggests that connexin 43-based channels participate in wound healing, but the effect may depend on the cell type. These differences may be related to the metabolic state of a given cell, because it was shown that the addition of Gap27 in a skin organotypic model enhanced tissue repair in normal donors, but had less effect in diabetic donors [205]. However, the use of connexin 43 C-terminus 1 (αCT1 or ACT1) peptide, a 25–amino acid peptide that includes the zonula occludens-1 (ZO-1)–binding domain of connexin 43 and increases connexin 43 gap junction channel activity, accelerated the healing of chronic diabetic foot ulcers when incorporated into a standard care protocol [206]. These differences may be related to the action of a given peptide. Thus, Gap27 is mostly, if not only, a connexin-based channel blocker, while ACT1 has the connexin 43 PDZ binding sequence [207], it increases connexin 43 gap junction channel activity and could potentially affect intracellular connexin 43 protein-protein interactions. On the other hand, Gap19, which is a specific inhibitor for connexin 43 hemichannels, upregulates MMPs, TGF-β, and VEGF-A, while pro-fibrotic molecules were downregulated in a human gingival fibroblasts wound repair model [208]. Connexin 43 peptides have not only been used in skin wound healing, but also both Gap27 and ACT1 have been used in wound repair in ex vivo human and in vivo rat corneas [209,210] and for improving the outcome of silicone implants in rats [211]. In fact, a multicenter, randomized controlled trial of ACT1 in wound healing showed that ACT1 induced significant improvements in cutaneous scarring [212]. In summary, the use of peptides that modulate connexin 43 gap junction channel and hemichannel

activity have a promising future in wound healing and currently, some of them are being used in clinical applications [212–215].

4.3.8. Difficulties to solve

The development of mimetic peptides against connexins is much more developed than antibodies and small molecules. However, there are still several issues to be addressed. Although the mechanism of action of peptides that act intracellularly is relatively well characterized, the mechanism of action for peptides that act on the extracellular side is still unclear [216]. Then there is the problem of selectivity. Although mimetic peptides are designed from non-homologous sequences of each connexin to attain selectivity, we do not know to which extent mimetic peptides are selective. No study has tested mimetic peptides designed for a specific connexin against all connexins, or at least against those which are known to be associated to human diseases. Finally, one of the limitations for the use of peptides in medicine is their rapid degradation in vivo. Some studies have begun to evaluate whether modified peptides with long half-lives have a similar efficacy to their unmodified counterparts [217–219], but much more studies in this direction are needed.

5. Other connexin hemichannel modulators

There are several small molecules that are known to affect connexin hemichannel activity, although it is still unclear whether they exert their effects directly by interacting with connexin proteins, or indirectly, by activating intracellular pathways that modulate hemichannel opening. As connexin hemichannels open in response to increases in intracellular Ca^{2+} [42,43,163], molecules that activate receptors that increase intracellular Ca^{2+} or activate G proteins may induce hemichannel activation. Intracellular kinases can also affect connexin hemichannel open probability [84,220,221], so small molecules that can trigger the differential activation of intracellular protein kinases could also modulate indirectly connexin hemichannel activity. Although hemichannels made of only a few types of connexins have been studied so far, if generalized, they may be sensitive to nitric oxide [34,36,37,83,222], carbon monoxide [223], lipids [224,225], lipid peroxides [33], proinflammatory mediators [25,59,226], cannabinoids [95,227,228] and to changes in redox potential [55]. Undoubtedly, studies focused on the mechanisms of action of these molecules on the opening/closing of hemichannels are required.

Boldine, a naturally occurring antioxidant extracted from the Chilean tree *Peumus boldus*, has also been shown to decrease connexin 43 hemichannel activity, without blocking gap junction channels [229]. Boldine prevented renal alterations in Streptozotocin-induced diabetic rats by decreasing hemichannel activity induced by high glucose and proinflammatory cytokines in mesangial cells [229]. Similarly, in a murine model of Alzheimer disease, boldine prevented the increase of connexin 43 hemichannel opening in cultured astrocytes and in brain slices [230]. Interestingly, oral administration of boldine was shown to reduce inflammation and increase remyelination processes in brains from animals in which the corpus callosus was demyelinated by lysolecithin injections [231]. Boldine administration also prevented hemichannel opening in skeletal muscles, which drastically reduced cell permeabilization and increased muscle function recovery in mice models of endotoxemia and dysferin-deficiency [232,233]. The mechanisms by which boldine exerts its effects have not been elucidated, and there is currently no reported evidence to suggest that it induces direct effects on connexin hemichannels. Notwithstanding, based on current evidence, boldine could be a good therapeutic opportunity to threat skeletal muscle dysfunctions.

6. Final comments

Hemichannels may serve important roles in physiological processes and a deregulation of their open probability (mostly an increase) is associated to several diseases, worsening symptoms due to induction or enhancement of cellular malfunction and even inducing cell death. Until recently, the pharmacology of hemichannels was unspecific, as it did not discriminate between hemichannels and gap junction channels and could also potentially modify the activity of other types of channels and receptors. Fortunately, studies conducted in basic science have elucidated many aspects of the molecular basis that govern the opening and closing of connexin hemichannels. With this information, several laboratories have been able to develop tools that allow us today to selectively modulate the activity of specific connexin hemichannels, without affecting gap junction channel activity. This has allowed the study of their effects in multiple models of human diseases with promising results. Although most of current evidence comes from in vitro studies and preclinical animal models, some are being tested for experimental clinical use, such as the use of connexin 43 peptides in wound healing. Undoubtedly, several questions still remain, and there are several limitations to the studies so far. For example, most of the studies have focused on connexin 43 hemichannels, but there are several human diseases in which other connexins are involved. Therefore, novel small molecules, antibodies and peptides must be developed to target other connexin hemichannels. Also, more preclinical studies are needed in order to discharge those “drug candidates” against connexin hemichannels that do not have an effect in vivo and therefore, do not have the ability to eventually reach patients. Finally, more studies focused on the biophysical properties of small molecules, antibodies and peptides are necessary, in order to improve their selectivity, solubility, blood barrier permeability and pharmacodynamics.

CRediT authorship contribution statement

MAR; designed, wrote and edited. AFO; edited tables and figures and JS; wrote, and edited the manuscript.

Declaration of competing interest

The authors declare that there have not conflicts of interest.

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