Review

Connexin therapeutics: blocking connexin hemichannel pores is distinct from blocking pannexin channels or gap junctions

Monica L. Acosta[1, 5, 6, 7, *], Mohd N. Mat Nor[1, 2], Cindy X. Guo[1], Odunayo O. Mugisho[3, 4, 5], Frazer P. Coutinho[3], Ilva D. Rupenthal[3, 4, 5], Colin R. Green[3, 5]

Abstract

Compounds that block the function of connexin and pannexin protein channels have been suggested to be valuable therapeutics for a range of diseases. Some of these compounds are now in clinical trials, but for many of them, the literature is inconclusive about the molecular effect on the tissue, despite evidence of functional recovery. Blocking the different channel types has distinct physiological and pathological implications and this review describes current knowledge of connexin and pannexin protein channels, their function as channels and possible mechanisms of the channel block effect for the latest therapeutic compounds. We summarize the evidence implicating pannexins and connexins in disease, considering their homeostatic versus pathological roles, their contribution to excesive ATP release linked to disease onset and progression.

Key Words: connexin; gap junction; gap19; hemichannel; pannexin; retina; tonabersat

Introduction

Connexin proteins form cell-to-cell gap junction channels for intercellular communication and signaling, but also cytoplasmic membrane pores called hemichannels, with both channel types emerging as key therapeutic targets for diverse diseases including cancer, retinopathies, neurodegenerative diseases and skin wounds (Vicario et al., 2017; Mugisho et al., 2019). Connexin protein channels contribute to the maintenance of cell integrity, and the coordination in space and time of vital communication signals. Pannexin proteins also form membrane channels of biological importance, and although generally agreed that they do not form cell-to-cell channels, recent reports show pannexins may in some circumstances form intercellular channels. Targeting pannexin and connexin protein channels and blocking the function of the pores they form minimizes the signaling burden of the biological process in which they participate. However, there are distinct differences between connexin hemichannel pores and gap junction channels, especially with regard to the action and roles of connexin and pannexin channels with important long-term consequences when blocking any one of these three distinct channel types. For example, both pannexins and connexins form ATP-release channels for paracrine signaling but pannexin channels are vital for cell homeostasis and long-term blockage of these pores causes dysregulated inflammation (Chen et al., 2019). On the contrary, connexin pores are transiently active in response to injury and are quite distinct from pannexin or gap junction channels through being formed and opening under human relevant pathological conditions (Bernstein and Fishman, 2016). There is vast literature of the therapeutic benefit of blocking hemichannels in pathological conditions and in this review, we address some of the more important features of pannexin and connexin channels that assist interpretation of the true mode of action of current anti-connexin therapeutics.

A systematic review of articles published from January 2000 through December 2019 was conducted using terms such as gap junction, connexin, pannexin, tonabersat, probenecid using the PubMed database. A few selected seminal publications expanding 1981–1998 were used to substantiate key statements. The final version of the review includes citations recommended by reviewers. Many important recent as well as seminal articles were not included in this review but have been cited by Leybaert et al. (2017).

An Overview of Connexins

The amino acid sequence of the connexin protein has biological implications for therapeutic treatments, and it is pertinent to review its basic structure first. Each connexin has four membrane spanning domains, two extracellular loops, one intracellular loop and cytoplasmic carboxyl (C) and amino (N) termini. There are 21 connexin isotypes known, named according to their molecular weight as predicted from their primary amino acid sequence as proposed by Beyer et al. (1987). This diversity of connexins is determined by the different amino acid sequences primarily of the cytoplasmic loop and the C terminus (Laird, 2006). For a review of the different connexins in human and animal models in health and disease, see recent publications (Danesh-Meyer et al., 2016; Delvaeye et al., 2018; Laird and Lampe, 2018).

Six connexin proteins come together in the endoplasmic

[1]School of Optometry and Vision Science, University of Auckland, Auckland, New Zealand; [2]Faculty of Medicine, Universiti Sultan Zainal Abidin, Terengganu, Malaysia; [3]Department of Ophthalmology, University of Auckland, Auckland, New Zealand; [4]Buchanan Ocular Therapeutics Unit, Department of Ophthalmology, Auckland, New Zealand; [5]New Zealand National Eye Centre, University of Auckland, Auckland, New Zealand; [6]Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand; 7 Brain Research New Zealand–Rangahau Roro Aotearoa, Auckland, New Zealand

*Correspondence to: Monica L. Acosta, PhD, m.acosta@auckland.ac.nz.
https://orcid.org/0000-0002-5018-339X (Monica L. Acosta)

How to cite this article: Acosta ML, Mat Nor MN, Guo CX, Mugisho OO, Coutinho FP, Rupenthal ID, Green CR (2021) Connexin therapeutics: blocking connexin hemichannel pores is distinct from blocking pannexin channels or gap junctions. Neural Regen Res 16(3):482-488.

reticulum to form a connexon or hemichannel. The connexons are transported to the plasma membrane and migrate around the membrane until they meet a connexon on a neighbouring cell whereby the extracellular loops dock to form a gap junction channel. At this point the channel opens and the two cells can communicate. The connexon or hemichannel prior to docking with a connexon from a neighbouring cell needs to remain primarily closed or a large, non-specific membrane pore is formed (this is discussed further later in the review).

It has been noted that one of the attributes of therapeutic intervention is that the connexin protein half-life is short, demonstrated mainly in the heart and the liver (Fallon and Goodenough, 1981; Laird et al., 1991; Beardslee et al., 1998). There are functionally associated proteins that regulate the connexin half-life within a few hours of being formed by contributing to their internalization and degradation (Laird et al., 1991). Further regulating the life cycle of connexins, as shown for example for Connexin43 (Cx43), is therapeutic, but, as discussed by Laird (2006), it is possible that these interactions are not affecting the protein unit itself but could be regulating the gating properties of the channels.

Pannexins Form Membrane-Based Hemichannels

Pannexins were more recently discovered to form unopposed channels structurally similar to connexin hemichannels but different from them in many ways. At least one of the three known proteins, Pannexin1 (Panx1), Pannexin2 (Panx2) and Pannexin3 (Panx3), has been found in every organ in mammals (Bennett et al., 2012). In the retina, their location in neurons suggests a role in normal metabolic and electrical activity (Dvoriantchikova et al., 2006). In other tissues, most of the evidence available relates to Panx1 channels but there is also evidence for Panx1/Panx2 heteromeric channels (Penuela et al., 2009). Panx1 does not share sequence homology in either its extracellular loop or intracellular domains with connexins although they are comparable in size and overall appearance to members of the connexin family (connexins 46 and 50) (Ambrosi et al., 2010). They are reported to have unique extracellular loop structural motifs important for ion selectivity and channel inhibition (Michalski et al., 2020). Also, unlike connexins, Panx1 is glycosylated and this modification is important for its targeting to the plasma membrane and accounts for its generally reported inability to form cell-cell channels, as demonstrated in *Xenopus* oocytes experiments (Boassa et al., 2008). However, this is not universally accepted, and some reports suggest pannexin glycosylation may vary and cell-cell pannexin channels are viable (Ishikawa et al., 2011; Sahu et al., 2014). Hence the term pannexin channel is indicative of a membrane pore, not a cell-to-cell communication pathway. Once oligomerized, these large-pore channels are predicted to allow the passage of many small molecules (Lohman and Isakson, 2014). The ionic current through Panx1 channels in *Xenopus* oocytes has been reported to be about 550 pS in 150 mM KCl, almost double of what has been seen for some connexin channels (Bao et al., 2004; Contreras et al., 2003). As will become apparent, it is difficult to differentiate pannexin, connexin hemichannel and gap junction channel roles when many of the tools used are not specific, and when the effect of one channel type's opening on another is not always obvious. The sequence of events is often poorly understood (or not taken into account). While Panx1 is important during the early stages of development, it needs to be down-regulated in mature tissues to avoid negative effects of its expression on cell function (Penuela et al., 2014b).

One of the main functions ascribed to pannexins which also suggests these channels as a therapeutic target, is their role in ATP release, leading to the proposal that connexin hemichannels and pannexin channels may have similar functions. However, more recent evidence indicates that this may not be the case (**Figure 1**). One major difference from connexin hemichannels is that Panx1 hemichannel half-life at the cell surface is long and when removed from the plasma membrane it is internalized by a classical membrane protein recycling pathway (Gehi et al., 2011). The mechanism for closing and opening the channel is unclear; however, cysteine and tyrosine residues in the intracellular loop play a major role in Panx1 function (Begandt et al., 2017), suggesting that there is an established mechanism for its removal.

Both types of channels release ATP but there is a strong suggestion that Panx1 ATP release activates purinergic receptors as a physiological pathway for cellular communication (Lohman and Isakson, 2014), rather than in the pathological conditions that connexin hemichannels do (**Figure 1**). Pannexin channels seem to release ATP under physiological conditions in response to activation of purinergic receptors, bradykinin B2 receptors, histamine receptors and N-methyl-D-aspartate receptors among others (Lohman and Isakson, 2014). The function of these channels seems to be tightly regulated to allow them to act in connection with other cellular pathways (Esseltine and Laird, 2016). For example, pannexins release ATP but are also closed by extracellular ATP and are therefore self-regulating. The physiological role of pannexins is supported by experiments showing that Panx1 channels are active at physiological Ca^{2+} levels (Barbe et al., 2006). The experimental evidence implicates the role of these channels in intracellular Ca^{2+} flux, as they were found in the plasma membrane and in the endoplasmic reticulum, thus contributing to sustained modulation of Ca^{2+} (Vanden Abeele et al., 2006; Thompson et al., 2008).

As for connexin channels, pannexin mutations have been linked to human disease including oocyte death and system dysfunction (hearing loss, skeletal defects and primary ovarian failure) (Shao et al., 2016; Sang et al., 2019), and pannexin channels open in response to experimental mechanical stress or ischemia suggesting a possible role in pathology. Penuela et al. (2014a) has reviewed the evidence for contrasting roles of pannexins in diseases, showing that their activation leads to cell death in experimental brain ischemia/stroke. In their review, they note that spinal astrocytes and neurons in culture release ATP via Cx43 hemichannels for the activation of Panx1, triggering cell death in experimental neurotoxic conditions (Orellana et al., 2010, 2011; Bennett et al., 2012). Nevertheless, there is also a suggestion that the mechanism of ATP release is the other way around. Kim et al. (2018) showed that about one-third of ATP release was from pannexins during ischemia (and two-thirds from connexin hemichannels), but the entire ATP was released from connexin hemichannels upon reperfusion. This, together with the knowledge that pannexin channels are located close to purinergic receptors suggest a key role for pannexin channels in ATP release. Opening Panx1 channels releases ATP leading to autocrine activation of P2X7 receptors (Alberto et al., 2013). P2X7 then activates the NOD-like receptor pyrin domain-containing protein 3 inflammasome and enhances the inflammation effect. This activation can be either harmful or helpful depending on the degree of severity and length of exposure to insult (Silverman et al., 2009). The relative role of each channel type and the interrelationship between them are yet to be fully determined. However, since pannexin channels are also regulated by extracellular ATP, and it is likely that whilst pannexin release of ATP may trigger inflammation, it is perpetuated in chronic disease by connexin hemichannels (Mugisho et al., 2019); and discussed further in the final section of this review. This means that activation of the inflammasome (**Figure 1**) leads to further induction of cytokine release; in turn opening hemichannels, increasing Cx43 expression and resulting in more ATP release. The effect crosses to other cells, as it has been shown that
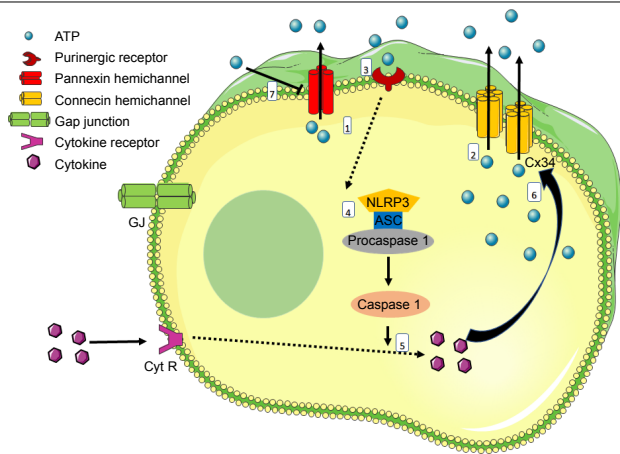
Review

Figure 1 │ Simplified diagram of the cell membrane channels associated with ATP release and the inflammasome pathway activation in pathological conditions.

(1) Pannexin channels are active under normal physiological conditions but contribute to ATP release during inflammation/ischemic damage. (2) Opening of pathologic Cx43 hemichannels results in additional ATP release into the extracellular environment. (3) Extracellular ATP binds to its receptors and results in autocrine activation of P2X receptors. (4) Purinergic receptors then activate the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome (5) which enhances pro-inflammatory cytokines release (6) perpetuating further an inflammatory environment. (7) Excess ATP blocks pannexin channels, with more ATP release perpetuating the NLRP3 inflammasome activation via open Cx43 hemichannels. Cell outline courtesy of Les Laboratories Servier Medical Art licensed under a Creative Commons Attribution 3.0 modified from Mugisho et al. (2018).

pro-inflammatory agents have an effect on gap junctions and hemichannels, within the central nervous system for example, not only in neurons but on the activation of Cx43 in astroglial cells (Orellana et al., 2009, 2016; Xing et al., 2019). The proposed mechanisms of disease spread has been reviewed (Orellana et al., 2012b), suggesting a fundamental role in the nervous system for metabolic and functional astrocyte-neuron coupling via Cx43. This also highlights the need to review the physiological and pathologic role of pannexin and connexin channels in different cell types, and their relationship to gap junctions, as discussed below.

Connexins Form Membrane Channels – The Physiological Roles of Opening the Gap, but the Therapeutic Target of Closing the Pathologic Pore

Connexin protein channels serve many roles in our body from cell proliferation and patterning to electrical coupling and in organ functions such as coordinated heart muscle contraction; however, they also form pathologic membrane pores in injury and disease. The specific location of the channels in neurons, glia and endothelial cells highlights their role in normal physiological functions and in pathologic conditions may impact upon neurodegenerative diseases (Orellana et al., 2016). Connexins contribute to two distinct structures at the plasma membrane: gap junctions and connexin hemichannels (connexons). The connexon is closed until docking with another connexon. Once they dock, these double membrane channels tightly couple the cytoplasm of connecting cells and allow for coordinated cellular metabolism and signaling functions. Gap junctions have transient communication between cells. Once docked they stay open but they do have a short half-life, which may be associated with their quick response to physiological requirements resulting in up- or down-regulation of cell-to-cell coupling.

From a few to several hundred gap junction channels in close proximity to each other, linking adjacent cells, have been

seen in electron microscopy and negative stain studies with this arrangement known as gap junction plaques (Kumar and Gilula, 1996). Within the plaque, there is a highly coordinated removal of older channels from the centre and continual accrual of new gap junctions to the periphery of the plaque arrangement (Gaietta et al., 2002). The turnover process involves intracellular recycling via lysosome or proteolytic pathways. As each gap junction channel allows passive diffusion of molecules up to 1000 Da (Evans and Martin, 2002), including nutrients, metabolites, second messengers, cAMP, Ca^{2+}, adenosine, ATP and ADP (Goldberg et al., 2004), the plaque is a hot spot for intercellular exchange (Decrock et al., 2009; Danesh-Meyer et al., 2016). Plaques show different properties to the dynamics of single, isolated gap junction channels.

Conductance through connexin channels is different for each connexin isotype, with dye transfer experiments showing different permeability depending also upon the perfused molecule charge, size and shape (Kanaporis et al., 2008). A gap junction also allows electrical coupling, with dual patch clamp experiments showing synchronized and rapid responses of the connecting cells. The active process of communication can be regulated by fast acting mechanisms, such as protein phosphorylation. Phosphorylation alters the gating activity of connexins by changing the surface charge of the pore lining as well as the pore size, as shown for the regulation of Cx43 (Lampe and Lau, 2000). The passage of ions can also be regulated by an increase in extracellular alkalinization that increases intracellular Ca^{2+} resulting in an increased gating mechanism (Bennett et al., 1991). In some organs, such as the heart, cell depolarization is fundamentally linked to the electrical communication function of gap junctions. Heart contraction employs differential expression of connexin isotypes depending upon specific functional requirements. The working myocardial cells, for example, are linked by Cx43 channels, but the fast conducting pathway utilizes larger Cx40 channels and the atrioventricular node has lower conductance Cx45 channels to induce a slight delay between atrial and ventricular contraction. In another example, Goliger and Paul (1994) identified that an absence of intercellular gap junctions may stimulate a cancerous phenotype in cells.

Much of the early literature, however, has attributed roles to gap junctions that are now being revised in light of the increasing understanding of connexin hemichannels. For example, tissue inflammation roles were often extrapolated from migration and contraction, or wound healing studies. While in pathological conditions where gap junctions were thought to be responsible for cellular alterations in inflammation, it is now understood that inflammation is more likely mediated by the opening of unopposed Cx43 hemichannels (Bennett et al., 2012; Davidson et al., 2014; Willebrords et al., 2016; Kim et al., 2017).

The connexin hemichannel appears to be a fundamental channel forming a pathologic membrane pore in injury and disease, proving a prime therapeutic target, especially for chronic disease conditions (Orellana et al., 2016; Laird and Lampe, 2018). This will be discussed more fully in the following sections.

Connexins Form Hemichannels That Are Therapeutic Targets

A hemichannel is a connexon that remains uncoupled. The open or closed status of hemichannels depends on the requirements of cells, such as the activity of Ca^{2+} ion channels and glutamate receptors in protecting against apoptosis. There is evidence that within the nervous system, the opening of glial cell hemichannels under physiological conditions may be crucial for some biological processes and it is important

to keep this possibility in mind when targeting connexin hemichannels therapeutically (Huckstepp et al., 2010; Orellana et al., 2012a; Chever et al., 2014; Meunier et al., 2017). However, the extent to which brain slices with associated lesion spread and neuronal death, or *in vitro* models, may reflect normal physiological conditions is uncertain. In clinical trials, tonabersat, now known to be a hemichannel blocker, showed no adverse neuronal effects in over 1000 patients with once daily dosing for up to 12 weeks (Bialer et al., 2009). In normal physiological conditions, hemichannels would seem to remain primarily closed (Contreras et al., 2002; Giaume et al., 2013) and Cx43 channels only open at a very low rate under physiological conditions (Contreras et al., 2003; Decrock et al., 2009). However, the undocked hemichannel may take on a prolonged or more frequently open state following an insult, whether it be mechanical stress, ischemia including deprivation of oxygen or glucose, metabolic inhibition, inflammation, ethanol or high extracellular Ca^{2+} (Contreras et al., 2003; Goodenough and Paul, 2003; Decrock et al., 2009; Froger et al., 2010; Guo et al., 2014; Gomez et al., 2018; Kim et al., 2018; Saez et al., 2018). When open in an undocked state, these hemichannels provide a pathway for the release of paracrine and autocrine signals in a relatively uncontrolled manner. Uncontrolled activation of hemichannels disrupts tissue homeostasis through the passage of molecules and ions between the cell cytoplasm and the extracellular milieu, and by preventing docking of hemichannels and so later also affecting gap junction communication in certain cell types (Abudara et al., 2014; Orellana et al., 2016).

Whilst some studies have indicated that injury, such as that causing chronic inflammation, may alter connexin expression in tissues, in particular Cx26, Cx32 and Cx43, most report that hemichannel numbers increase in response to injury or inflammation (Willebrords et al., 2016). Furthermore, failure of gap junction communication as well as hemichannel activation can be attributed to cumulative oxidative stress, as has been suggested for age-related macular degeneration (Danesh-Meyer et al., 2016). Increased Cx43 expression in vascular endothelium and astrocytes has also been reported in association with human central nervous system injuries (O'Carroll et al., 2008; Danesh-Meyer et al., 2012; Davidson et al., 2012b; Guo et al., 2016; Mao et al., 2017), and recent evidence suggests that ATP release from hemichannels is likely a key part of the mechanism, involving neurons and glia in neurodegenerative diseases (Orellana et al., 2016; Gajardo-Gomez et al., 2017; Mugisho et al., 2018). Upon inflammation, oxidative stress and glutamate excitotoxicity, dysregulation of both connexin and pannexin channels is seen coupled with the up-regulation of both channel types (Orellana et al., 2011), but connexin hemichannel mediated ATP release is the leading mechanism (Bennett et al., 2012; Kim et al., 2018).

Hemichannels can open in response to cytoplasmic Ca^{2+} changes but appear to open mainly in response to pathological signals and metabolic inhibition. The relationship between extracellular and intracellular Ca^{2+} levels and hemichannel opening has been extensively discussed elsewhere (Orellana et al., 2012b) but it is of note that an escalation in intracellular Ca^{2+} can increase homomeric connexin hemichannel activation via complex intracellular signalling cascades (camodulin, MAP kinases, phospholipases) in response to metabolic load (reactive oxygen species, nitric oxide). The Ca^{2+} and Mg^{2+} sensitivity of hemichannels and their large ionic current determine their effect on the intracellular ionic balance. Specifically, one type of hemichannel formed by Cx43 has large anion conductance and constitutes 'pathologic pores' in diseases because of its function balancing ions levels (Decrock et al., 2009). That the opening of Cx43 hemichannels directly contributes to rises in intracellular Ca^{2+} mobilized from the extracellular space has been shown by increasing extracellular pH which triggers hemichannel opening, but not pannexin

opening, leading to calcium influx (Schalper et al., 2010).

Danesh-Meyer et al. (2016) argued that with increased Cx43 expression, there is an increase in protein recruitment to the cell surface resulting in an increase in undocked hemichannels in the plasma membrane, likely through a feedback mechanism due to ATP and Ca^{2+} susceptibility. A novel function of hemichannels, associated with its formation in pathological conditions is that they form feedback loops that amplify the open state of the hemichannel over time. This is consistent with an autocrine feedback loop in the inflammasome pathway as proposed by Mugisho et al. (2018). Indeed, undocked connexin hemichannels and pannexins may be involved in paracrine and autocrine signaling between the intracellular and extracellular environment but the differences between them in pathological conditions are vast and specific to the cell type involved (Evans et al., 2006; Wang et al., 2013; Orellana et al., 2016; Mugisho et al., 2019).

There is ample evidence for a role of hemichannels in disease, especially with the involvement of Cx43. Several of these reports have shown that changes in connexin expression in the central nervous system, including the eyes, are associated with cell and tissue dysfunction and inflammatory processes that lead to retinal degeneration (Laird, 2006). For example, our own investigations have emerged from a knowledge of connexin channels in the pathology of retinal degeneration (Guo et al., 2014), with later studies using connexin hemichannel specific blockers establishing the role of connexin hemichannels in retinal disease (Danesh-Meyer et al., 2012; Guo et al., 2016; Kim et al., 2017; Mat Nor et al., 2018, 2020). These and other studies all indicate that opening of undocked hemichannels is associated with pathology (Davidson et al., 2014; Davidson et al., 2015; Mugisho et al., 2018; O'Carroll et al., 2008). The current evidence for connexin hemichannel-mediated ATP release in particular points toward a role for these channels during disease conditions such as ischemia and inflammation, pathologies that are often associated with a decrease in extracellular Ca^{2+} levels and large fluctuations in membrane potential, but with the inflammasome pathway being activated.

Pathologies of the central nervous system add a layer of complexity to the mechanism of connexin hemichannel action, as it involves not only neurons but also astrocytes in the release of cytokines and gliotransmitters. Upon calcium increase, communication between astrocytes and neurons enhance the feedback mechanisms that lead to neuronal injury, reviewed in (Bennett et al., 2012; Chever et al., 2014; Freitas-Andrade and Naus, 2016; Meunier et al., 2017; Mayorquin et al., 2018).

The most interesting and widely reported role of Cx43 hemichannels in pathology seems to be associated with purinergic receptors and the NOD-like receptor pyrin domain-containing protein 3 inflammasome pathway of the innate immune system with opening of Cx43 hemichannels being an early event in the inflammatory process. Sustained hemichannel opening and ATP release, in conjunction with released inflammatory cytokines, provide a mechanism for the amplification and perpetuation of the inflammasome response as described in cell cultures by Mugisho et al. (2018).

Therapeutic Approaches in Clinical Trials Designed to Target Connexin and Pannexin Channels

It has been reported that therapeutic effects could be achieved at four levels: the pannexin, the connexin gap junction, or the connexin hemichannel in the plasma membrane, as well as in mitochondrial hemichannels (Naus and Giaume, 2016). While some therapeutics have been specifically design to block

these channels, a vast number are also re-purposed drugs (**Additional Table 1**). For example, probenecid was a widely used drug for the treatment of gout and is employed clinically to augment the efficacy of antibiotics, chemotherapeutics and other drugs. The proposed mechanism of action is similar to that of carbenoxolone. However, carbenoxolone is unrestrained, affecting both connexin and pannexin channels whereas probenecid interacts with the first extracellular loop of Panx1 and specifically inhibits Panx1 channels at a concentration that does not inhibit connexin hemichannels (Silverman et al., 2008). The antiviral drug tenofovir, used in the treatment of viral hepatitis, reversing hepatic fibrosis/cirrhosis in patients with chronic hepatitis B, is an inhibitor of Panx1-mediated ATP release, by downregulating adenosine levels in the liver and skin (Feig et al., 2017). Feig et al. (2017) also demonstrated in human cell cultures that the effect is due to block of unstimulated ATP release through Panx1 channels, but not Cx43 hemichannels. The therapeutic effect is achieved by blocking the function of the pannexin hemichannel, closing it permanently or transitorily and this strategy, widely used for blocking connexin channels has now started to be investigated systemically for pannexins.

Drugs that act on connexins can be differentiated between those acting on gap junctions for therapeutic use to maintain the gap junction open, contrary to the desired action on hemichannels (Wang et al., 2013). An example of a clinically used therapeutic that acts through modulation of Cx43 gap junctions is the peptide α-connexin carboxyl terminal (ACT1) that maintains Cx43 at gap junction sites at cell-cell membrane borders of breast cancer cells and augments gap junction activity in functional assays (Grek et al., 2015). The increase in Cx43 gap junctional activity achieved by ACT1 impairs proliferation and survival of breast cancer cells. This peptide also re-establishes a normal wound repair cascade when applied on cutaneous wounds, as ACT1 is said to restore gap junctions that were lost in diabetes related injured tissues (Ghatnekar et al., 2015). However, it is more likely that ACT1 is acting as a hemichannel blocker in an identical manner to Gap19, which mimics the cytoplasmic loop to inhibit loop-C terminus interactions (Jiang et al., 2019). Other gap junction activators that have entered clinical trials include rotigaptide for the treatment of cardiac arrhythmia, a peptide that increases gap junction electrical conductance (Macia et al., 2011). Meclofenamate is clinically used to treat moderate pain and to reduce swelling, and is a gap junction inhibitor. It has also been trialed for the inhibition of tumour growth (Gleisner et al., 2017).

For blocking connexin hemichannels, a range of monoclonal antibodies and synthetic molecules including antisense oligonucleotide approaches and peptidomimetic strategies are now available, the latter building on the first peptides recognising the connexin extracellular loop sequences, a very significant contribution to the field by the late W. Howard Evans (Evans, 2015). [Howard died in 2019 and was a key player in the field, the first to isolate gap junctions and the first to make connexin peptide antibodies. He was the first to describe functional mimetic peptides]. A Cx43 antisense oligodeoxynucleotide was first applied in a series of compassionate use cases to treat severe ocular surface burns (Ormonde et al., 2012). Originally taken into clinical trials by CoDaTherapeutics, Inc, USA, it was subsumed into OcuNexus Therapeutics Inc. and has been licensed to Eyevance Pharmaceuticals, USA. It is currently in stage 3 clinical trials for ocular surface indications.

Some drugs that inhibit connexin channels may be non-specific, and can also inhibit pannexins and gap junctions, such as anaesthetics, fatty acids, alcoholic substances, carbenoxolone and glycyrrhetinic acid (Willebrords et al., 2017). Peptides, on the other hand recognize the primary

protein specific sequence and interfere directly with the channel function. The majority of compounds with therapeutic potential are peptides and organic compounds that block the interaction between the cytoplasmic loop and the C terminus of Cx43, or interact directly with the extracellular loops. Low concentrations of some peptides can be used to specifically block Cx43 hemichannels (such as Peptide5) without impacting significantly on gap junction coupling, and at least one peptide, Gap19, mimics the cytoplasmic loop to inhibit loop-C terminus interactions. Cx43 antisense oligonucleotides and Peptide5, a Cx43 hemichannel blocker, and more recently Xentry-Gap19, a construct of Gap19 with increased efficacy at low concentrations and disease targeting potential, also appear to provide viable therapeutic options for the treatment of chronic ocular surface and retinal inflammatory diseases associated with Cx43 hemichannel opening as well as both acute and chronic inflammation. The dose of the compound is critical for the therapeutic effect as high dose systemic Peptide5 have been associated with greater brain cell swelling after hypoxic-ischemic brain injury in fetal sheep (Davidson et al., 2012a). These data suggest that higher doses of extracellular acting connexin mimetic peptides are not beneficial to treat brain ischemia, most likely due to uncoupling of gap junctions. However, for example, intravitreal injection of low doses of Peptide5, and the benefits that intracellular acting Gap19 and Xentry-Gap19 offer with their hemichannel block specific mode of action (Abudara et al., 2014), has large potential, especially for acute injuries (for example in retinal stroke such as vein or artery occlusion) where the pharmacokinetics of an oral treatment such as Tonabersat may not be ideal.

The known involvement of ATP in inflammasome activation in many diseases, and the prospect of hemichannel opening mediating the release of this 'activation signal' that triggers assembly of the inflammasome complex in primed cells, has increased the interest in connexin hemichannels as a drug target candidate (Laird and Lampe, 2018; Mugisho et al., 2019). Blocking hemichannels requires lower drug concentrations than blocking or uncoupling gap junctions and this has been exploited for therapeutic use. Among all the therapeutic compounds, those that block Cx43 hemichannels have proven to be most efficient at preventing disease onset, and in shutting down disease perpetuation. Tonabersat is one of these compounds, originally proposed for the treatment of migraine with aura, migraine prophylaxis and as a treatment for epilepsy. Tonabersat is now recognised as a connexin specific hemichannel blocker. It is a benzopyran derivate with a unique stereo-selective binding site originally reported to inhibit gap junction communication (Read et al., 2000). Other studies, however, suggested that its mode of action is as a connexin hemichannel blocker and it has been re-purposed for this use in the treatment of inflammasome-related diseases (Kim et al., 2017). It has been shown recently that tonabersat directly reduces opening of hemichannels under pathological conditions (Kim et al., 2017; Mat Nor et al., 2020), which at low concentrations effectively acts only on undocked hemichannels. Tonabersat treatment has also proven to be effective in glioma (Aasen et al., 2019; De Meulenaere et al., 2019), again indicating a review of connexin channel effects in the biology of the glioma is overdue and that the involvement of hemichannel mediated effects may be understated. Since Tonabersat has proven safe in low and medium dose toxicity studies and has been used in humans for up to twelve weeks, it cannot be acting by uncoupling gap junctions, which would have severe adverse effects. This is one of the most clinically advanced compounds, available as an oral tablet and Phase II ready with safety evidence in over 1000 patients. In over 25 animal disease models, connexin hemichannel regulation has shown therapeutic benefit (Kim et al., 2017; Mugisho et al., 2019; Mat Nor et al., 2020) and it remains now to be seen if this translates into the clinic with an appropriate target indication.

Conclusion

The mechanism of diseases and the target for therapeutic interventions has evolved from gap junctions to connexin hemichannels, with a debatable role for pannexins in ATP-mediated disease mechanisms. Further evidence implicating pannexins in disease should ideally be regarded in light of what appears to be a key link between ATP-connexin hemichannels and pannexin channel activation in disease onset. Nevertheless, therapeutic interventions for inflammatory diseases still have Cx43 hemichannels as key therapeutic targets that can prevent disease spread while allowing ATP homeostasis and purinergic signaling to remain via pannexin channels. Cx43 hemichannels are an upstream target for a range of inflammatory diseases with promising therapies currently in clinical trials evaluating the use of peptides and small compounds specific for connexin hemichannels.

Additional file:
Additional Table 1: Selected therapeutics targeting connexins, pannexins and gap junctions.

References

Aasen T, Leithe E, Graham SV, Kameritsch P, Mayán MD, Mesnil M, Pogoda K, Tabernero A (2019) Connexins in cancer: bridging the gap to the clinic. Oncogene 38:4429-4451.

Abudara V, Bechberger J, Freitas-Andrade M, De Bock M, Wang N, Bultynck G, Naus CC, Leybaert L, Giaume C (2014) The connexin43 mimetic peptide Gap19 inhibits hemichannels without altering gap junctional communication in astrocytes. Front Cell Neurosci 8:306.

Alberto AV, Faria RX, Couto CG, Ferreira LG, Souza CA, Teixeira PC, Froes MM, Alves LA (2013) Is pannexin the pore associated with the P2X7 receptor? Naunyn Schmiedebergs Arch Pharmacol 386:775-787.

Ambrosi C, Gassmann O, Pranskevich JN, Boassa D, Smock A, Wang J, Dahl G, Steinem C, Sosinsky GE (2010) Pannexin1 and Pannexin2 channels show quaternary similarities to connexons and different oligomerization numbers from each other. J Biol Chem 285: 24420-24431.

Bao L, Locovei S, Dahl G (2004) Pannexin membrane channels are mechanosensitive conduits for ATP. FEBS Lett 572:65-68.

Barbe MT, Monyer H, Bruzzone R (2006) Cell-cell communication beyond connexins: the pannexin channels. Physiology (Bethesda) 21:103-114.

Beardslee MA, Laing JG, Beyer EC, Saffitz JE (1998) Rapid turnover of connexin43 in the adult rat heart. Circ Res 83:629-635.

Begandt D, Good ME, Keller AS, DeLalio LJ, Rowley C, Isakson BE, Figueroa XF (2017) Pannexin channel and connexin hemichannel expression in vascular function and inflammation. BMC Cell Biol 18:2.

Bennett MV, Barrio LC, Bargiello TA, Spray DC, Hertzberg E, Saez JC (1991) Gap junctions: new tools, new answers, new questions. Neuron 6:305-320.

Bennett MV, Garre JM, Orellana JA, Bukauskas FF, Nedergaard M, Saez JC (2012) Connexin and pannexin hemichannels in inflammatory responses of glia and neurons. Brain Res 1487:3-15.

Bernstein S, Fishman G (2016) Connexins and Heritable Human Diseases. In: Ion Channels in Health and Disease. Perspectives in Translational Cell Biology (Pitt GS, ed). pp331-343. Elsevier, Cambridge, MA.

Beyer EC, Paul DL, Goodenough DA (1987) Connexin43: a protein from rat heart homologous to a gap junction protein from liver. J Cell Biol 105:2621-2629.

Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS (2009) Progress report on new antiepileptic drugs: a summary of the Ninth Eilat Conference (EILAT IX). Epilepsy Res 83:1-43.

Boassa D, Qiu F, Dahl G, Sosinsky G (2008) Trafficking dynamics of glycosylated pannexin 1 proteins. Cell Commun Adhes 15:119-132.

Chen W, Zhu S, Wang Y, Li J, Qiang X, Zhao X, Yang H, D'Angelo J, Becker L, Wang P, Tracey KJ, Wang H (2019) Enhanced macrophage pannexin 1 expression and hemichannel activation exacerbates lethal experimental sepsis. Sci Rep 9:160.

Chever O, Lee CY, Rouach N (2014) Astroglial connexin43 hemichannels tune basal excitatory synaptic transmission. J Neurosci 34:11228-11232.

Contreras JE, Saez JC, Bukauskas FF, Bennett MV (2003) Gating and regulation of connexin 43 (Cx43) hemichannels. Proc Natl Acad Sci U S A 100:11388-11393.

Contreras JE, Sánchez HA, Eugenín EA, Speidel D, Theis M, Willecke K, Bukauskas FF, Bennett MVL, Sáez JC (2002) Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. Proc Natl Acad Sci U S A 99:495-500.

Coutinho FP, Green CR, Rupenthal ID (2019) Targeting Drugs to Diseased Ocular Cells. ONdrugDelivery Magazine, 94:10-12.

Danesh-Meyer HV, Kerr NM, Zhang J, Eady EK, O'Carroll SJ, Nicholson LF, Johnson CS, Green CR (2012) Connexin43 mimetic peptide reduces vascular leak and retinal ganglion cell death following retinal ischaemia. Brain 135:506-520.

Danesh-Meyer HV, Zhang J, Acosta ML, Rupenthal ID, Green CR (2016) Connexin43 in retinal injury and disease. Prog Retin Eye Res 51:41-68.

Davidson JO, Drury PP, Green CR, Nicholson LF, Bennet L, Gunn AJ (2014) Connexin hemichannel blockade is neuroprotective after asphyxia in preterm fetal sheep. PLoS One 9:e96558.

Davidson JO, Green CR, Bennet L, Gunn AJ (2015) Battle of the hemichannels--Connexins and Pannexins in ischemic brain injury. Int J Dev Neurosci 45:66-74.

Davidson JO, Green CR, Nicholson LF, Bennet L, Gunn AJ (2012a) Deleterious effects of high dose connexin 43 mimetic peptide infusion after cerebral ischaemia in near-term fetal sheep. Int J Mol Sci 13:6303-6319.

Davidson JO, Green CR, Nicholson LF, O'Carroll SJ, Fraser M, Bennet L, Gunn AJ (2012b) Connexin hemichannel blockade improves outcomes in a model of fetal ischemia. Ann Neurol 71:121-132.

De Meulenaere V, Bonte E, Verhoeven J, Kalala Okito JP, Pieters L, Vral A, De Wever O, Leybaert L, Goethals I, Vanhove C, Descamps B, Deblaere K (2019) Adjuvant therapeutic potential of tonabersat in the standard treatment of glioblastoma: A preclinical F98 glioblastoma rat model study. PLoS One 14:e0224130.

Decrock E, Vinken M, De Vuyst E, Krysko DV, D'Herde K, Vanhaecke T, Vandenabeele P, Rogiers V, Leybaert L (2009) Connexin-related signaling in cell death: to live or let die? Cell Death Differ 16:524-536.

Delvaeye T, Vandenabeele P, Bultynck G, Leybaert L, Krysko DV (2018) Therapeutic targeting of connexin channels: new views and challenges. Trends Mol Med 24:1036-1053.

Dvoriantchikova G, Ivanov D, Panchin Y, Shestopalov VI (2006) Expression of pannexin family of proteins in the retina. FEBS Lett 580:2178-2182.

Esseltine JL, Laird DW (2016) Next-generation connexin and pannexin cell biology. Trends Cell Biol 26:944-955.

Evans WH (2015) Cell communication across gap junctions: a historical perspective and current developments. Biochem Soc Trans 43:450-459.

Evans WH, De Vuyst E, Leybaert L (2006) The gap junction cellular internet: connexin hemichannels enter the signalling limelight. Biochem J 397:1-14.

Evans WH, Martin PE (2002) Gap junctions: structure and function (Review). Mol Membr Biol 19:121-136.

Fallon RF, Goodenough DA (1981) Five-hour half-life of mouse liver gap-junction protein. J Cell Biol 90:521-526.

Feig JL, Mediero A, Corciulo C, Liu H, Zhang J, Perez-Aso M, Picard L, Wilder T, Cronstein B (2017) The antiviral drug tenofovir, an inhibitor of Pannexin-1-mediated ATP release, prevents liver and skin fibrosis by downregulating adenosine levels in the liver and skin. PLoS One 12:e0188135.

Freitas-Andrade M, Naus CC (2016) Astrocytes in neuroprotection and neurodegeneration: The role of connexin43 and pannexin1. Neuroscience 323, 207-221.

Froger N, Orellana JA, Calvo CF, Amigou E, Kozoriz MG, Naus CC, Saez JC, Giaume C (2010) Inhibition of cytokine-induced connexin43 hemichannel activity in astrocytes is neuroprotective. Mol Cell Neurosci 45:37-46.

Gaietta G, Deerinck TJ, Adams SR, Bouwer J, Tour O, Laird DW, Sosinsky GE, Tsien RY, Ellisman MH (2002) Multicolor and electron microscopic imaging of connexin trafficking. Science 296:503-507.

Gajardo-Gomez R, Labra VC, Maturana CJ, Shoji KF, Santibanez CA, Saez JC, Giaume C, Orellana JA (2017) Cannabinoids prevent the amyloid beta-induced activation of astroglial hemichannels: A neuroprotective mechanism. Glia 65:122-137.

Gehi R, Shao Q, Laird DW (2011) Pathways regulating the trafficking and turnover of pannexin1 protein and the role of the C-terminal domain. J Biol Chem 286:27639-27653.

Ghatnekar GS, Grek CL, Armstrong DG, Desai SC, Gourdie RG (2015) The effect of a connexin43-based Peptide on the healing of chronic venous leg ulcers: a multicenter, randomized trial. J Invest Dermatol 135:289-298.

Giaume C, Leybaert L, Naus CC, Saez JC (2013) Connexin and pannexin hemichannels in brain glial cells: properties, pharmacology, and roles. Front Pharmacol 4:88.

Gleisner MA, Navarrete M, Hofmann F, Salazar-Onfray F, Tittarelli A (2017) Mind the gaps in tumor immunity: impact of connexin-mediated intercellular connections. Front Immunol 8:1067.

Goldberg GS, Valiunas V, Brink PR (2004) Selective permeability of gap junction channels. Biochim Biophys Acta 1662:96-101.

Review

Goliger JA, Paul DL (1994) Expression of gap junction proteins Cx26, Cx31.1, Cx37, and Cx43 in developing and mature rat epidermis. Dev Dyn 200:1-13.

Gomez GI, Falcon RV, Maturana CJ, Labra VC, Salgado N, Rojas CA, Oyarzun JE, Cerpa W, Quintanilla RA, Orellana JA (2018) Heavy alcohol exposure activates astroglial hemichannels and pannexons in the hippocampus of adolescent rats: effects on neuroinflammation and astrocyte arborization. Front Cell Neurosci 12:472.

Goodenough DA, Paul DL (2003) Beyond the gap: functions of unpaired connexon channels. Nat Rev Mol Cell Biol 4:285-294.

Grek CL, Rhett JM, Bruce JS, Abt MA, Ghatnekar GS, Yeh ES (2015) Targeting connexin 43 with alpha-connexin carboxyl-terminal (ACT1) peptide enhances the activity of the targeted inhibitors, tamoxifen and lapatinib, in breast cancer: clinical implication for ACT1. BMC Cancer 15:296.

Guo CX, Mat Nor MN, Danesh-Meyer HV, Vessey KA, Fletcher EL, O'Carroll SJ, Acosta ML, Green CR (2016) Connexin43 mimetic peptide improves retinal function and reduces inflammation in a light-damaged albino rat model. Invest Ophthalmol Vis Sci 57:3961-3973.

Guo CX, Tran H, Green CR, Danesh-Meyer HV, Acosta ML (2014) Gap junction proteins in the light-damaged albino rat. Mol Vis 20:670-682.

Huckstepp RT, id Bihi R, Eason R, Spyer KM, Dicke N, Willecke K, Marina N, Gourine AV, Dale N (2010) Connexin hemichannel-mediated CO2-dependent release of ATP in the medulla oblongata contributes to central respiratory chemosensitivity. J Physiol 588:3901-3920.

Ishikawa M, Iwamoto T, Nakamura T, Doyle A, Fukumoto S, Yamada Y (2011) Pannexin 3 functions as an ER Ca(2+) channel, hemichannel, and gap junction to promote osteoblast differentiation. J Cell Biol 193:1257-1274.

Jiang J, Hoagland D, Palatinus JA, He H, Iyyathurai J, Jourdan LJ, Bultynck G, Wang Z, Zhang Z, Schey K, Poelzing S, McGowan FX, Gourdie RG (2019) Interaction of alpha carboxyl terminus 1 peptide with the connexin 43 carboxyl terminus preserves left ventricular function after ischemia-reperfusion injury. J Am Heart Assoc 8:e012385.

Kanaporis G, Mese G, Valiuniene L, White TW, Brink PR, Valiunas V (2008) Gap junction channels exhibit connexin-specific permeability to cyclic nucleotides. J Gen Physiol 131:293-305.

Kim Y, Davidson JO, Green CR, Nicholson LFB, O'Carroll SJ, Zhang J (2018) Connexins and Pannexins in cerebral ischemia. Biochim Biophys Acta Biomembr 1860:224-236.

Kim Y, Griffin JM, Nor MNM, Zhang J, Freestone PS, Danesh-Meyer HV, Rupenthal ID, Acosta M, Nicholson LFB, O'Carroll SJ, Green CR (2017) Tonabersat prevents inflammatory damage in the central nervous system by blocking connexin43 hemichannels. Neurotherapeutics 14:1148-1165.

Kumar NM, Gilula NB (1996) The gap junction communication channel. Cell 84:381-388.

Laird DW (2006) Life cycle of connexins in health and disease. Biochem J 394:527-543.

Laird DW, Lampe PD (2018) Therapeutic strategies targeting connexins. Nat Rev Drug Discov 17:905-921.

Laird DW, Puranam KL, Revel JP (1991) Turnover and phosphorylation dynamics of connexin43 gap junction protein in cultured cardiac myocytes. Biochem J 273:67-72.

Lampe PD, Lau AF (2000) Regulation of gap junctions by phosphorylation of connexins. Arch Biochem Biophys 384:205-215.

Leybaert L, Lampe PD, Dhein S, Kwak BR, Ferdinandy P, Beyer EC, Laird DW, Naus CC, Green CR, Schulz R (2017) Connexins in cardiovascular and neurovascular health and disease: pharmacological implications. Pharmacol Rev 69:396-478.

Lohman AW, Isakson BE (2014) Differentiating connexin hemichannels and pannexin channels in cellular ATP release. FEBS Lett 588:1379-1388.

Macia L, Dolmatova E, Cabo C, Sosinsky AZ, Dun W, Coromilas J, Ciaccio EJ, Boyden PA, Wit AL, Duffy HS (2011) Characterization of gap junction remodeling in epicardial border zone of healing canine infarcts and electrophysiological effects of partial reversal by rotigaptide. Circ Arrhythm Electrophysiol 4:344-351.

Mao Y, Tonkin RS, Nguyen T, O'Carroll SJ, Nicholson LF, Green CR, Moalem-Taylor G, Gorrie CA (2017) Systemic administration of connexin43 mimetic peptide improves functional recovery after traumatic spinal cord injury in adult rats. J Neurotrauma 34:707-719.

Mat Nor M, Rupenthal I, Green C, Acosta M (2019) Connexin hemichannel block using orally delivered tonabersat improves outcomes in animal models of retinal disease. Neurotherapeutics 17:371-387.

Mat Nor N, Guo CX, Rupenthal ID, Chen YS, Green CR, Acosta ML (2018) Sustained connexin43 mimetic peptide release from loaded nanoparticles reduces retinal and choroidal photodamage. Invest Ophthalmol Vis Sci 59:3682-3693.

Mayorquin LC, Rodriguez AV, Sutachan JJ, Albarracin SL (2018) Connexin-mediated functional and metabolic coupling between astrocytes and neurons. Front Mol Neurosci 11:118.

Meunier C, Wang N, Yi C, Dallerac G, Ezan P, Koulakoff A, Leybaert L, Giaume C (2017) Contribution of astroglial Cx43 hemichannels to the modulation of glutamatergic currents by D-serine in the mouse prefrontal cortex. J Neurosci 37:9064-9075.

Michalski K, Syrjanen JL, Henze E, Kumpf J, Furukawa H, Kawate T (2020) The Cryo-EM structure of pannexin 1 reveals unique motifs for ion selection and inhibition. Elife doi: 10.7554/eLife.54670.

Mugisho OO, Green CR, Kho DT, Zhang J, Graham ES, Acosta ML, Rupenthal ID (2018) The inflammasome pathway is amplified and perpetuated in an autocrine manner through connexin43 hemichannel mediated ATP release. Biochim Biophys Acta Gen Subj 1862:385-393.

Mugisho OO, Rupenthal ID, Paquet-Durand F, Acosta ML, Green CR (2019) Targeting connexin hemichannels to control the inflammasome: the correlation between connexin43 and NLRP3 expression in chronic eye disease. Expert Opin Ther Targets 23: 855-863.

Naus CC, Giaume C (2016) Bridging the gap to therapeutic strategies based on connexin/pannexin biology. J Transl Med 14:330.

O'Carroll SJ, Alkadhi M, Nicholson LF, Green CR (2008) Connexin 43 mimetic peptides reduce swelling, astrogliosis, and neuronal cell death after spinal cord injury. Cell Commun Adhes 15:27-42.

Orellana JA, Froger N, Ezan P, Jiang JX, Bennett MV, Naus CC, Giaume C, Saez JC (2011) ATP and glutamate released via astroglial connexin 43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels. J Neurochem 118:826-840.

Orellana JA, Hernandez DE, Ezan P, Velarde V, Bennett MV, Giaume C, Saez JC (2010) Hypoxia in high glucose followed by reoxygenation in normal glucose reduces the viability of cortical astrocytes through increased permeability of connexin 43 hemichannels. Glia 58:329-343.

Orellana JA, Retamal MA, Moraga-Amaro R, Stehberg J (2016) Role of astroglial hemichannels and pannexons in memory and neurodegenerative diseases. Front Integr Neurosci 10:26.

Orellana JA, Saez PJ, Cortes-Campos C, Elizondo RJ, Shoji KF, Contreras-Duarte S, Figueroa V, Velarde V, Jiang JX, Nualart F, Saez JC, Garcia MA (2012a) Glucose increases intracellular free Ca(2+) in tanycytes via ATP released through connexin 43 hemichannels. Glia 60:53-68.

Orellana JA, Saez PJ, Shoji KF, Schalper KA, Palacios-Prado N, Velarde V, Giaume C, Bennett MV, Saez JC (2009) Modulation of brain hemichannels and gap junction channels by pro-inflammatory agents and their possible role in neurodegeneration. Antioxid Redox Signal 11:369-399.

Orellana JA, Sanchez HA, Schalper KA, Figueroa V, Saez JC (2012b) Regulation of intercellular calcium signaling through calcium interactions with connexin-based channels. Adv Exp Med Biol 740:777-794.

Ormonde S, Chou CY, Goold L, Petsoglou C, Al-Taie R, Sherwin T, McGhee CN, Green CR (2012) Regulation of connexin43 gap junction protein triggers vascular recovery and healing in human ocular persistent epithelial defect wounds. J Membr Biol 245|:381-388.

Penuela S, Bhalla R, Nag K, Laird DW (2009) Glycosylation regulates pannexin intermixing and cellular localization. Mol Biol Cell 20:4313-4323.

Penuela S, Harland L, Simek J, Laird DW (2014a) Pannexin channels and their links to human disease. Biochem J 461:371-381.

Penuela S, Kelly JJ, Churko JM, Barr KJ, Berger AC, Laird DW (2014b) Panx1 regulates cellular properties of keratinocytes and dermal fibroblasts in skin development and wound healing. J Invest Dermatol 134:2026-2035.

Read SJ, Smith MI, Hunter AJ, Upton N, Parsons AA (2000) SB-220453, a potential novel antimigraine agent, inhibits nitric oxide release following induction of cortical spreading depression in the anaesthetized cat. Cephalalgia 20:92-99.

Saez JC, Contreras-Duarte S, Gomez GI, Labra VC, Santibanez CA, Gajardo-Gomez R, Avendano BC, Diaz EF, Montero TD, Velarde V, Orellana JA (2018) Connexin 43 hemichannel activity promoted by pro-inflammatory cytokines and high glucose alters endothelial cell function. Front Immunol 9:1899.

Sahu G, Sukumaran S, Bera AK (2014) Pannexins form gap junctions with electrophysiological and pharmacological properties distinct from connexins. Sci Rep 4:4955.

Sang Q, Zhang Z, Shi J, Sun X, Li B, Yan Z, Xue S, Ai A, Lyu Q, Li W, Zhang J, Wu L, Mao X, Chen B, Mu J, Li Q, Du J, Sun Q, Jin L, He L, et al. (2019) A pannexin 1 channelopathy causes human oocyte death. Sci Transl Med doi: 10.1126/scitranslmed.aav8731.

Schalper KA, Sanchez HA, Lee SC, Altenberg GA, Nathanson MH, Saez JC (2010) Connexin 43 hemichannels mediate the Ca2+ influx induced by extracellular alkalinization. Am J Physiol Cell Physiol 299:C1504-1515.

Shao Q, Lindstrom K, Shi R, Kelly J, Schroeder A, Juusola J, Levine KL, Esseltine JL, Penuela S, Jackson MF, Laird DW (2016) A germline variant in the PANX1 gene has reduced channel function and is associated with multisystem dysfunction. J Biol Chem 291:12432-12443.

Silverman W, Locovei S, Dahl G (2008) Probenecid, a gout remedy, inhibits pannexin 1 channels. Am J Physiol Cell Physiol 295:C761-767.

Silverman WR, de Rivero Vaccari JP, Locovei S, Qiu F, Carlsson SK, Scemes E, Keane RW, Dahl G (2009) The pannexin 1 channel activates the inflammasome in neurons and astrocytes. J Biol Chem 284:18143-18151.

Thompson RJ, Jackson MF, Olah ME, Rungta RL, Hines DJ, Beazely MA, MacDonald JF, MacVicar BA (2008) Activation of pannexin-1 hemichannels augments aberrant bursting in the hippocampus. Science 322:1555-1559.

Vanden Abeele F, Bidaux G, Gordienko D, Beck B, Panchin YV, Baranova AV, Ivanov DV, Skryma R, Prevarskaya N (2006) Functional implications of calcium permeability of the channel formed by pannexin 1. J Cell Biol 174:535-546.

Vicario N, Zappala A, Calabrese G, Gulino R, Parenti C, Gulisano M, Parenti R (2017) Connexins in the central nervous system: physiological traits and neuroprotective targets. Front Physiol 8:1060.

Wang N, De Vuyst E, Ponsaerts R, Boengler K, Palacios-Prado N, Wauman J, Lai CP, De Bock M, Decrock E, Bol M, Vinken M, Rogiers V, Tavernier J, Evans WH, Naus CC, Bukauskas FF, Sipido KR, Heusch G, Schulz R, Bultynck G, et al. (2013) Selective inhibition of Cx43 hemichannels by Gap19 and its impact on myocardial ischemia/reperfusion injury. Basic Res Cardiol 108:309.

Willebrords J, Crespo Yanguas S, Maes M, Decrock E, Wang N, Leybaert L, Kwak BR, Green CR, Cogliati B, Vinken M (2016) Connexins and their channels in inflammation. Crit Rev Biochem Mol Biol 51:413-439.

Willebrords J, Maes M, Crespo Yanguas S, Vinken M (2017) Inhibitors of connexin and pannexin channels as potential therapeutics. Pharmacol Ther 180:144-160.

Xing L, Yang T, Cui S, Chen G (2019) Connexin hemichannels in astrocytes: role in CNS disorders. Front Mol Neurosci 12:23.

C-Editors: Zhao M, Li JY; T-Editor: Jia Y

Additional Table 1 Selected therapeutics targeting connexins, pannexins and gap junctions

Target	Drug effect	Drug (formula)	Description	Condition	References
Connexins, pannexins	Blocks connexins and pannexins	Carbenoxolone ($C_{34}H_{50}O_7$)	Glycyrrhetinic acid derivative with a steroid-like structure	Lip sores, mouth ulcers	Willebrords et al. (2017)
Pannexins	Several pharmacological targets, including blocking pannexins	Probenecid ($C_{13}H_{19}NO_4S$)	4 -Dipropylsulfamoyl benzoic acid derivative	Chronic gout, gouty arthritis	Silverman et al. (2008)
Pannexins	Inhibitor of pannexin channel function	Tenofovir ($C_9H_{14}N_5O_4P$)	Acyclic nucleotide analogue of adenosine	Chronic hepatitis virus infection	Feig et al. (2017)
Connexins	Gap junction blocker	Meclofenamate ($C_{14}H_{10}Cl_2NNaO_2$)	2-(2,6-Dichloro-3-methylanilino) benzoate	Osteoarthritis	Gleisner et al. (2017)
Cx43	Cx43 hemichannel blocker	Tonabersat (Xiflam^{TM}) ($C_{20}H_{19}ClFNO_4$)	Benzopyran derivative	Retinal ischemic injury, inflammation disease	Kim et al. (2017)
Cx43	Decreases Cx43 levels by inhibiting protein translation	Cx43 oligonucleotide (Nexagon®) (5′-GTA-ATT-GCG-GCA-GGA-GGA-ATT-GTT-TCT-GTC-3′)	Cx43 antisense oligo deoxynucleotide	Corneal/skin wounds	Ormonde et al. (2012)
Cx43	Increases Cx43 gap junction function	Rotigaptide (YPXGAG)	Antiarrhythmic peptide analogue	Ischemic injury of the heart	Macia et al. (2011)
Cx43	Decreases Cx43 gap junction formation and decreases Cx43 hemichannel formation	α-Connexin carboxyl terminal peptide (ACT1) (RQPKIWFPNRRKPWKK – RPRPDDLEI)	Peptide incorporating the zonula occludens-1 (ZO-1)–binding domain of Cx43	Skin scars	Ghatnekar et al. (2015); Jiang et al. (2019)
Cx43	Decrease Cx43 levels	Peptide5 (Peptagon^{TM}) (VDCFLSRPTEKT)	Cx43 extracellular loop 2 mimetic peptide	Retinal diabetic injury	O'Carroll et al. (2008)
Cx43	Cx43 hemichannel blocker	Gap19 (YGRKKRRQRRR-KQIEIKKFK	Cx43 intracellular loop mimetic peptide	Gap Junctional Communication in Astrocytes	Abudara et al. (2014)
Cx43	Cx43 hemichannel blocker	Xentry–Gap 19 LCLRPVGG-KQIEIKKFK	Cx43 intracellular loop mimetic peptide	Choroidal neovascularisation (wet AMD model)	Coutinho et al. (2019)