Chapter 6

Involvement of Gap Junction Proteins in Infectious Diseases Caused by Parasites

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Abstract

Parasitic diseases affect low-income nations with health consequences that affect the economy of these countries. Research aimed at understanding their biology and identification of potential targets for drug development is of the highest priority. Inhibitors of channels formed by proteins of the gap junction family such as suramin and probenecid are currently used for treatment of parasitic diseases caused by pathogenic protozoan. Gap junction proteins are present in both vertebrates and invertebrates permitting direct and indirect cellular communication. These cellular specializations are formed by two protein families corresponding to connexins (vertebrates) and innexins (invertebrates). In addition, a third protein family composed by proteins denominated pannexins is present in vertebrates and shows primary sequence homology to innexins. Channels formed by these proteins are essential in many biological processes. Recent evidences suggest that gap junction proteins play a critical role in bacterial and viral infections. Nonetheless, little is known about the role of these channels in parasitic infections. In this chapter, we summarized the current knowledge about the role of gap junction family proteins and channels in parasitic infections.

Keywords: connexins, pannexins, innexins, cellular communication, parasites

1. Introduction

The gap junction protein families include connexin, pannexin, and innexin proteins [1]. Connexin and innexin proteins form gap junction channels, which connect the cytoplasm of neighbouring cells, or connexin, pannexin and innexin proteins form channels (a half of gap junction

channel) that connect the intra- and extracellular milieu [1]. In humans, connexins and pannexins are encoded by 21 and 3 genes, respectively [1]. Moreover, it has been identified 25 and 8 innexin genes in *Caenorhabditis elegans* and *Drosophila melanogaster*, respectively [2, 3]. It is known that Panx1 channels participate in response to bacterial and viral infections; however, little is known about the role of Panx1 channels and gap junction channels in infections caused by parasites [4–7] (**Table 1**). For example, *Shigella flexneri*, which is a causative agent of bacillary dysentery, causes opening of hemichannels formed by connexin 26 [4], which favours its spread and invasion [4]. Also, blockade of Panx1 channels has been shown to inhibit HIV replication in CD4(+) T lymphocytes [6]. In this chapter, we summarized the current knowledge about how the parasite infections modulate channels formed by gap junction proteins in host cells and the cellular pathways involved in this phenomenon. We also comment on channel blockers currently used in medicine for treatment of parasitic diseases caused by pathogenic protozoan (**Table 2**).

Gap junction proteins	Parasite	Cell type	Effects	References
Cx43	*Trypanosoma cruzi*	Cardiomyocytes	Downregulated	[43]
		Astrocytes	Downregulated	[44]
		Leptomeningeal cells	Downregulated	[44]
		Cardiomyocytes	Downregulated	[48]
		Cardiomyocytes	Downregulated	[51]
		Cardiomyocytes and heart human biopsies	Downregulated	[50]
	Toxoplasma gondii	Astrocytes	Downregulated	[44]
		Leptomeningeal cells	Downregulated	[44]
Cx26	*Toxoplasma gondii*	Astrocytes	Downregulated	[44]
Cx37	*Trypanosoma cruzi*	Heart from chagasic mouse	Upregulated	[52]
Cx40	*Trypanosoma cruzi*	Heart from chagasic mouse	Not change	[52]
Cx45	*Trypanosoma cruzi*	Heart from chagasic mouse	Not change	[52]
	Trypanosoma cruzi	Cardiomyocytes	Upregulated	[51]
Panx1	*Plasmodium falciparum*	Human erythrocytes	Increased ATP release	[54]
	Entamoeba histolytica	Human monocytic cells	Increased ATP release	[60]
AGAP001476	*Plasmodium falciparum*	Midgut tissues from *Anopheles gambiae*	Upregulated	[61]
	Plasmodium berghei	Midgut tissues from *Anopheles gambiae*	Upregulated	[61]

Table 1. Summary of published works on the effect of parasite infections on the gap junction proteins.

Drug	Commercial name	Presentation and quantity	Company	Country production
Probenecid	Probalan	Tablets 500 mg	Lannett	USA
Probenecid	Probenecid & Colchicine	Tablets 500 mg	Watson	INDIA
Probenecid	Probenecid	Tablets 500 mg	Mylan	USA
Probenecid	Probenecid & Colchicine	Tablets 500 mg	Ingenus	USA
Suramin	Germanin	Vial 1 g	Bayer	Germany

Table 2. Commercial drugs.

2. The family of gap junction proteins

Gap junction proteins are present in both vertebrates and invertebrates from mesozoa to mammals [8]. In chordate animals, gap junction channels are encoded by a family of genes called connexins (Cxs) [9] (**Table 3**). In addition, gap junction communication of invertebrate is mediated via another family of proteins called innexins (Inxs) [8]. Inx homologues have been identified in vertebrates and were termed pannexins (Panxs) [10]. Members of the same protein family oligomerize in hexamers forming channels, which are inserted into the plasma membrane connecting the intra- and extracellular milieu [8]. Whereas, docking of two channels forms intercellular channels (gap junction channels) that connect the cytoplasm of two cells [8]. It has been proposed that Panx-based channels do not form gap junction channels due to their post-translational glycosylation [11]. However, this theoretical prediction might be proved wrong because in exogenous cells systems forms functional gap junctions. In support to this possibility is the fact that Panx1 expressed in exogenous cell systems forms functional gap junctions [12, 13].

2.1. Genes

The first Cx gene was cloned in 1986, and there are at least 21 Cx isoforms in the human genome [8, 14]. Most Cx genes have a first exon containing only 5′-untranslated region (UTR) sequences and a large second exon containing the complete coding region sequence (CDS) as well as all remaining untranslated sequences [8]. Exceptions to this gene structure are the Cx32, Cx36, and Cx45 genes [8]. Panx are termed as Panx1, Panx2, and Panx3 and are present both in invertebrate and chordate genomes [15, 16]. The human and mouse genome contain three Panx-encoding genes [10]. The genomic sequence revealed that human Panx1 contains five exons with four introns [10]. Moreover, Panx2 and Panx3 contain four exons [10]. The first Inx gene was identified in 1998 as a result of genome sequencing of nematode *C. elegans* [17]. Actually, 25 and 8 Inx genes in *C. elegans* and *D. melanogaster* have been identified, respectively [2, 3]. Usually, Inx genes are encoded on multiple exons and have the potential to produce more than one protein by differential splicing [18]. Recently, viral homologs of Panxs/Inxs were identified in *Polydnaviruses* and denominated vinnexins (Vinx) [19].

Abbreviations	Definitions
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CDS	Coding region sequence
Cx	Connexin
DCSF	Divalent cation solution free
HC	Hemichannel
Inx	Innexin
Panx	Pannexin
P.Falciparum	*Plasmodium falciparum*
PMA	Phorbol 12-myristate 13-acetate
T. cruzi	*Trypanosoma cruzi*
UTR	Untranslated region
Vinx	Vinnexin

Table 3. Abbreviations.

2.2. Secondary structure

Cx, Inx, and Panx proteins share the same membrane topology, characterized by four transmembrane domains connected by two extracellular loops and a single cytoplasmic loop [20]. These extracellular loops contain 2 (for Panxs and Inxs) or 3 (for Cxs) highly conserved cysteine residues [21]. Moreover, the intracellular loop is highly variable [21]. The four transmembrane domains are well-conserved among members of the same family of proteins and form alpha-helical sheets that contribute to the wall of the HC and line its central hydrophilic space [21]. All members of the 3 families have their NH_2- and COOH-terminal region within the cytoplasm [21]. The COOH-terminal region differs in length and sequence in all gap junction proteins [21]. Inx proteins have a highly conserved pentapeptide YYQWV close to, or at, the beginning of the second transmembrane domain [22].

2.3. Gap junctional channels

Gap junctions are specialized cell-to-cell junctions that mediate direct intercellular communication between cells [8]. Depending on whether the two interacting channels are made of the same or different Cxs, gap junction plaques are formed by homo- and heterotypic channels, respectively, with distinct biophysical characteristics [21]. These intercellular channels are essential in several Physiologic tissue functions such as electrical conduction between cardiomyocytes [23], development and regeneration of skeletal muscle [24], endocrine gland secretion [25], and ovarian folliculogenesis [26]. They are also implicated in pathophysiological conditions including hereditary deafness [27], cataract [28], ectodermal dysplasias [29], tumorigenesis [30], and neuroinflammatory responses [31].

2.4. Hemichannels (HCs)

Several studies have shown that HCs allow the bidirectional passage of ions and cytosolic signaling molecules, such adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD^+), glutamate, glutathione, and prostaglandins [32]. Under physiological conditions, HCs are involved in the regulation of cell volume [33], vascular tone [34], hemostasis [35], and neuroglia paracrine interactions [36], among others. However, HCs have been the focus of interest because of their relevance in pathological conditions, including metabolic inhibition [37], stroke [38], myocardial infarction [39, 40], ischemic neuronal death [41], spinal cord injury [42], diarrhoea during infectious enteric disease [5], and keratitis-ichthyosis-deafness syndrome [43].

The presence and functional HCs in the plasma membrane have been determined through several techniques such as electrophysiology, uptake of fluorescent dyes, and release of adenosine triphosphate (ATP) [44]. Due to the existence of non-selective channels in the plasma membrane, there are significant considerations for studying HCs [45]. These criteria are as follows: (i) cell expression of at least one Cx/Panx isoform at the plasma membrane, (ii) the ability of the cells to incorporate or release molecules, (iii) to mediate membrane currents with conductance associated to Cx/Panx HCs, (iv) the abolishment of HC function using a pharmacologic approach (e.g. La^{3+}, probenecid, or carbenoxolone) or mimetic peptide blockers (Gap19, Gap26, Gap27 for specific Cx HCs or ^{10}Panx1 for Panx1 HCs), and (v) to demonstrate that blockade of HCs affect physiological responses [44, 45].

3. Gap junction proteins in parasitic infections

3.1. Connexins (Cxs)

3.1.1. Functional studies

Pioneering studies in the 1990s by de Carvalho et al., 1992 showed that *Trypanosoma cruzi* induces a gap junction alteration in cardiac myocytes [46] (**Table 1**). They showed that *T. cruzi* infection reduces the junctional conductance and Lucifer yellow transfer in cardiomyocytes, revealing that this parasite infection reduces the channel function of host cells [46]. The same researchers also showed that infection caused by *Toxoplasma gondii* reduces intercellular communication in astrocytes and leptomeningeal cells [47]. Recently, we demonstrated that *T. cruzi* increases dye uptake via HCs in non-confluent Cx43-HeLa cells [7]. Suramin, an anti-protozoa drug, inhibits the activity of HCs [48]. Suramin causes a concentration-dependent inhibition of a divalent cation-free solution (DCSF)-induced dye uptake in a rat kidney epithelial cell line [48]. Also, suramin blocks the DCSF-induced ATP release in a rat kidney epithelial cell line [48]. Interestingly, the suppressive effect of suramin on the influx of dye and efflux of ATP was not reproduced by PPADS, a broad-spectrum antagonist of P2 receptors, suggesting that the action of suramin on HCs is independent of its action on P2 purine receptors [48]. Also, suramin (300 μM for 12 h) did not affect the total Cx43 level [48]. Moreover, prolonged incubation of *T. cruzi*-infected LLC-MK2 cells in the presence of suramin (500 μM) causes morphological

changes on trypomastigote forms characterized by an accentuated decrease on parasite motility [49]. In trypomastigotes, suramin causes a decrease in ~5% in cell length and an increase in ~43% in cell width [49]. Also, it was observed that 95% of trypomastigotes exposed to suramin present a partial or even total detachment of the flagellum from the cell body [49].

3.1.2. Protein expression alterations

At the protein level, *T. cruzi* reduces Cx43 levels at junctional membrane regions in neonatal rat cardiomyocytes [46, 47]. Other studies in mouse cardiomyocytes showed that *T. cruzi* reduces Cx43 levels at 24-h post-infections [50]. Interestingly, cardiomyocytes with pronounced decrease in Cx43 protein levels showed an increased number of intracellular amastigotes, suggesting a direct relationship between host cell parasitism and Cx43 downregulation in *vitro* [50]. Also, it has been described that infection with *T. cruzi* or *T. gondii* reduces the levels of Cx43 and Cx26 protein in astrocytes or leptomeningeal cells [47]. *In vivo* model of *T. cruzi* infection showed a significant reduction in myocardial Cx43 protein levels [50]. *Swiss Webster* mice infected with *T. cruzi* showed a reduction in Cx43 levels in atrium and ventricles at 11- or 30-day post-infection, respectively [50]. Moreover, brain slices prepared from mice infected with *T. gondii* showed complete absence of Cx43 immunoreactivity within the cysts and marked reduction in the surroundin tissue [47]. The same study described a reduction of Cx43 protein levels in whole brains of *T. cruzi*-infected mice [47]. In monkeys, *T. cruzi* infection causes significant Cx43 loss in the cardiac tissue [51]. Clinical studies described that samples from chagasic patients showed alterations of cardiac Cx levels [52]. Immunohistochemical analysis of left ventricle biopsies from subjects with chronic chagasic disease showed reduction in both mean number (<20%) and size (<2.2 fold) of Cx43 plaques [52].

3.1.3. Gene expression regulation

Gene profiling of *T. cruzi*-infected cardiomyocytes revealed downregulation at 48 h after infection of *GJA1* and *GJC1* genes, which encode for Cx43 and Cx45, respectively [53]. Upregulation of *GJA4* gene encoding Cx37, a major endothelial cell Cx, was also described [54].

3.1.4. Cx knock-out mice and parasitic infections

Hepatic granulomas induced by *Schistosoma mansoni* infection in Cx43 deficient mice showed a higher degree of fibrosis and a reduced index of cell proliferation at 8 and 12 weeks after infection [55]. However, no differences in the average area of granulomas or number of cells per granuloma were observed [55]. The authors of the above mention work suggested that deletion of one allele of Cx43 gene could be the cause of reduced gap junction channels that modifies the interactions between granuloma cells, thereby modifying the characteristics of granuloma [55].

3.2. Pannexins (Panxs)

It has been demonstrated that *Plasmodium falciparum* infection induces ATP release via Panx1 channels in human erythrocytes [56]. A mixture of isoproterenol (β-adrenergic agonist),

forskolin (adenylate kinase activator), and papaverine (phosphodiesterase inhibitor) induce cyclic adenosine monophosphate (cAMP)-dependent ATP release in human erythrocytes, and this effect was 3.8-fold higher in trophozoite-infected erythrocytes compared to uninfected erythrocytes [56]. Interestingly, this effect was reduced by 100 μM carbenoxolone or 100 nM mefloquine, two Panx1 channel blockers [54]. These authors suggest that the increased ATP release from infected red cells could be mediated by by Panx1 channels [56]. Several studies have shown that probenecid has a marked antimalarial effect [57–59]. The incubation of *P. falciparum* with probenecid shows antimalarial activity at concentrations >150 μM at day 2 of treatment [57]. However, probenecid at concentration <150 μM increases the *P. falciparum* sensitivity to antifolate drugs [57]. For example, in the presence of 50 μM probenecid, the IC_{50} (nM) was reduced from 1.42 ± 0.52 to 0.52 ± 0.36, from 215 ± 150 to 36.50 ± 26.80 and from 33.53 ± 12.30 to 1.77 ± 2.70 for pyrimethamine, sulfadoxine, and dapsone, respectively [57]. Probenecid also reverses the chloroquine resistance of *P. falciparum* and increases piperaquine activity *in vitro* [57]. Also, probenecid chemosensitize a multidrug-resistant strain V1S of *P. falciparum* to piperaquine [59]. Moreover, antimalarial drugs such as artemisinin and artesunate also inhibit Panx1 channel [60]. For example, artesunate causes a concentration-dependent inhibition of membrane current mediated by Panx1 channels with an IC_{50} of 450 μM, while 200 μM artemisinin causes a membrane current reduction of about 20% in *Xenopus* oocytes [60]. Moreover, artemisinin also inhibits dye uptake with an IC_{50} of 0.14 μM in frog erythrocytes [60]. Moreover, 100 nM mefloquine significantly reduces voltage-activated Panx1 channel currents in astrocytes from Cx43-null mice [61]. Also, mefloquine blocks dye uptake induced by ATP in astrocytes from Cx43-null mice [61]. In addition, it has been described that *Entamoeba histolytica* induces ATP release into the extracellular space through opening of Panx1 channels in macrophages [62]. Incubation with 500 μM ^{10}Panx1, a mimetic blocking peptide of Panx1 channels, abolished ATP release in response to *E. histolytica* in phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 human monocytic cells [62]. The same results were observed with 100 μM carbenoxolone or 250 μM probenecid [62].

3.3. Innexins (Inxs)

It has been demonstrated that Inx proteins have a critical role for mediating anti-*Plasmodium* responses in *Anopheles gambiae* [63]. It has been shown that AGAP001476 mRNA levels were induced during *Plasmodium* infection in *Anopheles* midguts [63]. The carbenoxolone-treated mosquitoes showed an increase in both *Plasmodium* oocyst number and infection rate [63].

4. Possible role of gap junction proteins in parasite infections

Although the role of gap junction proteins in parasitic infections has not been fully elucidated, they could participate in responses that include changes in plasma membrane permeability, signalling, and inflammasome activation.

4.1. Alteration of the host cell membrane permeability

A common condition and often necessary for infection is the alteration of the host cell membrane permeability [64, 65], and hemichannel activity can considerably affect the permeability of the cell membrane in mammalian cells [66]. For example, *T. cruzi* alters the plasma membrane permeability in host cells during different stages of the disease [65, 67–69]. Another parasite that alters the plasma membrane permeability is *P. falciparum*. This parasite invades and replicates asexually within human erythrocytes and enhances plasma membrane permeability in different stages of the disease [70, 71]. The apicomplexan *Babesia divergens* also increases the membrane permeability of erythrocyte [64]. The mechanism for such erythrocyte permeabilization is different in transport rates, solutes selectivity, and temperature dependence compared with the alteration induced by *P. falciparum* [64].

4.2. Intracellular Ca^{2+} mobilization

Gap junction proteins participate in Ca^{2+} signalling, and they constitute one pathway for intercellular Ca^{2+} wave propagation in cardiomyocytes, astrocytes, and osteocytes, among other cell types [72]. In addition, Cx26, Cx32 and Cx43 HCs are permeable to Ca^{2+} [73–76] and might be involved in initiation of intracellular rise in Ca^{2+} signals. In protozoan infections, a key process in early stages of invasion is the rise in cytosolic Ca^{2+} concentration [77]. For example, when *T. cruzi* comes into contact with the host cell, triggers a transient increase in cytosolic Ca^{2+} concentration that induces lysosome exocytosis in host cells [65, 77]. This process is required for cell invasion, because chelating the intracellular Ca^{2+} transients in host cells reduces the entry of the parasite into the cell [78]. **Figure 1** shows a model of the possible participation of pannexin channel in intracellular Ca^{2+} mobilization during the invasion by *T. cruzi*.

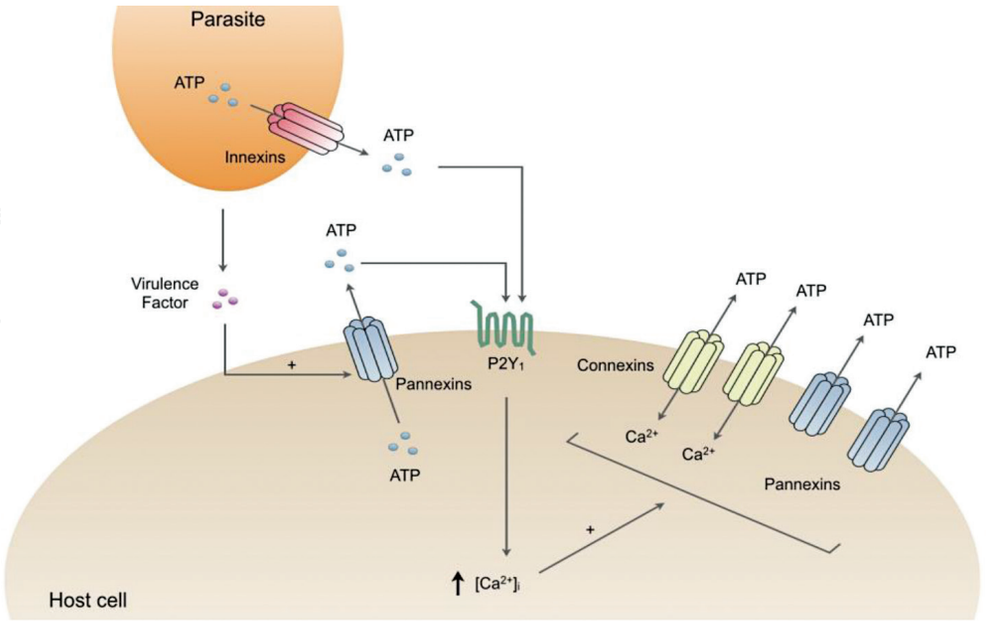

Figure 1. Model of the possible participation of gap junction proteins in the invasion of host cells by *Trypanosoma cruzi*. Parasites release a virulence factor, which opens Pannexin 1 channels allowing the release of ATP to the extracellular milieu. The ATP activates $P2Y_1$ receptors and promotes Ca^{2+} release from intracellular stores generating intracellular Ca^{2+} transients, which induces the opening of new hemichannels formed by connexin or pannexins. These effects promote the *Trypanosoma cruzi* invasion.

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4.3. Activation of the inflammasome

The inflammasome activation triggers innate immune defence by inducing the processing of pro inflammatory cytokines, such as IL-1, in a caspase 1-dependent manner [79]. Panx1 channels play a key role in inflammasome activation [79]. It has been proposed that small pathogen-associated molecule patterns (PAMPs) can gain cytosolic access via the $P2X_7$ receptor/Panx1 ($P2X_7R$/Panx1) complex and activate the inflammasome [79].

5. Conclusions

Parasitic infections affect predominantly underprivileged areas of the world and represent serious life-threatening conditions in high-risk groups such as young children, elderly, and immune deficient subjects. Also, therapeutic options include a wide variety of compounds with considerable toxic and undesirable side effects. The introduction of knockout animals and specific inhibitors has increased our understanding about the role of Cx, Panx, and Inx proteins in the pathophysiology of many infectious conditions. However, their participation in infections caused by parasites is not completely elucidated. A variety of methods have been used to evaluate changes in gap junction protein expression during parasite infections. These methods include Western blot, immunofluorescence, or functional studies such dye uptake, dye coupling, or current measurements with electrophysiological techniques. In summary, the available data suggest that the parasite infections modulate gap junction proteins in host cells. In this context, characterization of gap junction proteins and their functions in protozoan parasites might facilitate the design of effective new therapies to fight protozoan infections such as malaria and Chagas disease.

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