Chapter

Possible Role of Gap Junction Channels and Non-Junctional Channels in the Infection Caused by *Trypanosoma cruzi*

José Luis Vega, Camilo Juyumaya, Luis Rodríguez, Juan Güiza, Camila Gutíerrez, Iván Barría and Juan C. Sáez

Abstract

Chagas disease affects low-income nations with health consequences that impact the economy of those countries. Interestingly, inhibitors of channels formed by proteins of the gap junction family, such as suramin and boldine, exhibit trypanocidal activity. Gap junction proteins are integral membrane proteins present in both vertebrates and invertebrates that participate in cellular communication. These proteins form gap junction channels, which connect the cytoplasm of neighboring cells or non-junctional channels that connect the intra- and extracellular milieu. Interestingly, *Trypanosoma cruzi* modulates the expression of proteins of the gap junction family or modify the activity of the channels formed by these proteins in host cells. Moreover, Lucifer yellow microinjected into fibroblast was incorporated into associated trypanosomes of *Trypanosoma musculi*, suggesting the possibility of direct communication via gap junction channels between them. In this chapter, we summarized the current knowledge about the possible roles of gap junction family proteins in Chagas disease.

Keywords: connexin, pannexin, innexin, hemichannels, infection

1. Introduction

Chagas disease affects low-income nations with health consequences that impact the economy of these countries [1]. Research aimed at understanding their biology and identification of potential targets for drug development is the highest priority [1]. Interestingly, inhibitors of channels formed by proteins of the gap junction family such as suramin and boldine have trypanocidal activity and some of them are currently used for treatment of parasitic diseases such Human African Trypanosomiasis [2–5]. Also, studies have shown that infections caused by *Trypanosoma cruzi* (*T. cruzi*) modulate the expression of proteins of the gap junction family or modify the activity of the channels formed by these proteins in host cells [6–11]. Moreover, previous studies have shown gap junction type structures in *Trypanosoma musculi* [12]. In this chapter, we summarized the current knowledge about the role of gap junction family proteins in Chagas disease.

2. Gap junction proteins

Gap junction proteins are present in both vertebrates and invertebrates from mesozoa to mammals [13]. The protein families include connexins (Cxs), innexins (Inxs) and pannexins (Panxs) [14, 15]. They are integral membrane proteins that participate in cellular communication playing a relevant role in several physiological processes [15]. In vertebrates, Cxs and Panxs are present, while in invertebrates, only Inxs are present [15]. In humans, 21 and 3 genes encode Cx and Panx proteins, respectively [14]. Most Cx genes contain two exons and an intron of variable length [14]. The Panx1 and Panx2 genes contain 5 exons, while the Panx3 gene contains 4 exons [16]. Moreover, Inx genes have been found in the phylum *Arthropoda* [17–20], *Nematoda* [21], *Chordata* [22], *Annelida* [23], *Platyhelminthes* [24], *Cnidaria* [25], and *Mollusca* [26]. In *Drosophila melanogaster*, the Inx genes have the potential to be differentially spliced [18], while in *C. elegans*, 15% of genes are found in operons and three pairs of the innexins are polycistronic such as inx-12 and inx-13, inx-16 and inx-17, and inx-21 and inx-22 [27]. Regarding the structure of the protein topology, hydropathy plots of several Inx, Cx and Panx proteins have predicted the presence of four hydrophobic domains with transmembrane spanning regions and the extracellular loops with several highly conserved residues [28]. In contrast, the cytoplasmic loop and the carboxy terminus vary extensively in length and amino acid composition [28].

2.1 Gap junction channels

Cx and Inx proteins form gap junction channels, which connect the cytoplasm of neighboring cells [14, 15]. Moreover, Cx, Panx and Inx proteins form non-junctional channels that connect the intra- and extracellular milieu [14]. Gap junction plaques are formed by a variable number of homo- and/or heterotypic gap junction channels with distinct biophysical characteristics [29]. Structurally, they are formed by oligomers of Cx, Inx, or Panx proteins, which co-oligomerize into the same (homomeric) or mixed (heteromeric) channels [30]. Gap junction channels are essential in several physiologic functions such as electrical conduction between cardiomyocytes [31], development and regeneration of skeletal muscle [32], endocrine gland secretion [33], and ovarian folliculogenesis [34]. Also, they are implicated in pathophysiological conditions such as hereditary deafness [35], cataract [36], and tumorigenesis [37].

Gap junction proteins can also form non-junctional channels, which play important roles as autocrine/paracrine cellular communication pathways [14]. They are permeable to ions and several metabolic and signaling molecules such as glucose, glutamate, glutathione, adenosine, NAD+ (superindice) and ATP among others [14]. It has been proposed that Panxs do not form gap junctions, however, they form plasma membrane channels (named pannexons) with some properties similar to those of the non-junctional channels formed by Inxs or Cxs also called hemichannels [15].

The pannexons are permeable to ATP when are activated with certain stimuli such a low oxygen, mechanical stress, and elevated extracellular potassium ion concentration [38]. Otherwise, the Panx1 channel is selective for chloride ions and exhibits no ATP permeability when stimulated simply by depolarization to positive potentials or the C-terminal is cleaved by proteolysis [38]. Since we found increased Panx1 channel activity and increase in ATP release in cells infected with *T. cruzi* [11], it suggested that under this condition the Panx1 channel does not undergo proteolysis and adopt the large channel configuration [11, 38]. In addition, it has been described that Panx1-based channels are regulated by mechanical stress [39].

Inx-based hemichannels are activated by increased extracellular potassium ion concentration and by membrane depolarization [40]. Furthermore, Panx1- and Inx-based channels are inhibited by low concentration of carbenoxolone (<5 μM) or high probenecid concentrations (>500 μM) [41, 42].

Moreover, Cx-based hemichannels are regulated by intracellular acidification [43], intracellular Ca^{2+} [44], intracellular Na^{+} [45], pro-inflammatory cytokines [46], positive membrane potentials [47], phosphorylation [48], and S-nitrosylation [49]. They are inhibited by lanthanum chloride, carbenoxolone (>50 μM), and Cx mimetic peptides GAP16, GAP27, and GAP19 [15].

3. Gap junction and Chagas

3.1 Connexins

In 1992, Spray's group showed for the first time that gap junctions were altered between rat neonatal cardiomyocytes infected with the Tulahuen strain of *T. cruzi* (**Table 1**) [6]. They showed that junctional conductance and intercellular transfer of Lucifer yellow was reduced between cardiomyocytes infected with *T. cruzi* (**Figure 1**) [6]. In 1998, the same group described that Tulahuen strain of *T. cruzi* reduced gap junction communication between rat astrocytes and between rat leptomeningeal cells as well [7]. Also, these authors demonstrated through immunocytochemistry studies that Cx43 reactivity was significantly reduced in whole brains from rats acutely infected with *T. cruzi* [7]. In 2008, *in vitro* studies showed that Y strain of *T. cruzi* increased the amount of Cx43 at 1 hour postinfection and reduced it at 72 hour postinfection in mouse cardiomyocytes (**Figure 1**) [8]. Moreover, they demonstrated through immunoblotting analysis that the amount of Cx43 was significantly reduced in heart atria and ventricles from mice infected with Y strain of *T. cruzi* at 11 days postinfection [8]. In 2009, Waghabi and collaborators demonstrated that the number and length of Cx43 plaques were reduced in heart biopsies of human chronic chagasic patients [9]. In 2013, we described that CL Brener strain of *T. cruzi* increased the Cx43 hemichannel activity in HeLa cells stably transfected with Cx43 (**Figure 1**) [15]. Also, we observed that the number of amastigotes was 3 times higher in

Year	Strain	Cell type	Effect	Ref.
1992	Tulahuén	Cardiomyocytes	Decrease gap junction channels activity	[6]
1998	Tulahuén	Astrocytes	Decrease gap junction channels activity	[7]
1998	Tulahuén	Leptomeningeal cells	Decrease gap junction channels activity	[7]
2008	Y	Cardiomyocytes	Increase Cx43 amount at 1 h p.i.	[8]
2008	Y	Cardiomyocytes	Decrease Cx43 amount at 72 h p.i.	[8]
2009	n.d.	Heart biopsies	Decrease Cx43 levels at 11 days p.i.	[9]
2013	CL Brener	HeLa-Cx43	Increase Cx43-hemichannel activity	[15]
2014	Brazil	White adipose tissue	Increase Cx43 amount at 30 and 90 days p.i.	[10]
2014	Brazil	Brown adipose tissue	Decrease Cx43 amount at 30 and 90 days p.i.	[10]
2018	H510	Cardiomyocytes	Increase Panx1 channel activity	[11]

n.d.: not determinated; p.i.: post-infection.

Table 1.
Summary of the studies that describe the effect of T. cruzi on gap junction protein family.

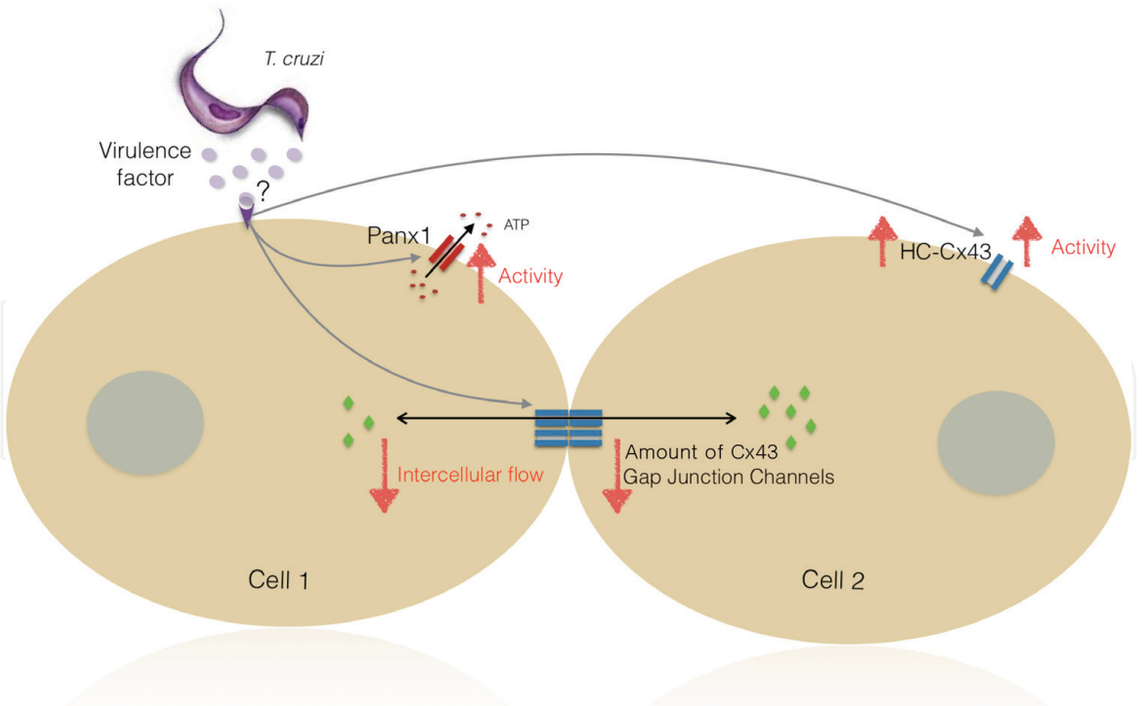

Figure 1.
Model that summarizes the main effects of the Trypanosoma cruzi on cellular communications mediated by gap junction channels. Parasites release a virulence factor that opens Panx1 channels allowing the release of ATP to the extracellular milieu [11]. Also, the parasite causes a reduction of intercellular communications mediated by gap junctions [6]. Contrarily, the parasite increases the activity of the hemichannels present in the plasma membrane of the infected cells [15].

HeLa-Cx43 as compared to HeLa parental cells at 48 hours postinfection [15]. In 2014, Burke and collaborators demonstrated that Brazil strain of *T. cruzi* reduced the amount of Cx43 in brown adipose tissue at 30 and 90 days postinfection (**Figure 1**) [10]. Moreover, *T. cruzi* infection caused an increased the amount of Cx43 protein in white adipose tissue at 30 and 90 days postinfection [10].

3.2 Pannexins

In 2018, we found that H510 strain of *T. cruzi* increased the Panx1 channel activity in rat neonatal cardiomyocytes at 1 hour postinfection [11]. Interestingly, the increased pannexon activity induced by the parasite was directly related to an elevated ATP release [11]. This is relevant because ATP has been proposed as a key molecule in *T. cruzi* host cell invasion [11, 50]. For example, blockade of $P2Y_1$ receptors with a MRS2179, a selective $P2Y_1$ antagonist, reduced *T. cruzi*-evoked Ca^{2+} transients in rat cardiomyocytes [11]. Moreover, inhibition of mitochondrial ATP production by treating parasites with rotenone plus antimycin A reduced the infectivity of the parasites [50]. Also, pre-treatment with pannexon activity-blocking drugs significantly reduced the number of intracellular parasites in cardiomyocytes infected with H510 strain of *T. cruzi* [11]. For instance, cells exposed to 100 μM ^{10}Panx1 or 400 μM probenecid showed a 114 ± 2 and 71 ± 2 parasites/500 cells, respectively, versus 5001 ± 2 parasites/500 cells in control condition [11]. Interestingly, scanning and transmission electron microscopy studies have demonstrated the presence of reminiscent gap junction at physical interactions between *Trypanosoma musculi* and mice spleen-derived adherent fibroblasts [12]. Also, Lucifer yellow microinjected into fibroblast was incorporated into associated trypanosomes, suggesting that those gap junctions were functional [12].

4. Gap junction channel blocking compounds as antiparasitic drugs

Interestingly, several drugs that block the activity of gap junction channels have been described as potent anti-parasitic agents. Among them are: probenecid, boldine and suramin, among others (**Table 2**) [2–4].

4.1 Probenecid

Human clinical trials have shown that probenecid, an inhibitor of a non-junctional channel formed by Panx1 has an antimalarial effect [2]. The authors described that 50 μM probenecid caused an increase in the sensitivity of highly resistant V1S strain of *Plasmodium falciparum* to pyrimethamine, sulfadoxine, chlorcycloguanil, and dapsone by seven-, five-, three-, and three-folds, respectively [2].

4.2 Suramin

Suramin, a general antagonist of purinergic receptors (P2Y and P2X) [51] and blocker of Panx1 channels [52], exhibits anti-parasitic properties [3]. Culture of LLC-MK2 cells treated with suramin (500 μM) during the intracellular development of *T. cruzi*, caused morphological changes in the parasites; increase in parasite width, and partial or complete detachment between flagella and cell body [3]. Interestingly, suramin is one of the 5 approved drugs for treatment of sleeping sickness [5].

4.3 Boldine

Boldine, an alkaloid obtained from Boldo tree, which blocks the activity of Cx43-formed hemichannels, and Panx1 hemichannels exhibit, an anti-parasitic activity [53, 54]. Boldine at concentrations above 500 μM reduces the epimastigotes growth of Tulahuen, LQ and DM28c strains of *T. cruzi* [4]. Since the active compound also inhibited cell respiration, it was suggested that these drugs may block the mitochondrial electron transport [4].

Agent	Chemical Formula	Class	Diseases	Target
Antiparisitic drugs				
Suramin	$C_{51}H_{40}N_6O_{23}S_6$	Acid	HAT	Cx43
Nifurtimox	$C_{10}H_{13}N_3O_5S$	Nitrofurans derivates	Chagas	n.d.
Benznidazol	$C_{12}H_{12}N_4O_3$	Nitroimidazole derivates	Chagas	n.d.
Pentamidine	$C_{19}H_{24}N_4O_2$	Amidine	HAT	n.d.
Melarsoprol	$C_{12}H_{15}AsN_6OS_2$	Arsenical	HAT	n.d.
Channel blocker drugs				
Probenecid	$C_{13}H_{19}NO_4S$	Sulfoamida	Malaria	Panx1
Carbenoxolone	$C_{34}H_{50}O_7$	Terpenes	n.d.	Panx1, Cx43, Inxs
Oleamide	$C_{18}H_{35}NO$	Amide	n.d.	Panx1
Mefloquine	$C_{17}H_{16}F_6N_2O$	Quinolines	n.d.	Panx1
Boldine	$C_{19}H_{21}NO_4$	Alkaloid	n.d.	Panx1, Cx43

HAT: Human African Trypanosomiasis; n.d.: not determined.

Table 2.
Antiparasitic drugs and channel blocker drugs.

5. Conclusions

Chagas diseases affect predominantly underprivileged areas of Latin America, but attention has been increasing lately due to the rise in people migration habits, intercontinental travels, and immune suppressed patients [1]. Unfortunately, current therapeutic options include only two compounds (nifurtimox and benznidazole) with considerable toxicity and side effects, so the new drug development is of the highest priority [1]. Hemichannels are involved in the regulation of plasma membrane permeability in ischemic insults or metabolic inhibition [55, 56]. Moreover, alterations of plasma membrane is a common phenomenon in parasite-induced infections such malaria and *T. cruzi*, among others [57, 58]. Thus, hemichannels could be key players in parasite-induced plasma membrane permeabilization. All the above data support the importance of studying the possible role of hemichannels in parasitic infections. They could be potential targets for the development of new compounds to limit parasite infections or tissue/organ damage induced by their presence.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author details

José Luis Vega[1*], Camilo Juyumaya[1], Luis Rodríguez[1], Juan Güiza[1], Camila Gutíerrez[1], Iván Barría[2] and Juan C. Sáez[3,4]

1 Gap Junction and Parasites Laboratory (GaPaL), Antofagasta Institute, Universidad de Antofagasta, Antofagasta, Chile

2 Centro Interdisciplinario de Neurociencia de Valparaíso, Valparaíso, Chile

3 Instituto de Neurociencias, Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Chile

4 Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile

*Address all correspondence to: joseluis.vega@uantof.cl

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