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Trypanosoma cruzi Infection Induces Pannexin-1 Channel Opening in Cardiac Myocytes

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Abstract. *Trypanosoma cruzi*, the etiological agent of Chagas diseases, invades the cardiac tissue causing acute myocarditis and heart electrical disturbances. In *T. cruzi* invasion, the parasite induces $[Ca^{2+}]_i$ transients in the host cells, an essential phenomenon for invasion. To date, knowledge on the mechanism that elicits transients of $[Ca^{2+}]_i$ during the infection of cardiac myocytes has not been fully characterized. Pannexin1 (Panx1) channel are poorly selective channels found in all vertebrates that serve as a pathway for ATP release. In this article, we demonstrate that *T. cruzi* infection results in the opening of Panx1 channels in cardiac myocytes. We show that pharmacological blockade of Panx1 channels inhibits *T. cruzi*–induced $[Ca^{2+}]_i$ transients and invasion in cardiac myocytes. Our results indicate that opening of Panx1 channels are required for *T. cruzi* invasion in cardiac myocytes, and we propose that targeting Panx1 channel could provide new potential therapeutic approaches to treat Chagas disease.

INTRODUCTION

The protozoan *Trypanosoma cruzi* is the etiological agent of Chagas disease, which is considered one of the most important neglected tropical diseases that causes approximately 10,600 deaths per year and 0.6 million disability-adjusted life per year.[1] *Trypanosoma cruzi* is an obligatory intracellular parasite, and therefore, it must activate mechanisms that enable its entrance into the host cells.[2,3] Depletion of intracellular free calcium concentration ($[Ca^{2+}]_i$) in the host cell inhibits parasite invasion, indicating that increase in $[Ca^{2+}]_i$ is essential for *T. cruzi* infection.[4,5] Previous studies have described that *T. cruzi*–induced $[Ca^{2+}]_i$ transients are not mediated by adrenergic receptor or L-type Ca^{2+} channel activation in cardiac cells.[6] However, our understanding of the cellular mechanism involved in *T. cruzi*–induced $[Ca^{2+}]_i$ transients in cardiac myocytes remains unknown. Pannexin1 (Panx1) is a member of the gap junction family proteins and forms a plasma membrane channel that allows passage of anions, cations, dyes, and ATP.[7] Panx1 channels are involved in a variety of cellular responses that includes apoptosis,[8] inflammation,[9] and innate immune response.[10] Cardiac cells expresses Panx1, and other members of the gap junction family proteins such as connexin (Cx)40, Cx43, and Cx45.[11] Several publications indicate that *T. cruzi* infection reduces gap junctional communication due to a decrease in the amount of Cx43 protein and mRNA in cardiac myocytes.[12,13] In agreement with these studies, we found that *T. cruzi* infection increases Cx43 hemichannel (a half of gap junction channel) activity in nonconfluent Cx43-HeLa cells.[14] In addition, we demonstrated that *T. cruzi* invasion depends on host hemichannel types.[14] Normally, Panx1 channels are mainly in a closed state; however, recent studies have demonstrated that pathogens such as *Chlamydia trachomatis* and HIV can induce opening of Panx1 channels during infection.[15,16] In the current study, we provide first evidence that a pathogenic protozoan *T. cruzi* induces opening of Panx1 channels. In addition, our data provide evidence that opening of Panx1 channels is necessary for Ca^{2+} transients during *T. cruzi* infection of cardiac myocytes. Furthermore, we show that pharmacological blockade of Panx1 channels inhibits *T. cruzi* invasion in cardiac myocytes.

MATERIALS AND METHODS

Antibodies and reagents. Polyclonal anti-Panx1 (#HPA016930), monoclonal anti-α-actin (#A5044), polyclonal anti-Cx43 (#C6219), and monoclonal anti-vimentin (#V6630) antibodies were purchased from Sigma-Aldrich (St. Louis, MO). Anti-rabbit (#111-095-003) or anti-mouse (#115-095-003) IgG antibodies coupled to FITC were purchased from Jackson ImmunoResearch (West Grove, PA). Carbenoxolone disodium salt (#C4790), adenosine 5'-triphosphate disodium salt hydrate (#A9187), probenecid (#P8761), and cytosine beta-D-arabinofuranoside (#C1768) from Sigma-Aldrich. MRS2179 tetrasodium salt (#0900), *iso*-PPADS tetrasodium salt (#0683), and ^{10}Panx1 peptide (#3348) were purchased from Tocris (Bristol, United Kingdom). The Fluo-3 AM calcium indicator (#F1242) and cell viability assay kits (#L34969) were obtained from Thermo Fisher Scientific (Cambridge, MA). Ethidium bromide was obtained from Winkler (Santiago, Chile).

Parasites. H510 strain of *T. cruzi* was isolated in 1968 from a domiciliary *Triatoma dimidiata* found in an area endemic for Chagas disease in Costa Rica and cloned.[17] *Trypanosoma cruzi*–virulent clone (H510 C8C3***hvir***) were maintained for 30 years in an alternate mouse-cell culture passage, and avirulent clones (H510 C8C3***lvir***) were maintained for 30 years in an axenic culture passage.[18] Epimastigote forms were grown at 27°C in LIT medium (containing 5.4 mM KCl, 150 mM NaCl, 24 mM glucose, 5% [v/v] liver extract, 0.02% [w/v] hemin, 2% [w/v] yeast extract, and 1.5% [w/v] tryptose) and supplemented with 5% fetal bovine serum. For preparation of supernatant (SN) fraction, freshly harvested trypomastigotes were washed with phosphate-buffered saline (PBS) and resuspended in DMEM medium containing 0.5% FBS at 2×10^8/mL. Trypomastigotes were then removed (15.000 rpm for 10 minutes at 4°C) and supernatants were stored at −80°C.

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Cell culture. HeLa cells stably transfected with Panx1 attached to YFP fluorescent protein (HeLa-Panx1-YFP cells) were kindly provided by Dr. Felixas Bukauskas (Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY). HeLa Panx1-YFP or parental cells were grown in DMEM medium (Gibco) supplemented with 10% FBS (Gibco, Carlsbad, CA) at 37°C with 5% CO_2. The cells were seeded at a density of 10^5 cells/mL and then starved for 12 hours with serum-free DMEM medium.

Isolation of neonatal cardiac myocytes. Neonatal rat cardiac myocytes were prepared as described previously.[19] Animals were decapitated, hearts removed, atria excised, and the ventricles then minced in ice-cold PBS without Ca^{2+}/Mg^{2+}. The solution of ventricular tissue was then transferred to a new tube and subjected to overnight digestion (4°C, constant shaking) with 0.05% trypsin-EDTA solution (Gibco). A pellet fraction was then subjected to digestion with collagenase type II (0.75 mg mL^{-1}, 20 min) (Gibco). The collected enzyme solution was centrifuged, supernatant discarded, and the cardiac myocytes fraction was resuspended in culture medium (DMEM high glucose/M199, 4:1) and preplated into a 60 mm dish for 1–3 hours. This preplating step removed fibroblasts and endothelial cells. Nonadherent cells were then collected, quantified, and plated in 2% gelatin-coated 35-mm glass bottom microdish (Ibidi, GmbH, Munich, Germany). Cytosine beta-D-arabinofuranoside (3 µg/mL) was added to the culture medium during first 12 hours to eliminate residual fibroblasts. Cultures were maintained in a humidified incubator at 37°C and 5% CO_2. Culture medium was changed every day.

Dye uptake and fluorescence imaging. For visualization of the ethidium uptake by snapshot, control and treated cells were exposed to 5 µM ethidium bromide for 5 minutes at 37°C in Locke's solution (in mM: 154 NaCl, 5.4 KCl, 2.3 $CaCl_2$, and 5 HEPES, pH 7.4). The cells were then washed with PBS, fixed with 4% paraformaldehyde and mounted with ProLong Gold Antifade reagent, and examined on an inverted confocal microscope (Leica TCS SP8, ON, Canada). Ethidium fluorescence intensity (518 nm excitation and 605 nm emission) was measured at regions of interest placed on the cells using Image J software (NIH software, Bethesda, MD). Fluorescence intensity was expressed as arbitrary units and for each value, the background value was subtracted.

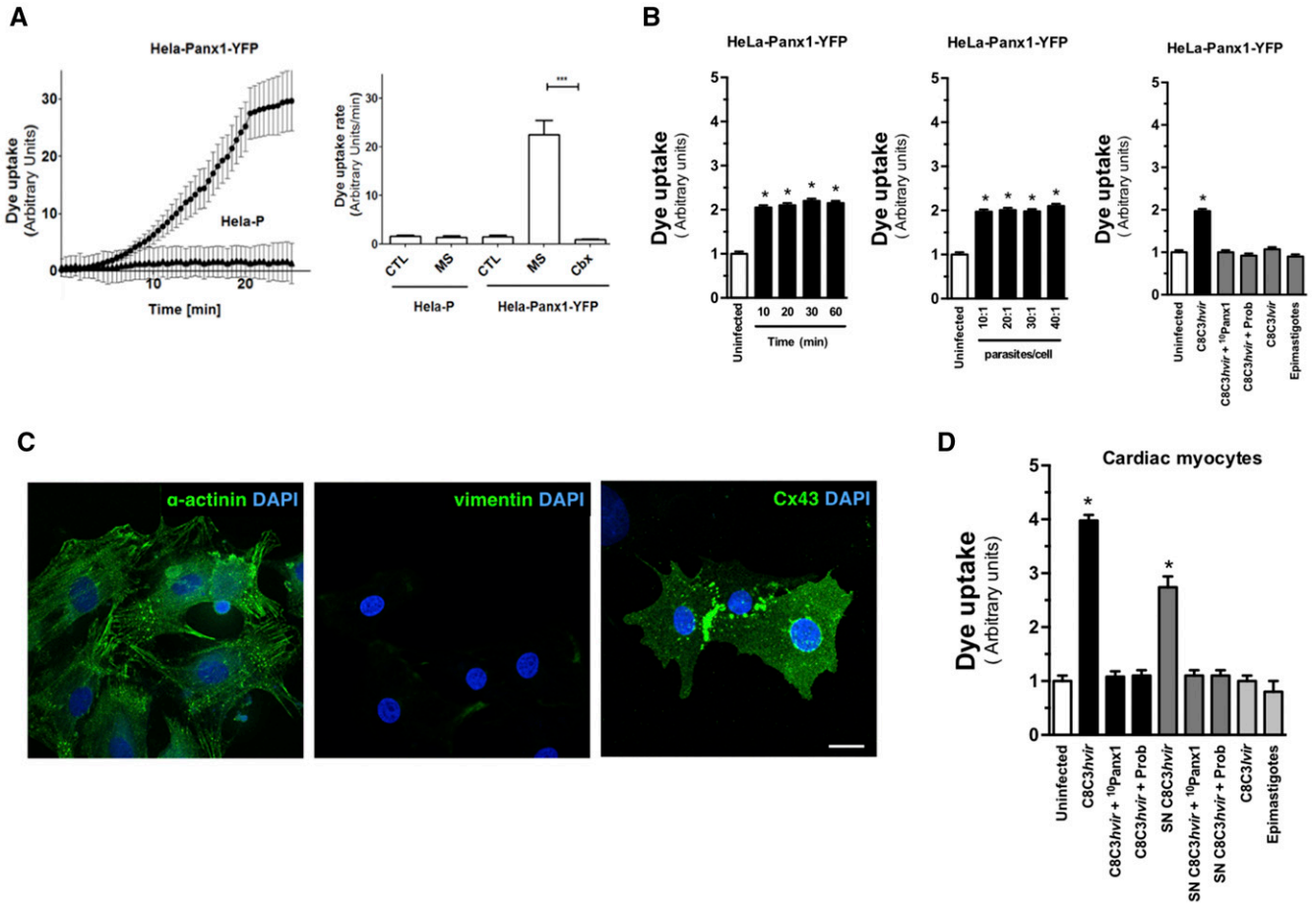

FIGURE 1. *Trypanosoma cruzi* induces pannexin1 (Panx1) channel opening. (**A**) Dye uptake rate of HeLa cells stably transfected with Panx1 attached to YFP fluorescent protein (HeLa-Panx1-YFP) or parental cells (HeLa-P) exposed to mechanical stimulation (MS). (**B**) Dye uptake of HeLa-Panx1-YFP cells uninfected or infected with virulent cell line (C8C3***hvir***) or avirulent cell line (C8C3***lvir***). (**C**) Representative confocal images of cardiac myocyte immunostaining for sarcomeric α-actinin, vimentin, and Cx43. In blue are shown nuclei stained with DAPI. Scale bar = 20 µm. (**D**) Dye uptake of cardiac myocytes uninfected or infected with virulent cell line (C8C3***hvir***), avirulent cell line (C8C3***lvir***), or supernatant (SN) fraction of virulent cell line (C8C3***hvir***). ^{10}Panx1: mimetic peptide blocker of Panx1 channels (100 µM); Prob: probenecid (400 µM). Data are shown as mean ± SEM ***$P < 0.001$ vs. control; *$P < 0.05$ vs. uninfected. $N = 3–5$. This figure appears in color at www.ajtmh.org.

Monitoring cytosolic Ca^{2+} signal. Cells were loaded with Fluo-3/AM (10 μM) in DMEM without serum (37°C for 30 minutes) and then washed three times in PBS and incubated for 15 minutes at 37°C by deesterification. Fluo-3 fluorescence (506-nm excitation, 526-nm emission) was monitored every 5 seconds using a Leica TCS SP8 confocal microscope (Leica, ON, Canada). Fluorescence intensity was analyzed by the Image J software (NIH software), which measured the selected region on cardiomyocytes that showed no spontaneous contraction. Fluorescence was expressed as arbitrary units, and for each value, the background was subtracted.

***Trypanosoma cruzi* invasion.** The effects of Panx1 on parasite invasion were evaluated by plating rat neonatal cardiac myocytes into a chamber slide (Thermo Fisher Scientific, Cambridge, MA) with trypomastigotes (10:1) for 4 hours at 37°C, 5% CO_2 in the absence or presence of ^{10}Panx1 or probenecid. The cells were then washed with PBS, fixed with PAF (4%), washed three times with PBS, and then permeabilized with 0.25% Triton X-100 (Winkler) in PBS. The coverslips were mounted with ProLong Gold Antifade with DAPI (Thermo Fisher Scientific). Fluorescence for DAPI (358 nm excitation, 461 nm emission) was measured using a Leica TCS SP8 confocal microscope (Leica). The numbers of intracellular parasites were quantified by Image J software (NIH software).

Cytotoxicity assay. The cytotoxic effect of drugs was tested using fixable dead cell stain kit (Thermo Fisher Scientific) according to the manufacturer's recommendation. Epimastigotes (3×10^6) were incubated with ^{10}Panx1 (100 μM), probenecid (400 μM), MRS2179 (0.2 μM), or *iso*-PPADS (100 μM) for 24 hours. Parasites were then washed with PBS and incubated with green fluorescent reactive dye (Thermo Fisher Scientific) for 30 minutes. After three washes with PBS, the parasites were fixed (4% PAF), washed, and resuspended in FACS buffer for fluorescence measurements (488 nm excitation, 530 nm emission) in BD FACSJazz (BD Biosciences, San Jose, CA) flow cytometer. The fluorescence of 10.000 parasites was acquired. Data were analyzed using BD FACS™ Sortware software (BD Biosciences).

Statistical analysis. Data are expressed as mean ± SEM and compared by one-way analysis of variance, followed by Tukey's test for multiple comparison analysis. Student's *t* test was used for single comparison analysis. Statistical significance was established at $P \leq 0.05$.

Ethics statement. All animal experiments were compliant with the animal ethics requirements of the Universidad de Antofagasta. Certification of Animal Protocol Approval was provided by the Ethics Committee of the Universidad de Antofagasta (to Fondecyt#11130013).

RESULTS

Trypanosoma cruzi* induces Panx1 channel opening.** To determine whether *T. cruzi* modifies Panx1 channel activity, we used HeLa cells stably transfected with Panx1 attached to YFP fluorescent protein in the C terminal (HeLa-Panx1-YFP cells). Mechanical stimulation (condition known to induce Panx1 channel opening) increased the dye uptake rate in HeLa-Panx1-YFP but not in HeLa parental cells (Figure 1A). To determine whether the parasite affects the functional state of Panx1 channel, we incubated HeLa-Panx1-YFP cells with virulent trypomastigotes (C8C3hvir***) for different times. Virulent *T. cruzi* was found to significantly increase the dye uptake of HeLa-Panx1-YFP at 10 minutes (~2 times) post-exposure,

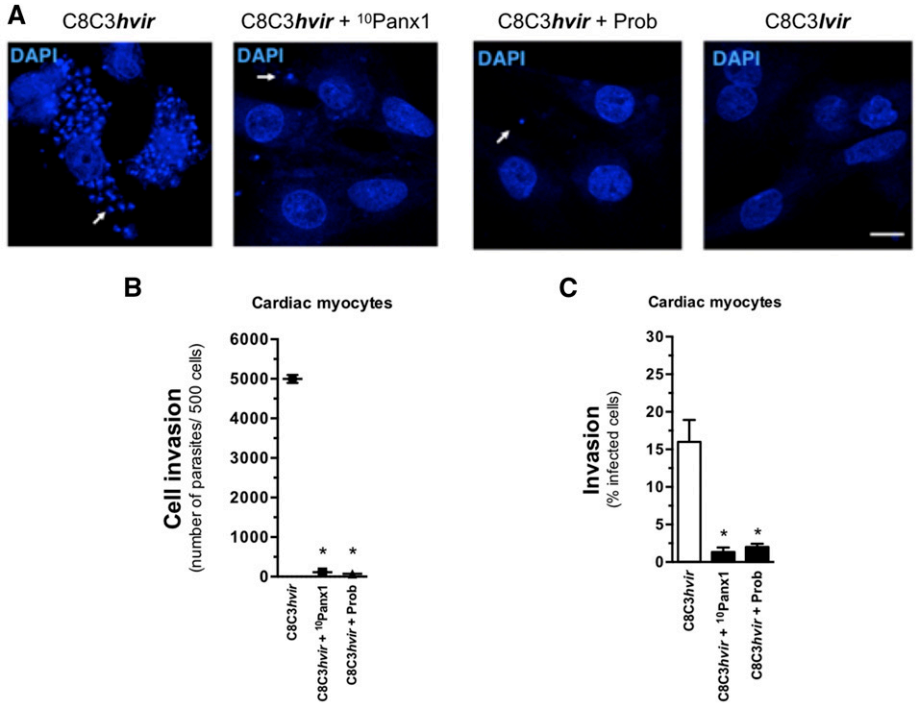

FIGURE 2. Pannexin1 channels are required for *Trypanosoma cruzi* invasion in cardiac myocytes. (**A**) Representative confocal images of cardiac myocyte immunostaining for DAPI. Cardiac cells were incubated with virulent cell line (C8C3***hvir***) for 4 hours. Scale bar = 20 μm. (**B**) Graph showing quantification of the number of parasites per 500 cells. (**C**) Graph showing the percentage of infected cells. Data are shown as mean ± SEM $^*P < 0.05$ vs. infected (control). $N = 5$. This figure appears in color at www.ajtmh.org.

and this response persisted at 60 minutes post-exposure to the parasite (Figure 1B). We then incubated (60 minutes) the HeLa-Panx1-YFP cells with several parasites per cell ratios. It was found that *T. cruzi* in a 10:1 ratio significantly increased the dye uptake (~2 times), and this response of HeLa-Panx1-YFP cells was similar to 40:1 ratio of parasites per cell (Figure 1B). The maximum effect was observed at 10 minutes with a ratio of 10 parasites per cell. To determine if Panx1 channel mediated these changes, we preincubated for 30 minutes the cells with specific Panx1 channel blockers. Under this condition, the *T. cruzi*–induced dye uptake was not observed in HeLa-Panx1-YFP cells pretreated with 100 μM ^{10}Panx1 or 400 μM probenecid (Figure 1B). To determine whether the *T. cruzi*–induced Panx1 channel opening was mediated by a virulence factor, we exposed HeLa-Panx1-YFP cells with avirulent trypomastigotes (C8C3***lvir***) or epimastigotes. In both cases, the increase in uptake was not observed (Figure 1B).

Subsequently, we evaluated the effect of *T. cruzi* in cardiac myocyte cultures. To evaluate the purity of our primary cardiomyocyte cultures, we used immunofluorescence detection of sarcomeric α-actinin (myocytes) and vimentin (for fibroblast) proteins. After 5 days in culture, confluent cultures of cardiac myocytes strongly and uniformly expressed sarcomeric α-actinin but did not present vimentin reactivity (Figure 1C). Because Cx43 protein is highly expressed in cardiac cells, forming gap junctions, we performed immunostaining using anti-Cx43 antibody as a control. The results showed that cardiac cells expressed Cx43, and gap junction plaques were observed (Figure 1C). The incubation of cardiac myocytes with virulent trypomastigotes (C8C3***hvir***) for 60 minutes increased the dye uptake (~4 times) as compared with uninfected cells (Figure 1D). This increase in dye uptake was not observed in cardiac myocytes pretreated with 100 μM ^{10}Panx1 or 400 μM probenecid (Figure 1D). To investigate the involvement of a virulence factor, we incubated cardiac myocytes with avirulent trypomastigotes (C8C3***lvir***), epimastigotes, or supernatant fraction of virulent trypomastigotes (C8C3***hvir***). Interestingly, the SN fraction increased the dye uptake (~2.6 times) (Figure 1D). These effects were blocked by 100 μM ^{10}Panx1 or 400 μM probenecid (Figure 1D). Moreover, epimastigotes and avirulent trypomastigotes (C8C3***lvir***) did not modify the dye uptake of HeLa-Panx1-YFP cells (Figure 1D).

Opening of Panx1 channel is required for efficient *T. cruzi* invasion in cardiac myocytes. In addition, we tested whether ^{10}Panx1 or probenecid blocks the *T. cruzi* invasion in cardiac myocytes. Treatment with 100 μM ^{10}Panx1 or 400 μM probenecid significantly reduced the number of amastigotes (C8C3***hvir***) to 114 ± 2 parasites/500 cells and 71 ± 2 parasites/500 cells, respectively, versus 5001 ± 2 parasites/500 cells under controlled conditions (Figure 2A). Amastigotes were not observed in cultures incubated with avirulent trypomastigotes (C8C3***lvir***) (Figure 2A). Moreover, 100 μM ^{10}Panx1 or 400 μM probenecid reduced the number of infected cells to 1.3% ± 0.5% and 2.0% ± 0.5%, respectively, versus 16.0% ± 2.9% of control (Figure 2C).

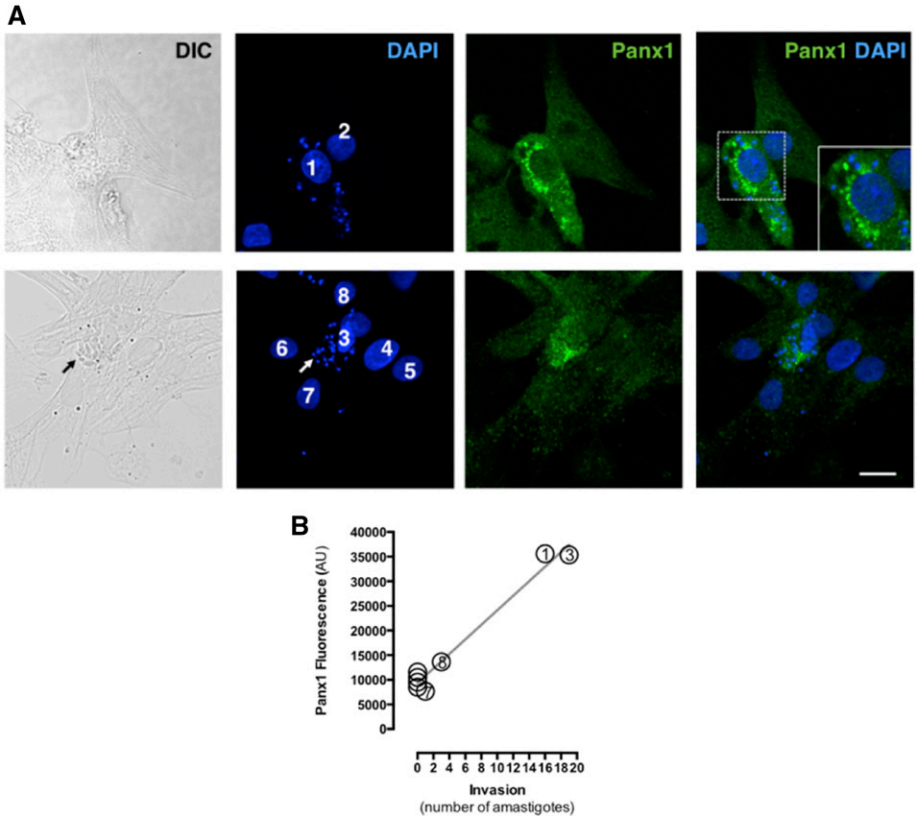

FIGURE 3. *Trypanosoma cruzi* invasion increases pannexin1 (Panx1) immunoreactivity in cardiac myocytes. (**A**) Representative confocal images of cardiac myocyte immunostaining for Panx1 and nuclei with DAPI. Numbers identify each cell. (**B**) Graph showing Panx1 fluorescence vs. number of amastigotes in each cell. The arrow indicates intracellular parasites (amastigotes). Scale bar = 20 μm. This figure appears in color at www.ajtmh.org.

***Trypanosoma cruzi* invasion increases Panx1 immunoreactivity in cardiac myocytes.** To determine if the increase in the activity of the Panx1 channel is associated with changes in the amount of Panx1 protein, we evaluated the amount of Panx1 protein by immunofluorescence. Infected cells showed greater immunoreactivity for Panx1 than uninfected cells (Figure 3). Moreover, antibody against Panx1 did not colocalize with DAPI, which labels the amastigotes (inset Figure 3).

Panx1 channel participates in Ca^{2+} signal transients evoked by *T. cruzi*. To evaluate whether Panx1 channel participates in Ca^{2+} signal transients evoked by *T. cruzi*, we evaluated Ca^{2+} signal in cardiac myocytes using Fluo-3 probe. Virulent trypomastigotes (C8C3***hvir***) were found to induce repetitive Ca^{2+} signal transient in cardiac cells (Figure 4). This response of cardiac myocytes was blocked by 100 μM ^{10}Panx1 peptide, 400 μM probenecid, or 5 μM carbenoxolone (Figure 4). To evaluate the involvement of extracellular ATP and P2 receptor activation in Ca^{2+} signal transients evoked by *T. cruzi,* we used MRS2179, a selective $P2Y_1$ antagonist, and *iso*-PPADS, a P2X antagonist. Treatment with 0.2 μM MRS2179 blocked repetitive Ca^{2+} signal transients promoted by exposure to virulent trypomastigotes (C8C3***hvir***) (Figure 4). In contrast, treatment with 100 μM *iso*-PPADS did not prevent *T. cruzi*–evoked repetitive Ca^{2+} signal transients (Figure 4).

Viability of *T. cruzi*. To analyze the effects of Panx1 channel blockers and P2 antagonist on *T. cruzi* viability, we incubated epimastigotes (3×10^6) with 100 μM ^{10}Panx1, 400 μM probenecid, 0.2 μM MRS2179, or 100 μM *iso*-PPADS for 24 hours. These drugs did not affect the *T. cruzi* viability (Figure 5). As a positive control, DMSO (10%, for 3 hours) was used, which killed 80.3% of the cells (Figure 5).

DISCUSSION

In the present work, we found that *T. cruzi* causes opening of Panx1 channel during the infection of cardiac myocytes.

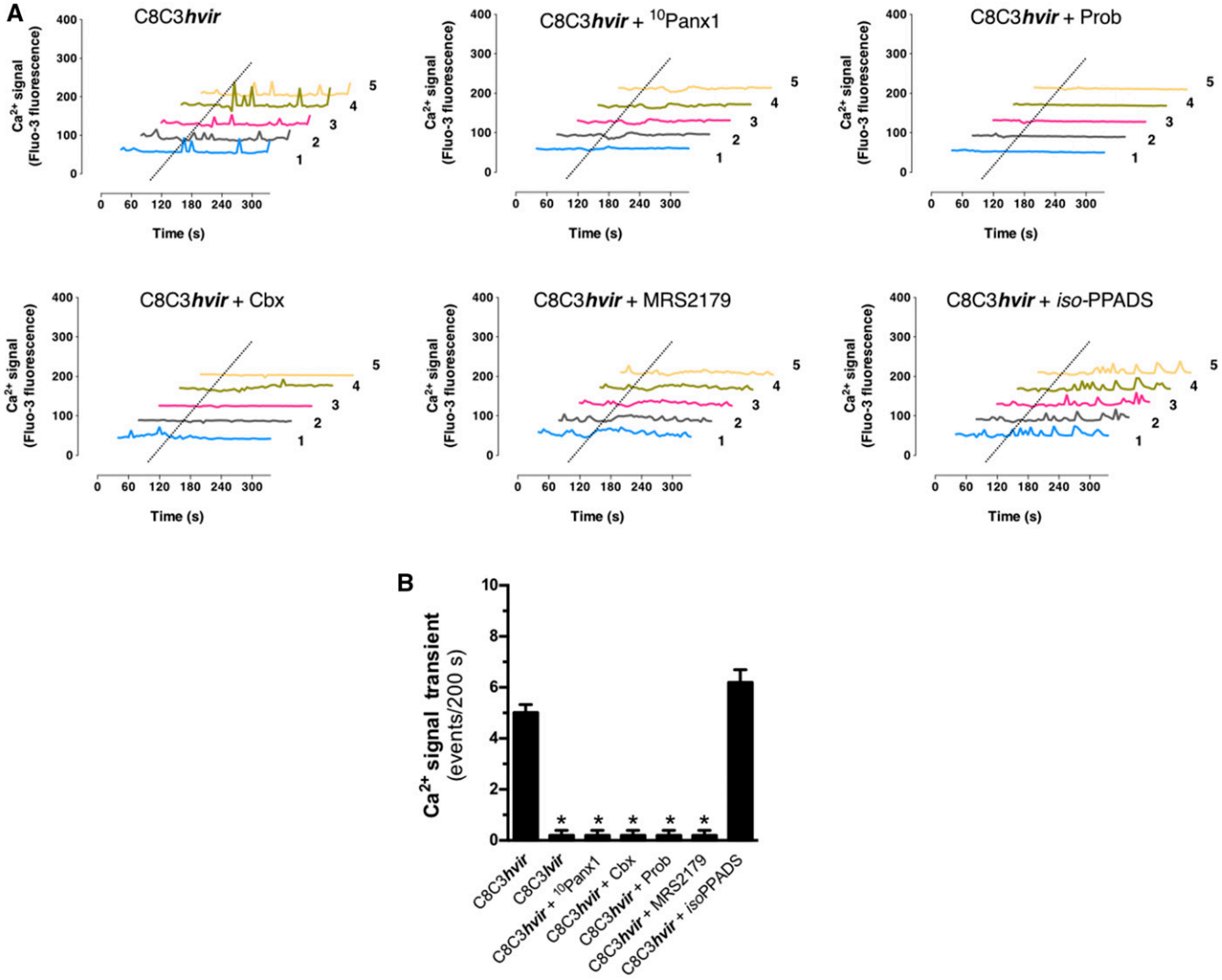

FIGURE 4. Pannexin1 (Panx1) channels participate in Ca^{2+} transients evoked by *Trypanosoma cruzi*. (**A**) A representative trace of Fluo-3 fluorescence (Ca^{2+} signal) measured in cardiac myocytes incubated with virulent cell line (C8C3***hvir***) (dotted line). ^{10}Panx1: mimetic peptide blocker of Panx1 channels (100 μM); Prob: probenecid (400 μM); Cbx: carbenoxolone (5 μM); MRS2179 (100 μM); and iso-PPADS (100 μM). (**B**) Frequencies of the records displayed in (**A**). ATP (100 μM) was used as a positive control. Data are shown in mean ± SEM $^*P < 0.05$ vs. control. This figure appears in color at www.ajtmh.org.

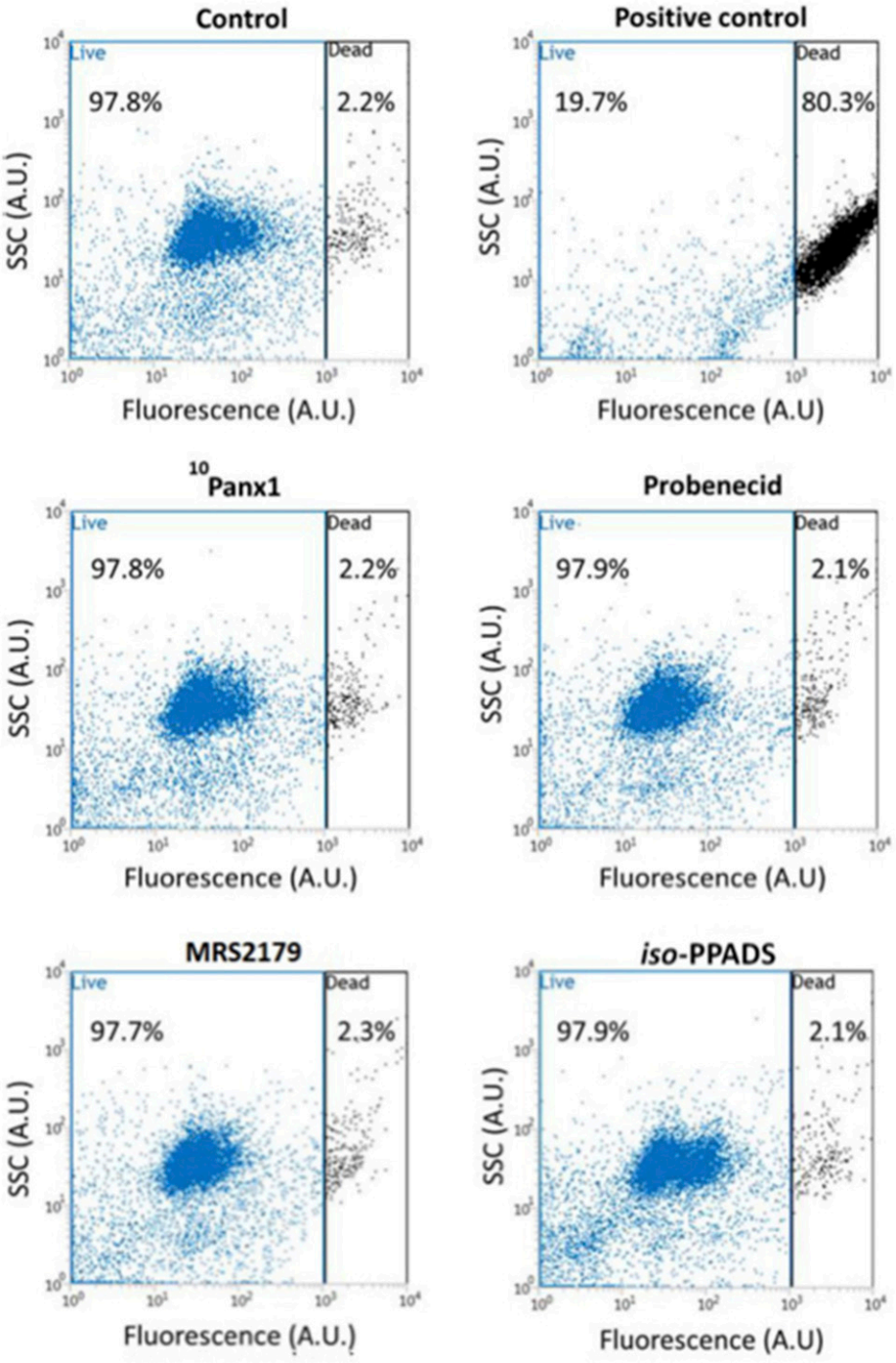

FIGURE 5. Effect of pannexin1 channel blockers or P2 antagonist drugs on *Trypanosoma cruzi* viability. Epimastigotes were incubated with each drug for 24 hours, and cell viability was evaluated by Dead Cell Stain kit (Thermo Fisher Scientific). Fluorescence was determined at 530 nm by flow cytometry. This figure appears in color at www.ajtmh.org.

Chagas disease is found mainly in endemic areas of Latin American countries, where it is mostly vectorborne transmitted to humans by contact with feces or urine of triatomine bugs.[20] We used H510 strain that was isolated in 1968 from a *T. dimidiata*, the main vector of Chagas disease in Costa Rica.[21,22] This strain is classified in the group TcIa of DTU.[18] A clone of this strain was maintained in alternate mouse-cell culture passage whereas another clone was maintained in axenic culture passage, which generated two clones of the same strain, one highly virulent (C8C3***hvir***) and one with very low virulence (C8C3***lvir***).[18] This allowed us to infer that the effect on the Panx1 channel must be mediated by a virulence factor because avirulent cell line (C8C3***lvir***) did not modify Panx1 channel activity. We have recently published that avirulent trypomastigotes (C8C3***lvir***) express lower levels of cruzipain and gp85/trans-sialidase compared with virulent trypomastigotes

(C8C3***hvir***); therefore, these factors could be responsible for the observed effects.[18] Experimental studies will be required to confirm the participation of these virulent factors on Panx1 regulation.

Moreover, we found that Panx1 channel opening was required for *T. cruzi* invasion in cardiac myocytes. The involvement of the Panx1 channel in infections caused by obligate intracellular pathogens has already been demonstrated. For example, it has been described that probenecid (1.5 mM) inhibits *Chlamydia* growth in HeLa cells.[15] In addition, ^{10}Panx1 (200 μM) has been demonstrated to abolish HIV replication in $CD4^+$ T lymphocytes.[16] *Trypanosoma cruzi* invades the cells, where it is transformed into amastigotes (replicative form) and begins to replicate intracellularly.[20] This invasion can be quantitatively examined via enumeration of intracellular amastigotes.[23] In agreement with previous studies, we found that ^{10}Panx1 or probenecid reduced the number of intracellular amastigotes in cardiac myocytes. Interestingly, we found that the cells with the greatest number of amastigotes presented greater immunoreactivity to Panx1. In *Chlamydia* infection, recruitment of Panx1 into *Chlamydia*-containing parasitophorous vacuole membrane has been observed.[15] The authors of the latter work suggested that Panx1 could contribute to maintaining an intracellular niche.[15] Panx1 has been detected preferentially in the surface membrane but has also been found in intracellular compartments in rat atrial and ventricular primary cardiomyocytes.[24,25] Unfortunately, our results do not allow us to identify if this greater expression occurs in the plasma membrane or at intracellular compartments of the cells.

Moreover, opening of Panx1 channel results in the release of ATP, which is a signaling molecule that can activate purinergic and adenosine receptors in an autocrine and paracrine manner.[26] Interestingly, ATP is a key molecule in *T. cruzi* host cell invasion. It has been demonstrated that depletion of ATP content decreases *T. cruzi* infectivity in HeLa cells.[27] In agreement with these studies, we found that ^{10}Panx1 or probenecid reduced *T. cruzi*–evoked Ca^{2+} signal transients in cardiac myocytes. Although it is unknown whether Panx1 channel is permeable or not to Ca^{2+}, it is well documented that Panx1 channel is permeable to ATP allowing its release to the extracellular milieu, which can induce transient Ca^{2+} signal by the activation of P2Y and/or P2X receptors.[28–32] In addition, we found that *T. cruzi*–evoked Ca^{2+} signal transients were inhibited by a $P2Y_1$ but not a P2X antagonist, suggesting that Ca^{2+} signal transients resulted from Ca^{2+} release from intracellular stores. In HIV infection, it has been described that inhibition of any of the constituents of Panx1, ATP, or P2Y affected HIV replication, suggesting that this signaling may be important in the intracellular pathogen infection.[32] Finally, we evaluated the cytotoxic effect of Panx1 blockers and P2 antagonist in *T. cruzi*, because probenecid has been demonstrated to induce chemosensitization of *Plasmodium falciparum* to antimalarial drugs[33] and suramin (a general P2 antagonist) used as an anti-trypanocidal drug.[34] For all drugs tested, none showed cytotoxic effects in *T. cruzi*.

In summary, our results strongly suggests that a virulence factor(s) from *T. cruzi* induces Ca^{2+} signal transients via Panx1 channel opening, putative ATP release, and subsequently activation of $P2Y_1$ receptors. Because cardiac myocytes are targets for *T. cruzi* in the vertebrate host, our study suggests that Panx1 channel may play a critical role in the pathogenesis of Chagas disease.

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