Implication of purinergic signalling pathways in clinical management of Chagas disease

EC Santos¹, RD Novaes², SA Cardoso³, LL Oliveira¹*

Abstract
Introduction
Chagas disease is a neglected tropical disease that affects about 12 million people in American countries and, owing to migration, infected individuals are distributed worldwide. The disease is caused by an intracellular protozoan Trypanosoma cruzi, a hemoflagellate protozoan (family Trypanosomatidae, order Kinetoplastida), transmitted by triatomine insect (Triatoma, Panstrongylus, Rhodnius). The parasite can also be acquired by blood transfusion, organ transplant, ingestion of food contaminated with triatomines or their faeces, or by congenital transmission. The life cycle of T. cruzi involves mandatory passage through vertebrate (mammals, including man) and invertebrate (haematophagous triatomine insect) hosts in a series of stages. Once the infection has been established in the vertebrate host, the parasite migrates to systemic organs such as liver, kidneys, spleen, bone marrow, intestine, pancreas and heart. Although the parasite has the ability to infect many tissues and organs, it has intense tropism for the cardiac muscle. Thus, Chagas disease is characterised as the most common cause of non-ischemic cardiomyopathy worldwide, leading to the death of thousands of patients every year, mainly due to dilated cardiomyopathy, congestive heart failure, arrhythmias and thromboembolic events that occur in approximately 30% of infected individuals.

Conclusion
The drug association mechanisms involving competitive antagonists of P2 receptors represent a good therapeutic strategy, inhibiting the essential processes for T. cruzi. Thus, the association between suramin and classical trypanocidal drugs is a promising tool in clinical management.

Introduction
Chagas disease is a neglected tropical disease, which is distributed worldwide affecting about 12 million people in American countries. Over the years, several therapeutic targets have been investigated in vitro and in vivo in the attempt to develop a safe and efficient chemotherapy for this disease. However, despite countless efforts in this regard, the methods are still failing and new therapeutic targets represent an urgent reality. We believe that ecto-nucleoside triphosphate diphosphohydrolase, a crucial enzyme for Trypanosoma cruzi survival, is a good target. The present review aims to provide an overview about the activation mechanisms of purinergic signalling pathways based on the activity of ecto-enzymes located on T. cruzi surface and the role of inhibitors of these enzymes in the clinical management of Chagas disease.

Conclusion
The drug association mechanisms involving competitive antagonists of P2 receptors represent a good therapeutic strategy, inhibiting the essential processes for T. cruzi. Thus, the association between suramin and classical trypanocidal drugs is a promising tool in clinical management.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

Discussion

Activation of purinergic receptors by pathogens

Until 1970s, it was believed that extracellular diphosphate (NDPs) and triphosphate (NTPs) nucleosides were generated by the process of cellular damage, and no biological function was assigned to them. Currently, it is known that these molecules represent key messengers that mediate diverse biological processes through the activation of the receptosome, specifically purinergic receptors. The extracellular nucleotides are involved in an array of cell-specific responses, but the integrative view of purinergic signalling as a multistep coordinated cascade has emerged recently.

Activation of the purinergic signalling pathway involves two types of specific receptors distributed in the body [the families of type 1 (P1) and type 2 (P2) purinergic receptors]. According to molecular, biochemical, and pharmacological evidence, Adenosine/P1 receptors have been further subdivided into four subtypes: A1, A2A, A2B, and A3; all coupled to G proteins and adenosine is the main activator of these receptors.

The P2 receptors are divided into two families: P2X and P2Y receptors. Based on differences in molecular structure and signal transduction mechanisms, P2X receptors consist of a trimeric ligand-gated ion channel, and there are seven subunits of the P2X numbered P2X₁ through P2X₇, which involve heterotrimers and homotrimers. These receptors respond to several extracellular mono- and dinucleotides, but are activated principally by ATP. The P2Y receptors, in general, are G protein-coupled and they are categorized into two subfamilies. The first includes P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁ receptors that are predominantly coupled to the subunit Gq of the G protein, and both are activated by phospholipase C-β. The second involves P2Y₁₂, P2Y₁₃, and P2Y₁₄ receptors coupled to the Gs subunit of G protein, which can be inhibited by adenylyl cyclase and regulate ion channels. In general, P2Y receptors can be activated by ATP, ADP, UTP, UDP, ITP, and nucleotide sugars, albeit agonist specificity varies between subtypes and different animal species.

It has been reported that a variety of pathogens are capable of synthesising ectonucleotidases that hydrolyse extracellular NTPs and NDPs, activating purinergic receptors. This activation begins a series of biological responses in the microenvironment of the infected organism, which can favour the survival and replication mechanisms of the parasite. An important ectonucleotidase described in trypanosomatids, which activates purinergic receptors, is the extracellular diphosphate diphosphohydrolases (E-NTPDases) member of the CD39 family. These enzymes are characterised by the presence of five ‘apyrase conserved regions’ (ACR1 to ACR5).

There is strong evidence that the parasite-associated E-NTPDases imply in the biological responses. In general, these responses contribute to enhance cell adhesion mechanisms, intracellular survival, and modulation of host immunity, and consequently enhance the virulence mechanisms of the parasite.

Implication of *T. cruzi* E-NTPDase for purines acquisition

NTPDase enzymatic activity has been described for several protozoan parasites, especially trypanosomatids. At the moment, just one ectonucleotidase has been discovered for *T. cruzi*. This was named Tc-ENTPDase-1, a homologue to CD39 family enzymes, characterised as an important virulence biomolecule located on the parasite surface, capable of hydrolysing extracellular NTPs and NDPs, especially extracellular ATP.

It is known that the extracellular ATP represents a danger signal that can be induced by pathogen infection. This way it is able to trigger cellular events such as proliferation, differentiation, chemotaxis, release of cytokines or lysosomal constituents, and generation of reactive oxygen or nitrogen species. All those mediators are highly toxic to *T. cruzi*. It has been described that Tc-ENTPDase-1 might play a modulatory role in response to these toxic mediators and in consequence affect parasite recovery pathways, which are necessary for nutrition and to maintain parasite viability; beyond this, they can be involved in inflammatory processes, modulation of the host immune system and consequently increase the virulence mechanisms of *T. cruzi*.

A virulence key mechanism related to Tc-ENTPDase-1 presents a relation with enzyme capacity that interferes with extracellular ATP signals and interrupts purinergic signalling, limiting mechanisms of host defences. This activity seems to be dependent on the presence of divalent cations such as calcium and magnesium. The main consequence of extracellular ATP hydrolysis by *T. cruzi* catalysed by Tc-ENTPDase-1 is purine recovery from their hosts. Purines represent precursor molecules fundamental for DNA and RNA synthesis, which are carriers of high-energy phosphate bonds, constituents of co-enzymes and modulators of certain enzymatic reactions. This makes the parasite to develop an absolute need for purines once *T. cruzi* does not have the machinery to synthesise its own purine ring *de novo*.

The inability of *T. cruzi* to produce nucleobases (adenine, guanine, xanthine, hypoxanthine) and nucleosides (adenosine, guanosine and inosine) makes this parasite to transport these compounds across its plasma membrane. This way initiates the acquisition of the purine ring and therefore nutrients. There are strong evidences indicating the presence of specific transporters.
associated with the plasma membrane of the parasite, that are configured to accommodate a repertoire of nucleosides by the parasite, essential for its metabolism and survival\textsuperscript{22}.

In general, specific nucleobase transporters have been considered as an important pharmacological target and have been extensively studied, especially in Trypanosomatidae. Applications of molecular and genetic approaches have allowed the identification and functional characterisation of a cohort of transporters and activation of the associated genes in these parasites\textsuperscript{23}. However, molecular investigations to specify the transporter associations to \textit{T}. \textit{cruzi} are still required\textsuperscript{22}. Some important transporters that act in trypanosomatids, especially \textit{T}. \textit{cruzi}, \textit{T}. \textit{brucei} and \textit{Leishmania} spp., presenting specificity substrates had been investigated and can serve as starting points for more extensive reading (Table 1).

Till date, it is known that these receptors are essential for nutrient acquisition and to increase \textit{T}. \textit{cruzi} virulence. A number of studies have been developed to demonstrate the presence and to define the specificity of purine transporters. However, despite advances in understanding the molecular processes between transporters and parasites, further investigations are still needed for a complete explanation, especially in \textit{T}. \textit{cruzi}\textsuperscript{23}.

**Table 1** Relation of transporters that act in trypanosomatids, according to substrate specificity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Parasite</th>
<th>Substrate</th>
<th>Transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetaud et al.\textsuperscript{27}</td>
<td>\textit{T}. \textit{brucei}</td>
<td>Hexoses</td>
<td>THT1, THT2</td>
</tr>
<tr>
<td>Tetaud et al.\textsuperscript{28}</td>
<td>\textit{T}. \textit{cruzi}</td>
<td>Hexoses</td>
<td>TcHT1</td>
</tr>
<tr>
<td>Ortiz et al.\textsuperscript{29}</td>
<td>\textit{T}. \textit{brucei}</td>
<td>Purines</td>
<td>TbAT1, TbNT2-12</td>
</tr>
<tr>
<td>Ortiz et al.\textsuperscript{30}</td>
<td>\textit{L}. \textit{major}</td>
<td>Purines</td>
<td>LdNT1-4, LmaNT1-4</td>
</tr>
<tr>
<td>Ligtenberg et al.\textsuperscript{31}</td>
<td>\textit{T}. \textit{brucei}</td>
<td>Iron</td>
<td>ESAG6/ESAG7 heterodimer</td>
</tr>
<tr>
<td>Huynh &amp; Andrews\textsuperscript{32}</td>
<td>\textit{L}. \textit{amazonensis}</td>
<td>Iron</td>
<td>LIT1</td>
</tr>
<tr>
<td>Hasne &amp; Ullman\textsuperscript{33}</td>
<td>\textit{L}. \textit{major}</td>
<td>Polyamines</td>
<td>LmaPOT1</td>
</tr>
<tr>
<td>Hasne et al.\textsuperscript{34}</td>
<td>\textit{T}. \textit{cruzi}</td>
<td>Polyamines</td>
<td>TcPOT1.1, TcPOT1.2</td>
</tr>
<tr>
<td>Shaked-Mishan et al.\textsuperscript{35}</td>
<td>\textit{L}. \textit{donovani}</td>
<td>Amino acids</td>
<td>LdAAP3</td>
</tr>
<tr>
<td>Dean et al.\textsuperscript{36}</td>
<td>\textit{T}. \textit{brucei}</td>
<td>Carboxylates</td>
<td>PAD1, PAD2</td>
</tr>
<tr>
<td>Montalvetti et al.\textsuperscript{37}</td>
<td>\textit{T}. \textit{cruzi}</td>
<td>Water, glycerol and osmolytes</td>
<td>TcAQP1</td>
</tr>
</tbody>
</table>

**Surrum, a potential strategy for Tc-NTPDase inhibition**

Evident therapeutic implications have been directed in parasitology, especially in relation to purine recovery pathways and activity of specific \textit{T}. \textit{cruzi} transporters\textsuperscript{21}. It has been described that drugs capable of acting on the biology of \textit{T}. \textit{cruzi} represent a promising tool to reduce the infectivity by inhibiting evasion mechanisms used by the parasite to escape the immune system\textsuperscript{23}. Trypanocidal drugs that are able to influence the biochemical activity of purinergic receptors, acting like competitive antagonists of P2X and P2Y receptors, may be promising in the clinical management of Chagas disease\textsuperscript{24}. It has been shown that there is a strong correlation of ecto-ATPase activity inhibition with reduced capacity of adhesion and internalisation of \textit{T}. \textit{cruzi} in the host cell\textsuperscript{27,29}.

An important ecto-ATPase inhibitor, capable of interfering with purinergic receptors and consequently in the biology of \textit{T}. \textit{cruzi}, is suramin, a polysulphonated naphthylurea compound, derivative of urea, that functions as a competitive antagonist of P2 receptors, used for the prophylactic treatment of African tripanosomiasis\textsuperscript{25}.

After decades of clinical studies with suramin, it was proved that it reduces the activity of enzymes such as retroviruses (reverse transcriptase, protein kinase C, DNA polymerase, protein tyrosine phosphatase and Mg\textsuperscript{2+} ATPase)\textsuperscript{36,37}. It has been reported that in \textit{T}. \textit{cruzi}, suramin can inhibit various enzymes, the endocytosis of some molecules, and interfere with the process of cell division\textsuperscript{26}.

Bisaggio et al.\textsuperscript{26} showed modulatory effect of suramin on enzymatic activity of the ecto-ATPase Mg\textsuperscript{2+} dependent on the surface of \textit{T}. \textit{cruzi}. This inhibition was dose-dependent, and an important biological consequence reported is the accentuated reduction of the ATP hydrolysis rate and a significant reduction in the electron-dense reaction product observed on the cell surface of epimastigote and trypomastigote forms of the parasite. Moreover, the drug inhibits the growth of epimastigote forms without affecting their cell viability or metacyclogenesis rate. Although these authors pointed the involvement of suramin on biological mechanisms related to Tc-NTPDase inhibition, due to binding to P2 receptors, this activity has been demonstrated only in vitro. Relative ineffectiveness of suramin has been demonstrated in studies in vivo and there are urgent necessities of controlled preclinical and clinical studies to define the molecular mechanisms involved in the modulation of parasite biology\textsuperscript{19,20}.

Thus, we considered suramin and purinergic signalling inhibition as a promising target in the clinical management of Chagas disease. However, it is worth mentioning the relevance of nucleoside and nucleobase transporters used by \textit{T}. \textit{cruzi} and are also
strongly associated with the mechanisms of virulence of the parasite.

**Conclusion**

Chagas disease is a reality worldwide and a challenge to the health sector. The drug association mechanisms involving competitive antagonists of P2 receptors represent a good therapeutic strategy, inhibiting the essential processes for T. cruzi. Thus, the association between suramin and classical trypanocidal drugs is a promising tool in clinical management.

**Acknowledgement**

This work was supported by FAPEMIG (APQ-00176-13).

**Abbreviations list**

ACR, apyrase conserved region; ADP, adenosine diphosphate; ATP, adenosine triphosphate; E-NTPDase, ecto-nucleoside triphosphate diphosphohydrolase; ITP, inosine triphosphate; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate; UTP, uridine diphosphate; UTP, uridine triphosphate.

**References**

Critical review

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)


