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Inositol Metabolism in *Trypanosoma cruzi:* Potential Target for Chemotherapy Against Chagas' Disease*

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ABSTRACT

Chagas' disease is a debilitating and often fatal disease caused by the protozoan parasite *Trypanosoma cruzi*. The great majority of surface molecules in trypanosomes are either inositol-containing phospholipids or glycoproteins that are anchored into the plasma membrane by glycosylphosphatidylinositol anchors. The polyalcohol *myo*-inositol is the precursor for the biosynthesis of these molecules. In this brief review, recent findings on some aspects of the molecular and cellular fate of inositol in *T. cruzi* life cycle are discussed and identified some points that could be targets for the development of parasite-specific therapeutic agents.

Key words: inositol, *Trypanosoma cruzi*, Chagas' disease, chemotherapy.

INTRODUCTION

CHAGAS' DISEASE

American trypanosomiasis or Chagas'disease, whose etiologic agent is *Trypanosoma cruzi*, was discovered by Carlos Chagas who characterized the parasite, its life cycle and its vector as well as the transmission process (Chagas 1909). Chagas disease ranks high in the group of infectious and parasitic diseases in terms of disability-adjusted years of life lost in Latin America. Only acute respiratory infections, diarrhoeal diseases and HIV / AIDS account for a greater burden (Schmunis *et al.* 1996). World Health Organization estimates that 15 to 20 million people are infected with *T. cruzi*. The disease is acquired by invasive trypomastigotes which are transmitted by insect vectors, blood transfusion, transplacental and organ transplantation (Brener & Gazzinelli 1997). *T. cruzi* is now viewed as an emerging human pathogen of HIV-infected individuals, since it induces dramatic brain pathology and faster death in the affected patients (Del Castillo *et al.* 1990).

The progression of Chagas'disease even today defies the attempts at efficient and safe chemotherapy. There are no vaccines and the drug presently in use to control the acute stage of the disease shows restrict applicability in chronic patients, besides presenting severe side effects (De Castro 1993). The best hope of finding new therapies rests in the development research programs that explore the differences between the parasite and its host, as it has been done by several research groups (Rodriguez & Gross 1995). This article focuses on segments

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of the known and suggested differences between *Trypanosoma cruzi* and mammalian inositol metabolism.

Inositol Lipids

Inositol, found in animals and plants, has been cited as essential in the growth of animals and microorganisms, including *T. cruzi* (Eagle *et al.* 1957, Holub 1982, Einicker-Lamas *et al.* 1999). Its action rests in the formation of a complex set of inositol-containing lipids, whose parent compound is the phospholipid phosphatidylinositol (PI), which can be further phosphorylated or glycosylated forming new classes of bioactive molecules, as depicted in Figure 1.

The glycosylated inositol derivatives are found in the outer leaflet of the plasma membrane facing the external medium, whereas the phosphorylated phosphatidylinositols are found on the inner leaflet of the lipid bilayer and play an important role on signaling pathways, modulating vital functions of the parasite. Glycosylphosphatidylinositol (GPI) -anchored or GPI-related molecules are present in the cell surface of the trypanosomatids at all stages of their life cycles. The detailed structure and the biosynthesis of the GPI anchors in African trypanosomes led the way for the development of this area of study. The cell coat of *Trypanosoma brucei* comprises approximately 10^7 copies/cell of variant specific glycoproteins anchored to the membrane by a GPI moiety (Ferguson 1999). Although GPI-anchors are present in many membrane proteins of mammalian cells, the biosynthetic pathways and particularities of this anchor in African trypanosomes is now being considered as a therapeutic target for African trypanosomiasis (Ferguson *et al.* 1999). In *T. cruzi*, most of the GPI structures are not attached to proteins, but form glycoinositol phospholipids (GIPLs) (Previato *et al.* 1990, Lederkremer *et al.* 1991, Carreira *et al.* 1996). The cell coat of *T. cruzi* is made up of a layer of GIPLs (2×10^7 copies/cell) and small mucin-like GPI-anchored glycoproteins (2×10^6 copies/cell) that project above this layer (Almeida *et al.* 1994, Previato *et al.* 1995, Serrano *et al.* 1995). The mucins from the bloodstream trypomastigote stage of the parasite are extremelly potent inducers of proinflammatory cytokines, and this activity resides in GPI-anchor component. Thus, some parasite GPI-anchors appear to be bioactive and to modulate de host immune system (Brener & Gazzinelli 1997).

The phosphorylated inositol derivatives in *T. cruzi* are found in fewer copies per cell than the GIPLs, amounting in approximately 12% of all phospholipids (Antunes & Oliveira 1981, Kaneda *et al.* 1986). Phosphoinositides in addition to their structural function, play active roles in cell signaling pathways as precursors of the potent second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Nishizuka 1984, Berridge 1984) or directly mediating cell functions through the products of the phosphoinositide-3-OH-kinase (PI-3-K) (Toker & Cantley 1997). These signaling pathways were found to modulate different steps of *T. cruzi* life cycle.

Inositol-lipids signaling pathways

Activation of IP_3/DAG system by exogenous $CaCl_2$ in digitonin-permeabilised epimastigotes was observed by Docampo and Pignataro (1991), and it was also reported an alteration of the phosphoinositides metabolism after cholinergic stimulation (Machado de Domenech *et al.* 1992). Earlier work from our laboratory suggested a phosphoinositide role in *T. cruzi* proliferation (Antunes & Oliveira 1981). Further investigation cleared the role of phosphoinositide signaling pathway, as we have shown that the exposure of *T. cruzi* to mitogenic factors in foetal calf serum (FCS) stimulate a PI-specific phospholipase C (PI-PLC) leading to the accumulation of IP_3 and DAG (Oliveira *et al.* 1993). Among all the phospholipids only the phosphatidylinositol(4, 5)P_2 (PIP_2) was mobilised by serum, and the PIP_2 hydrolysis as well as epimastigotes proliferation were hindered by specific PI-PLC inhibitors. The other phosphoinositides had no significant modification, as well as the major phospholipids such as phosphatidylcholine and phosphatidylethanolamine. Lysophos-

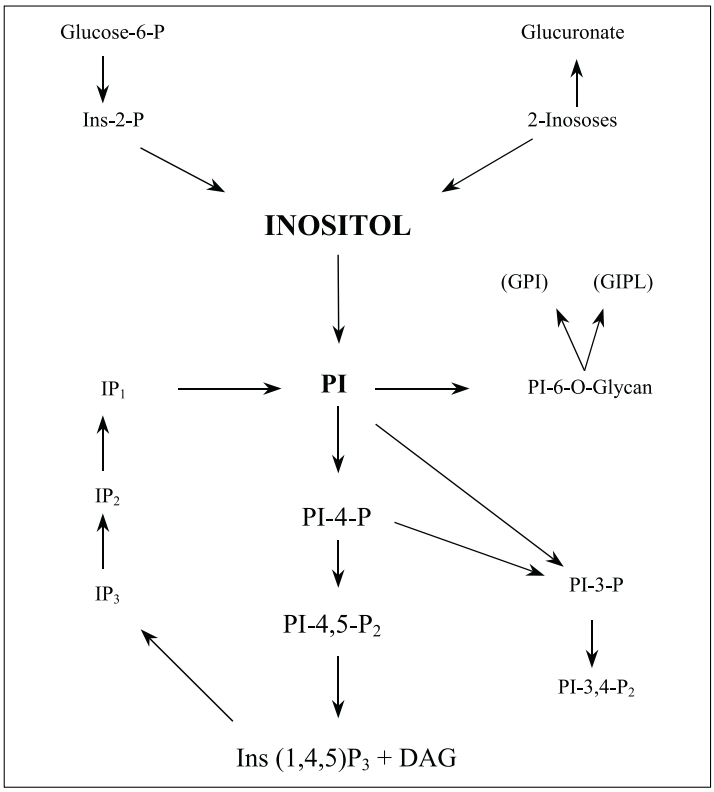

Fig. 1 – *myo*-inositol metabolic pathways. Symbols: **PI** - phosphatidylinositol; **PI-4-P** - phosphatidylinositol-4-phosphate; **PI-4,5-P_2** - phosphatidylinositol-4,5-bisphosphate; **Ins(1,4,5)P_3** and **IP_3** - inositol trisphosphate; **IP_2** - inositol bisphosphate; **IP_1** - inositol monophosphate; **DAG** - diacylglycerol; **PI-3-P** - phosphatidylinositol-3-phosphate; **PI-3,4-P_2** - phosphatidylinositol-3,4-bisphosphate; **PI-6-O-Glycan** - phosphatidylinositol-6-ortho-glycan; **GPI** - glicosylphosphatidylinositol; **GIPL** - glicosylphosphatidylinositol lipid; **Ins-2-P** - Inositol-2-phosphate.

phatidic acid (LPA), reported to be the active agent in some serum effects, had no mitogenic activity when added to epimastigotes cultures. The intracellular signaling downstream of PI-PLC activation was mediated by Ca^{2+}/phospholipid -dependent protein kinase and Ca^{2+}/Calmodulin dependent kinase II (Malaquias & Oliveira 1999).

A subversion of the host cell phosphoinositide signaling pathway was reported. In the invasion of *T. cruzi* into fibroblasts. Andrews and colleagues observed activation of the PI-PLC leading to IP_3 formation, followed by calcium mobilization into the host cell cytosol (Rodriguez *et al.* 1995). Results from our laboratory showed that a different phosphatidylinositol signaling pathway is activated during the infection of *T. cruzi* in macrophages, the first step in the naturally occurring Chagas disease. The entry of trypomastigotes strongly stimulated the formation of the lipid products of the PI-3-OH-kinases: phosphatidylinositol-3-phosphate (PI-3-P), phosphatidylinositol-3,4-bisphosphate (PI-3,4-P_2) and phosphatidylinositol-3,4,5-trisphosphate

(PI-3,4,5-P_3), but not the other phosphoinositides. Pre-treatment of the macrophages with the PI-3-K inhibitor, Wortmannin, markedly arrested *T. cruzi* infection. Using specific antibodies for each of the three classes of PI-3-K, and assaying the immunoprecipitates for enzymatic activity, we found different levels of stimulation in each PI-3-K class, thus suggesting differential roles for these inositol lipids in the process of parasite/host-cell interaction (Todorov *et al.* 2000). Further studies are necessary to clarify these points.

Fluorinated Inositol Analogues

To further evaluate the functional responses coupled to the inositol metabolism in *T. cruzi*, we have used the six isomers of *myo*-inositol in which a single hydroxyl group was replaced by a fluorine and evaluated their role on *T. cruzi* cell biology (Einicker-Lamas *et al.* 1999). The inclusion of fluorine in enzyme substrates has been recognised as a useful technique for the generation of enzyme inhibitors. Fluorinated analogues of *myo*-inositol were used as potential inhibitors of the phosphoinositide metabolism in different cells (Moyer *et al.* 1988, Kozikowski *et al.* 1990, McPhee *et al.* 1991, Offer *et al.* 1993, Cosulich *et al.* 1993). The rationale was that these monodeoxyfluoro-*myo*-inositols (nFIns) might be taken up into cells by the same uptake process as *myo*-inositol, and either be incorporated into phosphoinositides or prevent their formation, depending on the position of the fluorine substituent. We found differences between *T. cruzi* and mammalian systems in response to the fluorinated inositols. The inositol transport system in *T. cruzi* has different features than the one operating in thymocytes (Offer *et al.* 1993). All the [^3H]-labeled fluoroinositols were taken up by thymocytes whereas in epimastigotes only the analogues with fluor in 1, 2 and 4 position on the inositol ring, entered the parasite and were weak substrates for the PI synthases. The [^3H]-3Fins, [^3H]-5Fins and [^3H]-6Fins were not internalised, indicating that they were not recognized by the parasite inositol transport system. This was further confirmed in competitive assays for the uptake of [^3H]-inositol and its incorporation into phosphoinositides (Einicker-Lamas *et al.* 1999).

One major difference between the parasite and the mammalian pathways was the action of the 6-deoxy-6-fluoro-inositol, that although fully permeable to fibroblasts was innocuos to these cells, while having the strongest inhibitory effect on amastigotes and epimastigotes proliferation (Einicker-Lamas *et al.* 1999). The other cell-impermeable 3Fins and 5Fins analogues also hampered *T. cruzi* cell division, as they did in fibroblasts (Cosulich *et al.* 1993). The inhibitory mechanism displayed by the 3Fins and 5Fins were different in the parasite and in fibroblasts, where they interfered directly with the phosphoinositide metabolism (Cosulich *et al.* 1993).

The selective action of these inositol analogues between *T. cruzi* and mammalian system opens a line of research leading to a chemical formulation of a parasite specific inhibitor with therapeutic potential.

INOSITOL TRANSPORT IN *TRYPANOSOMA CRUZI*

Adaptation of protozoan parasites to hostile environments within their vectors and hosts depends on their ability to maintain intracellular homeostasis of ions and nutrients. The level of expression and function of membrane transporters is therefore critical for parasite survival inside their hosts. Transport processes through the parasite plasma membrane are potential targets for new chemotherapeutic drugs.

The results with fluoro-inositols led us to investigate the inositol transport system in *T. cruzi*. Inositol can be synthesized from glucose, as depicted in Figure 1, but most cells possess a specific transport system for the uptake of inositol from the medium. In *T. cruzi* it is not known whether the biosynthesis of inositol is occurring, but its absence from the culture medium impairs epimastigotes growth (Einicker-Lamas *et al.* 1999). Inositol transport in *T. cruzi* is an active transport process, as it is almost completed ablated by inhibitors of the energy metabolism. Also there is a partial dependence on extracellular sodium for this transport, implying the operation of a Na^+/inositol symport

(Einicker-Lamas *et al.* 2000). In view of the Na^+-requirement for the inositol transport we investigated the Na^+ homeostasis in *T. cruzi*. We found two different Na^+ pumps in the parasite: a classical ouabain-sensitive (Na^++K^+)-ATPase (Caruso-Neves *et al.* 1998) and a Na^+-ATPase (ouabain-insensitive) furosemide-inhibitable (Caruso-Neves *et al.* 1999). The inhibition of the inositol transport by furosemide, but not by ouabain suggested that the Na^+-ATPase may have a more important role in the creation and maintenance of the Na^+ gradient across the *T. cruzi* cell membrane, thus enabling an Na^+/inositol symport.

The characteristics of inositol transport system in *T. cruzi* are different from the ones found in the closely related trypanosomatid *Leishmania donovani*, as this organism features an H^+/inositol co-transport (Drew *et al.* 1995) and from the well studied trypanosome glucose carriers (Barrett *et al.* 1998). The inositol transported into epimastigotes is used for phosphoinositides and inositol-phosphates synthesis. These are not products of the PI-PLC action since we found inositol-phosphates with different degrees of phosphorylation (Einicker-Lamas *et al.* 1999, 2000). The function of these inositol-polyphosphates in *T. cruzi* cell biology is unknown at present, and further studies are necessary to elucidate this point. It is possible that the *T.cruzi* inositol carrier may represent either a chemoterapeutic target or a gateway, which allow the targeting of other toxic molecules to these parasites.

PERSPECTIVES

Some progress has been made on the biochemistry of the inositol metabolism in *T. cruzi*. Despite the overall conservation of the GPI anchors and the phosphoinositide signaling pathways, significant differences in the specificities of trypanosome and mammalian inositol metabolism have been demonstrated. The effects of 6-deoxy-6-fluoro-inositol constitute a promising research avenue to the design of specific drugs anti-*T. cruzi*. The biosynthetic routes involving inositol and principally the inositol transporter as the primary target, could be specific and potential therapeutic points to be explored in order to develop new drugs against Chagas disease. The clonning of *T. cruzi* inositol transport genes and structural information on this protein remain important goals for the future.

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