The effects of nitric oxide on the immune system during *Trypanosoma cruzi* infection

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Trypanosoma cruzi infection triggers substantial production of nitric oxide (NO), which has been shown to have protective and toxic effects on the host’s immune system. Sensing of trypanomastigotes by phagocytes activates the inducible NO-synthase (NOS2) pathway, which produces NO and is largely responsible for macrophage-mediated killing of *T. cruzi*. NO is also responsible for modulating virtually all steps of innate and adaptive immunity. However, NO can also cause oxidative stress, which is especially damaging to the host due to increased tissue damage. The cytokines IFN-γ and TNF-α, as well as chemokines, are strong inducers of NOS2 and are produced in large amounts during *T. cruzi* acute infection. Conversely, TGF-β and IL-10 negatively regulate NO production. Here we discuss the recent evidence describing the mechanisms by which NO is able to exert its antimicrobial and immune regulatory effects, the mechanisms involved in the oxidative stress response during infection and the implications of NO for the development of therapeutic strategies against *T. cruzi*.

Key words: *Trypanosoma cruzi* - nitric oxide - myocarditis - cytokines - regulatory T cells

Nitric oxide (NO) and the respiratory cycle: the beginnings of oxidative stress

Nitrogen monoxide, also called NO, is a low-molecular weight radical (30 kDa) that performs multiple biological activities. The biological importance of this ubiquitous intra- and intercellular signalling molecule was first described in the early 1980s as being part of the endothelial derived relaxing factors (Furchgott & Zawadzki 1980). NO was named “Molecule of the Year” in 1992 by the journal *Science* and, later that decade, studies were conducted to demonstrate its cardinal mechanism of action on vascular smooth muscle cells (Murad 1986). These studies made it clear that generation of NO by endothelial cells causes smooth muscle relaxation through activation of guanylate cyclase by nitrosation of its heme group. This work resulted in the Nobel Prize in Physiology and Medicine being conferred to Murad, Furchgott and Ignarro, in 1998 (Murad 1986).

It is hypothesised that NO may have originated in metazoans as an ancient mechanism of first-line defence against intracellular pathogens. This theory has been confirmed by the wide occurrence of the enzyme responsible for NO production, NO-synthase (NOS2), in several species, ranging from invertebrates (Ribeiro et al. 1993) to mammals and non-mammalian vertebrates. In mammals, NO production is upregulated in response to infection by a wide range of unicellular organisms such as bacteria, yeast and parasites (i.e., *Trypanosoma cruzi*) (Cardoni et al. 1990). Evidently, evolutionary diversity has induced NO synthesis to be performed in response to different kinds of stress stimuli.

Under homeostatic conditions, NO is produced at low concentrations from constitutive NOS2 and acts as an intracellular messenger and a cytoprotective (antioxidant) factor. Indeed, overexpression of NOS3 blocks the exocytosis of inflammatory mediators by endothelial cells, thus preventing blood vessel inflammation. Conversely, exposure to inflammatory stimuli leads to the production of substantial amounts of NO in a variety of cell types, as well as modifications of the cellular microenvironment, which by its turn upregulates NO effects. These effects are a consequence of the formation of dinitrogen trioxide and peroxynitrite at sites of simultaneous superoxide formation, as occurs in phagocytes (Chen & Deen 2001).

The old paradigm stating that NO is a mere “unspecific” cytostatic mediator of defence has been challenged by the recent discovery that NO has a large variety of effects on the biology of leukocytes. These effects can be direct or indirect and can influence several physiological processes, ranging from DNA transcription and replication to protein synthesis and secretion (Marnett et al. 2003). Under physiological conditions, NO mediates homeostatic anti-inflammatory reactions, such as inhibition of neutrophil adhesion (Dal Secco et al. 2006), cyclooxygenase activity (Gilroy 2005), cytokine production (Livonese et al. 2009), osteoclast bone resorption (Fukada et al. 2008), among others, in order to prevent autoimmunity.
The broad spectrum of effects performed by NO can be exerted through two main mechanisms: the activation of guanylate cyclase (which can be soluble in the cytosol or coupled to the cell membrane) (Poulos 2006) or through its interaction with the major cellular source of superoxide anion, the NO/Cytochrome C oxidase, which is found in mitochondria. The guanylate cyclase-dependent effects of NO mainly affect the vascular tonus thereby affecting the inflammatory reaction. Other effects pertaining to mitochondrial functions involve the respiratory burst (Ghaforifar & Cadenas 2005). Mitochondria can produce NO through its own Ca\(^2+\)-sensitive synthase (mitochondrial, mtNOS). This enzyme regulates mitochondrial oxygen consumption and transmembrane potential via a reversible reaction with cytochrome C oxidase. The intramitochondrial reaction of NO with superoxide anion yields peroxynitrite, which irreversibly modifies susceptible targets within the mitochondria, inducing oxidative and/or nitrative stress.

In addition to their primary role in the production of energy (ATP), mitochondria generate reactive oxygen species (ROS) that can directly or indirectly affect the NO response (Poderoso 2009). Since NO and ONOO- can inhibit cellular respiration at the level of cytochrome C oxidase and complexes I-III, respectively, it has been suggested that mitochondrial function can influence the balance between apoptosis and necrosis induced by NO (Lizasoain et al. 1996). In addition, NO can stimulate the biogenesis of new mitochondria in a guanosine 3',5'-monophosphate (cGMP)-dependent manner (Nisoli et al. 2003).

These findings are of particular relevance for T. cruzi infection, since it has been described that T. cruzi causes an energetic impairment in myocardial mitochondria, without altering the organelle ultra structure (Uyemura et al. 1995). Hence, it is possible that T. cruzi can control the central machinery responsible for energetic metabolism in the host in order to access metabolites that are crucial to its proliferation (Schwarz de Tarlovsky et al. 1995, Baez et al. 2008). This possibility is crucial and warrants further research in order to understand the mechanisms that induce oxidative stress during T. cruzi infection.

Iron-proteins constitute a predominant scavenger mechanism of NO (Angelo et al. 2008, Richardson & Lok 2008). As iron is mainly provided by the heme group, it constitutes an additional link between the functions of NO and the respiratory cycle (Chung et al. 2008). Oxygen drives the conversion of nitrosylhemoglobin in the “tense” structure (or partially nitrosylated, deoxy) to S-nitrosohemoglobin in the “relaxed” structure (or ligand-bound, oxy). In the absence of oxygen, nitroxy anion (NO-) is liberated in a reaction which produces methemoglobin. The yields of both S-nitrosohemoglobin and methemoglobin are dependent on the NO/Hb ratio. These recently discovered reactions have provided new insights into the origin of S-nitrosothiols, methemoglobin and its related valence hybrids.

Mechanistic re-examination of the interactions of NO with other heme proteins containing allosteric thiol sites may be warranted (Gow & Stamler 1998). In addition, it is well established that, in the Haber-Weiss reaction (a reaction that generates hydroxyl radicals [•OH] from hydrogen peroxide and superoxide [•O\(_2\)\(^-\)]), iron has a catalytic role, which leads to the propagation of damaging ROS. Thus, NO appears to be involved in cellular defence against iron-mediated ROS generation, mainly by the induction of cellular iron removal (Larrainzar et al. 2008, Trujillo et al. 2008). The role of these mechanisms in the pathogenesis of T. cruzi-induced myocarditis is currently unknown.

**NO and the immune response**

As previously stated, one of the most important functions of NO in the immune system is in antimicrobial defence (De Groote & Fang 1995, Fang 1997, Nathan & Shiloh 2000). Reactive oxygen and nitrogen species derived from NO are essential for protection against various intracellular pathogens including viruses, bacteria, fungi and protozoans. More specifically, NO has been demonstrated to protect against infection from T. cruzi (Figs 1, 2) and other protozoa as Toxoplasma gondii, Leishmania major, Leishmania donovani, Plasmodium sp and Schistosoma mansoni (Adams et al. 1990, Vespa et al. 1994, Wynn et al. 1994, James 1995, Stenger et al. 1996, Murray & Nathan 1999, Brunet 2001). Furthermore, the killing activity of NO has also been shown to be effective in host defence against tumour cells (Huerta et al. 2008) and alloantigens (Shi et al. 2008).

NO is perhaps the most important among the group of early mediators produced by cells of the innate immune system. Phagocytes constitute the first line of microbial defence and they function by sensing the presence of different types of infectious agents (Carneiro-Sampaio & Coutinho 2007) through pattern recognition receptors, including Toll-like receptors (TLRs) and the most recently described NOD- (NLRs) and RIG-like receptors. These receptors recognise multiple microbial patterns; therefore, they are critical for triggering the production of inflammatory mediators and essential for activation of the adaptive immune response (Schnare et al. 2001, Kanneganti et al. 2007, Underhill 2007). In fact, several antigens derived from intracellular parasites can be recognized by innate immune receptors on macrophages, triggering NOS2 activity (Xie et al. 1992, MacMicking et al. 1997).

NOS2 is produced by antigen-presenting cells (APC) during antigen processing and presentation to T cells and it can modulate various functions of APCs. It can inhibit the expression of major histocompatibility complex class II molecules in activated macrophages and, at high concentrations, may also inhibit IL-12 synthesis, thus contributing to the desensitization of macrophages after exposure to inflammatory stimuli (van der Veen 2001). Indeed, NO induces transcription of IL-12 p40, but not of IL-12 p35, in human macrophages (Salvucci et al. 1998). The IL-12 p40 homodimer is an antagonist for IL-12 and this antagonism might be at least partially responsible for the reduced Th1 reactivity in the presence of NO (Pahan et al. 2001). However, a new report has indicated that the IL-12 p40 homodimer can also induce...
NO production by microglia (Jana et al. 2009), revealing the complex functions of NO in innate immunity.

Furthermore, NO affects the immune profile of Th1 cells, as mice with a disrupted NOS2 gene exhibit enhanced Th1 activity, which in turn, can affect the Th1/Th2 balance (Singh et al. 2000). It has been shown that high amounts of NO prevent apoptosis and, given that Th1 cells are more susceptible to apoptosis than Th2 cells, this represents an additional regulatory mechanism of the Th1/Th2 balance (Xiao et al. 2008).

NO can also affect immune responses through its ability to regulate S-nitrosylation of several components of the apoptotic machinery (Okuda et al. 1996, Melino et al. 1997, Johann et al. 2007). Apoptosis is an important process in lymphocyte homeostasis and maturation in the thymus, as well as in lymphocyte proliferation in the periphery. Decreased S-nitrosylation of caspase-3 increases its intracellular enzymatic activity. In addition, Fas-mediated activation of caspase-3 is induced not only by cleavage of the zymogen to its active subunits, but also by denitrosylation of its active thiol site. The regulation of apoptosis by NO has an obvious impact on the strength of effector immune responses.

The cytoprotective properties of low/intermediate levels of NO may limit tissue damage during inflammation (Cattell & Jansen 1995, Okuda et al. 1996, Niedbala et al. 1999, De Gouw et al. 2001). Interestingly, NO significantly increases the proliferation, division and viability of regulatory T cells (Sakaguchi 2004), a lymphocyte subset which has been shown to be involved in acute experimental *T. cruzi* infection (Mariano et al. 2008). Indeed, regulatory T cells induced by NO stimulation (NO-Treg) are as efficient as natural Tregs in suppressing the differentiation of different effector lymphocyte subsets (Niedbala et al. 1999, Packard & Khan 2003). Furthermore, exposure of murine lymphocytes to NO suppresses IL-2 transcription, reducing clonal expansion and indirectly favouring a Th2 response (Taylor-Robinson et al. 1994).

Other important feedback mechanisms mediated by NO, which prevent dysregulated immune responses, include downregulation of cell adhesion and migration, which unchecked, would result in serious and overwhelming inflammatory injury (Biffl et al. 1996, Hokari et al. 1998, Staykova et al. 2003, Dal Secco et al. 2006). Of note, inactivation of P-selectin expression by NO, which affects leukocyte adherence, may also preferentially affect Th1 cell migration (van Wely et al. 1998).

Upon stimulation by cytokines or bacterial lipopolysaccharide (LPS), endothelial cells exhibit increased expression of ICAM-1 in vitro, which contributes to the transmigration of all classes of leukocytes, but mainly neutrophils (Biffl et al. 1996). In addition, NOS2 deficiency or inhibition of NOS or sGC by pharmacological inhibitors, leads to enhanced LPS-induced ICAM-1 expression on mesenteric microcirculation (Dal Secco et al. 2006).

The migration of inflammatory cells may also be affected by the chemical modifications of matrix metalloproteinases (MMPs) (Ridnour et al. 2007). Recently, it was demonstrated that the activities of MMP-2 and MMP-9 are increased during acute myocarditis in experimental *T. cruzi* infection and that the inhibition of these enzymes leads to reduced myocarditis and improved survival in mice (Gutierrez et al. 2008). Accordingly, MMPs are activated in inflammatory or ischemic/reperfusion conditions (Gu et al. 2002).
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NO may also affect lymphocyte migration by altering cell motility. In vitro, NO induces actin polarization in T cells, inhibiting their trans-endothelial migration in a p70S6 kinase-independent manner (Staykova et al. 2003). Moreover, NO may also inhibit the expression of integrins, such as CD11a/CD18, in neutrophils (Banick et al. 1997, Grisham et al. 1998). Since NOS2 is involved in peroxynitrite-dependent tyrosine nitration (Sato et al. 2000, Yeh et al. 2007), it also regulates chemokine production and affects the inflammatory response mediated by IP-10, MCP-1, MIP-1α and MIP-2, and IL-8 (Mach et al. 1999, Pfiefschitter et al. 2001).

NO also participates in the maintenance of inflammatory diseases (such as arthritis, ulcerative colitis and Crohn’s disease) and in the pathogenesis of *T. cruzi*-induced myocarditis (Silva et al. 2003, Machado et al. 2008). Indeed, several classic inflammatory symptoms, for example erythema and vascular leakiness, are related to the production of NO and can be reverted by NOS inhibition (Cuzzocrea et al. 2002). In chronic immune responses to intracellular pathogens, NO is reported to play a regulatory role and may promote parasite persistence. For these reasons, it is suggested that NO is cytostatic rather than cytotoxic for parasites (Klotz et al. 1995).

The dual role of NO during *T. cruzi* infection

Intracellular protozoans have infected vertebrates since ancient times and are usually able to establish chronic infection. A spontaneous cure is uncommon in these diseases, suggesting that potent mechanisms have been developed by these pathogens in order to evade immune detection or destruction. Among these keystone mechanisms, which attest to their remarkable strength, is the capacity of *T. gondii*, *T. cruzi* and *Leishmania* spp. to invade and replicate within many different cell types (Leiriao et al. 2004, Denkers & Butcher 2005, Gregory & Olivier 2005).

Infection with *T. cruzi* in humans can lead to the development of Chagas disease, the clinical features and evolution of which are determined by a combination of parasite factors (i.e. tissue tropism and evasion mechanisms), mode of inoculation (i.e. the mode of contamination or transmission and the size of the inoculum), as well as by host-derived factors (i.e. exacerbated immune response) (Coura 2007).

During *T. cruzi* infection, NO can directly or indirectly modulate the effector leukocyte machinery through diverse mechanisms. This process involves microbicidal effects derived from toxic-free radicals (peroxynitrite and superoxide) generated after NO production, as well as regulation/enhancement of the inflammatory response induced during this type of infection, a dual role in the immunity that is usually observed for NO. This well-known immune duality is usually dependent on concentration and, once dysregulated, may lead to host cell toxicity, autoimmunity or parasite persistence due to immune evasion, all of which can lead to pathology (FR Gutierrez et al. 2009, unpublished observations).

NO is involved in the control of *T. cruzi*-induced parasitemia and directly kills the parasite in vitro (Vespa et al. 1994) (Figs 1, 2). NO affects *T. cruzi* by chemically modifying cysteine-containing proteins and/or by binding to metalloproteins that mediate crucial metabolic processes. Recently, it was reported that NO or NO donors can inhibit the catalytic activity of cruzipain, the major papain-like cysteine proteinase in *T. cruzi*. Analogous to a similar protein in *Plasmodium*, this dose-dependent effect was attributed to S-nitrosylation of Cys25, a catalytic residue present in the active site of cruzipain (Venturini et al. 2000).

The strength of NO toxicity is dependent on the sensitivity of the parasite, which differs among parasite strains and according to the physiological microenvironment. NO has been demonstrated to be the principal effector molecule involved in macrophage-mediated killing of *T. cruzi* amastigotes (Nathan & Shiloh 2000, Colasanti et al. 2002, Silva et al. 2003). Contradictory evidence suggests that susceptible mouse strains display increased macrophage activation after contact with the parasite, which may be due to the fact that, in these animals, infection with *T. cruzi* induces an overwhelming production of both NO and •O₂⁻ (Russo et al. 1989, Cardoni et al. 1990, Arantes et al. 2004).

An additional mechanism by which NO can affect the metabolism of *T. cruzi* is through the reduction of available growth factors. For example, iron is an important growth factor for *T. cruzi* (Ciccarelli et al. 2007). NO induces nitrosilation of the heme group from haemoglobin, haematin or haemin, the main sources of iron. The main target of oxidative stress during *T. cruzi* infection is the erythrocyte, as it is the major principle site of the antioxidant chemical machinery. The nature and extent of oxidative injury depends on three factors: (i) the induction of NOS2 and, thus production of NO in response to infection (Alvarez et al. 2004); (ii) the oxidative stress generated outside of the erythrocyte, particularly phagocyte-derived •O₂⁻ and (iii) the rate of reaction between NO and either haemoglobin or •O₂⁻.

The imbalanced counteraction of the oxidative response leads to haematological disorders (i.e., anaemia), which are observed in the acute phase of *T. cruzi* infection (Malvezi et al. 2004).

Oxidative stress is also observed in myocarditis during experimental *T. cruzi* infection. As myocarditis progresses, a substantial decline in cardiac mtDNA content (54-60%) and mitochondria-encoded transcripts (50-65%) indicate that alterations in mtDNA contribute to the quantitative deficiencies in respiratory chain activity of infected individuals (Vyatkina et al. 2004). In fact, during chagasic cardiomyopathy, mitochondrial dysfunction occurs as a consequence of intense oxidative stress (Wen et al. 2006) and is evidenced by deficiencies in respiratory chain complexes (CI-CV) (Garg 2005).

As previously suggested, the accuracy of initial pathogen recognition by the immune system is crucial for the production of NO in order to mount an appropriate immune response. TLRs can sense the presence of *T. cruzi* (Campos & Gazzinelli 2004), however, because it is an intracellular protozoan, *T. cruzi* has an extremely complex antigenic repertoire (Buscaglia et al. 2006). This
makes it difficult to determine the exact mechanism by which the large diversity of cell-surface molecules on \textit{T. cruzi} are recognised by the innate immune system (Tartleton 2007). Although other molecules may be involved, it is known that innate recognition of glycosphingolipid-dylinositol-anchored mucin-like glycoproteins from \textit{T. cruzi} are potent inducers of NO biosynthesis by IFN-\(\gamma\)-activated macrophages (Camargo et al. 1997).

Early after infection, IL-12 is required for the induction and maintenance of IFN-\(\gamma\) production by innate and adaptive immune cells (Silva et al. 1998). IFN-\(\gamma\) production by Th1 effector cells has consistently been implicated in the pathogenesis of Chagas disease and is an important factor for maintaining \textit{T. cruzi}-mediated pathology. During acute experimental \textit{T. cruzi} infection in mice, the parasite induces a profound suppression of the lymphoproliferative response to mitogens and \textit{T. cruzi} antigens. This process is largely mediated by increased NO synthesis and decreased IL-2 production (Abrahamsohn & Coffman 1995). Our group demonstrated that NO induces apoptosis of cells from BALB/c mice acutely infected by \textit{T. cruzi}. Splenocytes from infected mice displayed reduced viability and elevated levels of spontaneous apoptosis after 48 h in culture. Inhibition of NO production, by the addition of the L-arginine analogue NG-monomethyl-L-arginine or the addition of monoclonal antibodies (mAbs) against IFN-\(\gamma\) or TNF-\(\alpha\) partially restored viability and decreased apoptosis of splenocytes from infected mice (Martins et al. 1998). In addition, the production of IL-17 has recently been implicated in mediating regulatory responses against \textit{T. cruzi} (Monteiro et al. 2007). Of note, IL-17 markedly augments NOS2 mRNA and subsequent NO production. Additionally, \textit{T. cruzi} infection induces the expression of chemokines (MIG, IP-10, RANTES, MIP) and adhesion molecules at sites of CD4+ and CD8+ T cell infiltration (Teixeira et al. 2002). Cytokines and NO can modulate the production of chemokines and adhesion molecules in vivo and in vitro, influencing the course of infection (Savino et al. 2007, Machado et al. 2008). Chemokine receptors are also involved in cellular activation during parasitic infections and this G-protein-coupled signaling pathway is implicated in NO production as well (Benevides et al. 2008).

One mechanism by which the innate immune response can affect the activation of T cells is through the macrophage-mediated reduction of available L-arginine in the microenvironment. The levels of this metabolite depend on the cytokine milieu. For example, macrophages stimulated with IL-4 and IL-13 (but not IFN-\(\gamma\)) up-regulate arginase I and the L-arginine receptor CAT-2B, thus inducing a rapid reduction in the concentrations of L-arginine; this, in turn, down-modulates the expression of CD3e in T lymphocytes reducing their activation (Rodriguez et al. 2003). Arginine is also required for the synthesis of NO, thus this can constitute a feedback mechanism to regulate the immune system. Recently, recognition of intracellular pathogens by TLRs has been implicated in the downregulation of NO production, through increased arginase I activity, in a STAT6-independent manner, which favours parasite growth and survival (El Kasmi et al. 2008).

As one of the most successful parasitic protozoans, \textit{T. cruzi} has evolved active strategies to evade host defences (Eckmann et al. 2000). Interestingly, epimastigote forms of \textit{T. cruzi} synthesise their own NO through a partially characterized NOS enzyme which displays regulatory and immunoochemical properties resembling those of endogenous NOS1 (Pereira et al. 1999, Goldstein et al. 2000, Piacenza et al. 2001).

Furthermore, \textit{T. cruzi} can also exploit the removal of apoptotic cells by professional phagocytes, which is an important mechanism by which some pathogen-induced cell alterations are ultimately detected and which is involved in the recycling of cellular constituents. Uptake of apoptotic cells does not induce an inflammatory response. Accordingly, macrophages upregulate arginase II after phagocytosis of apoptotic cells, which regulates NO production by NOS2 (Freire-de-Lima et al. 2000, Johann et al. 2007). Additionally, L-arginine, the substrate for NO production, can inhibit the programmed cell death of epimastigotes, either by NOS2-dependent production or by the activity of arginine decarboxylase, which produces polyamines that support parasite proliferation (Paveto et al. 1995).

**Implications of NO in therapeutic treatment against Chagas disease**

The current pharmacological agents available to treat Chagas disease include benznidazole (Rochan and Rodanil; Roche, Brazil) and nifurtimox (Lampit; Bayer, Germany). These drugs are relatively effective in the acute and sub-chronic stages of Chagas disease (Rossomando et al. 1998, Sosa Estani et al. 1998, Cançado 2002, Altelas et al. 2005). However, both drugs have significant side effects, including anorexia, vomiting, peripheral polyneuropathy and allergic dermopathy (Rassi et al. 1999). Moreover, several parasite strains are resistant to these treatments, even during the acute phase of the disease (Filardi & Brener 1987, Galvao et al. 1993, Urbina 1999). The rate of cure observed in patients with these drugs is 50-70% during the acute phase and 0-20% during the chronic phase (Guedes et al. 2006). This situation is severely aggravated by the absence of a diagnostic standard, which makes the parameters for a cure, in order to evaluate the outcome of trypanocidal therapies, debatable. Thus, there is an imperative requirement for the development of novel, safe therapeutic agents to treat Chagas disease.

As stated before, parasite elimination largely depends on the production of pro-inflammatory cytokines, such as IFN-\(\gamma\), TNF-\(\alpha\) and IL-12, as they act in concert to activate macrophages to kill the intracellular parasite through the production of NO and its derived nitrogen and oxygen radicals (Aliberti et al. 1999, 2001, Machado et al. 2000). Studies using experimental models of acute \textit{T. cruzi} infection have demonstrated that the anti-parasitic activity of benznidazole involves the participation of these cytokines (Michailowsky et al. 1998, Molina et al. 2000), as well as covalent modifications of macromol-
ecules by nitreducer intermediates (reductive stress). Conversely, nifurtimox acts by reducing the nitro group to unstable nitro anion radicals, which, in turn, react to produce highly toxic reduced oxygen metabolites (superoxide anion and hydrogen peroxide) (Docampo 1990).

NO donor compounds have low toxicity in vitro and in vivo and are stable in aqueous media in the presence of oxygen and NO released by reducing agents that are present in the host inflammatory microenvironment (Bogdan 2001, Silva et al. 2007). These donor compounds have recently emerged as an interesting and important alternative treatment to experimental T. cruzi infection (Silva et al. 2007). We recently reported that a series of ruthenium nitrosyls, trans-[Ru(NO)(NH₃)₄](NO₃)₂⁺ complex ([15]aneN₄ = 1,4,8,12-tetrazacyclopentadecane, a macrocyclic quadridentate amine ligand) induced parasitological cure in a therapeutic schedule that involved a 20-day treatment of mice infected with the Y strain of T. cruzi. We evaluated the parasitological cure of mice treated with trans-[RuCl([15]aneN₄)NO]⁺ and compared it to treatment with benzimidazole or treatment with both drugs. Benzimidazole or trans-[RuCl([15]aneN₄)NO]⁺ alone resulted in a 40% and 20% parasitological cure, respectively. However, when administered together, 80% of the treated animals were considered cured. These findings were associated with reduced or absent cardiac damage during the acute phase of T. cruzi infection (PMM Guedes et al., unpublished observations).

These studies provide evidence that NO donors help to improve the efficacy of current trypanocidal drugs, reducing the time of treatment and preventing adverse reactions. Hence, administration of NO donors and other drugs in conjunction can constitute a promissory therapeutic approach that can affect the biology of T. cruzi by direct toxicity, by affecting essential metabolites, or by enhancing the immune response against the parasite.

Nonetheless, this broad spectrum of activity of NO can also be responsible for extensive damage to the tissues of infected hosts and for manifestation of the disease. These data have led investigators to propose NO as a crucial target for the immunotherapy of this infectious disease. However, additional studies are required to further understand the multiple roles of NO and to establish the risks and benefits of such therapeutic approaches during parasitic infection in patients.

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