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COMMENTARY

New drugs for neglected infectious diseases: Chagas' disease

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Chagas' disease (CD) is caused by the protozoan *Trypanosoma cruzi* (Tc) and remains an important cause of morbidity and mortality. Most researchers in the field now agree that chronic low grade parasite persistence in tissue drives tissue damage and the autoimmune component of CD. Current therapy relies on two compounds: benznidazole and nifurtimox. Despite their long history in the treatment of CD, both compounds induce significant side-effects. In the current issue of the *BJP*, two contributions demonstrate that NO-donors are active, especially in combination with benznidazole, against Tc *in vitro* and in experimental models *in vivo*. The basic concept used by the authors to develop novel anti-Tc compounds relied on the demonstrated ability of nitric oxide to kill the parasite. There are several issues still to be resolved but the reported studies are a clear advance to the field and should be considered for further pre-clinical development.
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This article is a commentary on Silva *et al.*, pp. 260–269 and Guedes *et al.*, pp. 270–282 of this issue. To view these papers visit http://dx.doi.org/10.1111/j.1476-5381.2010.00524.x and http://dx.doi.org/10.1111/j.1476-5381.2010.00576.x

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Abbreviations: CD, Chagas' disease; iNOS, inducible NO synthase; NOS, NO synthases; Tc, *Trypanosoma cruzi*

Chagas' disease (CD) is caused by the protozoan *Trypanosoma cruzi* (Tc) and remains an important cause of morbidity and mortality. Chronic CD is considered a neglected disease that affects the lives of millions of people, and the chronic cardiomyopathy is the most severe form of human infection.

Most researchers in the field now agree that chronic low grade persistence of the parasite in tissue is the major driver of tissue damage and the autoimmune component of CD and this has generated new interest in the development of novel compounds that target the parasite. Current therapy relies on two compounds: benznidazole and nifurtimox. Despite their long history in the treatment of CD, both compounds induce significant side-effects. Moreover, treatment failure is known to occur even during acute infection, the stage in which anti-parasitic drug therapy is most effective. Most experts believe that once the disease has progressed to the chronic clinical stage, current anti-parasitic treatment is not justified as structural damage to the heart appears to be irreversible. The modern management of established chronic Chagasic heart disease is complex and includes clinical staging, use of anti-arrhythmic drugs, pacemakers and anti-coagulants (Rocha *et al.*, 2007). Heart transplantation (Godoy *et al.*, 2009) and stem cell therapy (Tanowitz *et al.*, 2009) have been recently reported with some success in patients with the most severe forms of Chagasic cardiomyopathy, but at considerable cost. Because of these costs, much interest has been placed in trying to understand the role of therapy during the indeterminate phase of the disease. Indeed, treatment during this stage is controversial and the value of anti-parasitic treatment to enhance quality of life and prevent disease progression is currently being evaluated in large clinical trials. Factors that associate with progression from the indeterminate phase to clinical disease and prognostic factors of progression are not known. Moreover, although the immunology of CD in experimental models and humans has been extensively investigated (Dutra *et al.*, 2005), these studies have not led to the development of vaccines or immunomodulatory drugs to ameliorate the consequences of this infection. It requires therefore the concerted effort of the scientific community to develop new therapeutic agents and understand their usefulness in the treatment of acute, indeterminate and chronic CD.

In the current issue of the *BJP*, two contributions by Dr Silva's group (Guedes *et al.*, 2010; Silva *et al.*, 2010) describe potential new molecules which are active against Tc *in vitro* and in experimental models *in vivo*. The basic concept used by the authors to develop novel anti-Tc compounds relied on the demonstrated ability of nitric oxide (NO) to kill the parasite (Silva *et al.*, 2003). NO is a gas initially characterized as a vasodilator and is a product of the oxidation of L-arginine to L-citrulline by a family of NADPH dependent enzymes,

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collectively known as NO synthases (NOS). NO production by inducible NOS (iNOS or NOS2) has antimicrobial actions, depending on its concentration, and can also interfere with cell proliferation and death by apoptosis. In experimental acute infection with Tc, the cytokines IFN-γ, TNF-α and several chemoatractants are produced and thought to be relevant for iNOS induction and NO-dependent killing of Tc (Machado *et al*., 2000; Talvani *et al*., 2002; Machado *et al*., 2005). While NO contributes to intracellular killing of Tc, it may also cause myocardial dysfunction (Silva *et al*., 2003). Therefore, the generation of NO from cardiac NOS may be a 'double-edged sword' (Huang *et al*., 1999). Moreover, it should be noted that the role of NO in the context of human infection by Tc is not understood. This is similar to the situation with infection by *Mycobacterium tuberculosis*, for which there are good experimental data in mice suggesting a beneficial role for NO but no definitive data in humans (Chan and Flynn, 2004). Thus, the suggestion of the Silva group to employ NO-donors for therapy against Tc infection should be viewed with caution and further studies in other experimental systems and in humans are needed.

Despite these potential caveats and need for further studies, results from the Silva group are an advance to the field. In the first study (Guedes *et al*., 2010), they demonstrate that treatment with *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ releases NO inside macrophages and has significant trypanocidal effect *in vitro*, especially in the presence of benznidazole. Significantly, combined treatment with *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ and benznidazole prevented death, greatly decreased parasitemia and cardiac inflammation, and resulted in 80% cure rates, as assessed by the summation of accepted techniques. In the second study, the authors report a series of ruthenium NO-donors, based on the structure *cis*-[Ru(NO)(bpy)2L]Xn. These compounds possessed anti-Tc activity *in vitro* and *in vivo* at doses substantially lower those necessary to cause toxicity. Significantly, the studies suggest that one of the mechanisms of action of *cis*-[Ru(NO)(bpy)2L](PF6)n compounds was via S-nitrosylation of the active site Cys166 of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC1.2.1.12). Bloodstream forms of trypanosomatids have no functional tricarboxylic acid cycle and are highly dependent on glycolysis for ATP production (Verline *et al*. 2001 and Souza *et al*. 1998). GAPDH is a key glycolytic enzyme for the parasite and has important structural differences from the homologous protein of the mammalian host (Guido *et al*. 2008).

There are many issues to be resolved before the reported compounds reach the clinics. Although, the compounds were effective against the *Y* strain of Tc, these authors have not tested the efficacy of their compounds against other parasite strains and in other models of this infection, including models of Tc infection in other species. This is a relevant issue as the effect of anti-Tc drugs may be strain-specific. For example, not all strains of Tc respond similarly to treatment with benznidazole even during acute human infection (Filardi and Brener, 1987). Furthermore, pharmacokinetic studies of compounds and full toxicological profiling need to be performed to define ideal drug candidates. Despite these concerns, results in these two papers demonstrate that NO-donors are active both *in vitro* and *in vivo*, especially in combination with benznidazole, and should be considered for further pre-clinical development.

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