Present situation and new strategies for Chagas disease chemotherapy - a proposal

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Treatments for Chagas disease have been administered since the first attempts by Mayer & Rocha Lima (1912, 1914) and up to the drugs currently in use (nifurtimox and benznidazole), along with potential drugs such as allopurinol and first, second and third-generation antifungal agents (imidazoles and triazoles), in separate form. Several diseases such as tuberculosis, leprosy and AIDS only came under control after they were treated with associations of drugs with different mechanisms of action. This not only boosts the action of the different compounds, but also may avoid the development of parasite resistance .To this end, over the short term, we propose experimental studies on laboratory animals and clinical trials with the following associations: (i) nifurtimox (8 mg/kg/day) + benznidazole (5 mg/kg/day) x 60 consecutive days; (ii) nifurtimox (8 mg/kg/day) or benznidazole (5 mg/kg/day) + allopurinol (8-10 mg/kg/day) x 60 days and (iii) nifurtimox (8 mg/kg/day) or benznidazole (5 mg/kg/day) + ketoconazole, fluconazole or itraconazole (5-6 mg/kg/day) x 60 consecutive days. The doses of the drugs and the treatment schedules for the clinical trials must be adapted according to the side effects. From these, other double or triple associations could be made, using drugs with different mechanisms of action. This proposal does not exclude investigations on new drugs over the median and long terms, targeting other aspects of the metabolism of Trypanosoma cruzi. *Until such time as the ideal drug for specific treatment of Chagas disease might be discovered, we need to develop new strategies for achieving greater efficacy with the old drugs in associations and to develop rational experimentation with new drugs.*

Key words: Chagas disease - chemotherapy - present situation - new strategies - a proposal

Since 1912, just three years after the discovery of Chagas disease, many attempts have been made to treat the disease. Mayer and Rocha Lima (1912) experimentally tested atoxyl (arsenic), fuchsine (rosaniline dye) and tartar emetic and soon afterwards (1914) tried mercury chloride; all without favourable results. Carlos Chagas himself, together with Evandro Chagas in their Manual on Tropical and Infectious Diseases Volume I (1935), highlighted in just one paragraph of seven lines the ineffectiveness of the attempts that had been made to treat the disease. In this passage, they stated: "So far, no specific treatment for American trypanosomiasis exists. Medications with trypanosomicidal action have been tried out experimentally by many researchers without any success. Some clinical syndromes may experience symptomatic therapeutic action, according to their manifestations and evolution."

A review conducted by Coura and Silva (1961) showed that, up to that time, the following groups of substances had been used clinically or experimentally: quinoline derivatives; various other antimalarial agents; arsenobenzols and other arsenical compounds; phenanthridines; gold, bismuth, copper and zinc salts; sodium iodide; gentian violet; aminopterin; para-amino salicylic acid; hydrazides; antihistamines; sulfonamides; adrenocorticotropic hormone and cortisone; stylomycin derivatives; amphotericin B; more than 30 antibiotics and nitrofurans. Some nitrofurans had shown promising results experimentally, according to Packanian (1952, 1957) and Brenner (1961). Brenner (1968) highlighted that, among the 36 groups of medications that had been used up to that time, the following had shown activity against experimental Chagas disease: bis-quinaldine (Bayer 7602); phenanthridines; amino-quinolines; trivalent arsenical compounds (Bayer 9736) and spirotrypan; acromycin or stylomycin and stylomycin aminonucleoside; nitrofurans; 2-acetamide-5-nitrothiazole and imidazole (Flagyl). However, Cançado (1968, 1973) criticised various results considered "good" or "excellent" in treating human cases of Chagas disease with these medications because they were contradictory to each other or because the methodology was inadequate for cure verification. For example, Mazza et al. (1937, 1942) and Pifano (1941) obtained "good" results using Bayer 7602 to treat acute cases of the disease, based on their observations of a reversal of the clinical manifestations and a shift in smear drop results to negative; however, these signs were also observed in untreated cases. The treated cases with "good results" subsequently presented positive xenodiagnosis, which is indicative of therapeutic failure.

The first experimental results that showed real effectiveness were observed with the use of nitrofurans, in a line of research initiated by Pakchanian (1952, 1957). These results led to the introduction of nitrofurazone

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(5-nitro-2-furaldehyde-semicarbazone), which was put on the market in Brazil by the Eaton laboratory as Furacin ointment for topical use. Subsequently, this drug was used by Brener (1961) at a dose of 100 mg/kg/day orally for prolonged periods (53 days) in mice infected with *Trypanosoma cruzi* and it was observed that 95.4% of the animals treated achieved parasitological cure (62/65). Ferreira (1961, 1962) and Ferreira et al. (1963) observed "good results" in the first acute cases of human disease in children, with few side effects. During this same period, Coura et al. (1961, 1962) treated 14 chronic cases of Chagas disease with nitrofurazone at progressive doses from 10-30 mg/kg/day over prolonged periods and observed significant side effects (particularly polyneuropathy), which made it necessary to halt the treatment in most cases. By reducing the dose to 10 mg/kg/day, they were able to treat 10 patients for 60 days. Three of the patients experienced parasitological cure. Of the three treated patients, one had the early form of the disease, acquired in Goiás 18 months earlier when he made a journey to that state as a truck driver and the other two were in the late chronic phase.

A review by Brener (1984) on recent advances in the knowledge of Chagas disease, published in a special issue of the Memórias do Instituto Oswaldo Cruz to mark the 50th anniversary of the death of Carlos Chagas (1889-1934), highlighted several new drugs for treating this disease, along with their mechanisms. Among these drugs were fexinidazole (HOE239), MK-436, ketoconazole, megazole (CL 64'855) and allopurinol. More recently, Coura and De Castro (2002) published an extensive "Critical Review on Chagas Disease Chemotherapy", in which they analysed the experimental and clinical studies carried out since 1970, including the rules and recommendations for clinical treatment, the prospects of new drugs in clinical trials and promising new targets. The experimental studies of Andrade et al. (1977, 1991, 2000) on the resistance of *T. cruzi* strains to treatment and the persistence of the parasite's antigen, which delays the change to negative serological reactions for cure verification, were highlighted.

The objectives of the present paper are to review the present situation regarding specific treatments for Chagas disease and to propose new treatment strategies using existing drugs of different mechanisms of action in combination, as is done in the treatment for tuberculosis, leprosy and AIDS, among other infectious diseases, with the aims of avoiding resistance and increasing treatment efficacy.

Present situation regarding specific treatments for Chagas disease - At present, there are only two effective drugs for the treatment of Chagas disease, particularly in the acute and chronic, early phase of the infection: nifurtimox and benznidazole. However, neither of these therapeutics meets the precepts for a good drug in accordance with the criteria of the World Health Organisation: (i) parasitological cure of acute and chronic cases of the infection; (ii) effective in a single dose or with few doses; (iii) accessible to patients, i.e., low cost and easy to obtain; (iv) no side effects or teratogenic effects; (v) no need for hospitalisation for treatment and (vi) no resistance shown or induced in the etiological agent. Both of these drugs cure around 80% of acute cases and 20% of chronic cases. Patients require 60 days of treatment, with 2-3 doses per day. The drugs are not accessible for patients; at least not in Brazil, where nifurtimox is unavailable and the distribution of benznidazole is restricted to specialised clinics that require medical monitoring during the course of treatment. Both drugs induce significant side effects and some strains of *T. cruzi* are resistant to treatment.

Nifurtimox - Nifurtimox is 5-nitrofuran [3-methyl-4-(5'-nitrofurfuridene-amine) tetrahydro-4H-1,4-thiazine 1,1-dioxide (Bayer 2502). The mechanism of action of nifurtimox involves the production of nitro-anion radicals, which, in the presence of oxygen, leave *T. cruzi* incapable of detoxifying free radicals (Do Campo & Moreno 1986). Nifurtimox was put on the market in Brazil under the name of Lampit and was subsequently withdrawn from the market in Brazil, Argentina, Chile and Uruguay. Today, this medication is produced and used predominantly in Central America. Nifurtimox is the most active of the 5-nitrofurfuridenes tested experimentally by Bock et al. in 1969 and clinically by several other researchers (Bocca-Tourres 1969, Cançado et al. 1969, 1975, 1976, Rubio & Donoso 1969, Schenone et al. 1969, 1972, 1981, Rassi & Ferreira 1971, Prata et al. 1975, Ferreira 1990, Rassi & Luquetti 1992, Coura 1996, Coura et al. 1997). All research shows the best results are obtained when treatment is started during the acute phase; results are highly variable for patients in the chronic phase. It is important to note that some strains of *T. cruzi* present natural resistance to treatment with nifurtimox, which has also been proven experimentally (Andrade et al. 1977).

The nifurtimox treatment regimen from which the best results have been obtained and which continues to be recommended, is 8-10 mg/kg/day, divided into 2-3 doses per day, for 60 days. The side effects most frequently observed have been anorexia, weight loss, psychological changes, excitability, muscle tremors, somnolence, hallucinations and digestive manifestations such as nausea, vomiting and, occasionally, abdominal pain and diarrhoea. In rare cases, localised convulsions have been observed. These side effects can be controlled with diazepam, cimetidine, metoclopramide, antihistamines and other medications.

Benznidazole - Benznidazole is 2-nitroimidazole (N-benzyl-2-nitroimidazole-acetamide (RO-1051). The action of benznidazole is related to the nitroreduction of components of the parasite and the binding of metabolites to the nuclear DNA and k-DNA of *T. cruzi* and the lipids and proteins of the parasite (Polack & Richle 1978, Diaz de Taranzo et al. 1988). Benznidazole was put on the market in Brazil by Roche (which recently transferred the patent to LAFEPE, the Pharmaceutical Laboratory of the state of Pernambuco) under the name Rochagan and in Argentina as Rodanil. Experimentally, benznidazole shows high levels of in vitro and in vivo activity against *T. cruzi* (Richle 1973). Clinically, benznidazole has been tested on both the acute and the chronic phase of Chagas disease by many researchers, particularly in Argentina, Brazil and Chile (Schenone et al. 1975, 1981, Ferreira

1976, Coura et al. 1978, 1997, Cançado & Brenner 1979, Rassi & Luquetti 1992, Viotti et al. 1994, Andrade et al. 1996, Coura 1996, Cançado 1997, Sosa-Stani et al. 1998, Rassi et al. 1999). Coura et al. (1978) analysed 309 cases treated with benznidazole (54 acute and 255 chronic cases) by 10 groups of Brazilian researchers with therapeutic regimens of 5-8 mg/kg/day for 30 or 60 days and observed that the degree of suppression of parasitaemia (as assessed by xenodiagnosis) and the side effects were identical after 30 or 60 days of treatment. Further, there was no treatment advantage with doses greater than 5 mg/kg/day. Subsequently, Coura et al. (1997) found from a randomised, controlled field study treating groups of patients with nifurtimox, benznidazole and placebo, that benznidazole was better at suppressing parasitaemia in chronic cases of Chagas disease.

The best regimen for treating Chagas disease with benznidazole is 5 mg/kg/day, divided into 2-3 daily doses, for 60 days. The main side effects observed have been (i) hypersensitivity (dermatitis, generalised oedema, ganglionic infarction and joint and muscle pains; (ii) bone marrow depletion (neutropaenia, thrombocytopaenic purpura and agranulocytosis) and (iii) peripheral polyneuropathy. These side effects can be controlled with antihistamines, corticosteroids and, in severe cases of agranulocytosis, thrombocytopaenic purpura or Stevens-Johnson syndrome, suspension of the treatment.

Other drugs used in experimental and clinical trials for the treatment of Chagas disease - Other drugs such as allopurinol, a hypoxanthine analogue that inhibits xanthine oxidase and is used as an antihyperuricaemic agent for treating gout and antifungal agents such as ketoconazole (a derivative of imidazole), fluconazole and itraconazole (derivatives of triazole) and, more recently, posaconazole (also an azole derivative) have been shown to be active in vitro against *T. cruzi*. Nonetheless, the experimental and clinical results have been controversial.

Allopurinol - Allopurinol acts as an alternative substrate for the hypoxanthine-guanidine phosphoryl transferase system; it is incorporated into the RNA and leads to the formation of a non-physiological nucleotide that blocks *de novo* synthesis of purines. According to Marr et al. (1978, 1984) and Marr (1991), allopurinol could act as a therapeutic agent against leishmaniasis and American trypanosomiasis. Studies conducted by Lauria-Pires et al. (1998) showed that allopurinol was ineffective during the acute phase of Chagas disease. Gallerano et al. (1990) treated two groups of patients with 600 and 900 mg/day of allopurinol for 60 days and compared these patients to two other groups treated with nifurtimox and benznidazole. They found that the xenodiagnosis became negative (ranging from 75-92%) in all four groups, with the lowest toxicity exhibited in the group treated with allopurinol. Apt et al. (1998) treated 104 chronic patients with allopurinol (8.5 mg/kg/day for 60 days), monitoring the patients by clinical examination, serology, xenodiagnosis, haemoculture and electrocardiogram. Parasitological cure was achieved in 44% of the allopurinol-treated patients. However, this study needs to be better evaluated prospectively. Allopurinol has also been indicated in heart transplantation cases with reactivation of infection due to post-surgical immunosuppression. Erythematous lesions on superior and inferior members were observed in one patient, but the lesions disappeared within three weeks (Tomimori-Yamashita et al. 1997).

Ketoconazole - Ketoconazole is an antifungal agent and acts by altering the permeability of the cytoplasmic membrane, thereby inhibiting sterol synthesis by the membrane and the formation of ergosterol, with degradation of the fatty acids and endogenous steroids of the cells. Brener et al. (1993) demonstrated that ketoconazole was incapable of eradicating parasitaemia from six of the eight Chagas patients treated with doses of 3.1-8.7 mg/kg/day during 51-96 days by oral route and followed up for 60 months. Ketoconazole was the first imidazole to show in vitro activity against *T. cruzi* in the acute phase of experimental infection, but it was shown to be ineffective against the chronic phase (De Castro 1993).

Fluconazole, itraconazole and other triazoles - The triazoles fluconazole and itraconazole present significant action against filamentous fungi and yeasts, acting in the same way as other azoles, i.e., by inhibiting the cytochrome P450 enzyme, which is responsible for synthesising ergosterol in the cytoplasmic membrane, thereby leading to increased permeability and rupturing of the membrane. Second-generation triazoles, including terconazole, saperconazole (R 66905), electrazole (BAY R-3783), genaconazole (SCH 39304) and D0870, along with third-generation triazoles such as variconazole (UK109-496), posaconazole (SCH 56592), ravuconazole (ER 30346) and TAK-187, are drugs that show potential for the treatment of Chagas disease. Some of these drugs have already been shown to have in vitro action against *T. cruzi* (Urbina et al. 1996, 1999, 2000, Molina et al. 2000, 2001, Do Campo 2001). Posaconazole is currently the greatest hope for the treatment of Chagas disease and is already in its initial phase of experimentation in human subjects.

Indications and contraindications for treating Chagas disease - Over the last 10-12 years, several meetings on developing treatments for Chagas disease have been held in Brazil and abroad. Two of these meetings had a prominent role in defining the position of specialists on this subject. The first such meeting was held in Brazil by the Brazilian Ministry of Health, with the participation of 13 specialists and was very well summarised by Luqueti (1997). The second was held at the Oswaldo Cruz Institute (Fiocruz) by OPS/OMS, from April 23-April 25 1998; at this meeting, the rules for Chagas disease treatment using the drugs that are still in use today were established. At the latter meeting, the indications for treatment in the acute phase of the disease, the congenital form, laboratory accidents, chronic phase, recent cases, late chronic phase, organ transplantation cases and acute reactivation of the chronic phase were defined, as described in the critical review by Coura and De Castro (2002). The indications for treating Chagas disease are summarised: (i) acute cases of any nature; (ii) acute reactivations due to immunosuppression; (iii) recent cases: cases among children up to 12 years of age or among

adults infected recently and (iv) indeterminate or benign chronic form, at the discretion of the attending physician. Patients in the severe acute phase and symptomatic congenital patients should be hospitalised for treatment. Asymptomatic or oligosymptomatic acute cases and chronic cases can be treated at the outpatient level, with follow-up by an experienced physician. The contraindications for specific treatment are: pregnancy, liver failure, kidney failure, neurological diseases unrelated to Chagas disease, advanced Chagas disease with grade III or IV cardiopathy (OPS/OMS) and other diseases that might be worsened by this treatment.

Cure verification for Chagas disease - To verify that cure has been achieved, parasitological cure needs to be considered, with evaluation by means of serological tests, polymerase chain reaction (PCR), blood culturing and xenodiagnosis (Coura & De Castro 2002) as well as by observation of the clinical evolution of the disease using conventional electrocardiography, dynamic electrocardiography (Holter), echocardiography, myocardial scintigraphy, ergonometry, assessment of the autonomic system and radiological and manometric evaluations of the digestive system (Rezende-Filho et al. 2005, Rocha et al. 2005). Verification of parasitological cure in the acute phase is relatively easy and fast and is achieved by observing no parasites in direct blood tests, blood cultures, xenodiagnosis, PCR and serological tests. In the chronic phase, verification that parasitological cure has been achieved becomes much more complicated and much slower, considering the low levels of parasitaemia (with treatment) and the long time required for serological tests to become negative (10, 15, 20 or more years), because of the sensitisation of dendritic cells by the antigens of *T. cruzi* (Andrade et al. 1991). Serological tests continue to be positive for many years, even though there is a gradual decline in the titres of anti-*T. cruzi* antibodies. On the other hand, evaluation of the clinical evolution of Chagas disease or of its regression is extremely complex and there is no unanimity on this in studies conducted so far. This is partially due to the small number of cases evaluated and the different evaluation methods used by different authors. A project currently in progress, called BENEFIT (supported by TDR/WHO and several other institutions) is evaluating 1,500 patients treated with benznidazole in three countries (Argentina, Brazil and Colombia) and the respective controls, to define the benefits of treatment on the evolution of Chagas cardiopathy.

Proposal for a new strategy for treating Chagas disease - Several diseases such as tuberculosis, leprosy and AIDS only came under control after they were treated with combinations of drugs with different mechanisms of action. Combinatorial treatments can not only boost the action of the different therapeutic compounds, but may also aid in avoiding the development of parasite chemotherapeutic resistance.

To this end, over the short term, we propose experimental studies on laboratory animals and clinical trials with the following associations: (i) nifurtimox (8 mg/kg/day) + benznidazole (5 mg/kg/day) x 60 consecutive days; (ii) nifurtimox (8 mg/kg/day) or benznidazole (5 mg/kg/day) + allopurinol (8-10 mg/kg/day) x 60 consecutive days; (iii) nifurtimox (8 mg/kg/day) or benznidazole (5 mg/kg/day) + ketoconazole, fluconazole or itraconazole (5-6 mg/kg/day) x 60 consecutive days.

The dosage of the drugs and the treatment schedules for the clinical trials must be adapted according to the observation of any side effects.

Based on data obtained from these studies, other double or triple associations could be designed, using drugs with different mechanisms of action. This proposal does not exclude investigations of new drugs over the medium and long terms, targeting other aspects of the metabolism of *T. cruzi*. Until the ideal drug for the specific treatment of Chagas disease is discovered, we need to develop new strategies for achieving greater efficacy with the old drugs by using combinatorial treatments and develop rational experimentation courses for novel drugs.

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