# A Critical Review on Chagas Disease Chemotherapy

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*In this "Critical Review" we made a historical introduction of drugs assayed against Chagas disease beginning in 1912 with the works of Mayer and Rocha Lima up to the experimental use of nitrofurazone. In the beginning of the 70s, nifurtimox and benznidazole were introduced for clinical treatment, but results showed a great variability and there is still a controversy about their use for chronic cases. After the introduction of these nitroheterocycles only a few compounds were assayed in chagasic patients. The great advances in vector control in the South Cone countries, and the demonstration of parasite in chronic patients indicated the urgency to discuss the etiologic treatment during this phase, reinforcing the need to find drugs with more efficacy and less toxicity. We also review potential targets in the parasite and present a survey about new classes of synthetic and natural compounds studied after 1992/1993, with which we intend to give to the reader a general view about experimental studies in the area of the chemotherapy of Chagas disease, complementing the previous papers of Brener (1979) and De Castro (1993).*

Key words: Chagas disease - chemotherapy - review - *Trypanosoma cruzi* - drug development - clinical treatment

## BACKGROUND

Chagas disease is endemic in Latin America, affecting 16-18 million people, with more than 100 million exposed to the risk of infection (WHO 1997). Its etiological agent is *Trypanosoma cruzi*, an hemoflagellate protozoan (family Trypanosomatidae, order Kinetoplastida) (Hoare & Wallace 1966), whose life cycle involves obligatory passage through vertebrate (mammals, including man) and invertebrate (hematophagous triatomine bugs) hosts, in a series of stages. The trypomastigote ingested by the insect differentiates into the proliferative epimastigote form that, on reaching the posterior intestine, evolves to metacyclic trypomastigotes. This latter form, following invasion of vertebrate host cells, undergoes differentiation into amastigotes, which after several reproductive cycles transform to trypomastigotes, the form responsible for the dissemination of the infection. The transmission of the disease occurs mainly by the vector (80 a 90%), blood transfusion (5 a 20%) and congenital routes (0.5 a 8%) (Dias 2000).

In humans, after infection and a subsequent incubation period, the acute phase of Chagas disease begins, and in the absence of specific treatment, the symptoms persist for about two months, with a mortality rate of 2 to 8%, especially among children. *T. cruzi* is able to invade and multiply within different host cells, including macrophages, smooth and striated muscles, fibroblasts and even neurons. The first reaction to *T. cruzi* is focal mononuclear inflammation due to rupture of parasitized cells. Within days to two weeks can be detected in the serum the presence of immune complexes, decrease in C3 level, besides necrosis in the inflammatory foci. Severe inflammation is usually accompanied by necrosis of parasitized and non-parasitized cells, especially in the heart. Platelet aggregation, eosinophils degranulation, microvascular pathology, edema, thrombosis, blood stasis and ischaemia have also been demonstrated (Andrade & Andrade 1999).

After the acute infection, the patient presents strong evidence of immunity, but has a tendency to remain infected. Some parasites evading the immune response and focal inflammatory lesions are seen in several organs. Amastigote forms can be detected by conventional histology, by immunofluorescence and genomic markers by *in situ* hybridization. The combination of rising immunity against the parasite with specific immunological suppression of hypersensibility and reduction of inflammatory reaction seem to be the main pathways to the indeterminate phase of Chagas disease (Andrade 1999).

In the chronic phase that follows, most patients remain asymptomatic, with about 20 to 50% of the cases, accordingly to the endemic area analyzed, developing the characteristic symptoms of this phase, namely cardiac, digestive or neurological disturbances (reviwed in Brener et al. 2000). Chronic, active, fibrosing myocarditis have been attributed to hypersensibility to parasite antigens, neoantigens or autoimmunity. The presence of cross-reactive antigens between heart muscle and *T. cruzi* has been demonstrated, but the autoimmunity does not entirely explain Chagas heart disease. The high positivity of xenodiagnosis and hemoculture, and reactivation of chronic disease by immunosuppression demonstrate the presence of the parasite in chronic cases. High frequency of parasites and/or antigens associated with myocardial inflammation is an important guide to the therapeutic procedures in the chronic phase.

The pathogenesis of Chagas disease is not yet completely defined and understood, with two basic inflammatory lesions, one focal and the other diffuse. The focal lesion is associated with the parasite, and occurs when parasitized cells are disrupted. The diffusion lesion is out of proportion in relation to the presence of parasites. During the acute infection there are diffuse lesions in the heart and focal lesions in several other organs. In the chronic heart disease, severe fibrosis and inflammatory lesions seem not to be related only to the presence of *T. cruzi*, but

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also to a strong delayed hypersensibility host response and ischaemic lesions (Andrade 1999, Andrade & Andrade 1999, Higuchi 1999). Two mechanisms are proposed for pathogenesis in chronic chagasic infections: the persistence of the parasite results in chronic inflammatory reactivity and it induces immune responses targeted at self-tissues (reviewed in Tarleton 2001). Several clinical reports reinforces the first mechanism (Higuchi et al. 1993, 1997, Jones et al. 1993, Anez et al. 1999), while the main support of the second one is that signs of the disease are evident in tissues in the apparent absence of parasites.

Several reviews about clinical and/or experimental Chagas disease treatment have been published as articles (Coura & Silva 1961, Prata 1963, Brener 1975, 1979, 1984, De Castro 1993, Coura 1996, Urbina 1999, Stoppani 1999) and as book chapters (Brener 1968, 2000, Cançado & Brener 1979, Cançado 1968, 1985, 1997, 1999, 2000, Rassi & Luquetti 1992, Storino et al. 1994). We will detail here just some of the publications and refer readers to the reviews cited above.

The aim of this "Critical Review" is to analyse drugs employed in the clinics since the 70s bringing attention to the recommendations about treatment, and evaluation of cure, and the studies about the development of new drugs, considering potential targets in the parasite and summarizing the experimental studies performed with new compounds assayed against *T. cruzi* after 1992/1993. For a more complete coverage of experimental in vitro and in vivo studies we suggest the reviews of Brener (1975, 1979) and De Castro (1993).

## EXPERIMENTAL AND CLINICAL TREATMENT

### Drugs assayed up to the decade of 70

The first compounds assayed experimentally for the treatment of Chagas disease, soon after its discovery by Carlos Chagas in 1909, were atoxyl (arsenical), fucsin (rosanilin dye), tartar emetic (or antimony potassium tartarate, a pentavalent antimonial) and mercury chloride, employed experimentally by Mayer and Rocha Lima (1912, 1914) and all of them without effective results. Until the publication of the "Manual de Doenças Tropicaes e Infectuosas" by Carlos Chagas and Evandro Chagas (1935) "there was no specific treatment for American trypanosomiasis. Drugs with trypanocidal activity have been assayed by a great number of researchers, but without success", affirm the authors (p. 144).

Among the chemotherapeutic agents employed until 1962 stand out quinolein derivatives, several other antimalarials, arsenobenzoles, and other arsenicals, phenantridines, salts of gold, bismuth, copper and tin, sodium iodide, gentian violet, aminopterin, para-amino salicylic acid, nicotinic acid hydrazide, antihistaminics, sulfonamides, ACTH, cortisone, stylomycin derivatives, amphotericin B, more than 30 antibiotics and some nitrofurans (reviewed in Coura & Silva 1961, Brener 1968, Cançado 1968).

Brener (1968) made a meticulous evaluation of the experimental drugs assayed in vitro and in vivo against *T. cruzi*, registering 27 compounds and more than 30 antibiotics, assayed between 1912 and 1962, that were inactive. He also considered that the following compounds had a suppressive effect on the parasitemia but were not curative: the bisquinaldine Bayer 7602 (Cruzon, Imperial Chemical Industry, UK), the phenatridine carbidium sulfate (74C48), aminoquinolines (pentaquine, isopentaquine and primaquine), trivalent arsenicals (Bayer 9736 and Bayer 10557 also named spirotrypan), aminoglycoside of stylomycin, nitrofurans and antibiotics.

Packchanian (1952, 1957) opened a new and promising line of potential drugs with the nitrofurans that led to nitrofurazone (5-nitro-2-furaldehyde-semicarbazone). This derivative administered by oral route for 53 days consecutively in the dose of 100 mg/kg/day mice experimentally infected with *T. cruzi* cured 95.4% of the animals (62/65) (Brener 1961)

Ferreira (1961, 1962) and Ferreira et al. (1963) treated the first ten cases of acute Chagas disease with this nitrofuran, obtaining "good clinical results" with few collateral effects but the xenodiagnosis became positive in five cases after treatment Coura et al. (1961, 1962) treated 14 chronic cases with this drug in long-term schemes, observing in the first four patients, that received progressive doses of 10 to 30 mg/kg/day, important side effects that led to suspension of the treatment due to severe sensitive polyneuropathy, that began at the third week of nitrofurazone administration. With reduction of the dose to 10 mg/kg/day and association with complex B, administered by parenteral route, five patients tolerated the treatment for 60 days, in spite of the side effects (anorexia, weight loss, paresthesia, and sensitive polyneuropathy). Another patient was treated with 20 mg/kg/day and presented paresthesics manifestations only at the 53th day of treatment, evolving to a severe sensitive polyneuropathy, with termination of the treatment by his own decision (informed consent). From the six patients submitted to long-term treatment, two of them were considered cured, based on xenodiagnosis and serology (complement fixation) persistently negative. Cançado et al. (1964) also treated five chronic patients with 10 mg nitrofurazone/kg/day during 10 to 34 days when the treatment had to be suspended due to polyneuritis, being all the five patients considered as therapeutic failures.

In a critical analysis of the literature about the clinical experiences, Cançado (1968) emphasized the lack of methods in the execution, preferential selection of acute cases, based on the remission of the symptoms and signs that could also be spontaneous. He cited, as example, the reports of Mazza et al. (1937, 1942) and Pifano (1941) about the results with Bayer 7602 and Bayer 9736 that were considered "excellent results" only based on the reduction of the symptoms and signals. Both compounds were in fact ineffective, since the xenodiagnosis after treatment remained positive and untreated cases had also reduction of the symptomatology. In subsequent works (Cançado et al. 1973, Cançado 1981) reviewed the results of therapeutic trials in the period of 1936 to 1965 and proposed basic criteria for the evaluation of the specific treatment, that were later updated by 15 experts from Latin America (OPS/OMS 1998).

Following the requirements of the World Health Organization (WHO) the ideal drug for the treatment of Chagas disease should fulfill the following requirements: (i) parasitological cure of acute and chronic cases; (ii) effective in single or few doses; (iii) accessible to the patients, in other words, of low cost; (iv) no collateral or teratogenic effects; (v) no need of hospitalization for the treatment; (vi) no induction of resistance.

As we will see bellow this ideal drug does not exist and possibly it will take a long period of time to be obtained. Since the end of 1960 beginning of the 70s two drugs have

been used for the treatment of Chagas disease: nifurtimox and benznidazole.

**Nifurtimox and benznidazole**

The drugs and Chagas disease treatment

Nifurtimox (Nif) is a 5-nitrofuran (3-methyl-4-(5'-nitrofurfurylideneamine) tetrahydro-4H-1,4-tiazine-1,1-dioxide (Bayer 2502) and benznidazole (Bz) is a 2-nitroimidazole (N-benzyl-2-nitroimidazole acetamide (RO 7-1051), commercialized, respectively, with the names Lampit and Rochagan in Brazil (Radanil in Argentina) (Fig. 1a,b). Nif that was the most active 5-nitrofurfurilidene derivative experimentally assayed (Bock et al. 1969) and Bz showed a high in vitro and in vivo activity against *T. cruzi* (Richle 1973). Since the 80s Nif had its commercialization discontinued, first in Brazil, and then in Argentina, Chile and Uruguay. The mode of action of Nif involves generation of nitroanion radical by nitroreductases that, in the presence of oxygen, led to reactive intermediates and being *T. cruzi* is partially deficient in free radical detoxification mechanisms, it is susceptible to such intermediates (reviewed in DoCampo & Moreno 1986). On the other hand, this oxidative damage was not the key action of Bz, the detection of corresponding nitroanion radical occurred only at concentrations much higher than those that killed the parasite. The action of Bz could involve covalent bond or other interactions of nitroreduction intermediates with parasite components (Polak & Richle 1978), or binding to DNA, lipids and proteins (Diaz de Toranzo et al. 1988).

Nif and Bz have been employed by different authors, especially in Brazil, Chile and Argentina (Cançado et al. 1969, 1973, 1975, Cançado & Brener 1979, Bocca-Tourres 1969, Rubio & Donoso 1969, Schenone et al. 1969, 1972, 1975, 1981, Rassi & Ferreira, 1971, Rassi & Luquetti 1992, Cerisola et al. 1972, 1977, Prata et al. 1975, Ferreira 1976, 1990, Coura et al. 1978, 1997, Macêdo & Silveira 1987, Viotti et al. 1994, Andrade et al. 1996, Sosa Estani et al. 1998).

The results obtained with both drugs varied according to the phase of Chagas disease, the period of treatment and the dose, the age and geographical origin of the patients. Good results have been achieved in the acute phase, in recent chronic infection (children under 12 years old), congenital infection and laboratory accidents. For the acute phase treatment and congenital cases it is recommended 8 to 10 mg/kg/day of Nif or 5 to 7.5 mg/kg/day of Bz during 30 to 60 days consecutively, and divided in two or three daily doses. Patients with less than 40 kg can take up to 12 mg/kg/day of Nif and up to 7.5 mg/kg/day for Bz during 30 to 60 days (OPAS/OMS 1998). For recent chronic infection (children under 12 years old) or individuals infected in the last 10 years, the treatment should be made with 8 mg/kg/day of Nif or 5 mg/kg/day of Bz during 30 to 60 days. In the case of accidental infection the treatment must begin immediately and last for only 10 to 15 consecutive days. Cases of late chronic infections without clinical manifestation or with mild cardiac or digestive manifestations should be treated during 60 to 90 days, in accordance with the tolerance to the drugs, aiming to prevent or reduce the evolution of Chagas disease to more severe forms, a fact that is not yet definitely proved.

Side effects and contraindications

The more frequent collateral effects with Nif treatment are anorexia, loss of weight, psychic alterations, excitability or sleepiness and digestive manifestations, such as nausea, vomit and occasionally intestinal colic and diarrhea. The adverse reactions with Bz could be classified in three groups: (i) symptoms of hypersensibility, dermatitis with cutaneous eruptions (usually appearing between the 7th and 10th day of treatment), generalized edema fever, lymphadenopathy, articular and muscular pain; (ii) depression of bone marrow, thrombocytopenic purpura and agranulocytosis, the most severe manifestation; (iii) polyneuropathy, paresthesia and polyneuritis of peripheric nerves.

The two most serious complications induced by Bz are agranulocytosis, initiated by neutropenia, sore throat, fever and septicemia, and thrombocytopenic purpura, characterized by reduction of platelets, petechiae, hemorrhagic blister and even mucosal bleeding. At the first signs of such manifestations, medication must be immediately suspended and should be started a treatment with antibiotics in the case of septicemia plus corticosteroids for the control of the agranulocytosis and of the thrombocytopenic purpura. Other manifestations of intolerance and hypersensibility could be circumvented with the reduction of the dose or suspension of the drug, depending on their intensity, introduction of symptomatic medication and eventually of anti-histaminics and corticosteroides.

Teixeira et al. (1990, 1994) have been alerting for the appearance of lymphomas and mutagenic and carcinogenic activities in experimental animals (rabbits and mice) treated with Bz. However, a broad review of thousands of patients treated with these drugs by several authors has not demonstrated such effects.

Bz and Nif should not be indicated for pregnant patients, in cases of severe diseases associated with Chagas disease, such as systemic infections, cardiac, respiratory, renal or hepatic insufficiency, hemopathies and neoplasies without the possibility of treatment, old-aged and very debilitated persons.

Effect of the treatment on the evolution of Chagas disease

Since 1969 several therapeutic experiences have been performed in acute and chronic cases of Chagas disease using Nif (Bocca-Tourres 1969, Rubio & Donoso 1969, Schenone et al. 1969, 1972, Rassi & Ferreira 1971, Cerisola et al. 1972, 1977, Silva et al. 1974, Prata et al. 1975, Cançado et al. 1975, 1976, Cançado & Brener 1979), Bz (Schenone et al. 1975, Ferreira 1976, Coura et al. 1978, Viotti et al. 1994, Andrade et al. 1996, Sosa Estani et al. 1998) and also comparing the efficacy and tolerance of both drugs in different groups of patients, therapeutic schemes and periods of follow-up and cure evaluation criteria (Schenone et al. 1981, Ferreira 1990, Coura et al. 1997, Lazzari & Freilig 1998).

The results of such experiences showed a great variability, according to the authors, and the type of casuistic and of cure control employed. In general, results obtained were good for acute phase and recent infection cases, especially among children, who, besides tolerating a long-term treatment much better, presented high indexes of cure, as demonstrated in a randomized field study with Bz in Brazil (Andrade et al. 1996) and in Argentina (Sosa Estani et al. 1998). For acute cases and recent infections an average index of parasitological cure around 60% is estimated. In relation to chronic infection cases, results have been poor, for the Brazilian experience, while in the South Cone, they were much better, possibly due to the type of parasite strain (Silva et al. 1974, Cerisola et al. 1977).

Studies on the clinical evolution of Chagas disease after specific treatment are controversial and results are not convincing, due to differences in casuistics, methods

of evaluation, time of follow-up and interpretation of the data. Macêdo and Silveira (1987) studying 171 adults with chronic disease treated with Nif or Bz with a follow-up of 7 years, observed electrocardiographic (ECG) evolution of cardiopathy in 6.7% of the cases, against 8.8% for untreated patients, indicating no significant differences between the two groups. Ianni et al. (1993) monitored 33 adults in the indeterminate phase for 8 years, reported the evolution in 13.3% of the cases treated with Bz (n = 15) and 0% of the cases that received placebo (n = 18), not allowing a definitive conclusion, due to the small casuistic. Miranda et al. (1994 *apud* OPAS/OMS 1998), in 120 patients (adults and children), observed ECG evolution in 10.5% of those treated with Bz and 63.6% of the placebo group. Although these authors monitored the patients for 10 to 16 years, the combination of the data obtained with adults and children and the ECG interpretation made the analysis of the results obtained difficult.

Viotti et al. (1994) in a well-designed study with 201 adult patients monitored for 8 years, observed ECG evolution in 7/131 cases (5.3%) treated with Bz (5 mg/kg/day for 30 days) and 16/70 (22.8%) in the control group. In patients with more than 50 years old, the ECG alterations occurred in 3/36 (8.3%) of the treated cases and 7/40 (17.5%) of the untreated ones; differences were not statistically significant. For those under 50 years old, such alteration occurred in 4/95 (4.2%) and 9/30 (30%), respectively, for treated and untreated patients, and were significantly different. Two patients died during the follow-up, one treated and one untreated. Although the study has been well conducted, after 8 years of follow-up, 68% of the untreated patients presented positive serology against 48.2% of the Bz-treated group. The low number of ECG alterations and their frequent mutability in chronic cases make the interpretation of the data difficult.

On the other hand, Fragata Filho et al. (1995) reported, in a study with 120 chronic patients with follow-up for 7-8 years, ECG evolution in 7% for Bz-treated cases (n = 71) and 14.3% for the untreated group (n = 49).

Andrade et al. (1996) performed a randomized field study in the State of Goiás (Brazil) treating children between 7 and 12 years old with positive serology for Chagas disease. Sixty-four patients received 7.5 mg/kg/day of Bz for 60 days and 65 received placebo. From these 129 children, 88.7% (58 treated and 54 control) were monitored for three years. The authors considered the treatment effective in 55.8% of the treated cases (most of them with a significant decrease in serological titers). However, no significant results were observed when the ECG abnormalities were compared. In a similar way, in Salta (Argentina), Sosa Estani et al. (1998) treated 55 children from 6 to 12 years old with 5 mg Bz/mg/day for 60 days and 51 children with placebo, and monitored the study for four years. They observed negativation or significant decrease of the specific serology in 62% of the treated group and none for the control group. In relation to xenodiagnosis the positivity was 4.7% and 51.2%, respectively for treated and untreated children, indicating an important suppressive activity of the treatment, however, the ECG alterations were similar for both groups: 2.5% (1/40) and 2.4% (1/41).

**Rules and recommendations for the clinical treatment**

A meeting of 13 specialists promoted in Brasília by the Ministry of Health of Brazil summarized by Luquetti (1997) and another one by the Pan American Health Organization and World Health Organization (OPAS/OMS 1998) that took place in Instituto Oswaldo Cruz (Fiocruz) in April 23-25 of 1998, established some rules and recommendations for the etiologic treatment of Chagas disease, with the present available drugs, that are summarized bellow:

Acute phase

In this phase the parasite is detected by direct examination of peripheral blood by weat smear or in stained cover slides or by concentration methods (centrifugation of the blood and microscopic examination of leukocyte cream or stained quantitative buffy coat techniques). With or without clinical manifestations, the detection of the parasite by direct methods or determination of IgM levels allows the diagnostic of the acute phase. Independently of the mechanism of transmission (vectorial, transfusional, by oral route, or laboratory accident) the patients must be treated, as indicated previously, since about 60% of them could be cured in the acute phase.

Congenital infection

The diagnostic of congenital infection is based in cases of children from infected mothers, serologically positive, who presented *T. cruzi* in the blood of umbilical cord, specific IgM in the serum soon after birth, or IgG after 6 months, when the possibility of vectorial, transfusional and oral transmission are absent. The treatment is similar to that of the acute phase patients.

Accidental infection

The person, technician or researcher, that working with *T. cruzi*, was accidentally punctured by infected needle, ingested or had any contact with infected materials in lesions, wounds, mucosal, or any other form indicative of the possibility of the parasite penetration is considered infected. In these cases, blood is collected for serological test and treatment immediately begins, during 10 to 15 days, repeating the serology after 15, 30, and 60 days after the accident. It is recommended that all the laboratories that work with *T. cruzi* have the drug available.

Chronic phase

*Recent chronic phase* - Recent chronic phase is considered when the infection was acquired in the last 10 years, including children up to 12 years old or adults that have been infected occasionally in endemic areas of by blood transfusion in a known interval of time. The published work indicates that the results obtained by the etiologic treatment in this phase are much better than that performed in late chronic patients.

*Late chronic phase* - Patients with more than 10 years of infection are considered late chronic cases. There is no agreement about the clinical evolution of such cases, and parasitological cure is obtained in 10 to 20% of the patients, according to different experiences. The treatment must be elective, with priority to cases in the indeterminate form or with minor pathology that may be monitored by long periods of time, after treatment.

*Transplant of organs* - In cases of transplants it is always necessary to perform specific serological reaction both in the donor and the receptor. The transplant of organs from patients with infection by *T. cruzi* could transmit the parasite to the receptor, especially during the immunosuppression phase. On the other hand, in the infected receptor a reactivation of the disease could occur through the immunosuppression, compromising even the transplanted organ, mainly in cardiac cases. Jatene et al. (1997) refer to reactivation of Chagas disease in 40% of

cases derived from immunosuppression in heart transplants. Both the donor and the receptor must be treated with Bz in the dose of 5 mg/kg/day for at least 60 days. Tominori-Yamashita et al. (1997) considered that "allopurinol seems a safe and effective treatment for reactivated Chagas disease after heart transplantation, although it is not recommended as a post-transplantation prophylaxis because reactivation of the disease is unpredictable".

Reactivation of Chagas infection

Reactivation of the chronic disease can occur due to immunosuppression by several diseases, such as leukemia, lymphoma and other neoplasies, infection by HIV/AIDS and in the cases of transplants with immunossupression. Meningoencephalitis and acute myocarditis are the most frequent manifestations (reviewed in Ferreira et al. 1997a). In chronic patients parasites were detected in cutaneous lesions after transplant with immunosuppressive treatment and in the smooth muscle after cancer chemotherapy. Ferreira et al. (1997a,b) made an extensive review about this topic, recommending as first choice the treatment with Nif or Bz, indicating as alternatives triazole derivatives and allopurinol. Long-term secondary prophylaxis should be recommended for patients who respond to therapy, although it is uncertain which drug to use for this purpose (Ferreira et al. 1997b). Anyway all HIV-positive cases, patients with neoplasies or candidates to transplants must be thoroughly investigated for the possibility of concomitance with chagasic infection. There is no consensus about the prophylatic indication of the etiologic treatment of the infection, in cases without clinical reactivation, but with xenodiagnosis or hemoculture positive for *T. cruzi*.

Patients in the chronic phase and receiving corticoid because of concomitant diseases were treated with Bz since the beginning of the use of corticoid (n=12) or 15 days afterwards (n = 6) (Rassi et al. 1999). The authors observed that Bz prevented an increase of parasitemia, and suggested that in immunocompromized patients with chronic Chagas disease this drug could be useful.

Where and who should treat the patient

The patients in severe acute phase and the congenital symptomatic cases with diagnostic at birth, must be hospitalized for treatment. The acute oligosymptomatic or chronic cases can be treated in basic health care units under the supervision of an experienced physician. Bz and Nif must be considered drugs of high complexity, and recommended only by professionals with solid information about the side effects and the disease in itself. The acute phase and the accidental infection are emergency situations, and treatment must start immediately even with a professional without experience that must search a colleague or a qualified institution for orientation.

**Evaluation of cure**

The evaluation of cure of Chagas disease is certainly the more complex aspect of its treatment, leading several times to diverse and controversial results, in relation to both parasitological cure and clinic cure. The term parasitological cure itself is of difficult interpretation and the evaluation is almost impossible, since it would mean the total elimination of the parasite not only from the blood but also from all tissues. So, in humans it is not viable to be confirmed. On the other hand, clinic cure is the long-term evaluation and several times uncertain due to the pathogenesis of the disease, which involves the action of parasite and the immune and autoimmune response of the patient and in antigenic complexes deposition, generation of antibodies, inflammatory reactions, tissular lesions with cellular degeneration, ischaemia, fibrosis and their consequent clinical manifestations, sometimes for long periods of time.

For the evaluation of experimental animals, mostly in drug assays, the situation is less complex. The in vitro tests (tissue culture) are not necessarily reproduced in vivo. On the other hand, as occurs in humans, a suppressive effect on the parasitaemia does not correspond exactly to the effect of a drug in tissues. In his pioneer work Brener (1961) demonstrated parasitological cure in 94.5% of the mice treated with nitrofurazone. Later, Brener et al. (1969) demonstrated by electron microscopy that 13.5% of the amastigotes of the Y strain were intact in heart cells of the treated animals. A question remained: were these findings due to populations of the parasite with primary resistance or the drug did not reach all the infected cells? The group of Andrade reported development of resistance to both nitroheterocycles and the influence of *T. cruzi* strain in the cure rate, for example, Bz cured 87% of the mice infected with the Peru strain, and only 16.7% in the case of Colombian strain (Andrade et al. 1975, 1977, Andrade & Figueira 1977). Resistance to both drugs, including cross resistance, was also observed in animals infected with the Y strain (Costa Silva et al. 1990). Could this be due to a mechanism similar to that questioned above?

Parasitological evaluation

The suppressive activity on the parasitaemia is almost immediate after the beginning of the treatment when the strain (population) of *T. cruzi* is susceptible to the drug employed. In acute cases this fact could be verified by the direct examination of blood in fresh or stained preparations or by concentration methods. In chronic cases, the usual methods are xenodiagnosis standardized with 40 nymphs of 3rd/4th stage of *Triatoma infestans* or by 20 nymphs of this species and another 20 from *Panstrongylus megistus* or of other species that could give similar yield. The nymphs are distributed in four boxes (10 per box); two are placed in the internal face of each arm for about 30 min (Coura et al. 1991). In this study we observed a positivity of 50.7% in 570 xenodiagnosis performed in 246 patients. Nowadays, due to ethical questions and comfort for the patients, the preferential method is the artificial xenodiagnosis that consists in collection of 10 ml of the blood that are placed in a condom type membrane, with external heating, and then the nymphs are added and the reading done after 30, 45 and 60 days. The yield obtained is similar to that of the natural test (Pineda et al. 1998).

In a multicenter study that involved researchers from 10 Brazilian institutions and 312 Bz-treated patients monitored by a media of 12 xenodiagnosis performed after treatment, suppression of parasitaemia was demonstrated in 78% of the cases (Coura et al. 1978). In another comparative controlled study with 77 chronic patients treated with Bz (n = 26), Nif (n = 27) or placebo (n = 24), suppression of the parasitaemia monitored xenodiagnosis by one year after treatment was achieved in 98.1% (2/110) of the Bz group, and in 90.4% (75/83) of the Nif group (Coura et al. 1997). However, this result does not imply that cases with xenodiagnosis negative are cured since only 34.3% of the control group was positive.

The hemoculture is the second parasitological method of choice for the control of cure in chronic Chagas disease, being equivalent in sensibility to xenodiagnosis

(Chiari & Brener 1966). Both methods have a tendency to increase the positivity with the number of tests performed, amount of blood employed, cultivation medium, interval of time between blood collection and cultivation and other factors emphasized by Chiari et al. (1989) aiming standardization of the assay. Using 30 ml of blood seeded in six test tubes with LIT medium and readings up to 60 days, Chiari et al. (1989) obtained a positivity up to 50%, while with small modifications of the technique such as direct seeding soon after blood collection, refrigerated centrifugation for a short time, gentle homogenization and up to 120 days led Luz et al. (1994) to a positivity of 94%, not achieved by any other author, for assays with chronic cases of Chagas disease.

Polymerase chain reaction (PCR) was a major advance for the parasitological control of the cure of Chagas disease, with positivity 2 to 3 times higher for chronic cases when compared to routine xenodiagnosis and hemoculture. By this technique it is possible to detect one parasite or a fragment of *T. cruzi* DNA in 20 ml of blood (Ávila et al. 1991).

Sturm et al. (1989) amplifying minicircles of DNA of *T. cruzi* obtained fragments of 83 and 22 pairs of base (bp) from variable regions that were employed for detection of the parasite (Moser et al. 1989). Ávila et al. (1991) using a solution of 6 M guanidine plus EDTA and equal amount of blood of chronic patients, promoted lysis of proteins and maintained the integrity of the DNA sample at room temperature. The treatment of this lysate with phenantroline-copper (OP-$Cu^{2+}$) led to the cleavage of DNA and liberation of minicircles, allowing the identification of a single parasite in 20 ml of blood when three initiators for the fragments of 83, 122 and 330 bp from the variable and constant regions of the minicircles. By this technique, the authors identified *T. cruzi* in samples of 10 ml of blood of five chronic patients, four of them with negative xenodiagnosis.

Several authors (Wincker et al. 1994, Britto et al. 1995, 2001, Junqueira et al. 1996) have demonstrated the efficiency of PCR for the diagnosis of chronic disease and for the control of cure after treatment. However, Junqueira et al. (1996), in a comparative study among PCR, xenodiagnosis and hemoculture in 101 chronic cases, observed positivity of, respectively, 59.4%, 35.6% and 25.7%, but in five cases with positive xenodiagnosis and/or hemoculture, the result of PCR was negative. Recently, Britto et al. (2001) comparing PCR and xenodiagnosis in the control of cure of 85 chagasic patients submitted to specific treatment and 15 chronic assymptomatic cases that received placebo, reported that in all the cases of positive xenodiagnosis, positivity was obtained also by PCR. On the other hand when xenodiagnosis was negative, PCR was positive in 18.5% of the acute phase group (n = 37), 29% of the chronic phase group (n = 45) and 57.1% of the control group. These results demonstrate the advantage of PCR over conventional techniques to demonstrate persistent infections in patients that underwent chemotherapy.

Serological evaluation

This evaluation is certainly the most simple, more broad and reliable for the control of cure of chagasic infection after treatment, especially in the chronic phase when the serology is positive in almost 100% of the untreated cases. Whereas the positivity of parasitological methods depends on the random presence of the parasite in the blood sample, the presence of antibodies is almost warranted in all the samples. On the other hand, the serology since the Guerreiro and Machado reaction (1913) until the qualitative and quantitative reaction of complement fixation (Kelser 1936, Freitas & Almeida 1949), the indirect immunofluorescence assay (Fife & Muschel 1959, Camargo 1966), the hemagglutination assay (Neal & Miles 1970), the conventional ELISA (Voller et al. 1975) and with recombinant antigens (reviewed in Silveira 1992, Silveira et al. 2001), the lysis mediated by complement (Krettli & Brener 1982, Kretlli et al. 1984) and finally techniques using immunoblots have been improved as confirmatory tests due to their sensibility and specificity (Umezawa et a1. 1996, 2001).

The three basic serological reactions for the diagnosis of Chagas disease are indirect immunofluorescence, hemagglutination and ELISA. During the years, the great polemics is their negativation in cases of parasitological cure. Some authors as Cançado (1963, 1997) consider cure as "definitive post-therapeutic reversion to negativity of parasitological and serological tests", whereas others, like Rassi and Luquetti (1992), Andrade et al. (1996) and Sosa Estani et al. (1998) admit a long period of negativation of the reactions and even low serological titers as criteria of cure. Andrade et al. (1991) demonstrated that, in mice infected with *T. cruzi* and parasitologically cured by chemotherapy, parasite antigens persist in interstitial dendritic cells in the spleen and the animals present positive serology. Recently Andrade et al. (2000) reported the importance of the presence of parasite antigens in the same type of cells in the heart of infected dogs and the pathogenesis of the chagasic myocarditis probably by presentation of *T. cruzi* antigens to immune-competent cells, and, as consequence, maintenance of the response to the infection.

Clinical evaluation

This type of the cure evaluation after chemotherapy is perhaps the most difficult and long topic to be addressed. In this review, we have already discussed some aspects of the clinical evaluation when analyzing the evolution of Chagas disease after treatment with Nif or Bz, so, in this topic we will discuss only some essential clinical tests for monitoring the disease, before, during and after treatment.

In the clinical evaluation of the treatment we must consider, besides the anamnesis and clinical examination, the electrocardiographic and radiologic aspects together with other non-invasive tests with high sensibility, such as dynamic electrocardiography (Holter) for the study of arrhythmias and echocardiography for the anatomophysiological evaluation of the cardiac function, the endoscopy and manometry for the anatomofunctional study of the digestive system and some other tests for evaluation of the autonomous nervous system and neuronal lesion, besides biopsies for histological and histochemical studies, that will not be evaluated here in depth.

A careful clinical examination after a detailed anamnesis, especially analyzing the cardiovascular, digestive and neurologic systems, before, during and after treatment, monthly in the first year and at least once a year subsequently is fundamental importance for the evolutive study of the patients. The ideal condition, when ethics allows, would be the monitoring of a control group, of the same age and sex, untreated or receiving a placebo, with aspect similar to the drug, for evaluation of collateral effects and a comparative study between the treated and the control groups.

The standard electrocardiogram, with the limb leads (D1, D2, D3, aVR, aVL and aVF) and chest leads from V1 to V6 is the most simple and most important examination in the clinical evaluation. This test must be associated with the anamnesis and the physical examination in each consultation during all the follow-up period.

The radiological examination is a less sensible method and more expensive than ECG, so it should be performed once before treatment, 6 and 12 months later and then once per year of monitoring. This examination should consist of a chest RX with postero-anterior and lateral views with esophagus contrasted immediately after ingestion of barium and also after 1 min for the evaluation of the time necessary to drain the contrast (Rezende et al. 1960). The barium enema with previous preparation and radiography of colon is the only test capable of evaluating an established megacolon (Rezende 1959).

The dynamic electrocardiography and the echocardiography are suitable techniques for evaluation of arrhythmias and anatomophysiology of the heart and must be performed by a cardiologist. In the same way, endoscopic and manometric tests need an experienced gastroenterologist. The examination of the autonomous and peripheric nervous systems, with or without stimulation with cholinergic drugs, such as pilocarpine, can be done by a physician (Macêdo 1997).

The histopathology study of biopsies fragments of the digestive system or endomyocardic with conventional microscopy or analysis by immunoperoxidase or immunofluorescence are indicated only in some research cases and could only be performed following strict protocols from the ethics point of view.

**New drugs in clinical tests**

For several years in Brazil, and more recently in Argentina, Chile and Uruguay, only Bz is commercialy available as the development of drugs for tropical diseases is of little interest for the pharmaceutical industry (Fairlamb 1999). After the introduction of Nif and Bz, few compounds were assayed in chagasic patients.

Allopurinol

The results obtained with allopurinol (4-hydroxypyrazolo (3,4-*d*) pyrimidine HPP, Fig. 1c) in experimental animals and the knowledge about its mode of action led to its clinical assays for the treatment of Chagas disease. This compound is a hypoxanthine analog that acts as an alternative substrate of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and is incorporated into the RNA. This incorporation leads to formation of non-physiological nucleotides and to blockade of the synthesis *de novo* of purine nucleotides (reviewed in Marr 1991).

In the treatment of six acute phase patients, allopurinol was ineffective, with maintenance of positive xenodiagnosis and serology (Lauria-Pires et al. 1988). In a study, with chronic patients, Galleano et al. (1990) treated two groups of patients with 600 and 900 mg/kg/day of allopurinol for 60 days, compared with other two treated with Nif and Bz. In the four groups the percentage of negativation of xenodiagnosis was in the range of 75-92%, and those treated with allopurinol presented less collateral effects. Allopurinol (600 mg/day for 2 months) was administered to two cases of reactivation of Chagas disease due to cardiac transplant. Erythematous lesions on the superior and/or inferior members characterized this reactivation. In one patient the lesions disappeared in 3 weeks, and in the sec-

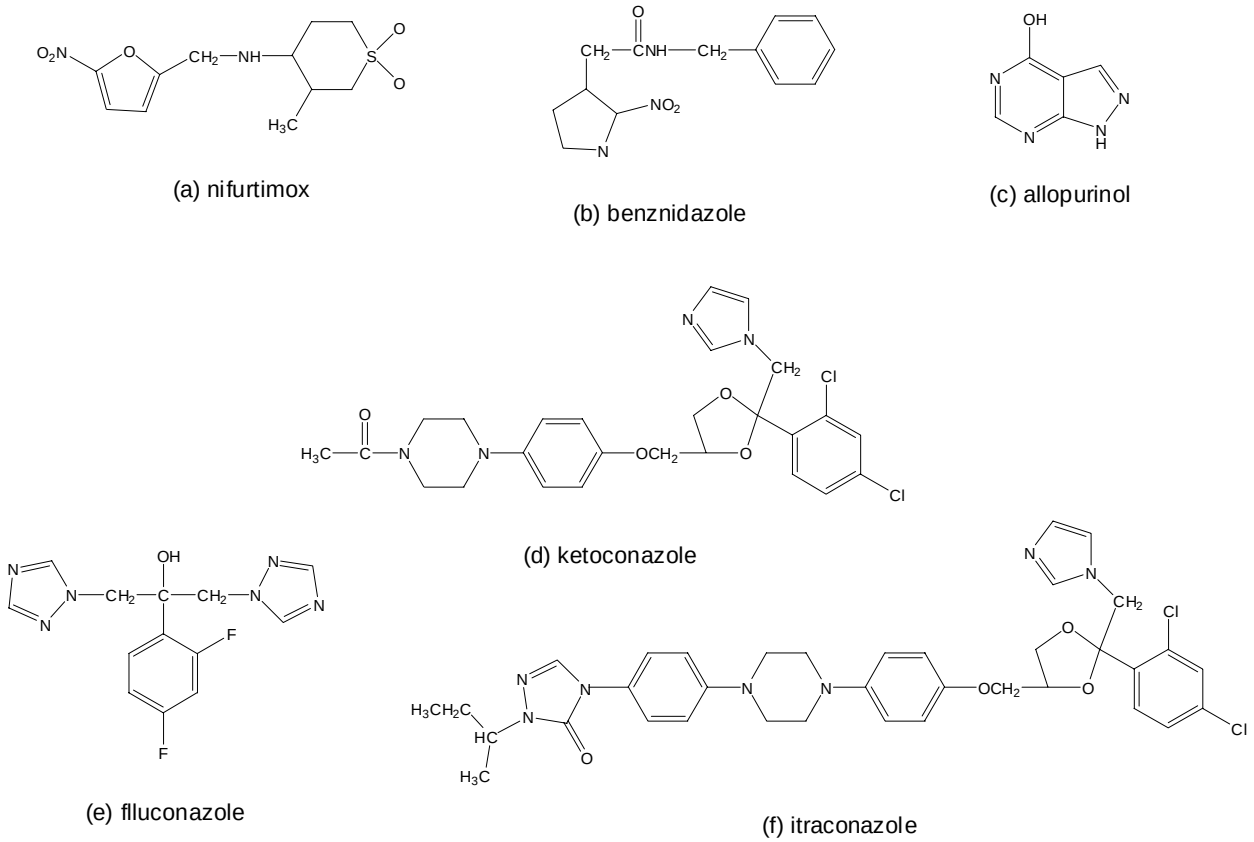


Fig. 1: structure of compounds used in the treatment of Chagas disease after the 70s.

ond one, after 2 weeks there was a clinical improvement of the lesions. In both cases after treatment, xenodiagnosis and hemoculture tests were negative and in the follow-up of 38 and 17 months, respectively, no reactivation of Chagas disease occurred, even with continued immunosuppression (Tomimori-Yamashita et al. 1997). Apt et al. (1998) treated 104 chronic patients with allopurinol (8.5 mg/kg/day for 60 days) that were monitored by clinical examination, serology, xenodiagnosis, hemoculture and electrocadiogram. Parasitological cure was achieved in 44% of the allopurinol-treated patients. The criteria for parasitological cure were maintenance of negative xenodiagnosis and/or complement-mediated lysis for at least four years. A double blind randomized longitudinal study must be performed to reevaluate the efficacy of this drug for the treatment of Chagas disease.

Ketoconazole

In eight chronic patients, ketoconazole (*cis*-(*dl*)-1-acetyl-4-[4-[[(2-2,4-dichlorophenyl)-2-(1H-imidazol-1-yl-methyl)-1,3-ioxalan-4-yl]methoxy]-phenyl]-piperazine, Fig. 1d) was administered in doses between 3.1 and 8.7 mg/kg by oral route during 51 to 96 days and cure evaluation was performed by hemoculture, conventional serology and complement-mediated lysis. The patients were monitored up to 60 months and it was observed that the drug was unable to erradicate the parasites, from 6 out of 8 patients with positive hemoculture and two others with positive serology (Brener et al. 1993).

In a case of reactivation of Chagas disease in a patient in the inderteminate phase due to infection by HIV, ketonazole (400 mg/day) was administered for 70 days leading to a negative xenodiagnosis. The treatment was suspended by the patient´s own decision and after one month occurred signs of reactivation of the disease, including development of myocarditis. Bz was introduced (200 mg/day) and after four days, although negativation of parasitemia, the patient presented signs of neurological deterioration. This drug was maintained for 45 days and then replaced by ketoconazole (400 mg/day) as a suppressive treatment. However, after a general clinical improvement, there was a new neurologic deterioration and the patient died (Galhardo et al. 1999).

Ketoconazole was one of the first imidazoles that showed in vitro activity against *T. cruzi*, with accumulation of metabolites of sterol metabolism in epimastigotes. In vivo ketoconazole led to parasitological cure in experimental animal in the acute phase, but was ineffective in the chronic phase (reviewed in De Castro 1993). A synergic effect of ketoconazole and Bz was observed in mice infected with the CL or Y strain, what did not occur in the case of the Bz-resistant Colombian strain (Araújo et al. 2000).

Fluconazole and itraconazole

An haemophylic patient was infected by blood transfusion with HIV and *T. cruzi* and brain biopsy revealed the presence of amastigotes inside glial, macrophages and endothelial cells. Initially he was treated with Bz (400 mg/day) but due to general worsening of his clinical condition, the medication was changed to itraconazole (200 mg/day), and latter, aiming a better CNS penetration, to fluconazole (400 mg/day). The azoles were administered for 11 weeks, and during this period the fever resolved and neurological symptoms stabilized. No significant collateral reaction was observed and three months after treatment the xenodiagnosis was negative and the titer of indirect hemagglutination test was 1:16 (Solari et al. 1993). Following the same methodology described for the treatment with allopurinol, Apt et al. (1998) treated 135 chronic patients with itraconazole (6 mg/kg/day for 120 days) observing parasitological cure and normalization of ECG in 36.5% of the treated patients but new abnormalities of the ECG appeared in 48.2% after treatment. As in the case of allopurinol these azoles must be further investigated in a well-designed protocol for treatment of chagasic patients.

The azoles fluconazole (α-(2,4-difluorophenyl)-α-(1H-1,2,4,-triazol-1-ylmethyl)-1H-1,2,4-triazol-1-ethanol, Fig. 1e) and itraconazole (*cis*-4[4-4-4[[2-(2-4-dichlorophenyl)-2-(1H-1,2,4,triazol-1-methyl)-1,3-dioxolan-4-yl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methyl-propyl)-3H-1,2,4-triazol-3-one, Fig. 1f) have been previously assayed in experimental animals, and their mechanism of action against *T. cruzi* involve interference on ergosterol synthesis (reviewed in De Castro 1993). A more recent study showed potent effect of the D(+) isomer of fluconazole – compound D0870 – in both acute and chronic mice models, with 30-50 times higher activity than ketoconazole and Nif and leading to 60-70% of parasitological cure (Urbina et al. 1996). A formulation of D0870 as loaded nanospheres administered by intravenous route to mice, showed also a significant cure rate (Molina et al. 2001). It is important to stress that this compound, based on several cure parameters was also active in a chronic phase model. We believe that the pharmacokinetics of D0870 is now being investigated.

## DEVELOPMENT OF NEW DRUGS

Development of anti-parasite chemotherapy could emerge from screening of synthetic or natural libraries, of compounds with structural similarities, with a drug with recognized activity, of assays with agents already approved for other diseases or through the determination of a specific target, identified in a key metabolic pathway. Although several putative targets have been presented, there is a need for their validation. The criteria for such validation was discussed by Wang (1997), who suggested that preliminary verifications can be indicated by in vitro activity of an inhibitor of the putative target, but before a major effort is directed to the design of specific inhibitors, three approaches should be used: (i) correlation of the target inhibition and anti-parasite activity among a series of drug derivatives; (ii) the comparison of the target between drug-sensitive and drug-resistant parasites; (iii) the knock out of the gene encoding such target in the parasite. In this paper Wang (1997) also pointed out the inherent difficulties of such approaches of target validation.

### Promising targets

Recent developments in the study of the basic biochemistry of *T. cruzi* have allowed the identification of novel targets for chemotherapy that include sterol metabolism, enzymes such as trypanothione reductase, cystein proteinase, hypoxanthine-guanine phosphoribosyltransferase, glyceraldehyde-3-phosphate dehydrogenase, DNA topoisomerases, dihydrofolate reductase and farnesylpyrophosphate synthase (reviewed in DoCampo 2001 and in Rodriguez 2001).

Sterol synthesis

The knowledge about sterol synthesis on fungi opened the possibility of interference in this pathway, leading sev-

eral pharmaceutical companies to develop drugs for the treatment of different types of superficial mycosis and systemic fungal infections. Since the main sterol of *T. cruzi* is ergosterol, an intensive and fruitful investigation about the potential effect of inhibitors of this sterol, especially by the group of Urbina in Venezuela. Parallel to clinical studies with azole derivatives, the trypanocidal activity and mechanism of action of new compounds is under intensive investigation.

The triazole posaconazole (SCH56592, Schering-Plouch), inhibited epimastigote proliferation and ergosterol synthesis at levels 30 to 100 times higher than ketoconazole and D0870. In experimental infections, this compound led to a cure rate of 50% in animals infected with strains resistant to Nif, Bz and ketoconazole (Molina et al. 2000). Another triazole derivative UR-9825 was very active against epimastigotes and intracellular amastigotes. At the minimum inhibitory concentration for epimastigotes occurred also depletion of 4,14-desmethyl endogenous sterols, such as ergosterol, and their replacement by methylated sterols, indicating inhibition of C14-alpha demethylase, as previously reported for other azoles. This drug induced also alteration in the phospholipid profile of the parasite (Urbina et al. 2000).

The induction of resistance of *T. cruzi* to azoles, such as fluconazole, and also the cross resistance between ketoconazole, miconazole and itraconazole, observed in in vitro experiments point to difficulties in the use of such compounds as chemotherapeutic agents (Buckner et al. 1998). In a subsequent work, Buckner et al. (2001) reported the development of inhibitors of a key enzyme in sterol biosynthesis, oxidosqualene cyclase, which converts 2,3-oxidosqualene to lanosterol. The lead compound, N-(4E,8E)-5,9, 13-trimethyl-4,8, 12-tetradecatrien-1-ylpyridinium, was shown to cause an accumulation of oxidosqualene and decreased production of lanosterol and ergosterol in *T. cruzi*. This compound and 27 related derivatives were tested against *T. cruzi*, and 12 of them were highly active against trypomastigotes.

Trypanothione reductase

Trypanosomatids present trypanothione ($N^1$,$N^8$-bis(glutationyl)spermidine) and of specific enzymes for this cofactor, trypanothione reductase (TR) and trypanothione oxidase (reviewed in Fairlamb & Cerami 1992). TR is an NADPH-dependent flavoprotein that maintains trypanothione in its reduced form and able to be oxidized by trypanothione oxidase, leading to reduction of free radicals levels and contributing to the maintenance of an intracellular reducing environment. TR has been used as a target for rational drug design against trypanosomiasis and leishmaniasis in a number of laboratories, since this enzyme and the mammalian counterpart (gluthatione peroxidase/glutathione reductase system) differ on the substrate specificity (reviewed in Augustyns et al. 2001). The determination of the structure of the active center of TR (Krauth-Siegel et al. 1987) allowed the search of inhibitors of this enzyme, being assayed different classes of compounds. In most cases the studies analyzed the effect of a putative inhibitor on the purified enzyme, and depending on the results obtained, new compounds based on molecular modeling were developed. A first group of inhibitors reported were the so-called "subversive substrates", due to the futile-cycling of TR induced by redox-damaging drugs, such as nitrofurans, and naphtoquinones (Henderson et al. 1988, Salmon-Chemin et al. 2001). Subsequently the structure of tricyclic neuroleptic showed to be a promising backbone class of TR inhibitors, and based on computational design techniques several tricylcic compounds were investigated (Chan et al. 1998, Gutierrez-Correa et al. 2001). Some compounds of the series of 2-amino diphenylsulfides, that have lower neuroleptic activity than phenothiazines, were potent inhibitors of TR (Girault et al. 1998). Polyamine derivatives (Bonnet et al. 1997, Li et al. 2001), bisbenzylisoquinoline alkaloids (Fournet et al. 1998) and platinum II complexes (Bonse et al. 2000) were also studied in their capacity of inhibiting TR of *T. cruzi*.

Cystein protease

Cruzipain, also known as cruzain or GP57/51, is a member of the papain C1 family of cystein proteinases (CPs). The *T. cruzi* enzyme consists of a catalytic moiety with high homology to cathepsins S and L, and is absent in all other C1 families described so far (reviewed in Cazzulo et al. 2001). Irreversible inhibitors of cruzipain, such as several peptidyl diazomethylketones, peptidyl fluoromethylketones and peptidyl vinyl sulphones interfered with the in vitro intracellular cycle of *T. cruzi*, killing the parasite (reviewed in McKerrow 1999).

The treatment of acutely infected mice with the vinyl sulphone N-piperazine-Phe-hPhe-vinyl sulphone phenyl led to the absence of myocardial lesions, lymphocyte infiltration and intracellular amastigote clusters. This drug kills *T. cruzi* by inducing an accumulation of unprocessed cruzipain in the Golgi cisternae, interfering with the secretory pathway (Engel et al. 1998a,b). Cruzipain exposed to biotin-labelled peptidyl diazomethane inhibitors with a spacer arm showed a stronger reaction than the counterparts without such spacer, probably due to differences in the topologies of the binding sites of proteinases, differences that could be exploited to improve specificity against trypanosomal CP (Lalmanach et al. 1996). Roush et al. (2000) substituting the *L*-leucine residue of the natural peptidylepoxysuccinate E-64, a selective irreversible inhibitor of CP, by a *D*-threonine obtained a derivative with much higher activity against cruzipain than against bovine cathepsin B. Yong et al. (2000) commented that a possible limitation of CP as a target would be the emergence of parasite populations developing resistance to inhibitors. These authors reported a phenotypically stable *T. cruzi* cell line (R-Dm28) that displays increased resistance to Z-(SBz)Cys-Phe-$CHN_2$, an irreversible cysteine proteinase inhibitor, which preferentially inactivates cathepsin L-like enzymes.

Hypoxanthine-guanine phosphoribosyltransferase

Trypanosomatids must rely upon the salvage of exogenous purines for nucleotide synthesis, while in mammals these nucleotides are synthesized both *de novo* and salvaged from recycled purine bases. These protozoa convert purine bases to ribonucleotides, by the single enzyme HGPRT. This enzyme can also initiate in these parasites the metabolism of certain cytotoxic purine base analogs, such as allopurinol. This implies that either inhibitors or substrates of HGPRT have the potential of being effective and selective chemotherapeutic agents. The hgprt genes from *T. cruzi* and other pathogenic trypanosomatids have been cloned, sequenced and overexpressed in *Escherichia coli*, and the recombinant proteins have all been purified and characterized (reviewed in Ullman & Carter 1997).

The purine analogs 3'-deoxyinosine, 3'-deoxyadenosine and allopurinol inhibited the proliferation of amastigotes in HeLa cells, being the latter the most active. Among the pyrimidine analogs, 3'-azido-3'-deoxythymidine showed high activity against *T. cruzi* (Nakajima-Shimada et al. 1996). Purine analogs were assayed for their interaction with the HGPRTs from *T. cruzi* and man and some of them showed affinity for the trypanosomal enzyme (Eakin et al. 1997). A structure-based docking method identified 22 potential inhibitors of the enzyme. Three compounds (2,4,7-trinitro-9-fluorenyl-idenemalononitrite, 3-(2-fluorophenyl)-5-(phenoxy)-1,2,4-triazolo (4,3-C)-quinazoline and 3,5-diphenyl-4´-methyl-2-nitrobiphenyl) were effective against intracellular amastigotes and one [6-(2,2-dichloro-acetamido)chrysene] was a potent inhibitor of the trypanosomal HPRT (Freymann et al. 2000).

DNA topoisomerases

DNA topoisomerases II are enzymes that alter the topology of DNA and in kinetoplastids have been the focus of considerable study in the areas of molecular and cellular biology and also experimental chemotherapy. The gene encoding *T. cruzi* type II topoisomerase was isolated and the comparison with the amino acid sequence of the corresponding enzymes of *T. brucei* and *Crithidia fasciculata* showed a high degree of conservation (Fragoso & Goldenberg 1992). The enzyme is expressed in epimastigotes but not in trypomastigotes, although both forms of the parasite present the mRNA encoding the enzyme and is localized exclusively in the nucleus of the parasite (Fragoso et al. 1998).

Several inhibitors of bacterial DNA topoisomerase II showed activity against *T. cruzi*, inhibiting both proliferation and differentiation processes, and causing damage to kinetoplast and/or the nucleus of epimastigotes (Kerschmann et al. 1989, Gonzales-Perdomo et al. 1990), suggesting that both organelles could be the targets of the drugs. Camptothecin, inhibitor of eukaryotic DNA topoisomerase I, induced cleavage of nuclear and mitochondrial DNA in *T. cruzi* (Bodley & Shapiro 1995).

Dihydrofolate reductase

Dihydrofolate reductase (DHFR) and thymidylate synthetase exist as a bifunctional protein in different species of protozoa. This enzyme has successfully been used as a drug target in chemotherapy of cancer, malaria and bacterial infections. The gene coding for the DHFR domain from *T. cruzi* was cloned and expressed (Reche et al. 1996). Zucotto et al. (1998) described the modelling of *T. cruzi*'s DHFR based on the crystal structure of *Leishmania major* enzyme. From methotrexate, inhibitor of the human enzyme, among several derivatives synthesized, some of them showed a greater selectivity for the parasite enzyme than for the human counterpart (Zuccotto et al. 1999). In the same line, Chowdhury et al. (2001) designed and synthesized novel inhibitors of DHFR of trypanosomatids, however the compounds showed weak activity against both the enzyme and intracellular amastigotes of *T. cruzi*.

Glyceraldehyde-3-phosphate dehydrogenase

Since intracellular amastigotes possibly derive its energy from glycolysis, inhibition of glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may be a novel approach for the development of anti-*T. cruzi* drugs. The structure of GAPDH from glycosomes was reported and comparison with that of the mammalian counterpart led to the group of Oliva to consider the possibility of development of specific inhibitors of the parasite enzyme (Souza et al. 1998). In a subsequent work the isolation of flavonoids from the fruits of *Neoraptua magnifica* led to the compound 3',4',5',5,7-pentamethoxyflavone that showed the highest activity over flavones and pyrano chalcones against the GAPDH of the parasite (Tomazela et al. 2000). Crystal structure of trypanosomatids and human GAPDHs provided details about the interaction of adenosyl moiety of $NAD^+$ with proteins. Although adenosine is a very poor inhibitor, addition of substituents to the 2' position of ribose and the N6-position of adenosine led to a series of disubstituted nucleosides, and [N6-(1-naphthalenemethyl)-2'-(3-chlorobenzamido) adenosine] inhibited the proliferation of amastigotes without effect on the corresponding human enzyme (Bressi et al. 2001).

Farnesylpyrophosphate synthase

In pathogenic protozoa the pathway responsible for the synthesis of a variety of sterols and polyisoprenoids involves the enzyme farnesylpyrophosphate synthase, leading to the formation of farnesylpyrophosphate that marks the branching point of these synthetic routes. A gene encoding the farnesylpyrophosphate synthase of *T. cruzi* (TcFPPS) was cloned and sequenced and the enzyme was inhibited by the nitrogen-containing bisphosphonates, such as pamidronate and risedronate, but was less sensitive to the non-nitrogen-containing bisphosphonate etidronate, which does not affect parasite growth (Montalvetti et al. 2001). Pamidronate caused a decrease in the parasitemia of *T. cruzi*-infected mice and inhibited the in vitro intracellular replication of amastigotes (Urbina et al. 1999). Risedronate inhibited the proliferation of epimastigotes and sterol biosynthesis at a pre-squalene level and based on sterols analysis in treated parasites Martin et al. (2001) associated these results with the inhibition of farnesylpyrophosphate synthase. The effect of a series of bisphosphonates derived from fatty acids were assayed against *T. cruzi* and some of these drugs were potent inhibitors of the proliferation of intracellular amastigotes, but all of them were devoid of activity against epimastigotes (Szajnman et al. 2000). The selective action of nitrogen-containing bisphophonates against *T. cruzi* in comparison to mammalian cells could result from the preferential drug accumulation in parasite acidocalcisomes, acidic organelles rich in calcium, pyrophosphate, magnesium, sodium, zinc and polyphosphates (reviewed in DoCampo & Moreno 2001).

**Experimentally assayed drugs after 1992/1993**

The present review offers a survey of the available literature about new classes of compounds and also new derivatives from compounds previously assayed in the search for new drugs against *T. cruzi*. Recently other reviews have also been published, most of them exploring selected groups of compounds or inhibitors for *T. cruzi* targets (Rodriguez & Gros 1995, Urbina 1999, DoCampo 2001, Rodriguez 2001). As we have already mentioned with the present review, together with those of Brener (1979) and De Castro (1993), we intend to give the reader a general view of the experimental studies in the area of the chemotherapy of Chagas disease.

Synthetic drugs

*Thiadiazine derivatives* - In assays with epimastigotes, most of the 1,3,5-thiadiazine-2-thione derivatives were more active than Nif, while among 1,2,6-thiadiazin-3,5-dione 1,1-

dioxides; although active against the parasite, most of them were also toxic to mammalian cells (Ochoa et al. 1999, Di Maio et al. 1999). Among the less cytotoxic derivatives of the second series, one compound showed activity against intracellular amastigotes similar to the standard drug (Muelas et al. 2001).

*1,2,5-oxadiazole N-oxide derivatives* - A series of 1,2,5-oxadiazole N-oxide, benzo[1,2-c]1,2,5-oxadiazole N-oxide, and of quinoxaline di-N-oxide derivatives were synthesized and the activity against epimastigotes was associated with N-oxide radical formation (Cerecetto et al. 1999).

*1,4-dihydropyridines* - Among nitro-aryl-1,4-dihydropyridines, nicardipine, isradipine and lacidipine inhibited epimastigote proliferation and oxygen uptake in intact parasite, and the first compound showed also a similar effect in mitochondria *in situ* (Nunes-Vergara et al. 1997). For a series of 3-chloro-phenyl-1,4-dihydropyridine derivatives a positive correlation between trypanocidal effect and easiness of oxidation of the dihydripyridine ring was found (Maya et al. 2000).

*Acridine derivatives* - Since the 80s several acridine and acridinone derivatives have been presented a correlation of activity against epimastigotes and DNA binding (reviewed in De Castro 1993). Among a series of *bis*(9-amino-6-chloro-2-methoxyacridines), a bisacridine containing piperazine as central amine showed co-localization with kDNA of epimastigotes (Girault et al. 2000), reinforcing that their activity is associated with DNA interaction. Several 9-thioalkylacridines were active against *T. cruzi* (Bsiri et al. 1996). 9-Amino and 9-thioacridines have been reported to inhibit the enzyme TR (Bonse et al. 2000).

*Nitroimidazoles and nitrofurans derivatives* - Several nitroimidazoles such as megazol (CL 64,855), MK-436 and fexinidazole presented high activity in infected animals (reviewed in De Castro 1993). The current investigation about the use of nitroimidazoles for therapy of African trypanosomiasis, especially megazol (Barrett et al. 2000), renewed the interest in the area of Chagas disease. The coupling of 5-chloro-4-nitro-1-methylimidazole with heterocycles led to the synthesis of two compounds with activity against trypomastigotes (Boechat et al. 2001). More recently Cerecetto et al. (1999) synthesized nitrofurazones (5-nitro-2-furaldehyde semicarbazones) and tyophenes (5-nitrothiophene-2-carboxaldehyde) in which $N^4$-semicarbazone moiety was replaced by different of amines, aiming to mimic the spermidine part of trypanothione. These compounds presented lower activity against epimastigotes than the parent compound, while some nitrofurazones bearing $N^4$ other substituents produced complete survival in infected mice (Cerecetto et al. 2000).

*Phenothiazines* - Before the period mentioned in this review several groups investigated the effect of phenothiazines, tricyclic compounds employed clinically as antidepressants (reviewed in De Castro 1993). More recently it was reported that this class inhibited the enzyme TR. Promethazine and thioridazine assayed in vivo decreased parasitemia levels and mortality (Paglini-Oliva et al. 1998). When mice infected with low innoculum of *T. cruzi* and treated with thioridazine were checked 135 days post-infection, the heart histology and density of cardiac β-receptors were similar to those of uninfected, untreated controls (Rivarola et al. 1999), suggesting to the authors that this drug could prevent the evolution to the chronic phase of the infection.

*Metal chelating agents and metallic complexes* - Several chelating agents and derivatives were active against epimastigotes; being proposed that they act by interfering with the essential metabolism of iron, copper, or zinc (Rodrigues et al. 1995). Several $Fe^{3+}$ chelating agents showed activity against epimastigotes, that could be decrease in the presence of iron (Jones et al. 1993, Singh et al. 1997), and tetraethyl derivative of aminothiol was active against trypomastigotes (Deharo et al. 2000). The metal chelator sodium diethylamine-N-carbodithioate showed, in relation to Bz a similar activity against epimastigotes and intracellular amastigotes but lower activity against trypomastigotes (Lane et al. 1996). Another chelator, 1,10-phenanthroline inhibited epimastigote proliferation and led to electron-dense deposits in the kinetoplast, mitochondrion, and endoplasmic reticulum, identified as containing predominantly calcium and suggesting to the authors the involvement of disruption of calcium homeostasis in the trypanocidal activity (Lane et al. 1998).

Among several osmium(III) complexed with carbamates and different metals complexed with 1,2,4]triazolo [1,5a]pyrimidines some compounds were active against epimastigotes (Castilla et al. 1996, Luque et al. 2000).

*Propene-1-amine derivatives* - A series of 3-(4'-bromo-[1,1'-biphenyl]-4-yl)-3-(4-*X*-phenyl)-*N*,*N*-dimethyl-2-propen-1-amine derivatives were active against the three forms of *T. cruzi* (De Conti et al. 1996, Oliveira et al. 1999). The two compounds – the unsubstituted ($X$ = H) and the bromine ($X$ = Br) analogs – with highest activity against bloodstream forms, and lowest toxicity to mammalian cells, were assayed in vivo. The bromo-derivative displayed a strong suppressive effect on the parasitemia, and led to the survival of all the treated mice, whereas its unsubstituted analog was ineffective under the same conditions (Pereira et al. 1998).

*Aminoquinoline derivatives* - Quinolines have been assayed as potential drugs for Chagas disease since the 50s (reviewed in Brener 1979). Against epimastigotes the action of primaquine involves the formation of free radicals and this drug presented synergistic effect with ketoconazole decreasing the parasitemia of experimentally infected mice (reviewed in De Castro 1993). More recently, dipeptide derivatives of this aminoquinoline were synthesized as prodrugs, and inhibited the infection of LLC-MK2 cells with *T. cruzi* (Chung et al. 1997). Among 77 primaquine analogues, one of them reduced the parasitemia in mice in levels 14 and 4 times higher than Nif and primaquine, respectively (Kinnamon et al. 1996). In the same model, among 40 8-aminoquinolines, non-related to primaquine, 6 were more active than Nif, and for one of them, the activity was 13 times higher than this standard drug (Kinnamon et al. 1997).

*Dinitroanilines* - These compounds are microtubule-disrupting herbicides and one of the most studied is trifluralin. This compound was active against epimastigotes, trypomastigotes, and axenic and intracellular amastigotes. In trypomastigotes, trifluralin led to alterations at the surface analyzed by transmission and scanning electron microscopy, with membrane waving not associated with subpellicular microtubules. Treated epimastigotes showed alterations of shape, and some parasites presented two or three flagella and kinetoplasts, suggesting interruption of the cytokinesis process. Trifluralin also inhibited endocytosis in epimastigotes, monitored by complexes of gold with bovine serum albumine (Dantas et al. 1998, Dantas

2000). This dinitroaniline presented also in vivo effect in mice model (Zaidenberg et al. 1999) and inhibited the differentiation of epimastigotes to trypomastigotes (Bogitsh et al. 1999).

Recently we reviewed the effect of dinitroanilines on pathogenic protozoa and observed that *Leishmania* spp. and *Trypanosoma brucei* were more susceptible to trifluralin than *T. cruzi* and also compared sequences of tubulins of susceptible organisms (plants and trypanosomatids) and resistant (mammals) (Traub-Cseko et al. 2001).

*Lysophospholipid analogs* - These compounds are under clinical studies for cancer chemotherapy. The most studied derivatives are the alkylglycerophosphocholine edelfosine, a thioether substituted phosphatidylcholine analog ilmofosine and the alkylphosphocholine hexadecylphosphocholine (mitelfosine) (reviewed in Wieder et al. 1999). Clinical trials with mitelfosine for the treatment of Indian visceral leishmaniasis gave high percentage of cure including in cases resistance antimony therapy (Sundar et al. 2000). These LPAs were active against epimastigotes, intracellular amastigotes and trypomastigotes and led to damage of the flagellar membrane of epimastigote, and edelfosine inhibited the in vitro metacyclogenesis process (Santa-Rita et al. 2000). Previous in vivo experiments showed that LPAs had only a suppressive effect on the parasitaemia of *T. cruzi*-infected mice (Croft et al. 1996). It was found that LPAs are potent inhibitors of phosphatidylcholine synthesis in epimastigotes, and that ergosterol and its 24-ethyl analog were replaced by its $\Delta^{22}$-saturarted analogs, indicating inhibition of sterol C-22 desaturase (Lira et al. 2001).

Drugs derived from natural sources

Natural products account for about half of the pharmaceuticals in use today, but there has been a shift away from their use with the increasing predominance of molecular approaches to drug discovery (Clark 1996). However, in the last decades occurred a movement named by some as "back to Nature", with the revival of phytotherapy, needing a multidisciplinary group with expertise in botany, chemistry, biochemistry, molecular and cellular biology, and pharmacology. This trend was accentuated by the success obtained, for example, by taxol in cancer chemotherapy and artemisinin for malaria. A complementary approach is the development of synthetic analogs of the lead natural compound, aiming the improvement of properties such as pharmacokinetics, compatibilities and stability. Such intensification in the search of drugs from natural sources was also observed in the area of trypanosomiasis and leishmaniasis. In the literature several recent reports deal with the investigation of trypanocidal activity of a wide variety of crude natural extracts, especially vegetal ones, compounds isolated and semi-synthetic analogs.

*Alkaloids* - A great number of alkaloids have been assayed against *T. cruzi*. The activity of a series of alkaloids against epimastigotes was associated to the inhibition of cell respiration, and the most active compound was apomorphine (Morello et al. 1994). Using epimastigotes of Nif-resistant, the activity of β-carboline alkaloids was also associated with respiratory chain (Rivas et al. 1999). Several glycoalkaloids were tested against epimastigotes, bloodstream and metacyclic trypomastigotes, and a-chaconine and a-solamargine showed higher activity than ketoconazole (Chataing et al. 1998). Another group studied was bisbenzylisoquinoline alkaloids and daphnoline and cepharanthine were active against the parasite and inhibited enzyme TR (Fournet et al. 1998). In acute infections, dapholine led to a significant decrease in parasitemia and increase in cure rate in comparison with Bz-treated mice, and in chronic infections, in 70% of the treated mice no parasite was detected (Fournet et al. 2000). Five new bisbenzylisoquinoline derivatives were isolated from the stem bark of *Guatteria boliviana* and funiferine, antioquine and guatteboline were active against trypomastigotes (Mahiou et al. 2000). The alkaloid anti-microtubule agents vinblastine and vincristine obtained from *Vinca rosea* interfere with the proliferation of epimastigotes, inhibiting both nuclear division and cytokinesis, leading to giant cells with multiple nuclei, kinetoplasts and flagella (Grellier et al. 1999).

*Taxoids* - Taxol obtained from the bark of *Taxus brevifolia* is an anti-microtubule drug. This compound and synthetic derivatives are employed in cancer chemotherapy. Taxol was active against epimastigotes and trypomastigotes leading to significant alterations in the morphology of the parasites. Taxol also inhibited the endocytosis of proteins by epimastigotes (Dantas 2000).

*Stilbenoids and derivatives* - Among natural dihydrostilbenoid isonotholaenic acid and several simple derivatives some compounds showed activity similar to Bz against epimastigotes and others were more active than crystal violet against trypomastigotes (Olmo et al. 2001).

*Snake venom* - Venom from *Cerastes cerastes* and *Naja haje* inhibited the proliferation of epimastigotes of *T. cruzi* at levels similar to Bz, and that of *C. cerastes* was also active against trypomastigotes (Fernandez-Gomez et al. 1994).

*Gangliosides* Treatment with gangliosides of mice during acute infection promoted survival and clearance of parasites from the bloodstream and organs, and it was suggested that the effect of gangliosides could be due to interference of parasite penetration into the host cells due to inhibition of phospholipase $A_2$ (Lujan et al. 1993), but since the compounds had no direct effect on the parasite, Bronia et al. (1999) consider that the in vivo effect could be due to modulation of the host immune system.

*Juvenile hormone inhibitors and analogs* - The juvenile hormone fenoxycarb and methoprene inhibited the proliferation of epimastigotes (Stoka et al. 1995). Fenoxycarb and analogs were synthesized, and assays with epimastigotes suggested that an allyl ether moiety bonded at the polar extreme is important for the trypanocidal effect (Cinque et al. 1998). Some analogs were also active against bloodstream forms and reduced the parasitemia and mortality levels in relation to untreated controls (Fichera et al. 1995). 4-Phenoxyphenoxyethyl thiocyanate was very active against epimastigote and accumulation of low molecular weight metabolites from mevalonate to squalene was observed (Szajnman et al. 2000), leading the authors to suggest that the effect is associated with interference in the synthesis of ergosterol. Also sulfur-containing derivatives showed high activity against epimastigotes and amastigotes of *T. cruzi* (Rodriguez et al. 2000).

*Propolis* - It is a natural resin produced by honey bees has been used in folk medicine, since it displays strong anti-microbial activity, associated mainly with flavonoids and derivatives of hydroxycinamic acid (reviewed in De Castro 2001). Ethanolic extract prepared from a North American commercial sample were active in vitro against epimastigotes, trypomastigotes and intracellular

amastigotes (Higashi & De Castro 1994). However, in vivo experiments using different propolis formulations, showed no effect on the course of acute infection (De Castro & Higashi 1995). A Bulgarian sample has been assayed against trypomastigotes, and the ethanolic and acetonic extracts showed activity, respectively, similar and two times higher than crystal violet. The chemical composition of both extracts showed a high content of flavonoids, with differences in relative individual concentrations (De Castro et al. 2001). Recently Brazilian propolis became a subject of increasing scientific and commercial interest, and due to significant differences between the composition samples form tropical and temperate zones (reviewed in Bankova et al. 2000), we isolated from a sample collected in the State of Paraná (Brazil) four derivatives of hydroxycinnamic acid than when assayed against trypomastigotes showed activity bellow that of crystal violet (Marcucci et al. 2001).

*Naphthoquinones and synthetic derivatives* - Quinones are present in different families of plants and in some of them have been used in folk medicine for the treatment of diseases, especially cancer. Among bioactive natural naphthoquinones, we found lapachol, isolated from the heartwood of Tabebuia ("ipês") and also α- and β-lapachone obtained as contaminants in the process of lapachol isolation. Recently, lapachol derivatives were assayed against trypomastigotes and the triacetoxy derivative of reduced lapachol showed relevant trypanocidal activity (Santos et al. 2001). Due to the increasing number of reports about the activity of β-lapachone against a wide variety of tumor cells, by inhibition of topoisomerases and induction of apoptosis, Dubin et al. (2001) pointed out the potential of clinical applications of this naphthoquinone. Previous studies with b-lapachone showed activity against epimastigotes that was associated to generation of free radicals and inhibition of nucleic acids and protein synthesis (reviewed in De Moura et al. 2001). In a study of naphthoquinones isolated from Tabebuia, we synthesized 50 heterocyclic derivatives. The overall analysis of the structures indicated the tendency of trypanocidal activity in compounds with an imidazole or oxazole ring linked to a naphthopyrane structure (Pinto et al. 1997, Neves-Pinto et al. 2000). Among these compounds two naphthoimidazoles showed activities against trypomastigotes was 14.5 and 34.8 times higher than the standard crystal violet and both have an aromatic moiety linked to the imidazole ring (reviewed in De Moura et al. 2001).

Other groups of quinones isolated from natural products were also assayed against *T. cruzi*: (*a*) the trihydroxylated anthraquinone purpurin, obtained from the roots of *Rubia tinctorum* (Rubiaceae), showed an activity against trypomastigotes 1.5 times higher than crystal violet (De Castro et al. 1994); (*b*) among 1,4-naphthoquinones isolated from *Calceolaria sessilis*, 2,3,3-trimethyl-2-3-dihydronaphtho[2,3-*b*]furan-4,9-quinone showed against epimastigote and tumor lineages a high cytotoxicity and a temporary increase of oxygen consumption (Morello et al. 1995); (*c*) the polyprenylated benzoquinone 7-epiclusianone, isolated from *Rheedia gardneriana* (Clusiacease) was active *in vitro* against trypomastigote, but showed no effect on experimentally infected (Alves et al. 1999).

*Crude plant extracts and components* – (i) The sesquiterpene lactone dehydroleucodine isolated from *Artemisia douglasssiana* was active against epimastigotes, that presented pycnotic nucleus and a decreased number of ribosomes (Brengio et al. 2000); (ii) cryptofolione isolated from the fruits of *Cryptocarya alba* was active against trypomastigotes, however showed moderate effect for both amastigotes and macrophages, indicating little selectivity for *T. cruzi* (Schmeda-Hirschmann et al. 2001); (iii) from the seeds of *Annona glauca* (Annonaceae) nine acetogenins were isolated, and among them glaucanisin, squamocin, annonacin A and annonacin showed activity against trypomastigotes (Waechter et al. 1998); (iv) megalomicin is a macrolide antibiotic produced by *Micromonospora megalomicea*, that inhibits vesicular transport in Golgi apparatus, resulting in the undersialylation of cellular proteins in mammalian cells. This compound was active against epimastigotes and intracellular amastigotes at concentrations bellow those that interfere with the mammalian organelle (Bonay et al. 1998); (v) from the leaves of *Zanthoxyllum naranjillo* (Rutaceae), the lignan, methylpluviatolide was highly effective in vitro and in vivo (Bastos et al. 1999); (vi) *Terpenes:* (*a*) among diterpenoids isolated from *Azorella compacta*. (Llareta) azorellanol and mulin-11,13-dien-20-oico acid were active against intracellular amastigotes and the cytotoxicity to mammalian cells was lower than that of Nif (Neira et al. 1998); (*b*) from the aerial parts of *Wedelia paludosa* (Asteraceae), the isolated diterpenes ent-kaur-16-en-19-oic acid, ent-kaur-9(11),16(17)-dien-19-oic acid and 3 alpha-angeloiloxy-ent-kaur-16-en-19-oic acid showed activity against trypomastigotes (Batista et al. 1999); (*c*) in a study in Bolivia, 14 plants used in folk medicine to treat cutaneous leishmaniasis, extracts from 53 medicinal plants used for other diseases and different parts from 43 plants were screened and some of these material showed activity against epimastigotes of different strains of *T. cruzi* (Fournet et al. 1994); (*d*) extracts used in popular medicine in Guatemala from several plants such as *Neurolaena lobata* (Asteraceae), *Tridax procumbens* (Asteraceae), *Petiveria alliacea* (Phytolaccaceae) and *Byrsonima crassifolia* (Malpighiaceae) showed high activity against trypomastigotes. *N. lobata* and *Solanum americanum* showed in vitro and in vivo trypanocidal activity (Caceres et al. 1998). In a subsequent work, extracts, fractions and isolated sesquiterpene lactones and germacranolides from *N. lobata* showed high activity against epimastigotes (Berger et al. 2001); (*e*) among 79 extracts from plants of the families Asteraceae, Araceae, Moraceae, Solanaceae, Rhamnaceae, Zingiberaceae, Leguminosae and Sapotaceae, nine of then were active against epimastigotes (Muelas-Serrano et al. 2000); (*f*) from 32 crude plant extracts of nine species of Rutaceae eight of them showed significant activity trypomastigote. Fractionation of the active extracts provided 25 fractions, and the two fractions more active were obtained from the leaves of *Almeidea coerulea* and from *Conchocarpus inopinatus* (Mafezoli et al. 2000).

**Prophylatic drugs**

Although recent advances in vector control in the Southern Cone countries, by initiative of the Pan American Health Organization (PAHO) and World Health Organization (WHO), have decreased the incidence of new infections (Schofield & Dias 1999), we are still challenged by two critical problems: the treatment of chronic cases of the disease and the high level of acute cases in some Latin American countries, such as Bolivia and Mexico, where the incidence of infection in some regions reaches levels

above 80% of the population (Medrano-Mercado et al. 1996). In endemic areas the transfusional transmission of Chagas disease, due mainly to urbanization and migration processes, represents a great threat (Wendel & Dias 1992). For example, in 1990 in the USA, 7 million persons emigrated from countries in which Chagas disease is endemic (Schmunis, 1994, 2000) a preoccupying fact for the health authorities of non-endemic countries.

There is a recommendation from the WHO (1984) for the use of crystal violet in hemotherapic centers in endemic areas to eliminate the parasite in the blood used for transfusion (Nussenzweig et al. 1953). This dye presents no substantial side effect, although there are reports about blood micro-agglutination and potential mutagenicity (Thomas & McPhee 1984). Its main disadvantage is the bluish color that it confers to blood and tissues, which is not well accepted by the population. The development of trypanocidal drugs also involves the search for alternatives for crystal violet, when the assayed were performed with trypomastigotes in the presence of blood. These studies are included in the above item, but it must be kept in mind that when looking for an alternative to crystal violet its is fundamental to perform the assays with bloodstream forms in the presence of blood and at 4°C. The presence o blood is essential, since several trypanocidal compounds that are inactivated by blood components, such as gossypol (Rovai et al. 1990) and naphthoquinones and their heterocyclic derivatives (Lopes et al. 1978, Pinto et al. 1997). Another problem associated with drugs for prophylaxis of blood is, besides eventual toxicity, the poor solubility in aqueous medium and binding to plasma protein.

## CONCLUDING REMARKS

Nowadays infectious diseases are still an important cause of mortality and morbidity and the rising incidence of emerging or re-emerging diseases can be explained, at least partly, by the deterioration of health care systems and diverse socio-economic and ecological disorders. The cost of investments and the lack of market potential and market security in developing countries have dampened interest in developing drugs for tropical diseases. Among the 1061 new drugs developed from 1975 to 1994, less than 2.7% concern tropical diseases (Trouiller et al. 2000).

In relation to Chagas disease, great advances are being made in parts of South America to control the transmission by insect vectors or blood transfusion, but more effective chemotherapy is needed for the millions who are already infected. The demonstration of parasite in chronic patients indicates its importance in the maintenance of Chagas disease (reviewed in Tarleton 2001) and open the discussion about the etiologic treatment during this phase (OPAS/OMS 1998), reinforcing the need of finding more efficient and less toxic drugs.

In relation to experimental studies, a dichotomy has arisen between a rational and an empirical approach for drug development (Croft 1994). However, both are important routes to achieve a potential drug and sometimes are even used in conjunction. For example, having in hands a potential parasite target, the finding of an inhibitor could be searched by empirical tests or by modeling and synthesis that could interfere with the special regions of the target, such as the active center of an enzyme. The advent of genomics, rapid DNA sequencing, bioinformatics, combinatorial chemistry and automated high-throughput screenings had strength the interaction among groups with different expertise in order to find compounds with efficacy, including for immunosuppressed patients, without or at least with low toxicity, favorable pharmacokinetics and permeability properties and low cost of production.

Most of the studies with designed drugs against potential targets in *T. cruzi* involves inhibition tests using purified enzymes such as TR, CP and HGPRT, and it is possible that a compound discarded due to a negative result in this assay could be effective against the intact parasite. The continuous research of TR as a target could lead to an inhibitor that is a trypanocidal agent itself or that compromising the redox defenses of the parasite act in synergy with redox-damage drugs. However since the identification of trypanothione and although several groups are involved in finding inhibitors of TR, no drug emerged as effective and with low toxicity in experimentally infected animals.

After the introduction of Nif and Bz, among the extensive list of classes of compounds with in vitro and in vivo activity against *T. cruzi*, with exception of allopurinol, itraconaozle and fluconazole, none was submitted to clinical assays. This is due is some cases, to the absence of strong indication of their curative effect, to their potencial toxic and/or teratogenic effect (usually assayed only in vitro). This analysis emphasizes the need of a better choice of experimental models and standardization of protocols. In *in vitro* tests, it is of fundamental importance the use of trypomastigotes in the presence of blood both at 4°C (blood prophylaxis) and at 37°C (treatment) and comparison with crystal violet, and the effect against intracellular amastigotes using a suitable host cell and comparison with Nif or Bz. Still today several drugs are screened only against epimastigotes, although several authors have already reported different sensibilities to drugs among different forms of the parasite. In relation to in vivo tests a standardization of both acute and chronic phase models and of parameters to monitor the cure are of outmost importance. Also toxicity and mutagenicity studies in animals are needed. Otherwise we will continue to employ a lot of effort in experimental studies without achieving a drug that could be submitted to clinical trials.

After the presentation of potential targets in the parasite and active drugs in experimental models, which are the compounds that could potentially be employed in clinical tests? Among them stand out the nitroimidazoles megazol (CL-64855) (Filardi & Brener 1982) and MK-436 (Andrade et al. 1989), drugs studied before 1993, but that afterwards, at least to our knowledge, no new papers dealing with such promising drugs appeared in the literature. We believe that it could be important to review all the data obtained about megazol and MK-436, and develop new in vivo protocols to monitor their pharmacokinetics and bioavailability properties and also any potential toxic, mutagenic and/or teratogenic effects. It is possible that the discontinuity of such studies was due to the toxicity of the compounds or no interest of pharmaceutical companies in their synthesis. Drugs for Chagas disease are not in the interest of such industries and only the effort and persuasion of researchers in establishing partnerships could change the current scenery. An interesting case is megazol, which although previously shown by the group of Brener as an active drug even against strains of *T. cruzi* resistant to Nif and Bz, was not further investigated (Filardi & Brener 1982). However, several recent works pointed to its usefulness for the treatment of African trypanosomia-

sis (Barrett et al. 2000) and it was communicated in the 4th CostB9 Meeting in Portugal (2001) that a pharmaceutical industry will probably begin to synthesize megazol with this objective.

What must be implemented to achieve new effective and less toxic drugs for the treatment of Chagas disease? With all the knowledge accumulated about the biology and biochemistry of *T. cruzi* a lot of effort must be directed to the understanding of the mechanism of action of selected compounds. Another line that is beginning to be studied is the preparation of different formulations of drugs, which would allow its delivery to the right places. Excellent results have been obtained in the case of formulations of amphotericin for the clinical treatment of leishmaniasis (Sundar et al. 1998) and also experimental Chagas disease (Yardley & Croft 1999). Also in studies with *T. cruzi*, it was already been shown to increase in vitro and in vivo effect of different drugs. For example the use of ethylcyanoacrylate nanoparticles prepared by an emulsion polymerization process together with allopurinol or Nif and surfactants led to higher activity against epimastigotes than the corresponding free compound (Gonzalez-Martin et al. 1998, 2000). Also Molina et al. (2001) incorporated inhibitors of sterol biosynthesis into long-circulating polyethyleneglycol-polylactide nanospheres improving the bioavailability of these poorly soluble compounds and increasing the cure rate in experimental animals.

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