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RECENT ADVANCES IN THE CHEMOTHERAPY OF CHAGAS' DISEASE

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The accumulated experience on specific treatment of human acute Chagas' disease shows that it usually prevents lethality and in a certain percentage of cases it is even able to cure patients (Cerisola, Alvarez & De Rissio, 1970; Krettli, Cançado & Brener, 1982). This treatment is then useful for the clinical management of the acute cases which occur in endemic area, the acute cases induced by blood transfusion, the congenital cases (Moya, 1984) and, finally, those infected by laboratory accidental infections (Brener, 1984). A few recent chronic infections can probably also be cured (Krettli, Cançado & Brener, 1982). Specific therapy is also indicated for preventing transmission by kidney transplantation in the peculiar situation of a chagasic organ donor being the suitable match for the recipient patient (Dias, Brener & Macedo, 1984). Treatment of patients in the indetermined form of the disease with the available drugs is still controversial. The discovery of new active, non-toxic compounds would probably expand treatment, then including those patients in which clinical manifestations are absent or can only be disclosed by more elaborate medical procedures.

Historical background: after the discovery of the disease in 1909 and until 1937 no compounds have been tested in humans with Chagas' disease (reviewed by Gutteridge, 1980). Since then a number of new *in vivo* active compounds had been discovered as a result of more extensive screening programs by pharmaceutical industries. In addition, empirical attempts to use new derivatives which proved to be active against unrelated parasitic diseases (such as arsenobenzene, 8-aminoquinolines and phenanthridinium derivatives) increased the arsenal of compounds active against *Trypanosoma cruzi*. Most of those drugs failed to cure and were poorly tolerated.

Packchanian (1952) screened a number of 5-nitrofurans and reported suppressive effect *in vivo* of a number of 5-nitrofurans. Brener (1961) was the first to demonstrated that long-term treatment with a nitrofuran (nitrofurazone) had a consistent curative effect on *T. cruzi* experimental infections, a finding that stimulated this approach in the treatment of the human disease. The old widespread concept that parasitological cures could not be achieved because the sequestrated intracellular forms were not accesible to active drugs was proved to be wrong by a series of experiments *in vitro* and in animals (review: Brener, 1975). A gradual decline and disappearance of the acquired immunity in animals cured from their *T. cruzi* infection was also reported (Brener, 1962). The implications of this finding in the criteria of cure in human Chagas' disease is discussed elsewhere in this Symposium (Krettli, 1984). The only drugs used clinically at present are a nitrofuran derivative [3-methyl-4 (5'-nitrofurfurylidene-amino)-tetrahydro-4H-1, 4-thiazine-1, 1-dioxide] ("nifurtimox") and a 2-nitroimidazol (N-benzyl-2-nitro-1-imidazolacetamide (benznidazole).

Present situation of clinical treatment: as above mentioned, only nifurtimox (Bock et al., 1972) and benznidazol (Grunberg et al., 1968) are being used in chagasic patients. Both compounds are far from the ideal medicine for the treatment of Chagas' disease whose most important requirements are: cure parasitologically acute and chronic cases, be effective by oral route in a single of few doses, be affordable by the patients and free of significant side effects and teratogenicity, not require hospitalization and, finally, not cause parasite resistance (WHO, 1981).

Both drugs given according to long-term schedules of drug administration (30-90 days) induce side-effects such as hypersensitivity reactions, peripheral neuritis, loss of weight and gastrointestinal disturbances. Patients treated with benznidazol may present marked decrease in the number of leukocytes. The efficacy of the drugs is low and only a very small percentage of patients are actually cured. Treatment at the acute phase, however, is indicated because of the risks involved at this stage of the disease (Cançado & Brener, 1979). The reported side-effects and the general concern about toxicity of nitroheterocyclic compounds suggest that both nitro derivatives, nifurtimox and benznidazol, should be reserved for the management of acute cases or for the treatment of limited numbers of chronic patients (preferably in the indetermined form) under careful medical supervision.

New drugs for the treatment of Chagas' disease: the lack of extensive drug screening and the incipient stage of the rational approach for the development of compounds ative against *T. cruzi* supports the guess of Gutteridge (1980) that "it seems that the nitroheterocycles currently in use or under investigation will remain the mainstay of our efforts to treat cases of Chagas' disease for the rest of the decade". Actually, as we will see by the following list of new drugs active against *T. cruzi*, this seems to reflect the poor prospects for the treatment of Chagas' disease:

SQ 18506: (trans-5-amino-3-[2-(5-nitro-2-furyl) vinyl]-1, 2, 4-oxadiazole) was studied by Gutteridge, Cover & Gaborak (1975). It presents a suppressive effect on *T. cruzi* infections in mice but has not curative action. Of 8 analogues tested against *T. cruzi* in tissue culture, 3 showed some degree of activity.

Fexinidazole (HOE 239): is a 1-methyl-2-(4-methylthiophenoxymethyl)-5-imidazole (Raether & Deutschländer, 1979) active against *T. cruzi* infections in mice. Apparently no further data on its activity have been reported.

MK-436: 3 (1-methyl-5-nitroimidazol-2-yl)-3a, 4, 5, 6, 7, 7a-hexahydro-1, 2-benzisaxazole) is a 2, substituted 5-nitroimidazole active against *T. cruzi*. Studies carried out in mice demonstrated a curative effect not only at the acute stage of the disease but also in mice chronically infected (Murray, Habbersett & Meurer, 1983). An interesting observation was that high percentages of cure in the chronic disease are detected even at low dose levels, provided that the drug is administered over prolonged periods of time. The efficacy of this compound was confirmed by brazilian authors but the drug did not reach the stage of clinical trial.

Ketoconazole: <2-S, (R)-2, 4-(dichlorophenyl)-2-(imidazol-1-Y1 methyl)-1, 3-dioxolan-4-R, (S)-Y methyl >-(2 2(4-chlorophenyl)-ethyl >-1, 2, 3, 4-tetra-hydro-isoquinolin-6-Y1)-ether is a potent imidazole derivative active against topic and systemic fungal infections as well as a large number of protozoa (Raether & Seidenhat, 1984). Interestingly, it is also effective on chloroquine-resistant *Plasmodium falciparum* strains. The drug protected mice inoculated with lethal inocula of *T. cruzi Y* strain but failed to cure the animals (McCabe et al., 1984).

Megazol (CL 64'855): 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole. This compound has a broad spectrum of activity as an anti-bacterial and anti-parasitic agent (Berkelhammer & Asato, 1968). The possible curative action of CL 64'855 in experimental Chagas' disease was investigated by Filardi & Brener (1982) who reported that in their experience this compound "is the most active compound so far tested and it can be added to the existing list of nitroheterocycle drugs with marked activity against *T. cruzi*". This drug is extremely active against intracellular stages in the vertebrate host (Maria, Filardi & Brener, 1984) and cures a high percentage of infections induced by *T. cruzi* strains highly resistant to nifurtimox and benznidazol (Filardi & Brener, 1982).

Allopurinol: [4-hydroxypyrazolo (3, 4-d) pyrimidine] (HPP) is a hypoxanthine analogue which decreases the production of uric acid by inhibiting conversion of hypoxanthine into xanthine; in addition it interfers with the "de novo" purine synthesis. Allopurinol is extensively used for the treatment of gout, a disease characterized by deposition of uric acid in joint bones and organs. This drug is remarkable well tolerated by humans.

HPP, but not oxipurinol (the most important metabolite in humans), was demonstrated to strongly inhibit growth of *T. cruzi* epimastigotes in acellular medium (Marr, Berens & Nelson, 1978). *In vivo* effect of HPP was reported by Ávila & Ávila (1981) who treated *T. cruzi* infected mice with 8-64 mg/kg/day and observed a clear suppressive effect on the parasitemia. Avila, Avila & Munoz (1981) further reported that some *T. cruzi* strains were insensitive to HPP. Since HPP is converted by *T. cruzi* (and also by *L. donovani*) into 4-aminopyrazolo (3, 4-D) pyrimidine (APP) which is about 15-fold more effective on epimastigotes than HPP, Ávila et al. (1983) administered APP to experimentally infected mice and demonstrated that suppressive effect could be detected with doses 400-fold lower than that found active for HPP. An *in vitro* screening with 42 different pyrazolo (3, 4-D) pyrimidines produced only one active derivative less potent than the parent drug (Ávila, 1983).

More recently, Lauria-Pires, Castro & Prata (1984) treated 6 patients in the acute phase of Chagas' disease with allopurinol in the dose of 20-30 mg/kg/day for 45-60 days. The evaluation of drug activity was performed by blood examination (Strout method) and xenodiagnosis. The drug was in general well tolerated. Nevertheless, in all patients parasites had been detected by xenodiagnosis after the long-term treatment. The conclusion was that allopurinol had neither suppressive nor curative effect on human acute Chagas' disease.

Allopurinol riboside (BW 28U) has been given to 5 dogs acutely infected with *T. cruzi* (5-25 mg/kg, 30 days). All dogs survived the acute phase but the results were not conclusive in relation to curative effect (Tanus et al., 1984).

Gossypol: this is a substance isolated from cotton plant that chinese investigators demonstrated to display an antifertility action in men by inducing reversible azoospermia. The chemical structure is (1, 1', 6, 6', 7, 7'-hexahydroxy-5, 5'-diisopropyl-3, 3'-dimethyl-(2, 2'-binaphthaleno)-8, 8'-dicarboxaldehyde). This drug inhibits selectively the lactate dehydrogenase isozyme X which is involved in sperm metabolism and provides energy to spermatozoa. Montamat et al. (1982) reported that gossypol also inhibits α-hydroxyacid dehydrogenase and malate dehydrogenase of *T. cruzi* culture epimastigotes. In higher doses the drug induces morphological changes detected by electron microscopy. No data on its effect in *T. cruzi* infected hosts are available. Although gossypol has been used as a contraceptive in humans and other species there is still a controversy on its potential mutagenic activity.

Mode of action of anti-*T. cruzi* drugs – The metabolism of nitroheterocyclic derivatives in *T. cruzi* has recently been investigated mostly with nifurtimox (for review see Stoppani, 1983; Docampo & Moreno, 1984). Experimental data provided by Docampo & Stoppani (1979) and Docampo et al. (1981) strongly suggest that the effect of this 5-nitrofuran derivative is mediated by free radical intermediates generated by the drug, such as superoxide anions and hydrogen peroxide. Basically, this effect is carried out according to step-wise reactions as described by Docampo et al. (1981) (Fig. 1). When homogenates of *T. cruzi*

$$1 - 2\,Ar\,NO_2 + NAD(P)H \xrightarrow{NR} 2\,Ar\,NO_2^{\cdot -} + NAD(P)^+ + H^+$$

$$2 - Ar\,NO_2^{\cdot -} + O_2 \xrightarrow{NE} Ar\,NO_2 + O_2^{\cdot -}$$

$$3 - O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

$$4 - O_2^{\cdot -} + H_2O_2 \xrightarrow{Fe^{+3}} HO^{\cdot} + OH^- + O_2$$

Fig. 1: mechanism of free radical generation by nitrocompounds (Apud Docampo et al., 1981). Ar NO_2 = nitrocompound (ex. nifurtimox); NR = nitroreductase; NE = nonenzymatic reaction; SOD = superoxide desmutase.

containing NAD(P)H are incubated with nifurtimox the reduction of the drug occurs (apparently initiated by a flavin-linked nitroreductase) and a nitro radical is formed (1). In the next step the nitro radical react with O_2 and generates the superoxide anion ($O_2^{\cdot -}$) (2). This anion will then produce hydrogen peroxide (H_2O_2) either spontaneously or under the action of the enzyme superoxide dismutase (SOD) (3). Finally, in a Fe catalyzed reaction, hydroxy radicals are formed (4). The free radicals are extremely deleterious to the cells and once generated they may interact with many cell components and propagate their harmful effects. Similar mechanism of toxicity against *T. cruzi* has been reported in relation to naphtoquinones related compounds such as β-lapachone (Docampo et al., 1978), which are active *in vitro* but not in the living host.

Peroxide production by nifurtimox is detected in homogenates of all *T. cruzi* developmental stages (epi-, trypo- and amastigotes) but its production is higher with the amastigotes (Docampo et al., 1981), a finding consistent with the described damaging effect of nitrofurans on the parasite intracellular stages.

Intringuinly, benznidazol does not generate free radical intermediates in *T. cruzi* despite its effectiveness in Chagas' disease and also the fact that it generates these radicals in mammal cells as demonstrated in experiments with rat liver microsomes (Moreno et al., 1982). Thus, it is possible that the lethal effect of benznidazol does not depend on the reduction of the nitro group and free radical generation.

Gutteridge (1980), suggested that generation of free radicals as an explanation for the action of 5-nitrofurans on *T. cruzi* "cannot be the whole of the story". The current concept is that whereas mammal cells have a number or enzymes (superoxide dismutase, catalase and peroxidases) which prevent the lethal effect of free radicals, *T. cruzi* has poor detoxification mechanism (it has not catalase and has a very limited glutation peroxidase activity) and therefore is rather sensitive to nitrofuran derivatives. Nevertheless, as described by Gutteridge et al. (1982), *Crithidia fasciculata,* a monogenetic catalase-containing trypanosomatid is also sensitive to nitroheterocyclic drugs. This finding led Stoppani (1983) to speculate that either the presence of catalase is not essential to prevent the harmful effects of the oxigen derivatives or, then, generation of free radical intermediates is not the only mechanism involved in the trypanocide effect induced by nitrofurans.

More recently, Goijman, Frasch & Stoppani (1984) investigated the effects of nifurtimox and benznidazol on DNA, RNA and protein biosynthesis of *T. cruzi* epimastigote stages using labeled precursors. The results suggest, in relation to nifurtimox, a relationship between free radical generation, inactivation of DNA biosynthesis by these radicals and the trypanocide effect. Again, the authors concluded that "oxygen radicals generation was not essential to inhibit protein biosynthesis in *T. cruzi*".

In relation to allopurinol, Marr, Berens & Nelson (1978) were the first to demonstrate that *T. cruzi* epimastigotes metabolize this drug by converting it to HPPR-MP (allopurinol ribonucleoside monophosphate) which is sequentially converted to APPR-TP (4-aminopyrazolopyrimidine ribonucleoside triphosphate). The conversion to APPR-TP and the incorporation into *T. cruzi* RNA is apparently a mechanism of selective toxicity to the parasite (Ávila, 1983). Actually, these conversion and further incorporation are carried out only by *T. cruzi* and leishmania, but not by mammal cells. This event turns the flagellates into suitable targets for the drug. Transformation of allopurinol into APPR-TP is detected not only in *T. cruzi* epimastigotes but also in trypomastigote and amastigote stages (Ávila, 1983). The great potential advantage of allopurinol is, therefore, its differential metabolism in the parasite and the mammal cell. Whereas in *T. cruzi* the drug is converted by sequential steps to APPR which is toxic for the cell, in the man about 90% is converted into oxipurinol which is readily excreted.

Since allopurinol ribonucleoside is an inosine analog, Marr et al. (1984) tested six modified inosine analogs in *T. cruzi* epimastigotes and reported that all of them were active *in vitro.*

Natural resistance of *T. cruzi* to chemotherapeutic agents – Hauschka (1949) has already reported differences in the natural resistance of two *T. cruzi* strains to a bisquinaldine derivative. Since then this phenomenon has been confirmed by many authors who treated animals with the two standard drugs used in patients, namely, nifurtimox and benznidazol. Some experiments have been carried out determining drug sensitivity of strains isolated from a defined geographical area. Andrade et al. (1981) and Andrade, Maga-

lhães & Pontes (1984) reported that strains isolated from patients living in an endemic area in Minas Gerais (Montalvania) were significantly more resistant to both drugs than those isolated from humans in Bahia (São Felipe) and Goiás (Mambaí). Schlemper (1982) detected resistance to benznidazol and nifurtimox in 2 out of 7 *T. cruzi* strains isolated from chronic chagasic patients in Minas Gerais.

Filardi & Brener (1984) described a rapid method for testing *in vivo* the susceptibility of different *T. cruzi* strains to active compounds. The rational for this method was based on previous observations on the pharmacokinetics of nifurtimox (Medenwald, Brandan & Schlossman, 1972) and benznidazol (Raaflaub & Ziegler, 1979) which demonstrated that in humans the maximum concentration of the drugs in the blood was reached within 1-2 hours after "per os" administration (Fig. 2). In fact, single doses of active drugs given to *T. cruzi* infected mice permitted to detect in a short period of time (4-6h) the effects on the blood forms. With the sensitive strains a rapid and marked decline on the number of circulating parasites is observed whereas with resistant populations the number of flagellates is kept at the same level observed before treatment. A good correlation was detected between the results obtained with this rapid method and the long-term treatment schedules.

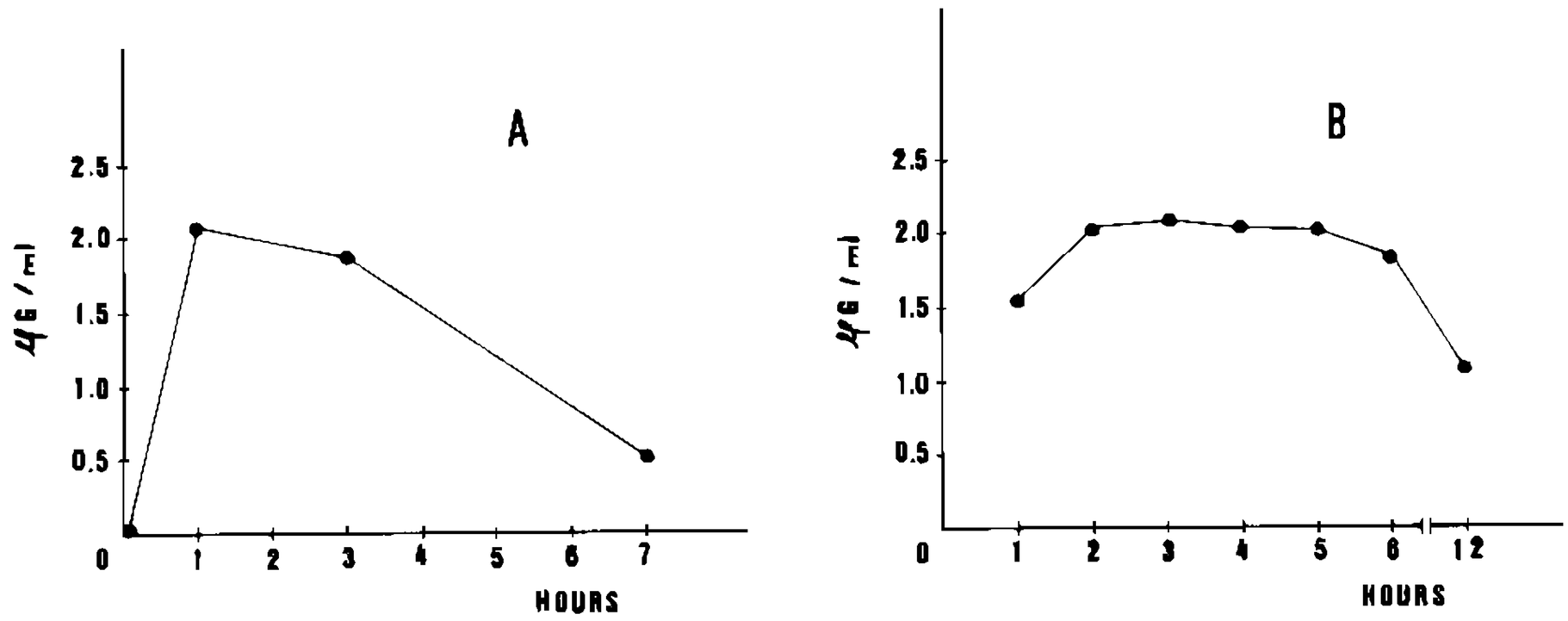

Fig. 2: pharmacokinetics of trypanocide compounds is man.
A: blood concentration of nifurtimox, 1,000mg, single dose, oral route (Medenwald et al., 1972);
B: blood concentration of benznidazol, 100mg, single dose, oral route (Raaflaub & Ziegler, 1979).

The occurrence of natural resistance in a number of strains isolated from human patients or domestic vectors and deposited in the cryobank of trypanosomes from the Centro de Pesquisas René Rachou has been investigated by Filardi & Brener (unpublished data). Blood trypomastigotes from 30 different *T. cruzi* strains had been inoculated into mice which were then treated with nifurtimox and benznidazol. Evaluation of the treatment was based on fresh blood examination, hemocultures and serology. The main conclusions were the following: 70% of the strains are fairly sensitive to the drugs as demonstrated by the rates of 80-100% cures in the treated animals; 4 strains were extremely resistant to treatment (0% cure) and 7 strains induced infections in which less than 50% of the animals could be cured. With only one exception, all strains were equally sensitive or resistant to both compounds.

Ávila, Ávila & Munoz (1981) treated groups of mice inoculated with 6 different *T. cruzi* strains with allopurinol (32 mg/kg) and detected differences in the sensitivity to the drug, which were independent of the parasitemia characteristics and strain origin. When trypomastigotes collected from mouse blood were incubated with allopurinol *in vitro* (in LIT medium) they demonstrated to be more resistant than epimastigotes to drug action, suggesting that the parasite multiplying intracellular stages are those more sensitive to allopurinol.

The mechanism of the natural resistance of *T. cruzi* strains to the nitroheterocyclic derivatives is not yet known. Defficient drug absorption is a possibility but has not been demonstrated. Strains deffective in the nitroreductase (which is essential for the reduction of the nitro group and further generation of free radicals) could be an alternative explanation. Actually, oxygen-insensitive nitroreductases apparently unable to transfer electrons to nitroderivatives have been already described in *Escherichia coli* and *Salmonella typhimurium* (*in* Docampo & Moreno, 1984). This phenomenon can not, however, explain *T. cruzi* resistance to benznidazol whose action is not likely to depend on enzymatic nitro reduction. The role played by catalase and other enzymes which protect cells from oxygen reduction intermediates has been already discussed and does not seem to be essential in this phenomenon of resistance.

In relation to allopurinol the resistance of some of the *T. cruzi* strains is due to a slower uptake and metabolism of the drug by the blood forms (Ávila, 1983).

Agosin et al. (1976) described in *T. cruzi* epimastigotes a detoxifying system that has been suggested to be important in the phenomenon of the insensitivity of *T. cruzi* to chemotherapeutic agents. Basically, this is a monooxygenase system linked to P-450 cytochrome. However, the participation of this system in the detoxification and metabolism of drugs active against *T. cruzi in vivo* has not been surely established.

Influence of specific treatment in the course of Chagas' disease – The lack of a reliable criteria of cure in the human disease, the difficulties in carrying out prolonged follow-up of treated cases and the possible participation of auto-immune reactions in the pathogeny of Chagas' disease has prevented an assessment of the influence of specific treatment in the outcome of the disease. There are, however, some experimental data and a few clinical investigations in this field which are worth mentioning.

Laguens et al. (1983) studied the effect of nifurtimox and benznidazol in mice chronically infected with *T. cruzi*. The drugs were given for 30 days (100 mg/kg and 200 mg/kg, respectively) and the animals were investigated 30 and 60 days after treatment. Although the incidence and severity of the electrocardiogram alterations declined after treatment, the myocarditis has not significantly changed. Andrade & Andrade (1976) had previously studied the effects of treatment with nifurtimox in the anatomo-pathological aspects of mice with chronic infection of long duration (215 to 575 days) induced by the highly treatment-resistant *Colombiana* strain. The animals had been sacrified 50 to 100 days after treatment. The prevalence of the inflammatory process was similar in both treated and untreated animals but the lesions were less intense and frequent in the treated group; fibrosis was present respectively in 50% and 36.3% of both groups. Since intracellular parasites were detected in 18.1% and 62.2% of the treated and control animals the authors suggest that the decrease in the parasitism are responsible for the diminution of the pathological changes.

Lima Pereira, Filardi & Brener (1983) treated mice with LC 64'855 whose curative action was reported by Filardi & Brener (1982). The animals were chronically infected with the *Colombiana* strain and had been sacrificed about 10 months after treatment. All untreated controls presented severe myocarditis, focal endocarditis, ganglionitis and myositis. In the treated but uncured animals inflammation was less frequent and fibrosis seldom observed whereas in the cured mice only small foci of mononuclear cells in the heart and reparative fibrosis (in 4 out of 15 animals) were observed. These results suggest that specific treatment reduces the severity of the inflammatory lesions.

Data on the influence of specific treatment in the course of the human disease are even more scarce. Chiale (1980) was able to follow-up two groups of chronic chagasic patients for 18-48 months: 30 untreated patients and 57 treated with nifurtimox (8-10 mg/kg for 60 days). In the untreated group of patients no significant electrocardiographic changes occurred whereas in the second group three patients developed electrocardiographic and clinical alterations despite the treatment.

Manzullo & Darraidon (1983) conducted a longitudinal study of 185 chronic patients treated with nifurtimox (8 mg/kg, 60-90 days). Only patients who had normal ECG and X-ray and had been followed-up for at least 8 years were included in the investigation. Electrocardiographic changes emerged at an annual rate of 6.6% and 6.7% in, respectively, the treated and untreated patients, indicating that this treatment has not prevented the occurrence of ECG alterations.

Although those clinical data are rather clear on suggesting that specific treatment with nifurtimox does not influence the outcome of the disease, we should mention that because the lack of a dependable criterion of cure, parasite erradication could not be surely established in these patient samples.

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