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Rational Approaches to Chagas' Disease Chemotherapy (**)

Rational drug design is still in its infancy, partly because fundamental knowledge of many areas of the parasites' metabolism is still lacking. No doubt, as more differences become apparent, new classes of chemotherapeutic compounds will be developed against Chagas' disease (***). The few existing active drugs are usually detected in experimentally infected animals by their suppressive effects on parasitemia and the decrease in mortality rates. Animals treated with active drugs may have prolonged periods of inapparent parasitemia, that indicate either a parasitological cure or conversion to the chronic phase of the infection. Criteria of cure involve a number of laboratory methods to detect "cryptic" infection, such as repeated fresh blood examination, hemoculture, reinoculation, subinoculation, xenodiagnosis, circulating antibody or antigen levels, etc. What criteria should be used to ascertain whether American trypanosomiasis has been cured?

Since one is dealing with a parasitic disease, the evidence of cure would rest on the demonstration of the presence of parasites before treatment and its disappearance after treatment. Extinction of the parasites themselves, i.e., cure of the infection, would, of course, bring as a consequence completely negative results by xenodiagnosis and, as a corollary, progressively negative results in immunologic tests. If the antigenic complex represented by *T. cruzi* disappears from blood and tissues, it is reasonable to assume there has been an interruption in formation of its specific antibodies, the titers of which would progressively decrease. This would mean a decrease in the positivity of immunologic reactions, up to the point of complete annihilation of the infection at the end of a given time.

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(***) In this paper, I shall review what is now known about the chemotherapy of Chagas' disease, highlighting with detail and references the information that has emerged in the last five years. Earlier references can easily be found elsewhere (Goble, 1961; Brener, 1975, 1979; Guteridge, 1975).

A priori, therefore, we think that once the chagasic patient is cured, serologic reactions would also become negative. We do not know how long it would take for this to occur. For example, in the acute phase of Chagas' disease, the suppressive action of nifurtimox is followed by negative results in the serological tests or a fall in their titers (Cerisola *et al.*, 1970). As far as xenodiagnosis is concerned, there is no doubt that extinction of parasitism results in a negative test. Nevertheless, some patients with chronic disease show spontaneously negative monthly xenodiagnoses for as long as four years (low parasitemia). Therefore, even when we choose for a trial patients with a high level of parasitemia, we cannot state with certainty how long it would be necessary to extend the post-therapeutic period of observation of monthly negative xenodiagnoses, since high and low parasitemia may be only phases in the long natural history of Chagas' disease. It seems necessary to interpret concomitantly both parameters: xenodiagnosis and serologic reactions in placebo and drug groups, in order to draw any conclusions as to the curative value of a drug given a clinical trial. How to interpret the results of experiments showing definite negative xenodiagnosis results during the period of treatment and a decrease in seropositivity in the post-therapy period? One hypothesis is that the drug kills only the circulating forms of *T. cruzi* and does not affect tissue parasitism, despite experimental evidence to the contrary.

The use of *in vitro* tests with culture forms for establishing sensitivity or resistance to a given drug has been favored by establishing new defined culture media (Avila *et al.*, 1979b, 1983a), although differences in drug metabolism between culture and blood forms of *T. cruzi* have recently been demonstrated (Avila *et al.*, 1981b, 1983b), suggesting the difficulties in extrapolating results obtained on epimastigote forms to those achieved on trypomastigote forms of *T. cruzi*. On the other hand, the use of tissue cultures infected with *T. cruzi* permits the study of direct effects on intracellular stages (Brener, 1973).

According to the World Health Organization (1981) "the ideal drug for treatment of Chagas' disease would achieve parasitological cure of both acute and chronic cases from all parts of Latin America. It would be active by both the oral and, for cases of megaesophagus, the parenteral route; it would be effective in one or a small number of daily doses and be affordable by those who suffer from the disease. At the doses used, it would be free of serious side effects (including those that are only transient) and of teratogenicity. It would not require patients to be hospitalized, and drug resistance would not develop readily".

This review will deal only with purine analogues and their conjugates, and free radical-generating drugs. Such different types of drugs seem to be promising chemical compounds as tested in experimental and human infections.

Purine analogues

The actual rationale for the use of purine analogues is to synthesize compounds very closely related structurally to the naturally occurring metabolites,

assuming that perhaps the analogs will react with specific enzymes involved in cellular metabolism of naturally occurring metabolites in such a way as to interfere with cellular metabolism, hopefully specifically in an abnormal cell. This can be effected by having either the analog *per se* inhibit a specific enzyme, or by having the analog function as a substrate for an enzyme and the concerted analog product then exerts the desired effect. The optimum effect would be for the analog to exhibit some selectivity of action as regards the host (normal) cell and an invading (abnormal) cell (*T. cruzi*). However, whether an analog will achieve this specificity is difficult to predict, due to the many effects which the metabolites (heterocycle, nucleoside, nucleotide and polymer compounds) exert on various cellular processes. It is of considerable interest, in the nucleic acid area, that the enzymic conversion of purine and pyrimidine nucleoside analogues to their nucleotide forms appears to be a prime requisite for biological activity. While this conversion may not be essential for biological or chemotherapeutic activity in every instance, it does seem to be of particular importance for anti-trypanosomal activity (Marr and Berens, 1983). If the nucleoside of concern is a structural analog of adenosine, it can be directly phosphorylated to the 5'-monophosphate level in most mammalian systems by the enzyme adenosine kinase, which has low substrate specificity. However, if the compounds of interest are analogs of inosine and guanosine, the metabolic route leading to the 5'-monophosphate level is a bit more complicated. To reach the nucleotide stage, the guanosine analog must be cleaved by purine nucleoside phosphorylase to produce the apparent heterocycle, which must then act as a substrate for hypoxanthine-guanine phosphoribosyl transferase (HGPRT), to afford the 5'-phosphate derivative, by a condensation of the heterocycle with phosphoribosyl-pyrophosphate (PRPP).

The structural modifications implemented in the search for biological active purine nucleoside analogs can be grouped into two basic approaches: a) those that involve the carbohydrate moiety, and b) those that involve the heterocyclic moiety. Modifications of the heterocycle can be accomplished by replacement or modification of the functional groups on the naturally occurring purine, or by the replacement, interchange, deletion, etc., of the carbon and nitrogen ring atoms of the naturally occurring ring.

Pyrazolopyrimidine Derivatives

Allopurinol (4-hydroxypyrazolo-(3,4-d)-pyrimidine (HPP), a structural analog of hypoxanthine, was synthesized in 1956 as a possible antineoplastic agent (Robins, 1956). Although ineffective as such, it was found to be a potent inhibitor of xanthine oxidase (Spector and Johns, 1970) and effective in the treatment of gout and other hyperuricemic states (Yu and Gutman, 1964). Allopurinol is believed to diminish uric acid production primarily by inhibiting the conversion of hypoxanthine to xanthine, but it also acts to depress *de novo* purine synthesis through feedback inhibition of amidophosphoribosyltransferase (Caskey *et al.*, 1964) and by depletion of the essential substrate, phosphoribosyl-

pyrophosphate (McCollister *et al.*, 1977). In humans it has been demonstrated that 60% of the ingested drug will be converted to oxipurinol (a metabolite which is also an inhibitor of xanthine oxidase), with the remaining 30% being excreted as such in the urine and 10% as HPP-1-ribonucleoside (HPPR). More recently, Hande *et al.* (1978) have demonstrated that concentrations of about 3 µg/ml are reached within 1-2 hours of oral administration of 300 mg allopurinol, serum half-lives of HPP and oxipurinol being 39 ± 11 minutes and 14 ± 3 hours, respectively.

As regards the effects of HPP on *T. cruzi*, studies on epimastigote forms of several strains have demonstrated a strong inhibitory effect of low concentrations, ID_{50} (inhibitory dose causing a 50% inhibition of cell growth) being of about 3 µg/ml (Marr *et al.*, 1978; Avila and Avila, 1981a). Noteworthy, the major mammalian metabolic product of HPP, oxipurinol, had no effect on epimastigotes (Marr *et al.*, 1978).

Subsequent studies showed that among the several purines tested, only adenine was effective in reversing the HPP trypanostatic effects (Marr *et al.*, 1978; Avila *et al.*, 1981c). Biochemical investigation of the metabolism of HPP by *T. cruzi* has shown that this compound is metabolized by a sequential conversion to HPPR-MP (allopurinol ribonucleoside monophosphate), this metabolite being aminated to 4-aminopyrazolo-(3,4-d)-pyrimidine-5'-monophosphate (APPR-MP), then converted to the di- and triphosphates APPR-DP and APPR-TP, respectively, and the latter incorporated into the cellular RNA (Marr *et al.*, 1978; Avila *et al.*, 1981c). The incorporation of APPR-TP into RNA of *T. cruzi* is unique; as mammalian cells show, neither this conversion nor the incorporation (Nelson *et al.*, 1973). This process is believed to be the basis of the selective toxicity of HPP to *T. cruzi* as compared to animal cells, thus fulfilling (Himmelweir, 1960), the aim of a specific chemotherapy, that is, to exterminate an invading organism without injuring the host.

The incubation of *T. cruzi* epimastigotes with HPP caused no depletion of nucleotide pools (Marr *et al.*, 1978). This fact, plus the observation that *T. rangeli* culture forms are insensitive to the inhibitory effects of HPP but sensitive to APP, suggests that in *T. cruzi* the succino-AMP synthetase activity is the enzyme responsible for aminating HPPR-MP to APPR-MP. In fact, the *T. cruzi* enzyme is 100-fold more active on HPPR-MP than the *rangeli* enzyme (Avila *et al.*, 1981c). In the case of *Leishmania*, a second enzyme has been demonstrated as participating in this metabolic pathway: succino-AMP lyase (Spector *et al.*, 1979). This latter group described the kinetic properties of this enzyme, which have in *T. cruzi* the same broad substrate specificity that seems to be characteristic of this activity from most organisms (Spector *et al.*, 1982).

Interestingly, HPPR-MP is a substrate of succino-AMP synthetase from *T. cruzi* (Avila *et al.*, 1981c) but not from a mammalian source (Spector and Miller, 1976). In the case of *Leishmania* the synthetase appears to be the rate-limiting step, this enzyme being unique in its ability to accept HPPR-MP as an alternate substrate for IMP. Evidence for the formation of succino-APPR-MP, APPR-MP or their metabolic products has been sought but not found in mam-

malian tissues treated with HPP (Nelson and Elion, 1975). The low V_{max} for HPPR-MP (about 2% that for IMP) is consistent with the metabolic studies described above, which showed that APPR-MP is formed very slowly after the rapid accumulation of HPPR-MP. In fact, it has been shown that APP is about 15-fold more active on *T. cruzi* epimastigotes (Avila *et al.*, 1981d).

HPP and APP inhibit the incorporation of 3H uracil and ^{14}C leucine into RNA and protein. At a concentration of 10 $\mu g/ml$, APP inhibits RNA and protein synthesis completely, and HPP inhibits both by approximately 40% (Marr and Berens, 1983). However, using ^{14}C serine incorporation, there are no differences between control and 10 $\mu g/ml$ APP or HPP-treated epimastigotes. Furthermore, HPP suppresses the synthesis of all classes of RNA, and this does not appear to be related to a rapid breakdown of RNA. *T. cruzi* treated for 24 hours with 25 $\mu g/ml$ HPP shows also a decrease in the maximum number of ribosomes per strand of RNA, from 12 to 7. This suggests a premature termination of protein synthesis.

Since the pathogenic forms of *T. cruzi* are the amastigote and trypomastigote, it is important to know whether pyrazolopyrimidine metabolism in these is the same as in the epimastigote. Avila *et al.* (1983b) have demonstrated that trypomastigotes are able to transform ^{14}C HPP into APPR-MP, APPR-DP and APPR-TP, and also that the uptake and metabolism of ^{14}C HPP and ^{14}C APP were significantly slower in trypomastigotes as compared to epimastigotes. Additionally they found that amastigotes are also sensitive to HPP and APP.

As discussed before, APP is about 15-fold more active on *T. cruzi* epimastigotes than HPP. These results suggested the possibility of testing several different HPP or APP derivatives in order to increase the antitrypanosomal efficacy of the parent compounds. Among 40 additional pyrazolo-(3,4-*d*)-pyrimidines tested on *T. cruzi* epimastigotes only 1- β -D-ribofuranosyl-4-aminopyrazolo-(3,4-*d*)-pyrimidine was active, although slightly less potent than the parent 4-aminopyrazolo-(3,4-*d*)-pyrimidine.

In vivo antitrypanosomal effects of purine analogs

Avila and Avila (1980; 1981a) demonstrated that allopurinol was effective in treating acute Chagas' disease in mice. However, a subsequent paper (Avila *et al.*, 1981b) demonstrated that some *T. cruzi* strains are insensitive to HPP treatment, this resistance being due to slower HPP uptake and metabolism by blood trypomastigotes as compared with those of sensitive *T. cruzi* strains (Avila *et al.*, 1983b; Avila *et al.*, 1983c) also demonstrated the effectiveness of low doses of APP (0.25-1 mg/Kg) on experimental acute Chagas' disease, this dose being about 400-fold lower than that found as effective for HPP. The striking therapeutic effect of APP could be explained by the ability of the parasite to concentrate the drug or to convert it to 4-aminopyrazolo-(3,4-*d*)-pyrimidine ribonucleotides as demonstrated in *L. brasiliensis* and *L. donovani* (Nelson *et al.*, 1979). Furthermore, the optimal dose 0.25 mg/Kg/day is about

40-100-fold lower than the LD₅₀ reported for APP-treated mice (Scholler *et al.*, 1956; Shaw *et al.*, 1960) and quite similar to that reported as nontoxic in human studies (Shaw *et al.*, 1960).

APP riboside has been revealed as a promising drug in the treatment of experimental acute Chagas' disease, the optimal dose being 1.5 mg/Kg administered during 10 consecutive days. However, it is evident that, on a molar basis, this drug is less active *in vivo* than the parent drug: APP, a situation similar to that found for HPPR (Marr and Berens, 1983). Avila *et al.* have found the ribofuranosyl-pirazo-lo-pyrimid-7-one derivative effective on experimental acute Chagas' disease, the optimal dose being 3 mg/Kg, although, again, activity depends on the *T. cruzi* strain under study.

Lysosomotropic approaches

As a possible explanation for a partial failure of pyrazolopyrimidines and purine analogs in arresting experimental acute Chagas' disease might be their short half-lives (Elion *et al.*, 1966; Hande *et al.*, 1978), Avila assayed two different approaches in order to obtain a sustained release of active drugs and a targeting to highly phagocytic cells, usually most parasitized cells (except the case of heart). The first was the use of carriers, the second encapsulation of active drugs inside nanoparticles.

Lysosomotropic conjugates

Regarding the first approach, for a drug-carrier conjugate to be effective, the link between drug and carrier must remain stable in the blood-stream and withstand the action of serine hydrolases. On the other hand, unless the drug is able to act in conjugated form at the cell's surface, it has to be released from the carrier after interaction of the conjugate with the target cell, and its mode of release must be such as to allow the drug to reach its biochemical target, situated intracellularly, and to interact effectively with it. Because the most general fate of macromolecules is to be interiorized by endocytosis and conveyed to the lysosomes for digestion, an obvious way of ensuring appropriate release of the drug is to rely on lysosomal hydrolysis. This approach is evidently limited to drugs that are not inactivated in the lysosomes and that can reach their biochemical target from the lysosomal compartment, as for example, amastigotes or trypomastigotes lying free in the cytoplasm. The principles governing this "lysosomotropic" chemotherapy have been developed in greater detail by de Duve *et al.*, 1974.

Avila *et al.* have developed and tested, *in vitro* and *in vivo*, a bond meeting the above requirements between 1-β-D-glucosamine-4-aminopyrazolo-(3,4-d)-pyrimidine and bovine serum albumin. They introduced glucosamine into APP, because this aminosugar has a primary amino group suitable for an amide type linkage. Albumin was selected as a model carrier because of its protein nature and ready availability.

The therapeutic effect of APP-gluNH₂ (APPGN) and its various conjugates on acute Chagas' disease may be summarized as follows. APPGN exerted a moderate activity with an increase in life-span of 53% at a dose of 0.15 mg/Kg, the 2 mg/Kg dose being toxic. This induced a weight loss of more than 25% on day 10 and the death of some animals before the controls. Albumin-APPGN and Albumin-leu-APPGN had no chemotherapeutic effect at 5-10 mg/Kg and proved to have little toxicity because no significant weight loss was observed on day 10 at highest dose (15 mg/Kg). The effects of albumin-ala-leu-APPGN at 0.10-0.50 mg/Kg were similar to the effect of APPGN at lower dosage. Albumin-leu-ala-leu-APPGN and albumin-ala-leu-ala-leu-APPGN had markedly higher therapeutic effects with an average of 100% survivors on day 100 and an increase in life-span of 500%. Additional experiments showed that incubation of the various APPGN conjugates with purified macrophagic lysosomal enzymes revealed no APP release from albumin-APPGN. However, the rate of APP release increased markedly when the peptide spacer arm was lengthened to three or four aminoacids. About 40% of the bound drug was released as free APP from albumin-ala-leu-APPGN and 80% from albumin-ala-leu-ala-leu-APPGN after 20 hours of incubation at pH 5.5. It is then evident that the chemotherapeutic efficiency of the conjugates paralleled closely their sensitivity to lysosomal hydrolysis.

Lysosomotropic nanoparticles

The second approach was the use of polyalkylcyanoacrylate nanoparticles as a drug carrier. The main advantage of these particles is their degradability at a rate depending on the length of the alkyl chain (Couvreur *et al.*, 1979). These ultrafine particles (diameter of 0.2 µm) are able to efficiently absorb a variety of drugs in a stable and reproducible way (Couvreur *et al.*, 1980a). It has also been shown that the binding of cytostatic drugs to nanoparticles modifies their distribution pattern in rat tissues and generally increases the tissue capture of these drugs (Couvreur *et al.*, 1980a, b).

The mortality and parasitemia of *T. cruzi*-infected mice, thirty days after injection of either free or nanoparticles-bound APP of the ribofuranosyl-pyrazolo-pyrimid-7-one derivative at doses of 0.50, 0.25 and 0.10 mg/Kg/day were given for 10.5 or 2 consecutive days of administration. The results obtained were as follows. At the doses of 0.25 and 0.10 mg/Kg the mortality in the free drug groups was significantly higher than that noted in the nanoparticles group ($P < 0.005$). At the dose of 0.10 mg/Kg/day the parasitemia in the free drug groups was fourfold higher than for the groups injected with the bound drug.

When the administration schedules of both free and bound APP or its pyrimidone derivative were limited to two interdaily successive injections of 0.10 mg/Kg/day, the nanoparticles group showed significant differences in both mortality and parasitemia ($P < 0.010$) (Avila, 1983).

In conclusion, these results show a significant increase in antitrypanosomal activity when APP or derivatives are fixed to nanoparticles, effects corresponding

to a diminution in mortality and parasitemia after administration of bound drug for various doses and administration schedules. It should be noted that the use of polyalkylcyanoacrylate nanoparticles as the drug carrier can reduce considerably the inherent toxicity and side effects of a cytotoxic drug and could be useful in Chagas' disease chemotherapy.

One must however be careful when testing these purine analogues in mammals, as effects of these drugs on the cellular immune system have clearly been demonstrated. Thus, for example Tritsch and Niswander (1983) have recently demonstrated that HPP inhibits superoxide production by elicited macrophages, and Nishida *et al.* (1980) have shown that HPPR inhibits purine nucleoside phosphorylase and consequently T cell immunity in mice. Thus, continuous evaluation of cellular and humoral immunity has to be carried out in human or animal trials using purine analogs.

Free-radical generating drugs

It is becoming increasingly apparent that many reactive intermediates are free-radicals; that is, they have an odd or spin-unpaired electron in their outer orbital. Because of the thermodynamic potential of the unpaired electron to form an electron pair, free radical intermediates are extremely reactive. As such, they can undergo a variety of reactions, including adding across unsaturated bonds, abstracting hydrogen from other molecules, or combining with themselves to form dimers. Another possibility to be considered is that the radical intermediate may be in an electronically excited state, which could then transfer its electronic energy to acceptor molecules or utilize this energy to oxidize target molecules. In addition, free radical intermediates can activate molecular oxygen by univalent reduction to the superoxide anion (O_2^-) which in turn can rapidly dismutate to produce H_2O_2 . The metal catalyzed reaction of these two species of oxygen metabolites results in the formation of an extremely powerful oxidant, the hydroxyl radical ($\cdot OH$). The generation of free radicals, including secondary molecular oxygen-derived radicals, presents a danger to cells because radicals are capable of interacting with, and subsequently damaging, the entire array of biomolecules which constitute cells. Moreover, radical-initiated processes are particularly deleterious because they are conservative and propagative; that is, radical interactions with cell constituents may produce secondary and tertiary free radicals derived from lipids, amino acids, glutathione, ascorbic acid and components of nucleic acids.

All these observations, plus the fact that protective enzymes such as glutathione peroxidase and catalase, which destroy H_2O_2 in mammalian cells, appear to be very low or absent in *T. cruzi*, present this cell as more susceptible to cellular damage by activated oxygen species than mammalian cells (Boveris *et al.*, 1980).

In recent years, several chemically different types of drugs have been linked by their capacity to generate very active oxygen-derived free radicals, thus pro-

ducing trypanocidal effects. Included in this group are nitrofurans and nitroimidazole derivatives.

Nitrofurans derivatives

Activity of nitrofurans against amastigotes of *T. cruzi* has been observed in tissue culture (Brener, 1966). The experimental demonstration that nitrofurazone and related compounds may actually cure animals treated according to long-term schedules (Brener, 1961) stimulated clinical trials with nitrofurans. This author demonstrated that in groups of mice treated with nitrofurazone for 53 consecutive days, 96% were parasitologically cured, whereas in a group treated with only 20 doses, 80% remained infected.

Haberkm and Gonnert (1972) confirmed the efficacy of this treatment schedule and demonstrated that cure of animals treated with nitrofurans depends more on the duration of treatment than on the total dosage of these drugs. Interruption of treatment even for short periods of time prevented the parasitological cures. Freeman *et al.* (1975) described an *in vitro* drug screen using trypanomastigotes released from infected tissue culture Vero cells; of 48 nitrofurans derivatives tested in this system, 56% were active. Effectiveness was increased by introduction of an electronreleasing methyl group at the furan 2-position; compounds presenting a 5-nitrofururylidene group were also very active. Among a number of nitrofururylidene compounds, 3-methyl-4-(5'-nitrofururylidene-amino)-tetrahydro-4H-1,4-thiazine-1,1-dioxide (nifurtimox) was selected as the most promising derivative (Bock *et al.*, 1969, 1972; Haberkm and Gonnert, 1972).

As regards *in vitro* effects, Cardoni *et al.* (1977) demonstrated that Nifurtimox interfered with the trypanomastigote to epimastigote shift found in liquid culture and that this effect was related to the dose of the drug, no transformation existing at 45 µg/ml of drug.

Regarding the biochemical basis of the trypanostatic effects of Nifurtimox, this point has been examined in depth by Stoppani and coworkers (Boveris *et al.*, 1980; Boveris and Stoppani, 1977; Docampo and Stoppani, 1979; Docampo *et al.*, 1981; Dubin *et al.*, 1983 and 1984; Moreno *et al.*, 1980 and 1982; Gojman *et al.*, 1985; Gojman and Stoppani, 1985).

Nifurtimox is active in different animal species (mice, rats, hamsters, guinea pigs, cats and dogs) acutely infected with *T. cruzi* (Haberkm and Gonnert, 1972).

Different *T. cruzi* strains displayed marked differences in their susceptibility to nifurtimox and related compounds. Animals inoculated with eight different strains showed cure rates between 0% (Tulahuen strain) and 100% (WBH strain) (Haberkm and Gonnert, 1972). Similar results have been reported by Andrade *et al.* (1975) and Brener *et al.* (1976).

As regards toxicity, high doses (over 100 mg/Kg) of nifurtimox administered for long periods in mice and rats caused complete inhibition of spermatogenesis with degeneration and atrophy of the testicular parenchyma. It must be emphasized that there is increasing concern about the mutagenicity and carcinogenicity of nitrocompounds (Shakin and Kilbey, 1974), especially now that

there is evidence that the mode of action of these drugs involves interaction with DNA (Sims and Gutteridge, 1978) Goffman *et al.* (1985) among other mechanisms. Docampo *et al.* (1981) have shown that mammalian tissues, in addition to *T. cruzi*, are able to reduce enzymatically nifurtimox to its anion free radical, the initial reduction of the drug being initiated by NADPH-cytochrome P-450(c) reductase. In the presence of air, the nitro anion-free radical is oxidized, resulting in catalytic superoxide generation, which may play an important role in the toxicity of nifurtimox to mammals. An enhanced formation of superoxide and the relatively low catalase activity of brain (Hochstein and Cohen, 1960), and testes cells (Burbuley and Ellis, 1973) may be factors in the organ specificity of nifurtimox toxicity if similar reactions occur *in vivo*. As regards humans, in 52 chronic patients treated with 8 mg/Kg nifurtimox for 60 days 54% had to interrupt treatment because of digestive, nervous, hematological and skin symptoms; reversible side effects appeared in 39% of children, 54% of juveniles and 69% of adults (Wegner and Rohwedder, 1972).

Nifurtimox has been widely used for treatment of chronic and acute Chagas' disease in several Latin American countries, with enormous discrepancies in clinical results. The reasons for these differences are not yet understood. The possible participation of *T. cruzi* strains with different susceptibility to chemotherapeutic agents is now quite clear (Avila *et al.*, 1981b) and might be related to the presence of different zymodemes in *T. cruzi* populations (Miles *et al.*, 1981).

Nitroimidazole derivatives

The best known of these compounds is benzimidazole, also called Radanil. It is exactly N-benzyl-2-nitro-1-imidazoleacetamide. Again, considerations on the biochemical basis of trypanocidal activity of benzimidazole were presented before (Stoppani, 1983). However, the difference noted between nifurtimox's and benzimidazole's capability to generate free radicals in mammalian cells may be significant in the treatment of Chagas' disease when these drugs are compared for their toxicity to the host.

In cultures of KB cells, concentrations of 0.4-0.8 $\mu\text{g/ml}$ of benzimidazole inhibited the growth of the parasite, whereas 3-6 $\mu\text{g/ml}$ were needed for killing both intracellular amastigotes and extracellular trypomastigotes within 4 days (Riehle and Rasflaub, 1980).

Pharmacokinetic evaluation of plasma obtained from 8 patients treated with daily doses of 7 mg/Kg revealed an average half-life of 13.8 hours, the highest individual level measured being of 16.4 $\mu\text{g/ml}$ and the lowest 5.4 $\mu\text{g/ml}$. Thus the plasma benzimidazole level of these patients was permanently above the minimal trypanocidal concentration of 3.6 $\mu\text{g/ml}$ as found in tissue culture (Rasflaub and Ziegler, 1979).

In patients suffering from parasitologically proven acute or chronic Chagas' disease, high parasitological cure rates (about 80% were achieved in Argentina; Barclay *et al.*, 1978) and Brazil (Coura *et al.*, 1978) with daily doses of 5-7

mg/Kg given for one month. Parasitological cure was established by repeated negative xenodiagnosis and, especially in acute cases, by a drop of the titers in three serological tests: hemagglutination, immunofluorescence and complement fixation tests. Cerisola (1977), using those criteria, assessed in Argentina the efficacy of benzimidazole in chronic Chagas' disease. Ninety-four percent of patients became parasitologically negative, the dose of 7-10 mg/Kg producing a high number of side-effects, such as polyneuropathy and "progressive purpuric dermatitis" (Cançado, 1969), and digestive symptoms; psychic disturbances, rash and peripheral neuritis being observed in chronic patients treated for 2 months with benzimidazole at 5-10 mg/Kg (Levi *et al.*, 1975).

Other Trypanocidal Agents

T. cruzi is sensitive to other free-radical generators, especially quinones and quinonoid compounds (Boveris *et al.*, 1978a and b; Cruz *et al.*, 1978; Docampo *et al.*, 1978; Goijman *et al.*, 1983; Goijman and Stoppani, 1985b). So far, there is no clinical experience with these substances.

General Conclusions

We can conclude from these previous considerations that:

a) Pyrazolopyrimidines are potentially useful in the treatment of at least acute Chagas' disease. Given the excellent results obtained in humans affected with visceral leishmaniasis (Kager *et al.*, 1981; Jia, 1983) as well as in experimental animals inoculated with American leishmaniae (Walton *et al.*, 1983) without any evidence of side effects, allopurinol was assayed on a small group of cutaneous leishmaniasis patients (1600 mg/Kg for 30 days), obtaining total healing of ulceration with concomitant disappearance of inflammatory signs, suggesting this drug as a candidate for a successful treatment of cutaneous leishmaniasis. The possibility of using albumin-APPGN conjugates or APP-loaded nanoparticles opens new ways for approaching the chemotherapy of Chagas' disease.

b) Nifurtimox and benzimidazole may be of value in the treatment of acute cases by shortening the course of infection and preventing deaths. Mass treatment or even treatment of large populations with chronic infection is not recommended because cures have not been unequivocally obtained and a high incidence of severe side effects has been reported.

Summing up, further studies are urgently needed for the development of new drugs for the treatment of Chagas' disease, capable to fulfill WHO requirements.

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