In Pursuit of Drugs for American Trypanosomiasis: Evaluation of Some "Standards" in a Mouse Model[1] (44192)

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Abstract. **Forty-nine "standard" compounds known to be useful in the treatment of other diseases were tested for their suppressive activity against the trypomastigotes of *Trypanosoma cruzi*–infected mice. The most active was the antidepressant protriptyline, which was almost three times as effective as the reference drug, nifurtimox. A major value of the present data is to demonstrate the refractoriness of the *T. cruzi* parasite against many of the drug standards that have known biological activity.**

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Chagas' disease, a condition caused by the protozoan *Trypanosoma cruzi,* places one-quarter (100 million) of the inhabitants of Latin America at risk (1). The 16 million to 18 million people infected result in the "disability-adjusted life years" (DALYs) to rank only behind malaria and schistosomiasis in the global burden. Nifurtimox and benznidazole are employed to treat the disease, but the malady remains essentially incurable.

The lack of interest in seeking new, more effective compounds is the high cost of drug development, then a poor market for the items produced (2, 3). It has been suggested that it would be a worthwhile undertaking to evaluate medications not designed for the treatment of Chagas' disease (3). Some of these may "incidentally" have significant antichagasic activity. The identification of such drugs would constitute a means of taking advantage of development costs already incurred.

We chose to test 49 compounds known to be active in one or more disease conditions. Compounds chosen for evaluation were from among more than 250,000 held by the Walter Reed Army Institute of Research. This large inventory has been amassed principally as a result of testing agents for activity against other parasitic diseases.

Materials and Methods

Animal Model and Procedures. The system employed was as described elsewhere (4) and was designed to determine the suppressive activity of test compounds against trypomastigotes of *T. cruzi* in the blood of infected mice.[3] More specifically, female albino mice (4–6 weeks old, CF_1 Strain, Harlan-Sprague Dawley, Madison, WI) and a Brazil strain of *T. cruzi* were used (5). The organism was maintained by passage every 2 weeks in 4- to 6-week-old mice. Blood was drawn into a heparinized syringe from a donor mouse by cardiac puncture. The appropriate dilution was then prepared in Hanks' balanced salt solution, pH 7.2, and 0.2 ml of this suspension containing 50,000 trypomastigotes was injected intraperitoneally into each test mouse. Twice daily oral administration of the experimental compound was begun on day 11 of the infection and continued through Day 14. Each compound was suspended in a 0.1% Tween 80 plus 0.5% hydroxyethylcellulose (HEC-Tween).

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[3] The experiments reported herein were conducted according to principles set forth in the Animal Welfare Act as amended and its promulgated regulations (7 USC, para. 2131 et. seq.) and the *Guide for the Care and Use of Laboratory Animals,* Institute of Laboratory Animal Resources, National Research Council, U.S.A., NIH Publication No. 85-23.

Drug dosage levels ranged from 0.8125 to 104 mg/kg/day using groups of six animals for each dose level, at least three groups per drug. Control groups consisted of mice receiving vehicle only (negative controls) and others receiving nifurtimox (positive controls). The negative controls were used to establish the baseline parasitemia in the untreated animals. This baseline level was then employed to compare the degree of suppression found for positive controls (nifurtimox) with that for animals receiving the test drugs. Blood smears were prepared on Day 15, and numbers of parasites per milliliter of blood were determined.

Handling of Data. A comparison of the antischizotrypanosomal activity of each test compound with that of the reference compound, nifurtimox (2-methyl-4-[5-nitro-2-furyiidene]amino-1,4-thiazane-1,1-dioxide), was made, and an index (relative activity of the test compound to that of nifurtimox for each test compound) was calculated by the following formula:

$$\text{Nifurtimox Index (NI)} = \frac{SD_{50} \text{ for Nifurtimox}}{SD_{50} \text{ for Test Compound}}.$$

In the expression, SD represents the amount of compound (mg/kg) that produced a specified degree of suppression of the infection, such as 50%. Since the compounds were tested "blind," each was administered on the basis of total compound weight, unadjusted for formula weight due to the acid component in salts. Final correction was made in salts by taking into consideration the acid combined with the base. This was done by multiplying the index obtained by the reciprocal of the fraction of base component in the salt.

The degree of suppression (e.g., SD_{50}) was estimated graphically from plots of percent parasite suppression and dose of compound administered (mg/kg/day) on log probit paper. When the SD_{50} could not be obtained because of low activity of the test compound, a lower SD value was used. A nifurtimox index of greater than 1 indicates that the test compound is more active than the reference compound.

Results

The compounds found to be active are shown in Table I. Inactives are listed in Table II. Preliminary data have been reported (6). The most suppressive substance was the antidepressant protriptyline (Compound 5, Table I). It was 2.92 times as effective as the reference drug, nifurtimox. Imipramine (Compound 1, Table I), also an antidepressant, was considerably less active than the former. Compounds 2–4 were one-fourth to one-half as effective as nifurtimox. Protriptyline, which was almost three times as effective as the reference drug, nifurtimox, was approximately six times as suppressive as the next most effective compound, ketoconazole (Compound 2), a broad spectrum antimycotic with known antichagasic properties (7), and almost nine times as effective as the analog antidepressant, imipramine (Compound 1).

Table I. Compounds Found To Be Active against *Trypanosoma cruzi* Infections in Mice

Compound	Common name	N index[a]	WR no.[b]
1	Imipramine	0.33	6180
2	Ketoconazole	0.50	248,310
3	Nigericin	0.33	124,906
4	Niridazole	0.27	5,950
5	Protriptyline	2.92	35,941
	Nifurtimox (reference compound)	1.0	205,632

Note. Source of compounds: Reference compound (nifurtimox), Plumpe, Bayer AG, 56 Wuppertal-1, Postfach 130105, West Germany; 1, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178; 2, K. Schellekens, Janssen Pharmaceutica, 2340 Beerse, Belgium; 3, D. M. Brennan, Department of Chemistry, Eli Lilly, Indianapolis, IN 46206; 4, C. H. Sullivan, Research Department, Ciba, Pharmaceutical, Summit, NJ 07901; 5, M. H. Fisher, Research Labs, Merck Sharpe & Dohme, Rahway, NJ 07065. Salt form of compound: 1 and 5, hydrochloride; 2–4, free base.

[a] Nifurtimox used as a reference drug for N index. N Index = suppressive dose (SD) for Nifurtimox/SD for Test Compound × 1/[(1 – Formula wt Due to Salt)/(Total Formula wt)].

[b] Number assigned by the Walter Reed Army Institute of Research.

Discussion

To our knowledge *in vivo* activity for *T. cruzi* has not been previously reported for the tricyclic antidepressant (TCA) protriptyline (Compound 5), the most active compound of the 49 evaluated. This amphiphilic cationic compound is structurally related to imipramine, which was among a series of iminodibenzyl derivatives synthesized in the late 1940s (8). *In vitro* action against *T. cruzi* has been found for it, as well as for imipramine (9, 10).

How the TCAs act to suppressive *T. cruzi* levels is not known. Their antichagasic effects and their properties when used as antidepressants in humans are not readily apparent. The primary hypothesis offered, known as the biogenic amine hypothesis, for the mechanism of action as antidepressants, was formulated in the mid-1960s (11). It is based on the theory that pathogenic depression is due to a deficiency of biogenic amines at postsynaptic sites in the brain. The TCAs are among drugs that have the ability to elevate synaptic concentrations of neurotransmitter amines, especially norepinephrine and serotonin, thus alleviating mental depression (12). Newer findings, however, have prompted a reassessment of this original hypothesis. This shifting of emphasis has been to examine the cellular site of action. How this might be related to the activity of TCAs against *T. cruzi* remains to be seen.

With respect to the antichagasic properties, the tricyclic antidepressants may be operating to effect lipidosis. It has been found that amphiphilic cationic compounds after gaining access to lysosomes form complexes with certain lipids within these minute cellular bodies (13). This intralysosomal entrapment results in an accumulation of lipids. Evidence for the direct interaction of the amphiphilic drugs with phospholipids has been seen (14). It has been suggested that this causes membrane destabilization (9, 10).

Table II. Compounds Found To Be Inactive against *Trypanosoma cruzi* in Mice

Compound name	WR no.[a]	
Antimalarial		
6 Artemisinin (qinghaosu)	249,309	
7 Clopidol	61,112	
8 Endochin	7,295	
9 Lapinone	26,041	
10 Menoctone	49,808	
11 Metachloridine	6,010	
12 Prodigiosin	25,187	
13 Quinacrine (atabrin)	1,543	
Antitrypanosomal		
14 Diminazine aceturate	27,800	
15 Ethidium	141,377	
16 2-Hydroxystilbamidine	30,457	
17 Pentamadine	4,931	
18 Phenamidine	25,977	
19 Stilbamidine	9,131	
Antischistosomal		
20 Astiban	7,035	
21 TAC pamoate	3,709	
22 Tartar emetic	6,980	
Antifilarial		
23 Cambendazole	102,866	
24 DEC (diethyl carbamizine)	7,744	
25 Flubendazole	242,630	
26 Thiabendazole	6,430	
Antibiotic		
27 Kanamycin	148,257	
28 Kanamycin A	35,913	
29 Minocycline	87,781	
30 Tetracycline	7,220	
31 Valinomycin	124,892	
Amino acid analog		
32 Difluoromethylornithine (DFMO)	247,222	
33 *O*-Methylthreonine	151,136	
Miscellaneous		
34 Acyclovir (zovirax:	255,797	anti-herpes
35 Amoscanate	234,927	anthelmintic antimicrobial
36 Amphotericin B	230,330	antifungal
37 AZT	255,708	anti-AIDS
38 Benzidazole	12,435	anti-adrenergic
39 Cyclosporin A	254,983	immunosuppressant
40 Cytimmune mouse interferon	254,534	immunoregulator
41 Glucantime	214,975	antileishmanial
42 Hetol	17,206	fasciolicide
43 Levamisole	194,184	anthelmintic
44 Litracene	254,734	antidepressant
45 Naloxone	250,642	narcotic antagonist
46 Parvaquone	68,226	antiprotozoal
47 Phenothiazine	16,191	anthelmintic
48 Promazine	37,579	tranquilizer
49 Trichlorfon (metrifonate)	42,640	anthelmintic

Note. Source of Compounds: 6, Starks Associates, Inc., 1280 Niagara Street, Buffalo, NY 14213; 7, 32 Hoechst Marion Roussel, 2110 E. Galbraith Road, Cincinnati, OH 45215-6300; 8 and 9, H. Koppel, Aldrich Chemical, 940 W. St. Paul Avenue, Milwaukee, WI 53233; 10 and 13, F. C. Nachod, Sterling-Winthrop Res. Inst., Rensselaer, NY 12144; 11, Robin O. Powell, Northwestern University, The McGaw Medical Center, Ward Memorial Building, 303 East Ohio St., Chicago, IL 60611; 12, A. J. Castro, Department of Chemistry, San Jose State University, San Jose, CA 95114; 14, 16, and 19, A. Markovac, Ash Stevens, 5861 John C. Lodge Freeway, Detroit, MI 48202; 15, CALBIOCHEM, 10933 N. Torrey Pines Road, La Jolla, CA 92037; 17, May & Baker, Dagenham Essex RMIO 7S, England; 18 and 21, E. F. Elslager, Pharmaceutical Research Division, Warner-Lambert, 2800 Plymouth Road, Ann Arbor, MI 48105; 20, G. Zbinden, Hoffman-Laroche, Nutley, NJ 07110; 22, GSA Customer Supply Center, 6810 Loisdale Road, Springfield, VA 22150-1910; 23 and 26, M. H. Fisher, Research Labs, Merck Sharpe & Dohme, Rahway, NJ 07065; 24, 30, and 45, Sigma, 3500 Dekalb St., St. Louis, MO 63118; 25, K. Schellekens, Janssen Pharmaceutica, 2340 Beerse, Belgium; 27, Mann Research Labs, 136 Liberty Street, New York, NY 10006; E. A. Steck, Division of Exp. Therap., Walter Reed Army Institute of Research, Washington, DC 20307; 29, E. H. Dearborn, Dome Labs., Division of Miles Labs, West Haven, CT 06516; 31, S. Kantor, Agricultural Division, American Cyanamid, P.O. Box 400, Princeton, NJ 08540; 33, General Biochemicals, Laboratory Park, Chagrin Falls, OH 44022; 34 and 37, Medical Department, Burroughs Wellcome, 1 Scarsdale Road, Tuckahoe, NY 10770; 35, Ciba-Giegy, Old Mill Road, Suffern, NY 10901; 36, S. J. Lucania, E. R. Squibb & Sons Inc., Squibb Inst. for Med. Research, P.O. Box 400, Princeton, NJ; 38, 42, 43, and 47, Aldrich Chemical, 2371 North 30th Street, Milwaukee, WI 53210; 39, A. Corletti, Biological & Medical Res. Div., Sandoz Pharma Ltd., Basle, Switzerland; 40, Lee Biomolecular Research Laboratories, 1211 Sorrento Valley Road, San Diego, CA; 41, Rhone-Poulenc, Centre De Recherches, De Vitry, France; 44, H. Lundback, Ottilia rej 7-9, DK 2500 Valby, Copenhagen, Denmark; 46, L. H. Schmidt, Department of Pharmacology, University of Alabama in Birmingham, University Station, Birmingham, AL 35294; 48, Park-Davis Pharmaceutical Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105; 49, G. Hanes, Chem. Coord. Unit, AEQI, U.S. Department of Agriculture, Building 1072, BARC-EAST, Beltsville, MD 20705. Salt form of compound: 6–12, 23, 25–27, 31, 33–37, 39, 40, 42, and 46–49, free base; 13, dihydrochloride dihydrate; 14, dimaleate; 15, bromide; 16, dihydrochloride hemihydrate; 17 and 18, diisethionate; 19, dimesylate; 20 sodium; 21, pamoate; 22, diantimonate dipotassium hemihydrate; 24, citrate; 28, dihydrogen sulfate; 29, 30, 38, and 43–45, hydrochloride; 32, hydrochloride hydrate; 41, antimonate.

[a] Number assigned by the Walter Reed Army Institute of Research.

In working with Leishmania, a member of the family Trypanosomidae like Trypanosoma, Zilberstein and Dwyer (15) made observations related to energy transduction processes after employing the TCA clomipramine. They suggest that the effects seen may be applicable to other parasitic protozoa. These investigators found evidence that clomipramine induces changes which would not allow *Leishmania donovani* and *Leishmania major* promastigotes and amastigotes to maintain pH homeostasis. Since it is known that this drug and imipramine uncouple oxidative phosphorylation in mammalian mitochondria (16), they reasoned that the drug might also disrupt H^+ transport across surface membranes of the Leishmania species with which they were working. This interference could lead to faulty transfer of certain solutes such as L-proline and D-glucose that could result in the death of the organism.

The "killing mechanism" for *T. cruzi,* initiated by some antichagasic compounds, perhaps the TCAs, may be similar to that found for a high-density lipoprotein of human serum which destroys the circulating trypomastigotes of the African species *Trypanosoma brucei brucei,* an animal parasite not pathogenic for humans (17, 18). The serum component is termed "trypanosome lytic factor" (TLF) and involves the trypanosome's lack of catalase expression. The latter, in turn, results in high intracellular concentrations of H_2O_2. This elevation of hydrogen peroxide makes the trypanosomes very susceptible to oxidative damage. The authors hypothesize that TLF binds with hemoglobin to form an active complex. After the latter is endocytosed by the trypanosome, the complex homes in on the lysosome (18). The low pH of the lysosome stimulates peroxidase activity of the complex which then reacts with H_2O_2 to cause lipid peroxidation of the lysosomal membrane. The disrupted lysosome then releases its enzymes and the trypanosome is digested from the inside.

Why protriptyline (Fig. 1) with its dibenzoheptene ring structure and terminal methylamino in the 5-position side chain was found to be nine times as effective as imipramine with its dibenzoazepine ring configuration and terminal dimethylamino group is not understood. Neither is it recognized why litracene (Compound 44, Table II), an antidepressant with a trihexyl ring structure, was not suppressive. Others employing *in vitro* means found it to be active (19, 20).

Hammond *et al.* (9) in considering various tricyclic ring structures found no obvious structure-activity relationship in the ability of the test compounds to lyse trypomastigote suspensions of *T. cruzi.* They utilized structures (i) having various substitutions on the tricyclic ring, (ii) with C, N, and O in the middle ring, and (iii) with groupings of different side chains. However, others have shown that addition of electron-withdrawing moieties such as Cl and NO_2 to the tricyclic ring of imipramine enhances biological activity (10).

Our finding that the antischistosomal agent niridazole (Compound 4, Table I) was suppressive but less so than

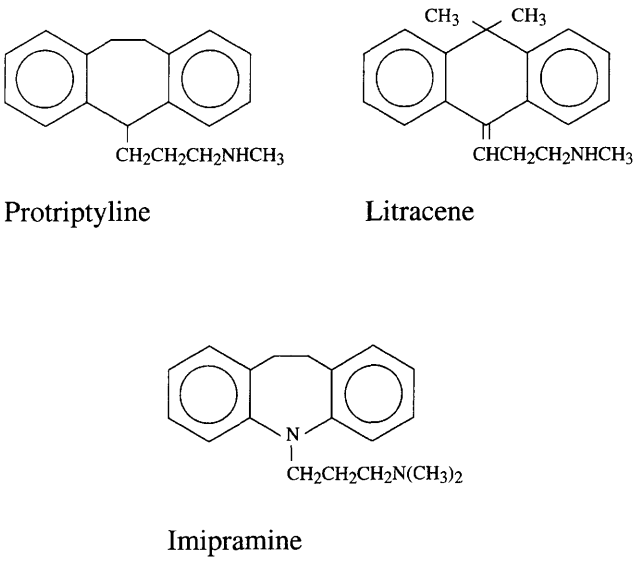

Figure 1. The structures of the TCAs protriptyline, litracene, and imipramine.

nifurtimox was in agreement with the *in vitro* findings of Gutteridge *et al.* (21). Our observation of antichagasic properties for the antibiotic nigericin (Compound 3, Table I) in our *in vivo* model is at variance with the report by Croft *et al.* (11) of inactivity in an *in vitro* system.

New drugs are sorely needed. The list of standard compounds given in Table II attests to the refractoriness of the organism. The activity of the tricyclic antidepressant drugs on *T. cruzi* is of particular interest because these compounds have been widely used without serious adverse side effects (8). Protriptyline with its suppressiveness three times as great as that for nifurtimox warrants further evaluation. Further, chemical modification of the tricyclic structure exemplified in Figure 1 may produce substances of even greater value in treating Chagas' disease.

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