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THE EFFECT OF DIAMINOALKYL-ANTHRAQUINONE DERIVATIVES ON THE GROWTH OF THE PROMASTIGOTES OF *LEISHMANIA TROPICA MINOR*, *L. T. MAJOR*, *L. DONOVANI* AND *L. AETHIOPICA*

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Abstract—By combining knowledge of polyamine biosynthesis and its inhibition by various analogues with that on the activity of synthetic anthraquinones, a series of six anthraquinone derivatives were synthesized. Their ability to inhibit the growth of leishmanial promastigotes *in vitro* was used as a preliminary screen to check their potential as new antileishmanial chemotherapeutics. They were tested against four strains, representing four different species; *Leishmania tropica major, L. tropica minor, L. aethiopica* and *L. donovani*, associated with four separate disease syndromes. All six derivatives exhibited a fair degree of antileishmanial activity, some being more effective than others. They all inactivated cultures at 100 μg/ml and some did so at 10 μg/ml and even 1 μg/ml; but taking different lengths of time to achieve this. Antileishmanial activity associated with anthraquinone derivatives might provide a new approach to the chemotherapy of leishmaniasis.

Steck [1], in reviewing the chemotherapy of leishmaniasis, states that it may be considered satisfactory in uncomplicated cases receiving prompt treatment; but stresses the considerable toxicity of the compounds that have been and are being used, i.e. antimonials, diamidines, berberine, amphotericin B, emitine, paromomycin and other antibiotics. It is clear that less toxic alternatives would be much preferred, and the search for new drugs must continue; especially as many cases seen are complicated, as in leishmaniasis recidivans [2], diffuse cutaneous leishmaniasis [3] and mucocutaneous leishmaniasis [4], and not easily cured with existing therapies.

Analysis of the molecular structure of various known antiparasitic drugs reveals that many are alkylamine derivatives of polycyclic compounds (Fig. 1). Others are acridine derivatives, e.g. quinacrine, which has been used in the treatment of cutaneous leishmaniasis, but was not very effective against mucocutaneous leishmaniasis [1].

Some anthraquinone derivatives have been synthesized and had their biological activity examined. Double and Brown [5] synthesized 2-amino-5-diethylaminopentane derivatives of anthraquinone and described their interaction with DNA. Amidine derivatives of anthraquinones have also been synthesized and their activity against *Entamoeba histolytica* demonstrated [6]. These, unlike metronidazole, are not mutagenic. More recently, bis (substituted amino-alkylamino) anthraquinones

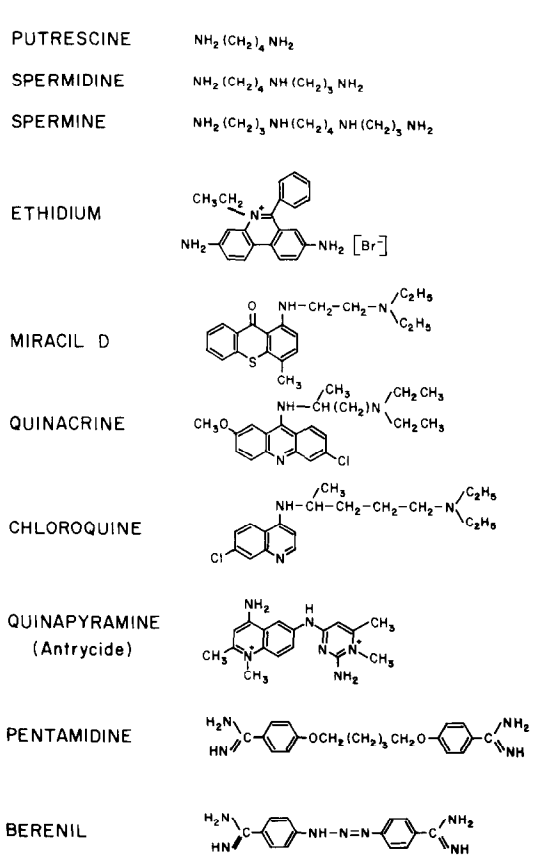

Fig. 1. The structure of polyamines and some antileishmanial drugs.

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have been synthesized [7, 8], at least one of which possessed antineoplastic activity against a leukemia and a melanoma system.

We have shown that leishmanial parasites produce polyamines, the levels of which fluctuate during the growth of both promastigotes [9, 10] and amastigotes [11]. Similar results were also reported by Morrow *et al.* [12]. Some antileishmanial compounds are polyamine analogues and act by competing with polyamines and impairing normal nuclear activity of the parasites [9, 11]. A similar analogy has also been reported for drugs which affect the growth of *Trypanosoma brucei* [13, 14].

In this study, we combined knowledge on polyamine biosynthesis and its inhibition by various analogues with that on the activity of synthetic anthraquinones, by synthesizing a series of anthraquinone derivatives and testing their ability to inhibit the growth of leishmanial promastigotes of various species, as a preliminary screen in the search for new antileishmanial chemotherapeutics.

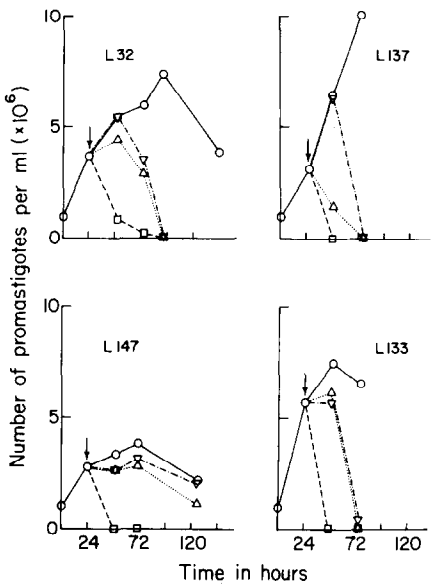


Fig. 2. The effect of different concentrations of 1,8-(di-3-aminopropyl-diamino)-anthraquinone·2HCl (compound IV) on the growth of the cultured promastigotes of *L. tropica minor* LRC-L32, *L. tropica major* LRC-L137, *L. aethiopica* LRC-L147 and *L. donovani* LRC-L133; ○---○ normal control, ▽·-·-·▽ 1 μg/ml, △···△ 10 μg/ml, □---□ 100 μg/ml. Arrow indicates the time of drug addition.

MATERIALS AND METHODS

Anthraquinone derivatives. Six derivatives were tested: (I) 1-(2-aminoethylamino)-anthraquinone·HCl; (II) 1-(3-aminopropylamino)-anthraquinone·HCl; (III) 1-(4-aminobutylamino)-8-chloroanthraquinone·HCl; (IV) 1,8-(di-3-aminopropyldiamino)-anthraquinone·2HCl; (V) 2-(2-aminoethylamino)-anthraquinone·HCl and (VI) 2-(3-aminopropylamino)-anthraquinone·HCl.

Their synthesis and general characteristics are described elsewhere [15, 16]. For use in experiments, these compounds were dissolved in sterile water, compound (III) being somewhat less soluble than the others. The anthraquinone solutions were adjusted to pH 6–7, using 1 M HCl and sterilized through millipore filters (pore size 0.45 μm).

Leishmanial parasites and their cultivation. The four leishmanial strains, representing four different Old World species associated with four separate disease syndromes, were obtained from the culture collection of the World Health Organization's International Reference Center for Leishmaniasis (WHO-LRC), housed in the Department of Medical Protozoology, the Hebrew University-Hadassah Medical School, Jerusalem. They are *Leishmania tropica major* LRC-L137 (oriental sore), *L. tropica minor* LRC-L32 (oriental sore and leishmaniasis recidivans), *L. aethiopica* LRC-L147 (oriental sore and diffuse cutaneous leishmaniasis) and *L. donovani* LRC-L133 (kala-azar). Their origins and intrinsic taxonomic characters have been described, as has their method of maintenance and culture [9, 10].

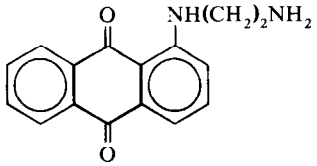
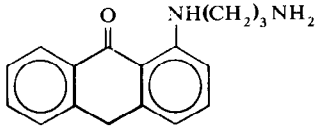
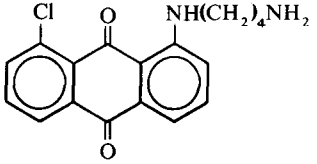
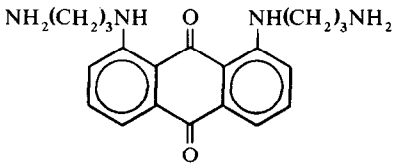
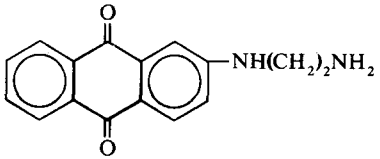
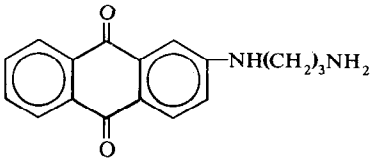
To test the effect of the anthraquinone derivatives on leishmanial growth, the compounds were added to fresh cultures of promastigotes grown in a Panmede (Paines and Byrne Ltd., Greenford, Middlesex) based medium containing 5% normal rabbit serum [9]. Such cultures, containing 10^6 promastigotes/ml at zero time, were permitted to undergo normal growth for 24 hr, were counted and soon after drugged, by adding the derivatives to give final concentrations of 100 and 10 μg/ml in all cases and 1 μg/ml in some cases. Daily number counts of living promastigotes were made using a hematocytometer. Experiments were done at least twice and each sample was counted in triplicates. Amastigotes were grown from promastigotes in cultured C_3H mouse macrophages as described [11].

RESULTS

Figure 2 shows that compound (IV), (1,8-(di-3 aminopropyldiamino)-anthraquinone·2HCl) was active against the promastigotes of all four strains, and 100 μg/ml inhibited the growth of the promastigotes and killed them in all cases. In the case of the *L. tropica major* strain LRC-L137, 10 μg/ml also caused total inhibition of growth and killing, and 1 μg/ml shortened the life span of promastigotes, although what seemed like normal growth occurred for almost 48 hr after administering the drug. Untreated cultures survived for much longer. A similar, but lesser effect was seen in the case of the *L. tropica minor* strain LRC-L32 and the *L. donavani* strain LRC-L133, where 10 μg/ml permitted some initial growth and where total killing of cultures took longer, in the case of the *L. tropica minor* strain. The least effect seen with this compound was on the *L. aethiopica* strain LRC-L147, where concentrations of 1 μg and 10 μg/ml had not killed cultures within the experimental period, but growth was suppressed.

The other anthraquinone derivatives also affected the growth of *Leishmania tropica major* LRC-L137. Table 1 shows that compound (II) was most active

Table 1. Effect of various anthraquinone derivatives on the growth of *Leishmania tropica major* LRC-L137

	Compounds	Average number of promastigotes/ml 1 μg/ml	10 μg/ml	100 μg/ml	Control
I			3.75×10^6	0	7.5×10^6
II			0.70×10^6	0	7.5×10^6
III			6.00×10^6	0	7.5×10^6
IV		7.87×10^6	3.37×10^6	1.3×10^6	7.5×10^6
V		10.8×10^6	2.5×10^6	0	11.4×10^6
VI		8.9×10^6	1.3×10^6	0	11.4×10^6

Promastigotes were grown in a liquid medium as described under Materials and Methods. Drugs were added after 24 hr and number of viable parasites was determined, in triplicate, after 48 hr or 24 hr (compounds V and VI).

and 10 μg/ml inactivated 90% of the promastigotes within 48 hr.

A similar inactivation (89%) was also observed when *L. tropica major* LRC-L137 was treated with compound (VI). This anthraquinone derivative is an isomer of compound (II), both have an aminopropylamino side chain which is substituted either at position 1 or position 2 of the anthraquinone molecule. It appears that a three-carbon diamine derivative is more active than the two- or four-carbon diamine analogues. These exhibited a lower antileishmanial activity (Table 1).

Substitution of two diaminoalkyl chains at positions 1 and 8 (compound (IV)) did not increase the antileishmanial activity.

It is also evident that chlorine at position 8 (III) hardly affected the antileishmanial activity. *L. donovani* LRC-L133 was slightly more sensitive to compounds (V) and (IV) and a total inactivation of the promastigotes was achieved by 10 μg (results not shown).

The fact that some of the anthraquinone derivatives inactivate promastigotes at a concentration of 10 μg/ml, is certainly interesting. The use of these compounds as possible antileishmanial drugs depends on their selective toxicity and their ability to kill the intracellular parasite, the amastigote, without affecting the host. When compound (II) was added to cultured glioma C_6BUI cells in tissue culture 90.8% of the cells tolerated a dose of 25 μg/ml. Cultured chick embryo fibroblasts also tolerated compound (II) and exposing the culture for 48 hr to 12.5 μg/ml only caused the death of 11% of the cells. Chick embryo fibroblasts transformed by Rous sarcoma virus showed a similar resistance to the drug. The effect of compound (IV) was also tested on the development of embryonated eggs. A normal development of the embryo was observed when 100 μg were injected into the allantoic fluid of a 10-day old embryonated egg.

Compound (II) was toxic for cultured C_3H mouse macrophages at 100 μg/ml. This drug at lower concentrations had no deleterious effect on the cultured cells. Intracellular *Leishmania tropica major* LRC-L137 amastigotes were affected by 10 μg/ml of compound (II) and their number declined by 70% within one day. A similar reduction in the number of viable intracellular amastigotes was observed when 5 μg/ml of compound (I) was added to the cultured macrophages. This dose of compound (I) was not toxic for the host.

DISCUSSION

The results recorded here show that the six anthraquinone derivatives synthesized and studied exhibit a fair degree of antileishmanial activity against the culture promastigotes of the four leishmanial species used. Preliminary studies also demonstrated that at least two compounds (Nos. I and II) caused a significant reduction in the number of viable intracellular amastigotes without affecting the host.

Of the six anthraquinone derivatives, compounds (II), (V) and (VI) were the most effective. It should be noted that diaminoalkyl derivatives substituted at position 2 of the anthraquinone molecule were more active than the 1-diaminoalkyl derivatives (Table 1).

Concerning the mode of action of these compounds, one can only speculate. On the one hand, they have a cyclic structure of the type shared by acridine derivatives, some of which are said to be active against certain types of leishmanial organism, e.g. quinacrine [1]. On the other hand, they carry alkylamino side-chains, e.g. compound (III) has a diamino side-chain that is identical to putrescine and compounds (I), (II) and (IV) contain aminopropyl side-chains like the polyamines spermidine and spermine (Fig. 1). Thus, these anthraquinone derivatives might act through their cyclic structure, or their polyamine side-chains, or both. We have recently observed (unpublished data) that the antiviral activity of compound (II) can be reduced significantly by adding either putrescine or spermidine to a suspension of T_2 coliphages. The anthraquinone derivatives, possibly act as polyamine analogues, competing with naturally occurring polyamines as intercalators between the DNA double helix, blocking and disrupting normal nuclear function and, thus, the growth and multiplication of the leishmanial organisms. Either way, these anthraquinone derivatives might, with further study, provide a new approach to the chemotherapy of leishmaniasis.

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