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COMMENTARY

BIOCHEMICAL AND PHARMACOLOGICAL EFFECTS IN RELATION TO THE MODE OF ACTION OF ANTISCHISTOSOMAL DRUGS

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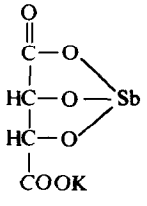
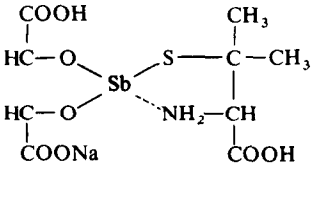
Schistosomiasis is a major human parasitic disease conservatively estimated to affect some 200 million people. As a result of water resources development its prevalence is increasing in most endemic areas and is a serious public health problem. The chemotherapy of schistosomiasis began in 1918 with the introduction of tartar emetic (antimony potassium tartrate) as a specific remedy. This drug and other antimonial compounds subsequently developed were troublesome to administer and severe side effects were frequent, so limiting their usefulness. During the last forty years much research effort has been expended in the search for new and better agents and in recent years a number of non-antimonial drugs have become commercially available.

In the strictest sense of the word, none of the antischistosomal drugs in current use are schistosomicidal *per se*. Their efficacy appears to stem from a common property in that they all induce a shift of worms from their preferred site in the body, either the mesenteric blood vessels or those of the vesical plexus, to organs such as the liver and lungs where they may become trapped and eventually killed through host tissue reactions. Although the precise mechanism of action of these drugs is difficult to define, the shift of worms appears ultimately to result from immobilisation of the suckers possessed by these parasites which causes them to lose their attachment to the venous walls and to be passively swept away. The variety of biochemical and pharmacological effects produced by antischistosomal drugs which may be related to their differing modes of action is the subject of this Commentary.

ANTIMONIAL COMPOUNDS



APT



NAP

The medicinal properties of antimony have been known since 4,000 B.C. and antimonials were commonly prescribed by Paracelsus, the celebrated 16th century physician. Since the introduction of antimony potassium tartrate (APT) as a specific remedy for schistosomiasis, a host of different antimony-containing compounds have been evaluated. Antimonial drugs are undoubtedly effective antischistosomal agents; however, the need for lengthy treatment and their high toxicity, assumed to be a consequence of inhibition of sulphydryl groups of cellular enzymes [1], has limited their use. The toxic effects of antimony can be partially antagonised by agents such as mercapto compounds. Thus co-administration of dimethylcysteine (penicillamine) was found to reduce the toxicity of APT in mice and hamsters [2, 3] without seriously affecting cure rate [4, 5]. The observations led to the development of new antimonial drugs such as NAP (A. H. Robbins Co.) a dimethylcysteine chelate of antimony sodium tartrate, which is better tolerated than APT and which is clinically effective [6, 7].

Probably the best documented effect of antimonials on schistosomes is their effect on carbohydrate metabolism. In schistosoma, glycolysis is the major pathway for the provision of metabolic energy [8]. Bueding [9] reported that fouadin (antimony pyrocatechin disulphonate) inhibited glycolysis in *Schistosoma mansoni*, an affect subsequently shown to result from inhibition of phosphofructokinase (PFK) [8], the enzyme controlling the rate of glycolysis in this parasite [10].

A high rate of glycolysis appears to be essential to continuous attachment of the worms to the venous walls. Buttle and Khayyal [11] reported that the loss of attachment and a passive shift of worms to the liver from the mesenteric blood vessels occurs within 2 hours following injection of APT to infected mice. At this time an accumulation of both glucose-6-phosphate and fructose-6-phosphate and a decrease in fructose-1,6-diphosphate are demonstrable in the worms, indicative of PFK inhibition [12]. Maintenance of worms in the liver is dependent upon continued administration of the antimonial as inhibition of PFK is reversible and migration back to the mesenteric veins occurs after a few days when PFK activity and hexosephosphate levels have returned to normal. There is thus a likely causal link between inhibition of PFK and the hepatic shift induced by antimonials.

The inhibitory action of antimonials on PFK is remarkably selective, the schistosomal enzyme being about 80 times more sensitive than mammalian PFK [12]. Whereas schistosomal PFK is activated markedly by thiols such as cysteine, glutathione, penicillamine and mercaptoethanol, the inhibitory effect of antimonials is not reduced by such compounds [13]. Hence it

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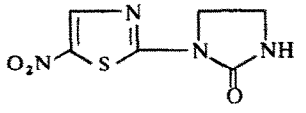
appears that sulphydryl group inactivation is not the mechanism of PFK inhibition and offers an explanation for the selective protection afforded by drugs such as NAP.

Accumulation of antimony is greater in female worms than in the males [14–16] and astiban (sodium dimercaptosuccinate) is reported to have a toxic effect on the vitelline cells of *Schistosoma mansoni* females [17]. The cytotoxic changes show some selectivity of drug action. Cells at a stage of granular endoplasmic reticulum development are rapidly destroyed. More mature cells are less affected, whilst those in the first stages of development are unaffected and continue to develop when the drug is withdrawn.

It has been reported that APT reversibly inhibits DNA, RNA and protein synthesis in *Escherichia coli* [18]. It is not known whether these inhibitory effects occur in schistosoma but they might account for the toxic changes seen in vitelline cells actively engaged in ribosome synthesis.

NIRIDAZOLE



The antischistosomal properties of niridazole (1-(5-nitrothiazolyl)-2-imidazolidinone, Ambilhar, Ciba Ltd.) were first reported by Lambert *et al.* [19] and since then it has been widely used. Although it displays activity against all three major species responsible for schistosomiasis in man it is most effective against *S. haematobium* while the lowest cure rates occur in cases of infection with *S. mansoni* [20].

Niridazole is a relatively slowly acting drug. In contrast to treatment with antimonials an hepatic shift of worms does not begin to occur until 72 hours following oral administration of chemotherapeutically effective doses to mice infected with *S. mansoni* [13]. An earlier indication of drug action is seen in its effect on the glycogen stores of male worms.

In the male worm, stored glycogen accounts for more than 25 per cent of its dry weight, whereas in the female worm values of about 5 per cent of dry weight are found [21]. Daily administration of niridazole brings about a progressive dose related reduction in the level of glycogen. Following a single high dose (200 mg/kg) glycogen is significantly reduced within 18 hours, and 24 hours after three such doses given at daily intervals the glycogen levels fall to approximately 50 per cent of that present in untreated controls [10, 22].

Glycogen is apparently lost from the musculature of the worms, but there is little if any loss from the dorsal tubercules, tegumental structures which are also known to contain glycogen in large amounts. This contrasts with the effects of *p*-rosaniline, a compound which is effective against experimental *S. mansoni* infections in mice and monkeys [23] and which causes a progressive depletion of glycogen from the tubercules [24].

Loss of glycogen appears to result from potentiation of the activity of schistosomal glycogen phosphorylase brought about through inhibition of phosphorylase phosphatase, the enzyme which catalyses the conversion of phosphorylase to an inactive form [22]. This action of niridazole is not entirely selective. Although it has no inhibitory activity against the phosphorylase-inactivating enzymes in the liver, brain and heart of the mouse it inhibits phosphorylase phosphatase of mouse skeletal muscle though to a lesser degree than in the parasite. It also causes a marked depletion of skeletal muscle glycogen in the rhesus monkey [25]. It is interesting to note that another antischistosomal nitroheterocyclic compound *trans*-5-amino-3(2-(5-nitro-2-furyl)-vinyl-1,2,4-oxadiazole, a nitrovinylfuran derivative (SQ 18506) with similar effects against worm phosphorylase phosphatase, showed no toxicity towards the host muscle enzyme [26].

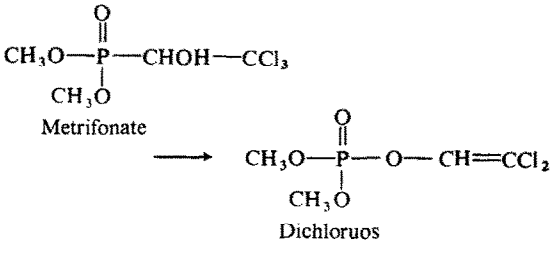
Another effect of niridazole is damage to the female reproductive system [27] which follows a time course similar to that of phosphorylase inactivation and this may be a more important effect as niridazole appears to be more lethal in the female worm [28].

The significance of glycogen loss with regard to the antischistosomal properties of niridazole or indeed of other antischistosomal agents which also cause glycogen loss (e.g. *p*-rosaniline, hycanthone, oxamniquine) is not clear. Bueding and Fisher [22] have suggested that glycogen, besides providing a store of utilisable glucose for energy provision, may fulfil a role in maintaining the structural and functional integrity of the parasite.

The precise identity of the enzyme inhibiting substance is unknown. Faigle and Keberle [29] considered that the antischistosomal effects of nitridazole were due to the unchanged drug as the parasites are unable to take up metabolites [30]. However, 24 hours after a single dose these workers could detect only metabolites of niridazole in the tissues of both parasite and host, yet inhibition of phosphorylase phosphatase increased progressively for at least 72 hours under these conditions.

Many nitro heterocyclic compounds e.g. nitrothiazoles, nitroimidazoles and nitrofurans possess antibacterial and antiprotozoal properties and it has been assumed that the nitro group is essential for activity. It is interesting to note that in each of these structural types there is an electron withdrawing atom adjacent to the nitro group. This would serve to lower the electron density in the nitro group and facilitate its reduction to a highly reactive and potentially toxic hydroxylamino species. Possibly this may be the basis for the antischosomal effects of niridazole.

METRIFONATE



Metrifonate, dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate, has been commercially available for many years as a recommended pesticide (Trichlorfon; Dipterex) for use against household pests [31] and also as an ectoparasiticide for veterinary use [32]. Since

Beheyt *et al.* [33] provided evidence of its low toxicity in man it has been used as an antischistosomal agent.

It is effective against infections with *S. haematobium* [34–36] but displays poor activity against *S. mansoni* [37, 38]. There appear to be no reports of its effect on *S. japonicum* infections in humans but it is reported to have little effect against experimental infections in hamsters [39].

The ability of metrifonate and many other organophosphorous compounds to inhibit acetylcholinesterase enzymes in various species has long been recognised. Schistosomes possess acetylcholinesterase [40] and in these parasites acetylcholine appears to function as an inhibitory neurotransmitter [41]; consequently inhibitors of acetylcholinesterase have a paralysing effect. Bueding *et al.* [42, 12] studied the activity of acetylcholinesterase in male and female *S. mansoni* and *S. haematobium* previously exposed to varying concentrations of metrifonate. The concentration of metrifonate required to produce 50 per cent inhibition of enzyme activity (I_{50}) was found to be identical for both species (4×10^{-6}M). There was also no sex difference in susceptibility. These results are incompatible with the strikingly different therapeutic results obtained in infections with these two species.

James *et al.* [43] studied the susceptibility to metrifonate of *S. mansoni* and *S. haematobium* in the hamster. As in man the drug was more active against *S. haematobium*, and also the female worms of both species appeared to be more susceptible than the males.

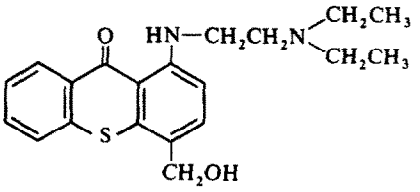
Both species shifted from the mesenteric veins as a result of treatment. *S. mansoni*, which occupies the inferior mesenteric veins, was shifted to the liver, whereas a high proportion of *S. haematobium*, which is distributed mainly in the posterior inferior mesenteric veins, was swept into the lungs. When the effects of the drug wore off there was a reverse migration of worms from the liver to the mesentery. The lung shift of worms appeared however, to be irreversible, as worms shifted to the small arterioles of the lung are readily trapped, become encased and die.

It is suggested that the lung shift of *S. haematobium* results from pathological anastomoses between the posterior inferior mesenteric veins and the inferior *vena cava*, as this species deposits the greater proportion of its eggs at this site with marked blockage of blood vessels [44]. In man, *S. haematobium* occupies the vesical blood vessels from where it would also shift to the lungs as a result of treatment.

Opinion is divided as to whether metrifonate itself is the proximal enzyme inhibitor. It has been reported that metrifonate is devoid of anticholinesterase activity but that at neutral or alkaline pH it undergoes a rapid and spontaneous rearrangement to form dichlorvos [45], a potent inhibitor of acetylcholinesterase. These authors believe that there is little doubt that metrifonate is converted non-enzymatically to dichlorvos *in vivo* under physiological conditions of pH and that dichlorvos is the agent responsible for phosphorylation of the active site of acetylcholinesterase.

This idea gains some support from the results of Bueding *et al.* [42] who found that dichlorvos administered to infected hamsters caused a greater degree of inhibition in *S. haematobium* than in *S. mansoni*. In addition, when worms were incubated with dichlorvos *in vitro*, inhibition of acetylcholinesterase of female *S. haematobium* was significantly greater than for both sexes of *S. mansoni* and male *S. haematobium*. This is in accord with the observed greater sensitivity of female worms to metrifonate *in vivo*.

HYCANTHONE



Hycanthone 1-2(2-(diethylamino) ethylamino)-4-hydroxymethylthioxanthen-9-one (Etrenol, Sterling Winthrop) is a potent antischistosomal agent effective against *S. mansoni* and *S. haematobium* but with negligible activity against *S. japonicum* [19]. Rosi *et al.* [46–48] showed that hycanthone is a hydroxy metabolite of lucanthone (Miracil D) an antischistosomal drug available since the late 1940's but of limited value and with undesirable side effects. Hycanthone was found to be considerably more potent than its parent drug [49] and since its introduction more than a million patients have received it.

Like niridazole, hycanthone is a slowly acting drug. Although Yarinsky *et al.* [50] reported that a major shift of worms from the mesenteric veins to the hepatic sinuses occurred within 24 hours after a single i.m. injection of 80 mg/kg to infected mice, their results have not been confirmed by other workers. Other investigators [51, 52] found no pronounced shift of worms until 5 days after treatment and a complete shift of worms does not occur until 9 or 10 days treatment [53].

In contrast to niridazole, hycathone is more active against male worms [28, 54] though female worms appear able to concentrate drug-related material to a greater extent than the males [50]. It is not known for certain whether it is unchanged hycanthone that is ultimately responsible for the antischistosomal action of hycanthone or a metabolite. Yarinsky *et al.* [50] found that after exposure to tritiated hycanthone, male worms contained only unchanged drug. However, they gave no details of their methodology and did not examine the worms at any time points later than 24 hours after initial exposure.

The reported effects of hycanthone on schistosomes are several. One such effect is interference with normal neuromuscular transmission. Hycanthone, at low concentrations (10^{-5}–10^{-6}M) stimulates the motor activity of *S. mansoni* and partially blocks the paralytic effects of carbachol (cholinomimetic) and physotigmine (AChE inhibitor) [55] responses which are also induced by atropine. Hycanthone is also an inhibitor of acetylcholinesterase in *S. mansoni* and at higher concentrations (10^{-3}–10^{-4}M) it depresses rather than stimulates motor activity [56]. Thus hycanthone appears to have an affinity for schistosomal cholinergic systems.

Further support for this idea has been gained by the use of a fluorescent compound (DNS-Chol) resembling acetylcholine which binds to ACh receptors permitting their visualisation by fluorescence microscopy [57].

Prior treatment of worms with a range of concentrations of hycanthone (10^{-5}–10^{-7}M) or with atropine caused a graded reduction in fluorescence when the worms were afterwards exposed to DNS-Chol. These observations suggest that hycanthone and DNS-Chol may compete for the same binding site on the acetylcholine receptors. Interestingly, Hillman *et al.* [58] found that the blocking effect of hycanthone on DNS-Chol uptake was much stronger in *S. mansoni* than in *S. japonicum*, a fact which correlates with the clinical usefulness of the drug in infections with these species.

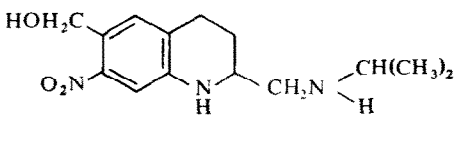
There is evidence that suggests that serotonin (5-HT) functions as an excitatory neurotransmitter in schistosoma [4, 59–61]. Thus hycanthone stimulation of motor activity might result from a direct serotoninergic effect rather than by an anticholinergic mechanism. Chou *et al.* [62] reported that hycanthone treatment of infected mice stimulates the uptake of 5-HT by *S. mansoni* causing an accumulation which becomes evident at the time of an hepatic shift. This effect was not seen in hycanthone-resistant worms. Other workers [5] have been unable to confirm enhanced uptake of 5-HT. Perhaps of greater significance is the additional finding of Chou *et al.* that the neurones of hycanthone treated worms lose their ability to store 5-HT and in these worms the amine is located only in extraneuronal sites.

Hycanthone like niridazole increases glycogenolysis. Rogers and Bueding [63] found that 1–2 hr after a single i.m. dose of hycanthone to infected animals the glycogen content of recovered worms was reduced by up to 25 per cent, but later recovered to control levels. This may result from an increased demand for energy due to cholinergic blockade, as the amount of drug in the worms is close to maximal at this time [50].

Tomosky-Sykes and Bueding [64] consider that the anticholinergic and anti-serotonin effects of hycanthone are unrelated to the mode of action for one or more of the following reasons: (a) the effect is only seen after and not prior to the hepatic shift; (b) they are not demonstrable with antischistosomal structural analogues of hycanthone; or (c) the same effects are found in hycanthone-resistant worms.

Several morphological changes in hycanthone-treated worms have been reported. One is a deterioration of the tegument which occurs in *S. mansoni* but not in *S. japonicum* [58]. Other changes include loss of body weight and derangement of the vitellaria [52]. The mechanism of these morphological effects is as yet unknown.

OXAMNIQUINE



Oxamniquine (6-hydroxymethyl-7-nitro-2-isopropylaminomethyl,1,2,3,4-tetrahydroquinoline, Mansil, Pfizer Ltd.) is a cyclic analogue of Mirasan [65] and structurally related to hycanthone. The common structural feature necessary for biological activity in compounds of this type is an alkylaminoethylamino group para to a hydroxymethyl group on an aromatic ring [66]. Despite structural similarity to hycanthone there is a notable contrast in the antischistosomal properties of these two drugs. A single intramuscular or oral dose of oxamniquine is very effective against *S. mansoni* infections [67–70] but has little effect against *S. haematobium* [71, 72].

In man, in contrast to *S. mansoni* which prefers the mesenteric veins, *S. haematobium* occupies the vesicular blood vessels, which could result in exposure to lower concentrations of orally administered drug due to first pass metabolism in the liver. This difference in distribution cannot explain the difference in susceptibility of the two species, as oxamniquine is inactive when given intramuscularly when first pass effects would not apply. Other possible reasons for the species difference may be differences in the uptake and/or the subsequent elimination of drug or to specific differences in protein receptors.

Oxamniquine, like hycanthone, is a slowly acting drug and is more active against male worms [73]. *In vitro* there is no significant sex difference either in susceptibility to or in uptake of oxamniquine. However the amount of drug related material found in male worms recovered from [^{14}C]oxamniquine-treated mice was four times greater than in females paralleling their observed greater sensitivity [74]. The amount of unmetabolised drug in male worms was only one and a half times greater than that present in females, the major proportion of the material in both sexes being metabolites. It thus appears that the antischistosomal effects of oxamniquine may be due to a metabolite(s). Oxamniquine metabolites can be taken up by both sexes [74] but it is not known whether either sex has itself the ability to metabolise the drug.

Like hycanthone, oxamniquine stimulates motor activity *in vitro*. At concentrations of 10^{-4}–10^{-5}M the motor activity of both male and female *S. mansoni* and *S. haematobium* is increased but higher concentrations (10^{-3}M) caused paralysis [75]. The onset of these responses is rapid. Worms incubated for two hours with [^{14}C]oxamniquine, when analysed, contained only unchanged drug [74] so these effects must be due to oxaminiquine itself. If the antischistosomal activity of oxamniquine is due to a metabolite then this finding gives support to the proposal that the anticholinergic effects of the Mirasan-related compounds are unrelated to their mode of action.

CONCLUSION

Whilst real advances in the chemotherapy of schistosomiasis have been made, none of the drugs presently available satisfies the criteria for an ideal schistosomicide, and their precise modes of action remain obscure. The discovery of new antischistosomal agents has resulted from structural modifications of existing chemical types displaying such activity or from the random screening of large numbers of novel compounds synthesised for a host of different reasons. The success rate of these approaches is low and the development costs are high and it is a sad fact that many of the major pharmaceutical companies have ceased to be active in this important area of research.

What is urgently needed is a better knowledge of the biochemistry of schistosomes and of their pharmacological responses. In particular, we need to know how

these processes differ from those in human tissues. Armed with this knowledge suitable targets for our "magic bullets" can be identified and the search for the design of new drugs can then become more rationally based. At the present time a serious decline in the profitability of the pharmaceutical industry has placed many companies in a financial strait-jacket. They are thus unable to involve themselves in fundamental research of this nature. The initiative must come from researchers in the universities and research institutes.

The WHO Scientific Working Group on Schistosomiasis [76] have recently declared that chemotherapy will play an increasing role in control of the disease. There is thus a clear challenge to researchers in comparative biochemistry and pharmacology to provide promising leads for the pharmaceutical companies which might encourage them to re-enter the field. It is to be hoped that both WHO and responsible governments of countries where the disease is endemic will not be slow in offering necessary material and logistic support.

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