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Inhibition of in vitro RNA synthesis by hycanthone, oxamniquine and praziquantel

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Summary. The schistosomicides, hycanthone, oxamniquine and praziquantel, were found to inhibit the in vitro RNA synthesis using isolated hamster liver nuclei. Preincubation of the nuclei with these drugs revealed that the inhibitory effect of oxamniquine was irreversible and progressed with time, whereas that of hycanthone and parziquantel was reversible. On the other hand, hycanthone and praziquantel have a high affinity for DNA but oxamniquine does not. The data indicate that the mechanism of inhibition by oxamniquine is different from that of hycanthone and praziquantel.
Key words. Schistosomicides; hycanthone; oxamniquine; praziquantel; RNA synthesis; drug resistance.

Hycanthone, oxamniquine and praziquantel have been used as therapeutic drugs against schistosomiasis, although the use of hycanthone has recently been discontinued[1,2]. The rapid absorption and distribution of the drugs throughout all tissues contributed to the extremely fast onset of drug action against the three important strains of human schistosomes, *S. hematobium*, *S. mansoni* and *S. japonicum*[3,4]. A single intramuscular or oral dose is generally effective[2]. However, continued usage of hycanthone and oxamniquine has been found to induce drug resistance in *S. mansoni*[5,6].

Despite the routine use of these drugs for clinical treatment, none of them are without side effects. Symptoms like coughing, headaches, vomiting, nausea, anorexia, gastrointestinal disturbances, skin rashes, arthralgia and myalgia are common[2]. Hycanthone, for example, is a hepatotoxic drug which causes severe hepatic injury[1]. In addition to these toxic side effects, the antischistosomal agents are also carcinogenic and mutagenic[7,8]. The mutagenicity of hycanthone was detected in Salmonella, Drosophila, *E. coli* T4 bacteriophage and various cell cultures[9–11]. Oxamniquine, on the other hand, produced a weak response in the frame-shift mutant TA1538 of *Salmonella typhimurium*[11].

The mechanism of action of hycanthone against *S. mansoni* has been investigated. Mattocci et al.[12,13] reported that hycanthone inhibited the macromolecular synthesis of *S. mansoni*, both in vitro and in vivo. We[14] have found enzymatic differences between hycanthone resistant and sensitive strains of *S. mansoni*, suggesting the important effect of hycanthone in altering gene expression. In this paper we report the inhibition of hycanthone on RNA synthesis in a mammalian system and compare its effects to that of oxamniquine and praziquantel.

Materials and methods

Materials. Calf thymus DNA, CTP, GTP, UTP, ATP, dithiothreitol and sucrose were purchased from Sigma Chemical Co. [5,6-^{3}H]UTP (36 Ci/mmole) was from ICN Chemical Co. Hycanthone was generous gift of Dr. Ernest Bueding, Johns Hopkins University. Oxamniquine was obtained from Pfizer Pharmaceuticals Company and praziquantel from Miles Pharmaceuticals. Hamster liver nuclei were isolated according to the modified procedure of Ernest et al.[15]. All other chemicals used were of reagent grade.

In vitro RNA synthesis. The procedure used for in vitro RNA synthesis was that of Reeder and Roeder[16] and Marzluff et al.[17]. The reaction mixture contained 25 mM tris-chloride, pH 7.6, 0.25 M sucrose, 0.25 mM calcium acetate, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.05 mM EDTA, 2.5 mM dithiothreitol, 0.15 M KCl, 0.4 mM of each of ATP, CTP and GTP, 0.05 mM [5,6-^{3}H]UTP and about 10^7 isolated liver nuclei. After incubation at ambient temperature for 30 min, the reaction was stopped by adding equal volume of 20% trichloroacetic acid. An

aliquot was spotted onto a piece of Whatman No. 3 filter paper and washed with 5% cold trichloroacetic acid followed by 5 ml acetone. After drying, the paper was measured for its radioactivity content in a Beckman LS 1801 scintillation counter.

The effect of drugs on RNA synthesis was investigated by including the drug in the incubation mixture described above. The drugs were initially dissolved in either ethanol or dioxane and aliquots were added to the reaction mixture. Control experiments showed that the presence of diluted ethanol or dioxane under our assay conditions had no effect on the RNA synthesis.

Flow dialysis. The drug-DNA interaction was measured by the technique of flow dialysis described by Klapper[18]. Calf thymus DNA in 0.1 M tris-chloride buffer, pH 7.6, was placed on one side of a dialysis membrane and the buffer was pumped over the other side at a flow rate of 40 ml/h. Drug was added to the DNA solution and its absorbance in the buffer was monitored by a Perkin-Elmer Hitachi 200 spectrophotometer. The flow dialysis measurements were analyzed by the Scatchard plot.

Results

Inhibition of RNA synthesis. The effect of the schistosomicides on the RNA synthesis machinery in isolated hamster liver nuclei is shown in figure 1. Hycanthone seems to be the most potent inhibitor of the three drugs, followed by praziquantel and then oxamniquine. Only about 1.7 mM of hycanthone was sufficient to reduce 50% of the RNA synthesis whereas 2.7 mM of praziquantel was required to attain the same effect. For oxamniquine, even higher concentrations might be necessary.

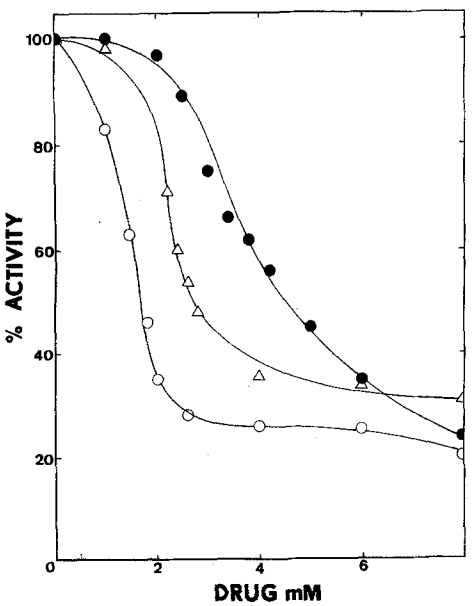
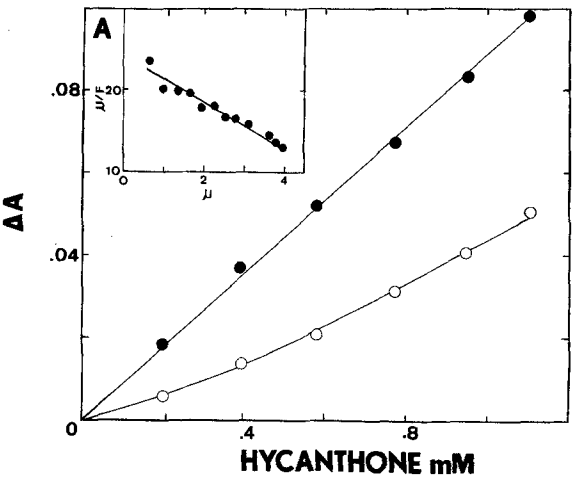
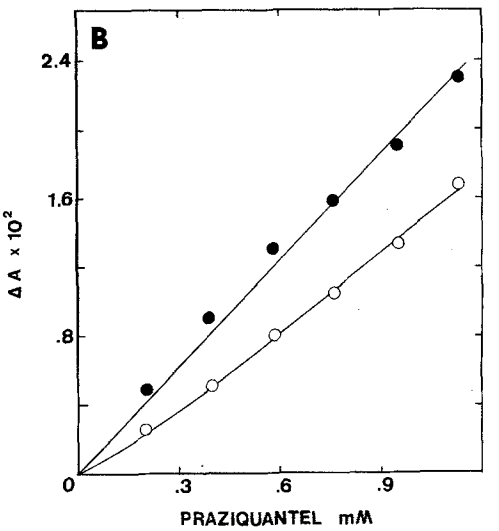


Figure 1. Inhibition of in vitro RNA synthesis. The isolated hamster liver nuclei were assayed for their ability to incorporate uridine into macromolecules in the presence of: hycanthone, ○——○; oxamniquine, ●——●; praziquantel, △——△.





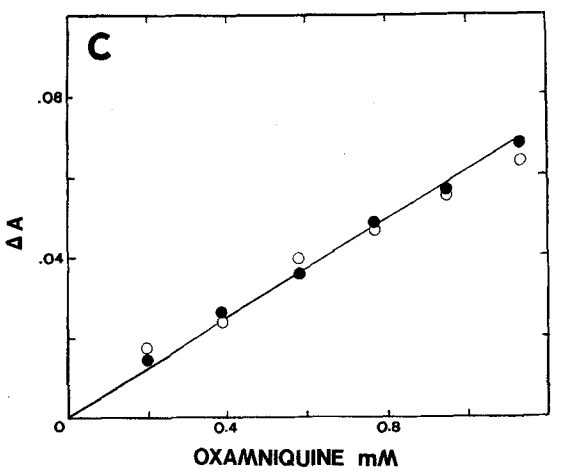


Figure 2. Flow dialysis analysis of drug-DNA interaction. The drugs used are: *A* hycanthone; *B* praziquantel; and *C* oxamniquine. Closed circles (●) indicate the absence of DNA; open circles (○) indicate the presence of 0.1 mg/ml DNA. Insert: Scatchard plot of the flow dialysis data, μ is the ratio of μmoles of drug bound per mg of DNA, and F is unbound drug concentration.

Effect of preincubation of hamster liver nuclei with schistosomicides on RNA synthesis. The nuclei were incubated with 5 mM of the drug at ambient temperature. At the indicated time intervals an aliquot was removed and assayed for its ability to incorporate radioactive uridine into macromolecules, that at zero time was taken as 100%. The final concentration of the drug in the assay mixture is 0.5 mM.

Drug	Incubation time (min)	% UTP incorporation
Hycanthone	0	100
	10	122
	20	90
	30	105
Oxamniquine	0	100
	10	76
	20	58
	30	55
Praziquantel	0	100
	10	97
	20	100
	30	106

Both hycanthone and praziquantel failed to completely inhibit the in vitro RNA synthesis. There was about 20–30% of residual activity even at 8 mM concentrations. This may indicate that the possible effects of these drugs is on elongation rather than on initiation such that there is always a certain amount of incorporation of radioactive UTP. The case of oxamniquine is different. There is a progressive inhibition as the concentration is increased. This is probably due to the irreversible effect of the drug as shown below.

In order to investigate if the inhibitory effect of the drugs is reversible, the nuclei were preincubated with 5 mM of these drugs for various durations. RNA synthesis was then measured after 10-fold dilution. If the drug inhibition is an irreversible process, dilution will not have any effect on the inhibition. As it is shown in the table, preincubation of the nuclei with hycanthone and praziquantel had no effect on RNA synthesis, indicating that the inhibition at higher drug concentrations is reversible. With oxamniquine, however, the RNA synthesis was decreased with increasing time of preincubation, suggesting that the effect of oxamniquine at higher concentrations is irreversible on dilution of the drug.

Drug-DNA interaction. The ability of these drugs to bind to DNA was studied by the flow dialysis method. As shown in figure 2, hycanthone has the highest affinity for DNA with a dissociation constant of 0.37 mM compared with a value of 1.6 mM for praziquantel. Oxamniquine, on the other hand, does not seem to bind at all.

Discussion

As shown in figure 1, the presence of any of the three antischistosomal agents, hycanthone, oxamniquine and praziquantel, decreases the in vitro RNA synthesis of isolated hamster liver nuclei. Hycanthone is the most efficient inhibitor followed by praziquantel and then oxamniquine. This trend seems to follow the degree of binding of the drugs to DNA. Hycanthone has the highest affinity for DNA followed by praziquantel, whereas oxamniquine does not bind at all (fig. 2). Although it is not clear whether the basic mechanism of inhibition of RNA synthesis is due to the interaction of these drugs with DNA, the correspondence of inhibitory potency and DNA affinity is remarkable.

Our finding is consistent with the observation that hycanthone is the most potent mutagenic and hepatotoxic agent of the three drugs investigated[2,7]. Mattoccia et al.[12,13] have reported the inhibition of hycanthone on the macromolecular synthesis in *S. mansoni*. Our present study reveals that similar effect may occur in the mammalian system. Thus the side reactions of the schistosomicides may have a common basis. The ability of hycanthone to intercalate into DNA was reported by Hirschberg and Weinstein[19] and is confirmed by our present study. Such a DNA-drug interaction has been speculated to play a role in the mutagenicity and toxicity of the drug[20]. The affinity of praziquantel for DNA is much lower and it is found to be less toxic[2]. Oxamniquine has been reported not to affect the melting temperature of calf thymus DNA[21] consistent with our failure to detect any association of the drug and DNA (fig. 2C). This may partly explain its very weak mutagenicity[11].

The table reveals that the inhibitory action of oxamniquine on RNA synthesis is mediated by a mechanism different from that of hycanthone and praziquantel. The irreversible nature of oxamniquine inhibition on the RNA synthesis suggests its extreme tight binding to or covalent modification of some component of the RNA synthesis machinery. This speculation is in agreement with the suggestion that oxamniquine forms a DNA-drug adduct on metabolic activation[21]. Whether such mode of action is really operative in our in vitro studies is not known.

The therapeutic range of schistosomicides is generally in the order of μg/ml. A concentration of 1 μg/ml of praziquantel, for example, is lethal to adult *S. mansoni*[22]. In this study, we have found that the inhibition of RNA synthesis in isolated hamster nuclei requires mM concentrations of the drugs. This suggests that the schistosomicidal activity of these drugs is not due solely to inhibition of RNA synthesis and may be multifactorial. For instance, exposure of schistosomes to praziquantel causes immediate tetanic muscle contraction[23]. It is also possible that in vivo metabolism of the drugs may transform them into more potent metabolites. The original compounds may be much more inferior in their binding to DNA and nuclear membrane permeability. Nevertheless, our findings strongly suggest that the mode of action of oxamniquine is different from that of hycanthone and praziquantel, at least in the in vitro RNA synthesis.

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Glycerylphosphorylarsenocholine and phosphatidylarsenocholine in yelloweye mullet (*Aldrichetta forsteri*) following oral administration of arsenocholine

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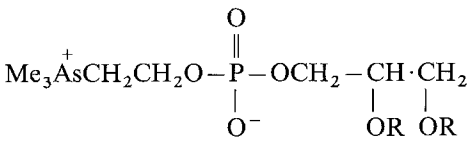
Summary. The novel arsenical glycerylphosphorylarsenocholine has been isolated from yelloweye mullet (*Aldrichetta forsteri*) following oral administration of arsenocholine. Ether-soluble arsenic was shown to be present as phosphatidylarsenocholine.
Key words. Glycerylphosphorylarsenocholine; phosphatidylarsenocholine; arsenocholine; arsenobetaine.

Although arsenobetaine (**1**) is recognised as the major form of arsenic in marine animals, the biosynthetic pathway for this compound is not fully understood[1]. Evidence suggests that arsenobetaine is accumulated by marine animals via the food chain rather than directly from seawater[2]. Arsenic is present in marine macroalgae, at levels comparable to those found in marine animals, primarily as dimethylarsinylribosides, and these algal arsenic compounds are a possible source of arsenobetaine[3]. Arsenocholine (**2**) is a likely intermediate in the conversion of dimethylarsinylribosides to arsenobetaine[4].

(1)

(2)

In a recent study[5] on the accumulation of arsenic compounds by the yelloweye mullet (*Aldrichetta forsteri*) we showed that fish readily metabolised administered arsenocholine to arsenobetaine. Other metabolites of arsenocholine were a weakly basic water-soluble compound and some ether-soluble arsenic, but the small quantities precluded further examination. Additional experiments have now provided larger quantities of these two arsenic fractions and we here report the isolation and identification of glycerylphosphorylarsenocholine (**3**) from yelloweye mullet fed arsenocholine. Ether-soluble arsenic (lipid arsenic) was shown to be present as phosphatidylarsenocholine (**4**) by the isolation and identification of compound 3 following alkaline hydrolysis of the lipids.



(3) R = H
(4) R = $CO(CH_2)_nCH_3$

Juvenile yelloweye mullet were fed meat (beef) dosed with arsenocholine as previously described[5]. Yelloweye mullet were used as experimental fish because of their ease of maintenance in aquaria. Furthermore, the small amount of native arsenic in juvenile yelloweye mullet was insignificant relative to the quantity accumulated during experimentation. Arsenocholine bromide (163 mg ≡ 50