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THE EFFECT OF INDOMETHACIN ON LIPID METABOLISM OF *SCHISTOSOMA MANSONI*

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Abstract—Incubation of adult *Schistosoma mansoni* with 10^{-4}M indomethacin induced instant and general paralysis of the parasites. This effect of indomethacin was not associated with the inhibition of alkaline phosphatase, ATPase, acetylcholinesterase or non-specific esterases. However, indomethacin was able to activate the schistosome phospholipase and/or lipases, and simultaneously inhibit the synthesis of triglycerides. It was concluded that the toxicity induced by indomethacin is probably associated with an action on the surface membranes of the parasites.

During investigations on prostaglandin biosynthesis by adult *Schistosoma mansoni*, it was noticed that indomethacin, a non-steroidal anti-inflammatory agent and inhibitor of prostaglandin synthesis, was able to induce general paralysis in the worms at concentrations of 10^{-4}M. The toxic effects of indomethacin were observed soon after addition to the parasites *in vitro* and appeared to be reversible. The observed paralysis was not due to any prostaglandin mediated action since schistosomes lack the ability to synthesize prostaglandins [1]. Because indomethacin appeared to be interfering with an important metabolic pathway, probably associated with the surface membranes of the parasites, we have studied the effects of indomethacin on enzymatic systems known to be present in the tegument and which are likely to participate in ion and nutrient transport, nervous transmission and membrane structure.

MATERIALS AND METHODS

[1-^{14}C]Oleic acid 56 mCi/mmole, [1-^{14}C]linoleic acid 51 mCi/mmole and [^{14}C]glycerol 13 mCi/mmole were purchased from the Radiochemical Centre, Amersham, U.K. [^{3}H]Phosphatidyl choline obtained through hydrogenation was a gift from Dr. M. Green of this Institute. After further purification using silicic acid chromatography it was found to have a specific activity of 104 μCi/mmole. Indomethacin and ATP were from Sigma Chemical Co., London, U.K. and plastic-backed silica gel plates were from Merck, Darmstadt, West Germany. Foetal calf serum was from Gibco Biocult, Paisley, U.K.

Parasites

Adult *S. mansoni* were perfused from Syrian hamsters infected 6 weeks previously with 1000 cercariae, as described by Smithers and Terry [2].

Enzymatic assays

Typically, 20 pairs of adult worms were incubated with or without indomethacin, washed, homogenized and added to the enzymatic assay mixtures. Alternatively, homogenates of adult worms were added directly to tubes containing the assay mixture and indomethacin.

ATPase. The incubation medium (final volume 200 μl) consisted of 50 mM 2-aminomethylpropane-1, 3-diol pH 9.5, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM ATP. Indomethacin (0.1 mg/ml) was dissolved in ethanol and added to the assay mixture. The reaction was started by adding the homogenate or membrane preparation of adult schistosomes and this was incubated at 37° for 1 hr. The tubes were then transferred to an ice-bath and 1.8 ml of water added. Inorganic phosphate was estimated according to Carson [3].

Alkaline phosphatase. The incubation medium (final volume 0.2 ml) contained 50 mM amnediol buffer pH 9.5, 1 mM MgCl and *p*-nitrophenyl phosphate 1 mM and 10 μl of the homogenate. Incubation was carried out at 37° for 10 min and then 0.8 ml of 0.5 N NaOH added. Optical densities were read at 410 nm.

Monoacylesterase. The assay system (final volume 1 ml) contained 100 mM phosphate buffer pH 8, 1 mM *p*-nitrophenyl caprylate and *p*-nitrophenyl palmitate were used. They were dissolved in absolute ethanol, whereas *p*-nitrophenyl acetate was dissolved in water. Whole homogenates of adult schistosomes were added in concentrations of 80 μg protein/ml. Incubation was carried out for 40 min at 37°, and then 5 ml of chloroform:methanol 2:1 (v:v) were added. The tubes were stirred and centrifuged and the upper phase collected, diluted 1:1 with 0.3 N NaOH and used for estimation of the optical density at 405 nm.

Acetylcholinesterase. The method employed was that of Ellman *et al.* [4].

Lipid metabolism

Live schistosomes or whole homogenates were incubated for the times indicated, with the different labelled lipid substrates. After incubation the parasites or the homogenates were extracted with 2 ml of chloroform:methanol 2:1 (v:v) and the chloroform phase was concentrated under N_2 and then applied to plastic-backed silica gel thin layer plates. The plates were developed using the following system:

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Table 1. Effect of indomethacin on tegumental enzymes of adult schistosomes

Enzyme	% inhibition by indomethacin
Alkaline phosphatase	16.6
ATPase	21.3
Monoacylesterase	0
Acetylcholinesterase	0

Homogenates or live worms were incubated with indomethacin ($2.5–5 \times 10^{-4}$M) and assayed for enzymatic activity. The results are expressed as a percentage of reduction in specific activity of indomethacin-treated groups in relation to non-treated controls. Live worms were pre-incubated with the drug for 2–5 hr.

isopropyl ether:acetic acid 96:4 (v:v) up to 2/3 of the plate, and then petroleum ether:diethyl ether acetic acid 90:10:1 (v:v). The plates were dried, cut into small rectangles and counted directly in Packard scintillator 299 fluid, on an Intertechnique SL-4000 scintillation counter. Radiolabelled lipids were added to the culture medium as sonicated emulsions in Earles lactalbumin hydrolysate (ELAC) medium containing 0.1% Tween 80.

Fatty acid acyl transferase activity

Adult worms (50 pairs) were incubated for 6 hr at 37° with [^{14}C]glycerol (0.22 μCi/tube) in ELAC medium containing 10% foetal calf serum in the presence or absence of indomethacin. After incubation the parasites were washed, homogenized and extracted with chloroform:methanol 2:1 (v:v). The organic and the aqueous phases were collected, evaporated under N_2 and counted for radioactivity.

RESULTS

The results in Table 1 summarize the experiments in which the effect of indomethacin was tested on several enzymatic systems present in the tegument of schistosomes.

The results show that none of the above enzymatic systems was substantially affected by indomethacin in concentrations which were able to induce general paralysis of the parasites. Lipid uptake and metabolism however, was affected by indomethacin, as was shown by the following series of experiments.

Homogenates

In these experiments adult worms were homogenized and incubated for 2 hr at 37° in the presence of indomethacin and [^{3}H]phosphatidyl choline at pH 4.2 or 7.4. After incubation the lipids were extracted and separated as described in Materials and Methods. The results are shown in Table 2.

The results in Table 2 deserve some special comments. First, no triglyceride synthesis is observed when homogenates are incubated with labelled lipid precursors, thus indicating that triglyceride biosynthesis requires intact tissue compartments. Second, it can also be seen that at pH 4.2, only phospholipase C activity is apparent since mainly diglycerides are produced and that indomethacin does not affect this enzyme. At pH 7.4, however, besides diglycerides, free fatty acids are generated. This result indicates the action of lipases and/or phospholipase A. Furthermore, indomethacin seems to activate this enzyme since it promoted approximately a three-fold increase in the amount of free fatty acids released from phosphatidyl choline.

In order to test whether indomethacin was activating the schistosome lipases, [^{14}C]linoleic acid was used as a substrate instead of [^{3}H]phosphatidyl choline, since this fatty acid should undergo no metabolic changes when using homogenates. The results are shown in Table 3.

The results in Table 3 show that indomethacin does not affect the utilization of [^{14}C]linoleic acid, thus supporting the idea that in homogenates, the effects of the drug are restricted to an activation of lipases or phospholipases.

Live worms

Previous results have shown that when live worms are incubated in the presence of radiolabelled fatty acids, phospholipids and triglycerides, the major end products are triglycerides [1]. In the present work we have shown that when live worms are incubated in the presence of indomethacin and [^{3}H]phosphatidyl choline there is a decreased synthesis of triglyceride, as seen in Table 4. The results also show that when the parasites are pre-incubated with indomethacin, washed and then incubated with the radiolabelled lipid, the inhibition of triglyceride

Table 2. Effect of indomethacin on lipid metabolism of homogenates of *S. mansoni*

Lipid class	Control pH 7.4	Ind. pH 7.4	Control pH 4.2	Ind. pH 4.2	B pH 7.4	B pH 4.2
	cpm	cpm	cpm	cpm	cpm	cpm
Phospholipids	45434 (82.5)	53725 (73.2)	49146 (86.7)	50672 (88.1)	123694 (97.3)	114021 (97.3)
Diglycerides	1684 (3)	2900 (3.95)	2420 (4.2)	2099 (3.6)	610 (0.49)	714 (0.48)
Fatty acids	2075 (4.9)	6263 (8.54)	256 (0.45)	360 (0.62)	349 (0.27)	372 (0.25)

Parasites were homogenized and 100 μl of the homogenate incubated for 2 hr at 37° with 0.14 mmoles [^{3}H]phosphatidyl choline (PC) (104 μCi/mM) and indomethacin 5×10^{-5}M. [^{3}H]PC was added to 50 μl Triton X-100 (in chloroform:methanol) 1:1) and dried under N_2. Tubes incubated at pH 4.2 had 50 μl of 1 M Na acetate buffer pH 4.2 added + 50 μl of H_2O. Blanks (B) contained no homogenate. Homogenates which had previously been heated at 70° for 25 min gave the same results as the blanks. After incubation the homogenates were washed, extracted for lipids and those were applied on t.l.c. The figures in brackets represent the percentage of total lipids present on the plate.

Table 3. Effect of indomethacin on homogenates incubated with [^{14}C]linoleic acid

Lipid class	Control	Indomethacin	Bl
Phospholipids	748	747	566
Diglycerides	4530	4300	4336
Fatty acids	40869	40163	46748

Adult worms were homogenized and incubated with indomethacin (5×10^{-4}M) and [^{14}C]linoleic acid (51 mCi/mM) in neutral pH, for 90 min at 37°.

After incubation the homogenates were extracted for lipids which were separated on t.l.c. as described in the text.

Bl = blanks which contained no homogenate.

Table 4. The effect of indomethacin added prior to or simultaneously with [^{3}H]phosphatidyl choline on lipid metabolism by live schistosomes

Lipid class	Pre-incubated with indomethacin		Incubated with indomethacin and ^{3}H-phosphatidyl choline simultaneously	
	Indo. cpm	Control cpm	Indo. cpm	Control cpm
Phospholipids	1160	1013	1559	1686
Diglycerides	256	288	379	515
Fatty acids	492	673	907	1411
Triglycerides	392	462	312	738

Live adult worms (20 pairs/group) were either incubated with indomethacin (13 mM) for 2 hr, washed and incubated with [^{3}H]phosphatidyl choline, or simultaneously with indomethacin and [^{3}H]phosphatidyl choline for 2 hr. They were then homogenized, extracted for lipids and separated as described in the text.

synthesis is not as pronounced, suggesting a reversible effect. Moreover, the worms gradually recover their motility after removal of the drug.

Incubation of live schistosomes with [^{14}C]linoleic acid and indomethacin was also carried out. The results in Table 5 show that indomethacin produces the same degree of inhibition of triglyceride formation as shown with [^{3}H]phosphatidyl choline.

The results in Tables 4 and 5 indicate that indomethacin inhibits acylation of fatty acids to glycerol.

DISCUSSION

Investigation on the mechanism of the anti inflammatory activity of indomethacin has revealed that as well as inhibiting prostaglandin synthesis and cyclic AMP mediated reactions, the drug can affect a series of other metabolic pathways including inhibition of mucopolysaccharide synthesis and inhibition of collagen synthesis [5, 6]. Although indomethacin might have similar effects on schistosomes,

Table 5. Effect of indomethacin on the uptake and metabolism of [^{14}C]linoleic acid by live adult schistosomes

Lipid class	Indomethacin	Control cpm
Phospholipids	717	871
Diglycerides	316	246
Free fatty acids	524	414
Triglycerides	1575	3126

Adult worms (20 pairs/group) were incubated for 5 hr with [^{14}C]linoleic acid (0.0 11 μmoles) in the presence or absence of indomethacin (2×10^{-4}M). The worms were then washed, extracted with chloroform:methanol 2:1 and the lipids separated as described above.

suggest that any effects on biosynthetic mechanisms would only be secondary. It is more likely that the indomethacin-induced toxicity to schistosomes is directly connected with membrane-associated effects. In this context, the drug has also been shown to be effective in inhibiting the activity of phospholipases [7] and diglyceride lipases of human platelets [8], inhibiting the release of lysosomal enzymes [9] and inhibiting monocyte phagocytosis [10]. The last two actions may also reflect an interference with lipid metabolism, since both would depend on membrane function. Therefore it is plausible that the indomethacin-induced accumulation of free fatty acids in schistosomes produced by a concomitant activation of phospholipases and inhibition of fatty acyl transferases would be indicative of an interference with membrane function, possibly by altering surface charges and hence cellular activity. That surface membranes, rather than internal cell structures, were affected, was indicated by the rapidly reversible effect of the drug on schistosome motility and by the fact that pre-incubation of the parasites with indomethacin followed by washing with fresh medium did not alter the parasite's ability to metabolise phospholipids. If the drug had been absorbed and stored in intracellular organelles a more delayed action would be expected. Furthermore, unpublished experiments by our group have shown that isolated surfaces of adult schistosomes contain high phospholipase activity which strongly suggests that these enzymes constitute the primary target for indomethacin action.

The simultaneous effect of indomethacin on the activation of lipases and/or phospholipases and inhibition of fatty acyl transferases indicates that these enzymatic systems are in close proximity on the surface membranes of the schistosomes. Such localisation can be predicted on a functional basis, since it was shown that incorporation of phospholipids and triglycerides by schistosomes takes place sequentially through hydrolysis into the constituent fatty acids, followed by reacylation of the fatty acids to glycerol.

that whole homogenates of adult worms were not able to synthesize triglycerides. Possibly in schistosomes, the synthesis of triglycerides takes place through the glycerol monophosphate pathway [11], which requires that glycerol be phosphorylated by glycerol kinase, followed by acylation with the activated fatty acids (in the form of fatty acyl coenzyme A). It is probable that in live worms glycerol is segregated and phosphorylated in some organelles and then reacts with the fatty acids, whereas in homogenates, the presence of free phosphatases would block the acylation reaction by dephosphorylation of glycerol monophosphate, or alternatively free glycerol might be utilized in other metabolic pathways.

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