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In vitro effect of nitroxynil (Trodax R) and rafoxanide on adult *Fasciola gigantica*

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RÉSUMÉ

Effet *in vitro* du nitroxynil (Trodax R) et du rafoxanide sur *Fasciola gigantica* adulte

L'auteur a étudié les effets du nitroxynil et du rafoxanide sur les contractions de *Fasciola gigantica* adultes plongées dans un milieu liquide enrichi par l'une ou l'autre de ces drogues à des concentrations différentes.

L'amplitude des contractions est réduite à son minimum lorsque la concentration atteint le taux de 50 mg/ml pour le rafoxanide et de 20 mg/ml pour le nitroxynil. Elles cessent alors au bout de deux minutes d'un pareil traitement.

Des hypothèses sont émises sur le mode d'action des deux corps étudiés.

INTRODUCTION

Fasciolasis due to *Fasciola gigantica* is of considerable economic importance in the livestock industry in Nigeria (4). Chemotherapy is the widely used method of controlling the disease in the country.

The popular fasciolicides used are Rafoxanide (1) (3,5-diiodo-3'-chloro-4' (p-chlorophenoxy) salicylanilide) and Nitroxynil/(4-cyano-2-iodo-6-nitrophenol) (2). Although, these drugs are effective in the treatment of mature infections (1, 5), there is little information on the mode of action of the two drugs on adult worms.

MATERIALS AND METHODS

Adult *F. gigantica* were obtained freshly from infected cattle livers at the Ibadan Municipal abattoir. These were maintained in 199 medium prior to use.

The adult flukes were suspended vertically in an organ bath containing medium 199 (Welcome Laboratories, Beckenham). Each fluke was attached at both anterior and posterior ends by means of hooks in such a way that their contractions were traced on a revolving smoked drum. Normal contractions were observed before application of the drugs.

Thereafter, different drug concentrations in a mixture with 199 were added into the organ bath. Rafoxanide was used at 12,5, 25, 37.5 and 50 mg/ml of active ingredient while Nitroxynil was tested at between 20 and 80 mg/ml of active ingredient. Each drug was allowed to act for 2 min and observations continued at the same intervals for another 4 min. The drug concentrations were tested on 5 adult flukes each on two occasions. Because of the uniformity of results, the amplitude of contractions was graded using a plus scale with a negative representing cessation of contractions.

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RESULTS

There was decrease in the amplitude of contractions with increasing concentration of rafoxanide (table). However, there was a cessation of contraction at 50 mg/ml of the drug leading to a continuous line on the Kymograph (fig.). The effect of Nitroxynil was more pronounced. The lowest concentration of the drug used (20 mg/ml) produced an immediate cessation of muscular contraction after the first 2 min.

In vitro effect of rafoxanide and nitroxynil on adult *F. gigantica*

Drug Concentrations		Amplitude of contractions at 2-minute intervals		
		1	2	3
Rafoxanide	(mg/ml)			
	12.5	++	++	+
	25	++	++	+
	37.5	+	+	-
	50	-	-	-
	20	-	-	-
	40	-	-	-
	60	-	-	-
	80	-	-	-

++ = Strong contractions ; + = Weak contraction
- = No contraction.

DISCUSSION

The present results indicate that Nitroxynil has a more potent *in vitro* action on adult worms than rafoxanide. However, this could be due to differences in the mode of action of these drugs on adult flukes.

The decrease in the amplitude of contractions with increasing concentrations of rafoxanide supports the suggestion that the drug acts on the energy metabolism of flukes. For instance, administration of rafoxanide has been shown to lead to a depletion in glycogen, glucose-6-phosphate and ATP in adult flukes (3). Therefore, rafoxanide may have an indirect anthelminthic action leading to expulsion or death of weakened worms in treated hosts. This may explain its limitation in the treatment of prebiliary infections (1). On the other hand, the immediate cessation of contractions caused by nitroxynil indicates that the drug may act directly as a neuromuscular blocker or respiratory inhibitor. Adult flukes possess enzymes of the anaerobic sequence (6) and the cyano-moeity of nitroxynil may have little respiratory inhibitory effect. Therefore, it seems more plausible that nitroxynil is acting as a neuromuscular blocker.

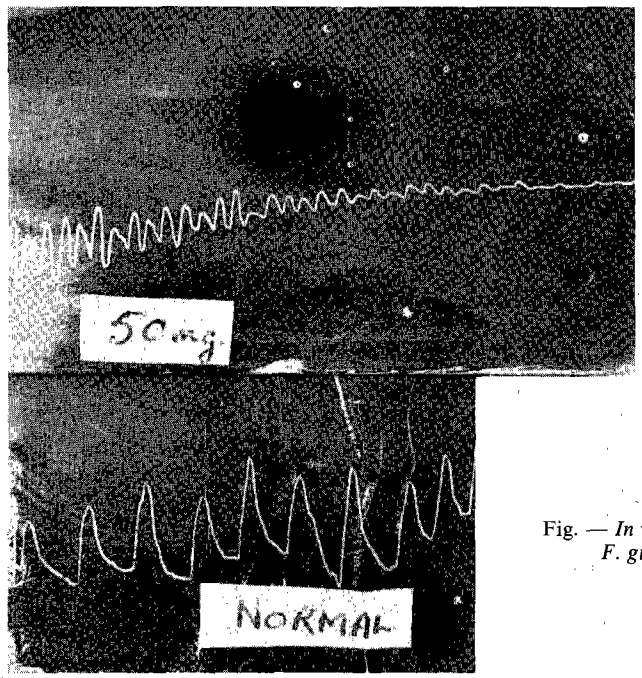

Fig. — *In vitro* effect of rafoxanide (50 mg/ml) on adult *F. gigantica* (below : normal contractions).

SUMMARY

We studied the effect of two popular fasciolicides (Nitroxynil and Rafoxanide) on adult *Fasciola gigantica* maintained in cell-free culture media. With the aid of a kymograph, it was shown that both drugs caused a reduction in the amplitude of muscular contractions of adult worms. This effect was maximal at drug concentrations of 50 mg/ml Rafoxanide and at a lower concentration of 20 mg/ml Nitroxynil leading to a cessation of muscular contractions after a 2 min treatment period. An hypothesis is presented for the mode of action of these drugs.

RESUMEN

Efecto *in vitro* del Nitroxynil (Trodax^{R}) y del Rafoxanide sobre *Fasciola gigantica* adulto

El autor estudió los efectos del Nitroxynil y del Rafoxanide sobre contracciones de *Fasciola gigantica* adultos bañadas en un medio líquido enriquecido por uno o otro de los productos con concentraciones diferentes.

Se reduce al minimo la amplitud de las contracciones cuando la concentración del Rafoxanide llega a la proporción de 50 mg/ml y la del Nitroxynil 20 mg/ml y las dichas desaparecen al cabo de dos minutos.

Se hacen hipotesis sobre el modo de acción de los dos medicamentos.

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