

## The Chemotherapeutic Activity of Selected Fasciolicides Against Immature *Fasciola hepatica* in Mice

Several reports dealing with the activity of fasciolicidal drugs against the major trematode parasite, *Fasciola hepatica*, in rats are available<sup>1-6</sup>, but no comparable published information exists for mice. It was the object of the present study to determine the efficacy of four prominent fasciolicidal agents against immature stages of *F. hepatica* in mice.

**Methods.** Animals: Male, CF-1 (Carworth, N.Y.) mice, 18–22 g at the time of infection, were used throughout.

**Metacercariae:** Infective cysts of *F. hepatica* were obtained from a laboratory maintained culture of *Lymnaea tomentosa* infected with miracidia obtained from eggs of *F. hepatica* recovered from the bile of experimentally infected sheep. Each mouse was infected by gavage with 1 or 2 selected metacercariae, as shown in the Table. The experiments were terminated at 21 days post infection, except for the 21- to 31-day treatment-period groups, which were terminated at 31 days post infection.

**Medication:** All medication was administered via the diet. The drugs were mixed with ground commercial mouse chow (Purina), and this was available ad libitum for 7 to 14 days during the course of infection as shown. The drugs used were: rafoxanide<sup>7</sup> [3, 5-Diiodo-3'-chloro-4'-(*p*-chlorophenoxy) salicylanilide]; oxyclozanide<sup>8</sup> [2, 2'-Dihydroxy-

3, 3', 5, 5', 6-pentachlorobenzanilide]; nitroxylin<sup>9</sup> [4-Cyano-2-iodo-6-nitrophenol (25.4% H<sub>2</sub>O)]; diamphenethide<sup>10</sup> [ $\beta$ ,  $\beta'$ -bis-(4-Acetamidophenoxy)ethyl ether). The concentrations of rafoxanide and nitroxylin used were selected on the basis of a toxicity study, so as to be high but at subtoxic levels. For oxyclozanide and diamphenethide, 0.1% was used as previous experience has shown

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Chemotherapeutic activity of selected fasciolicidal agents against immature stages of *F. hepatica* in mice

Medication	Conc. in diet (%)	Treatment period (days p. i.)	No. of mice	No. cysts per mouse	Infection (%)	No. flukes recov.	Fluke reduction (%)
Rafoxanide	0.0125	7–14	5	2	80	2	60
		7–21	20	1	55	0	100
		14–21	20	1	80	1*	100
		21–31	10	1	70	0	100
	0.025	–1– 6	12	2	92	17	6
		7–14	5	2	60	0	100
		7–21	20	1	85	0	100
		14–21	20	1	80	0	100
		21–31	10	1	50	0	100
Oxyclozanide	0.1	–1– 6	12	2	92	16	11
		7–14	5	2	60	3	40
		7–21	19	1	53	1	90
		14–21	20	1	75	0	100
		21–31	10	1	40	0	100
	0.4	7–14	5	2	80	2	60
		7–21	19	1	47	0	100
		14–21	19	1	58	1	93
		21–31	10	1	60	0	100
Nitroxylin	0.1	–1– 6	12	2	100	18	0
		7–14	4	2	75	1	75
		7–21	20	1	85	0	100
		14–21	20	1	85	0	100
		21–31	10	1	100	0	100
Diamphenethide	0.1	–1– 6	12	2	42	4	78
		7–14	5	2	100	0	100
		7–14	18	1	33	2	87
		14–21	15	1	60	4	62
IUC	0.0	–1– 6	12	2	100	18	0
		7–14	5	2	80	5	0
		7–14	17	1	82	14	0
		7–21	19	1	58	10	0
		14–21	37	1	76	26	0
		21–31	9	1	67	4	0

\*Dead. Combined results from 4 experiments. IUC = infected, unmedicated control.

this to be a desirable level for compounds of unknown activity.

**Toxicity:** Three of the drugs were fed to uninfected mice at doubling concentrations in the diet, and the method of REED and MUENCH<sup>11</sup> was used to calculate the projected concentration resulting in 50% mortality over a 7-day period ( $LD_{50}$  - 7 days). The values obtained were: rafoxanide 0.038%; oxyclozanide > 0.4%; nitroxylinil 0.163%. The toxicity of diamphenethide was not tested but could be expected to be very low<sup>12</sup>.

**Chemotherapeutic effect:** Five periods of medication covering the whole of the immature phase of migration of the parasite were used. Activity was based on comparison of parasite recovery (flake burden) of medicated versus infected unmedicated control (IUC) groups for a particular period, as determined by piece-meal dissection of the liver. Percent reduction of flukes equals the IUC value minus the medicated group value divided by the IUC value  $\times 100$ , where the values are in terms of number of flukes per animal. During the medication period, IUC animals consumed a mean of 4.5 g of feed per mouse per day (range 4.0 to 5.1), while medicated feed was consumed at a mean of 4.6 g per mouse per day (range 4.1 to 5.7).

**Results.** The results will be given for each drug separately below and are shown in the Table.

**Rafoxanide:** It was active for all doses used for all periods except -1 to 6 days post infection. Complete elimination of all parasites was seen for the periods 7 to 21, 14 to 21, and 21 to 31 days post infection. It was inactive when given from days -1 to 6 post infection and highly active only at 0.025% for the period 7 to 14 days post infection.

**Oxyclozanide:** It was active at the dosages tested for the periods 7 to 21, 14 to 21, and 21 to 31 days post infection but only at the higher dose level for the 7- to 14-day period. It was inactive when given at 0.1% for -1 to 6 days post infection.

**Nitroxylinil:** It was active for all periods tested except -1 to 6 days post infection. It was less active for the period 7 to 14 days post infection than for later periods.

**Diamphenethide:** It was active for all periods tested. This was the only drug that exhibited a marked degree of activity against the very early stages of *F. hepatica* when given in the feed at 0.1% for the period -1 to 6 days post

infection. There was a marked fluke reduction for the period 7 to 14 days post infection, with a lesser degree of reduction for the 14- to 21-day post infection period.

**Discussion.** It is evident from the results that the fasciolicidal activity of the drugs tested was clearly shown in a *F. hepatica* mouse system. Though not shown by the data, it is also notable that flukes recovered from medicated animals were generally retarded in size, and there was an obvious reduction in gross liver pathology that paralleled drug activity. As the drugs tested are the 4 most prominent, new fasciolicidal agents available, it is considered that the mouse-*F. hepatica*-system should lend itself well to the detection of such activity in unknown compounds. Thus, murine fascioliasis could be used for the screening of potential fasciolicidal agents, and the drugs used in this study could be used as standards in such testing. In fact, a method for the primary empirical screening of chemicals for activity against *F. hepatica*, utilizing mice medicated from 14 to 21 days post infection and changes in pathologic status as a parameter of activity, has recently been presented<sup>13</sup>.

**Zusammenfassung.** Die Wirkung von vier faszioziden Agentien (Rafoxanid, Oxyklozanid, Nitroxylinil und Diamphenethid) wurde im Mäuseversuch verglichen. Alle vier Substanzen waren gegen unreife *Fasciola hepatica* wirksam und entfalteten ihre höchste Wirksamkeit gegen spätere unreife Stadien, mit Ausnahme von Diamphenethid, welches gegen die Frühstadien der unreifen Parasiten am stärksten war.

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## Molecular Weight of Virus-Induced Tortoise Interferon in Cell Cultures

In an earlier work<sup>1</sup> we reported the interferon production in cell cultures of tortoise (*Testudo graeca*) kidney after infection with arboviruses (Semliki Forest, West Nile). In addition to the physico-chemical characteristics of the tortoise interferon, an investigation was carried out to determine its molecular weight. For this purpose, the method of Sephadex gel filtration was used.

The 'crude' interferon was obtained by infecting primary monolayer cell cultures of tortoise kidney (TGK) with Semliki Forest virus (SFV), and subsequent treatment of the cultural liquid harvested at the 48th h after the virus inoculation, by techniques previously described<sup>1</sup>. After a 72 h dialysis against 30 volumes of PBS, pH 7.4, the interferon preparation was concentrated 15-30 times with polyethyleneglycol 6000 (Carbowax 20M, Serva).

The molecular weight was determined by column chromatography in Sephadex gel according to the method of ANDREWS<sup>2,3</sup>. A column 110  $\times$  1.5 cm was packed with hydrated Sephadex G-100 beads (Pharmacia, Uppsala) in PBS, pH 7.4, containing 0.02 sodium azide, and left

for 48 h at 10°C to achieve equilibration. Calibration of the column was performed with the following proteins used as standard markers, with known molecular weight: human serum albumin (69,000) Koch-Light, ovalbumin (45,000), soybean trypsin inhibitor (21,500) Miles-Sera-vac, cytochrome c (13,000) Miles-Sera-vac. Proteins were dissolved in PBS and layered in 2 ml volume, containing a mixture of human serum albumin and soybean trypsin inhibitor 3 mg each and ovalbumin and cytochrome c 2 mg each. 0.2% Blue dextran 2000 (2,000,000) Pharmacia, Uppsala, was used as an additional marker to determine the exclusion volume of the column. The flow rate was about 7 ml/h/cm<sup>2</sup>. 4-ml-effluent fractions were collected with fraction collector Nowator 301 B (Poland).

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