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Evaluation of broad-spectrum anthelmintic activity in a novel assay against *Haemonchus contortus*, *Trichostrongylus colubriformis* and *T. sigmodontis* in the gerbil *Meriones unguiculatus*

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Abstract

The gerbil *Meriones unguiculatus*, infected with three species of nematodes, each located in a separate part of the gastrointestinal tract, provided a reliable laboratory assay for the evaluation of broad-spectrum anthelmintic activity. Gerbils harbouring 6-day-old infections of *Haemonchus contortus*, *Trichostrongylus colubriformis* and *T. sigmodontis* were given selected broad-spectrum anthelmintics by gavage. Three benzimidazoles, thiabendazole, oxfendazole and albendazole, a tetrahydropyrimidine, morantel, an imidazothiazole, levamisole hydrochloride, a macrocyclic lactone, ivermectin and an experimental natural product, paraherquamide, were active against all three nematodes at various dosages. *Trichostrongylus colubriformis* was most sensitive to levamisole hydrochloride, morantel, thiabendazole and paraherquamide whereas ivermectin, oxfendazole and albendazole were more effective against *H. contortus*. All compounds were active against the caecal nematode *T. sigmodontis* although it was less sensitive than *T. colubriformis. Haemonchus contortus* was more sensitive than *T. sigmodontis* to all anthelmintics tested except thiabendazole.

Introduction

Various nematode infections in gerbils have been used to study host–parasite relationships and evaluate gastrointestinal anthelmintic activity. These studies include those of Leland (1963) on *Trichostrongylus axei* infections and Williams & Palmer (1964) who used Libyan gerbils infected with *T. colubriformis*. Kates & Thompson (1967), Ostlind & Cifelli (1981), Panitz & Shum (1981) and Court & Lees (1985) evaluated anthelmintics against various stages of *T. colubriformis* whereas Court *et al.* (1988) established *Ostertagia circumcincta* infections in gerbils. Conder *et al.* (1990) tested anthelmintics against *Haemonchus contortus* and more recently, Gonzalez *et al.* (2004) and Johnson *et al.* (2004) used variations of the gerbil model to evaluate *in vitro*-active compounds.

The present paper considers an evaluation of selected broad-spectrum anthelmintics in gerbils, each harbouring 6-day-old infections of three nematode species, the sheep stomach worm (*H. contortus*), the ovine small intestinal trichostrongylid (*T. colubriformis*) and the rodent trichostrongylid (*T. sigmodontis*).

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Materials and methods

Infections and treatments

Five-week-old male and female gerbils (Haley Farms, Hurt, West Virginia, USA) were orally infected with 500 ± 10% *H. contortus*, 500 ± 10% *T. colubriformis* and 500 ± 10% *T. sigmodontis* third stage larvae on day 0. When older larvae were used, the inoculum was

increased appropriately to compensate for reduced viability. Six days after infection the test compounds, dissolved or suspended in a polyethylene glycol 400-DMSO mixture (1:2 v/v) (Ostlind & Cifelli, 1981), were administered by gavage to randomly selected gerbils using a ball-tipped, bent, 50 mm, 18 ga needle on a 1 ml tuberculin syringe. The compounds tested were three benzimidazoles, thiabendazole, oxfendazole and albendazole, a tetrahydropyrimidine, morantel, an imidazothiazole, levamisole hydrochloride, a macrocyclic lactone, ivermectin and an experimental natural product, paraherquamide. Drug concentrations were adjusted so that dosing each gerbil at 0.5 ml per 100 g of body weight would deliver the desired dosage in $mg\,kg^{-1}$. Six to 10 gerbils, given the solvent mixture at the same rate, served as placebo-treated controls while treated groups consisted of combined data from 3–6 animals.

Necropsy and worm recovery

On day 9, gerbils were necropsied to recover the stomach, small intestine and caecum. Each organ was slit open and placed in separate 4 oz (stomach) or 8 oz plastic cups and allowed to incubate in saline at 50°C for 2 h. After incubation, saturated aqueous iodine was added to each cup to stain the worms and cease bacterial action. The contents of each cup were collected and washed on a 200 mesh (75 μm) seive. The retained material was backwashed into its original cup and filled to a 100 ml mark prior to counting worms, either by 10 ml aliquots or totally, under a dissecting microscope. Efficacy was determined as the per cent reduction from the average worm burden of placebo-treated controls. All *in vivo* procedures were approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories.

Statistical analysis

Dunnetts one-sided test was used to determine statistical significance.

Results

The efficacy of six commercial anthelmintics and an experimental natural product against 6-day-old infections of *H. contortus*, *T. colubriformis* and *T. sigmodontis* in gerbils is shown in table 1.

Efficacy against Haemonchus contortus

Against *H. contortus* a high activity (>90% reduction) was attained by ivermectin at $0.125\,mg\,kg^{-1}$. It was followed by oxfendazole in potency at or below 3.125 mg kg^{-1} (minimum effective >90% endpoint not established) and albendazole at $3.125\,mg\,kg^{-1}$. Levamisole hydrochloride was highly active at $12.5\,mg\,kg^{-1}$. Morantel and thiabendazole were highly active at 200 and 400 mg kg^{-1}, respectively. Paraherquamide was highly active at 12.5 mg kg^{-1}.

Efficacy against Trichostrongylus colubriformis

Against 6-day-old *T. colubriformis*, ivermectin was the most potent compound tested (>95% effective at $0.0625\,mg\,kg^{-1}$). Paraherquamide at $0.781\,mg\,kg^{-1}$ was 96.5% effective. Albendazole and levamisole hydrochloride were 89.2 and 100% effective at $6.25\,mg\,kg^{-1}$, respectively. Morantel reduced the worm burden by 99.8% at $12.5\,mg\,kg^{-1}$. A minimum effective level (>90% reduction) was not determined for morantel. Oxfendazole was 92.4% efficacious at $25\,mg\,kg^{-1}$. Thiabendazole was the least potent compound against *T. colubriformis* as $200\,mg\,kg^{-1}$ was required to produce >90% reduction.

Efficacy against Trichostrongylus sigmodontis

Ivermectin at $0.2\,mg\,kg^{-1}$, the highest level tested, was 89.4% effective against the caecal nematode, *T. sigmodontis*. Albendazole was 95.8% effective at $6.25\,mg\,kg^{-1}$. Oxfendazole and levamisole hydrochloride were 93.4 and 98.0% effective at $12.5\,mg\,kg^{-1}$, respectively. Morantel and thiabendazole were 97.3 and 90.3% effective at $200\,mg\,kg^{-1}$, respectively. A $50\,mg\,kg^{-1}$ dose of paraherquamide reduced the caecal worm burden by 89.2%.

Discussion

The three-species–gerbil assay as described clearly demonstrated its ability to assess broad-spectrum anthelmintic activity as well as a reasonable ranking of their respective potencies with respect to their commercial use levels. The relative potencies of the anthelmintics tested in common with those reported by Campbell (1983) and Court & Lees (1985) were similar. The combined data for each compound against all three parasites showed ivermectin to be the most potent anthelmintic tested whereas thiabendazole was the least potent as expected. Ivermectin was followed by albendazole, levamisole hydrochloride, paraherquamide, oxfendazole and morantel. Paraherquamide, an experimental natural product, was active against all three nematodes, and its broad-spectrum activity was confirmed in sheep (Shoop *et al.*, 1990) and cattle (Shoop *et al.*, 1992). The large sensitivity difference between *T. colubriformis* and *T. sigmodontis* for paraherquamide predicted less sensitivity against large bowel worms. This was confirmed by Shoop *et al.* (1990), as an extrapolated dosage close to 18× the dose efficacious against the abomasal and intestinal species would be needed to remove *Oesophagostomum columbianum* in sheep.

The vast majority of the global anthelmintic market value for food animals is generated by broad-spectrum products. Therefore, assessment of potential broad-spectrum activity is highly desirable as well as being accomplished as economically as possible. The described assay has the ability to predict broad-spectrum anthelmintic activity because it covers nematodes in the three most commonly parasitized regions of the gastrointestinal tract as well as being in a well-established laboratory rodent. Also, two of the three nematode species involved are economically significant parasites of food-producing domestic livestock.

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Table 1. Efficacy of ivermectin, oxfendazole, albendazole, levamisole hydrochloride, morantel, thiabendazole and paraherquamide against 6-day-old *Haemonchus contortus* (H.c.),*Trichostrongylus colubriformis* (T.c.) and *T. sigmodontis* (T.s.) in the gerbil.

	Efficacy%		
Dosage (mg kg^{-1})	H.c.	T.c.	T.s.
Ivermectin			
Placebo	146.4[a] (98–178)	293.6[a] (209–391)	392.0[a] (354–479)
0.2	100.0*	99.8*	89.4*
0.15	97.6*	98.7*	79.7*
0.125	100.0*	100.0*	11.9
0.0625	83.6*	96.5*	0.0
0.03 125	79.0*	56.5	0.0
0.01 562	35.3	0.0	0.0
Oxfendazole			
Placebo	124.4[a] (70–186)	256.3[a] (198–373)	429.4[a] (349–524)
25.0	96.5*	92.4*	96.6*
12.5	97.9*	87.2*	93.4*
6.25	98.9*	79.8*	67.0
3.125	97.1*	65.2*	26.9
Albendazole			
Placebo	156.5[a] (89–182)	250.8[a] (178–322)	344.3[a] (239–449)
12.5	100.0*	97.8*	97.5*
6.25	97.7*	89.2*	95.8*
3.125	92.3*	79.5*	59.5*
1.562	74.5*	28.3	0.0
0.781	31.8	0.0	0.0
Levamisole HCL			
Placebo	98.6[a] (54–126)	275.7[a] (190–358)	358.6[a] (306–413)
12.5	100.0*	100.0*	98.0*
6.25	82.7*	100.0*	49.8*
3.125	13.4	83.7*	3.0
1.562	0.0	28.1	0.0
Morantel			
Placebo	224.5[a] (131–283)	320.8[a] (251–390)	753.5[a,b] (563–924)
200.0	100.0*	99.8*	97.3*
100.0	68.9	99.1*	22.1
50.0	69.5	95.8*	49.1
25.0	50.5	98.0*	0.0
12.5	46.4	99.8*	0.0
Thiabendazole			
Placebo	120.4[a] (90–162)	309.9[a] (256–368)	437.8[a] (388–513)
400.0	97.5*	94.5*	95.8*
200.0	65.7*	91.8*	90.3*
100.0	26.9	78.4*	52.4
50.0	3.1	56.9*	23.8
Paraherquamide			
Placebo	74.8[a] (52–94)	286.7[a,c] (119–370)	528.7[a,d] (455–616)
200.0	99.5*	100.0*	99.3*
100.0	95.9*	100.0*	98.9*
50.0	100.0*	100.0*	89.2*
25.0	100.0*	99.5*	78.8*
12.5	95.9*	100.0*	63.6*
6.25	77.0	100.0*	35.6*
3.125	20.4	99.7*	0.0
1.562	0.0	98.1*	13.9
0.781	0.0	96.5*	11.7
0.39	0.0	66.3*	11.7

[a] Average worm burden of 6–10 animals; (Range).
[b] Given 1200 ± 10% infective larvae.
[c] All *T. colubriformis* data for paraherquamide reproduced from *Research in Veterinary Science* (1990) **48**, 260.
[d] Given 800 ± 10% infective larvae.
* $P < 0.05$ (Dunnetts one-sided test).

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Trichostrongylus sigmodontis was first used for anthelmintic evaluation in the early 1960s by J.R. Egerton (personal communication) as a potential indicator of activity against worms that inhabit the large intestine. It was the least sensitive of the three nematode species, but it has the advantage of laboratory propagation in rodents whereas *H. contortus* and *T. colubriformis* require an ovine host. Consistently reproducible worm burdens were obtained without the need of immunosuppression or exsheathment of larvae prior to inoculation. The three-species–gerbil assay was employed at the Merck Research Laboratories from 1979 through the 1990s and was operated using the one animal per treatment group concept from its inception. During this period, the assay was used to evaluate the avermectins (Ostlind & Cifelli, 1981) and their synthetic derivatives (Mrozik *et al.*, 1982; Schulman *et al.*, 1985; Meinke *et al.*, 1992).

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