Veterinary Parasitology, 39 (1991) 123–136
Elsevier Science Publishers B.V., Amsterdam

Effect of parasitism with *Nematodirus battus* on the pharmacokinetics of levamisole, ivermectin and netobimin

Q.A. McKellar[a], F. Jackson[b], R.L. Coop[b], E. Jackson[b] and E. Scott[a]
[a]*Department of Veterinary Pharmacology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH, UK*
[b]*Department of Parasitology, Moredun Institute Gilmerton Road, Edinburgh EH17 7JH, UK*

(Accepted 20 November 1990)

ABSTRACT

McKellar, Q.A., Jackson, F., Coop, R.L., Jackson, E. and Scott, E., 1991. Effect of parasitism with *Nematodirus battus* on the pharmacokinetics of levamisole, ivermectin and netobimin. *Vet. Parasitol.*, 39: 123–136.

The pharmacokinetics of levamisole, ivermectin and netobimin administered orally and by subcutaneous injection were compared in lambs exposed to a moderate challenge with *Nematodirus battus* and in parasite naive lambs. There were no significant differences ($P > 0.05$) in the bioavailability of any of the anthelmintics tested between parasitized and non-parasitized animals. Levamisole reduced nematode faecal egg output by more than 99% when administered by either route. Ivermectin was also highly effective (> 99%). Orally administered netobimin reduced egg output by more than 98% seven days after administration. However egg output was only reduced by 89% 21 days after administration, suggesting poor activity against the early parasitic stages of *N. battus*. Netobimin was not effective against *N. battus* when administered by the subcutaneous route and this was probably because very low plasma concentrations of its active albendazole metabolites were achieved.

INTRODUCTION

The pharmacokinetics of levamisole (Bogan et al., 1982), ivermectin (Prichard et al., 1985; Marriner et al., 1987) and netobimin (Steel et al., 1985; Delatour et al., 1986, Lanusse and Prichard, 1990) have been assessed in sheep which did not have significant burdens of gastrointestinal parasites. Levamisole and ivermectin are highly efficacious against abomasal and intestinal nematodes of sheep when given by the oral or subcutaneous route although ivermectin is not licensed for subcutaneous administration in the UK (Roberson, 1977; Benz et al., 1989). Netobimin administered orally has also been shown to have high activity against gastrointestinal nematodes of sheep (Duncan et

0304-4017/91/$03.50

al., 1985; Herd et al., 1985; Richards et al., 1987). Netobimin is a nitrophenylguanidine prodrug of albendazole. As a salt it is highly water soluble which may confer some advantages on this drug in terms of administration over the benzimidazoles which are inherently insoluble (Ngomuo, 1983; Townsend and Wise, 1990). Following oral administration in sheep, netobimin is reduced by gut microflora and subsequently cyclized into albendazole. It then undergoes the same metabolic steps as albendazole and anthelmintic activity is principally attributed to these metabolites (Delatour et al., 1986).

The solubility of netobimin has made it appropriate for administration in drinking water (Downey, 1987) and for parenteral administration. Following subcutaneous administration to cattle, netobimin is thought to be secreted into the intestine, cyclized to albendazole metabolites which are reabsorbed. Abomasal activity is attributed to the subsequent plasma concentrations of the albendazole metabolites (Steel and Hennessy, 1987). Netobimin is currently registered for parenteral therapy in cattle in South America at a dose rate of 12.5 mg kg^{-1} but is only available as an oral preparation for sheep and cattle in the UK.

Most pharmacokinetic studies on anthelmintics have been carried out in non-parasitised animals. However it seems likely that parasitic burdens could influence the plasma kinetics and tissue and gastrointestinal disposition of anthelmintic drugs (Prichard, 1985). The liver is the major organ for the metabolism of xenobiotics and infection with *Fasciola hepatica* may affect the drug metabolizing capacity of the host which would be of particular importance where metabolites are active (Galtier et al., 1986; Tufenkji et al., 1988). Infection with the abomasal parasite *Ostertagia circumcincta* has been shown to affect the plasma and abomasal concentrations of fenbendazole and its metabolites in sheep and it was suggested that this could be attributed to the pathophysiological changes associated with this parasite (Prichard, 1980; Marriner et al., 1985). During ostertagiasis there is an elevation in abomasal pH and an increased permeability of the mucosa to macromolecules (Anderson et al., 1965; Armour et al., 1966). It may be that the reduced bioavailability of fenbendazole and its metabolites was a result of reduced solubility of the parent molecule at the elevated abomasal pH. It is also apparent from the relative proportions of parent drug and metabolites that the metabolic capacity of the parasitized animals was reduced (Marriner et al., 1985).

Intestinal nematode parasitism also may affect the pharmacokinetics of anthelmintics. Infection with *Trichostrongylus colubriformis* has been shown to affect gut transit time markedly with a general reduction of intestinal motility (Gregory et al., 1985). It is also possible that the extensive intestinal villous atrophy associated with *N. battus* infection (Coop et al. 1973) could influence local absorption, since the intestine is considered to be the major site of absorption for most orally administered drugs.

The present study was carried out to investigate the effect that natural par-

asitism with *N. battus* would have on the pharmacokinetics of orally and parenterally administered levamisole, ivermectin and netobimin, and to determine the best route of administration for these drugs during parasitism.

MATERIALS AND METHODS

Eighty-four Greyface Suffolk cross twin lambs which were born indoors in mid March were used. Forty-two of the lambs with their dams were turned out onto two 1-ha paddocks (Paddocks A and B) on 10 April 1989. The paddocks had initially been contaminated using lambs artificially infected with *N. battus* in 1985 and the infection had been maintained during the following years by lambs or calves in their first grazing season. The 42 outdoor lambs were divided into two groups of 21 which were rotated weekly between the paddocks in an effort to equalize infection. The remaining 42 lambs were reared indoors under conditions designed to reduce the likelihood of infection with gastrointestinal helminth parasites. Pasture larval samples were taken by the method of Taylor (1939) and the larvae recovered, counted and identified using the methods described by M.A.F.F. (1986). Faecal nematode egg output was monitored in the outdoor lambs and when *N. battus* larval and faecal egg numbers suggested moderate to high burdens of *N. battus* the lambs were housed (15 May) and subsequently maintained under the same conditions as the indoor lambs. All housed sheep had free access to water and were offered hay ad libitum. The lambs had access to creep feed (ad libitum) and the ewes were offered concentrate (0.5 kg per head day^{-1}).

Eighteen sets of twin lambs which had been at pasture were allocated into groups of 12 animals on the basis of bodyweight (BW) such that each group contained six sets of twins and each group had an approximately even distribution of weights. The groups of 12 were then subdivided in two with one twin of each set being allocated to a different group of six animals. Eighteen sets of naive twin lambs were allocated to groups on the same basis. Drugs were administered to groups such that each pair of lambs was given the same drug, with one lamb receiving the drug orally and the other parenterally. Each group containing six lambs was administered levamisole, ivermectin or netobimin by either the oral or subcutaneous routes on 16 May according to the experimental design shown in Table 1. The remaining three sets of lambs which had been at pasture were allocated to a single group as were the remaining three sets which had been reared indoors. These two groups (4A and 4B) received netobimin at 12.5 mg kg^{-1}. The anthelmintics were administered according to the actual weight of each lamb and the following preparations and dosage rates were used. Levamisole (Levicide, Norbrook Laboratories Ltd) was administered orally at a dose rate of 7.5 mg kg^{-1} BW and by subcutaneous injection (Worm Guard Injection, Smith Kline) at a dose rate of 7.5 mg kg^{-1} BW (Groups 1A–1D). Ivermectin (Oramec Drench, MSD

TABLE 1

Protocol for the administration of anthelmintics to lambs infected with *N. battus* and control lambs

Group	Lambs (*n*)	Anthelmintic used	Dose (mg kg^{-1})	Route of administration	Previous management
1A	6	Levamisole	7.5	Oral	Pasture
1B	6	Levamisole	7.5	Subcutaneous	Pasture
1C	6	Levamisole	7.5	Oral	Housed
1D	6	Levamisole	7.5	Subcutaneous	Housed
2A	6	Ivermectin	0.2	Oral	Pasture
2B	6	Ivermectin	0.2	Subcutaneous	Pasture
2C	6	Ivermectin	0.2	Oral	Housed
2D	6	Ivermectin	0.2	Subcutaneous	Housed
3A	6	Netobimin	7.5	Oral	Pasture
3B	6	Netobimin	7.5	Subcutaneous[a]	Pasture
3C	6	Netobimin	7.5	Oral	Housed
3D	6	Netobimin	7.5	Subcutaneous[a]	Housed
4A	6	Netobimin	12.5	Subcutaneous[a]	Pasture
4B	6	Netobimin	12.5	Subcutaneous[a]	Housed

[a]These groups were retreated 14 days later with netobimin, administered orally at a dose rate of 7.5 mg kg^{-1} bodyweight.

Agvet) was administered orally at a dose rate of 0.2 mg kg^{-1} BW and by subcutaneous injection (Ivomec Injection for Cattle, MSD Agvet) at a dose rate of 0.2 mg kg^{-1} BW (Groups 2A–2D). Netobimin (Hapadex Drench for Sheep, Kirby Warrick Animal Health) was administered orally at a dose rate of 7.5 mg kg^{-1} BW and by subcutaneous injection (Hapadex Injection for Cattle, Schering Corporation) at a dose rate of 7.5 mg kg^{-1} BW (Groups 3A–3D). Netobimin (Hapadex Injection for Cattle, Schering Corporation) was also administered by subcutaneous injection at a dose rate of 12.5 mg kg^{-1} BW, the recommended dose rate for cattle (Groups 4A and 4B). Groups 3B, 3D, 4A and 4B which were treated with netobimin by subcutaneous injection were retreated 14 days later with netobimin administered orally at a dose rate of 7.5 mg kg^{-1} BW. All subcutaneous injections were administered on the right side of the neck. Ivomec injection for cattle and Hapadex injection for cattle are not registered for use in sheep in the UK.

Faecal consistency was assessed on Days −14, −7, −1, 7, 14 and 21 and scored on a scale of one to seven where one was blood/mucus and seven was dry, hard pellets. Faecal samples were also examined for the presence of trichostrongyle eggs by a flotation technique (Christie and Jackson, 1982) on Days −1, 7, 14 and 21.

Blood samples were collected from all lambs in each group into lithium heparin vacutainers (Becton–Dickinson) at regular intervals for 24 h after

the administration of levamisole, for 28 days after the administration of ivermectin and for 36 h after the administration of netobimin. Blood was centrifuged immediately at 1700 *g* and plasma collected and frozen at −20°C until time of drug analysis. Analysis of plasma by high performance liquid chromatography for the parent molecules was carried out for levamisole (Groups 1A–1D) by the method of Marriner et al. (1980) and for ivermectin (Groups 2A–2D) by the method of Fink (Scott et al., 1990). Plasma from lambs administered netobimin (Groups 3A–3D and 4A and 4B) was analysed by high performance liquid chromatography for albendazole sulfoxide and albendazole sulfone (Bogan and Marriner, 1980). Netobimin is thought to owe much if not all, of its activity to albendazole metabolites which have been demonstrated in the plasma of sheep following oral administration (Delatour et al., 1986, Lanusse and Prichard, 1990).

The area under the plasma concentration time curve (AUC) was determined by the trapezoidal rule using observed values and analysis of variance was used to compare data from each group, results were considered significant when $P<0.05$.

RESULTS

Pasture *N. battus* larval counts in Paddock A steadily rose from March through to May reaching maximum numbers of 1547 L_3 kg^{-1} dry herbage on 9 May. In Paddock B the number of *N. battus* larvae rose to a maximum of 4050 L_3 kg^{-1} dry herbage on 2 May but had fallen to 1861 L_3 kg^{-1} dry herbage by 9 May.

None of the individual lambs exposed to *N. battus* at pasture had faecal consistency scores of 2 or less and only two lambs (in Group 3B on Day −7) had scores of 3. The mean ±SE scores ranged from 4.66±0.56 (Group 3B Day −7) to 6.00±0.00 (many occasions). These scores are not typical of severe acute nematodiriasis in which liquid faeces with very low dry matter content is common (Coop et al., 1973) and suggested that the lambs had a moderate parasite burden only.

Mean faecal egg counts from each of the groups of lambs exposed to nematode larvae on pasture (Groups 1A, 1B, 2A, 2B, 3A 3B and 4A) are given in Table 2. The efficacy of the anthelmintic measured as the percentage reduction of faecal egg output compared with Day −1 egg counts is also given.

Orally and subcutaneously administered levamisole and orally administered ivermectin were greater than 99% effective against *N. battus* when assessed by faecal egg reductions on Days 7, 14 and 21 after treatment. Ivermectin given by subcutaneous injection and oral netobimin were also highly effective (>97%) when assessed on Days 7 and 14 after administration. However the percentage reduction in faecal egg count after oral netobimin had fallen to 89.04% by Day 21. Since the animals were housed between treat-

TABLE 2

Geometric mean faecal egg counts and percentage reduction in faecal egg counts (in parenthesis) following anthelmintic treatment on Day 0

Group[a]	Treatment	Day −1	Day 7		Day 14		Day 21	
1A	Levamisole oral	533.18	1.21	(99.77)	1.32	(99.75)	1.86	(99.65)
1B	Levamisole subcutaneous	394.07	0.29	(99.95)	0.91	(99.77)	0.26	(99.93)
2A	Ivermectin oral	471.24	0.20	(99.98)	0.20	(99.98)	0.00	(100.00)
2B	Ivermectin subcutaneous	566.05	13.70	(97.58)	9.21	(98.37)	5.18	(99.08)
3A	Netobimin oral	394.01	5.05	(98.72)	11.05	(97.20)	43.17	(89.04)
3B	Netobimin subcutaneous	372.73	1324.68	(−255.40)	772.60[c]	(−107.28)	0.15[d]	(99.98)[b]
4A	Netobimin subcutaneous	473.54	445.39	(5.94)	444.86[c]	(6.06)	0.00[d]	(100.00)[b]

[a] $n=6$ for each group.
[b] Calculated following oral retreatment on Day 14 using means c and d.

ment and Day 21 this suggested that netobimin either had a temporary suppressive effect on the egg output of female *N. battus* or was not fully effective against early parasitic larval stages of *N. battus* which subsequently matured to the egg laying adult stage. Netobimin was ineffective when given by subcutaneous administration at a dose rate of 12.5 mg kg^{-1} BW. Both groups treated with netobimin administered subcutaneously (Groups 3B and 4A) were retreated 14 days later with netobimin administered orally at a dose rate of 7.5 mg kg^{-1} BW. This oral treatment reduced faecal egg output by more than 99% in both groups 7 days later.

The mean ±SE concentration of levamisole in Groups 1A–1D are given in Table 3 and mean concentrations are shown in Fig. 1. Pharmacokinetic parameters for the mean maximum concentration (C_{max}), time of C_{max} (t_{max}) and AUC are given in Table 6. C_{max} values were lower and were achieved later in both groups of lambs receiving levamisole orally. However, there were no significant differences ($P>0.05$) in AUC values between any of the groups receiving levamisole. The C_{max} values obtained after subcutaneous administration in the present study (1.67 ± 0.24 μg ml^{-1} and 1.41 ± 0.58 μg ml^{-1}) were much lower than the mean maximum concentration (approximately 3

TABLE 3

Mean (±SEM) concentration ($\mu g\ ml^{-1}$) of levamisole in plasma of sheep after administration of levamisole at a dose rate of 7.5 mg kg^{-1} by the subcutaneous and oral routes

Time (h)	Group			
	1A	1B	1C	1D
0	0	0	0	0
0.17	0.30±0.07	1.24±0.16	0.47±0.14	0.95±0.24
0.50	0.41±0.09	1.67±0.24	0.55±0.16	1.19±0.23
1.00	0.42±0.09	1.48±0.25	0.76±0.16	0.92±0.26
2.00	0.71±0.13	1.01±0.24	0.83±0.20	0.73±0.19
4.00	0.54±0.15	0.45±0.13	0.58±0.20	0.32±0.08
6.00	0.43±0.11	0.26±0.09	0.42±0.15	0.15±0.05
8.00	0.27±0.08	0.10±0.06	0.29±0.11	0.09±0.04
12.00	0.11±0.05	0.06±0.03	0.16±0.07	0.04±0.02
24.00	0.01±0.01	0	0.01±0.01	0

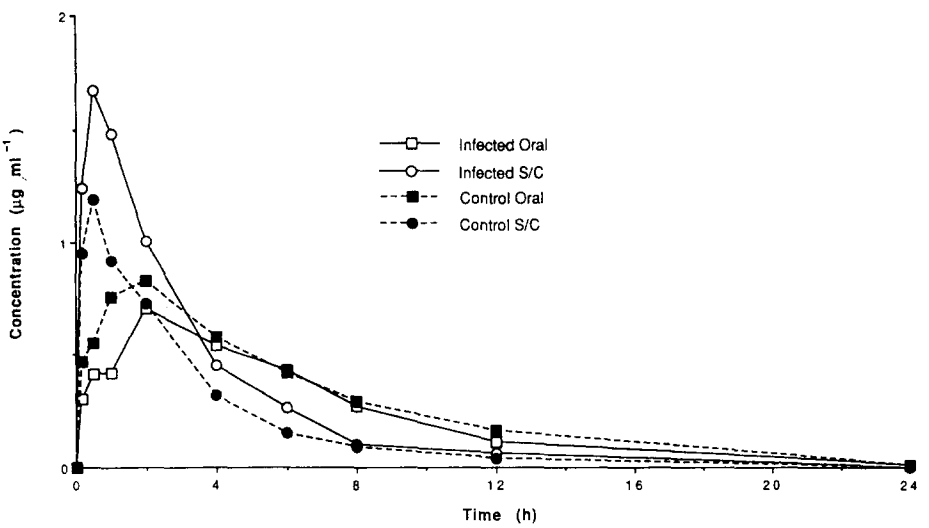

Fig. 1. Mean plasma levamisole concentrations ($\mu g\ ml^{-1}$) following oral administration to parasitized lambs –□– (Group 1A), subcutaneous administration to parasitized lambs –○– (Group 1B), oral administration to control lambs ---■--- (Group 1C) and subcutaneous administration to control lambs ---●--- (Group 1D).

$\mu g\ ml^{-1}$) obtained by Bogan et al., (1982) in non-parasitized sheep. In the previous study subcutaneous administration produced significantly higher bioavailability than oral administration. The mean ±SEM concentrations of ivermectin in Groups 2A–2D are given in Table 4 and mean concentrations are shown in Fig. 2. C_{max}, t_{max} and AUC data are given in Table 6. There was a very large inter-animal variation in the plasma concentration of ivermectin

TABLE 4

Mean (±SEM) concentration (ng ml^{-1}) of ivermectin in plasma of sheep after administration of ivermectin at a dose rate of 200 $\mu g\ kg^{-1}$ by the subcutaneous and oral routes

Time	Group			
	2A	2B	2C	2D
Pre	0	0	0	0
1 h	2.24±1.09	8.24± 4.70	1.62±0.87	2.28± 2.20
2 h	6.84±2.78	12.53± 3.91	6.87±1.86	5.63± 4.78
4 h	14.65±4.85	11.65± 3.74	21.26±4.64	7.71± 3.93
8 h	18.69±6.06	22.38±11.33	24.88±6.78	17.79±11.25
12 h	16.32±5.11	25.50±12.19	23.62±4.25	21.33±14.12
24 h	14.34±2.86	23.60± 7.54	20.71±3.39	25.11±12.05
36 h	11.55±0.08	25.52± 6.33	19.57±4.97	22.96± 6.30
2 days	11.01±2.72	22.64± 6.53	17.28±2.60	21.45± 7.06
3 days	9.51±2.53	21.61± 5.48	10.50±1.36	17.38± 3.65
5 days	6.27±1.74	15.41± 2.17	3.97±0.74	7.16± 3.05
8 days	3.93±2.09	8.53± 2.31	0.30±0.30	1.51± 0.86
11 days	1.77±1.77	4.03± 1.85	0	0.58± 0.37
14 days	1.19±1.19	1.58± 0.79	0	0.09± 0.09
21 days	0.52±0.52	0.26± 0.26	0	0
28 days	0	0	0	0

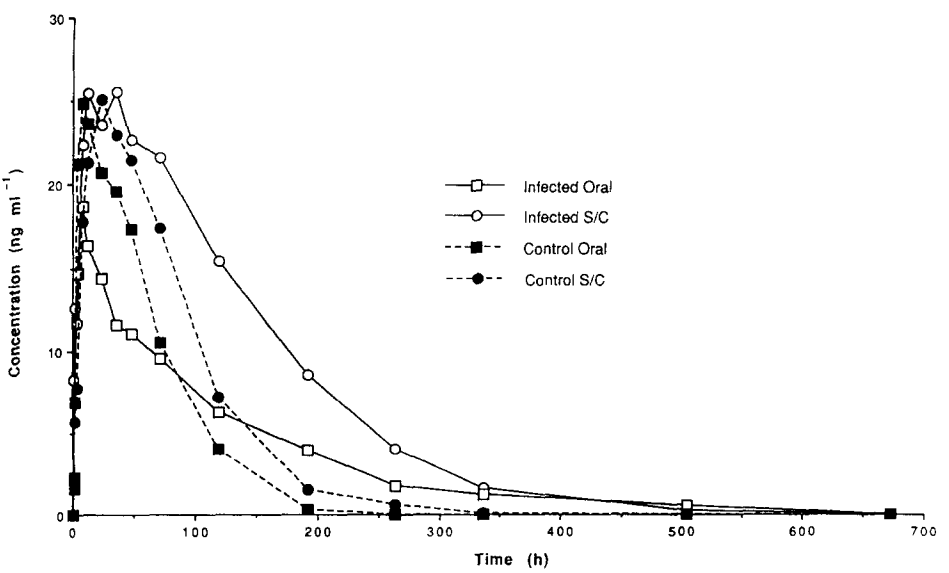

Fig. 2. Mean plasma ivermectin concentrations (ng ml^{-1}) following oral administration to parasitized lambs -□- (Group 2A), subcutaneous administration to parasitized lambs -○- (Group 2B), oral administration to control lambs ---■--- (Group 2C) and subcutaneous administration to control lambs ---○--- (Group 2D).

TABLE 5

Mean (±SEM) concentration ($\mu g\ ml^{-1}$) of albendazole sulphoxide and albendazole sulphone in plasma of sheep after administration of netobimin at a dose rate of 7.5 mg kg^{-1} and 12.5 mg kg^{-1} by the subcutaneous and oral routes

Time (h)	Group					
	3A	3B	3C	3D	4A	4B
Albendazole sulphoxide						
0	0	0	0	0	0	0
2	0.58±0.10	0	0.41±0.12	0	0	0
4	1.06±0.20	0	1.00±0.18	0	0	0
8	1.14±0.14	0.03±0.03	1.13±0.20	0.14±0.04	0.18±0.03	0.11±0.06
12	0.95±0.10	0.03±0.03	0.90±0.17	0.15±0.05	0.27±0.05	0.13±0.05
16	0.66±0.09	0	0.59±0.12	0.08±0.04	0.11±0.04	0.16±0.07
20	0.24±0.06	0	0.21±0.07	0.02±0.02	0	0.11±0.10
24	0.01±0.01	0	0.01±0.01	0	0	0
36	0	0	0	0	0	0
Albendazole sulphone						
0	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0.07±0.20	0	0.06±0.01	0.01±0.01	0	0
8	0.21±0.04	0	0.17±0.03	0	0.01±0.01	0.06±0.05
12	0.36±0.05	0.01±0.01	0.27±0.05	0.05±0.01	0.08±0.01	0.10±0.03
16	0.46±0.04	0	0.36±0.07	0.05±0.02	0.11±0.02	0.14±0.04
20	0.42±0.05	0.01±0.01	0.29±0.08	0.04±0.02	0.05±0.02	0.08±0.03
24	0.08±0.05	0	0.12±0.04	0.01±0.01	0	0
36	0	0	0	0	0	0
	0					

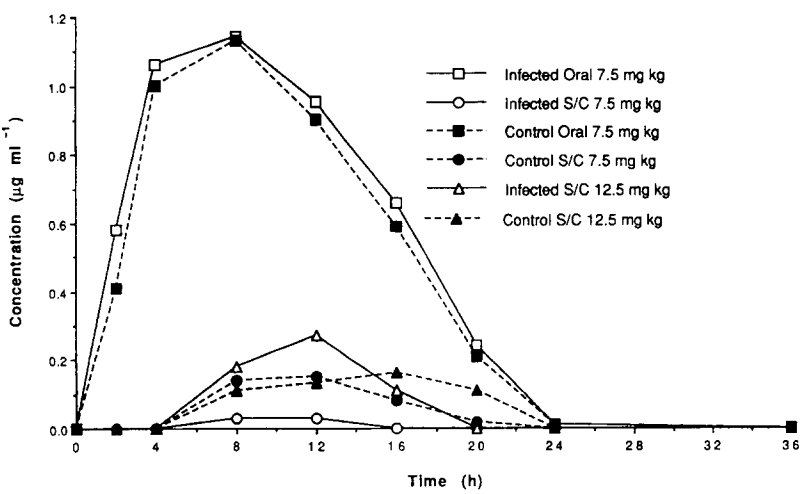

Fig. 3. Mean plasma albendazole sulfoxide concentrations ($\mu g\ ml^{-1}$) following oral administration of netobimin 7.5 mg kg^{-1} to parasitized lambs –□– (Group 3A), subcutaneous administration of netobimin 7.5 mg kg^{-1} to parasitized lambs –○– (Group 3B), oral administration of netobimin 7.5 mg kg^{-1} to control lambs ---■--- (Group 3C) and subcutaneous administration of netobimin 7.5 mg kg^{-1} to control lambs ---●--- (Group 3D). Plasma concentrations are also shown following subcutaneous administration of netobimin 12.5 mg kg^{-1} to parasitized lambs –△– (Group 4A) and control lambs ---▲--- (Group 4B).

TABLE 6

Mean ± SE pharmacokinetic parameters for levamisole, ivermectin and netobimin in lambs

Group	C_{max} ($\mu g\ ml^{-1}$)	t_{max} (h)	AUC ($\mu g\ mol^{-1}\ h^{-1}$)
Levamisole			
1A	0.82 ±0.10	2.42± 0.55	5.38±1.28
1B	1.67 ±0.24	0.50± 0.00	5.97±1.52
1C	0.84 ±0.20	1.83± 0.17	6.20±2.09
1D	1.41 ±0.58	0.58± 0.28	4.66±1.21
Ivermectin			
2A	0.021±0.005	20.00± 6.45	2.13±0.84
2B	0.035±0.011	38.17±17.30	4.20±0.93
2C	0.029±0.004	19.33± 7.41	1.79±0.26
2D	0.030±0.011	46.00± 8.44	2.44±0.73
Netobimin (albendazole sulfoxide) × 7.5 mg kg^{-1}			
3A	1.21 ±0.15	7.33± 1.23	16.49±2.09
3B	0.03 ±0.03	8.00± 0.00	0.28±0.28
3C	1.14 ±0.20	7.33± 0.67	15.23±2.71
3D	0.17 ±0.05	10.40± 0.89	1.65±0.49
Netobimin (albendazole sulfoxide) × 12.5 mg kg^{-1}			
4A	0.29 ±0.05	11.33± 0.67	2.26±0.38
4B	0.23 ±0.09	13.60± 1.46	2.08±0.90

as can be seen from the standard errors of the C_{max} data, and this was particularly apparent following subcutaneous administration to either infected or control lambs. Large inter-animal variation has been demonstrated previously for ivermectin following subcutaneous administration (Marriner et al., 1987) and the C_{max}, t_{max} and AUC data obtained in the present study are in broad agreement with previous reports (Prichard et al., 1985; Marriner et al., 1987; Fink and Porras, 1989). There were no significant differences ($P > 0.05$) in bioavailability of ivermectin in parasitized or non-parasitized animals following administration by either route.

Mean ± SEM concentrations of the major netobimin metabolites, albendazole sulfoxide and albendazole sulfone, in Groups 3A–3D and Groups 4A, 4B, are given in Table 5 and mean concentrations of albendazole sulfoxide are shown in Fig. 3. C_{max}, t_{max} and AUC data for albendazole sulfoxide are given in Table 6. The plasma concentrations of the sulfoxide metabolites were very similar in parasitized and non-parasitized animals following oral administration of netobimin.

The C_{max} was slightly higher and AUC lower for this metabolite than previously reported (Delatour et al., 1986). However the previous study was

carried out following a higher dose (8.4 mg kg^{-1}) in only one sheep. The sulfoxide metabolite had a similar plasma versus time profile following administration of netobimin as it does following albendazole (Marriner and Bogan, 1980). Albendazole sulfoxide and albendazole sulfone were detected only at very low concentrations following the subcutaneous administration of netobimin. Even when given parenterally at a dose rate of 12.5 mg kg^{-1} the AUC values in infected and housed animals were only 2.26 ± 0.38 and 2.08 ± 0.90 μg ml^{-1} h respectively compared with 16.49 ± 2.09 and 15.23 ± 2.71 for similar animals given 7.5 mg kg^{-1} orally. There was no significant difference between the AUC of netobimin given orally to parasitized and non-parasitized animals.

DISCUSSION

The present study indicates that the plasma kinetics of levamisole, ivermectin and netobimin are not affected significantly by the presence of moderate infections of the intestinal nematode *N. battus*.

Levamisole was highly effective against *N. battus* after both oral and subcutaneous administration. However, the maximum plasma concentration and AUC values following subcutaneous administration were lower than those demonstrated previously (Bogan et al., 1982) and this may reflect a difference in the preparations used, a difference in the breed of sheep, or a difference in the site of injection.

In the present study Wormguard Injection (Smith Kline) was used and the sheep were Greyface Suffolk Cross. In the study by Bogan et al. (1982) Nemicide (ICI) injection was used and the sheep were Finn–Dorset Cross. It is unlikely that differences in injection site were important since no significant differences were observed in the plasma concentrations of levamisole following subcutaneous administration in the neck, gluteal or thoracic regions (Bogan et al., 1982). In the present study the drug was administered in the neck.

Ivermectin was highly effective against *N. battus* when administered by both oral and subcutaneous routes. There is evidence from critical trials to suggest that ivermectin may be more effective when given orally for intestinal *Nematodirus* spp. (Benz et al., 1989) and this is surprising since in the present study the bioavailability of ivermectin was shown to be greater following subcutaneous administration. It seems likely, therefore, that maximum gastrointestinal concentrations are important for activity against gut parasites and that plasma pharmacokinetics may be less meaningful for this anthelmintic than for the benzimidazoles, where activity is thought to be related to prolonged plasma persistence (Prichard et al., 1978) and where parenteral administration may be equally or more effective than oral administration (Hennessy and Prichard, 1981). However, it is interesting that such differences in efficacy exist because very high concentrations of ivermectin are

known to be excreted in the bile and to be present in the duodenal and ileal fluid and mucus following subcutaneous administration (Bogan and McKellar, 1988).

Seven days after oral administration netobimin had reduced nematode faecal egg output by 98.72%. However in faecal samples collected 21 days after administration 43.17 (geometric mean) eggs per gram were demonstrated indicating only 89.04% reduction. There was not a statistically significant difference ($P > 0.05$ using Mann–Whitney) between the faecal egg counts at Day 7 or 14 and Day 21. However the relatively high number of eggs in faecal samples collected on Day 21 indicated either that netobimin temporarily inhibits egg output by *N. battus* females, or that it is not highly effective against early parasitic stages of *N. battus* which subsequently mature. Anthelmintics may inhibit egg production by nematodes (McKellar et al., 1988) and may have an ovicidal action on voided eggs (Niec et al., 1980). However the authors are unaware of any data to suggest that netobimin or its benzimidazole metabolites affect egg production by the parasites. It seems more likely that netobimin is not fully effective against the early parasitic stages of *N. battus*. Similar variable activity has been recorded for earlier benzimidazoles and probenzimidazoles such as thiabendazole and thiophanate, and for these drugs it is advisable to increase the dose rate when treating acute nematodiriasis (Anonymous, 1983). Netobimin was shown to be effective against third and fourth stage larvae of *N. battus* in one critical trial (Richards et al., 1987). However the number of immature parasites in the control groups from this study were very small and further data would be useful to determine the efficacy of netobimin against immature *N. battus*. It would also be interesting to examine the efficacy of other benzimidazoles against early parasitic stages of *N. battus* to determine whether all members of the group have such variable activity.

Netobimin was not effective when given by the subcutaneous route. This was probably because of the very low concentrations of the albendazole metabolites achieved following administration by this route. Netobimin is metabolized to active moieties following subcutaneous administration in cattle (Steel and Hennessy, 1987) and is effective against pulmonary and intestinal nematodes when administered by this route in cattle (Duncan et al., 1985). The reason for the reduced concentrations of albendazole metabolites detected in the plasma of sheep in the present study is unknown. It may be that the parenteral cattle formulation of netobimin is not absorbed from the subcutaneous site of administration in sheep, or it may be that it is metabolized and excreted by different pathways following parenteral administration. The latter explanation seems more likely since Lanusse and Prichard (1990) have recently demonstrated more efficient conversion of netobimin to albendazole metabolites in the gastrointestinal tract following oral rather than subcutaneous administration.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. M. Michael for technical assistance and the farm staff at Firth Mains for assistance with animals.

REFERENCES

Anderson, N., Armour, J. Jarrett, W.H.F., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M., 1965. A field study of parasitic gastritis in cattle. Vet. Rec., 77: 1196–1204

Armour, J., Jarrett, W.H.F. and Jennings, F.W., 1966. Experimental *Ostertagia circumcincta* infections in sheep: development and pathogenesis of a single infection. Am. J. Vet. Res., 27: 1267–1278.

Anonymous, 1983. Anthelmintics for cattle, sheep, pigs and horses. MAFF Publications, Booklet, 2412(83): 33–51.

Benz, G.W., Roncalli, R.A. and Gross, S.J., 1989. Use of ivermectin in cattle, sheep, goats and swine. In: W.C. Campbell (Editor), Ivermectin and Abamectin. Springer-Verlag, New York, NY, pp. 215–230.

Bogan, J.A. and Marriner, S.E., 1980. Analysis of benzimidazoles in body fluids by high performance liquid chromatography. J. Pharm. Sci., 69: 422–423.

Bogan, J.A. and McKellar, Q.A., 1988. The pharmacodynamics of ivermectin in sheep and cattle. J. Vet. Pharmacol. Ther., 11: 260–268.

Bogan, J.A., Marriner, S.E. and Galbraith, E.A., 1982. Pharmacokinetics of levamisole in sheep. Res. Vet. Sci., 32: 124–126.

Christie, M.G. and Jackson, F., 1982. Specific identification of strongyle eggs in small samples of sheep faeces. Res. Vet. Sci., 32: 113–117.

Coop, R.L., Angus, K.W. and Mapes, C.J., 1973. The effect of large doses of *Nematodirus battus* on the histology and biochemistry of the small intestine of lambs. Int. J. Parasitol., 3: 349–361.

Delatour, P., Cure, M.C., Benoit, E. and Garnier, F., 1986. Netobimin (Totabin-SCH): preliminary investigations on metabolism and pharmacology. J. Vet. Pharmacol. Ther., 9: 230–234.

Downey, N.E., 1987. Action of netobimin in drinking water on nematode parasites in calves. Vet. Rec., 121: 275.

Duncan, J.L., Armour, J. and Bairden, K., 1985. Netobimin (Totabin-SCH). Efficacy in ruminants in U.K. In: Proceedings of the 11th Conference of the World Association for the Advancement of Veterinary Parasitology, Rio de Janeiro Abstract No. 135: 31.

Fink, D.W. and Porras, A.G., 1989. Pharmacokinetics of ivermectin in animals and humans. In: W.C. Campbell (Editor), Ivermectin and Abamectin. Springer-Verlag, New York, pp. 113–131.

Galtier, P., Larrier, G., Tufenkji, A.E. and Franc, M., 1986. Incidence of experimental fascioliasis on the activity of drug-metabolising enzymes in lamb liver. Drug Metab. Dispos., 14: 137–141.

Gregory, P.C., Wenham, G., Poppi, D., Coop, R.L., Macrae, J.C. and Miller, S.J., 1985. The influence of a chronic sub-clinical infection of *Trichostrongylus colubriformis* on gastrointestinal motility and digesta flow in sheep. Parasitology, 91: 381–396.

Hennessy, D.R. and Prichard, R.K., 1981. The role of absorbed drug in the efficacy of oxfendazole against gastrointestinal nematodes. Vet. Res. Commun., 5: 45–49.

Herd, R.P., Schwarz, W.R. and Heider, L.E., 1985. Netobimin (Totabin-SCH). Efficacy in Ruminants in Ohio, U.S.A. In: Proceedings of the 11th Conference of the World Association for the Advancement of Veterinary Parasitology, Rio de Janeiro, Abstract No. 129: 30.

Lanusse, C.E. and Prichard, R.K., 1990. Pharmacokinetic behaviour of netobimin and its metabolites in sheep. J. Vet. Pharmacol. Ther., 13: 170–178.

M.A.F.F., 1986. Manual of Veterinary Parasitological Techniques. Reference Book 418, HMSO 3rd edn., pp. 36–37.

Marriner, S.E. and Bogan, J.A., 1980. Pharmacokinetics of albendazole in sheep. Am. J. Vet. Res., 41: 1126–1129.

Marriner, S.E., Galbraith, E.A. and Bogan, J.A., 1980. Determination of the anthelmintic levamisole in plasma and gastro-intestinal fluids by high performance liquid chromatography. Analyst, 105: 993–996.

Marriner, S.E., Evans, E.S. and Bogan, J.A., 1985. Effect of parasitism with *Ostertagia circumcincta* on pharmacokinetics of fenbendazole in sheep. Vet. Parasitol., 17: 239–249.

Marriner, S.E., McKinnon, I. and Bogan, J.A., 1987. The pharmacokinetics of ivermectin after oral and subcutaneous administration to sheep and horses. J. Vet. Pharmacol. Ther., 10: 175–179.

McKellar, Q.A., Bogan, J.A., Horspool, L. and Reid, K., 1988. Effect of ivermectin on the reproductive potential of *Cooperia curticei*. Vet. Rec., 122: 444.

Ngomuo, A.J., 1983. Pharmacokinetic studies of fenbendazole and oxfendazole in cattle. M.V.M. Thesis, University of Glasgow, pp. 72–89.

Niec, R., Lukovich, R., Bonazzie, E., Eddi, C., Nurez, J.L., Cobenas, M.E.M. and Moltedo, H.L., 1980. Ovicidal action of oxfendazole on sheep nematodes. Vet. Rec., 107: 248–249.

Prichard, R.K., 1980. Host influences on the efficacy of benzimidazole anthelmintics. In: Van den Bossche (Editor), The Host Invader Interplay. Elsevier, Amsterdam, pp. 731–734.

Prichard, R.K., 1985. Interaction of host physiology and efficacy of antiparasitic drugs. Vet. Parasitol., 18: 103–110.

Prichard, R.K., Hennessy, D.R. and Steel, J.W., 1978. Prolonged administration: a new concept for increasing the spectrum of effectiveness of anthelmintics. Vet. Parasitol., 4: 309–315.

Prichard, R.K., Steel, J.W., Lacey, E. and Hennessy, D.R., 1985. Pharmacokinetics of ivermectin in sheep following intravenous, intra-abomasal or intraruminal administration. J. Vet. Pharmacol. Ther., 8: 88–91.

Richards, L.S., Zimmerman, G.L., Hoberg, E.P., Schons, D.J. and Dawley, S.W., 1987. The anthelmintic efficacy of netobimin against naturally acquired gastrointestinal nematodes in sheep. Vet. Parasitol., 26: 87–94.

Roberson, E.L., 1977. Antinematodal drugs. In: N.N. Booth and L.E. McDonald (Editors), Veterinary Pharmacology and Therapeutics, Ames, Iowa State University Press, IA, 5th edn; pp. 803–851.

Scott, E.W., Kinabo, L.D. and McKellar, Q.A., 1990. Pharmacokinetics of ivermectin after oral or percutaneous administration to adult milking goats. J. Vet. Pharmacol. Ther., 13: 432–435.

Steel, J.W. and Hennessy, D.R., 1987. Dose response pharmacokinetics and metabolism of parenterally administered netobimin in cattle. In: Proceedings of the 12 Conference of the World Association for the Advancement of Veterinary Parasitology, Montreal, Abstract No. 11B-2: 39.

Steel, J.W., Hennessy, D.R. and Lacey, E., 1985. Netobimin (Totabin-SCH) metabolism and pharmacokinetics in sheep. In: Proceedings of 11th Conference of the World Association for the Advancement of Veterinary Parasitology, Rio de Janeiro, Abstract No. 166: 38.

Taylor, E.L., 1939. Technique for the estimation of pasture infestation by strongyloid larvae. Parasitology, 31: 473–478.

Townsend, L.B. and Wise, D.S., 1990. The synthesis and chemistry of certain anthelmintic benzimidazoles. Parasitol. Today, 6: 107–112.

Tufenkji, A.E., Alvinerie, M., Pineau, T., Boulard, C. and Galtier, P., 1988. Incidence of a subclinical fascioliasis on antipyrine clearance and metabolite excretion in sheep. Xenobiotica, 18: 357–364.