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# Interaction between Fenbendazole and Piperonyl Butoxide: Pharmacokinetic and Pharmacodynamic Implications

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## Abstract

The effect of the cytochrome P450 inhibitor, piperonyl butoxide on the pharmacokinetics and anthelmintic efficacy of the benzimidazole compound fenbendazole was studied in sheep and goats.

Pretreatment of goats with the inhibitor caused a greater than three-fold increase in the relative bioavailability of fenbendazole and fenbendazole sulphoxide. A pharmacokinetic dose titration study was carried out in sheep with fenbendazole (5 mg $kg^{-1}$) and piperonyl butoxide administered orally at 0, 15, 31, 63, 125 and 250 mg $kg^{-1}$. The AUC of fenbendazole and the sulphoxide were significantly increased when fenbendazole was co-administered with piperonyl butoxide at dose rates equal to or higher than 31 mg $kg^{-1}$. Peak plasma concentrations ($C_{max}$) and mean residence time (MRT) were also significantly increased.

The efficacy of the combination was assessed in sheep against two species of benzimidazole-resistant abomasal nematodes; *Ostertagia circumcincta* and *Haemonchus contortus*. The percentage reduction in the total number of *O. circumcincta* worms was 7·9% (fenbendazole) and 97·8% (fenbendazole-piperonyl butoxide). For *H. contortus*, the percentage reduction was 84·8% (fenbendazole) and 99·0% (fenbendazole-piperonyl butoxide).

The in-vitro *S*-oxidation of fenbendazole and fenbendazole sulphoxide was studied using microsomal preparations from rat liver. Piperonyl butoxide inhibited significantly the sulphoxidation and sulphonation of fenbendazole.

It was concluded that piperonyl butoxide inhibited the oxidative conversion of fenbendazole into inactive metabolites and this resulted in a potentiated anthelmintic action.

Fenbendazole is a thiosubstituted benzimidazole widely used in veterinary medicine for the control and treatment of helminth infections (Campbell 1990). In the UK, fenbendazole is licensed for use in dogs, cats, horses, sheep, goats, cattle and birds either alone or in combination with other anthelmintic molecules. The increasing problem of nematode benzimidazole-resistance in sheep and goat flocks, and the slow introduction onto the market of novel anthelmintics has led to diverse attempts to preserve the efficacy of benzimidazole derivatives. Previous attempts to reduce the number of benzimidazole-resistant gastrointestinal nematodes in sheep and goats by increasing the dose have shown marginal increases in efficacy (Sangster et al 1991; Jackson et al 1993). Dividing the dose over an extended period has shown some success but is not practical when a large number of animals is to be treated. Another alternative, which is considered in the present paper, is the inhibition of benzimidazole metabolism. A number of studies have been published where benzimidazole potentiation has been investigated. In man, the clinical efficacy of mebendazole (Bekhti & Pirotte 1987) and albendazole (Wen et al 1994) in the treatment of echinococcosis was enhanced by concomitant administration of cimetidine. This potentiation was believed to be the result of higher and more persistent concentrations of active drug or metabolites achieved as a consequence of benzimidazole metabolism inhibition by cimetidine. The potentiation of fenbendazole sulphoxide by combination with another benzimidazole, parbendazole, resulted in a higher efficacy against benzimidazole-resistant gastrointestinal nematodes (Hennessy et al 1985). Sulphur-containing benzimidazole derivatives are extensively metabolized in the liver to form sulphoxide, sulphone and hydroxylated metabolites (Fig. 1). However, the hydroxy-metabolites are excreted directly in the bile as free or conjugated metabolites and are therefore only detected to a limited extent, if at all, in blood (Short et al 1987a, b; Hennessy et al 1993). Activity against abomasal nematodes is attributed to the parent drugs and the sulphoxide metabolites. Inhibition of sulphonation and hydroxylation can therefore increase the plasma concentration of and prolong the parasite exposure to the pharmacologically active moieties.

The involvement of hepatic microsomal cytochrome P450 and flavine monooxygenase enzyme systems in the *S*-oxidation and hydroxylation of fenbendazole have been previously reported by Murray et al (1992). There is evidence from in-vitro inhibition studies that the sulphoxidation of fenbendazole involves both the flavine-monooxygenase and the cytochrome P450 enzyme systems whereas for the sulphonation and hydroxylation, evidence only exists to implicate cytochrome P450. The P4501A subfamily has been shown to be induced by repeated treatments with fenbendazole sulphoxide (Gleizes et al 1991a, b). The flavine monooxygenase inhibitor methimazole resulted in inhibition of fenbendazole sulphoxidation both in-vitro and in-vivo (Murray et al 1992; Lanusse et al 1995). The participation of the cytosolic fraction in the metabolism of fenbendazole has not been explored so far, although Short et al (1988) who studied the in-vitro oxidation of fenbendazole using *S*9 fractions from different animal species, considered it possible. In the present study, we report the effect of piperonyl butoxide, an inhibitor of cytochrome P450 (Franklin 1977), on

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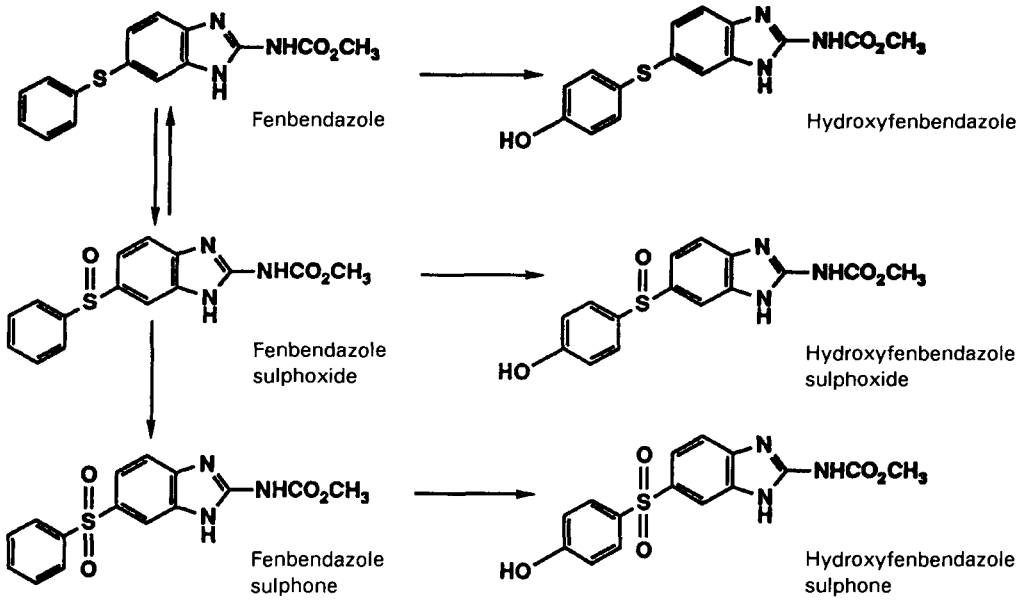


FIG. 1. Structure of fenbendazole and it's metabolites.

the pharmacokinetic and anthelmintic efficacy of fenbendazole in ruminants and the in-vitro *S*-oxidative metabolism of fenbendazole in rat liver microsomes.

## Materials and Methods

*Chemicals*

Fenbendazole, fenbendazole sulphoxide and fenbendazole sulphone were obtained from Hoechst Ltd (Gebaude, Germany). Piperonyl butoxide was purchased from Aldrich Chemicals Ltd (UK). Other chemicals and biochemicals were bought from commercial sources.

*Animals*

Scottish Blackface sheep, 25–29 kg, and mixed breed goats, 24–44 kg, were used for the pharmacokinetic studies. Parasite-naive Suffolk cross sheep, 27–44 kg, were used for the efficacy trial. Animals were maintained indoors with hay and water freely available and cereal concentrate (0·5 kg) twice a day. Female Sprague–Dawley rats, 300–400 g, were used as a source of liver tissue for in-vitro drug metabolism studies.

*Pharmacokinetic studies*

*Experiment 1.* Goats were given fenbendazole (Panacur 2·5%) (7·5 mg $kg^{-1}$) alone or 1 hour after intramuscular administration of piperonyl butoxide (500 mg $kg^{-1}$). Blood samples were taken before drug administration and 0·25, 0·5, 1, 2, 4, 8, 12, 32, 48, 72 and 96 hours thereafter.

*Experiment 2.* A 6-way crossover dose titration study was carried out in sheep where a fixed dose of fenbendazole (5 mg $kg^{-1}$) was administered alone or with an oral dose of piperonyl butoxide given at 15, 31, 63, 125 and 250 mg $kg^{-1}$ body weight. In a pilot study piperonyl butoxide was given orally to sheep at a dose rate of 500 mg $kg^{-1}$ and blood samples were taken at different times post-administration.

*Efficacy study*

Twenty-four parasite-naive lambs were allocated into four groups (A, B, C and D) of six animals each. Each lamb received an oral dose of 6000 infective larvae [third stage larvae (L3)] of bemzimidazole-resistant *Ostertagia circumcincta* and 2400 benzimidazole-resistant *Haemonchus contortus* (both nematode strains were obtained from the Moredun Institute, Edinburgh, UK). Twenty-eight days after infection, group B was treated with fenbendazole at the recommended dose rate (5 mg $kg^{-1}$), group C received piperonyl butoxide at the selected dose rate of 63 mg $kg^{-1}$ body weight, group D was treated with the combination fenbendazole–piperonyl butoxide (5–63 mg $kg^{-1}$) and animals in group A were left untreated as controls. Seven days after treatment, faecal samples were taken, all animals were killed and their abomasa collected for parasite counting.

*In-vitro metabolism studies*

Sulphoxidation and sulphonation of fenbendazole and fenbendazole sulphoxide were studied using liver microsomal preparations. Microsomes were prepared according to the method described by Rutten et al (1987). Microsomal incubations were carried out aerobically in a shaking water bath at 37°C for a period of 1 hour. The incubation mixture contained 4 mg of microsomal protein, the substrate dissolved in 5 μL of dimethylsulphoxide (DMSO) and added at a final concentration of 50 μM, a NADPH-generating system consisting of 1 mM $NADP^+$, 0·15 M $MgCl_2$, isocitrate dehydrogenase (0·2 int. units $mL^{-1}$) and 15 mM trisodium isocitrate made up in a final volume of 1 mL 0·1 M Tris buffer (pH 7·4). Piperonyl butoxide was dissolved in DMSO and used at a final concentration of 100 μM. Microsomal proteins were measured according to the method of Lowry et al (1951). Cytochrome P450 was measured according to the method described by Omuro and Sato (1964). Tubes without microsomes were used as controls for possible non-enzymatic drug conversion.

Piperonyl butoxide was added as required. All microsomal incubations were carried out in triplicate.

*Drug analysis*

Benzimidazole analysis was by HPLC following solvent extraction. Plasma samples and microsomal mixtures were analysed following the same procedure. To 1 mL of sample were added 200 μL of 0·1 M ammonium hydroxide, 0·2 g sodium chloride and 6 mL chloroform. After shaking for 10 min on a slow rotary mixer, the samples were centrifuged for 20 min at 1700 *g*. The supernatant was carefully removed and discarded and 4 mL of the organic phase were transfered to a 10-mL thin-walled tube. Samples were evaporated to dryness under nitrogen, reconstituted in 150 μL methanol then processed on the HPLC system. Piperonyl butoxide was extracted following plasma precipitation. Briefly, 0·5 mL plasma was mixed with 1 mL acetonitrile. Four millilitres of chloroform were added and the tube was shaken on a slow rotary mixer. After centrifugation, the organic phase was transferred to conical tubes and evaporated to dryness. Samples were processed on a computerized (PC 1000, Spectra-Physics Analytical Inc., UK) HPLC system comprising a gradient pump (model P4000), a UV-detector (Spectra-Focus) set at 292 nm, an autosampler (model AS 3000) and a controller (model SN 4000). The mobile phase was a mixture of acetonitrile-water to which glacial acetic acid was added (0·5%, v/v). It was pumped through the column (C18 Nucleosil 5, 10 cm × 4·6 mm, HPLC Technology Ltd., Cheshire, UK) in a linear gradient fashion changing from 35 : 65 (acetonitrile : water) to 82 : 18 for 8 min, 82 : 18 to 35 : 65 for 1 min and the last ratio was maintained for 3 min. The flow rate was 1·5 mL $min^{-1}$. The retention times were 1·60 min (fenbendazole sulphoxide), 2·60 min (fenbendazole sulphone) and 5·0 min (fenbendazole). Mean recoveries achieved with a concentration range of 0·05–1·0 μg $mL^{-1}$ were 90·49% (inter-assay CV = 8·03%) for fenbendazole, 89·29% (inter-assay CV = 5·24%) for fenbendazole sulphoxide and 89·24% (inter-assay CV = 6·15%) for fenbendazole sulphone.

*Pharmacokinetic and statistical analyses*

Pharmacokinetic parameters were calculated from observed values (time zero to the last sampling time). The area under the concentration-time curve and derivatized values were determined using the trapezoidal rule.

Pharmacokinetic data were analysed using a Wilcoxon signed rank test. A Mann–Whitney *U* test was used for the efficacy trial data analysis. In-vitro results were compared using analysis of variance. Differences were considered significant when $P < 0.05$.

## Results

*Fenbendazole in goats*

Mean pharmacokinetic parameters for fenbendazole and its metabolites are shown in Tables 1, 2 and 3. The plasma profiles of fenbendazole metabolites with and without piperonyl butoxide pretreatment are shown in Fig. 2. The $C_{max}$ values for fenbendazole, fenbendazole sulphoxide and fenbendazole sulphone were increased by 137, 160 and 50%, respectively, when piperonyl butoxide was given before fenbendazole administration. Areas under the plasma concentrations versus time curves and MRT values were significantly increased ($P < 0.05$) for fenbendazole and metabolites with piperonyl butoxide pretreatment.

Table 1. Pharmacokinetic parameters of fenbendazole in goats following administration of fenbendazole (7·5 mg $kg^{-1}$).

| | Fenbendazole alone | Fenbendazole with piperonyl butoxide |
|---|---|---|
| $AUC_{obs}$ (μg h $mL^{-1}$) | 4·76 ± 0·65 | 15·6 ± 2·52* |
| $AUMC_{obs}$ (μg $h^2$ $mL^{-1}$) | 88·8 ± 16·0 | 423 ± 89·7* |
| MRT (h) | 18·4 ± 1·03 | 25·9 ± 1·85* |
| $C_{max}$ (μg $mL^{-1}$) | 0·19 ± 0·02 | 0·45 ± 0·07 |
| $T_{max}$ (h) | 8·00 (8·00–12·0) | 15·0 (8·00–24·0) |

Parameters are presented as mean ± s.e.m except for $T_{max}$ (median (range)). *Significantly different from fenbendazole alone ($P < 0.05$).

Table 2. Pharmacokinetic parameters of fenbendazole sulphoxide in goats following administration of fenbendazole (7·5 mg $kg^{-1}$).

| | Fenbendazole alone | Fenbendazole with piperonyl butoxide |
|---|---|---|
| $AUC_{obs}$ (μg h $mL^{-1}$) | 7·72 ± 0·99 | 24·4 ± 3·45* |
| $AUMC_{obs}$ (μg $h^2$ $mL^{-1}$) | 186 ± 28·57 | 773 ± 143* |
| MRT (h) | 23·9 ± 1·01 | 30·7 ± 2·02* |
| $C_{max}$ (μg $mL^{-1}$) | 0·25 ± 0·03 | 0·65 ± 0·08* |
| $T_{max}$ (h) | 24·0 (18·0–24·0) | 26·0 (24–32) |

Parameters are presented as mean ± s.e.m except for $T_{max}$ (median (range)). *Significantly different from fenbendazole alone ($P < 0.05$).

Table 3. Pharmacokinetic parameters of fenbendazole sulphone in goats following administration of fenbendazole (7·5 mg $kg^{-1}$).

| | Fenbendazole alone | Fenbendazole with piperonyl butoxide |
|---|---|---|
| $AUC_{obs}$ (μg h $mL^{-1}$) | 3·02 ± 0·40 | 6·03 ± 0·68* |
| $AUMC_{obs}$ (μg $h^2$ $mL^{-1}$) | 104 ± 15·9 | 262 ± 29·2* |
| MRT (h) | 34·4 ± 1·52 | 43·9 ± 3·20* |
| $C_{max}$ (μg $mL^{-1}$) | 0·08 ± 0·01 | 0·12 ± 0·01 |
| $T_{max}$ (h) | 30 (24–32) | 36 (12–48) |

Parameters are presented as mean ± s.e.m. except for $T_{max}$ (median (range)). *Significantly different from fenbendazole alone ($P < 0.05$).

*Dose titration in sheep*

The AUC of fenbendazole, fenbendazole sulphoxide and fenbendazole sulphone increased linearly with increasing doses of piperonyl butoxide (Fig. 3) ($r^2 = 0.98$ for fenbendazole, 0·93 for fenbendazole sulphoxide and 0·95 for fenbendazole sulphone). However, when the dose of piperonyl butoxide administered was 250 mg $kg^{-1}$, the AUC of fenbendazole and fenbendazole sulphoxide showed a 4-fold increase from the control value, while that of the sulphone metabolite only doubled therefore decreasing the ratio AUC (fenbendazole sulphone)/AUC (fenbendazole) from the control value of 0·79 to 0·33 and the ratio AUC (fenbendazole sulphone) (fenbendazole sulphoxide) from 0·56 to 0·29. The MRT of fenbendazole sulphone increased significantly ($P < 0.05$) when fenbendazole was given with doses of piperonyl butoxide equal to or higher than 15 mg $kg^{-1}$ (Table 4).

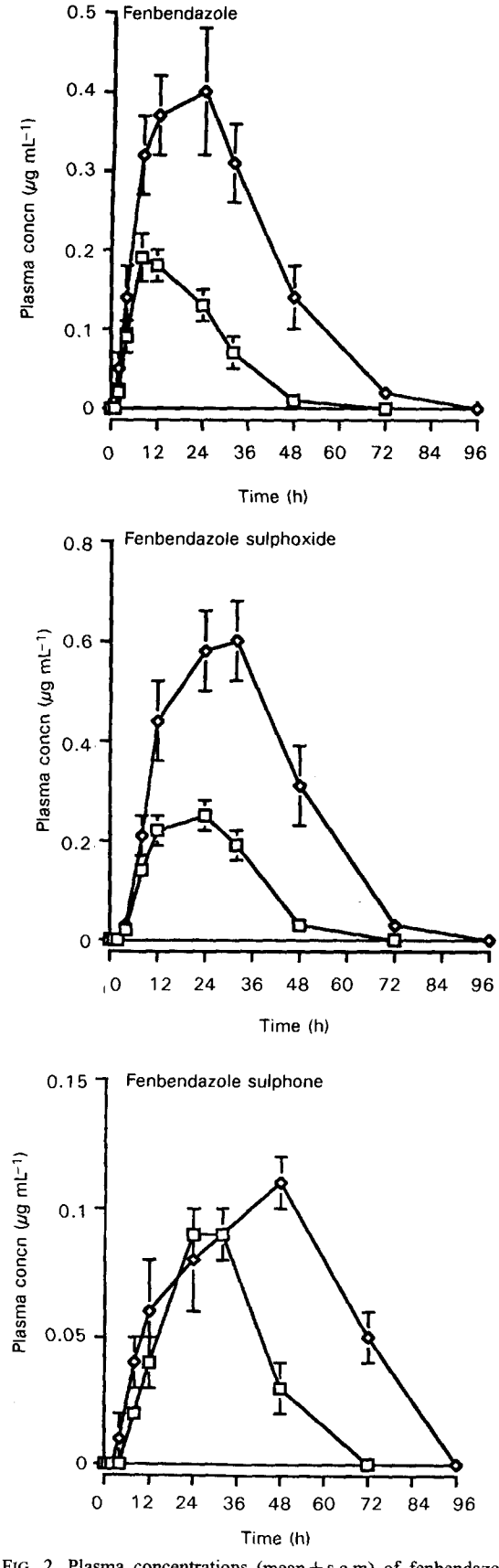


FIG. 2. Plasma concentrations (mean ± s.e.m) of fenbendazole, fenbendazole sulphoxide and fenbendazole sulphone following administration of fenbendazole alone (n = 6) (□) or with piperonyl butoxide (n = 6) (◇) in goats.

*Efficacy study*

The results of the efficacy trial are reported in Table 5. Piperonyl butoxide, given on its own had no effect on nematode infection. Fenbendazole given alone was not effective (less than 8% efficacy) in reducing the number of benzimidazole-resistant *0. circumcincta*, the administration of the combination showed a 98% efficacy against this strain. The combination was also more effective against benzimidazole-resistant *H. contortus* (99% reduction) than the conventional treatment (85% reduction). Only one animal showed a positive faecal egg count after treatment with the combination.

*In-vitro S-oxidation of benzimidazole*

The mean cytochrome P450 concentration in the microsomal preparations was 0·53 ± 0·05 nmols $mg^{-1}$.

The results of the in-vitro fenbendazole metabolism are displayed in Fig. 4. In the incubation mixtures, fenbendazole was converted to sulphoxide and sulphone metabolites. The conversion was slow with only 16% of the substrate being transformed into *S*-oxide metabolites. When fenbendazole sulphoxide was incubated for 1 hour it was only partially converted to fenbendazole sulphone (13%).

Sulphoxidation and sulphonation of fenbendazole were decreased by 65 and 67%, respectively, when fenbendazole was incubated with piperonyl butoxide. Also, the amount of unchanged fenbendazole was significantly higher with metabolic inhibition. Conversion of fenbendazole sulphoxide into fenbendazole sulphone was diminished by 53% when fenbendazole sulphoxide was incubated with piperonyl butoxide. Minimal reduction of fenbendazole sulphoxide into fenbendazole occurred and this was not affected by inhibition of cytochrome P450 with piperonyl butoxide (Fig. 4B).

## Discussion

The alteration of drug oxidative metabolism by pretreatment with piperonyl butoxide resulted in significant changes in the pharmacokinetic patterns of fenbendazole in sheep and goats. More than 3-fold increases in the AUC of fenbendazole and fenbendazole sulphoxide were observed in goats following piperonyl butoxide pretreatment. In sheep, the response to metabolic inhibition expressed in the alteration of fenbendazole metabolite bioavailability, increased with increasing doses of piperonyl butoxide and the linear increase observed in the AUC indicates the absence of saturation kinetics within the dosage range studied. The increase in the AUC of the end product fenbendazole sulphone was unexpected and could be the result of three mechanisms: firstly, piperonyl butoxide could divert the benzimidazole metabolism from the hydroxylation pathway in favour of the *S*-oxidation, this would lead to the production of lower levels of hydroxyfenbendazole and hydroxyfenbendazole sulphoxide resulting in higher substrate (fenbendazole and fenbendazole sulphoxide) concentrations available for sulphonation. The decreased fenbendazole sulphone/fenbendazole sulphoxide ratio suggests however that sulphonation is also inhibited by piperonyl butoxide. Secondly, it is reported that piperonyl butoxide causes cytochrome P450 inhibition for a period of 24 to 72 hours, after which an induction can occur (Skrinjaric-Spoljar et al 1971); this could explain the delay in the $T_{max}$ and the increase in AUC and

Table 4. Mean residence times (MRT), maximum concentrations ($C_{max}$) and times to maximum concentrations ($T_{max}$) of fenbendazole and its sulphoxide and sulphone metabolites following administration of fenbendazole (5 mg $kg^{-1}$) with different dose rates of piperonyl butoxide in sheep.

| | Dose of piperonyl butoxide (mg $kg^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 15 | 31 | 63 | 125 | 250 |
| Fenbendazole | | | | | | |
| MRT (h) | 25·5 ± 2·02 | 29·2 ± 1·36 | 29·3 ± 1·92 | 27·6 ± 2·56 | 29·3 ± 2·14 | 37·0 ± 1·71* |
| $C_{max}$ (μg $mL^{-1}$) | 0·24 ± 0·02 | 0·20 ± 0·00 | 0·35 ± 0·05 | 0·35 ± 0·03* | 0·57 ± 0·09* | 0·66 ± 0·05* |
| $T_{max}$ (h) | 9·00 (4·00–24·0) | 10·0 (2·00–32·0) | 18·0 (8·00–48·0) | 8·00 (4·00–24·0) | 24·0 (12·0–24,0) | 32·0 (24·0–48·0)* |
| Fenbendazole sulphoxide | | | | | | |
| MRT (h) | 28·7 ± 1·61 | 32·5 ± 1·10* | 33·8 ± 0·85* | 29·4 ± 4·03 | 33·8 ± 2·1 1 | 39·7 ± 1·60* |
| $C_{max}$ (μg $mL^{-1}$) | 0·27 ± 0·03 | 0·26 0·02 | 0·42 ± 0·05* | 0·53 ± 0·04* | 0·79 ± 0·10* | 0·79 ± 0·08* |
| $T_{max}$ (h) | 24·0 (8·00–32·0) | 24·0 (12·0–32·0) | 28·0 (24·0–48·0) | 24·0 (24·0–32·0) | 24·0 (24·0–32·0) | 32·0 (24·0–48·0) |
| Fenbendazole sulphone | | | | | | |
| MRT (h) | 46·7 ± 1·74 | 49·6 ± 2·35 | 49·8 ± 0·98 | 51·3 ± 2·14 | 54·4 ± 1·87* | 58·8 ± 1·19* |
| $C_{max}$ (μg $mL^{-1}$) | 0·11 ± 0·01 | 0·11 ± 0·01 | 0·13 ± 0·02 | 0·13 ± 0·01 | 0·17 ± 0·02* | 0·20 ± 0·02 |
| $T_{max}$ (h) | 32·0 (24·0–48·0) | 44·0 (32·0–72·0) | 36·0 (32·0–48·0) | 48·0 (24·0–48·0) | 48·0 (32·0–48·0) | 72·0 (48·0–72·0) |

Mean ± s.e.m., except for $T_{max}$ which is median range. *$P < 0.05$ compared with fenbendazole alone.

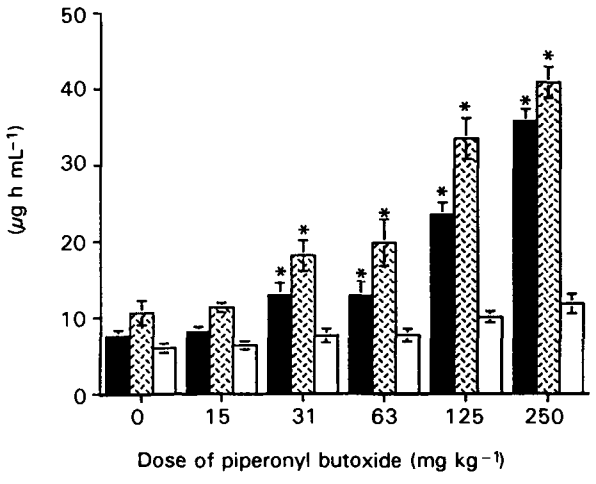


FIG. 3. Area under the plasma concentration–time curve (AUC) values of fenbendazole metabolites at various dose rates of piperonyl butoxide. *Significantly different from control values ($P < 0.05$). Fenbendazole (▨); fenbendazole sulphoxide (■); fenbendazole sulphone (□).

MRT of fenbendazole sulphone (Fig. 2). Thirdly, piperonyl butoxide could increase the gastro-intestinal absorption of fenbendazole; the properties of piperonyl butoxide as a possible enhancer of drug absorption are so far unexplored and the confirmation or rejection of this hypothesis require further investigation.

The pharmacokinetic behaviour of fenbendazole was similar to that described by Marriner & Bogan (1981) with fenbendazole sulphoxide being the main metabolite in plasma. A direct comparison of fenbendazole disposition in sheep with that observed in goats was not possible given the difference in dosage rates, it is of interest, however, that taking sheep as the reference species, the relative bioavailability corrected for dose achieved in goats was only 42%. Fenbendazole bioavailability was also found to be lower in goats than in cattle (Short et al 1987a,b).

*Table 5. Worm and faecal egg counts (geometric means with range) in control and treated sheep.*

| | *O. Circumcincta* | *H. Contortus* | Faecal egg counts |
|---|---|---|---|
| Group A | 2911 (1750–3610) | 340 (290–450) | 438 (250–900) |
| Group B | 2681 (1750–5400) | 52 (20–100)** | 28 (0–200)*** |
| Group C | 3760 (3000–4250) | 297 (160–520) | 373 (250–1000) |
| Group D | 65 (0–1500)†† | 4 (0–20)***,† | 2 (0–50)*** |

Group A (control), n = 6; Group B (fenbendazole), n = 6; Group C (piperonyl butoxide), n = 6; Group D (fenbendazole-piperonyl butoxide), n = 6; ††$P < 0.01$ (different from group A, B and C); **$P < 0.01$ (different from group A and C); ***$P < 0.005$ (different from group A and C); †$P < 0.01$ (different from group B).

The increased AUC of fenbendazole and its active metabolite fenbendazole sulphoxide following concomitant administration of fenbendazole and piperonyl butoxide markedly improved the efficacy of the benzimidazole drug against benzimidazole-resistant strains of *O. circumcincta*. Indeed, while it was almost totally refractory to fenbendazole given alone, this strain was highly susceptible to the combination ($P < 0.01$). The *Haemonchus* strain did not show a high degree of resistance since fenbendazole given alone at the recommended dose rate significantly removed this nematode ($P < 0.01$). Nevertheless, the coadministration of piperonyl butoxide with fenbendazole significantly improved the removal of *H. contortus* ($P < 0.01$). The faecal egg counts were significantly reduced by both fenbendazole and fenbendazole with piperonyl butoxide; the reduction, although much higher with the combination was not significantly different ($P = 0.06$) from that attributed to the benzimidazole given alone. Taking into account the much higher fecundity of *H. contortus* in comparison to *O. circumcincta*, a greater difference in terms of faecal egg production would have been

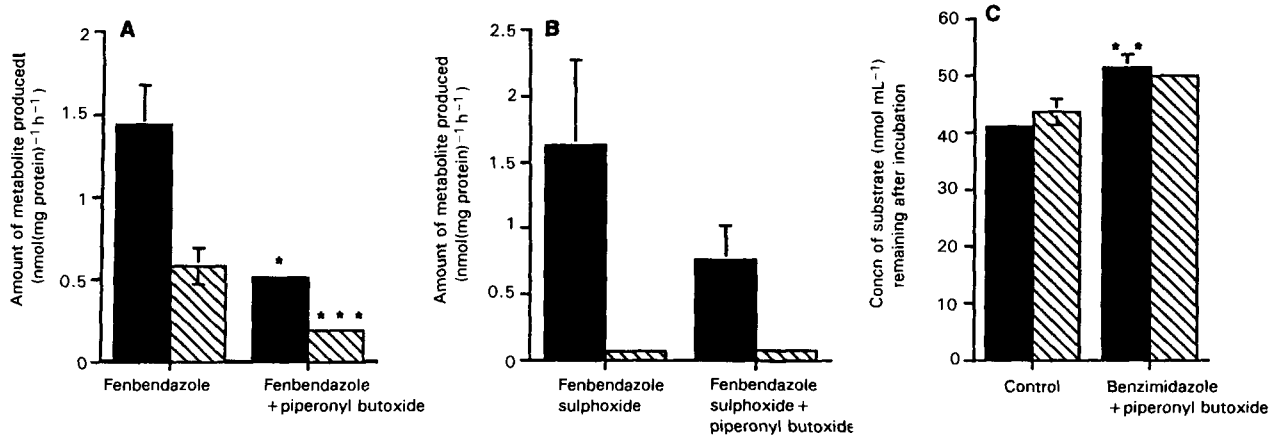


FIG. 4. The effect of piperonyl butoxide on benzimidazole in-vitro *S*-oxidation in rat liver microsomes. A. Metabolites (■ sulphoxide, ▧ sulphone) formed after fenbendazole incubation (n = 4). B. Metabolites (■ sulphone, ▧ fenbendazole) formed after fenbendazole sulphoxide incubation (n = 3). C. (■ fenbendazole, ▧ sulphoxide). Unchanged substrates remaining after incubation (A) and (B). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

expected between the group that received fenbendazole alone and the one that received the drug combination, had the level of resistance of *H. contortus* been higher. Piperonyl butoxide given alone had no effect on worm burden or faecal egg excretion and therefore the interaction between fenbendazole and piperonyl butoxide is not an additive one. Whether the interaction is more crucial at the parasite level or in the animal host is not known. However, previous studies on drug metabolism in helminths suggests that nematodes, unlike insects are not equipped with a microsomal system that would enable them to detoxify xenobiotics (Barrett, 1981). The synergism observed in the present study is most likely due to an improvement of the pharmacokinetic profile of the potentiated benzimidazole drug in the animal host and not in the parasite as is the case for the synergistic action of piperonyl butoxide on pyrethrins in insects (O'Brien 1967). Furthermore, given the lack of efficacy observed with twice the recommended dose of benzimidazole (Jackson et al 1993) and the enantioselective nature of fenbendazole metabolism (Delatour et al 1990), it may be that cytochrome P450 inhibition could shift the ratio (+)/(−) of sulphoxide metabolite in favour of a possible eutomere which is still to be characterized.

From the in-vitro studies in rat liver, the *S*-oxidation of fenbendazole appears to be slower than that described for albendazole in rat (Fargetton et al 1986) or in sheep and cattle microsomes (Galtier et al 1986; Lanusse et al 1993). This finding correlates well with several in-vivo pharmacokinetic studies. Indeed, whereas albendazole undergoes first-pass effect in all species studied and is absent or detected at trace amounts in the systemic circulation after oral administration (Marriner & Bogan 1980; Gyurik et al 1981; Hennessy et al 1989), fenbendazole is more slowly converted to its sulphoxide metabolite and persists for several hours in plasma following administration (Marriner & Bogan 1981).

Piperonyl butoxide was shown to diminish substantially the *S*-oxidation of fenbendazole in rat liver microsomes. Although the findings of the in-vitro inhibition study cannot be correlated to the pharmacokinetic results because of the species difference, they confirm the inhibition of benzimidazole metabolism by piperonyl butoxide.

In conclusion, the co-administration of piperonyl butoxide with fenbendazole affects significantly the pharmacokinetic disposition of the anthelmintic drug and results in a potentiation of the antinematodal activity.

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