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Clinical efficacy assessment of the albendazole–ivermectin combination in lambs parasitized with resistant nematodes

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Abstract

Combination of anthelmintic drugs from different chemical groups has been proposed as alternative parasite control strategies where failure of individual drugs is documented. The main goal of the current trial was to compare the clinical anthelmintic efficacy of albendazole (ABZ) and ivermectin (IVM) given either separately or co-administered to lambs naturally infected with gastrointestinal nematodes resistant to both molecules. Seventy (70) Corriedale lambs naturally infected with multiple resistant gastrointestinal nematodes were involved in the efficacy trial: the animals were allocated into 7 experimental groups ($n = 10$) and treated with either ABZ intravenously (iv) (ABZ_{IV}), IVM_{IV}, $ABZ_{IV} + IVM_{IV}$, ABZ intraruminally (ir) (ABZ_{IR}), IVM subcutaneously (sc) (IVM_{SC}) and $ABZ_{IR} + IVM_{SC}$ or kept as untreated controls. The indirect estimation of the efficacy of the different treatments was performed by the faecal egg count reduction test (FECRT). Additionally, four animals randomly chosen from the untreated control and ABZ_{IV}, IVM_{IV} and $ABZ_{IV} + IVM_{IV}$ experimental groups were sacrificed 15 days post-treatment to evaluate the efficacy against different adult resistant nematode parasites. The results were statistically compared by a non-parametric ANOVA (Kruskal–Wallis test). The following egg output reduction values were obtained: 73.4% (ABZ_{IV}), 79.0% (IVM_{IV}), 91.9% ($ABZ_{IV} + IVM_{IV}$), 43.5% (ABZ_{IR}), 79.8% (IVM_{SC}) and 70.8% ($ABZ_{IR} + IVM_{SC}$). The efficacy against *Haemonchus* spp. was 95.1 (ABZ_{IV}), 99.3 (IVM_{IV}) and 99.9% ($ABZ_{IV} + IVM_{IV}$), while the efficacy against *Trichostrongylus colubriformis* for the same treatment groups was 79.6, 100 and 99.9%. The data obtained on the assessment of the ABZ–IVM combination indicates that no potentiation synergism is observed. This work is complementary to a parallel study that demonstrated the lack of negative pharmacokinetic interactions between the two anthelmintics acting by different mode of action. Thus, an additive effect may be achieved against nematodes resistant to both compounds. Further work is required to understand the implications of potential pharmacokinetic/pharmacodynamic interactions between anthelmintics before drug combined formulations are developed to be introduced into the pharmaceutical market.

Keywords: Efficacy; Resistance; Albendazole; Ivermectin; Drug combination

1. Introduction

Helminth infections are the most important cause of productivity losses in livestock (Waller, 2003), being chemically based treatments the main tool to control

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parasitism. However, the development of resistance to the available anthelmintic drugs is a seriously increasing problem (Kaplan, 2004; Wolstenholme et al., 2004). A critical situation has been described in Brazil where nematode resistance to albendazole (ABZ) has been reported in about 90% of evaluated sheep farms. Similar situations were observed for levamisole (resistance was found in 84% of the tested farms), for the combination ABZ–levamisole (73%) and for ivermectin (IVM, 13%) (Echevarria et al., 1996). In other South American countries such as Uruguay (Nari et al., 1996) and northern Argentina (Eddi et al., 1996) the development of anthelmintic resistance in sheep nematodes is not an exception. In fact, current available data shows that almost 80% of the sheep farms in Argentina have cases of nematode resistance to anthelmintics (Caracostantogolo et al., 2005). Mixtures of drugs from different chemical families have been proposed as a valid strategy to delay the development of resistance (Anderson et al., 1988). It has been suggested that drug combinations may be efficacious against resistant nematode strains where the failure of individual drugs is documented. However, the limited available information is unclear on the potential additive or synergistic anthelmintic effect occurring after co-administration of two drugs with different mode of action.

Benzimidazoles (BZD), imidazothiazoles and avermectins/milbemycins are the most important chemical groups used to control helminth infections in ruminant species. ABZ is a BZD methylcarbamate anthelmintic compound effective against lungworms and gastrointestinal (GI) nematodes, tapeworms and liver flukes (Campbell, 1990; McKellar and Scott, 1990). The intrinsic anthelmintic action of BZD compounds on the parasite relies on a progressive disruption of basic cell functions as a result of their binding to parasite tubulin and depolimerization of microtubules (Lacey, 1990). Ivermectin (IVM), a member of the macrocyclic lactone antiparasitic drugs, exhibits a broad-spectrum of activity against GI and lung nematodes (Egerton et al., 1979) as well as against ectoparasites of domestic animals (Campbell et al., 1983). IVM acts primarily on glutamate-gated chloride channels, which are involved in nematode feeding, reproduction and locomotion (Yates et al., 2003). There are several drenches in the veterinary pharmaceutical market which combine BZD and avermectin-type compounds. Some broad spectrum antiparasitic combinations containing ABZ, IVM and levamisole (Triton®, Merial) or oxfendazole, abamectin and levamisole (Matrix®, Ancare) have been initially introduced into the Australian and/or New Zealand markets. A multi-combination drench for sheep, which combines ABZ, levamisole, closantel, and abamectin (Q-Drench®, Jurox) is approved for use in sheep in Australia. The use of these products are based in a lower resistance in individual worms to a preparation with multiple components (each one with different mechanism of action) compared to the treatment with a formulation with a single active component. However, potential pharmacokinetic (PK) and/or pharmacodynamic (PD) interactions between components may occur. A summarized overview of the pharmacological basis of drug to drug interactions is presented in Fig. 1.

Some of the following PD interactions could be expected following the co-administration of two molecules: indifference, antagonism, additive or synergistic action. A synergistic action takes place when the response obtained after the multiple administration is greater to that obtained after the administration of each drug alone. While it is more likely to obtain an additive effect after the administration of two drugs with similar mechanism of action, a synergistic effect may occur after the administration of drugs acting at different molecular levels. Evidence of synergist action between the BZD compound fenbendazole and levamisole has been reported (Miller and Craig, 1996). These authors reported a 62% of egg counts reduction after the combination of fenbendazole and levamisole compared to 1 and 23% of reduction when fenbendazole and levamisole were administered alone, respectively. Furthermore, Mehlhorn and Harder (1997), observed a synergistic effect after the combined administration of pyrantel and febantel against *Heterakis spumosa* developed in mice. On the other hand, an additive effect is apparently involved in the increased efficacy observed for the combination of ABZ and levamisole against BZD-resistant nematodes (Anderson et al., 1991a,b). The observed enhanced efficacy of the drug mixture may be based on the sum of the effects at different molecular targets.

The main goal of the current trial was to compare the clinical efficacy of ABZ and IVM given either separately or co-administered to lambs naturally infected with GI nematodes resistant to both molecules. The work reported here is complementary of a PK evaluation addressed to characterize the potential interaction between ABZ and IVM in sheep (Alvarez et al., 2008). Thus, the efficacy results reported here are discussed within the framework of the mentioned kinetic characterization of the anthelmintic combination.

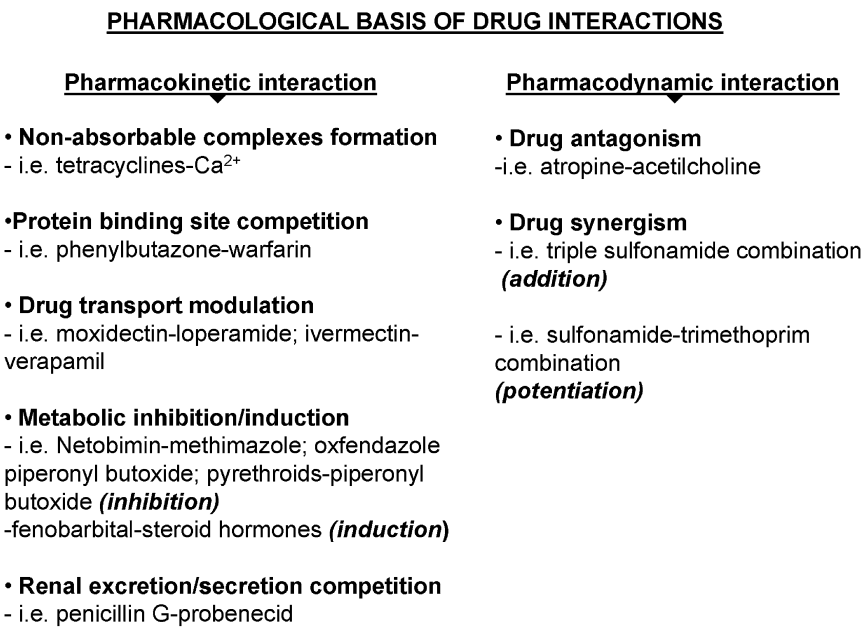

Fig. 1. Schematic representation of the main pharmacological basis of drug interactions.

2. Material and methods

2.1. Animals

Seventy (70) Corriedale lambs (27.3 ± 4.3 kg), naturally infected with resistant GI nematodes (*Haemonchus* spp. and *Nematodirus* spp. showed multiple resistance to both, ABZ and IVM), were involved in this trial. The selected farm is a sheep experimental unit with a parasite control program based on the intensive use of anthelmintics over the years, where failure of ABZ and IVM to control nematodes was previously demonstrated (Entrocasso, C., personal communication). The selection of the animals was based on worm egg per gram counts (epg). On day −1 all lambs were checked for epg, ear tagged and the individual body weights were recorded. Experimental animals had an average of 2028 ± 1111 epg counts ranging from 600 to 4860. Animals were allocated in a paddock and feed on a lucerne/white and red clover pasture during the experiment and for 20 days before start clinical efficacy study. All the animals had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar).

2.2. Chemicals

The commercial formulation of ABZ (Valbazen®, 10%, w/v, suspension) was provided by Pfizer Animal Health, Argentina and IVM (Ivomec®, 1%, w/v, solution) by Merial, Argentina. An ABZ solution was prepared as a 2% (w/v) solution in propylene glycol/dimethyl sulphoxide (80/20) (Anedra, Buenos Aires, Argentina) for intravenous administration to lambs.

2.3. Experimental design, treatments and sampling

All parasitized lambs were randomly allocated into seven groups (n = 10) to perform the clinical efficacy study. Each group received on day 0 the following treatments: ABZ_{IV}, ABZ (2% solution) intravenously (iv) administered at the dose rate of 3.8 mg/kg; IVM_{IV}, IVM administered by the iv route at 0.2 mg/kg; ABZ_{IV} + IVM_{IV}, ABZ (2% solution, 3.8 mg/kg) plus IVM (0.2 mg/kg), both administered by the iv route; ABZ_{IR}, ABZ intraruminally (ir) administered at the dose rate of 3.8 mg/kg; IVM_{SC}, IVM subcutaneously (sc) administered at the dose rate of 0.2 mg/kg; ABZ_{IR} + IVM_{SC}, ABZ (3.8 mg/kg) plus IVM (0.2 mg/kg), administered by the ir and sc route, respectively; and untreated control group. Faecal individual samples were collected pre-treatment (day −1) and at 13 days post-treatment to assess the epg counts. Additionally, at 15 days post-treatment, four (4) animals randomly chosen from groups ABZ_{IV}, IVM_{IV}, ABZ_{IV} + IVM_{IV} and untreated control group were sacrificed by captive bolt gun and rapidly exsanguinated. Abomasum and different gut sections were identified and isolated and the content analysed to record the different parasite stages following the World Association for the Advancement of Veterinary Parasitology guidelines (Wood et al., 1995).

2.4. Efficacy assessment

The anthelmintic efficacy of the treatments was evaluated by the faecal egg count reduction test (FECRT), calculated according to the formula (Coles et al., 1992):

$$\text{FECRT}\,(\%) = 100 \times \left[1 - \left(\frac{\text{Ct}}{\text{Cc}}\right)\right];$$

where Ct is the arithmetic mean epg counts in the treated group at 13 days posttreatment and Cc is the arithmetic mean epg counts in the untreated control group at 13 days posttreatment. The 95% confidence intervals were calculated as reported by Coles et al. (1992). Faecal cultures were performed according to previously described techniques (Coles et al., 1992). The genus and species of the nematodes recovered from parasitized lambs were determined by the identification of the third stage larvae recovered from faecal pool cultures obtained from each experimental group.

Direct adult nematode counts of animals from experimental groups ABZ_{IV}, IVM_{IV}, $ABZ_{IV} + IVM_{IV}$ and Control ($n = 4$ for each group) were determined 15 days after treatment according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood et al., 1995). The efficacy of each anthelmintic treatment was determined by the comparison of worm burdens in treated (groups ABZ_{IV}, IVM_{IV} and $ABZ_{IV} + IVM_{IV}$) versus untreated animals. The following equation expresses the percentage of efficacy ($\%E$) of a drug treatment against a given parasite species (S) in a single treatment group (T) when compared with an untreated control (C):

$$\%E = \frac{\text{Mean of } S \text{ in } C - \text{Mean of } S \text{ in } T}{\text{Mean of } S \text{ in } C} \times 100$$

The geometric mean was used as it most accurately represents the distribution of nematode populations within each group (Wood et al., 1995). The genus and species of the third stage larvae recovered from faecal pool cultures or adult nematodes recovered from parasitized lambs (groups ABZ_{IV}, IVM_{IV} and $ABZ_{IV} + IVM_{IV}$) were identified following the Ministry of Agriculture, Fisheries and Food, (1986) guidelines.

Statistical analysis of the data: Faecal eggs and nematode counts (reported as arithmetic mean ± S.D.) were analysed by non-parametric ANOVA (Kruskal–Wallis test). A value of $P < 0.05$ was considered statistically significant. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, CA, USA).

3. Results

The faecal egg counts (arithmetic mean) obtained for all experimental groups, including the results of the FECRT and upper and low confidence limits (95%), are shown in Table 1. The highest anthelmintic efficacy (91.9%) obtained by the FECRT was observed for the group $ABZ_{IV} + IVM_{IV}$. The overall low efficacy levels observed indicates the presence of GI nematodes resistant to both ABZ and IVM. The adult nematode counts and resultant clinical efficacy obtained for the ABZ_{IV}, IVM_{IV}, $ABZ_{IV} + IVM_{IV}$ treatment groups and nematode counts in untreated control are shown in Table 2. Since a rather low number of experimental

Table 1
Nematode egg counts[a] (range) and reduction percentage of faecal egg counts (FECRT) after administration of albendazole (ABZ) and ivermectin (IVM) alone or co-administered to parasitized lambs

Treatment group	Mean faecal egg counts (range)		FECRT[b] (%)	UCL	LCL
	Day 0	Day 13			
Untreated control group	2160 (600–3840)	3291 (1320–7800)[a]	–		
ABZ_{IV}	1733 (600–3840)	877 (150–2520)[a,b]	73.4	87	47
IVM_{IV}	2064 (600–2640)	690 (0–2520)[b,c]	79.0	91	53
$ABZ_{IV} + IVM_{IV}$	2142 (600–4800)	267 (0–1260)[b,d]	91.9	97	76
ABZ_{IR}	2170 (600–4860)	1860 (420–3600)[a,c]	43.5	65	9
IVM_{SC}	1962 (600–3840)	666 (0–1620)[b,d]	79.8	89	62
$ABZ_{IR} + IVM_{SC}$	1986 (600–3360)	962 (120–2480)[a,c,d]	70.8	85	43

UCL, Upper confidence limit 95%; LCL, lower confidence limit 95%. Nematode egg counts at day 13 posttreatment with different superscript letters (a–d) are statistically different at $P < 0.05$. Dosage regimens: ABZ 3.8 mg/kg, IVM 0.2 mg/kg.

[a] Arithmetic mean.

[b] FECRT estimated according to Coles et al. (1992).

Table 2
Adult nematode worm counts[a] (range) and efficacy[b] (%) obtained at 15 days posttreatment with albendazole (ABZ, 3.8 mg/kg, $n = 4$), ivermectin (IVM, 0.2 mg/kg, $n = 4$) and their combined use at the same doses rates (3.8 and 0.2 mg/kg, respectively, $n = 4$), by the intravenous (iv) route of administration

Parasites	Untreated control group	ABZ_{IV}		IVM_{IV}		$ABZ_{IV} + IVM_{IV}$	
	Worm counts	Worm counts	Efficacy (%)	Worm counts	Efficacy (%)	Worm counts	Efficacy (%)
Abomasum							
Haemonchus spp.	1600 (600–2900)	250 (0–500)	95.1	75 (0–200)	99.3	125* (0–500)	99.9
Teladorsagia circumcincta	2800 (1300–3600)	0*	100	0*	100	0*	100
Trichostrongylus axei	3200 (2000–4300)	75 (0–300)	100	0*	100	0*	100
Small intestine							
Trichostrongylus colubriformis	4475 (2400–6300)	1100 (500–2600)	79.6	0*	100	175* (0–700)	99.9
Cooperia spp.	50 (0–100)	0	100	25 (0–100)	98.4	0	100
Nematodirus spp.	600 (300–1300)	525 (100–1400)	34.9	125 (0–500)	99.7	0	100
Large intestine							
Oesophagostomun spp.	250 (100–400)	0*	100	0*	100	0*	100
Trichuris ovis	25 (0–100)	0*	100	0*	100	0*	100

Nematode worm counts recorded in the untreated control group are also shown.
[a] Arithmetic means.
[b] The percent of efficacy was calculated using geometric mean as suggested by Wood et al. (1995).
* Nematode counts are statistically different ($P < 0.05$) compared to worm counts in untreated control group.

animals were sacrificed in each group, the direct nematode counts were only indicative of the efficacy of each anthelmintic treatments. ABZ_{IV} and IVM_{IV} demonstrated high efficacy against *Haemonchus* spp. (95.1 and 99.3%, respectively). Due to variations in individual *Haemonchus* spp. counts, the observed differences did not reach statistical significance ($P > 0.05$) compared to the untreated control. However, the ABZ–IVM combination resulted in a significantly ($P < 0.05$) reduction in *Haemonchus* spp. counts and high efficacy (99.9%). ABZ given by the iv route fails to control *Trichostrongylus colubriformis* and *Nematodirus* spp., showing efficacies of 79.6 and 34.9%, respectively. The observed efficacy after the iv administration of IVM was 98.4% (*Cooperia* spp.) and 99.7% (*Nematodirus* spp.). The ABZ + IVM combination (iv route) achieved a high clinical efficacy against all gastrointestinal worms (Table 2).

The L_3 composition (%) observed after faecal culture of poolled samples collected from the untreated control animals and the different treated groups at 13 days posttreatment are shown in Table 3. Whereas *Haemonchus* spp. and *Trichostrongylus* spp. survive the ABZ + IVM combination given by the iv route ($ABZ_{IV} + IVM_{IV}$), *Haemonchus* spp., *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Cooperia* spp. were recovered from faecal cultures obtained from the $ABZ_{IR} + IVM_{SC}$ treatment group.

4. Discussion

A potential drug interaction refers to the possibility that one drug may alter the intensity of the pharma-

Table 3
Third stage (L_3) larvae composition (%) observed after faecal culture of pooled faecal samples obtained from untreated control and treated lambs at 15 days posttreatment

Treatment group	Larval identification (%)				
	Haemonchus spp.	*Teladorsagia circumcincta*	*Trichostrongylus* spp.	*Cooperia* spp.	*Nematodirus* spp.
Untreated control group		30	46	6	8
ABZ_{IV}	16	–	84	–	–
IVM_{IV}	100	–	–	–	–
$ABZ_{IV} + IVM_{IV}$	94	–	6	–	–
ABZ_{IR}	68	–	32	–	–
IVM_{SC}	64	14	20	2	–
$ABZ_{IR} + IVM_{SC}$	70	10	16	4	–

cological effects of another drug given concurrently (Nies and Spielberg, 1996). The modified effect may result from a change in the concentration of either one or both drugs in the organism (PK interaction) or from a change in the relation between drug concentration and response of the organism to the drug (PD interaction). A PK interaction occurs when one drug modifies the absorption, distribution, biotransformation and/or elimination of other drug; as a consequence, drug concentration at the biophase (the site of receptor localization) may be either increased or decreased. In fact, the PK interaction between ABZ and IVM co-administered to lambs has been reported (Alvarez et al., 2008). A PD interaction results when the altered pharmacological effect derived from a direct or indirect drug interaction at the receptor or effector mechanism level. If a drug combination causes in PD interaction, an additive effect is present when the combined effects of two drugs equal the sum of their independent activities measured separately. On the other hand, a synergistic effect is achieved when the combined effects of the drugs are significantly greater than the independent effects (Prescott, 2000). A similar approach may be assumed for anthelmintic drugs, in which a synergistic effect obtained after the co-administration of two drugs with different mechanism of action would be an ideal situation against resistant parasites. Since ABZ and IVM differ in their intrinsic anthelmintic mode of action, the co-administration in sheep may hypothetically induce a synergistic effect. The results obtained in the current experimental work will be discussed in terms of the potential PD interaction occurring between ABZ and IVM when these compounds are used in combination against resistant nematodes.

According to the criteria of Coles et al. (1992) which evaluates anthelmintic resistance by means of the FECRT, it is clear that multiple resistance against ABZ and IVM was present, since for all experimental groups the percentage of reduction in egg faecal counts was less than 95% (with 95% confidence levels $< 90\%$) (Table 1). The low efficacy levels observed in the current experiment confirms that the animals were naturally infected with nematodes resistant to both ABZ and IVM. After the iv co-administration of ABZ plus IVM, an egg count reduction of 91.9% was observed, in comparison to 73.4% (ABZ treated animals) and 79.0% (IVM treated animals). Additionally, the lower 95% confidence limit obtained for the $ABZ_{IV} + IVM_{IV}$ group (LCL 76%) resulted higher compared to that obtained for ABZ_{IV} (LCL 47%) and IVM_{IV} (LCL 53%) groups. The enhanced efficacy value observed for the $ABZ_{IV} + IVM_{IV}$ treatment in comparison to each drug administered alone, could be related to a PK interaction between ABZ and IVM which enhances the IVM plasma profile as recently shown by Alvarez et al., 2008. Furthermore, an additive effect of both anthelmintic molecules acting by different mode of action on different parasite genus/species could also account to explain the increased efficacy observed after the combined treatment. Following the WAAVP guidelines (Wood et al., 1995), ABZ (iv treatment) showed to be highly effective (>98%) against *T. circumcincta*, *Trichostrongylus axei*, *Cooperia* spp., *Oesophagostomun* spp. and *Trichuris ovis*, effective (90–98%) against *Haemonchus* spp. and failed to control *T. colubriformis*, and *Nematodirus* spp. IVM (IVM_{IV}) resulted highly effective against all nematode species assessed (Table 2). However, a lack of statistical significance in worm count reductions compared to the untreated control group was observed for *Haemonchus* spp. and *Cooperia* spp. and *Nematodirus* spp., likely due to a large individual variability in worm counts. It is valid to consider that after the combination of both compounds ($ABZ_{IV} + IVM_{IV}$) an enhanced nematode control may be achieved. This enhanced efficacy is likely derived from an additive effect of these two anthelmintics, as it can be observed from the efficacy data obtained against some resistant nematode species in the current trial. Similar results were reported by Anderson et al. (1991a) after evaluation of the efficacy of mixtures of ABZ–sulphoxide (active metabolite of ABZ) and levamisole against sheep nematodes resistant to both compounds, where the observed effect of the drug combination was due to the sum of the individual effect of each compound.

The egg count reductions observed after the ir or sc treatments, resulted in an overall efficacy of 43.5% (ABZ_{IR}) and 79.8% (IVM_{SC}). The combination of both molecules ($ABZ_{IR} + IVM_{SC}$) did not have a positive effect in eggs reduction. In fact, after the ABZ + IVM co-administration an efficacy of 70.8% was observed. Clearly, the combined action of ABZ and IVM on GI nematodes did not improve the efficacy of IVM administered alone by the sc route.

The faecal cultures showed *Haemonchus* spp. and *Trichostrongylus* spp. as the main parasites resistant to ABZ and IVM. Interestingly, after the iv treatments *Haemonchus* spp. appears to be almost exclusively implicated in IVM resistance. However, *Haemonchus* spp., *T. circumcincta*, *Trichostrongylus* spp. and *Cooperia* spp. contribute in the egg output observed after the sc administration of IVM. On the other hand, after both the iv and ir ABZ administration *Haemonchus* spp. and *Trichostrongylus* spp. were the parasite genus

involved in the overall egg output. However, while the greatest contribution of *Trichostrongylus* spp. was observed (84% of the larvae) after the iv treatment, *Haemonchus* spp. (68% of the larvae) was the main contributor to egg output after the ir treatment. The improved efficacy observed following the ABZ + IVM co-administration may be related with the enhanced plasma concentration observed after the iv administration of both anthelmintics (Alvarez et al., 2008). Although the time of parasite exposure to the active drug may be a limiting step on anthelmintic drug action, the level of drug concentrations appear to be an important factor on the final anthelmintic effect of the drug (Moreno et al., 2004; Sanchez Bruni et al., 2005).

ABZ anthelmintic efficacy, evaluated by the FECRT, tended to be higher after its iv administration compared to that observed after the ir treatment (Table 1). The anthelmintic action of BZD compounds not only depends on their affinity for parasite tubulin, but also on their ability to reach high and sustained concentrations at the site of parasite location (Lanusse and Prichard, 1993). A high correlation between the concentration profiles of ABZ and its active sulphoxide metabolite measured in the bloodstream and those recovered in tissues of parasite location has been demonstrated (Alvarez et al., 1999, 2000). Enhanced plasma and tissue ABZ/metabolites concentrations account for increased drug levels recovered within target helminths, as it has been shown in *Moniezia* spp., *Fasciola hepatica* and *H. contortus* recovered from ABZ-treated sheep (Alvarez et al., 2000). Thus, the increased plasma availability of ABZ/ABZSO following the iv treatment (see Fig. 2) correlates with the observed improved clinical efficacy compared to the ir treatment. The higher the concentration achieved at the tissue where the parasite is located, the higher the amount reaching the target parasite. Conversely, the efficacy of IVM was equivalent after the iv and sc administration. The pattern of IVM absorption from the site of sc injection has been shown to be excellent reaching a peak concentration (21.3 ± 13.3 ng/ml) at 2.8 ± 1.5 days (T_{max}) and AUC value of 131.1 ± 70.5 ng day/ml, which result very close to that observed after the iv administration of IVM (112.3 ± 37.4 ng day/ml) (Alvarez et al., 2008). This adequate absorption pattern and its extensive tissue distribution may assure a parasite exposure to the drug equivalent to that obtained following the iv administration of IVM. Thus, it is not surprising to obtain equivalent efficacy results for IVM given intravenously and subcutaneously in sheep. This clearly differs with the efficacy pattern observed for ABZ, where its iv administration as a solution assures and enhanced drug accumulation at the site of parasite location,

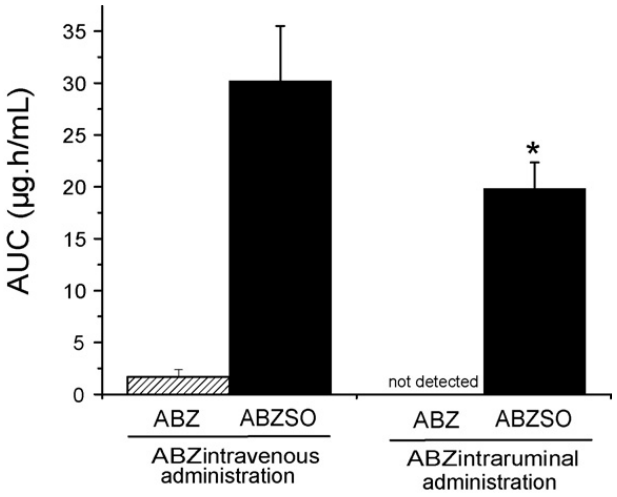

Fig. 2. Comparative systemic availability expressed as area under the plasma concentration vs. time curve (AUC) for ABZ and its sulphoxide metabolite (ABZSO) obtained after intravenous or intraruminal administration of ABZ (3.8 mg/kg) to parasitized lambs. The AUC value for ABZSO obtained after the iv administration of ABZ in lambs resulted significantly ($P < 0.05$) higher to that observed after ABZ ir administration. ABZ plasma concentrations were detected only after the iv administration of the parent drug. Adapted from Alvarez et al. (2008).

compared to that achieved following the ir administration of a drug suspension. Drug particles in the micronized ABZ suspension needs to be dissolved at the acidic abomasum pH to get absorbed in the small intestine, being the absorption the limiting process to achieve adequate systemic availability of the active anthelmintic drug/metabolites.

From the results reported in this article, it can be concluded that the efficacy of the ABZ and IVM co-administration would be related to the sum of each individual efficacy. The PK assessment of the drug combination does not show any negative interaction between both anthelmintic molecules (Alvarez et al., 2008). Oppositely, the co-administration results in enhanced ABZ sulphoxide systemic availability. However, in the presence of multiple ABZ and IVM resistance, the co-administration of both compounds did not result in a clinically significant enhanced anthelmintic effect, with the disadvantage of an increased resistance selection pressure over parasite populations. Moreover, recent findings indicate that macrocyclic lactones may select for three amino acids changes in beta-tubulin, being at least two of them involved in BZD resistance (Mottier and Prichard, in press). This work suggests that would be a critical error to choose a combination of compounds that induce overlapping mechanism of resistance in the target parasite. Therefore, further work is required to understand the potential PK/PD interactions between anthelmintics before drug

combined formulations are developed to be introduced into the pharmaceutical market.

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