A Review of the Pharmacological Interactions of Ivermectin in Several Animal Species

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Abstract: The antiparasitic activity of ivermectin depends on the presence of an active drug concentration at the site of parasites location for an adapted length of time. Ivermectin interactions with another concurrently administered drug can occur. Concomitant administration of some drugs can increase the bioavailability of simultaneously administered ivermectin. This can, in some cases, become a useful pharmacological strategy to improve its antiparasitic efficacy and to delay the development of resistance in livestock or, in other cases, lead to adverse drug reactions and toxicities. On the other hand, other interactions can result in lower levels of this drug, determining that moderate resistant residual populations of the parasites may persist to contaminate pastures. The characterisation of ivermectin interactions can be used to predict and optimise the value of the parasiticide effects. This article reviews the pharmacological interactions of ivermectin in several domestic animal species.

Keywords: Ivermectin, pharmacokinetic interactions, pharmacodynamic interactions, veterinary.

1. INTRODUCTION

Concomitant administration of different drugs can modify the systemic availabilities, disposition kinetics and resultant pharmacological effects of several molecules used in therapeutics. Although the knowledge of the interactions that can occur in animals is nowadays limited compared with humans, this is an important issue, since combination chemotherapy is usual in veterinary clinical practice.

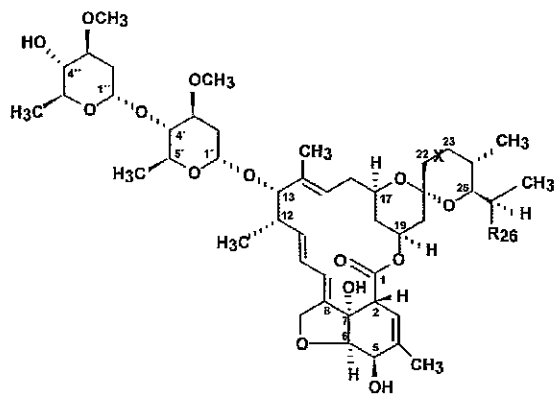
One of the most commonly used drugs in the pharmacotherapy of parasitic diseases in Veterinary Medicine is ivermectin, a potent antiparasitic agent effective against helminthes, arachnids and insects [1].

Ivermectin is a mixture of two chemically modified avermectins that contain at least 80% of 22,23-dihydroavermectin dihydroavermectin- B1a and > 20% 22,23-dihydroavermectin-B1b (Fig. 1).

Merck Sharp and Dohme brought this compound to the global animal health market in 1981 and within two years it had become a blockbuster drug of choice and market leader, quickly becoming the most successful broad-spectrum antiparasitic agent ever produced. Ivermectin proved active against endo- and ectoparasites, resulting in it being labelled as the first *endectocide.* All forms of livestock around the world are treated with it regularly (using dips, injections, feeds and other formulations) [2].

Many rumino-reticular delivery systems, as well as oral, topical, and injectable formulations of ivermectin are currently available at the dosage recommended by manufacturers, namely, 200 μg/kg in ruminants (500 μg/kg for topical application) and equines, 300 μg/kg in pigs, and 6 μg/kg in dogs.

The antiparasitic efficacy of this drug should be preserved, taking into account the serious problem that anthelmintic resistance represents for maintaining control of parasites in livestock worldwide and that there are few anthelmintics currently being developed (for an extensive review of the resistance situation see [3-6]). Resistance to avermectins, including ivermectin, is still low compared to other classes of frequently used anthelmintics (benzimidazoles and imidothiazoles-tetrahydropyrimidines, such as levamisole or pyrantel) although it is developing rapidly in some nematode parasites and appears to be increasing in incidence and spectrum of parasite species. Thus, it is necessary to take the appropriate measures to avoid the appearance and spread of resistant strains to this available drug.



C_{22}-X-C_{23}	R_{26}
-CH_2-CH_2-	>80% CH_2-CH_3 <20% CH_3

Fig. (1). Ivermectin structure.

In previous papers, we reviewed the pharmacokinetics and interactions of ivermectin in humans [7] and the pharmacokinetic properties of ivermectin in several animal species [8]. The general aspects of ivermectin pharmacokinetics are shown in Fig. (2). The purpose of the present manuscript is to provide useful information regarding the pharmacological (including pharmacokinetic and pharmacodynamic) interactions of ivermectin when concurrently administered with different drugs in several animal species.

2. INTERACTIONS OF IVERMECTIN WITH OTHER DRUGS

2.1. Pharmacokinetic Interactions

2.1.1. Interactions Mediated Through P-Glycoprotein

An important type of interaction involves several compounds that belong to different therapeutic drug classes. This interaction may arise through competition for binding sites on transport pro-

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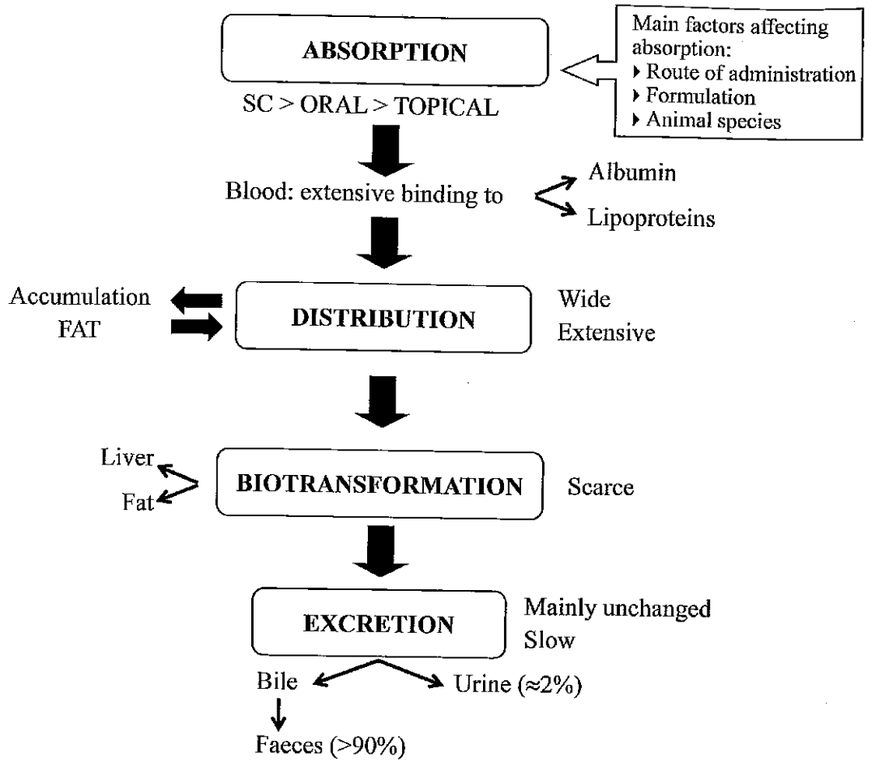

Fig. (2). General pharmacokinetics.

teins, such as P-glycoprotein (P-gp), a membrane protein located in blood-tissue barriers, intestine, liver and kidney, with broad substrate specificity [9, 10]. Due to the large number of substrates and modulators interacting with this protein, P-gp plays a clinically relevant role in drug-drug interactions [11].

Thus, drugs that interact with P-gp can alter the absorption, disposition and elimination of co-administered drugs, with a high potential for drug interactions.

As P-gp is linked to blood-tissue barriers, drug-drug interactions with P-gp may alter the absorption or excretion of ivermectin, resulting in higher levels of this drug. This modified drug pharmacokinetics by increasing bioavailability and organ uptake can be a useful tool to increase the efficacy of ivermectin or, on the contrary, give rise to toxic levels of the drug. Consequently, the identification and comprehension of these drug-drug interactions remain important keys to risk assessment.

2.1.1.1. Effect of Other Drugs on Ivermectin

Several studies have been conducted with **verapamil**, a calcium channel blocker used as antiarrhythmic and anti-hypertensive drug. This compound acts as a competitive inhibitor for the transport and extrusion of ivermectin by P-gp, giving rise to higher plasma levels of ivermectin.

The first of these studies [12] demonstrated that verapamil might act as a competitive inhibitor for the transport and extrusion of ivermectin by P-gp, when applied topically. This correlates with the presence of P-gp in polarized epithelial cell layers, indicating that it may be involved in the extrusion of ivermectin from the epithelial cell layer to the adjacent luminal space [13]. Thus, in rats topically treated with an ivermectin pour-on formulation [12], with the coadministration of verapamil the values of C_{max} (maximum plasma concentration) and AUC (area under the plasma-concentration-time curve) of ivermectin were significantly higher after combined treatment (Table 1).

In another study, and taking into account that the presence of P-gp in the intestine constitutes a barrier that limits the absorption of ivermectin administered orally, similar results (higher plasma levels of ivermectin) were found in sheep [14] after orally administering this compound and verapamil, as can be seen in Table **1**.

Moreover, as it was reported by [15], P-gp may also play a relevant role in the intestinal elimination of ivermectin, contributing to the high fecal excretion of this drug. Therefore, the higher plasma levels obtained after combined treatment (ivermectin-verapamil) may reflect an increase in the absorption of the antiparasitic compound from the gastrointestinal tract as well as a decrease of ivermectin intestinal elimination.

This enhanced ivermectin plasma availability obtained after its coadministration with this P-gp substrate may correlate with a greater exposure of tissue-located target parasites to active ivermectin concentrations, increasing the anthelmintic efficacy and having an important impact particularly against resistant- or rate-limiting parasites. This can be seen in the increase of the efficacy of ivermectin and moxidectin against *Haemonchus contortus* resistant to moxidectin in infected gerbils, following coadministration with verapamil [16]. Similarly, in sheep infected with resistant nematodes, ivermectin given alone had a 0% reduction in the number of parasite eggs in feces, confirming the strong resistance of those parasite strains. However, a 74.7% reduction in the egg fecal counts was achieved after the coadministration of ivermectin with verapamil [17].

Like verapamil, another P-glycoprotein substrate, **loperamide**, an opioid derivative used to reduce gastrointestinal secretions and motility, markedly modifies the pharmacokinetic behaviour of

ivermectin. When it was administered subcutaneously with ivermectin to rats, ivermectin plasma levels and AUC were higher than in animals treated with ivermectin alone (Table 1). In the presence of loperamide, higher ivermectin availabilities were also obtained in liver tissue and small intestine wall (60% higher AUC). The delayed intestinal transit induced by loperamide may have prolonged the time for reabsorption and enterohepatic circulation of ivermectin accounting for an extended plasma-intestine recycling-time and a potential competition between ivermectin and loperamide for the P-gp-mediated bile-intestinal secretion processes [18].

A similar enhanced systemic availability was obtained for ivermectin coadministered with a different P-gp substrate, the antifungal agent **itraconazole** [19] in intraruminally treated sheep (Table 1). Likewise, itraconazole enhanced ivermectin concentration profiles measured in different gastrointestinal tissues, as well as the levels detected in liver and bile. Meanwhile, the plasma disposition of ivermectin given intravenously was unaffected by the presence of itraconazole. Itraconazole-induced reduction on the P-gp efflux activity at the intestinal lining accounted for the greater absorption and enhanced systemic availability.

Regarding the findings of these authors [19], the low systemic availability of macrocyclic lactones (including ivermectin) after oral, compared to subcutaneous administration to sheep [20, 21], explained as a high association of ivermectin to particulate material of digesta [22] was corroborated in the cited study. Thus, the differential effect induced by itraconazole coadministration with ivermectin given either by the intraruminal or the intravenous route may be explained by the singular partition of this drug in the gastrointestinal tract. The low amount of frec drug available in the gastrointestinal lumen to be absorbed, following the intraruminal/oral administration of ivermectin, may favour the P-gp mediated gastrointestinal efflux which could contribute to its limited systemic availability. Thus, the presence of a P-gp modulator agent, itraconazole, induced a significant increment on the ivermectin absolute bioavailability obtained after its intraruminal administration to sheep.

The coadministration of ivermectin and itraconazole also resulted in an enhanced bioavailability in rats (Table 1) as well as significantly higher intestinal ivermectin concentrations, as a result of the lower intestinal secretion of ivermectin by inhibition P-gp [23]. In this same animal species, when studying sex-related differences in ivermectin disposition kinetics (with higher systemic availability in female rats, it was demonstrated that the presence of itraconazole induced drastic sex-related changes on the P-gp-mediated ivermectin gastrointestinal disposition. Coadministration with itraconazole resulted in a marked increase in ivermectin concentrations in the wall tissue from different portions of the gastrointestinal tract of male rats [24].

The effect of multiple oral daily dosing of the extensively used in dogs antifungal **ketoconazole** was investigated in animals that were given a subcutaneous injection of ivermectin. Concomitant administration of ketoconazole induced higher plasma levels and a longer mean residence time of ivermectin compared to ivermectin given alone (Table 1), and resulted in an increase in the elimination half-life ($t_{1/2}$), as well as in a decrease in the clearance of the drug. Altogether, these results suggest that the antifungal leads to a substantial increase in the overall exposure of the animal to the antiparasitic drug, by decreasing the elimination process. The authors concluded that clinicians should be aware of this interaction and caution must be exercised in the clinical practice, as both drugs are widely prescribed [25]. Regarding this, a review [26] that recopiled information from 632 dogs treated with ketoconazole (2.6-33.4 mg/kg) showed that adverse effects to ketoconazole were significantly more often recorded in dogs concurrently treated with cyclosporin or ivermectin.

Findings similar to those of dogs were observed in ruminants [27]. Sheep were given a single oral dose of ivermectin alone or in combination with ketoconazole. Ketoconazole modified the pharmacokinetics of ivermectin, inducing higher plasma concentrations, resulting in a significantly higher (1.75-fold) C_{max} and a twofold increased in AUC (close to statistical significance) (Table **1**). However, and in contrast to what was observed in dogs [25], ketoconazole did not change the elimination processes of ivermectin in sheep (MRT and clearance were similar and the production of the main ivermectin metabolite was not reduced). The increase in the systemic availability was explained due to a higher absorption of the parent drug resulting from ketoconazole-induced reduction of the ivermectin by P-gp at the level of cells lining the small intestine wall.

As a result of its tissue distribution, P-gp has been shown to reduce the uptake of lipophilic toxins and drugs from the gastrointestinal tract and to limit penetration into tissues such as the brain, testes or foetus.

As it is located at the blood-brain barrier, P-gp protects mammals against the penetration of ivermectin into the brain and its subsequent neurotoxicity [28-30]. In this sense, inhibition of the P-gp function at the blood-brain barrier during polytherapy using P-gp inhibitors must be taken into consideration. An increase in the acute neurotoxicity to ivermectin was found if injecting the immunosuppressive drug **cyclosporin A** (50 mg/kg) or the antipsychotic agent **trifluoperazine** (750 μg/kg) one hour prior to ivermectin (10-15 mg/kg) intraperitoneally to mice. The toxicological parameters were in agreement with the analysis of brain tissues, indicating that ivermectin brain concentrations were 2.5 fold higher when mice were previously treated with cyclosporin A [31].

This enhancement of ivermectin central nervous system toxicity was explained in a study carried out *in vitro*, using porcine brain endothelial capillaries. It was reported that **cyclosporin A** competed with ivermectin, decreasing ivermectin export process out of the capillaries, whereas ivermectin uptake remained unchanged. The same results were obtained with the combination **verapamil**-ivermectin [32].

Another work found that giving ivermectin (200 μg/kg) plus verapamil (3 mg/kg) during 8 weeks (intraperitoneal; once weekly), significantly induced hazardous effects on sperm characteristics of male rats. Meanwhile, ivermectin administration alone did not significantly change sperm characteristics. As P-gp is linked to the integrity of the blood-testis barrier, verapamil coadministration may have facilitated more passage of ivermectin, leading to its intracellular accumulation and retention, explaining the pronounced toxic effects of fertility [33].

P-gp can also enhance drug elimination by biliary, intestinal or renal secretion. Thus, attention should be given to the side effects of drugs that are substrates of this protein when they are concurrently administered with ivermectin.

2.1.1.2. Effect of Ivermectin on Other Drugs

Two studies examined the effect of ivermectin oral pretreatment on the pharmacokinetics of histamine H_1-antagonists in horses. A lower AUC (27%) for **fexofenadine** (10 mg/kg; orally) was detected when the animals were pretreated, 12 h before, with ivermectin, while the pharmacokinetics of fexofenadine administered intravenously was not affected. The authors suggested that ivermectin may induce intestinal P-gp in horses as well as cytochromes P450 during the pretreatment interval, resulting in a decreased uptake of the drug [34]. In another experiment [35], these authors found that ivermectin did not affect the pharmacokinetics of **cetirizine**, another antihistamine drug, administered *per os* (200 μg/kg) when given orally 1.5 h before cetirizine. However, ivermectin pretreatment 12 h before cetirizine increased AUC by 60%, and also C_{max}, MRT and $t_{1/2}$. The higher plasma levels of cetirizine were explained

Table 1. Pharmacokinetic Parameters of Ivermectin.

			C_{max} ($ng \cdot ml^{-1}$)	AUC ($ng \cdot d^{-1} \cdot ml^{-1}$)	MRT (d)
[12]	Rats	**Ivermectin: 500 µg/kg, T** **Verapamil: 5 mg/kg, T**			
		Ivermectin	4.6	14.9	
		Ivermectin + Verapamil	7.5	21.2	
[14]	Sheep	Ivermectin: 200 µg/kg, O Verapamil: 3 mg/kg, 3 times 12 h interval, O			
		Ivermectin	6.9	19.3	
		Ivermectin + Verapamil	12.6	29.7	
[18]	Rats	**Ivermectin: 200 µg/kg, SC** Loperamide: 1.5 mg/kg, SC			
		Ivermectin		23	
		Ivermectin + Loperamide		40	
[19]	Sheep	**Ivermectin: 50 µg/kg, IR** Itraconazole: 100 mg, 3 times 12 h interval, O			
		Ivermectin	2.09	6.95	
		Ivermectin + Itraconazole	6.85	26.5	
[23]	Rats	**Ivermectin: 200 µg/kg, IP** Itraconazole: 5 mg, two doses, IP			
		Ivermectin		20	
		Ivermectin + Itraconazole		37.9	
[25]	Dogs	**Ivermectin: 50 µg/kg, SC** Ketoconazole: 10 mg/kg over 5 days, O			
		Ivermectin		50.13	3.84
		Ivermectin + Ketoconazole		104.81	5.8
[27]	Sheep	**Ivermectin: 200 µg/kg, O** Ketoconazole: 10 mg/kg daily 3 days before and 2 days after ivermectin, O			
		Ivermectin	5.4	15.9	
		Ivermectin + Ketoconazole	10.6	28	

T: pour-on
O: oral
SC: subcutaneous
IR: intraruminal
IP: intraperitoneal

due to decreased renal secretion, related to ivermectin inhibition of the P-glycoprotein in the proximal tubular cells of the kidney.

On the other hand, the concurrent use of different **macrocyclic lactones** was evaluated in a study conducted by [36]. When ivermectin, doramectin or moxidectin alone, or a combination of two of these drugs were administered subcutaneously to sheep, AUC of doramectin was significantly higher when administered alone as compared with its coadministration with either moxidectin or ivermectin. No other significant differences in t_{max} (time to reach the maximum plasma concentration), C_{max} or AUC were detected between concurrent and individual administration for the three drugs. Nevertheless, the coadministration of ivermectin and moxidectin also produced a higher, although not significantly different, AUC for moxidectin (than moxidectin alone).

Similarly, [37] studied the pharmacokinetics of moxidectin in lambs treated only with this compound or concurrently with ivermectin (subcutaneous) and found that the coadministration of both drugs gave rise to an increment in the value of AUC (36%), although this increment was not significant.

Both the two previously cited authors [36-37] suggested that the increment in AUC was caused by the interfering action of ivermectin on P-gp.

2.1.2. Interactions Mediated Through P-Glycoprotein and Cytochromes P450

The elimination of macrocyclic lactones is, in part, governed by P-gp. In addition to this, and in parallel to the efflux activity of P-gp that mediates the extrusion of macrocyclic lactones out of the cells, the cytochromes P450 are the principal phase I enzymes involved in the metabolism of endectocides, including ivermectin [38-40].

2.1.2.1. Effect of Ivermectin on Other Drugs

In this sense, it was found [37], *in vitro*, that ivermectin increased significantly the quantity of moxidectin in cultured rat hepatocytes (these cells represent a unique experimental model for studying chemico-biological interactions because they spontaneously overexpress P-gp during culture and also express cytochromes).

Dealing with this, it has been proposed that both P-gp and cytochrome P450 3A4 act synergistically to increase the metabolism of macrocyclic lactones [41]. Transporter-enzyme interplay would complicate the interpretation of the underlying mechanisms of the interactions of drugs for which these proteins act as substrates [42].

As ivermectin is a P-gp interfering agent and cytochrome P450 3A substrate, some kind of interaction with albendazole metabolism is to be expected. Thus, in sheep, the oral administration of a combination of ivermectin (500 µg/kg) and the antiparasitary drug **albendazole** (7.5 mg/kg) gave rise to a significant increment of about 50% in the values of AUC determined for the active metabolite of albendazole: albendazole sulfoxide, with higher C_{max} and longer t_{max} values, compared with the data obtained when albendazole was administered alone. According to the authors, these results supported an increase of exposure of albendazole sulfoxide by a possible decrease of clearance and an increase of bioavailability by probably mixed effects of intestinal cytochrome P450 and drug efflux transporters. The relative contribution of cytochrome P450 3A and P-gp to overall interaction is still not completely clarified because the complex interplay involved between intestinal and hepatic cytochrome P450 3A and P-gp. In addition, drug efflux transporters could exist in locations other than the intestine (liver, kidney) and hence the observed increase in the albendazole sulfoxide systemic concentrations could be the result of a modification of the elimination processes at several sites [43]. Nevertheless, there is conflicting evidence for albendazole being a substrate for P-gp [44].

2.1.2.2. Co-Effects Ivermectin - Other Drugs

A later work evaluated, in deep, the interaction between ivermectin and albendazole. Lambs parasitized with gastrointestinal nematodes resistant to both anthelmintics received ivermectin and albendazole, either alone or concurrently. Firstly, the intravenous route of administration was used (for both compounds), while in the second part of the study ivermectin was injected subcutaneously and albendazole intraruminally [45]. After intravenous administration of both drugs, the presence of ivermectin did not affect the plasma disposition kinetics of albendazole and its metabolites. However, ivermectin plasma AUC was 88% higher after coadministration compared to treatment with ivermectin alone (Table **2**), and no other changes in the pharmacokinetic disposition of ivermectin was observed. On the other hand, after the concurrent administration of albendazole (intraruminal) and ivermectin (subcutaneous), and similar to the findings of the previous authors [43], albendazole sulfoxide AUC was significantly higher (42%) compared to that obtained with albendazole alone. Conversely, albendazole administered intraruminally did not affect the pharmacokinetics of subcutaneous administered ivermectin. According to the authors, two possible facts would help in explaining the observed pharmacokinetic changes: ivermectin induced inhibition of albendazole metabolism by mean of a cytochrome P450 3A and 1A competition / inhibition, or drug to drug interaction via drug efflux transporter-mediated mechanisms (P-gp). A synergistic effect of metabolic enzymes and efflux transporters at the intestinal level has been proposed, similar to [43], that attributed their results to a combined effect on metabolism and drug efflux transporter interactions.

Ivermectin is not effective against trematodes and, to extend its antiparasitic spectrum of activity, commercial preparations that include ivermectin combined with trematodicidal compounds are available in the veterinary pharmaceutical market. Thus, ivermectin and **triclabendazole**, a benzimidazole used to treat nematode and trematode infections in domestic animals, were administered [46], separately or concurrently, intravenously to sheep and found that the plasma concentration profile of both antiparasitic drugs was influenced by their coadministration. When combined with triclabendazole, ivermectin plasma availability was 3-fold higher and the elimination was significantly delayed (Table **2**). Complementary *in vitro* assays found that the rate and extent of ivermectin accumulation in the intestinal wall was significantly enhanced after coincubation with triclabendazole. Given that ivermectin is excreted through the biliary and intestinal tracts as the unchanged parent drug, it is likely that triclabendazole would modulate its biliary or intestinal P-gp-mediated elimination. On the other hand, the systemic availability of triclabendazole was decreased and higher peak plasma concentrations of triclabendazole metabolites were detected after coadministration. The authors hypothesized that a drug binding displacement may occur when triclabendazole and ivermectin are coadministered, as these drugs bind strongly to albumin in the circulation. Furthermore, significant interplay between transporter molecules and the cytochrome P450 system may occur.

2.1.3. Interactions Involving Metabolism

As indicated before, ivermectin is subjected to hepatic oxidative biotransformation typical of cytochrome P450 pathways. The major metabolites isolated *in vivo* in cattle, sheep and rats were 24-OH-$H2B_{1a}$ and 24-OH-$H2B_{1b}$ [47], whereas in pigs were O-demethylation derivatives (3"-O-desmethyl-$H2B_{1a}$ and 3"-O-desmethyl-$H2B_{1b}$) [48]. A 3-O-desmethyl metabolite has also been found in goats [49] and sheep [27]. In humans, the predominant isoform responsible for the biotransformation of ivermectin in the liver is cytochrome P450 3A4, converting the drug to at least 10 metabolites, most of them hydroxylated and demethylated derivatives [39].

In this sense, interactions can also occur when drugs are eliminated via the same biotransformation pathway. Considering this binding of ivermectin to cytochrome P450, the potential for induction or inhibition of cytochrome P450 isoenzymes could alter the elimination rate of a concurrently administered drug, leading to a decrease or increase in drug effect and / or toxicity. However, contradictory results have been obtained in this regard and the effect of ivermectin is not clear. Thus, in orally treated rats, 6 doses of 300 µg/kg did not have effect on any enzymes; higher doses (35 mg/kg or 1 mg/kg repeated in 6 consecutive days) induced enzymes 1A1 and 1A2 [50]; the same as in moufflons orally administered (500 µg/kg), whereas in fallow deers there was only a slight induction in 3A. Thus, it was considered a weak inducer only in moufflons [51].

In rats experimentally infected with *F. hepatica* and subcutaneously administered a preparation that contained ivermectin and **clorsulon**, a sulfonamide employed in the treatment of animals infected with adult liver flukes, (3.5 mg/kg) 8 weeks post-infection, the efficacy against the fluke was 100% in males and 53% in females. Such result was related to an overexpression of cytochrome P450 3A isoform in females attributed to a possible inductive activity of the formulation [52].

No relevant interaction was reported for **erythromycin** (15 mg/kg, intramuscular) or **antipyrine** (20 mg/kg, intravenously) when coadministered with ivermectin (200 µg/kg, intramuscular). This lack of effect of ivermectin may be indicative of an absence of influence on antipyrine hydroxylation pathway and erythromycin demethylation pathway [53]. Similarly, there was no substantial influence of ivermectin on **hexobarbital** sleeping time in rats treated with a single subcutaneous dose (200 or 400 µg/kg) [54].

In goats that were given a single subcutaneous dose, the *in vitro* hepatic activities of some drug-metabolizing enzymes (hexobarbitone oxidase, p-nitroreductase and UDP-glucuronyl transferase), a significant increase in the activities of the enzymes was found in animals infected with *Haemonchus contortus*, compared to infected untreated animals. Attention should be exercised when treating animals infected with haemonchiasis because the hepatic drug-metabolizing capacity is impaired and, accordingly, the dosage should be given due consideration [54].

2.1.4. Other Pharmacokinetic Interactions

The findings of El Banna *et al.* [55] (Table **3**), revealed that the combination of **rafoxanide**, a fasciolicide drug, with ivermectin increased the absorption of ivermectin and delayed its elimination in sheep and calves, after injecting ivermectin alone or in combination with rafoxanide. In sheep, rafoxanide induced a rapid absorption of ivermectin, manifested by an absorption half-life ($t_{1/2ka}$) 68.49% shorter. In addition, there was a significant increase in

Table 2. Pharmacokinetic Parameters of Ivermectin

			AUC ($ng \cdot d^{-1} \cdot ml^{-1}$)	$t_{1/2}$ (d)	MRT (d)
[45]	Sheep	**Ivermectin: 200 µg/kg, IV Albendazole: 3.8 mg/kg, IV**			
		Ivermectin	112.3		
		Ivermectin + Albendazole	210.3		
[46]	Sheep	**Ivermectin: 200 µg/kg, IV Triclabendazole: 5 mg/kg, IV**			
		Ivermectin	14.4	0.94	0.97
		Ivermectin + Triclabendazole	48.5	1.53	1.76

IV: intravenous

Table 3. Pharmacokinetic Parameters of Ivermectin [55].

	Ivermectin					Ivermectin + Rafoxanide				
	$t_{1/2ka}$ (d)	C_{max} ($ng \cdot ml^{-1}$)	t_{max} (d)	AUC ($ng \cdot d^{-1} \cdot ml^{-1}$)	$t_{1/2}$ (d)	$t_{1/2ka}$ (d)	C_{max} ($ng \cdot ml^{-1}$)	t_{max} (d)	AUC ($ng \cdot d^{-1} \cdot ml^{-1}$)	$t_{1/2}$ (d)
Sheep	0.73	19.4	1.5	83.0	2.04	0.23	18.6	0.7	95.9	3.3
Calves	0.16	19.1	1.1	165.4	4.95	0.13	22.4	1.0	188.9	5.8

Ivermectin: 200 µg/kg; subcutaneous; Rafoxanide: 2.5 mg/kg.
Sheep: differences in $t_{1/2ka}$, AUC, t_{max} and $t_{1/2}$ were significant. No statistical difference was found in C_{max}.
Calves: differences in C_{max}, AUC, and $t_{1/2}$ were significant. No statistical difference was found in $t_{1/2ka}$ and t_{max}.

AUC (15.5%) and $t_{1/2}$. Rafoxanide also induced, in calves, an earlier t_{max} and a $t_{1/2ka}$ 23.22% shorter, with a similar increase in AUC (14.2%) and also an increment in $t_{1/2}$.

Although we have described several pharmacokinetic interactions when ivermectin is administered with other drugs, it is important to remember that pharmacokinetic interactions do not always result in clinically significant effects [56].

2.2. Pharmacodynamic Interactions

Several authors evaluated the effect of ivermectin coadministration with different drugs employed to control helminthes (nematodes, trematodes and cestodes).

Some of these studies were conducted coadministering ivermectin and ***nematocide*** compounds.

It was reported that, in dogs, the combination of **pyrantel pamoate** and ivermectin, a medication intended for monthly use in the prophylaxis of heartworm caused by *Dirofilaria immitis*, and in the treatment and control of ascarids (*Toxocara canis*, *Toxascaris leonina*) and hookworms (*Ancylostoma caninum*, *Uncinaria stenocephala*), was completely effective against *Dirofilaria immitis* larvae and highly effective in the treatment of ascarids and hookworms, showing that pyrantel did not interfere with the activity of ivermectin against *D. immitis* and that ivermectin did not interfere with activity of pyrantel against intestinal nematodes, indicating a good safety and efficacy profile of the combination [57-61].

Regarding filarial infections, there is a pressing need for effective adulticide treatment and breaking transmission of heartworm disease early in the treatment. In this sense, **doxycycline**, a tetracycline used to treat infections, can enhance ivermectin effect, as it sterilizes adult female worms for a few months by depletion of the symbiotic endobacteria of filariae, *Wolbachia*, essential for their survival and reproduction [62]. In dogs, ivermectin (6 µg/kg *per os* weekly) combined with doxycycline (10 mg/kg/day orally from weeks 0-6, 10-12, 16-18, 22-26 and 28-34) resulted in a significantly faster decrease of circulating microfilariae of the causative agent of canine heartworm disease, *D. immitis*, and in higher adulticidal activity (the combination of the drugs caused adult worm death) compared with either the drugs given alone [63]. Moreover, McCall *et al.* [64] suggested that administration of doxycycline + ivermectin for several months prior to (or without) melarsomine will eliminate adult heartworm with less potential for severe thromboembolism than melarsomine alone. They conducted a study in dogs with adult heartworm infection, and evaluated the efficacy of ivermectin (6 µg/kg weekly for 36 weeks) and doxycycline (10 mg/kg day; orally weeks 1-6, 10-11, 16-17, 22-25, and 28-33) alone or together (with or without melarsomine: at week 24, 2.5 mg/kg intramuscular, followed 1 month later by two injections 24 h apart). Reduction of adult worms was 20.3% for ivermectin, 8.7% for doxycycline, 78.3% for ivermectin + doxycycline, 92.8% for ivermectin + doxycycline + melarsomine and 100% for melarsomine.

Other study proved that, for the control of brugian lymphatic filariasis in the cat reservoir, the combination of ivermectin (400 µg/kg) with **diethylcarbamazine**, a drug with activity against intestinal helminths and also used in preventive programs for heartworm infection, (6 mg/kg) as a single treatment offers advantage over ivermectin alone. Thus, in cats infected with *Brugia malayi* and treated orally, this combination demonstrated a microfilaricidal effect superior to that of either drug used alone, both in the initial rapid clearance of microfilariae, and in sustaining the effect for 8 months [65].

In contrast to these beneficial effects on filarial control, the simultaneous employ of ivermectin and **levamisole**, an imidazothiazole highly effective against nematodes, is not recommended in Collies, as 15 minutes after subcutaneously injecting levamisole (3 mg/kg) to a dog, the animal showed convulsions, vomitus and dyspnoe, and perished 2.5 h after the injection. Afterwards, high ivermectin concentrations, 25.5 $ng \cdot g^{-1}$, were detected in the liver [66]. This study elucidates that combination of low-dosed ivermectin and levamisole is no recommendable means against adverse effects of ivermectin, with respect to Collies. Moreover, the synergistic effects of ivermectin and levamisole suggest the same drug incompatibility in other dog breeds and animal species.

A recent work [67] compared a combination of **fenbendazole** (oral drench) and ivermectin (pour-on formulation) with the use of

the endectocide alone in heifers. The combination showed a higher effectiveness in reducing parasite burden and positively affected animal performance, heifers gained more weight and had heavier final body weights, when compared with ivermectin administered alone.

In a complementary and parallel work to the pharmacokinetic one [45], Entrocasso *et al.* [68] compared the clinical anthelmintic efficacy of **albendazole** (3,8 mg/kg; intraruminal) and ivermectin (200 µg/kg; subcutaneous) given either separately or coadministered to lambs infected with gastrointestinal nematodes resistant to both molecules. The efficacy against several nematodes and was higher with the combination. From the results reported in the article, it can be concluded that the efficacy of albendazole and ivermectin coadministration would be related to the sum of each individual efficacy (additive effect) and that no potentiation synergism was observed. These pharmacodynamic effects are in concordance with the modification of the pharmacokinetic parameters (higher values for AUC and C_{max}) obtained with the coadministration of albendazole and ivermectin, previously explained in the section *Interactions mediated through P-glycoprotein and cytochrome P450* [43, 45].

Another work evaluated the therapeutic efficacy of a combined formulation (2.25% ivermectin combined with 1.25% **abamectin;** 450 and 250 µg/kg, respectively) against *Rhipicephalus microplus*, *D. hominis* larvae and gastrointestinal nematodes parasitizing bovine in comparison with the 3.15% ivermectin commercial product [69]. The association showed higher anthelmintic activity and similar efficacy against arthropods and filaria.

Although the existence of side resistance between macrocyclic lactones has been reported [70, 71], this study [69] have shown that the combination of 2.25% ivermectin with 1.25% abamectin is pharmacologically interesting and could result in complementing the endectocide activity of avermectins, as ivermectin dosage limiting parasites are gastrointestinal nematodes, while for abamectin, they are ectoparasites.

On the other hand, other studies evaluated the consequences of the coadministration of ivermectin with ***flukicide*** drugs.

A recent work was focused on the efficacy of a novel product that contains **closantel** (5 mg/kg) and ivermectin (200 µg/kg) in clinical use. These authors [72] demonstrated the high efficacy against target cattle nematodes and trematodes, including the dose-limiting nematode *Cooperia oncophora*.

It was also reported that ivermectin (200 µg/kg, subcutaneous) did not impair the efficacy of **clorsulon** (7 mg/kg, oral) against *Fasciola hepatica* in cattle [73]. Such use should provide for control of *F. hepatica*, as well as most nematode and many arthropod parasites of cattle.

In addition, and dealing with resistance to macrocyclic lactones, some studies have shown that resistant parasites possessed an increased level of P-gp expression, providing evidence for the implication of P-gp in the efflux of ivermectin from parasites which may contribute to reduce activity of the drug [74,75]. In this sense, in an *in vitro* experiment, the influx/efflux balance for **triclabendazole** and triclabendazole sulfoxide (the active metabolite of triclabendazole), through the tegument of susceptible and resistant flukes in the presence or absence of ivermectin was assessed. An enhancement of triclabendazole and triclabendazole sulfoxide concentrations (30%) were observed in the resistant *F. hepatica* in the presence of ivermectin (compared to those measured in the absence of ivermectin), a fact that was explained on the basis of the ivermectin induced inhibition of Pgp activity, that decreased triclabendazole efflux from the resistant flukes. This could increase the efficacy of the flukicide drug [76]. In this sense, and as it was explained in the section *Interactions mediated through P-glycoprotein and cytochrome P450*, the increase efficacy correlates with higher plasma concentration profiles of both antiparasitic drugs when administered concurrently [46].

In connection with ***cestocide*** effects, the efficacy of ivermectin alone (1 µg/ml), **albendazole** alone (10 µg/ml) and a combination of both drugs against *Echinococcus granulosus* protoscolices was studied by means of *in vitro* incubation. The maximum protoscolicidal effect was obtained with the simultaneous use of both compounds, as 18 days postincubation the decrease in their viability was 65% compared with 50 and 18%, respectively, when using these drugs alone. Moreover, the protoscolices incubated with both compounds were the only ones that did not develop into hydatid cysts following their inoculation into mice. Finally, after the incubation (10 days) of cysts with both anthelmintics, they completely lose their infectivity to mice, a fact not achieved when employing each of the compounds independently [77]. With this preliminary study the authors showed the synergic effects of ivermectin and albendazole using cysts of 3-6 mm diameter, although the value of these drugs against larger cysts remains to be demonstrated. Nevertheless, these synergic effects could be of great interest in the chemotherapeutic treatment of hydatid disease, as the most commonly drug used in clinical practice against hydatidosis, albendazole, is necessary to be used for a long period of treatment.

The same year, Moreno *et al.* [78] demonstrated that the efficacy of a combination of ivermectin and albendazole, when used as a prophylactic treatment was 95.7% and 87% with respect to the number and the wet weight of cysts, respectively. When used in the treatment of secondary hydatidosis, the results were lower: 44.8% and 45.26%, respectively. Ivermectin treatment alone was not effective against *E. granulosus* in either case.

When an ivermectin-**praziquantel**, the latter being a high efficacy drug against cestode parasites, (200 µg/kg; 1 mg/kg, respectively) formulation appeared in the market, several studies were conducted in order to establish the efficacy and safety of this combined medicinal product in horses treated orally. It was concluded that the combination allowed efficacious treatment of horses for nematode and cestode infections [79, 80], being safe in horses treated under field conditions [79] and in pregnant mares and their foals even at 3 times the therapeutic dosage of both compounds [81].

Regarding the simultaneous use of ivermectin and ectoparasiticides, recently, the U.S. Food and Drug Administration Center for Veterinary Medicine announced that it has received reports of adverse reactions in dogs receiving Comfortis® (**spinosad**; a monthly oral, chewable tablet used for the prevention and treatment of flea infestations in dogs) concurrently with high, extra-label doses of ivermectin (used to treat dogs with non-responsive demodectic mange and other conditions). Veterinarians should be aware that some dogs have developed signs of ivermectin toxicity and that dogs receiving extra-label doses of ivermectin should not receive concurrent treatment with Comfortis® [82].

On the other hand, several authors studied the protective effects of ivermectin against convulsions caused by different drugs. In one study [83], an intravenous injection of ivermectin (1-10 mg/kg) dose-dependently antagonized the appearance of both **lidocaine** (20 mg/kg) and **strychnine** (0.75 mg/kg) -induced convulsions after their intravenous administration to rats. Convulsions in ivermectin pretreated animals appeared later and were weaker, in comparison to seizures in rats without ivermectin pretreatment. In a previous study [84], ivermectin (0.5-1 mg/kg, intraperitoneal) protected rats from the convulsant effects of **pentylenetetrazol** but it did not antagonized the seizures caused by **picrotoxin**. These results revealed that ivermectin has the pharmacological profile of an anxiolytic drug with GABAergic properties and that the site of action is different from that of the benzodiazepine drugs.

Moreover, as ivermectin enhances the binding of benzodiazepines to brain membranes, a possible enhancement of some of their

pharmacological effects was suggested [85-87]. Furthermore, when coadministered with **diazepam** (a compound used as anticonvulsant, anxiolytic, an adjunct to anesthesia, in the management of clinical signs of tetanus, in the treatment of functional urethral obstruction and urethral sphincter hypertonus in cats, and as appetite stimulants in cats), ivermectin prolonged the anticonvulsant action of this compound in genetically photosensitive epileptic chickens, providing effective protection against seizures [88]. Finally, ivermectin administered alone (15 mg/kg) prevented mortality in mice injected the convulsant monomethylhydrazine (30 mg/kg), while the concurrent administration of the antiparasitic drug (at a lower dose: 10 mg/kg) with diazepam (5 mg/kg) resulted in no seizures or deaths of the animals [89].

CONCLUSSION

Ivermectin interactions with another concurrently administered drug can occur. Many interactions may arise through competition for binding sites on transport proteins, such as P-glycoprotein. As a result, the absorption or excretion of ivermectin can be modified, resulting in higher levels of this drug. This fact can be a useful tool to increase the efficacy of ivermectin as observed with verapamil or, on the contrary, give rise to toxic levels of the drug (for example when administered with cyclosporin A). Other important interactions involve cytochrome P450 and metabolism due to ivermectin is subjected to hepatic oxidative biotransformation typical of cytochrome P450 pathways.

Thus, the factors responsible for modifying ivermectin pharmacokinetics and / or pharmacodynamics should be taken into account to ensure its clinical efficacy, prevent subtherapeutic levels, and minimize the development of resistance, allowing producers more time to adopt new strategies as parasite resistance to ivermectin increases.

In addition, it is important to take into account that the pharmacokinetics and / or pharmacodynamics of the drug co-administered with ivermectin can be altered. These interactions sometimes are beneficial, like the observed with diazepam (enhancement of its anticonvulsivant effects), but some of them are dangerous (as the increase in adverse reactions of spinosad).

LIST OF ABBREVIATIONS

AUC	=	Area under the plasma-concentration-time curve.
AUMC	=	Area under the first moment curve.
C_{max}	=	Maximum plasma concentration.
MRT	=	Mean residence time.
P-glycoprotein	=	P-gp.
$t_{1/2}$	=	Elimination half-life.
$t_{1/2ka}$	=	Absorption half-life.
t_{max}	=	Time to reach the maximum plasma concentration.

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