Pharmacological approaches towards rationalizing the use of endoparasitic drugs in small animals

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Parasitic diseases are an important health concern to small animal veterinarians worldwide, and their zoonotic potential is also of relevance to human medicine. The treatment and control of such conditions relies heavily on pharmaceutical intervention using a range of antiparasitic drugs and/or their biologically active metabolites. Broad spectrum agents have been produced, although narrow and even monospecific drugs are used in some situations. Their efficacy may depend on dosage, the target pathogen(s), the host species and/or the site of infection. Optimal use of antiparasitics requires a detailed consideration of the pharmacokinetic and pharmacodynamic properties of the drugs in specific clinical contexts. This review summarizes the present status of knowledge on the metabolism, and physicochemical and pharmacological properties of the major antiparasitic drugs currently used in small animal veterinary practice. In addition, data relevant to therapeutic dosage, efficacy and clinical indication/contraindication, particularly in relation to combination drug therapy, are included.

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INTRODUCTION

Internal parasitic diseases in small animals are relevant in clinical practice. Many are common and some considered zoonotic. The treatment and control of parasitic disease requires epidemiological knowledge of the parasite involved and an understanding of the pharmacological and therapeutic characteristics of the anthelmintic drugs available on the market. The therapy of parasitic diseases in small animals should be designed to optimize clinical efficacy and minimize the potential toxicity of anthelmintic drugs used, and decrease the risk of transmissibility to man. Although resistance to anthelmintics used in small animals does not appear to be a major problem at present, treatment strategies should be designed to minimize selection pressure.

Modern endoparasiticide therapy in dogs and cats utilizes a variety of drugs which are classified according to their spectrum of activity, physicochemical properties and mode of action, as follows: (i) piperazine and its derivative, diethylcarbamazine; (ii) tetrahydropyrimidines; (iii) imidazothiazoles; (iv) benzimidazole methyl-carbamates and pro-benzimidazoles (pro-BZD); (v) macrocyclic lactones; (vi) cyclic octadepsipeptides and (vii) narrow spectrum cestodicides [e.g. praziquantel (PZQ) and epsiprantel (EPQ)].

This classification is useful in selecting anthelmintics for treatment of parasitic disease where an empiric diagnosis is made and broad spectrum compounds are used (e.g. BZD or macrocyclic lactones), or where confirmed diagnosis indicates narrow spectrum compounds (e.g. piperazine, imidazothiazoles and cestodicidal compounds).

Some anthelmintic molecules have a low therapeutic margin. Moreover, toxicity may result from using inappropriate drug combinations in an effort to obtain broad spectrum efficacy. As a consequence, classification of antiparasitic drugs by their mode of action (pharmacodynamics) is highly relevant for the veterinarian to avoid unwanted interactions which could compromise patient health (Table 1). For example, the combination of levamisole (LVM) and pyrantel (PRT) is not recommended because their mode of action as cholinergic agonists in the target parasite is similar, and they share the same mechanism of toxicity. In combination, therefore, PRT increases the toxicity of LVM to the host (Martin, 1997). Pyrantel and piperazine may also be a combination to avoid because both have antagonistic pharmacological effects against the parasite.

Table 1. Pharmacodynamics and major pharmacological effects of some antiparasitic drugs available for use in small animals (Martin, 1997; Harder, 2002)

Antiparasitic drug	Mode of action	Pharmacological effect
Pyrantel/oxantel	Agonist of nicotinic receptors	Spastic paralysis
Levamisole	Agonist of nicotinic receptors	Spastic paralysis
Piperazine	Gamma aminobutyric acid agonist	Flaccid paralysis
Benzimidazole methyl carbamates	Depolymerization of microtubules	Decreased motility, flaccidity and degeneration of parasite gut
Avermectins/milbemycins	Opening the associated glutamate-gated Cl^- channels	Flaccid paralysis
Diethylcarbamazine	Metabolism of arachidonic acid	Immobilization
Praziquantel/epsiprantel	Increased Ca^{2+} influx	Parasite contraction
Cyclic octadepsipeptides	Activation of a latrophilin receptor causing Ca^{2+} influx	Relaxation of nematode

Pyrantel kills the parasite by inducing spastic paralysis whilst piperazine induces flaccid paralysis (Martin, 1997). The mode of action of the drug may contribute to other side effects of therapy. For example, intestinal obstruction can be a complication following the use of PRT in animals with large ascarid burdens.

PIPERAZINE AND DIETHYLCARBAMAZINE

Piperazine (MW 86.14 kDa) has a relatively simple chemical structure (Fig. 1a). It is a strong base soluble in water (1:18), glycerol and glycols, but only sparingly soluble in alcohol and totally insoluble in ether (Pharmaceutical Codex, 1979; Martindale: The Extra Pharmacopoeia, 1993). Piperazine, the active principle, is relatively unstable as the free base, and is usually formulated as a salt such as the adipate, citrate, phosphate, hexahydrate, to improve stability. Anthelmintic activity is directly related to the proportion of free base and this varies according to the salt form (e.g. adipate, 37% free base; chloride, 48%; citrate, 35%; dihydrochloride, 50–53%; hexahydrate, 44%; phosphate, 42% and sulphate, 46%) (Courtney & Roberson, 1995). Piperazine is classified as a narrow spectrum drug as its efficacy has been demonstrated against ascarids only in dogs and cats (Jacobs, 1987).

The mode of action of piperazine was initially thought to involve antagonism of cholinergic receptors located on the neuromuscular membrane, leading to parasite immobilization by flaccid paralysis and consequent removal from predilection site and death. This hypothesis has been refined following recent studies (Martin, 1997; Harder, 2002) which demonstrated selective agonism of gamma amino butyric acid (GABA) receptors, resulting in the opening of chloride channels and hyperpolarization of the membrane of the muscle cells of the parasites.

Piperazine is readily absorbed from the gastrointestinal (GI) tract and then extensively metabolized (60–70%). The remaining parent molecule is eliminated in urine, without modification, over the 24 h period following dosing. (Pharmaceutical Codex, 1979). The recommended dose in dogs and cats is 45–65 mg/kg of piperazine free base, given as a single oral dose. Efficacy has been demonstrated against *Toxocara* and *Toxascaris*, although piperazine is ineffective against *Trichuris vulpis* (Jacobs, 1987). In cats, piperazine was the anthelmintic for which toxicity (confirmed or suspected) was most frequently recorded by the Illinois Animal Poison Information Center (IAPIC) between January 1986 and August 1988 (Lovell, 1990), although this may reflect its widespread use as much as its toxic potential. Clinical signs of neurotoxicity in dogs and cats are manifested as muscle tremors, ataxia and alteration of the patient behaviour within 24 h after a daily dose of 100 mg/kg. The therapeutic index of piperazine has been established as three (cats) to six (horses) (EMEA/MRL/807, 2001).

DIETHYLCARBAMAZINE CITRATE (DEC)

Diethylcarbamazine citrate (MW 391.4; Fig. 1b) is a derivative of piperazine which is highly soluble in water, alcohol and chloroform, but insoluble in organic solvents (Pharmaceutical Codex, 1979) and it is stable in the environment. In veterinary medicine DEC is used in the control of filariasis in dogs, but only in combined commercial formulations also containing oxibendazole, ivermectin (IVM) or milbemycin-oxime (MBM-O). DEC was formerly used for the treatment of heartworm in dogs.

Diethylcarbamazine citrate appears to have different mode of action compared with other antiparasitic molecules, as its activity on microfilariae is absent *in vitro*, but extensive *in vivo*. It has been shown that DEC inhibits cyclic peroxide generation from arachidonic acid (AA) breakdown, specifically through its effects on the enzymes leucotriene (LT)A4 synthetase, LTC4 synthetase, prostacyclin (PGI)2 synthetase and prostaglandin (PG)E2 synthetase. However, DEC has no apparent inhibitory effect on thromboxane synthetase and, therefore, cannot be classified as an inhibitor of cyclo-oxygenase (Martin, 1997). Microfilariae produce PGI2 and PGE2 within endothelial cells of blood vessels of infested patients. As DEC can alter AA metabolism in both parasite and host, it is possible that vasoconstriction combined with amplified endothelial adhesion may be responsible for immobilization of the microfilaria with a complementary cytotoxic action provided by host platelets and granulocytes (Martin, 1997).

After oral administration, DEC is readily absorbed from the GI tract achieving maximum plasma concentration (C_{max}) at 3 h post-administration, with a subsequent detection period in

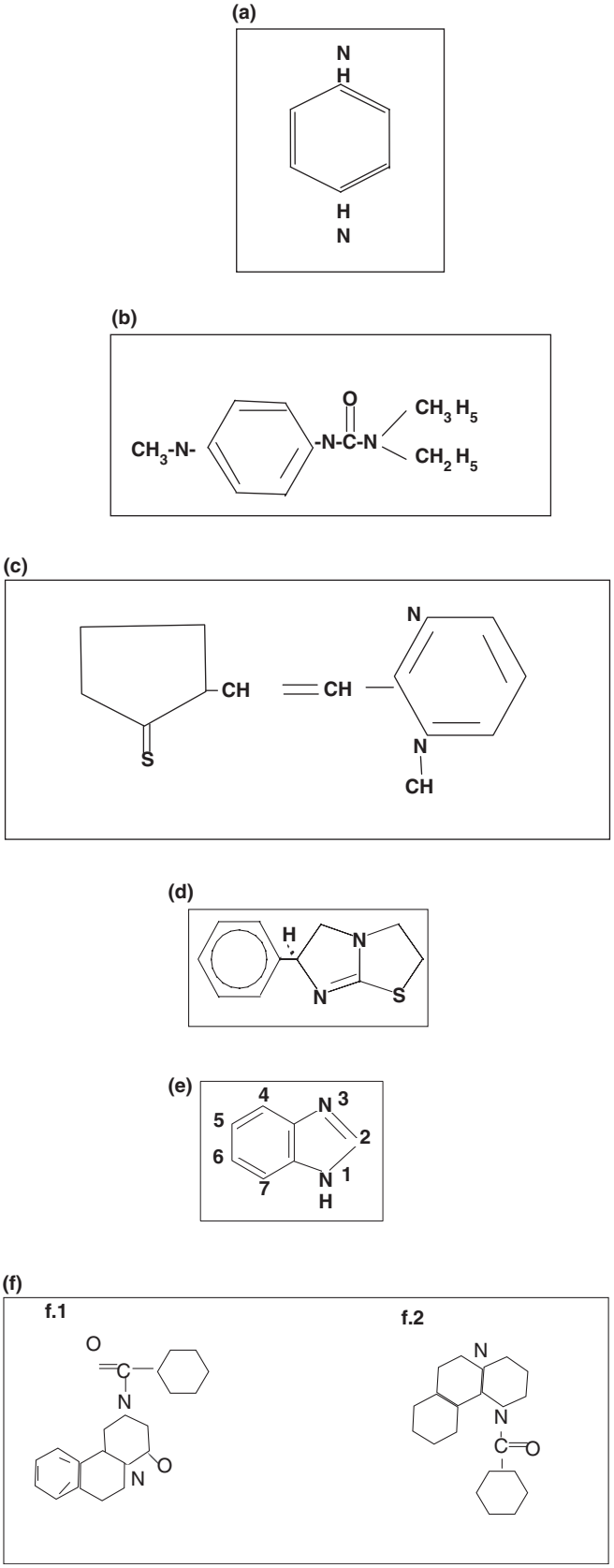

Fig. 1. Chemical structures of (a) piperazine, (b) diethylcarbamazine, (c) pyrantel, (d) levamisole, (e) benzimidazole central nucleus and (f1) praziquantel and (f2) epsiprantel.

plasma of approximately 48 h. The drug is widely distributed in tissues and is metabolized in the liver by N-dealkylation and N-oxidation into four metabolites. DEC is excreted in the urine either unchanged or as the N-oxide metabolite. Urinary excretion and, consequently, plasma half-life are dependent on urinary pH (Pharmaceutical Codex, 1979; Martindale: The Extra Pharmacopoeia, 1993). Studies with activated charcoal demonstrated that the latter significantly decreased the absorption and elimination of DEC by adsorption in GI tract (Orisakwe *et al.*, 2000, 2001). DEC was shown to have generally low toxicity at the therapeutic dose of 6.6 mg/kg given as tablets, or 2.5 mg/kg p.o. given in powder form once a month, although gastric irritation was observed as a side effect in a few patients (Courtney & Roberson, 1995). DEC is contraindicated in dogs parasitized with adult filaria as a hypovolemic shock type reaction occurs in a small percentage (0.3–5%) of such dogs after DEC administration, and this may lead to death within in a few hours (Courtney & Roberson, 1995).

TETRAHYDROPYRIMIDINES: PYRANTEL, OXANTEL

Pyrantel (1,4,5,6-tetrahydro-1-methil-2[2-(2-tieneil)etenil]-pyrimidine; MW, approximately 595 kDa; Fig. 1c) was the first tetrahydropyrimidine derivative used in small animals, followed by its phenol analogue, oxantel (*m*-oxiphenyl (3-[2-(1,4,5,6-tetrahydro-1-methyl-2-pyrimidyl)ethenyl] phenol)). Both drugs have been formulated as a variety of salts (pamoate, embonate, tartrate, citrate and fumarate) with the pamoate and tartrate forms being the most commonly used because of their more suitable kinetics. PRT pamoate is a yellow or dark yellow powder, which is practically insoluble in water or alcohol. PRT tartrate is similar, but more soluble in water than the pamoate. Each gram of the PRT pamoate is equivalent to approximately 347 mg (34.7%) of PRT free base.

Drugs belonging to this family exert antiparasitic action exclusively against adult GI nematodes (Katz, 1977) mimicking a cholinergic effect and depolarizing the parasite neuromuscular membrane, resulting in spastic paralysis (Harder, 2002). *In vitro* studies revealed that, in terms of potency, the effect was 100 times greater than the process mediated by acetylcholine and, whereas acetylcholine induced a reversible depolarization that associated with PRT was irreversible (Courtney & Roberson, 1995). Experiments in which mice and rats were given PRT intravenously resulted in death of the treated animals because of complete neuromuscular blockade. For that reason, the parenteral administration of PRT and its analogues is contraindicated in mammals (Martin, 1997). Drugs of this group demonstrate similar pharmacokinetic (PK) profiles and, after oral administration, differences in the proportion of drug absorbed are related to the particular salt form used. Other inconsistencies in absorption have been related to the particular characteristics of the GI tract of monogastrics compared with ruminants. PRT pamoate (16% free base) is poorly absorbed in dogs after oral administration and, consequently, it is eliminated largely in the faeces (Cadiergues & Franc, 1994). PRT citrate (41% free base) is

more extensively absorbed than the pamoate. Interestingly, there is a negative correlation between enhanced bioavailability and efficacy, as increased anthelmintic activity is achieved with PRT pamoate. This may be because a higher parasite exposure concentration is achieved during bulk flow of drug confined to the GI tract. Biotransformation studies indicated that PRT is mainly metabolized in the liver in most species studied. Hydroxylation of the thiophen ring appears to be the major route of metabolism, and several inactive polar metabolites are detected in the bile (Cadiergues & Franc, 1994). In dogs and cats, the tetrahydropyrimidines are exclusively indicated for the control of GI nematodes (*Toxocara*, *Toxascaris*, *Ancylostoma* and *Uncinaria*) (Jacobs, 1987). PRT has no efficacy against *Trichuris vulpis*, *Taenia* or microfilaria. PRT is relatively safe when administered p.o. as a salt, with the LD_{50} in dogs (690 mg/kg) being 138 times higher than the therapeutic dose (Courtney & Roberson, 1995). The use of tetrahydropyrimidines in combination with other anthelmintic drugs, with a similar mode of action, such as LVM, is not recommended.

IMIDAZOTHIAZOLE COMPOUNDS: LEVAMISOLE, BUTAMISOLE

Levamisole was first synthesized as a racemic mixture (tetramisole) containing 50% of each of two isomers, namely L-tetramisole (or levo tetramisole) and D-tetramisole (or dextro tetramisole) and, indeed, DL-tetramisole is still available on the market. However, when the isomers were studied individually, L-tetramisole (LVM) (Fig. 1d) was shown to exhibit more potent anthelmintic activity than D-tetramisole and as both isomers had similar toxic potency it was apparent that producing the levo isomer alone would confer greater potency for an equivalent safety. Consequently, LVM [(-)-2,3,5,6-tetrahydro-6-phenilimidazole[2,1b] thiazole] was marketed individually, as either its chlorhydrate or phosphate salt, in either liquid or tablet form.

Levamisole induces spastic paralysis in the target parasite as a result of permanent muscle contraction. This does not kill the parasite, which may be eliminated live from the body. Paralysis is associated with an agonistic cholinergic action on nicotinic receptors of susceptible parasite ganglia (Harder, 2002). LVM has a weak inhibitory effect on parasite acetylcholinesterase enzyme and has also been reported to inhibit fumarate-reductase which affects the oxidation of succinic acid and ultimately leads to impaired carbohydrate metabolism (Martin, 1997). However, in practical terms, the latter effect is of little clinical relevance as the concentrations of LVM required within the parasite are well in excess of the therapeutic dose. LVM is inactive against cestodes and external parasites, and is indicated only for the control of some larval stages and adult nematodes in dogs and cats. LVM is not ovicidal and has been shown to have no effect against inhibited stages of nematodes such as *Ancylostoma* (Jacobs, 1987).

Intravenous administration is contraindicated for the therapeutic use of LVM because of the danger that side effects may occur, although it has been given experimentally by this route to assess absolute bioavailability. In such studies, LVM showed an increased oral bioavailability (64%) when dogs were fasted for 12 h pre and post-treatment (i.e. a total fasting period of 24 h) compared with nonfasted animals (44%) (Watson *et al.*, 1988; Fig. 2). This suggests that a 24 h fasting period favours the absorption and efficacy of LVM in dogs as, unlike PRT, redistribution from plasma into the GI tract is thought to confer efficacy. LVM is widely distributed in the body achieving particularly high concentrations in the liver. It also accumulates in fat, kidney and blood, although some appears in the urine within 2 h after oral administration. LVM is extensively metabolized in the liver and 94% of the parent drug is excreted in the form of metabolites in urine (IPS INCHEM, 2003). After oral administration in dogs, concentrations of LVM rapidly decline over a period of 12 h, with 90% of the total dose excreted within 24 h (IPS INCHEM, 2003). *In vitro* biotransformation studies using hepatocytes isolated from dogs, pigs, cattle and man suggested qualitatively similar metabolic patterns in these species, with all metabolites seen in cattle also seen in dogs (IPS INCHEM, 2003).

Secondary effects were observed using supra-therapeutic doses of LVM, or when the drug was combined with organophosphates, as the latter also have a cholinomimetic mode of action. This explains the similarity between the clinical signs associated with LVM toxicity and organophosphate poisoning. Thus, dogs and cats exposed to high doses of LVM developed vomiting, diarrhoea, neurotoxicity, agranulocytosis, lung oedema, dyspnoea, immuno-mediated cutaneous eruptions and lethargy, amongst other disorders (Hsu, 1980). In weak animals, or those suffering from hepatic and/or renal failure, LVM should be used with extreme caution or, preferably, not at all. Although, no reliable or detailed information is available on the use of LVM in pregnant animals, it is indicated in situations where possible benefits overcome the risks of poisoning (Plumb, 1995). It should also be noted that the concomitant use of drugs with nicotinic action (e.g. PRT, morantel, diethylcarbamazine) or acetylcholinesterase inhibitors (e.g. organophosphates, neostigmine), may

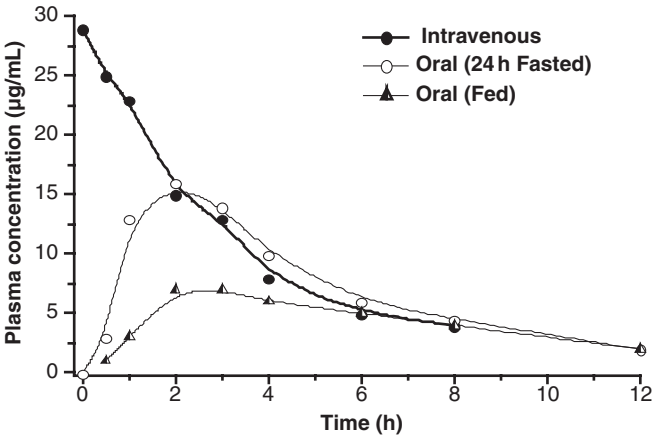

Fig. 2. Bioavailability of levamisole after i.v. or oral administration (10 mg/kg) in fed and 24-h fasted dogs. (Adapted from Watson *et al.*, 1988).

increase LVM toxicity. The therapeutic dose of LVM required depends on the target parasite involved. For example, in dogs, 5 mg/kg administered every 24 h for 2 days gives 94–100% efficacy against *Toxocara canis*, although a single dose of 10 mg/kg gives 95–100% efficacy against *Toxocara canis*, *Toxascaris leonina* and *Ancylostoma*. A dose of 7.5 mg/kg every 24 h for 3 days gives 95–100% efficacy against *Angiostrongylus*, but the same dose must be given for 10–30 days to be similarly effective against *Dirofilaria immitis*. LVM has no efficacy against *Trichuris vulpis*. In cats, a single dose of 5 mg/kg gives 95–100% efficacy against *Toxocara*, *Toxascaris* and *Ancylostoma*, whilst a similar dose (i.e. 5 mg/kg) must be given every 24 h for 14 days to achieve similar efficacy against nematodes of the respiratory tract.

Butamisole hydrochloride: propanamide, 2 methyl-N-[3-(2,3,5,6-tetrahydroimidazo [2,1-6] thiazol-6-yl) phenyl] monohydrochloride), is no longer sold as an anthelmintic in the USA. However, elsewhere butamisole (BTM) is used for the treatment of *T. vulpis* and *Ancylostoma* infections with 99% and 92% efficacy respectively. It is recommended at a dose of 2.4 mg/kg subcutaneously and has a threefold safety margin. The mode of action of BTM is apparently similar to that described for LVM with, consequently, the signs of toxicity (Courtney & Roberson, 1995).

BENZIMIDAZOLE COMPOUNDS

The benzimidazoles (BZDs) comprise a family of related anthelmintic compounds and their metabolites/derivatives which are widely used in antiparasite therapy in both veterinary and human medicine. Febantel (FBT), a pro-BZD, fenbendazole (FBZ) and mebendazole (MBZ) are the only methyl-carbamate BZDs approved for use in anthelmintic therapy in companion animals in Europe and the USA, although albendazole (ABZ) has also been approved for use in dogs and cats in a number of other countries worldwide. BZDs, used in correct dosage regimens, have a broad spectrum of activity against a variety of pulmonary and GI nematodes, and cestodes, in both cats and dogs (Jacobs, 1987). ABZ and FBZ may also be active against the flagellate protozoan, *Giardia* spp., in dogs (Barr *et al.*, 1993, 1994; Zajac *et al.*, 1998). BZDs are poorly soluble in water and this means that they are primarily administered as oral suspensions. As a consequence, their development into alternative pharmaceutical formulations has been limited.

There are marked species differences in the disposition of BZDs, although activity has been associated with drug reaching the GI tract by bulk flow and, more importantly, by redistribution from plasma. Higher and more sustained concentrations at the parasite target are achieved in ruminants and horses, compared with dogs and cats, after administration of a single dose. This was shown to be due to the reservoir role of the rumen, or the caecum/colon, in ruminants and horses respectively (Jacobs, 1987; Lanusse & Prichard, 1993; Baggot & McKellar, 1994). Several studies suggest that only limited rates of dissolution and absorption of BZD anthelmintics are achieved in the cat, dog and human. Consequently, these compounds may need to be given at a higher dose or as multiple administrations to provide therapeutic concentrations and, therefore, to achieve acceptable anthelmintic efficacy (Roberson & Burke, 1982; Jacobs, 1987; Edwards & Breckenridge, 1988).

BZDs and pro-BZD are considered to be one of the most important chemical families for the control of parasitic diseases in small animals because of their low cost, broad spectrum of activity and low toxicity. In 1961, the discovery of thiabendazole as a nematocide with a high therapeutic margin, encouraged the synthesis and development of several other anthelmintic derivatives from the same chemical nucleus (Lanusse & Prichard, 1993). BZDs are stable, crystalline substances which are all relatively insoluble in water, benzene and ether, but highly soluble in alcohol and other nonpolar solvents (Martindale, 1993). The chemical nucleus of BZDs is composed of a bicyclic ring formation in which a benzene group is attached at the 4- and 5- positions of an imidazole ring (Fig. 1e). Different chemical modifications at the 2- and 5-carbon positions of the basic BZD molecule resulted in the synthesis and development of a range of more potent drugs. Those which have been used therapeutically in small animals, are listed in Table 2.

BZDs have a high efficacy against adult and immature stages of GI and lung nematodes, including tissue arrested larvae of *Ancylostoma* (Jacobs, 1987). However, low GI absorption and lack of water solubility, have limited the development of pharmaceutical formulations, bioavailability and efficacy of BZDs. Pro-BZD, such as FBT, was developed in an attempt to overcome some of these problems, but they have had only a limited uptake. BZDs have a variety of pharmacological actions but their therapeutic effect is thought to depend primarily on the inhibition of parasite tubulin. Effects of structural modifications of the basic BZD molecule on the ability to alter the microtubular dynamic have been investigated (Lacey, 1990) and it has been shown that the carbamate group at position 2 of the BZD ring is pivotal for antimicrotubular activity. Substitution of the carbamate group by an amide reduces BZD activity by up to 10 times. Similarly, the replacement of the NH- group in the BZD chemical nucleus by -S- or -O-, completely abrogates the capacity for tubulin binding and the antitubular action (Lanusse & Prichard, 1993). BZDs with substitutions at position 5, namely ABZ, FBZ, oxfendazole (OFZ), which is fenbendazole sulphoxide (FBZSO) and MBZ are the compounds most commonly used in small animals.

Table 2. Benzimidazole compounds commonly used in small animals

Benzimidazole methylcarbamates:
Fenbendazole
Albendazole
Mebendazole
Oxfendazole
Oxibendazole
Pro-benzimidazoles
Febantel

Pharmacokinetic (PK) and pharmacodynamic (PD) relationships

The pharmacological effects of a drug can generally be observed a short time after its administration. However, between administration and pharmacological effect, the drug must cross biological barriers, and it can do this rapidly or slowly depending on the physicochemical properties of the molecule administered, and the nature of the biological barrier. A prerequisite for penetration of a biological barrier by any drug is its aqueous solubility. BZD compounds, such as ABZ or FBZ, formulated as tablets or suspensions for oral administration, must dissolve at low pH (stomach), and it has been demonstrated that the dissolution rate may be altered according to the size of the formulation particle (Hennessy, 1993). Subsequently, the dissolved drug is absorbed and distributed through the body, metabolized and eventually eliminated. BZDs distribute to the site where the target parasite is located (tissue/fluid) to a variable extent, according to the tissue concerned. Once there, they then have to penetrate the parasite itself to recognize and bind to the tubulin receptor to achieve their pharmacological effect.

FBZ, ABZ and MBZ are methyl-carbamate BZDs approved for use as broad spectrum anthelmintics in human medicine. In the UK, FBZ and MBZ are also approved for use in cats and dogs. Although they belong to the same chemical family, there are marked differences in their patterns of biotransformation and the resultant metabolite concentration profiles (Fig. 3). The parent drugs, FBZ and ABZ, contain a sulphur atom as a sulphide at position 5 of the BZD molecule. These sulphides are subjected, mainly in the liver, to phase I reactions (oxidation), catalysed by the flavine monooxygenase and the cytochrome P-450 (Cyt P-450) enzyme systems to form sulphoxide (SO) metabolites, with FBZSO (OFZ) and albendazole sulphoxide (ABZSO) being the primary, pharmacologically active metabolites generated (Murray *et al.*, 1992; Lanusse & Prichard, 1993). In a second metabolic reaction (sulphonation), catalysed by the Cyt P-450 system OFZ and ABZSO are transformed into inactive sulphone (SO_2) metabolites, namely $FBZSO_2$ and $ABZSO_2$ respectively (Lanusse & Prichard, 1993). MBZ has a keto-group at position 5 of the BZD ring and is also extensively biotransformed in the liver, in a phase I reduction process, in which pharmacologically inactive hydroxy-mebendazole (OH-MBZ) is produced as the major metabolite (Braithwaite *et al.*, 1982; Witassek *et al.*, 1983; Edwards & Breckenridge, 1988).

Unlike ABZ and FBZ, MBZ is poorly absorbed after oral administration and only low concentrations (representing <10% or less of the total drug administered) of MBZ parent drug and its inactive metabolite, OH-MBZ, were recovered in plasma of humans and dogs after oral treatment of MBZ (Witassek *et al.*, 1981; Edwards & Breckenridge, 1988; Plumb, 1995). This observation could explain the lack of efficacy of MBZ against lung parasites in humans and dogs. The pharmacokinetics of FBZ parent drug and its metabolites in dogs were characterized by McKellar *et al.* (1990, 1993, who detected FBZ, its active sulphoxide (OFZ) and inactive sulphone ($FBZSO_2$) metabolites in plasma for 48 h after administration of a single oral dose of 20 mg/kg (Fig. 4). The *AUC* ratio (FBZ:OFZ) showed considerable variation with dose even within the same study (see Fig. 8). Thus ratios of 1.61 (2.5 mg/kg), 1.26 (5.0 mg/kg), 0.86 (10.0 mg/kg), 1.55 (20.0 mg/kg), 1.30 (40.0 mg/kg) and 0.66 (80 mg/kg) were obtained (McKellar *et al.*, 1993). There is no obvious pattern to these ratios and they may simply reflect very large variations among animals which was certainly a feature of this study. This results in exposure of the parasites to the FBZ moiety which has greater potency than OFZ, and thus activity against the tissue arrested larvae and other immature parasite stages (Fig. 4; McKellar *et al.*, 1990). When ABZ was given as a single dose in tablet form, ABZSO and $ABZSO_2$ were

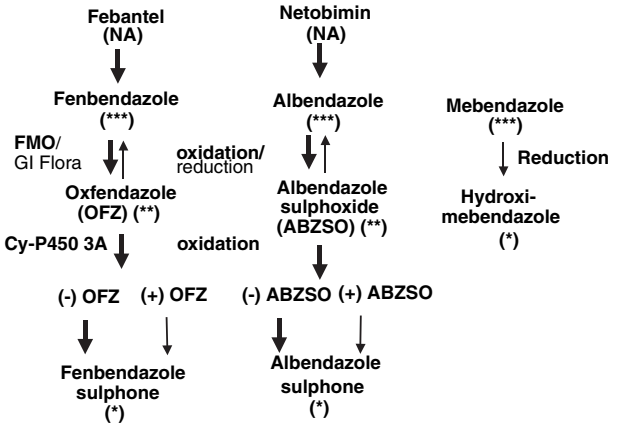

Fig. 3. Proposed metabolic pathway for methylcarbamate benzimidazoles. Oxidation processes are mediated by flavine monooxygenase (FMO) and cytochrome-P450 (Cyt P-450 3A). Reduction processes for oxfendazole (OFZ) and albendazole sulphoxide (ABZSO) are mediated by the gastrointestinal (GI) flora. Albendazole sulphoxide enantiomeric pathway: (−) and (+) enantiomers in dogs were demonstrated by Delatour *et al.*, 1990. The anthelmintic potency of parent drugs and metabolites are represented as NA, no activity; *, very poor or no activity; **, good activity and ***, very good activity.

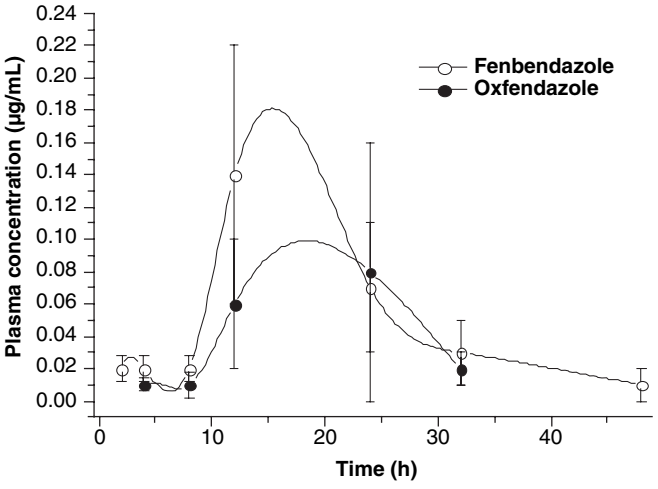

Fig. 4. Pharmacokinetic profile of fenbendazole (FBZ) and its metabolite oxfendazole after the oral administration of granulated FBZ (20 mg/kg) in dogs (Adapted from McKellar *et al.*, 1993).

the main metabolites detected in plasma for up to 16 h post-treatment (Fig. 5; Sánchez *et al.*, 2000).

BZD methyl-carbamates are more lipophilic compounds than thiabendazole and, therefore, have longer residence time in the systemic circulation. Consequently, the reversible distribution between plasma and the GI tract, which is crucial for the attainment of therapeutic concentrations in lung and digestive tract, is also longer and increases the exposure of the parasite to toxic levels of these drugs. Compared with ruminants, where the rumen acts as a drug reservoir, effective treatment of monogastrics requires more prolonged, multidose regimens of at least 3–5 days depending on the dose of drug used. For example, plasma concentrations of ABZSO were maintained in cattle for 36–72 h after treatment with 10 mg/kg ABZ, but for only 18 h in dogs given 25 mg/kg ABZ (Sánchez *et al.*, 1995; Sánchez *et al.*, 2000). Similar differences were obtained in ruminants, horses and dogs after FBZ and OFZ administration (McKellar *et al.*, 1990, 1993; McKellar *et al.*, 2002; Sánchez *et al.*, 2003). The results support the concept that, to achieve high efficacy against adult and immature parasites in monogastrics, repeated administration of BZDs is necessary to achieve sustained concentrations at the infection site (see Table 3).

As stated earlier, methyl-carbamate BZD anthelmintics are basic molecules ($pK = 7.8$) with low aqueous solubility, and this limits their formulation and potential routes of administration in both ruminants and nonruminant species (McKellar & Scott, 1990). It has been suggested that low aqueous solubility of BZDs may limit absorption during GI transit (McKellar *et al.*, 1990; McKellar & Scott, 1990; Baggot & McKellar, 1994) and, in dogs, this may be compounded by the short gut transit time compared with other domestic species (McKellar *et al.*, 1993) and man (Berardi & Camarero, 2003). The rate of dissolution of BZD anthelmintics in the stomach differs between animal species. This is thought to be crucial for achieving adequate absorption, consequent availability and retrograde GI secretion and, ultimately, high clinical efficacy (Hennessy, 1993; Lanusse & Prichard, 1993).

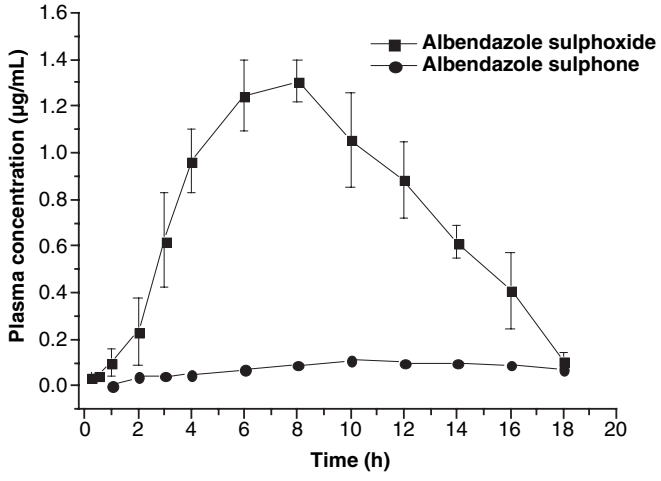

Fig. 5. Pharmacokinetic profile of albendazole sulphoxide (ABZSO) and its metabolite albendazole sulphone ($ABZSO_2$) after the oral administration of albendazole in tablets (25 mg/kg) in dogs. (Adapted from Sánchez *et al.*, 2000).

Table 3. Dose-efficacy relationship for fenbendazole (FBZ) in dogs (Adapted from Roberson & Burke, 1982)

FBZ dose	Efficacy	Genus
50 mg/kg (3 days)	>99%	*T. canis, T. leonina*
	>99%	*A. caninum, T. vulpis*
	100%	*Taenia* spp.
20 mg/kg (5 days)	>99%	*T. canis, T. leonina*
	>99%	*A. caninum, T. vulpis*
	73%	*Taenia* spp.
25 mg/kg (3 days)	67–72%	*A. caninum, T. vulpis*
	99%	*T. canis, T. leonina*
5 mg/kg (3 days)	67–72%	*A. caninum, T. vulpis*
	88%	*T. canis, T. leonina*
100 mg/kg (single dose)	2–27%	*A. caninum, T. vulpis*
	55%	*T. canis, T. leonina*
150 mg/kg (single dose)	57%	*A. caninum, T. vulpis*
	72%	*T. canis, T. leonina*

T. canis, Toxocara canis; *T. leonina, Toxascaris leonina*; *A. caninum, Ancylostoma caninum*; *T. vulpis, Trichuris vulpis.*

Factors affecting PK of BZD compounds in dogs

Historically, antiparasitic drugs have frequently been used inappropriately. Dosing regimens were not optimized as their PK and PD properties were poorly defined. Such misuse is likely to have favoured selection for resistance and/or the risk of toxicity. In the last decade factors that affect PK mechanisms have been characterized in several species. As previously mentioned, the disposition kinetics of LVM were significantly modified after a starvation period of 24 h (Watson *et al.*, 1988). However, food restriction had the opposite effect on the PK behaviour of FBZ and its metabolites in fed and fasted dogs (Fig. 6) (McKellar *et al.*, 1993). It was also reported that a fatty diet improved the dissolution and absorption of BZDs in man (Edwards & Breckenridge, 1988). However, in dogs, diets with differing fat contents had no statistically significant impact on the systemic bioavailability of FBZ and its metabolites (Fig. 7; McKellar *et al.*, 1993).

As sustained concentrations of BZDs in their therapeutic range, are necessary to achieve a good efficacy, the BZD compounds may be considered 'time-dependent' antiparasitic drugs. This implies that increasing dose above an essential minimum concentration does not improve efficacy against adult or immature stages of common parasites (Fig. 8, Table 3). It is well established, that BZD dissolution is rate-limited by the acidic pH of the stomach (Lanusse & Prichard, 1993; Sánchez *et al.*, 1999). As a consequence, the amount of drug absorbed above a minimum, associated with solubility, is constant and not dependent on the dose given. This was confirmed by the observation that the area under concentration–time curves (*AUC*) were similar in dogs given a single administration of FBZ at different doses over the range 25–100 mg/kg (McKellar *et al.*, 1993). More recently, attempts have been made to increase

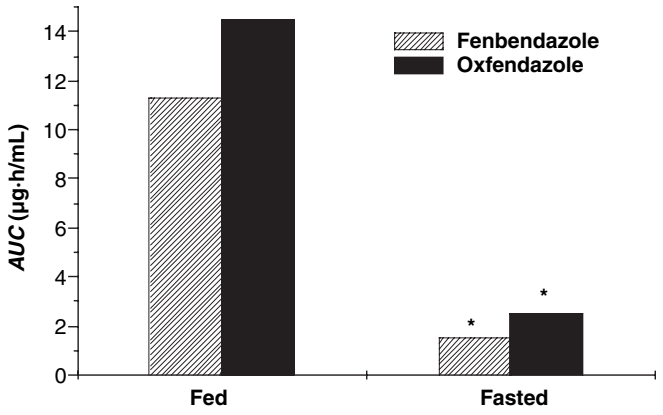

Fig. 6. Comparative area under the concentration vs. time curve obtained for fenbendazole (FBZ) and its metabolite oxfendazole in dogs, after the oral administration of FBZ at 20 mg/kg. *$P < 0.05$ (Adapted from McKellar *et al.*, 1993).

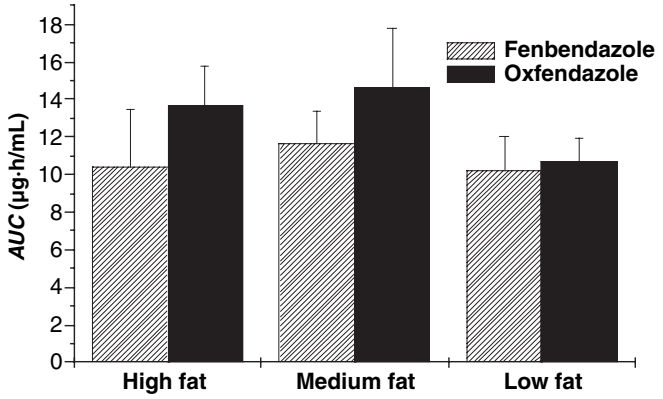

Fig. 7. Effect of diet on the pharmacokinetic profile of fenbendazole (FBZ) and its metabolite oxfendazole in dogs, after the oral administration of FBZ, 20 mg/kg (Adapted from McKellar *et al.*, 1993).

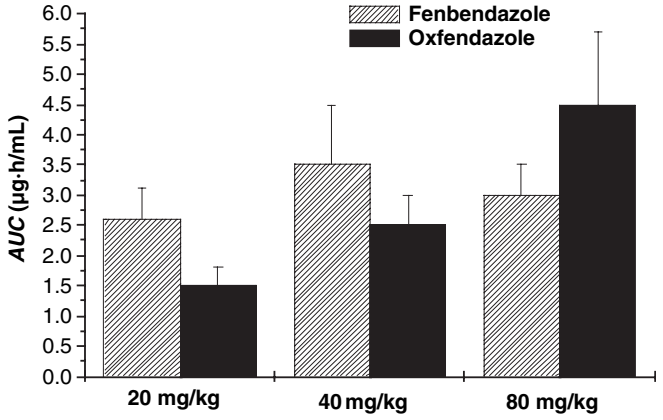

Fig. 8. Effect of dose on the pharmacokinetic profile of fenbendazole (FBZ) and its metabolite oxfendazole in dogs, after the oral administration of FBZ 20, 40 and 80 mg/kg (Adapted from McKellar *et al.*, 1993).

systemic bioavailability and to extend the dose intervals of the recommended therapy for anthelmintic BZD. Gelucire lipid matrixes, which increase the solubility and, therefore, absorption of hydrosoluble drugs have been developed for use in human medicine. A comparative study in dogs demonstrated that ABZ formulated as tablets was absorbed to a greater extent than when administered as gelucire lipid matrix in hard gelatine capsules (Sánchez *et al.*, 2000; Fig. 9). This may reflect a faster dissolution rate of the tablet formulation, possibly the limiting step for absorption in dogs in which there is a short transit time in the GI tract (García Sacristán *et al.*, 1995) compared with humans (Berardi & Camarero, 2003). These results re-emphasize the danger in extrapolating drugs, doses and pharmaceutical formulations between different mammalian species which, in turn, could compromise rational therapeutic use and treatment success. International dosage recommendations for the use of BZDs in small animals include: ABZ and MBZ 25 and 20 mg/kg, respectively, every 24 h for 4 days, confer similar efficacy to FBZ, 50 mg/kg given every 24 h for 3 days (see Table 3). The same dose of ABZ and FBZ given every 12 h is effective against *Giardia* spp. (Barr *et al.*, 1993, 1994; Zajac *et al.*, 1998). MBZ, at a total dose of 200 mg given at 12 h intervals for 4 days, is effective against adult *Echinococcus granulosus* (Jacobs, 1987).

ENDECTOCIDES: AVERMECTINS AND MILBEMYCINS

Endectocides are widely used for treatment of parasitic diseases in large animals and owe their popularity to high potency (effective at µg/kg doses), broad spectrum (against both endo- and ectoparasites), excellent clinical efficacy and long persistence in the body. The macrocyclic lactones used in small animals comprise the avermectins (AVM): ivermectin and selamectin (SMT) and the milbemycins: moxidectin (MOX) and MBM-O. Both groups share physicochemical properties including high molecular weight (approximately 800–900 kDa), solubility in organic solvents and insolubility in water. They are lipophilic compounds showing extensive tissue distribution. The mode of action of endectocides is mediated through the opening of

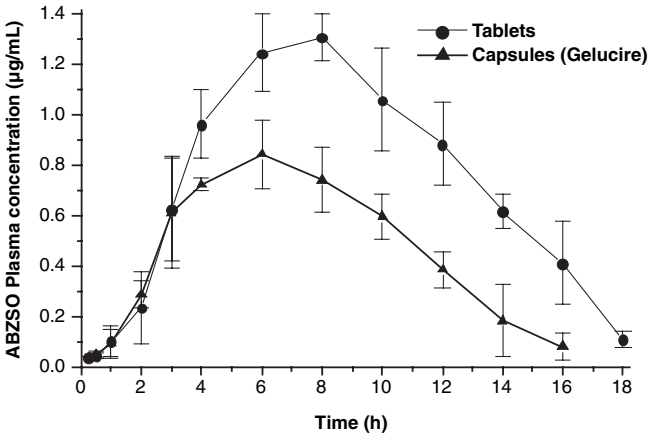

Fig. 9. Comparative pharmacokinetics profile of albendazole sulphoxide (ABZSO) after the oral administration of albendazole (25 mg/kg), formulated in tablets and capsules containing the lipid matrix gelucire (Adapted from Sánchez *et al.*, 2000).

chloride channels associated with glutamate and GABA neurotransmitters. Penetration of chloride ions into cells produces their hyperpolarization resulting in flaccid paralysis and, ultimately, the death of the parasite (Martin, 1997). Endectocide drugs are also characterized by high volume of distribution and minimal metabolism compared with BZD compounds. The high lipophilicity accounts for the extensive tissue distribution, and diffusion of drug into the parasite. This may underlie the high efficacy against certain tissue parasitic stages such as *Ascaris* larvae or arrested larvae of *Ancylostoma*, which are normally refractory to treatment with more hydrosoluble molecules such as LVM and/or PRT. The systemic persistence of the endectocides explains their prolonged pharmacological effect and their ability to protect against re-infestation. These properties are related both to their extensive tissue distribution and low hepatic capacity to metabolize the endectocides into more hydrosoluble compounds which would be more readily eliminated.

Avermectins (ivermectin and selamectin)

The 'extra-label' use of IVM at a dose of 200 μg/kg, extrapolated from the bovine commercial formulation, is a common practice used by veterinary surgeons in many countries worldwide. The i.v. PK profile of IVM given intravenously at 200 μg/kg in dogs was described by Lo *et al.* (1985) who reported a rapid decline in plasma IVM concentrations resulting in shorter elimination half-lives (1.8 days) compared with ruminants (approximately 2.8 days). However, the volume of distribution obtained was large (2.40 L/kg) and consistent with its lipophilic chemical nature (Fink & Porras, 1989). The efficacy of IVM was evaluated in studies using different commercial injectable formulations (Campbell, 1989). The results suggested that IVM activity against the more common parasites of dogs may be associated with parasite feeding habits: haematophagous species such as *Ancylostoma* or *Uncinaria* were sensitive to low doses of IVM given p.o. or by the s.c. route. Thus, the range 10–50 μg/kg of IVM given p.o. or s.c. conferred >95% efficacy against adult stages of *Ancylostoma* (Campbell, 1989). However, a higher dose of 100 μg/kg is required to obtain 100% efficacy against all stages of *Trichuris vulpis* in dogs (Campbell, 1989).

Ivermectin and other macrocyclic lactones are widely distributed throughout the body, particularly into highly perfused tissues. The intestinal mucosa and GI fluids may also receive large amounts of drug from exposure to biliary metabolites and by P-glycoprotein (P-gp)-mediated uptake processes (Hennessy & Alvinerie, 2002). Some target parasites (*Toxocara* and *Toxascaris*), which are thought to feed on detritus in GI fluids, require exposure to concentrations conferred by significantly higher dose levels (200–400 μg/kg) of IVM to be killed (Campbell, 1989). This is probably because drug concentrations are diluted in GI fluids, and access to parasite receptor targets occurs via the transcuticular rather than the oral route.

Pharmacokinetic studies with radiolabelled drug indicated that, when given p.o. at 6 and 100 μg/kg, IVM is rapidly absorbed by the GI tract with a C_{max} at around 4 h postadministration, and a plasma detection period of 14 days (Daurio *et al.*, 1992). In the same study it was also shown that, quantitatively, the C_{max} and *AUC* values obtained after treatment at 100 μg/kg were approximately 20- and 10-fold higher than after dosing at 6 μg/kg, suggesting dose-dependent PK.

Ivermectin is marketed in a chewable tablet form for the prevention of heartworm, at a recommended dose of 6 μg/kg. At this dose IVM has a relatively narrow spectrum of activity, and is less effective against other GI nematodes. For example, efficacy is only 52% against *A. caninum*, although this can be improved to 98% by doubling the dose (Daurio *et al.*, 1993). A chewable tablet formulation containing a combination of IVM (at 6 μg/kg) and PRT (at 5 mg/kg) has been shown to have high efficacy against *T. canis* (90%) and >98% efficacy against *T. leonina*, *A. caninum* and *Uncinaria stenocephala* (Clark *et al.*, 1992). Although no supporting data was presented, the same combination was reported to be ineffective against *T. vulpis*. The same study also confirmed that there was no interference in efficacy between the two active principles. In a separate study, Shoop *et al.* (1996) have since demonstrated that this combination has 100% efficacy against *A. brazilensis*. In the USA, IVM is also marketed as a prophylactic against *D. immitis* in cats, at a dose 24 μg/kg administered p.o. every 30–45 days.

IVM, SLM and MBMs appear to have a similar mode of action, which involves the potentiation of nematode and arthropod glumatate-gated channels, and possibly also the modulation of GABA-gated channels (Martin, 1997; Harder, 2002). Potentiation of these neurotransmitters is reflected in the stimulation of the influx of chloride ions into nerve cells causing a flaccid paralysis, and resulting in nematodicidal and arthropodicidal activity. IVM toxicity, manifested as a variety of neurological clinical signs including mydriasis, tremors, ataxia, blindness, seizure-like activity, coma and death, has been reported in Collie dogs (Pulliam & Preston, 1989; Hopper *et al.*, 2002) and related breeds. These findings contrasted with those of a previous study (Paul *et al.*, 1987) in which Collies given normal treatment dosages of IVM showed little adverse reaction. Dogs given 100–200 μg/kg IVM developed only mild signs of intoxication whilst those given 600 μg/kg developed no toxic signs at all. This suggests that there may be subpopulations of Collies either sensitive, or recalcitrant to IVM-induced neurotoxicity. The pivotal role of the P-gp in the central nervous system with regard to the neurotoxicity of AVM has recently been established. P-gp is a transmebrane glycoprotein which is an integral component of the blood–brain barrier, mucosa of GI tract and placenta. It modulates the intracellular to extracellular transport of certain types of molecule, including the AVM, and limits the accumulation of the several substrates, in potentially sensitive tissues (Shoop & Soll, 2002). At the blood–brain barrier, P-gp functions as a drug-transport pump mediating the recirculation of a variety of substrates from the brain back into the blood. In this context, the AVM and MBM are likely to be specific substrates for P-gp. A pharmacogenetic study, aimed at confirming the existence of an IVM-sensitive subpopulation of Collie dogs, concluded that alteration (by 4-bp deletion) of the multi-drug-resistance gene (*mdr1*) generated several stop codons that prematurely end P-gp synthesis (Mealey *et al.*, 2001). In the

same study, the authors concluded that dogs homozygous for the deletion mutation showed the IVM-sensitive phenotype, whilst the remaining homozygous normal and heterozygous animals did not. In conclusion, the presence of P-gp at the blood-brain barrier, and placental and GI tract mucosae appears to be an important protective biological barrier against adverse effects of the AVM (Shoop & Soll, 2002).

Selamectin, a new endectocide avermectin derived from doramectin, has recently been licensed for use in dogs and cats. SMT is specifically designed for use in companion animals, and has both anthelmintic and ectoparasiticidal activity at dosages which have good safety in Collies (Bishop *et al.*, 2000). The comparative pharmacokinetics of SMT given intravenously, p.o. or topically in dogs and cats has been described by Sarasola *et al.* (2002). In an i.v. titration study they concluded that SMT, like IVM, showed dose-dependent pharmacokinetics, with *AUC* values increasing as dosage was increased. In contrast, body clearance (Cl_b) and steady-state volume of distribution ($V_{d(ss)}$) were shown to be independent of dose in both dogs and cats. However, species differences were apparent in that values for elimination half-lives were substantially higher in cats (approximately 64 h) than dogs (approximately 14 h). A similar trend was observed for other parameters including Cl_b and $V_{d(ss)}$ and this supports the hypothesis that differences in SMT distribution, metabolism and excretion are species-related. Oral and topical formulations of SMT consistently resulted in higher bioavailability (F) in cats (74% and 109% respectively) compared with dogs (4.4% and 62% respectively). The higher oral bioavailability seen in cats, compared with dogs, may be the result of lower intestinal P-gp concentrations in the cat. Following topical treatment, similar observed species differences may be related to differences in transdermal flux of SMT through the skin of cats and dogs.

Canine heartworm is an important cause of morbidity and mortality in dogs and cats in many parts of the world (Jacobs, 2000). Applied topically at a dose of 6 mg/kg, SMT has been shown to have potent efficacy against infective third stage larvae of the causal organism, *D. immitis* L3, as well as other endoparasites including *T. Canis* and *A. tubaeforme* (Bishop *et al.*, 2000; McTier *et al.*, 2000a,b; Guerrero *et al.*, 2002). SMT has broad spectrum activity and high efficacy against a variety of external parasites including fleas and mites (Bishop *et al.*, 2000) and has been shown to be effective in experimental flea-associated allergic dermatitis in both dogs and cats (McTier *et al.*, 2000a,b; Dickin *et al.*, 2003).

Milbemycins

Milbemycins are unglycosylated AVM, which lack the bisole-androsyl moiety at the C-13 position. Milbemycin D (MBM-D) and the mixture of Milbemycin A4:A3 5-oxime (80:20) (MBM-A4-A3) were developed mainly for the control of heartworm and other endoparasites in dogs (Jung *et al.*, 2002). Using a liquid chromatographical method with UV detection, these authors were able to detect MBM-A4-A3 in the plasma of dogs, given a 5 mg tablet, for up to 48 h after administration. Rapid GI absorption (T_{max}, 1.00–2.4 h) and a high volume of distribution (6.6 L/kg) were also observed in dogs given either tablets, or injectable formulations by the i.v. route at a dose of 12.5 mg (Jung *et al.*, 2002). The same authors reported that the bioavailability of MBM-A4-A3, given as 12.5 mg tablets, was significantly higher in fed (77 ± 2.0%) compared with fasted (54 ± 6%) dogs. Efficacy studies demonstrated that MBM-A4-A3, given monthly, was highly effective against *D. immitis*, *T. canis*, *T. leonina*, *A. caninum*, *A. braziliense* and *T. vulpis* (Guerrero *et al.*, 2002). However, to date, there have been no reports of efficacy against *Uncinaria*.

Moxidectin is a synthetic macrocyclic lactone (MBM) derivative of nemadectin which has been shown to be effective against both endo- and ectoparasites of dogs and cats. It is chemically similar to IVM differing only in the presence of a disaccharide moiety at position C-13, a methoxime at C-23 and a dimethylbutenil side chain at C-25. MOX has lower molecular weight, and is more lipophilic, than other AVM, which allows it to be stored in adipose tissue and results in MOX being highly persistent with a long residence time (Alvinerie *et al.*, 1996; Lanusse *et al.*, 1997).

The pharmacokinetics of MOX, given p.o. as tablets, in dogs has been reported by Vanapalli *et al.* (2002). After administration of doses of 250 and 1000 μg/kg, MOX was detected in plasma for 28 days post-treatment and showed a biphasic curve with a rapid absorption half-life of 0.6 h and a time at peak plasma concentration (T_{max}) of 2.75 h. Moxidectin was also shown to be a low clearance drug (0.0222–0.0254 L/h/kg) with a distribution volume ranging from 8.69 to 15.65 L/kg, the values depending on the dose given (250–1000 μg/kg). In agreement with results from studies for other macrocyclic lactones in dogs, the PK profile of MOX was dose-dependent with *AUC* values of 12.7 and 45.9 μg·h/mL for doses of 250 and 1000 μg/kg respectively. The authors observed no sex-related differences in PK behaviour in male and female dogs given the same MOX treatments. MOX tablets, given once monthly at a dose of 1–3 μg/kg, have been reported to be effective in preventing natural infections with *D. immitis* (McTier *et al.*, 1992a,b; Genchi *et al.*, 2001). MOX is also formulated as sustained-release, injectable microspheres and a single dose of 0.17 mg/kg of the latter has been shown to confer complete protection, lasting for at least 180 days, against infection, following challenge-exposure to *D. immitis* L3 (Lok *et al.*, 2001). Moreover, injectable MOX formulations, given once every 6 months, not only controlled the development of *D. immitis* L3, but were also effective in the treatment of *A. caninum* (Lok *et al.*, 2001). Toxicity tests demonstrated that Collies which had mild to severe reactions to IVM treatment, did not develop any clinical signs of intoxication after MOX was given either p.o., at 30, 60 and 90 μg/kg, or by injection at dosages of 0.17, 0.51 and 0.85 μg/kg (Paul *et al.*, 2000). In contrast, in the USA, so many reports were received on potentially life-threatening, adverse drug reactions suspected to be associated with a specific sustained release injectable MOX formulation, that the FDA requested withdrawal of the product from the market in 2004.

CYCLODEPSIPEPTIDES

Cyclodepsipeptides are cyclic chains of amino acids with unique mode of activity (Jeschke *et al.*, 1993). Emodepside is a semisynthetic octadepsipeptide derived from a metabolite of *Mycelia sterilia*, a fungus found on the leaves of the plant *Camellia japonica* (Harder *et al.*, 2005). This compound is currently marketed in the EU as a spot-on formulation, in combination with PZQ, for endoparasite treatment in cats, although it is not yet approved by the FDA for use in the USA.

Emodepside is known to bind a latrophilin-like receptor in nematodes. Latrophilin receptors are those targeted by the neurotoxic protein of the black widow spider in vertebrates. In nematodes emodepside binds latrophilin-like receptors in the pharynx and in nematode musculature, with inhibitory concentration (IC50) values of 4.2 and 4.6 nM respectively. This causes inhibition of pharyngeal pumping and flaccid paralysis of the parasite (Harder *et al.*, 2005).

The latrophilin receptor is a seven trans-membrane helical G-protein coupled receptor. Stimulation by emodepside causes G-protein dissociation, activation of phospholipase-C and subsequent conversion of phosphatidylinositol into diacylglycerol. This in turn is thought to cause release of a transmitter (possibly a neuropeptide), which is the postsynaptic effector of pharyngeal pump inhibition and somatic muscle cell paralysis (Harder *et al.*, 2005).

The efficacy of emodepside in cats has been demonstrated against nematodes either following its administration alone or in combination with PZQ which has no nematodicidal activity. At a dose rate of 3.0 mg/kg (12.0 mg/kg PZQ) emodepside has excellent activity against *Toxacara cati* (mature and immature adults) 100%, (L4) 99.4% and (L3) 96.8%; *T. leonina* (mature adults) ≥98%, (immature adults) 100% and (L4) ≥93.4% and *Ancylostoma tubaeforme* (mature adults) 100%, (immature adults) >97% and (L4) >95% (Altreuther *et al.*, 2005a; Reinemeyer *et al.*, 2005).

The efficacy has been confirmed in controlled, blinded, randomized, multi-site clinical studies where faecal egg count reductions of >98% were demonstrated for nematode (including *T. cati*) eggs (Altreuther *et al.*, 2005b). In this study, the combination spot-on product was also shown to be well tolerated.

CESTODICIDAL DRUGS

Adult cestode tapeworms are not generally very pathogenic, although extensive infestation can give rise to clinical symptoms, including abdominal colic and diarrhoea in the host (Soulsby, 1987). The most common cestode parasites of small animals are *Taenia*, *Dipylidium*, *Echinococcus*, *Mesocestoides* and *Diphyllobothrium* spp. (Jacobs, 1987). When undertaking the pharmacological control of cestode infections (e.g. taeniasis) in animals, veterinary clinicians also have a pivotal role in addressing and preventing potential exposure of the human population to infection.

Echinococcus granulosus is an important zoonosis for which dogs and other wild canids are the definitive host (Urquhart *et al.*, 1991). However, humans act as aberrant intermediate hosts and can develop damaging hydatid cysts, mostly in the liver or lungs, because of harbouring immature (metacestode) forms of *E. granulosus*. Praziquantel is the only drug recommended for the treatment of *E. granulosus* infection in dogs. Other compounds do have high efficacies approaching 99%, but only PZQ ensures 100% elimination of the infesting parasites. Drugs which have been used for the treatment of cestodiosis include bunamidine, niclosamide (NSM), ABZ, FBZ, MBZ, PZQ and EPQ. However, PZQ is the drug of choice for programmes aimed at the control and eradication of *Echinococcosis*. The relatively low sensitivity of the parasite to some drugs could be related to the difficulty of the drug accessing the cestode site in suitable concentrations to kill the parasite. For example, *E. granulosus* adults locate in the intestinal crypts which may afford protection of the parasite from drug action.

Epsiprantel also has high activity against Echinococcosis, and both PZQ and EPQ show high efficacy against *Taenia*, *Dipylidium* and *Mesocestoides*. PZQ treatment usually has relatively few side effects, although injectable formulations can lead to transient discomfort at the injection site and in some cases may be associated with vomiting, diarrhoea, lethargy and/or loss of appetite. Cestodicidal activity is based on two associated mechanisms. Initial contact with the drug causes marked muscle contraction in the parasite as a result of increased permeability to calcium ions. Consequent tegument vacuolization then exposes antigens which render the parasite vulnerable to the host immune system and eventually results in the death and digestion of the cestode in the host gut (Martin, 1997).

The recommended therapeutic dose of PZQ for use in dogs is 3.75 mg/kg given p.o. The pharmacokinetics of PZQ in dogs was recently described by Giorgi *et al.* (2003), who used a much higher oral dose (30 mg/kg) to generate sufficiently high plasma concentrations for analysis. It was previously established in rats that PZQ undergoes oxidation in a first phase Cyt P-450 mediated metabolic step, generating the metabolite 4′-OH- PZQ (Masimirembwa & Haster, 1994). In the dog study (Giorgi *et al.*, 2003), PZQ and its metabolite were depleted in parallel from plasma which is supported by an $AUC_{PZQ/4'\ OH\text{-}PZQ}$ ratio of 1.3. The time of C_{max} was 0.75 and 1.58 h for PZQ and its metabolite, respectively, indicating rapid absorption and metabolism The plasma detection interval for both molecules indicated a short residence time (10 h post-treatment) in that compartment. It has been demonstrated in humans that grapefruit juice may be an inhibitor of the P-gp transport system and Cyt P-450-mediated oxidative drug metabolism in the GI tract and liver (Kane & Lipsky, 2000; Nagy *et al.*, 2002). Interestingly, in this context, increased levels of PZQ and its metabolite (approximately 2 and 4-fold *AUC* values respectively) were reported by Giorgi *et al.* (2003) after giving 100 mL of liquid or dry grapefruit juice combined with PZQ to dogs.

A combined formulation of the pro-BZD, FBT and PZQ is available on the veterinary market for use in the treatment of GI

nematodes (including *Trichuris vulpis*) and cestodes. A single dose of this product was ineffective against immature stages of nematodes and repeat treatments, on three consecutive days, at doses of 10 mg/kg FBT/1 mg/kg PZQ and 15 mg/kg FBT/1.5 mg/kg PZQ for adult and young dogs, respectively, have been recommended. It should be noted that this combination has been shown to have side effects (salivation, diarrhoea, vomiting and anorexia) in 3% of dogs and 10% of cats treated (Sharp & McCurdy, 1985). In Australia, a triple combination of PZQ, PRT and oxantel has been marketed as broad spectrum wormer for dogs. Oxantel is an N-subtype cholinergic receptor agonist, unlike PRT which is an L-subtype agonist (Martin *et al.*, 2004). These authors suggest a combination of the two is, therefore, likely to confer therapeutic advantages by increasing the spectrum of action and reducing the potential for the development of resistance.

In contrast to PZQ, the homologue, EPQ is only poorly absorbed in the GI tract and is thereby available to act against intestinal cestodes and is eliminated via the faeces. EPQ showed high *in vivo* and *in vitro* efficacy against *E. granulosus* producing tegumental damage in protoscoleces, immature stages and 7-day-old adults (Thompson *et al.*, 1991). The efficacy of EPQ reported at doses of 5 and 7.5 mg/kg was 99.9% against mature worms. Other studies carried out, using EPQ doses of 5.5 mg/kg and 2.5 mg/kg respectively, in dogs and cats infected with *E. multilocularis* indicated that EPQ is >99% effective, although residual worms may persist in some animals (Eckert *et al.*, 2001). The recommended dose of 5.5 mg/kg is also 100% effective against *Taenia* spp. and 99.8% against *Dipylidium* (Corwin *et al.*, 1989).

Bunamidine chlorhydrate (BNM) exhibits >90% efficacy against *Taenia*, *Dipylidium*, *Mesocestoides* and *Diphyllobothrium* spp. when given p.o. as a single dose of 25–50 mg/kg. It is most effective in fasted animals in which dissolution of tablets is improved and, therefore, subsequent contact of the drug with the parasite in the posterior gut is enhanced. Andersen *et al.* (1975), reported that BNM had efficacies ranging from 85.9% to 98.8% against the immature stages, and 100% against the mature stages of *E. granulosus*. Another study in which 122 dogs, experimentally infected with *E. granulosus*, were treated with a combination of BNM (50 mg/kg) and arecoline hydrobromide (4 mg/kg), reported comparable levels of efficacy against young worms (Trejos *et al.*, 1975). The same authors also found that, at these dose rates, worms were still present in almost 50% of the dogs at autopsy. BMN is considered the drug of choice for *Spirometra*, *Diphyllobothrium* and *Mesocestoides* (Georgi, 1987) and its potent cestodicidal activity is thought to be associated with effects on the parasite tegument, which alter glucose absorption and lead to subsequent parasite death.

The salicylanilide, NSM, is used as a cestodicide in both small and large animals and has been shown to be highly effective against *Taenia* spp. (Katz, 1977; Georgi, 1987), although it shows only low efficacy against *Dipylidium* and *E. granulosus*. In another study, NSM was reported to be effective at 32 and 64 mg/kg against *T. hydatigena* and, at 50 mg/kg, against *T. ovis* (Gemmell *et al.*, 1977). The mode of action of NSM involves interference with glucose absorption and oxidative phosphorylation mechanisms which leads ultimately to the death of the parasite, and its subsequent digestion in the host gut. In drug distribution studies, it was found that NSM, administered p.o. at doses of 100–157 mg/kg, produced only low plasma concentrations which correlate with low GI absorption and the known low toxicity of the drug (Courtney & Roberson, 1995).

BZD compounds, principally ABZ, FBZ and MBZ, are also used as cestodicides in small animals. They are given by the oral route because of their low solubility and the need to maintain a long drug-parasite contact time to have high efficacy (see earlier section on BZD). The mode of action of the BZD on cestodes is similar to that described for nematodes and involves interaction with the microtubule-tubulin equilibrium which provokes alterations in the structural and functional proteins of the parasite. Pharmacological effects against the cestode are not immediate and, for that reason, BZD must been given in repeated doses for 3–5 days.

CONCLUSIONS

Knowledge of the relationship between the PK and PD effects of the many different antiparasitic drugs available on the market is an essential prerequisite for establishing rational antiparasitic therapies in small animals. There are numerous examples of therapeutic failures as a consequence of inappropriate extrapolation of data obtained from studies in humans or other monogastric species which have distinct physiological differences. Integrated understanding of the pharmacological properties of antiparasite drugs and the epidemiological features of the parasitic diseases being treated are pivotal for rational therapy and successful control in both the Public and Animal Health sectors.

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