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Evaluation of pharmacological interactions after administration of a levamisole, albendazole and ivermectin triple combination in lambs

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ABSTRACT

The goals of the current trial were (a) to characterize the plasma disposition kinetics of levamisole (LEV), albendazole (ABZ) and ivermectin (IVM), each administered either alone (single active ingredient) or as a combined formulation to lambs; (b) to compare the clinical anthelmintic efficacy of the same drugs given either separately or co-administered to lambs infected with resistant nematodes. Fifty Corriedale lambs naturally infected with multiple resistant gastrointestinal nematodes were involved in the following experimental trials: (a) "*Pharmacokinetic trial*": the animals were allocated into five groups ($n = 10$ each) and intraruminally treated with either LEV (8 mg/kg), ABZ (5 mg/kg), IVM (0.2 mg/kg), or with a LEV + ABZ + IVM combined formulation, where each active ingredient was administered at the same dose. Blood samples were collected over 15 days post-treatment and drug plasma concentrations measured by HPLC. (b) "*Efficacy trial*": the same treated groups plus an untreated control group were used to assess the comparative anthelmintic efficacy by the faecal egg count reduction test (FECRT). Although the overall LEV disposition kinetics was unaffected, significantly lower (61%) ABZ-sulphoxide and higher (71%) IVM systemic availabilities were obtained after administration of the combined formulation in comparison to those obtained after treatment with each drug alone. A multiple drug resistance situation was observed for *Haemonchus* spp. The observed efficacies were 52% (LEV), 72% (ABZ), 80% (IVM) and 87% (triple combined formulation). The results reported here contribute to the pharmaco-therapeutic knowledge on drug combinations. This type of research is crucial before further development of combined anthelmintic preparations reaches the market to deal with resistant nematode control. The co-administration of LEV + ABZ + IVM did not result in a significant advantageous anthelmintic effect compared to the treatment with IVM alone. The simultaneous/combined administration of LEV, ABZ and IVM may account for a drug–drug pharmacological interaction in infected lambs. The pharmacokinetic interaction accounted for a reduced ABZ-sulphoxide and enhanced IVM systemic exposure following the combined treatment.

1. Introduction

The development of resistance to the available anthelmintic drugs is a serious constraint to the control of

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gastrointestinal (GI) nematodes in sheep, goats and cattle in many regions of the world (Kaplan, 2004; Wolstenholme et al., 2004; Jabbar et al., 2006). Currently, combinations of two or more anthelmintic active ingredients are primarily being used to manage anthelmintic resistance in ruminants, and to expand the spectrum of efficacy (Geary et al., 2012). Combination of anthelmintics with a similar spectrum of nematodicidal activity and different mechanism of action/resistance has been proposed as an alternative parasite control strategy, where failure of individual drugs is documented (Anderson et al., 1988; Barnes et al., 1995; Leathwick et al., 2009). The rationale behind the use of these combined preparations is based on a lower resistance in individual worms to a formulation with multiple components (each one with different mechanism of action/resistance) compared to the treatment with a single active component. Different drug combined formulations are available in Uruguay, a country with an economically relevant sheep industry and a widespread development of parasite resistance (Nari et al., 1996; Suarez et al., 2011, 2013), as in several others countries with a similar situation.

Among many others combinations, the mixture of levamisole (LEV), an imidazothiazole compound, albendazole (ABZ), a methyl carbamate benzimidazole compound and ivermectin (IVM), a macrocyclic lactone avermectin-type compound is already available in the market. The purpose of this combined is based on the different mechanism of anthelmintic action of each active ingredient. LEV causes a spastic paralysis of susceptible nematodes by selectively gating acetylcholine receptor ion channels on nerve and muscles (Robertson and Martin, 1993); the intrinsic anthelmintic action of ABZ relies on a progressive disruption of basic cell functions as a result of their binding to parasite β-tubulin and depolymerization of microtubules (Lacey, 1990). IVM acts on ligand-gated channels, including glutamate and GABA-gated chloride channels, which are involved on nematode feeding, reproduction and locomotion (Geary et al., 1993; Feng et al., 2002; Yates et al., 2003). The different mode of action/resistance of the active ingredients included in the LEV + ABZ + IVM combination, may complementary contribute to their efficacy against resistant nematodes. However, in spite of the fact that combined preparations are already being used in parasite control, there is a need for pharmacology-based research to assess the potential pharmacokinetic (PK) and/or pharmacodynamic (PD) interactions among the active ingredients in this combined anthelmintic formulation.

A drug–drug interaction refers to the possibility that one compound may alter the intensity of the pharmacological effects of another drug given concurrently. The modified/altered effect may emerge from a change on the relationship between drug concentration and response of the organism to the drug (PD interaction) or from a change on the concentration of either one or both molecules in the organism (PK interaction). PD interactions (at site of action) would account for indifference, antagonism, additive or synergistic effects. A synergistic pharmacological effect is achieved when the combined effect of the drugs is significantly greater than the independent effect of each molecule, which could be an ideal pharmacological situation in the control of resistant parasites. PK drug–drug interactions are mainly related to enzyme induction or inhibition, competition with drug transport proteins and/or protein binding. While most of the data obtained on the pharmacological assessment of different available anthelmintic combinations would indicate that only an additive anthelmintic effect is achieved by drug combined activity, the PK interactions among anthelmintic molecules may be more common than expected. A PK interaction between ABZ and IVM in sheep has been previously reported (Alvarez et al., 2008). Additionally, the presence of triclabendazole, an halogenated BZD compound used as flukicidal in sheep and cattle, increases the plasma concentrations of IVM in sheep (Lifschitz et al., 2009). The pharmaco-parasitological evaluation of drug interactions is becoming highly relevant since drug combinations are now widely used as an alternative to control resistant helminth parasites in livestock. Therefore, the potential PK and PD drug–drug interactions occurring among LEV, ABZ and IVM should be understood before this particular drug combined formulation is recommended to be used in sheep. The main goal of the current trial was to characterize the plasma disposition kinetics of LEV, ABZ and IVM administered either alone (a single active ingredient) or as a combined formulation in parasitized lambs. Additionally, the clinical efficacy of the same drugs given either separately or co-administered to lambs infected with multiple resistant nematodes was compared.

2. Materials and methods

2.1. Animals

Fifty male and female Corriedale lambs (7–8 month old), weighing 28.8 ± 4.0 kg and naturally infected with multiple resistant GI nematodes, were involved in this trial. All animals were subjected to a veterinary examination before inclusion in the study, and shown a FAMACHA (Van Wyk and Bath, 2002) score ≤2 and a body condition score between 2 and 4. The study was conducted on a farm (*Centro de Investigación y Experimentación "Dr. Alejandro Gallinal", Florida, Uruguay*) where the failure of different anthelmintics to control GI nematodes had been previously demonstrated by the faecal egg counts reduction test (FECRT) (Castells, 2002; Bonino et al., 2010). On day −1, all lambs were individually identified and the number of nematode eggs/gram of faeces (epg) was determined (modified McMaster technique). Experimental animals had an average of 1348 epg ranging from 50 to 9850. Throughout and 60 days before starting the experiment, animals grazed on a natural pasture and had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy of the Faculty of Veterinary Medicine, Universidad de la República (UDELAR), Montevideo, Uruguay.

2.2. Chemicals

Standards of ABZ, ABZ-sulphoxide (ABZSO), ABZ-sulphone ($ABZSO_2$), oxibendazole (OBZ, internal standard),