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Interference with P-glycoprotein improves ivermectin activity against adult resistant nematodes in sheep

A. Lifschitz[a,b,*], C. Entrocasso[c], L. Alvarez[a,b], M. Lloberas[c], M. Ballent[a,b], G. Manazza[c], G. Virkel[a,b], B. Borda[c], C. Lanusse[a,b]

[a] Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, UNCPBA, Campus Universitario, 7000 Tandil, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Balcarce, (7620) Balcarce, Argentina

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ABSTRACT

The *in vivo* co-administration of ivermectin (IVM) with P-glycoprotein (P-gp) modulator agents has been shown to enhance its systemic availability. However, there is no sufficient evidence on the impact that this type of drug–drug interaction may have on the *in vivo* efficacy against resistant nematodes in ruminant species. The current work reports on the effects of loperamide (LPM), a P-gp modulating agent, on both IVM kinetic behaviour and anthelmintic activity in infected lambs. Eighteen (18) lambs naturally infected with IVM-resistant gastrointestinal nematodes were allocated into three (3) experimental groups. Group A remained as untreated control. Animals in Groups B and C received IVM (200 μg/kg, subcutaneously) either alone or co-administered with LPM (0.2 mg/kg, twice every 12 h), respectively. Individual faecal samples were collected from experimental animals at days −1 and 14 post-treatment to perform the faecal eggs count reduction test (FECRT). Blood samples were collected between 0 and 14 days post-treatment and IVM plasma concentrations were determined by HPLC. Additionally, at day 14 post-treatment, lambs from all experimental groups were sacrificed and adult gastrointestinal nematode counts were performed. FECRT values increased from 78.6 (IVM alone) to 96% (IVM + LPM). *Haemonchus contortus* was highly resistant to IVM. The IVM alone treatment was completely ineffective (0% efficacy) against adult *H. contortus*. This efficacy value increased up to 72.5% in the presence of LPM. The efficacy against *Trichostrongylus colubriformis* increased from 77.9% (IVM alone) to 96.3% (IVM + LPM). The described favorable tendency towards improved anthelmintic efficacy was in agreement with the enhanced IVM plasma availability ($P < 0.05$) and prolonged elimination half-life ($P < 0.05$) induced by LPM in infected lambs. A LPM-induced P-gp modulation increases IVM systemic exposure in the host but also it may reduce P-gp efflux transport over-expressed in target resistant nematodes.

1. Introduction

Ivermectin (IVM) is the most used anthelmintic drug for parasite control in livestock animals. Since its introduction into the veterinary market in 1981, IVM has been extensively used against endo- and ectoparasites rapidly becoming in a leading drug compound worldwide (Crump and Otoguro, 2005). After several years of intensive use to optimize animal productivity, the inevitable appearance of

* Corresponding author at: Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, UNCPBA, Campus Universitario, 7000 Tandil, Argentina. Tel.: +54 2293 439850; fax: +54 2293 439850.
E-mail address: adrianl@vet.unicen.edu.ar (A. Lifschitz).

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IVM-resistant parasites has occurred (Geary, 2005). Currently, resistance to IVM and to other related macrocyclic lactones (MLs), is widespread in nematodes from small ruminants and it is becoming a serious concern in cattle nematodes (Kaplan, 2004; Anziani et al., 2004; Demeler et al., 2009a).

The ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp), have been implicated on the mechanisms of resistance in nematodes (Xu et al., 1998; Prichard and Roulet, 2007). P-gp is a transmembrane protein associated with a phenotype of multidrug resistance to certain anticancer drugs in mammalian cancer cells, which is able to pump a broad range of structurally and functionally unrelated compounds out of the cell by an ATP-dependent process (Ling and Thompson, 1974). IVM behaves as a P-gp substrate (Schinkel et al., 1994) and a drug efflux P-gp-mediated process reducing IVM concentrations at the parasite site of action was proposed as one of the resistance mechanisms in *Haemonchus contortus* (Blackhall et al., 1998; Prichard and Roulet, 2007).

Besides its contribution to resistance mechanisms, P-gp activity has also been observed in healthy tissues, particularly in organs relevant to drug disposition kinetics. According to its specific location at the apical side of epithelial cells, luminal surface of hepatocytes and ducts cells, kidney proximal cells and enterocytes, P-gp plays an important role in how drug compounds are absorbed, distributed to tissues and excreted from the body (Lin, 2003). The *in vivo* co-administration of MLs with P-gp modulator agents has been evaluated in different species (Lifschitz et al., 2002, 2004; Dupuy et al., 2003; Ballent et al., 2007; Alvinerie et al., 2008). Loperamide (LPM) is an opioid derivative that has been classified as a P-gp substrate (Schinkel et al., 1996). LPM induces favorable changes to the plasma kinetic disposition of moxidectin and IVM without any toxic effect (Lifschitz et al., 2002, 2004). However, there is not sufficient evidence on the impact that this type of drug–drug interaction may have on the *in vivo* field efficacy against resistant nematodes in sheep. It has been recently shown that modulation of P-gp activity improves the faecal egg count reduction induced by both IVM and moxidectin in cattle infected with resistant nematodes (Lifschitz et al., 2010). The current work describes for the first time a simultaneous pharmaco-parasitological assessment of the effects of LPM, a P-gp modulating agent, on IVM anthelmintic activity in sheep. LPM-induced changes on both IVM plasma disposition and efficacy (controlled test) were assessed in lambs naturally infected with highly resistant gastrointestinal nematodes.

2. Materials and methods

2.1. Animals

Eighteen (18) Corriedale lambs (26–35 kg), naturally infected with resistant GI nematodes were involved in this trial. The selected farm is a sheep experimental unit with a parasite control program based on the intensive use of anthelmintics over the years, where failure of IVM to control nematodes was previously corroborated (Entrocasso, C., personal communication). In fact, a 79.8% reduction on faecal egg counts was obtained after treatment with IVM the year prior to performing the trial described here (Entrocasso et al., 2008). The selection of the animals was based on worm egg per gram counts (epg). On day −1 all lambs were checked for epg, ear tagged and the individual body weights were recorded. Experimental animals had an average of 4538 ± 1370 epg counts ranging from 2520 to 6960. Animals were allocated in a paddock and feed on a lucerne/white and red clover pasture during the experiment and for 20 days before start clinical efficacy study. All the animals had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar).

2.2. Experimental design, treatments and samplings

Experimental lambs were assigned into three (3) experimental groups. Group A remained as untreated control. Animals in Groups B and C received IVM (Ivomec®, Merial Argentina) (200 μg/kg, subcutaneuosly) either alone or co-administered with LPM (0.2 mg/kg, two subcutaneous injections administered at 0 and 12 h post-administration of IVM) (Loperol, Argos, Argentina). LPM was administered at a therapeutic dose rate, and no adverse side effects were observed. Faecal samples were collected from all the lambs in each experimental group at days −1 and 14 post-treatment in order to estimate the epg counts. Jugular blood samples (7 ml) were collected into heparinised vacutainer tubes prior to and at 3, 6, 9 h and 1–4, 6, 8, 10 and 14 days post-treatment. Blood samples were centrifuged at 2000 × *g* for 20 min and the recovered plasma was kept in labeled vials. Plasma samples were stored at −20 °C until analyzed by high performance liquid chromatography (HPLC).

Additionally, at 14 days post-treatment, all the animals in each experimental group were sacrificed by captive bolt gun and rapidly exsanguinated. Abomasum and different gut sections were identified and isolated (small and large intestine) and the content analyzed to record the different parasite stages following the World Association for the Advancement of Veterinary Parasitology guidelines (Wood et al., 1995).

3. Analytical procedures

3.1. Parasitological techniques

The individual faecal egg counts were performed using the modified McMaster technique (Roberts and O'Sullivan, 1949). The anthelmintic efficacy of the treatments was evaluated by the faecal egg count reduction test (FECRT), calculated according to the formula (Coles et al., 1992):

$$\text{FECRT}(\%) = 100 \times \left(1 - \frac{T}{C}\right),$$

where T is the arithmetic mean epg counts in the treated group at 14 days post-treatment and C is the arithmetic