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Pharmacokinetic comparison of different flubendazole formulations in pigs: A further contribution to its development as a macrofilaricide molecule

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ABSTRACT

Despite the well established ivermectin activity against microfilaria, the success of human filariasis control programmes requires the use of a macrofilaricide compound. Different *in vivo* trials suggest that flubendazole (FLBZ), an anthelmintic benzimidazole compound, is a highly efficacious and potent macrofilaricide. However, since serious injection site reactions were reported in humans after the subcutaneous FLBZ administration, the search for alternative pharmaceutical strategies to improve the systemic availability of FLBZ has acquired special relevance both in human and veterinary medicine. The goal of the current experimental work was to compare the pharmacokinetic plasma behavior of FLBZ, and its metabolites, formulated as either an aqueous hydroxypropyl- β -cyclodextrin-solution (HPBCD), an aqueous carboxymethyl cellulose-suspension (CMC) or a Tween 80-based formulation, in pigs. Animals were allocated into three groups and treated (2 mg/kg) with FLBZ formulated as either a HPBCD-solution (oral), CMC-suspension (oral) or Tween 80-based formulation (subcutaneous). Only trace amounts of FLBZ parent drug and its reduced metabolite were measured after administration of the different FLBZ formulations in pigs. The hydrolyzed FLBZ (H-FLBZ) metabolite was the main analyte recovered in the bloodstream in pigs treated with the three experimental FLBZ formulations. The oral administration of the HPBCD-solution accounted for significantly higher ($P < 0.05$) Cmax and AUC (23.1 ± 4.4 μg h/mL) values for the main metabolite (H-FLBZ), compared with those observed for the oral CMC-suspension (AUC = 3.5 ± 1.0 μg h/mL) and injectable Tween 80-based formulation (AUC: 7.5 ± 1.7 μg h/mL). The oral administration of the HPBCD-solution significantly improved the poor absorption pattern (indirectly assessed as the H-FLBZ plasma concentrations) observed after the oral administration of the FLBZ-CMC suspension or the subcutaneous injection of the Tween 80 FLBZ formulation to pigs. Overall, the work reported here indicates that FLBZ pharmacokinetic behavior can be markedly changed by the pharmaceutical formulation.

1. Introduction

The control and eventual elimination of neglected tropical diseases (NTD) requires the expansion of interventions such as mass drug administration (MDA), vector control, diagnostic testing and effective treatment. Current efforts in this area of tropical public health have been aimed at onchocerciasis and lymphatic filariasis (LF) as well as loiasis as co-infection with this other filaria can cause severe adverse reaction in MDA areas. These diseases continue to cause widespread sickness and disability in many parts of the world. Of the total population requiring preventive chemotherapy for LF, 57% live in the South-East Asia Region and 38% live in the African Region. Onchocerciasis is endemic in 31 countries in Africa and 6 countries in Latin America, with 99% of cases of onchocerciasis-related blindness found in Africa (WHO, 2013).

Chemotherapy remains the main approach to treatment, control, and elimination of filarial infections aided where suitable by adjunct activities such as vector control and enhanced program management. Current onchocerciasis therapy employ ivermectin

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(IVM) against microfilariae (mf) in the skin and against further production of mf by female worms (Basáñez et al., 2008). These effects reduce the pathology of the disease and prevent transmission by blocking repopulation of the skin with mf for >6 months following a single dose. However, due to the long life-span of the adult parasites, eradication of the disease will almost certainly require a field-compatible macrofilaricide. Existing macrofilaricides have either unacceptable toxicity (e.g., suramin) (Awadzi, 2003) or induce severe adverse reactions contraindicating their use at doses required to be effective (e.g., diethylcarbamazine (DEC) (Bird et al., 1979). For LF, DEC or IVM in combination with albendazole (ABZ) are used as the basis for the global programme for elimination, but the availability of a single-dose macrofilaricide would provide chemotherapeutic options that could significantly reduce program duration (Taylor et al., 2010).

Although IVM has had an enormous impact on onchocerciasis and LF, this agent lacks the ability to kill the adult parasites. Since the adult worms can survive for many years, it has been necessary for control programs to continue drug distribution for more than a decade, for instance, until the adult worms eventually die (Mackenzie and Geary, 2011). As a consequence, the search for a macrofilaricide that can enhance elimination of filarial infections, and the diseases they cause, is a current and relevant goal. Different *in vivo* trials suggest that flubendazole (FLBZ), a benzimidazole methylcarbamate anthelmintic currently licensed for use in humans for treatment of infection by intestinal nematodes (EMEA, 1997), is a highly efficacious and potent macrofilaricide in experimental animals when given parenterally (Zahner and Schares, 1993; Mackenzie and Geary, 2011), including in the feline *Brugia pahangi* model, a host in which this parasite occurs naturally. In addition, FLBZ has been reported to be able to eliminate adult *Dirofilaria immitis* from dogs after a single injection (Mackenzie and Geary, 2011). It is important to stress that FLBZ is macrofilaricidal in these models only when given parenterally (as a consequence of its very low oral bioavailability in standard formulations). Studies in humans infected with *Onchocerca volvulus* reported problems associated with reactions at the intramuscular injection site where FLBZ, in its oil-based carrier, was administered.

As a chemical class, the benzimidazole methylcarbamates have very limited water solubility, which allows their preparation only as tablets/suspensions for oral administration in humans. Small differences in drug solubility may have a major influence on their absorption and resultant pharmacokinetic behavior (Lanusse et al., 1995). It has been reported that the use of complexing agents such as hydroxypropyl-β-cyclodextrins (HPBCD) increases the water solubility of FLBZ and ABZ (Ceballos et al., 2012) and their systemic drug exposure in different species (Evrard et al., 2002; Ceballos et al., 2009, 2014). Similar findings have been reported in humans (Rigter et al., 2004).

Prospects for an accelerated path to the elimination of onchocerciasis and LF would be much enhanced if a safe and effective macrofilaricide was available (Geary et al., 2010; Mackenzie and Geary, 2011). Bioavailability is a key pharmacokinetic parameter, defined as the proportion of a drug administered by a nonvascular route that gains access to the systemic circulation (Toutain and Bousquet-Melou, 2004). Bioavailability quantifies the proportion of a drug which is available to produce systemic effects. When pharmacological research cannot be done on humans for practical and ethical reasons, animal models constitute an acceptable alternative approach to understand the parasite-drug-host relationship.

In this context, the search for alternative pharmaceutical strategies to improve FLBZ oral bioavailability may be considered critical to optimize its pharmacological activity. The goal of the current experimental work was to compare the pharmacokinetic behavior of FLBZ, and its metabolites, formulated as either an aqueous hydroxypropyl- β -cyclodextrin-solution, an aqueous carboxymethyl cellulose-suspension or a Tween 80-based formulation, in pigs.

2. Materials and methods

2.1. Chemicals

Pure reference standards of FLBZ, reduced-FLBZ (R-FLBZ) and hydrolyzed-FLBZ (H-FLBZ) used to develop the analytical methodology were kindly provided by Janssen Animal Health (Beerse, Belgium). Oxibendazole (OBZ), used as internal standard, was obtained from Schering Plough (Kenilworth, NJ, USA). HPLC grade acetonitrile and methanol were from Sintorgan S.A. (Buenos Aires, Argentina) and J.T. Baker (Phillipsburg, New Jersey, USA), respectively. HPBCDs were from ISP Pharmaceuticals (Cavasol, New Jersey, USA). Low viscosity grade sodium CMC was purchased from Anedra (Buenos Aires, Argentina). Tween® 80 was purchased from Biopack (Buenos Aires, Argentina).

2.2. Preparation of FLBZ formulations

The FLBZ HPBCD-based solution was prepared by dissolving FLBZ (0.1%) and HPBCD (10%) in deionized water. The pH of the formulation was adjusted to 1.2 using HCl (25 mM). The formulation was shaken until total dissolution of the drug and then was filtrated through a 0.45 μm filter (Whatman, NJ, USA). The final FLBZ concentration was confirmed by HPLC. The Tween 80-based formulation was prepared by dissolving FLBZ (0.25%) in Tween 80. The FLBZ-suspension was prepared by addition of FLBZ (0.1%) and CMC (0.1%) in deionized water (pH = 6.0) with shaking for 6 h. The FLBZ-CMC suspension was vigorously shaken immediately before administration to pigs. FLBZ formulations were freshly prepared and maintained under refrigeration (3–5 °C).

2.3. Experimental animals

Sixteen (16) healthy pigs (45.2 ± 7.54 kg) were used in two different experiments. Pigs were fed *ad libitum* with a commercial balanced food and had free access to water. Animals were housed in pens with concrete floors, protected from rain and prevailing winds, but without temperature control. 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).

2.4. Experimental design

Two different experiments were undertaken to generate the data reported in this article. Experiment 1 was a crossover study involving two experimental phases. In phase I, pigs received the following treatments: FLBZ-HPBCD solution (Cavasol®, n = 4, oral treatment) and FLBZ-CMC suspension (n = 4, oral treatment). After a 21-day washout period, drug treatments were reversed for each drug (phase II). Additionally in experiment 2, eight pigs were dosed subcutaneously with the FLBZ-Tween 80 solution. All treatments were given as a single dose of 2 mg/kg. Blood samples (5 mL) were taken from all pigs by anterior vena cava venipuncture into heparinized Vacutainers® tubes (Becton Dickinson, Franklin Lakes, NJ, USA), prior to treatment and at 1, 2, 3, 6, 9, 12, 15, 24, 30, 34, 48 and 54 h post-treatment for oral treated groups (experiment 1) and up to 72 h post-treatment for subcutaneous administration (experiment 2). Plasma was separated by centrifugation at 2000 × *g* for