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Efficacy, safety and pharmacokinetics of 1,2,4-trioxolane OZ78 against an experimental infection with *Fasciola hepatica* in sheep

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ABSTRACT

The synthetic peroxide OZ78 is an effective flukicide in the rodent model, but the potential of OZ78 in target animals has not been studied to date. In the present study, OZ78 was administered at 50 mg/kg orally and subcutaneously to sheep harbouring an experimental *Fasciola hepatica* infection and the efficacy, tolerability and pharmacokinetic profiles were monitored. OZ78 given orally or subcutaneously revealed no effect neither on faecal egg counts nor on worm burdens. Apart from significant subcutaneous swelling at the injection sites of most of the treated animals, no other treatment related adverse events occurred. OZ78 had no significant effect on any haematological, coagulation or clinical chemistry variables tested. Following oral administration, a mean C_{max} of 45.8 ± 13 μg/ml was reached after 1 h. An estimated elimination half-life of 1.0 h and a mean AUC of 116.2 ± 47 μg min/ml was calculated for the oral administration. Following subcutaneous treatment with OZ78 C_{max} and t_{max} were 13.7 ± 6.1 μg/ml and 0.9 ± 0.4 h, respectively. The α and β half-lives were 4.5 ± 4.3 h and 56.5 ± 36 h, respectively and the mean AUC was 219.1 ± 74 μg min/ml. Further studies are needed to determine whether the excellent activity observed with OZ78 in the rat model can be translated into efficacy in larger mammals.

1. Introduction

Fasciola hepatica is a digenetic trematode flatworm of considerable agricultural and economic importance worldwide, with estimated losses as high as US$ 3 billion annually (Keiser and Utzinger, 2009; Mas-Coma et al., 2009). In addition, fascioliasis is a serious public health problem, with an estimated 17 million people infected and up to 180 million at risk (Keiser and Utzinger, 2005; Keiser and Utzinger, 2009). In the absence of an effective vaccine, chemotherapy remains the mainstay of control. The benzimidazole derivative triclabendazole currently is the treatment of choice for fascioliasis due to its high efficacy against juvenile and adult *F. hepatica*. However, development of resistance to triclabendazole is increasing such that alternative treatment options are warranted (Fairweather, 2009; Keiser et al., 2005).

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In the past few years, we have demonstrated that the semisynthetic artemisinin derivatives artemether and artesunate, today the most commonly used antimalarials, are effective fasciolicides in the treatment of rodent and sheep infections (Keiser et al., 2008, 2006a, 2010). Since the artemisinins have short half-lives and must be administered over a period of several days in the treatment of malaria, many synthetic antimalarial peroxides have been prepared with the ultimate goal to identify a more stable, rapid-acting, fully synthetic, artemisinin-like molecule (Tang et al., 2004). One promising class of potent antimalarial synthetic peroxides is the ozonides (1,2,4 trioxolanes (OZs)) (Tang et al., 2004; Vennerstrom et al., 2004). Several studies have characterized the trematocidal properties of these ozonides (Halferty et al., 2009; Keiser and Utzinger, 2007; Xiao et al., 2007; Zhao et al., 2010). For example, ozonide OZ78 is an effective flukicidal drug in the rodent model. Complete *F. hepatica* burden reductions were achieved with a single oral dose of 100 mg/kg in rats harbouring juvenile and adult *F. hepatica* infections (Keiser et al., 2006b). Importantly, OZ78 also resolved a triclabendazole resistant *F. hepatica* infection in rats (Keiser et al., 2007). Additionally, OZ78 showed an acceptable toxicological profile, based on an exploratory tolerance study in rats and *in vitro* (Vennerstrom et al., 2004).

The aim of this study was to determine the efficacy of OZ78 when administered orally and subcutaneously to sheep at 50 mg/kg for the control of mature *F. hepatica*. Additionally, the pharmacokinetic profiles and tolerability of OZ78 were investigated following both routes of drug administration.

2. Materials and methods

2.1. Study site and regulatory

The study was conducted in an indoor animal housing facility at Yarrandoo Research and Development Centre, Novartis Animal Health, Kemps Creek, NSW, 2178, Australia. The centre has a certificate of accreditation as an animal research establishment. The study was conducted in accordance with local and international guidelines (Anon., 2001a,b,c,d; Wood et al., 1995).

2.2. Study design

In week −13, twelve lambs were selected (3 months of age at commencement, body weights 20.8–30.2 kg). All lambs underwent a general health inspection by a veterinarian and only clinically healthy lambs were included in the study. Faecal examinations were performed to ensure all lambs were not infected with *F. hepatica*, and at most were harbouring only low levels of infection with nematodes. Each lamb was orally infected with 150–200 freshly harvested *F. hepatica* metacercariae mixed with water and dispensed over the back of the tongue (Veterinary Health Research [VHR] Sunny Corner susceptible strain) in week −10. Sufficient time was subsequently allowed to ensure that chronic *F. hepatica* had established. Faecal samples, collected directly from the rectum were sampled on days −6 and −2, processed using an in-house sedimentation (modified McMaster) method. Two grams of faeces was mixed and vigorously stirred with tap water. The sample was strained through a 75 μm mesh sieve into a 100 ml medicine bottle. The sample was allowed to stand for 4 min with the supernatant subsequently discarded. The sedimentation process was repeated and the supernatant discarded down to a level of 1.5 ml. Zinc sulphate (1.5 ml) was added to the sediment, the sample shaken and both chambers of a Whitlock paracytometer filled. All eggs seen in one field of each chamber were counted and multiplied by five to obtain the number of eggs per gram (epg).

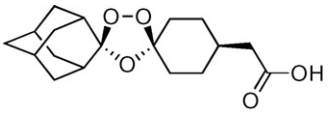

Fig. 1. Chemical structure of OZ78.

On the basis of faecal egg burden (day −2) and body weight (day −2), the animals were randomly allocated on day 0 to either the untreated control group (Group 1; $n = 4$) or the two treated groups (Groups 2 and 3; $n = 4$ lambs each) using a randomization software developed in-house. Treatment of Groups 2 and 3 occurred once on day 0.

Twenty days post-treatment another faecal sample was taken and the egg count determined. Lambs were euthanized on day 21 post-treatment. The liver, with gall bladder and bile duct intact with a portion of the small intestine, were removed from each animal. The bile ducts and small intestine were opened carefully and the flukes removed. Each liver was then sliced, squeezed and soaked in warm tap water. Individual slices were squeezed between the fingers to express flukes. Flukes were transferred to Petri dishes. Entire flukes as well as head and tail portions were counted. Total counts comprised the number of entire flukes and what ever was the greater, head or tail portions.

2.3. Treatment

OZ78 (Fig. 1) was obtained from Ranbaxy Laboratories Limited, India. The oral drug solution (20%, w/v) was prepared with 25% (w/v) solutol HS 15 (Macrogol 15 Hydroxystearate Ph.Eur./CAS-No. 70142-34-6), 20% (w/v) NMP (*N*-methylpyrrolidone Ph.Eur./CAS 70142-34-6) and Arlasolve DMI (Dimethylisosorbide/CAS 5306-85-4) ad 100 ml. For the subcutaneous administration, a 20% (w/v) solution was prepared with 20 ml Lipoid S 100, 10 ml ethanol, 30 ml NMP and Arlasolve DMI ad 100 ml.

Body weights of sheep were determined on calibrated scales two days prior to treatment. On the treatment day and before feeding, individual animals were treated once with 50 mg OZ78/kg orally (Group 2) and 50 mg OZ78/kg subcutaneously (Group 3) by the onsite veterinarian. All treatments were administered from disposable plastic syringes, measured by volume and rounded to the nearest 0.2–0.5 ml. Subcutaneous injections were administered on the neck behind the ear using new 18 G, 1 in. needles attached to disposable syringes. Six ml was the maximum volume administered at each injection site. Once the sheep had been treated they were returned to their pens and fed within 1 h.