Comparative Study of Albendazole and Oxfendazole in the Treatment of Cystic Echinococcosis in Sheep and Goats

Ernest Njoroge, PhD[*†]
Peter Mbithi, PhD[*]
Timothy Wachira, PhD[†]
Joseph Gathuma, PhD[*]
Peter Gathura, PhD[*]
T E Maitho, PhD[*]
Japhet Magambo, PhD[‡]
Eberhard Zeyhle, MS[†]

[*]*Departments of Clinical Studies and Public Health*
University of Nairobi
Nairobi, Kenya

[†]*African Medical and Research Foundation*
Nairobi, Kenya

[‡]*Institute of Tropical Medicine and Infectious Diseases*
Kenya Medical Research Institute
Nairobi, Kenya

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ABSTRACT

The objective of this study was to compare the efficacy of albendazole and oxfendazole in the treatment of cystic echinococcosis using naturally infected sheep and goats. Fifteen animals with natural infections of cystic echinococcosis were randomly divided into 3 groups of 5 animals each. Two groups were treated orally with either albendazole or oxfendazole at 30 mg/kg body weight twice a week for 4 weeks; a third group served as controls. Ultrasound and postmortem examination of the animals, as well as microscopic examination of protoscoleces for eosin dye exclusion and flame cell motility, were used to determine the efficacy of the 2 drugs. Ultrasound examination revealed that 4 animals in the albendazole group and 3 in the oxfendazole group had decreased cyst viability ($P < 0.05$). There were no changes in identifiable cysts of control animals. Microscopic examination showed that 60.9% (14/23) of the cysts from the albendazole group had dead protoscoleces, compared with 93.3% (14/15) and 27.3% (3/11) for oxfendazole and the control group, respectively. There were no significant differences between the effect of albendazole or oxfendazole on either sheep or goats. In the present study, oxfendazole has a higher efficacy (93.3%) than albendazole (60.7%) when administered at the same dosage rate (30 mg/kg body weight) and for the same period (twice weekly for 4 weeks). Based on the findings in this study, oxfendazole seems promising as an alternative drug for treatment of cystic echinococcosis.

INTRODUCTION

Cystic echinococcosis is a zoonotic disease caused by the larval stages of the parasite *Echinococcus granulosus*. However, there is no standard and effective chemotherapeutic

agent for the disease. In humans, albendazole has been recommended as the drug of choice.[1] In cases where albendazole has been reported to be successful in the treatment of cystic echinococcosis, very high dosage rates had to be administered for long periods of time (20 mg/kg body weight/d for 30–60 days).[2] Even in such cases, the efficacy rate of albendazole in humans has been reported to be 30%–60%.[1] In other studies oxfendazole has been found to have an efficacy rate of over 90% in sheep and goats.[3,4] It is important to compare the efficacy of albendazole and oxfendazole in the treatment of cystic echinococcosis. Only well-planned clinical trials in the same animal species can compare the efficacy of the 2 drugs. Conducting randomized studies in humans has been a problem in developing countries, where cystic echinococcosis is endemic.[5] This study sought to compare the efficacy of albendazole and oxfendazole in the treatment of cystic echinococcosis in naturally infected sheep and goats.

MATERIALS AND METHODS

Experimental Animals

Naturally infected animals used in this experiment were identified by scanning sheep and goats at various villages in Lokichogio Division, Northwestern Turkana, Kenya. All the animals identified as positive for *Echinococcus* cysts were purchased from their owners and transported to the African Medical and Research Foundation (AMREF) camp in Lopiding, Kenya. The animals were quarantined for 2 weeks before the start of the experiment. During the quarantine period, they were treated prophylactically against pneumonia with tetracycline at a dose of 20 mg/kg body weight for 4 days. They were also dewormed with a single dose of levamisole (Nilverm, Coopers Ltd, Nairobi, Kenya) (130 mg total dose). The animals were then assigned serial numbers and randomized to one of 3 groups: 2 treatment groups or a control group.

Treatment of Experimental Animals

The treatment groups were treated orally with either albendazole (Valbazen, Novartis East Africa Ltd, Nairobi, Kenya) or oxfendazole (Synanthic, Syntex Animal Health, Palo Alto, CA, USA) at 30 mg/kg body weight twice a week for 4 weeks, while the control group did not receive any treatment. All the animals were provided with feed and water ad libitum. Eight weeks after the start of treatment, the animals were euthanized and a complete postmortem examination carried out under blinded conditions. The lungs, liver, kidneys, and other abdominal organs were examined for *Echinococcus* cysts. The cysts were dissected and aspirated, and a portion fixed in formalin.

Evaluation of Hematological Changes

Hematological examination and liver and kidney function tests were carried out to determine the overall health of the animals. The tests were carried out as described by previous authors.[6] Blood was obtained from each animal once per week by jugular venipuncture using an 18-gauge needle. The samples were collected in 2 types of vials:

1. 5 mL of blood were placed in a vial without any anticoagulant for determination of aspartate aminotransferase (AST) and blood urea nitrogen (BUN).
2. 5 mL of blood were placed into a vial containing ethylenediaminetetraacetic acid (EDTA) for determination of hemoglobin (Hb) concentrations and packed cell volume (PCV).

AST activity was used to determine the functional status of the liver. A total of 0.1 mL of serum was added to 1.0 mL of AST reagent in a test tube. [AST reagent contains 200 mmol/L L-aspartate, 12 mmol/L 2-oxoglutarate, 600 U/L malate dehydrogenase, 0.25 mmol/L NADH, phosphate buffer (pH, 7.8 ± 0.1), and 0.05% sodium azide preservative.] The resulting solution was mixed by gently inverting the tube; the solution was then sucked into the spectrophotometer cuvette. The absorbance was read from the spectrophotometer at a wavelength of 340 nm.

BUN was used to determine the functional status of the kidney. A total of 0.01 mL of the serum sample was added to 1.0 mL of the BUN reagent in a test tube. [The active ingredients of BUN reagent include 8 mmol/L 2-oxoglutarate, 0.25 mmol/L NADH, 50,000 U/L urease, 1,500 U/L GLDH, phosphate buffer (pH, 8.0 ± 0.1), and 0.05% sodium azide preservative.] The solution was immediately mixed by gentle inversion and then sucked into the spectrophotometer cuvette. Absorbance was read from the spectrophotometer at a wavelength of 340 nm.

Hb concentration was determined by the cyanmethemoglobin method as reported by previous authors.[6] EDTA-treated blood was diluted 1:50,000 in isoton. Six drops of ZapOglobin (Coultronics, Luton, UK) were added to lyse the cells and convert hemoglobin to cyanmethemoglobin. The contents were then poured through the Coulter hemoglobinometer to read Hb concentration (g/100 mL or g/dL).

Packed Cell Volume

Packed cell volume (PCV) was determined in a high-speed microhematocrit centrifuge. A capillary tube was filled with EDTA-treated blood to three-quarters full and sealed on one end with plasticin. It was then centrifuged at 10,000 rpm for 5 minutes. The PCV was read using a microhematocrit reader.

Ultrasound Evaluation

Prior to treatment, views of the right lung, right lobe of the liver, and whole abdomen were obtained. The ultrasound examination was repeated 2, 4, and 8 weeks following the initial dose of either albendazole or oxfendazole. Cysts were observed for typical signs of degeneration, that is, decreased size, increased echogenicity, detachment of endocyst, and collapse.[7] The animals' treatment status was unknown to the ultrasonographer.

Pathology, Histology, and Viability Studies

A standard postmortem examination was performed. The lungs, liver, abdominal cavity, kidneys, and spleen were visually inspected and dissected. All surface cysts were dissected intact, and the cyst fluid removed by needle aspiration. Portions of liver cysts that appeared alive and intact were fixed in formalin and stained with hematoxylin and eosin. Eosin exclusion and observation for flame cell movement determined the viability of *E granulosus*.[8]

Data Analysis

Data were normalized by log transformation, and mean values and standard deviations were calculated and compared by the student *t*-test for paired data. Differences in efficacy were compared by the general linear models procedure. $P < 0.05$ was used to determine statistical significance.

RESULTS

Experimental Animals

A total of 472 animals were examined by ultrasound. Of these, 15 animals with cystic echinococcosis were entered into the experiment. The animals were randomly allocated into 3 groups of 5 animals each. Each group had 2 sheep and 3 goats. Two groups were subjected to treatment (with either albendazole or oxfendazole) while the third group was the control.

Hematological Findings

AST activity. AST activity was used to determine the status of liver function during the treatment period. In all 3 groups of animals (albendazole treated, oxfendazole treated, and control groups), there were no significant variations ($P > 0.05$) in AST activity. The AST levels were 24.28 ± 4.95 IU/L, 24.64 ± 4.40 IU/L, and 24.76 ± 5.17 IU/L in the albendazole, oxfendazole, and control groups, respectively (Table 1).

Blood urea nitrogen levels. The mean BUN levels in both the treatment groups and the control group are shown in Table 1. During the treatment period, there were no significant changes ($P > 0.05$) within and between different experimental groups. The mean BUN levels were 26.58 ± 6.57 mg/dL,

Table 1. Hematological Findings of Animals with Cystic Echinococcosis after Treatment with Either Albendazole or Oxfendazole

	Hematological Findings*							
	Mean AST (± SD) (IU/L)	Standard Error	Mean BUN (± SD) (mg/dL)	Standard Error	Mean Hb (± SD) (g/dL)	Standard Error	Mean PCV (± SD) (%)	Standard Error
Albendazole	24.28 ± 4.95	0.99	26.58 ± 6.57	1.31	9.86 ± 1.24	0.25	29.64 ± 3.74	0.75
Oxfendazole	24.64 ± 4.40	0.88	24.62 ± 6.52	1.30	9.22 ± 1.25	0.25	27.12 ± 3.77	0.75
Control	24.76 ± 5.17	1.03	24.91 ± 4.99	1.00	9.31 ± 1.45	0.29	27.48 ± 4.62	0.92

*AST indicates aspartate aminotransferase; BUN indicates blood urea nitrogen; Hb, hemoglobin; PCV, packed cell volume.

24.62 ± 6.52 mg/dL, and 24.91 ± 4.99 mg/dL in albendazole, oxfendazole and control groups, respectively.

Hemoglobin concentration. There were fluctuations in hemoglobin concentration within different experimental groups during the treatment period. The fluctuations were present in all 3 groups (albendazole, oxfendazole, and control). However, there were no significant differences between the groups ($P > 0.05$). The mean hemoglobin concentrations were 9.86 ± 1.24 g/dL, 9.22 ± 1.25 g/dL, and 9.31 ± 1.45 g/dL in the albendazole, oxfendazole, and control groups respectively (Table 1).

Packed cell volume. The mean PCV did not vary significantly within each group ($P > 0.05$). Additionally, there was no significant variation between the different experimental groups ($P > 0.05$). In the albendazole, oxfendazole, and control groups, the mean PCVs were 29.64 ± 3.74%, 27.12 ± 3.77%, and 27.48 ± 4.62%, respectively (Table 1).

Ultrasound findings. In animals receiving albendazole, 4 showed decreased cyst viability compared with 3 in the oxfendazole group ($P < 0.05$). The cysts showed increased echogenicity, complete or partial detachment of the endocyst, new calcification, or decrease in size. There were no changes in identifiable cysts of control animals.

Findings of postmortem examination, viability studies, and histology. A total of 49 cysts were harvested at postmortem. Twenty-three of the cysts were from the albendazole group while 15 and 11 were from oxfendazole and control groups, respectively. On gross examination 4 (17.4%), 7 (46.7%), and 1 (9.1%) cysts were degenerate from albendazole, oxfendazole, and control groups, respectively. The degenerate cysts had a yellow adventitial layer and were filled with turbid yellowish fluid. Additionally, there were significant differences ($P < 0.05$) in the viability of the cysts between the different experimental groups. Microscopic examination of protoscoleces for eosin dye exclusion and flame cell motility showed that 60.9% (14/23) of the cysts from the albendazole group had dead protoscoleces compared with 93.3% (14/15) and 27.3% (3/11) for oxfendazole and control groups, respectively (Table 2). The viable-appearing cysts showed evidence of marked host cell reaction consisting of infiltration of the adventitial layer with neutrophils, eosinophils, and plasma cells.

DISCUSSION

In the present study, the efficacy of albendazole was 60.9%. This is comparable with the efficacy of the drug in humans.[1] In humans, albendazole has been reported to have an efficacy of 30%–60% when administered daily at the dosage rate of 20 mg/kg body weight. In the present study, the dosage rate was 30 mg/kg body weight administered twice a week. Different administration rates (biweekly versus daily) and different dosage rates may have resulted in similar efficacy due to species differences. Sheep and goats are ruminants while humans are monogastric and therefore drug metabolism is expected to be different. However, there were not significant differences in the effect of either albendazole or

oxfendazole between sheep and goats of the same experimental group.

In the group treated with oxfendazole, protoscoleces were either dead or absent in 93.3% of the cysts. Protoscoleces were either absent or dead in 27.3% of the cysts in the control group. These findings are similar to those of other authors. In a recent study,[4] the mean aggregate of dead protoscoleces were reported to be 99%, 93%, and 68% for sheep treated daily, weekly, and monthly with oxfendazole at 30 mg/kg body weight. Similarly, they found dead protoscoleces in 32% of the cysts. The findings in the present study are, however, different from those found in an earlier study by the present authors.[3] In a previous study using 2 groups of animals (oxfendazole and control), protoscoleces were dead in 97.3% of the cysts from the treatment group while none were dead in the control group. The differences in the findings of the 2 studies may be difficult to explain because the later study tried to mimic the former one.

The findings of this study indicate that oxfendazole has a higher efficacy (93.3%) than albendazole (60.7%) when administered at the same dosage rate (30 mg/kg body weight) and for the same period (twice weekly for 4 weeks). These findings may be explained by the differences in pharmacokinetics of the 2 drugs. In a comparative analysis of albendazole and oxfendazole using high-performance liquid chromatography (HPLC), the plasma disposition of albendazole metabolites were found to be markedly different from that of oxfendazole derivatives.[9] Albendazole sulfoxide (the active metabolite in albendazole) exhibited faster absorption and a higher C_{max} than oxfendazole. Furthermore, while albendazole sulfoxide declined relatively rapidly in plasma, reaching nondetectable concentrations at 60 hours post-albendazole administration, oxfendazole was found in plasma for up to 144 hours post-treatment. However, more studies are necessary to determine the rate of penetration into *Echinococcus* cysts and levels of both albendazole sulfoxide and oxfendazole within the cyst.

Table 2. Microscopic Findings of *Echinococcus* Cysts from Animals Treated with Either Albendazole or Oxfendazole

Type of Treatment	Cysts with Viable/Nonviable Protoscoleces	
	Nonviable	Viable
Albendazole	60.9% (14/23)	39.1% (9/23)
Oxfendazole	93.3% (14/15)	6.7% (1/15)
Control	27.3% (3/11)	72.7% (8/11)

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