2-Hydroxypropyl-β-cyclodextrin improves the effectiveness of albendazole against encapsulated larvae of Trichinella spiralis in a murine model

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Objectives: We evaluated whether the effectiveness of albendazole against encapsulated larvae increases when 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) is added to improve bioavailability.

Methods: Mice were infected with Trichinella spiralis and treated with albendazole alone, albendazole plus HP-β-CD or not at all (controls) (Experiment I). Both immediately after treatment [76 days post-infection (p.i.)] and later (139 days p.i.) larvae were recovered, and the mean count was expressed in proportion to the larva count for controls. To evaluate the infectivity of the recovered larvae, the larvae recovered at 76 days p.i. and 139 days p.i. were used to infect another three groups (Experiments II and III, respectively).

Results: At 76 days p.i., the percentage of larvae recovered was 77.4% for mice treated with albendazole alone and 61.2% for those treated with albendazole plus HP-β-CD; at 139 days p.i., these percentages were 67.4% and 40.9%, respectively (Experiment I). In Experiments II and III, the percentage of larvae collected from the albendazole group and the combined-treatment group was 55.2% and 27.6%, and 53.1% and 26.6%, respectively. The ABZSO active metabolite was analysed to determine the bioavailability of albendazole. For the combined-treatment group, the area under the plasma concentration–time curve between 0 and 6 h was higher than that for the albendazole group.

Conclusions: These data suggest that HP-β-CD increases the bioavailability and consequently the effectiveness of albendazole against encapsulated Trichinella larvae.

Keywords: trichinellosis, treatment, bioavailability

Introduction

Albendazole is a wide-spectrum anthelmintic drug used for human and animal infections. When administered orally, albendazole is quickly biotransformed into its active intermediate metabolite albendazole-sulphoxide (ABZSO), which is then oxidized to the inactive form of albendazole-sulphone (ABZSO2). Because of their affinity for the parasite β-tubulin, both albendazole and ABZSO show anthelmintic activity. However, like other benzimidazole-carbamates, albendazole has a low water solubility, limiting its oral absorption and resulting in a lower bioavailability.

Cyclodextrins (CDs) are cyclic oligosaccharides that tend to form inclusion complexes. For this reason, in the late 1990s, 2-hydroxypropyl-β-cyclodextrin (HP-βCD) was used to improve the solubility of poorly water-soluble drugs, including albendazole.

The anthelmintic activity of albendazole combined with HP-βCD was first studied in mice infected with Trichinella spiralis, although the treatment used in the present study was considerably different in terms of the dosage and timing. García et al. used a higher single dose to improve the effectiveness of the drug (50 and 100 mg/kg of albendazole versus 15 mg/kg/day used in human treatment). In the present study, we attempted to simulate a human dose regimen to evaluate the feasibility of applying such treatment to humans. Trichinella sp. is the aetiological agent of trichinellosis, a widespread helminthic zoonosis acquired by ingesting undercooked meat containing...
Increased effectiveness of benzimidazoles in treating Trichinella infection

Infective larvae. The effectiveness of benzimidazoles in treating trichinellosis is strictly related to the time of administration; in fact, they are more effective in the early stages of infection, when worms are still present in the gut mucosa, or when newborn larvae are migrating from the gut vessels to the muscles. However, in most infected persons, diagnosis is made several weeks after being infected, when the larvae have already established themselves in the muscle cells and a collagen capsule has developed around them. Since anthelmintics have low water solubility and are poorly absorbed by the intestinal lumen, the bioavailability is low; consequently, only low amounts reach the encapsulated larvae in the muscles, at least when administered at the recommended doses. The objective of the present study was to determine whether HP-β-CD improves the oral bioavailability of albendazole and consequently the anthelmintic effectiveness at the muscular level using the highest recommended human dosage for albendazole.

Materials and methods

Parasite and animal model

T. spiralis muscle larvae (isolate code ISS3) were collected from infected mice by artificial digestion, following standard procedures. The larvae were then suspended in 0.25% agar in phosphate-buffered saline (PBS). The suspension was administered per os to 60 BALB/c female mice of 20 ± 3 g, which had been acclimated to the animal care facility for 1 week before being infected. Animals were housed and treated according to European directive 8/609EEC.

Drug formulations and administration

A commercial formulation of albendazole (Zentel, GlaxoSmithKline, Verona, Italy) was used. HP-β-CD was kindly supplied by Janssen Pharmaceutica N.V. (Beerse, Belgium). The metabolites (ABZSO and ABZSO₂) for HPLC analysis were kindly provided by GlaxoSmithKline (Brentford, Middlesex, UK). All of the formulations were administered daily in 0.2 mL of deionized water via a bucco-gastric tube. Albendazole was administered at a dose that has been recommended for humans (15 mg/kg/day). The formulation consisting of albendazole and HP-β-CD was prepared using the freeze-drying method of Castillo et al.

Experimental design

The effectiveness of treatment against encysted larvae was quantitatively measured by determining the number of larvae recovered after treatment for three groups of mice [one group treated with albendazole alone, another treated with albendazole plus HP-β-CD and a non-treated control group (Experiment I)] and the capacity of these recovered larvae to infect another three groups of mice (Experiments II and III).

Experiment I

At time zero, 60 mice were infected via bucco-gastric tube with 80 ± 8 T. spiralis muscle larvae (L1) suspended in 0.2 mL of a saline solution with 0.25% agar. Forty days after infection, at which time all larvae in the muscle tissues are encapsulated, the mice were randomly assigned to one of the three groups specified above (20 mice per group). The mice were treated daily for 2 weeks [from 41 to 54 days post-infection (p.i.)]; treatment was discontinued for 1 week (from 55 to 61 days p.i.), and then daily treatment was started again for another 2 weeks (from 62 to 75 days p.i.), according to a protocol used for human treatment. At 76 days p.i., 10 animals from each group were sacrificed by CO₂ and the whole-skinned and eviscerated carcass of each mouse was separately digested according to standard procedures. T. spiralis larvae from each mouse were then collected and counted in triplicate. This procedure was repeated at 139 days p.i. to evaluate whether or not additional larvae had been destroyed.

Experiments II and III

A total of 80 ± 8 of the larvae collected at 76 and 139 days p.i. from the three groups of mice in Experiment I were used to infect three new groups of mice (10 animals each). Experiment II consisted of infecting mice with the larvae collected at 76 days p.i.; Experiment III consisted of infecting mice with the larvae collected at 139 days p.i. The mice were sacrificed 45 days p.i., and larvae were collected and counted as described previously.

HPLC analysis

The plasma concentrations of albendazole, ABZSO and ABZSO₂ in infected mice were measured by HPLC analysis. Blood samples, gathered into 0.5% Na₂EDTA with PBS, were collected at 0, 0.25, 0.5, 0.75, 1.5, 3 and 6 h after administering the drugs. Chromatography was performed on a Waters chromatographic system equipped with a Waters 600 MS multisoliient delivery system and a Waters 717 Auto sampler as described previously. The limit of detection and of quantification values were, respectively, 1.27 and 3.82 ng/50 μL for ABZSO and 2.96 and 8.88 ng/50 μL for ABZSO₂.

Pharmacokinetics

The bioavailability of ABZSO was studied by evaluating the following parameters: maximum plasma concentration (Cₘₕₘₜ₅, μg/mL), time to achieve the maximum plasma concentration (Tₘₕₘₜ₅, h) and the area under the plasma concentration–time curve between 0 and 6 h (AUC₀ₖₘₜ₅, μg · h/mL). AUC₀ₖₘₜ₅ was calculated by applying the trapezoidal rule using the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). Statistical analysis

The number of recovered larvae from each group of mice was expressed as mean ± SD; this number was then expressed as a percentage in comparison with the number of larvae recovered from the control mice. The effectiveness of the two drug formulations was compared using the one-tailed ANOVA test. When a statistically significant difference was observed (P < 0.05), a post-test (Bonferroni) was used to compare single groups. The ABZSO plasma concentrations were expressed as mean ± SD (three mice per group per time). The pharmacokinetic parameters of ABZSO (Cₘₕₘₜ₅, Tₘₕₘₜ₅, AUC₀ₖₘₜ₅) were compared using the one-tailed Student’s t-test. The GraphPad Prism program was used to apply statistical tests.

Results

Experiment I

At 76 days p.i., the number of muscle larvae recovered from mice was 105 605 ± 6947 for the mice treated with albendazole, 83 610 ± 1588 for the mice treated with albendazole plus HP-β-CD, and 136 523 ± 6009 for the control mice (Figure 1). The difference between these means was highly significant (P < 0.001). The number of larvae recovered from mice treated
with albendazole alone and those treated with albendazole plus HP-βCD corresponded to 77.4% and 61.2%, respectively, compared with controls. Moreover, the Bonferroni post-test, which compared single groups, was highly significant in all cases \((P < 0.001)\). At 139 days p.i., the number of muscle larvae recovered from mice was 68,575 ± 3085 for the mice treated with albendazole, 41,570 ± 6520 for the mice treated with albendazole plus HP-βCD, and 101,785 ± 4226 for the control mice \((P < 0.001)\) (Figure 1). The Bonferroni post-test was highly significant in all cases \((P < 0.001)\).

Experiment II

The number of larvae collected at 45 days p.i. from the mice treated with albendazole and albendazole plus HP-βCD (infected with larvae collected at 76 days p.i. in Experiment I) was 63,083 ± 5082 and 31,543 ± 127, respectively, compared with 114,368 ± 4672 larvae recovered from control mice \((P < 0.001)\) (Figure 1). The number of larvae recovered from mice treated with albendazole alone and those treated with albendazole plus HP-βCD corresponded to 67.4% and 40.9%, respectively, compared with controls.

Experiment III

The number of larvae collected at 45 days p.i. from the mice treated with albendazole and albendazole plus HP-βCD (infected with larvae collected at 139 days p.i. in Experiment I) was 68,000 ± 1925 and 34,000 ± 2847, respectively, compared with 128,000 ± 6514 larvae recovered from control mice \((P < 0.001)\) (Figure 1). The number of larvae recovered from mice treated with albendazole alone and those treated with albendazole plus HP-βCD corresponded to 53.1% and 26.6%, respectively, compared with controls.

HPLC and pharmacokinetics

The ABZSO plasma concentrations are shown in Figure 2. ABZSO \(C_{\text{max}}\) values were similar when comparing the mice treated with albendazole alone with those treated with albendazole plus HP-βCD \((3.40 ± 0.10 \text{ versus } 3.84 ± 0.16 \mu g/mL, \text{ respectively}; P > 0.01)\). ABZSO \(T_{\text{max}}\) was achieved 0.5 h after administration for both the albendazole group and the group treated with albendazole plus HP-βCD. When comparing the two treatment groups in terms of ABZSO \(AUC_{0-6} \) \((2441 ± 0.13 \mu g \cdot h/mL \text{ for the albendazole group versus } 8384 ± 0.29 \mu g \cdot h/mL \text{ for the group treated with albendazole plus HP-βCD}), the difference was highly significant \((P < 0.001)\).

Discussion

The results of the present study suggest that albendazole combined with HP-βCD is significantly more effective than albendazole alone against encapsulated \(T. spiralis\) muscle larvae. The combined treatment significantly reduced the larva burden immediately after treatment (i.e. 76 days p.i.), and it was even more effective some weeks later (i.e. 139 days p.i.), possibly because of the death of additional larvae that had been damaged...
but not destroyed immediately following treatment. The combined treatment also significantly decreased the infectivity of the remaining larvae. In fact, not all the larvae collected from treated mice were able to infect mice in Experiments II and III, suggesting that lower infectivity is synonymous with lower viability and with a consequent reduction in the risk of developing chronic trichinellosis.\(^5\)

The increased effectiveness was probably a consequence of an increased drug bioavailability resulting from the use of HP-\(\beta\)CD in a liquid formulation. Since albendazole and ABZSO\(_2\) plasma concentrations were equal to or under the detection limit (data not shown), the bioavailability of albendazole was determined by evaluating the active ABZSO metabolite. With regard to ABZSO, \(t_{\text{max}}\) and \(C_{\text{max}}\), the values were similar when comparing the mice that had received the combined treatment with those treated with albendazole alone; however, the oral bioavailability, expressed as the AUC\(_{0-6}\) of the ABZSO plasma concentration, was four times higher for the combined-treatment group. Thus the increase in bioavailability can be attributed to the increased solubility and dissolution rate, which lead to better absorption. Given that the \(C_{\text{max}}\) values were similar for the two groups, we can suppose that the albendazole plus HP-\(\beta\)CD formulation does not have increased toxicity, even if the new formulation has a significantly longer half-life. In fact, no behavioural anomalies were noted in mice during treatment, and no organ anomaly or loss in body weight was observed at necropsy; only damp faeces were observed.

The dose of albendazole used in the study is one which has been recommended for the treatment of human trichinellosis and is commonly used.\(^6\) Regarding general toxicity, HP-\(\beta\)CD, depending on the dose and route of administration, is generally well tolerated in most species, especially when administered orally.\(^1\)

Moreover, it should be considered that ABZSO is a chiral molecule with two enantiomers [(+) and (–)] with different activities related to their metabolization. Enantiomer ABZSO(–) is dominant in the mouse and rat, whereas ABZSO(+) is dominant in the goat, sheep, dog, cattle and human. In previous studies, ABZSO enantiomers showed a divergence in the pharmacokinetic disposition after oral administration, possibly reflecting their selective metabolism,\(^1\) a sex-related difference in the pharmacokinetic behaviour\(^1\) and a selective binding to cytosolic proteins isolated from different helminth parasites.\(^1\) All these factors must be taken into account because they may contribute to the pharmacological properties of this chiral molecule. Indeed, Bolas-Fernandez et al.,\(^1\) comparing the anti-Trichinella larva activity of ABZSO(+) and ABZSO(–) in an ex vivo model, suggest that ABZSO(+) is likely to be responsible for the albendazole activity against Trichinella sp. We can thus expect albendazole plus HP-\(\beta\)CD to be more effective in treating humans, compared with a murine model.

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Transparency declarations

None to declare.

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