The Efficacy of Albendazole-Mebendazole Combination Against *Echinococcus granulosus* Protoscoleces (an *in vitro* evaluation)

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Summary

In order to determine the in vitro *efficacy of albendazole-mebendazole combination against protoscoleces of* E. granulosus *this study was undertaken. Hanks solution and DMSO were used as the maintaining medium and the drug solvent, respectively. The concentrations of albendazole and mebendazole were selected on the basis of the serum levels in patients who had undergone long term therapies with these drugs. Viability of protoscoleces was evaluated by eosin staining and flame cell motility methods. Additive or synergistic effects were observed when amounts of albendazole and mebendazole were combined. Certain regimens caused more than 90% mortality of protoscoleces. The best result was achieved when 150 µg/l mebendazole plus 750 µg/l albendazole were combined. This combination killed 99.9% of the protoscoleces, after 24 h incubation.*

Introduction

Prior to introduction of benzimidazole compounds, removal of hydatid cysts by surgery was the treatment of choice in patients with active viable cysts of *Echinococcus granulosus*. However, sometimes due to the extent or location of the cysts, complicated cysts or recurrence of the disease, patient's general condition or lack of adequate facilities, performance of surgery was impossible or impracticable. In such cases, attempts have been made to treat the disease with benzimidazole compounds such as albendazole and mebendazole. The first report on mebendazole, an effective drug against nematodes and cestodes, was encouraging (Bekhti *et al.*, 1977).

Albendazole, another benzimidazole compound, is also effective against several larval and adult stages of nematodes, trematodes and cestodes including *E. granulosus* (Euzeby, 1981; Rossignol *et al.*, 1981; Ramalingam and Krishman, 1983). In the present study, the *in vitro* efficacy of combined albendazole-mebendazole against *E. granulosus* protoscoleces was evaluated.

Materials and methods

Protoscoleces of *E. granulosus* were obtained from liver hydatid cysts of slaughtered sheep at abattoirs of Tehran. The surface of the cyst was sterilised with alcoholic iodine solution. To reduce intracystic pressure, the cyst wall was penetrated, using a large size needle, and the fluid of the cysts was withdrawn. The cyst wall then was cut open with scalpel and scissors and protoscoleces were transferred into a sterile container. Protoscoleces were treated with 0.5 mg/ ml pepsin at pH= 2 and were incubated at 37°C for 15-45 minutes. After removal, the protoscoleces were rinsed for 15 minutes 4 times in Hanks solution (Smyth and Davies, 1974). Initial viability of the protoscoleces was assessed following staining with 0.1% aqueous eosin solution and examination under a light microscope. Living protoscoleces did not take the dye up, whereas, the dead ones did. Flame cell activity also was used for the differentiation between the living and the dead protoscoleces (Smyth and Barret, 1980). Hanks solution and DMSO were used as the maintaining medium and drug solvent, respectively. Conditions such as pH, number of protoscoleces and the volume of media were standardised. One thousand protoscoleces were maintained in 10 ml of Hanks solution containing 100 µg/ml streptomycin and 100 I.U./ml penicillin.

The serum levels of albendazole and mebendazole following long term therapy in man (Brandimante *et al.*, 1980; Bryceson, 1980; Morris *et al.* 1983) were taken as a guide for selection of the concentration of these drugs in the experiments. Each drug was separately dissolved in DMSO (0.4%) and added to the medium. Viability of the protoscoleces were measured after 24 h incubation at 37°C.

Results

There were significant ($P< 0.001$) differences between mortality rates of *E. granulosus* in various concentrations of mebendazole and albendazole. The increase of mebendazole or albendazole content augmented the mortality rate of protoscoleces (Table 1). At the highest concentrations used

(330 µl mebendazole and 1000 µg/l albendazole), mortality of 68.2% and 86.6% were produced, respectively. When mebendazole and albendazole were combined, additive or synergistic effects were observed (Table 2). A combination of 150 µg/l mebendazole to 750 µ g/l albendazole killed 99.9% of protoscoleces.

Discussion

The purpose of this study was to determine, *in vitro*, the efficacy of 2 anthelmintic drugs, albendazole and mebendazole, when they were combined. Mebendazole, at concentrations studied *in vitro* and *in vivo* (Lorenzini and Ruggieri 1990; Schantz *et al.* 1982), had shown only partial lethal effects on protoscoleces of *E. granulosus.* Similarly, albendazole was, according to Morris *et al.* (1983), partially efficacious (61.7% at 1000 µg/l after 35 days incubation) against *E. granulosus.* In our study, the efficacy of the latter drug when used at the same concentration was also partial and did not exceed 86.6. Despite partial activity of mebendazole and albendazole, when separately used, the results, *in vitro*, were much better against protoscoleces when the two drugs were combined. Combination of different doses of the drugs caused additive or synergistic effects. By combining 150 µg/l mebendazole to 750 µg/l albendazole a mortality rate of 99.9% was achieved.

Table 1. *Mortality rate of* **Echinococcus granulosus** *in various concentration of mebendazole and albendazole, after 24 hours exposition (3 tests in triplicates).*

Mebendazole concen. (µg/l)	Mortality (%)	Albendazole concen. (µg/l)	Mortality (%)
0.00	0.72±0.43	0	0.84±0.30
12.50	8.78±0.64	25	16.14±0.99
25.00	10.66±0.71	50	26.86±1.54
50.00	14.84±1.30	100	32.02±1.83
100.00	48.46±1.60	250	44.98±1.49
150.00	49.78±1.48	500	50.14±1.85
200.00	55.70±1.90	750	58.27±2.20
300.00	68.20±2.42	1000	86.64±1.81

Table 2. *Synergestic or additive effects and mortality rates of* **Echinococcus granulosus** *protoscolesces in various concentration of mebendazole and albendazole after 24 hours exposition (three tests in triplicates).*

Mebendazole + Albendazole	Mortality (%)	Synergestic (S) or additive (A) effect
25 + 25	35.86 ± 2.07	S
50 + 50	41.16 ± 1.74	A
50 + 250	49.04 ± 2.37	A
100 + 250	70.88 ± 4.57	A
25 + 500	67.44 ± 1.84	S
50 + 500	94.80 ± 2.04	S
100 + 500	96.10 ± 0.72	A
150 + 500	97.59 ± 1.22	A
25 + 750	96.24 ± 0.72	S
50 + 750	97.20 ± 2.20	S
100 + 750	98.74 ± 1.33	A
150 + 750	99.92 ± 3.03	A

Acknowledgement

The authors express their sincere thanks to Dr. R. Hosseini for his valuable scientific cooperation in the course of this work.

References

Bekhti, A.; Schaaps, J.P.; Capron, M.; Dessainz, J.P.; Santoro, F.; Capron, A.(1977). Treatment of hepatic hydatid disease with mebendazole; preliminary results in four cases. British Medical Journal. **2**: 1047-1051.

Brandimante, C.; Robotta, G.; Fediani, T.; Lalloni, L. and Grunci, G. (1980). Effects of mebendazole treatment in 4 patients with hepatic hydatid disease. Italian Journal of Gastroentrology, **12**: 953-958.

Bryceson, A.(1980). Mebendazole and Hydatid disease. British Medical journa, 280,796.

Euzeby, J. (1981). Experimentation des proprietes preventives et curatives de l'Albendazole sur une cestodose larvaire animale. Mode e'ttude en vue la chimiotherapie du kyste hydatique. Annales pharmaceutiques Francaises, **39**: 45-49.

Lorenzini, R. and Ruggieri, A. (1990). An experimental *in vitro* model for evaluating drug against protoscoleces granulosus. Jouranal of Helminthology, **64**: 343-348.

Morris, D.L.; Dykes, P.W.; Dickson, B.; Marriner, S.E.; Bogan, J.A.; Burrows F.G.O. (1983). Albendazole in hydatid disease. British Medical Journal, **286**: 103-104.

Ramalingam, B.S. and krishman, S.U. (1983). Albendazole, and effective single dose broad spectrum antihelmintthic drug. American Journal of Tropical Medicine and Hygiene, **32**: 984-989.

Rossignol, J.F.; Friedman, J.C.; Arroueau, M. and Dicuolla, C.T. (1981). Albendazole toxicologia animal. Compendium de Ivestigaciones Latino Americanas, **1**: 56-60.

Schantz, P.M.; Van Den Bossche, H. and Eckert, J. (1982). Chemotherapy for larval echinococcosis in animals and human. Report of a workshop. Zeitschrift für Parasitenkunde, **67**: 5-26.

Smyth, J.D. and Barret, N.J. (1980). Procedures for testing the viability of human hydatid cysts following surgical removal, specially after chemotherapy. Transactions of the Royal Society of Tropical Medicine and Hygiene, **74**: 649-652.

Smyth, J.D., Davies, Z. (1974). *In vitro* culture of the strobilar stage of *Echinococcus granulosus* (sheep strain): A review of basic problem and results. International Journal for Parasitology, **4**: PP. 631-644.