J Med Microbiol Infect Dis, 2018, 6 (2-3): 53-56
DOI: 10.29252/JoMMID.6.2.3.53

Original Article

Increased Alkaline Phosphatase Activity in the Somatic Extract of Hydatid Cyst Protoscoleces upon Treatment with Albendazole, an Implication for *in vitro* Evaluation of Drug Efficiency

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Received Jun 19, 2018; Accepted Aug 01, 2018

Introduction: Hydatidosis is an endemic parasitic disease of humans in Iran, and Albendazole (ABZ) is a drug of choice for treatment of this infection. As the Alkaline phosphatase (ALP) is necessary for the metabolism of parasites, this study was aimed to evaluate the effect of ABZ on ALP enzyme activity in hydatid cyst parasite as a marker for drug efficiency. **Methods:** In the present study, the ALP activity level was estimated in the extracts of the untreated parasite (Hydatid cyst protoscoleces) as well as the ABZ-treated samples with a final concentration of 100 µg. The protein concentration and the protein bands in the extracted samples were analyzed by Bradford and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) methods, respectively. **Results:** The results showed that the mean value of the ALP activity level of the treated samples (0.474 U/ml/mg) was significantly higher than that of untreated samples (0.205 U/ml/mg) (P<0.05). SDS-PAGE analysis demonstrated the higher intensity of the 59 kDa protein band in ABZ-treated samples, compared to the untreated sample. **Conclusion:** Considering the effect of the ABZ drug on ALP activity in the hydatid cyst protoscoleces, this enzyme might be regarded as an indicator for the effectivity of drug on this parasite. *J Med Microbiol Infec Dis, 2018, 6 (2-3): 53-56*.

Keywords: Albendazole, Parasites, Alkaline Phosphatase, Hydatid cyst, Iran.

INTRODUCTION

Human Hydatid cyst **(**Echinococcosis**)** is caused by the larvae of the parasite *Echinococcus granulosus*. This parasite has a worldwide distribution and affects a wide range of intermediate hosts, including men and both domestic and wild animals [1-2]. Albendazole (ABZ), as a drug of choice, has shown to be highly effective in the treatment of this infection if administrated 10-15 mg/kg daily for several months [3]. This drug interferes with the uptake of glucose from the tegument of the parasite [4]. Understanding the biochemical and physiological functions of the parasites, when exposed to medications, would provide useful information on the prevention and treatment of the infection [5].

Normally, enzymes are necessary for survival, migration, and metabolism of parasites. The pivotal role of alkaline phosphatase (ALP) enzyme for the parasite feeding and its evasion of the immune system is known. Generally, ALP enzyme removes phosphate groups from the proteins in an alkaline environment and helps in breaking down the proteins [6-7]. This enzyme is produced by the liver and is bound to the bile capillaries cell surface [8]. The switching of ALP to Acid phosphates may be associated with the growth and development of the reproductive system in adult worms [9]. In the excretory-secretory products of hydatid cyst protoscoleces, ABZ caused more reduction of ALP activity in comparison to MBZ [10]. The present study aimed to determine the effect of ABZ on the secretion of ALP in protoscoleces and as a drug efficiency marker.

MATERIAL AND METHODS

Preparation of protoscoleces somatic extract solution. Hydatid cysts were obtained from livers of 10 infected sheep slaughtered at the local Solimani abattoir, Alborz, Iran. The protoscoleces were removed from the cysts by using a syringe in an aseptic condition, washed three times with PBS and divided in two groups of control and test. One hundred µg ABZ (Tolide Daruhai Dami Iran Company), was added in tubes of test group, and control tubes (without ABZ) were incubated in PBS media for 4-6 h at 37°C.

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The protoscoleces were frozen and thawed 3 times alternatively in liquid nitrogen and water bath of 37°C. The samples were then sonicated in a 150w ultrasonic disintegrator (Institute of Biochemistry and Biophysics, Tehran, Iran) on ice 3-5 times (10 seconds follwed by 10 seconds rest) until no intact parasite were visible microscopically. The suspension was centrifuged at 10000 g for 30 min at 4°C, and the supernatant was stored at -20°C [11-12].)

Measurement of Protein concentration and ALP activity. Protein concentration in the extracted solutions of parasites was measured by Bradford method with inclusion of Bovine Serum Albumin (BSA) as the standard [11]. The ALP activities in the samples were measured using a commercial enzyme assay kit (Ref. number 10-503R20; Ziest Chemo Company). The kit uses *p*-nitrophenyl phosphate (*p*NPP) as the substrate. The ALP converts *p*NPP and produces a color which is measurable spectroscopically at 405 nm. For measurement of the ALP activity, amounts of 20 µl of the parasites' extracts were added to a cuvette containing 1 ml of working solution (solution 1, Diethanolamine and Magnesium chloride; solution 2, P-nitrophenyl phosphate at 37°C), and the optical densities (OD values) were measured following 1, 2 and 3 min at 405 nm. Subsequently, the mean values of measured OD values were calculated, and the ALP activity of the test samples and controls were estimated according to the kit recommendations (http://Merko.ir/product/3530).

SDS-PAGE analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method with Coomassie blue staining were used to separate the protein components of the parasite extracts. The parasite extracts were mixed with a sample buffer and run on 12% acrylamide gels as described elsewhere [11]. The molecular weight of separated bands of protein samples was determined along a protein marker (peqGold; protein marker) and the ratio factor (ratio of the distance migrated by the molecule to that migrated by a marker dye-front) of appeared bands in the gel [11]. The proteins were identified by using the obtained molecular weights via the EXPASY protein database (http://www.expasy.org).

Statistical analysis. Independent two-sample t-test was performed to compare the mean values of enzyme activities between treated and untreated samples. The statistical comparisons were performed using the t-test Calculator for 2 Independent Means (http://www.socscistatistics.com/-tests/studenttest/).

RESULTS

Protein concentration and ALP activity. The mean values of protein concentrations and enzyme activities in samples treated with ABZ and untreated ones are presented in Table 1. The results showed that the mean value of the ALP activity of the treated samples (0.474 U/ml/mg) was significantly higher than that of untreated samples (0.205 U/ml/mg) (P<0.05).

SDS-PAGE analysis. Somatic extracted samples of the parasite were analyzed using SDS-PAGE (Fig. 1). The 59 kDa protein was recognized as the ALP according to the EXPASY in the two groups. The SDS-PAGE analysis exhibited a higher intensity of the 59 kDa protein band in ABZ-treated samples, compared to the untreated sample. Table 2 shows the proteins identified in this study.

Table 1. The mean values of protein amounts and ALP activity level in ABZ-treated and untreated protoscoleces

Samples	Protein amounts (mg/ml)	ALP total activity level (U/ml)	ALP Specific activity level (U/ml/mg)
Treated protoscoleces*	0.13	0.06	0.474
Untreated protoscoleces	0.16	0.03	0.205

* Treated with ABZ

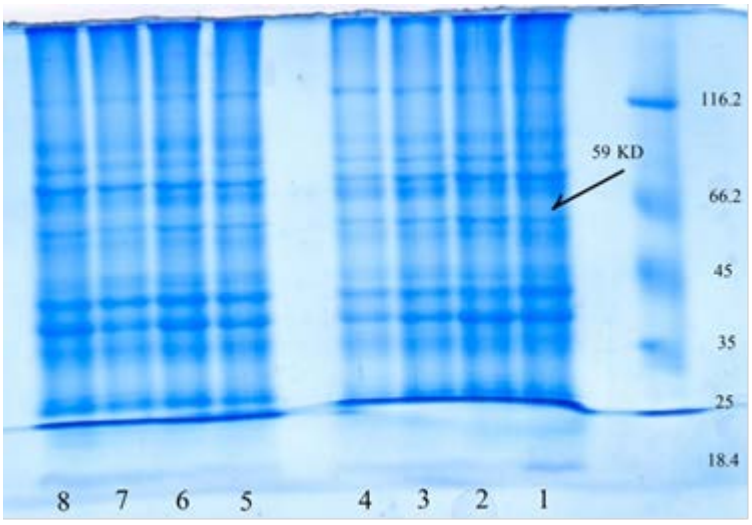

Fig. 1. SDS-PAGE analysis of proteins from the somatic extracted solution of the protoscoleces. Lanes 1-4, treated parasite samples with albendazole; lanes 5-8, untreated parasite samples.

Table 2. Recognized protein bands of parasite according to Expasy database

MW in gel	MW from Expasy database	Recognized proteins
144.130	147.297	Phosphoinositide phospholipase (W6UW42)
135.327	135.062	Tubulin beta-1chain (W6UGC9)
121.342	118.145	Eukaryotic translatin initiatin factor3 subunitic.(W6URL4)
93.167	90.881	Segment polarity protein dishevelled DVL-3.(w6UHQ9)
	98.741	Paramycin
84.763	85.788	Kataninp80 wD40repeat-containing subunit B1. (Wgutm7)
	84.086	Acetyl choline transferase
	83.600	Phosphatase and tensi
	83.742	Activated cdc 42 kinasse1
77.117		Propialy- coAcarboxylase alpha chain. (W6UBE5)
	78.318	UDP-N-acetyl-D-galactosamin :
	75.392	Popypeptide-acetylgalactosaminyl
	75.271	Transferase
		Phosphoenolpyrovate
		Carboxylase
70.160	71.675	78KDa glucose-regulated protein.(Q24895)
	72.554	Heat shock cognate 70 KDa protein
	72.271	Phosphoenol pyruvate carboxylase
62.910	61.474	Glucose -6-phosphate isomerase (Q56JA3)
57.374	57.017	Mitogen-activated Kinase Kinase (MKK2)
	59.522	**ALP (Alkaline phosphatase)**
47.490	47.022	(AST) Aspartate aminotransferase
	46.561	Enolase(DOVLV3)
	46.615	RAD9(A9QWR90
43.206	42.225	Putative calreticulin(Q56JAO)
	43.557	ERK-like protein
	45.339	Calreticulin(A5YTY7)
	45.347	B5ACH7-Beta-Tubulin
37.540	37.961	MKK1-like protein
	37.335	Smad C protein
	35.813	Putative paramyosin (Q56J95)
	35.437	Smad A protein
33.094	32.631	Malate dehydrogenase (Q56JA2)
	32.494	Putative MVP protein (Q56J97)
	34.484	Putative paramyosin (Q56J94)
25.410	25.115	Peptidyl-prolylcis-trans isomerase
	25.537	GolgiSNAP receptor complex member2
	26.006	Proteasome subunit alpha type
	28.324	Putative heat shock 70KDa protein

DISCUSSION

Hydatidosis is an endemic parasitic disease of humans in Iran and many parts of the world [13]. The derivatives of Benzimidazole including ALB and MBZ are the drugs of choice for the medical treatment of Echinococcosis [14]. These drugs are used not only in inoperable cases, but also as an aid in surgeries. Both combinations have limited absorption in the human small intestine, while plasma concentration of ABZ is more than MBZ. They are also low risk to the host and do not enter the food chain which is the best advantage of these drugs [15]. Some *in vitro* studies and clinical trials indicated that the activity of ABZ against hydatid cyst was more than MBZ. However, both drugs cause a decrease in the size of hydatid cysts, and in some cases, there are reports of sterilization of cyst contents [16-17]. Once absorbed, ALB rapidly metabolized in the liver and Albendazole Sulfoxide, the agent with the anti-worm activity, is produced. This compound passes the cyst wall and is detectable in cyst fluid. Administration of ABZ for ≥six months in 55.3% of the human cystic echinococcosis patients led to improvement and in 27.6% to cure the patients, while 17.1% showed no change [18].

Additionally, some studies have shown that ABZ and MBZ block polymerization into microtubules and inhibits the cell proliferation and cause degenerative changes in the intestinal cells of the worm [19-20]. Also, ABZ inhibits ATP, pyruvate kinase, phosphoenolpyruvate kinase, acid phosphatase, and alanine transferase and causes a decrease in the glycogen content of the cyst wall. ABZ induces cellular lysis and degeneration in microthrics and microtubules, resulting in the death of protoscoleces [21]. The prevention, treatment, and control of this disease require understanding the mechanisms of pathogenesis, drug resistance and the physiology of parasites [22]. The present study aimed to determine the effects of ABZ on the ALP activity in the parasite as an indicator of drug effects on the parasite. Our results showed a significant increase in the level of ALP activity of protoscoleces in the presence of ABZ compared with untreated samples. The pattern of protein bands in SDS-PAGE was almost the same in extracts from the ABZ-treated and untreated parasites. However, the 59 kDa protein band in ABZ-treated samples had a higher intensity compared to the untreated sample. The recent study has shown, ALP specific activity in ABZ

treated protoscoleces estimated as 19.22 U/mg protein/ml, however, ALP specific activity of control group of E/S products was 27.85 U/mg protein/ ml [10]. The authors revealed that the assayed AST specific activities of treated sample was lower than untreated sample by mebendazole [23]. Our identified proteins could be useful in future studies for the development of diagnostic tools or a new drugs.

Our findings show that administration of ABZ medicine leads to an increase in ALP activity. The increase of enzyme activity may be considered as an indicator for evaluation of drugs efficacy on this parasite.

ACKNOWLEDGEMENT

We are grateful to the staff of Soleimani abattoir in Alborz Province for providing infected livers. Tehran University of Medical Sciences founded this work (Project No. 24688).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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