In Vitro Study of Mebendazole (Anthelmintic drug) Effects on the Aspartate Aminotransferase (AST) Enzyme Activity of Hydatid Cyst Parasite

Zahra Naderloo [1], Ali Farahnak [1,*], Abolfasl Golestani [2], Mohamad Reza Eshraghian [3], Mohamad Bagher Molaei Rad [1]

[1] Department of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran
[2] Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding author**:** email address: Farahnak@sina.tums.ac.ir (A.Farahnak)

ABSTRACT

Hydatid disease is caused by the larva of *Echinococcus granulosus* parasite. Mebendazole (MBZ) is used as an alternative choice for the treatment of the disease. Aspartate aminotransferase (AST) is an essential enzyme in amino acid metabolism. The aim of the present study is to evaluate the effect of MBZ on AST activity of hydatid cyst parasite in order to detect enzymatic parameter for drug efficiency. In the present study, AST activity was estimated in the extracts of untreated parasite (hydatid cyst protoscolices) and treated samples by MBZ (100 µg final concentration). Samples' protein quantity and quality were detected by Bradford and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) methods respectively. For the purpose of determining the significant difference between the two independent samples, t-test was conducted. The values of the assayed AST specific activities of treated and untreated parasite samples were measured as 0.18 and 1.53U/ml/mg protein respectively. The difference between AST activities mean values of the two groups proved to be significant ($P<0.05$). SDS-PAGE demonstrated protein band of 50 kDa for AST enzyme. Considering the effect of the MBZ drug on AST activity in parasite, it can be concluded that this enzyme is useful for improving the drug efficiency.

Keywords: Aspartate aminotransferase, Hydatid Cyst; Protoscolices; Mebendazole

INTRODUCTION

Hydatid cyst disease is a general name used to equate to infection with the parasite larva in humans. Echinococcosis should be limited to infection with the adult stage in carnivores [1]. As an alternative choice, treatment with mebendazole (MBZ) 40-50 mg/kg B.W. daily for several months has been shown to be highly effective on patients [2]. Enzymes are compulsory for survival, feeding migration and metabolism of parasites. Aspartate aminotransferase (AST) catalyzes the reversible transfer of a α-amino group between aspartate and glutamate and therefore is an essential enzyme in amino acid metabolism. AST and alanine transaminase (ALT) are associated with liver parenchyma cells. The difference is that ALT is found principally in the liver, with clinically insignificant amounts found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells [3]. AST is commonly measured clinically as an indicator for liver health. It is confirmed that the elevation of AST activity during the liver disease progression should not be automatically referred to as liver disease [4]. In the present study, the effect of MBZ on the AST activity of parasite is discussed.

MATERIALS AND METHODS

Parasite somatic extract solution preparation

Parasites (10 samples) were obtained from 10 infected liver tissues with hydatid cysts of sheep slaughtered at a local abattoir (Soleimani, Tehran,Iran). The parasites were washed 3 times and cultured in PBS media, pH 7.2 with 100 µg

final concentration of MBZ (Tolid Darou Co., Iran) or without the drug for 4-6 hours in an incubator (37°C). After precipitation and washing samples, parasites were freeze-thawed 3 times in liquid nitrogen and water bath at 37 °C and sonicated in a 150 W ultrasonic disintegrator with10 sec shock and 10 sec rest on ice until no intact parasite were visible microscopically. Then suspensions were centrifuged (10000 ×g for 30 min at 4°C) and supernatants were stored at -20°C [5].

Protein assay

The concentration of protein in the extract solutions of parasite were estimated by the method of Bradford using bovine serum albumin (1mg/ml) as the standard [5].

Aspartate aminotransferase activity assay

AST activities in samples were assayed using the enzyme assay kit (Ref. number 92002; Pars Azmoon Company). The kit uses L-aspartate and 2-oxoglutarate as substrates and produces L-glutamate and oxoglutarate which finally leas to production of $NAD^+$and L- malate; the changes in absorbance can be measured at 340 nm. To enzyme assay, 100 µl of sample was added to each cuvette containing 1ml of working solution at 37°C. Immediatly, absorbances (Abs) at 1, 2, 3 minutes were measured at 340 nm with a spectrophotometer. After subtracting Abs values, mean value was calculated and AST activity of the test samples and controls were estimated [6].

SDS-PAGE preparation of samples

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method with coomassie blue staining was used to separate and stain the protein components of parasite samples. Samples were mixed with sample buffer and run on 12.5% acrylamide gels. Molecular weights of differentiated bands of sample proteins were determined with respect to the protein marker; the ratio factor was calculated afterwards [5]. Proteins were identified primitively by database protein [7].

Statistical analysis results

Independent two sample T-test was provided to compare the mean values of enzyme activities between the treated and untreated samples. Statistical comparisons were carried out using statistical softwar(http://www.socscistatistic.com)[8]

Abbreviations

Mebendazole (MBZ); Aspartate transaminase (ASP); Absorbances (ABS); Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); Alanine transaminase (ALT); Kilo Dalton (kDa); Molecular weight (MW)

RESULTS

Protein amounts, enzyme activities and statistical analysis results

The mean values of protein concentrations and enzyme activities for treated parasite and untreated samples by MBZ are presented in Table 1. Significant difference in AST activities of the two groups were observed($p<0.05$)

Table 1. The mean values of protein amount and AST activity in parasite samples

Samples	Protein concentration (mg/ml)	AST total activity(U/ml)	AST specific activity(U/ml/mg protein)
Treated with MBZ	**0.06**	**0.01**	**0.18**
Untreated parasite	**0.07**	**0.13**	**1.53**

***** Treated samples by Mebendazole (MBZ)

SDS-PAGE analysis results

Extract samples of parasites were analyzed using SDS-PAGE; the results are shown in Figure 1. SDS-PAGE shows protein band with 50 kDa for AST of samples. Identified proteins are presented in Table 2. Protein bands of 75, 58, 42, 20 and 19 kDa were observed in control group and were not observed in the test group.

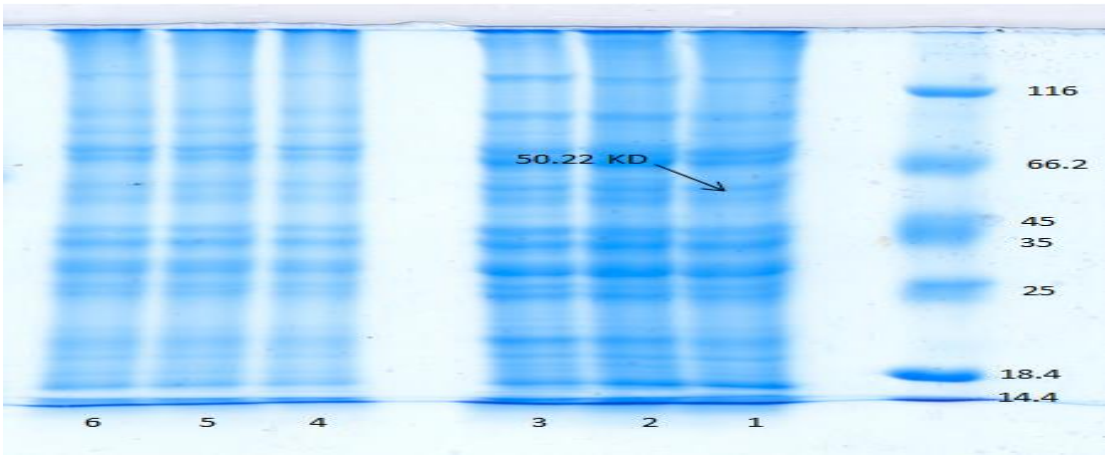

Figure 1 SDS- PAGE investigation of proteins from the extracts of parasite. First column is protein marker. Column 1-3 are treated parasite samples by MBZ and column 4-6 are untreated parasite samples.

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

MW in Gel	MW from Expasy database	Recognized proteins
126.610	126.748	Improtin 5
	126.892	Formin-like protein 3
104.341	104.334	DNA repair protein UVH 3
	104.694	Vacuolar protein sorting
83.340	83.600	Phosphatase and tensin
	83.742	Activated cdc 42 Kinase 1
	83.798	Transcriptional adapter 3
75.013	75.392	UDP-N-acetyl-D-galactosamin: popypeptide-acetylgalactosaminyl transferase
72.290	72.271	Phosphoenol pyruvate carboxylase
68.681	68.491	TGR : Thioredoxin glutathione reductase
	66.310	Serin /Threonine- protein phosphatase
58.733	57.017	Eg MKK 2
	59.187	NADH dehydrogenase subunit 5
	59.522	ALP (Alkaline phosphatase)
55.643	54.705	AMP activatedprotein kinase
	54.714	Phosphoenol pyruvate carboxylase
	54.849	α-amylase
	54.875	Ag 5 (serin-type endopeptidase activity)

50.226	**47.022** **50.235**	**(AST) Aspartate amino transferase** **AST:Aspartate amino transferase**
42.830	**42.151** **42.154** **43.557**	**P38-like protein** **ERK-like protein** **ERK-like protein**
40.003	**38.077** **37.961**	**EGMKK 1** **MKK1-like protein**
36.006	**35.437** **37.335**	**Smad A protein** **Smad C protein**
28.677	**27.778** **27.961** **28.358** **28.364** **28.396**	**14-3-3-protein homolog 2** **Eukaryotic translation factor 6** **Universal stress protein in QAH:OAS** **Proliferating cell nuclear antigen** **Proteasome sub unit alpha type**
25.886	**25.115** **25.537** **25.594** **26.006**	**Peptidyl- prolylcis-trans isomerase** **Golgi SNAP receptor complex member 2** **Peptidyl- prolylcis-trans isomerase** **Proteasome sub unit alpha type**
24.109	**23.881** **24.147** **24.226** **24.301**	**ZW 5** **COX 3** **Potative cysteine peptidase** **GST 2**
20.972	**20.908** **20.909**	**RasGTpase** **RasGtpase**
19.532	**19.046** **19.356** **19.677** **19.689** **19.691** **19.705** **19.733** **19.747**	**Calcineurine B (cal B)** **Calsium B like protein** **ZZKDa antigen 5** **ND1** **ND1** **ATPase sub unit 6** **ATP synthease F sub unit 6** **ND1**
18.557	**18.382** **18.991**	**Argenine N-methyl transferase 1** **Actin 1 (Act 1)**
16.990	**16.262**	**NADH dehydrogenase sub unit 1**
14.780 **14.042**	**14.218** **14.204**	**Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1**
13.303	**13.797** **13.823**	**Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1**
12.008	**11.951** **12.877** **12.861** **12.792** **12.438** **12.291**	**Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1** **Cytochrome C oxidase sub unit 1**

11.376	**10.585**	**Cytochrome C oxidase sub unit 1**
	10.641	**Cytochrome C oxidase sub unit 1**
	10.613	**Cytochrome C oxidase sub unit 1**
	11.058	**Cytochrome C oxidase sub unit 1**
	11.086	**Cytochrome C oxidase sub unit 1**
	11.114	**Cytochrome C oxidase sub unit 1**

DISCUSSION

Hydatidosis is an endemic parasitic disease which is known by its pathogen the *Echinococcus granulosus* larval parasite in Iran's human population and other parts of the world [9]. Numerous studies on the treatment methods of hydatid cyst have been done in the last few decades. Before 1961 and the discovery of the Benzimidazoles, the only treatment was surgery but a lot of patients who could not be operated on were victims of complications of the disease and even death. With discovery of Benzimidazoles, numerous studies were carried out on the chemotherapy of the Hydatid cyst. The first drug which was used was MBZ; yet, another medicine from this family named albendazole (ABZ) was used for treatment of distributed Hydatid cyst [10, 11]. Another medication for hydatid cyst is Praziquantel (PZQ) from Isoquinolins group. This drug has been mostly used in the treatment of adult gastrointestinal worms in dogs and canines but in recent years its therapeutic effects have been reported on the larval stage protosculex; however, it has had no significant inhibitory effect on the growth of cysts. Therefore, many studies on using ABZ alongside PZQ in vitro and animal models and human cases have been done which had promising results [12, 13].

This study aims to determine the effects of MBZ on the AST activity in the parasite which is important in the evaluation of the drug effects on parasite treatment.

As mentioned before, benzimidazole compounds (ABZand MBZ) are the basis for chemotherapy of hydatid cyst. Since 1977 which marks the introduction of Benzimidazole Carbamats, it has been proposed not only as the sole treatment method in inoperable cases, but as an aid in surgery. Since benzimidazoles are slightly soluble in water, their absorption in small intestine is limited (yet plasma concentrations of ABZ is more than MBZ) and they have low or even no risk of toxicity to the host and they do not enter the food chain which is the best advantage of these drugs [14]. When ABZ is absorbed in the intestine, it would rapidly be metabolized in the liver. In human and animals, its main metabolite is albendazole sulfoxide which is the cause of anti-worm activity of the medicine. This substance passes the cyst wall and can be detected in cyst fluid.

Treatment using the ABZ in 84 percent of the cases led to disappearance of the cyst and in more than 42 percent of the cases led to significant size reduction of the cyst [15]. According to above and similar pieces of evidence, the important point is the necessity of prevention, treatment, control and fighting against this disease that requires understanding the mechanisms of pathogenesis, drug resistance and the physiology of parasites [16]. According to review studies conducted in local databases, no documented research on comparison of the effects of the MBZ drug on the AST enzyme activity of parasite was found; therefore this study is the first in the country.The tests in this study indicated that there is a significant difference between average AST activity level with and without the presence of MBZ medicine so that average enzyme activity level in the samples with the presence of the

medicine is significantly lower than that of the control samples.

Also there are some differences between the protein bands in parasite with and without the presence of the medicine so that the following 5 bands (19, 20, 42, 58, 75 kDa) which were present in control samples are not formed in the test samples.

The similar and different protein bands in the parasite which were obtained from samples using SDS-PAGE method are very important in view of biochemical comparisons. With this method, 50 kDa protein bands were identified for the AST enzyme in parasitic samples [3].

The band intensity in test samples was weaker compared to controls, which confirms the activity reduction of the enzyme in test samples.

The identification of proteins obtained in the gel can be used in future studies. Enzyme activity in parasites of animal hydatid cysts due to the limited availability of human liver tissue can be used as a research model for studies of human cyst. Results obtained shows that the MBZ causes the decrease of AST activity. Decrease in enzyme is also evident in electrophoresis, leading to the decrease of colors in the bands which are formed in the test samples. The role of enzymes and enzyme systems in living organisms are quite impressive and clear and worms are associated with these enzymes; any deficiency or inhibition in them would prevent parasite life. Considering the effect of the MBZ on AST activity in parasite, it can be concluded that this enzyme is useful for treatment efficiency.

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