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Effect of mebendazole on free amino acid composition of cyst wall and cyst fluid of *Echinococcus granulosus* harbored in mice[1]

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ABSTRACT Eighteen and 23 FAA components were detected in the cyst wall and cyst fluid of *E granulosus*, respectively, by using automatic amino acid analyzer. The concentrations of most of the determined FAA were higher in the cyst fluid than those in the cyst wall, especially the taurine was 5-fold higher. Mebendazole treatment resulted in an increase in the concentration of alanine, valine, lysine, and taurine in both cyst wall and cyst fluid, the most notable being the alanine in the cyst wall. The results are interpreted as a coupling of glycolysis and amino acid metabolism, suggesting an involvement of FAA metabolism in the mechanism of Meb action.

KEY WORDS *Echinococcus*; cysts; amino acids; alanine; mebendazole

Our previous studies on the effect of mebendazole (Meb) on the glucose metabolism in the cyst wall of *Echinococcus granulosus* indicated that Meb exhibited an inhibition on the activity of pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK)[1], ATPase[2], and glucose transport[3]. Despite a lack of inhibition on lactate dehydrogenase of *E granulosus*, lactate content increased after Meb treatment[4]. Since *E granulosus* is glycophilic, it might utilize each glycogenic amino acid to derive glucose and pyruvate which could result in lactate production through lactate dehydrogenase. Based on this viewpoint, we intend to analyze the free amino acid (FAA) of *E granulosus*, which might help to gain an insight into the dynamic equilibrium between the FAA and glucose metabolism. This study was to obtain information on the biochemical differences in the FAA composition between the cyst wall and cyst fluid of *E granulosus* and on the action of Meb on them.

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MATERIALS AND METHODS

Parasite Protoscoleces of *E granulosus* were obtained from the sheep infected naturally with hydatid cysts in Xinjiang Uygur Autonomous Region. Cyst fluid containing protoscoleces was collected aseptically and stored at 4 ℃ after addition of penicillin and streptomycin 5×10^5 U·L^{-1} each, and amphotericin B 0.25 μg·ml^{-1}. The processing of the protoscoleces in the cyst fluid before inoculation was similar to that described previously[3].

Mice Kunming strain ♀ mice weighing 20±*s* 2 g ($n=38$) were inoculated ip with 2000 protoscoleces and fed on conventional rodent diet and water *ad lib.* At 11 months after infection, the treated groups of 5—7 mice each were given ig with Meb 25 mg·kg^{-1}·d^{-1}×14 d or 50 mg·kg^{-1}·d^{-1}×7 d. Seven untreated infected mice served as control.

Drug Meb was the product of Shanghai Institute of Pharmaceutical Industrial Research. A Meb suspension was prepared with 1 % tragacanth and used for intragastric gavage (ig).

Sampling At 24 h after the last medication, the mice were killed by bloodlerting. Intact cysts, turgid or collapsed, 3—8 mm in diameter were removed from the peritoneal cavity. The cyst fluid was obtained by needle aspiration. The cyst wall was weighed for preparing homogenate with normal saline. Sulphosalicylic acid (50 mg) was added into 1 ml of cyst fluid or cyst wall homogenate. The samples were kept at 4 ℃ for 1 h and then centrifuged at 3000 × *g* for 30 min. Its supernatant was stored at −20 ℃ before use. FAA in both cyst wall and cyst fluid was determined by using LKB 4151 automatic amino acid analyzer.

RESULTS

FAA composition in cyst wall and cyst fluid of the control group The cyst wall contained 18 FAA, whereas the cyst fluid, 23 FAA. Of the FAA, taurine constituted the major portion in the cyst wall, amounting to 16.7 % of the total FAA. The concentrations of threonine, serine, glutamine, glycine, alanine, citrulline, valine, methionine, leucine, and isoleucine in the cyst fluid were about 1—4 times higher than in the cyst wall (Tab 1, 2). Aspartic acid, asparagine, cystine, β-alanine, arginine, and α-aminobutyric acid were not detected in the cyst wall (Tab 1), while glutamic acid was absent in the cyst fluid. These results may reflect the fact that *E granulosus* is an entity rich in FAA and that the cyst fluid acts as both a reservoir for FAA and a discharge pool for Meb and metabolites.

FAA composition after Meb treatment Cyst wall: When infected mice were treated with Meb 25 mg·kg^{-1}·d^{-1} × 14 d, the concentrations of alanine, tyrosine, phenylalanine, and histidine increased 94.9 %, 46.7 %, and 48.1 %, respectively, whereas the concentrations of glutamine and citrulline decreased 40.1 % and 44.7 %, respectively (Tab 1).

Tab 1. Free amino acid contents (μmol·kg^{-1}) in cyst wall of *Echinococcus granulosus*. $n=7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control

	Control	After intragastric gavage of mebendazole		
		25 mg·kg^{-1}·d^{-1} × 14 d	50 mg·kg^{-1}·d^{-1} × 7 d	
	Full cyst	Full cyst	Full cyst	Collapsed cyst
Taurine	1 311±1 024	1 388±1 056^a	1 196±989^a	2 139±1 085^a
Threonine	272±25	370±198^a	328±107^a	437±65^b
Serine	366±105	275±96^a	372±100^a	488±107^a
Glutamic acid	358±191	524±233^a	419±154^a	965±385^b
Glutamine	684±151	410±290^b	455±189^b	619±284^a
Proline	472±387	492±128^a	552±123^a	459±530^a
Glycine	1 061±456	1 473±623^a	1 562±447^a	2 323±607^c
Alanine	955±232	1 862±639^c	1 839±500^c	2 796±441^c
Citrulline	141±38	78±48^b	151±35^a	181±75^a
Valine	497±129	511±188^a	666±126^b	909±193^c
Methionine	128±49	179±47^a	136±24^a	251±56^c
Leucine	388±61	324±101^a	361±78^a	456±94^a
Isoleucine	201±31	169±72^a	221±69^a	306±67^b
Tyrosine	152±51	223±56^b	165±12^a	284±84^b
Phenylalanine	92±21	133±35^b	107±7^a	153±56^a
Ornithine	172±40	166±96^a	138±23^a	193±83^a
Histidine	108±18	160±43^c	131±24^a	230±101^b
Lysine	469±87	648±199^b	610±68^c	892±217^c

Tab 2. Free amino acid contents (μmol·L^{-1}) in cyst fluid of *Echinococcus granulosus*. $n=7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

	Control	After intragastric gavage of mebendazole 25 mg·kg^{-1}·d^{-1} × 14 d	50 mg·kg^{-1}·d^{-1} × 7 d
Taurine	251±76	319±100^a	286±45^a
Aspartic acid	29±8	36±19^a	43±14^a
Threonine	571±325	717±378^a	776±220^a
Serine	1 042±327	531±315^b	953±180^a
Aspargine	211±61	141±139^a	187±78^a
Glutamine	1 904±1 008	1 031±595^a	1 587±406^a
Proline	338±114	258±95^a	356±230^a
Glycine	4 167±1 657	5 141±3 590^a	5 163±622^a
Alanine	3 985±926	5 417±3 074^a	5 491±946^c
Citrulline	418±147	212±130^b	551±171^a
α-Aminobutyric acid	30±13	41±33^a	42±13^a
Valine	1 691±612	1 591±282^a	2 207±505^a
Cystine	112±77	76±72^a	48±18^a
Methionine	377±121	369±237^a	478±114^a
Leucine	1 093±358	792±446^a	1 077±213^a
Isoleucine	703±265	450±216^b	758±172^a
Tyrosine	267±81	398±271^a	350±93^a
β-Alanine	75±59	114±101^a	92±86^a
Phenylalanine	111±52	163±105^a	124±60^a
Ornithine	104±50	176±127^a	127±62^a
Histidine	153±73	322±185^a	268±56^a
Arginine	64±58	—	—
Lysine	560±230	1 093±668^a	946±133^c

When the dose of Meb was increased to 50 mg·kg^{-1}·d^{-1} × 7 d, the concentration of alanine in the full and collapsed cyst wall increased 92.5 % and 192.8 %, respectively, whereas the concentration of glutamine decreased 33.5 %, being consistent with those observed in the cyst wall as compared to the 25 mg·kg^{-1} × d^{-1} group. The concentrations of glycine, lysine, valine, and taurine in the collapsed cyst wall also increased 118.9 %, 90.2 %, 82.9 %, and 63.2 %, respectively (Tab 1).

Cyst fluid: the concentrations of alanine and lysine in the 25 mg·kg^{-1}·d^{-1} × 14 d and 50 mg·kg^{-1}·d^{-1} × 7 d groups increased 35.9 %— 37.8 % and 68.9 %— 95.2 %, respectively (Tab 2). The alterations of the remaining FAA showed similar trend.

DISCUSSION

The present study has shown that both the cyst wall and cyst fluid of *E granulosus* in the FAA composition were similar except for the lack of glutamic acid in the cyst fluid and several minor amino acid components including β-alanine, α-aminobutyric acid, arginine, cystine, aspartic acid, and asparagine in the cyst wall. The results comply with those obtained by Hurd[5] in the cyst fluid from secondary equine cysts passaged in mice, and also provide an evidence that FAA play a role as structural components of the cyst wall, whereas the FAA in the cyst fluid result from an interaction of secretion, reabsorption, and transportation.

Noteworthy is the high concentration of

taurine in the cyst wall which is suited to an osmoregulatory function[6] essential for the preservation of osmolarity of the cyst fluid. In addition, this sulfur-containing amino acid possesses other functions such as cellular proliferation, membrane stabilization, calcium-flux modulation and neuronal excitability[7]. Thus, Meb induced increase in the collapsed cyst might be related to the damage of the normal function of the cyst wall.

Alanine in both the cyst wall and cyst fluid of *E granulosus* increased significantly after Meb treatment. As our previous papers showed that Meb inhibited pyruvate kinase of the cyst wall of *E granulosus*[1], hence pyruvate formation was reduced as a consequence of the inhibition. Thus, the increased alanine concentration in the cyst wall and cyst fluid could be considered as a compensation measure to counter the inhibition of the phosphoenolpyruvate interconversion in the glycolytic scheme for the reoxidation of NADH formed in the early process of glycolysis. It is reasonable to infer that FAAs, especially alanine, could be a preferential source of lactic acid production.

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甲苯达唑对小鼠细粒棘球蚴囊壁与囊液的游离氨基酸成分的影响[1]

R965.2

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摘要 感染11个月的小鼠细粒棘球蚴囊壁与囊液分别含有18种和23种游离氨基酸. 除牛磺酸外，囊液中的游离氨基酸含量均较囊壁的为高. 用甲苯达唑25 mg·kg^{-1}·d^{-1}×14 d 与50 mg·kg^{-1}·d^{-1}×7 d ig 治疗小鼠后，囊壁与囊液的丙氨酸、缬氨酸、赖氨酸及牛磺酸的含量均明显增高，以囊壁的丙氨酸的增高为最显著.

关键词 棘球属；囊；氨基酸；丙氨酸；甲苯达唑