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Effects of mebendazole, albendazole, and praziquantel on fumarate hydratase, pyruvate kinase, and phosphoenolpyruvate carboxykinase of *Echinococcus granulosus* cyst wall harbored in mice[1]

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ABSTRACT *Echinococcus granulosus* cyst wall exhibited activities of fumarate hydratase (FH), pyruvate kinase (PK), and phosphoenolpyruvate carboxykinase (PEPCK) with 911-1433, 151-215, and 54-98U, respectivity. The ratio of PK/PEPCK was 2.2—2.7, indicating that glycolysis is the main pathway of carbohydrate metabolism in the cyst wall. When infected mice were treated ig with mebendazole, albendazole or praziquantel at the respective daily dose of 25—50, 300, and 500 mg·kg^{-1} for 7—11 d, no apparent change of FH activity in the cyst wall was found, while PK and PEPCK activities in the cyst wall were markedly inhibited by mebendazole and albendazole. The inhibition rates of PK and PEPCK activities in mebendazole group were 85—88 % and 90—92 %, respectively, while in albendazole group were 55.3 and 71.6%, respectively. The results suggest that PK or PEPCK in the cyst wall may the important site attacked by effective antihydatid drugs.

KEY WORDS *Echinococcus*; mebendazole; albendazole; praziquantel; fumarate hydratase; phosphoenolpyruvate carboxykinases; pyruvate kinase

Since germinal layer of *Echinococcus granulosus* cysts is the only portion exhibiting life and proliferative activity, the aim of the chemotherapy is primarily to destroy this layer. Up to date no biochemical data concerning the carbohydrate metabolism of *E granulosus* cyst wall is available, although some relevant biochemical studies have been investigated with *E granulosus* protoscoleces[1]. We demonstrated that *E granulosus* cyst wall possessed malate dehydrogenase, fumarate reductase, and succinate dehydrogenase, which were related to a partial reversed tricarboxylic acid cycle[2], indicating that apart from glycolysis pathway, the parasite may fix CO_2 into the phosphoenolpyruvate (PEP) to form oxaloacetic acid for further metabolism. Thus, phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), a potential regulatory enzyme in glycolysis, and fumarate hydratase (FH), another enzyme occurred in partial reversed tricarboxylic acid cycle were determined and the effects of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on the 3 enzymes were also studied.

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MATERIALS AND METHODS

Parasites Cyst fluid containing protoscoleces of *E granulosus* was collected aseptically from hydatid cysts harbored in naturally infected sheep in Xinjiang. Having been added penicillin 500 IU·ml^{-1}, streptomycin 500 IU·ml^{-1}, and amphotericin B 0.25 μg·ml^{-1}, the cyst fluid was kept at 4 C. The processing of the protoscoleces in the cyst fluid before inoculation was similar to that described previously[3].

Mice NIH strain ♀ mice weighing 20 ± *s* 2 g were inoculated ip with 2000 protoscoleces. Mice were maintained on a rodent feed and water *ad lib*. Starting at 8—12 months after infection, the groups of 3—4 mice each were separately treated ig with Meb 25—50, Alb 300, or Pra 500 mg·kg^{-1}·d^{-1} for 7—14 d.

Drugs and reagents Meb, Alb, and Pra were the products of Shanghai Institute of Pharmaceutical Industrial Research, Hangzhou Pharmaceutical Factory, and Shanghai 6th Pharmaceutical Factory, respectively.

The suspensions of these 3 drugs were prepared with 1% tragacanth. PEP, sodium fumarate, and Commassie brilliant blue G-250 were the products of Sigma. Inosine diphosphate (IDP) and nicotinamide adenine dinucleotide-reduced (NADH, disodium salt with a purity of over 70%) were the products of Shanghai Dong Feng Biochemical Technique Company. Other reagents were all of AR grade.

Preparation of cyst wall homogenate At 24 h after the last medication, the mice were killed by blood letting. Ten collapsed cysts without fluid and 10 full cysts filled with fluid were harvested rapidly from the peritoneal cavity and placed in ice bath. After the endocysts were separated and the fluid was removed with filter paper, 400 mg of the cyst wall were homogenized in a glass homogenizer containing 2.0 ml Tris-HCl buffer (0.1 mol·L^{-1}, pH 7.4) in ice bath. The homogenate was centrifuged (22 000 × g, 20 min) at 4 ℃ and the supernatant was stored at 4 ℃ for use.

Fumarate hydratase measurement The activity of the enzyme which catalyzes the conversion of fumaric acid to malic acid was measured by the decrease of absorbance of fumaric acid at 300 nm[4]. The reaction system added into the testing tube was consisted of phosphate buffer (pH 7.4) 33 mmol·L^{-1} and sodium fumarate 17 mmol·L^{-1} with a volume of 3.0 ml. The reaction system without substrate was used as control. The testing tube was preincubated in water bath at 30 ℃ for 10 min and the absorbance of sodium fumarate at 300 nm was recorded. The cyst homogenate supernatant 0.2 ml was then added and the absorbance of sodium fumarate was measured again 10 min later. The value of difference between absorbances at 300 nm was calculated. One unit of enzyme activity represented consumption of 1 μmol fumaric acid per min per mg of protein, ie. $FH = \Delta OD_{300} \times 3.2/0.061 \times 10^{-3} \times$ mg protein × 10. In the formula, ΔOD_{300} was the decrease in absorbance at 300 nm 10 min after start of reaction; 3.2 was the total volume of reaction system (ml); 6.1×10^{-3} was the extinction coefficient of 1 μmol of fumaric acid; protein in the homogenate supernatant was expressed as mg and the reaction time was 10 min.

Pyruvate kinase measurement The enzyme activity was measured according to the consumption of NADH[1]. The testing tube containing substrate PEP, KCl, $MgSO_4$, and ATP with a volume of 3 ml was preincubated in water bath at 30 ℃. The control tube contained the same reaction system except PEP. Ten min after incubation the absorbance at 340 nm was recorded, and 0.2 ml cyst wall homogenate supernatant was then added. The absorbance at 340 nm was measured again 5 min later and the OD_{340} was calculated. One unit of enzyme activity represented consumption of 1 μmol NADH per min per mg of protein, ie. $PK = \Delta OD_{340} \times 3.2/6.2 \times 10^{-3} \times$ mg protein × 5. In the formula, ΔOD_{340} was the decrease in absorbance at 340 nm 5 min after start of reaction; 6.2×10^{-3} was the extinction coefficient of 1 μmol of NADH; 3.2 was the total volume of reaction system; protein in the homogenate supernatant was expressed as mg and the reaction time was 5 (min).

Phosphoenolpyruvate carboxykinase measurement The enzyme activity was also measured by the consumption of NADH method[1]. The testing tube containing the substrate PEP, KCl, $MgSO_4$, $NaHCO_3$, NADH, and IDP was incubated in water bath at 30 ℃ for 10 min. In order to eliminate the change of absorbance induced by the cyst wall homogenate supernatant itself, the reaction system without PEP was used as the corresponding control. The absorbance of the reaction system at 340 nm was recorded before start of reaction and 0.2 ml cyst wall homogenate supernatant was then added. The absorbance at 340 nm was measured again 10 min later and the OD_{340} was calculated. The expression of the enzyme activity was similar to that of PK activity, but the reaction time was replaced by 10 (min).

Protein measurement The protein in the cyst wall homogenate supernatant was assayed by the colorimetric method[5].

RESULTS

Fumarate hydratase When infected mice were treated ig with Meb 25 — 50 mg·kg^{-1}, Alb 300 mg·kg^{-1}, or Pra 500 mg·kg^{-1} daily for 7 — 14 d, no apparent change of the fumarase was found (Tab 1).

Pyruvate kinase In infected mice treated ig with Meb 25 mg·kg^{-1}·d^{-1} for 14 d, the PK activity of the cyst wall declined markedly with an inhibition rate of 85% (full cyst) or 86% (collapsed cyst) *vs* the control group,

Tab 1. Effects of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on fumarate hydroatase (FH), phosphoenolpyruvate carboxykinase (PEPCK), and pyruvate kinase (PK) activities in *Echinococcus granulosus* cyst wall harbored in mice. $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.01$ *vs* control. The figures in the parenthese are the inhibition rates (%).

Drug/ mg·kg^{-1}·d^{-1}	Cyst status	Cyst	FH activity, consumption 1 μmol fumaric acid·min^{-1}/ mg protein	PEPCK activity, consumption 1 μmol NADH·min^{-1}/mg protein	PK activity, consumption 1 μmol NADH·min^{-1}/mg protein
Control	Full	34	985±236 (—)	76±27 (—)	168±53 (—)
Meb 25×14	Full	20	868±189^{a}(12)	7±3^{b}(91)	25±15^{b}(85)
	Collapsed	16	894±164^{a}(9)	6±3^{b}(92)	23±18^{b}(86)
Control	Full	21	1 433±503^{a}(—)	98±29 (—)	215±106 (—)
Meb 50×7	Full	12	1 380±349^{a}(4)	9±5^{b}(91)	30±20^{b}(86)
	Collapsed	17	1 245±192^{a}(13)	10±4^{b}(90)	26±12^{b}(88)
Control	Full	22	1 102±200 (—)	88±29 (—)	215±48 (—)
Alb 300×14	Full	32	1 055±276^{a}(—)	25±17^{b}(72)	96±64^{b}(55)
Control	Full	11	911±165 (—)	54±11 (—)	151±58 (—)
Pra 500×14	Full	25	1 086±196^{a}(—)	54±13^{a}(—)	157±53^{a}(—)

and with 50 mg·kg^{-1}·d^{-1} for 7 d, the similar results were obtained. When infected mice were treated ig with Alb 300 mg·kg^{-1}·d^{-1} for 14 d, the PK activity of the cyst wall also declined markedly with an inhibition rate of 55% which was less than that of Meb group. No apparent effect of Pra on the PK activity of the cyst wall was noted when Pra was given ig to the infected mice at a daily dose of 500 mg·kg^{-1} for 14 d (Tab 1).

Phosphoenolpyruvate carboxykinase

When infected mice were treated ig with Meb 25—50 mg·kg^{-1}·d^{-1} for 7—14 d, the PEPCK activity of the cyst wall declined markedly with inhibition rates of 90.9—91.0% (full cyst) and 90—92 % (collapsed cyst) *vs* the corresponding control. Alb also showed inhibitory effect on PEPCK activity of the cyst wall when it was used ig at a daily dose of 300 mg·kg^{-1} for 14 d. However, the inhibition rate of Alb was less than that of Meb group, being 72 %. In infected mice treated ig with Pra 500 mg·kg^{-1}·d^{-1} for 14 d, the PEPCK activity was similar to that of the control group (Tab 1).

DISCUSSION

Our experimental results indicate that *E granulosus* cyst wall possesses the activity of PK, and PEPCK, and all the enzymes involved in a partial reversed tricarboxylic acid cycle, such as malate dehydrogenase, fumarate reductase, succinate dehydrogenase[3] and FH were detected in the cyst wall, indicating that the parasite could utilize fermentative pathways for alternative energy production, fix CO_2 via PEPCK and have a partial reversed tricarboxylic acid cycle. Since the PK/PEPCK ratio was 2.2—2.7, it is suggested that in the parasite glycolysis may play a more important role in carbohydrate metabolism and energy production[6].

Meb, Alb, and Pra exhibit no apparent effect on FH activity of *E granulosus* cyst wall harbored in infected mice. Meb exerts a potential effect on PEPCK activity in both full

and collapsed cysts, and the inhibitory rate was about 90% or so. Alb, which exhibits less therapeutic efficacy than Meb, also showed less inhibitory effect on the PEPCK activity than Meb. Similar tendency in inhibitory effect on PK activity induced by Meb and Alb is alos seen. Since Pra has no apparent effect on the two enzymes, and the order of efficacy on infected mice produced by Meb and Alb is in accordance with that of inhibitory effect on PK and PEPCK activities, it seems that these two enzymes as well as ATPase[17] of the cyst wall are the major links in fermentative pathways of carbohdrate metabolism and may serve as the targets for antihydatid-drug attack.

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甲苯达唑、阿苯达唑和吡喹酮对小鼠细粒棘球蚴囊壁延胡索酸酶、磷酸烯醇丙酮酸羧激酶和丙酮酸激酶的影响

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摘要 细粒棘球蚴囊壁的延胡索酸酶(FH)活力为911—1433 U, 磷酸烯醇式丙酮酸羧激酶(PEPCK)与丙酮酸激酶(PK)的活力之比为2.2—2.7, 表明囊壁的糖代谢以酵解途径为主. 感染小鼠用甲苯达唑、阿苯达唑或吡喹酮 ig 治疗, 剂量各为 25 — 50, 300 和 500 mg·kg^{-1}·d^{-1}, 连给7—14 d, 未见对 FH 有明显的影响, 而 PK 和 PEPCK 则可明显被前二种药物所抑制.

关键词 棘球属; 甲苯达唑; 阿苯达唑; 吡喹酮; 延胡索酸酶; 磷酸烯醇丙酮酸羧激酶; 丙酮酸激酶