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Effects of mebendazole, albendazole, and praziquantel on alkaline phosphatase, acid phosphatase, and adenosine triphosphatase of *Echinococcus granulosus* cysts harbored in mice[1]

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ABSTRACT Mice infected with protoscoleces of *Echinococcus granulosus* for 12-14 months were treated ig with mebendazole (Meb) 25-50 mg · kg^{-1} · d^{-1} for 7-14 d, albendazole (Alb) 200 mg · kg^{-1} · d^{-1}, or praziquantel (Pra) 500 mg · kg^{-1} · d^{-1} for 14 d. The mice were killed 24 h after the last medication, and acid phosphatase (ACP), alkaline phosphatase (AKP), and adenosine triphosphatase (ATPase) including (Na, K, Mg)-ATPase, (Na, K)-ATPase, and (Mg)-ATPase were determined and compared with those of untreated control group. The results showed that ACP activities of cyst wall in treated groups were lower than the control group. Whereas AKP activity of cyst wall in Pra group increased markedly, this is not the case in Meb and Alb groups. Three ATPase activities of cyst wall were inhibited in both Meb and Alb groups, Meb being more potent. No apparent changes in the ATPase activities were seen in Pra group.

KEY WORDS *Echinococcus*; mebendazole; albendazole; praziquantel; acid phosphatase; alkaline phosphatase; adenosine triphosphatase

Despite many studies made on the effect of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on metacestodes of *Echinococcus granulosus*, the action mechanism of these drugs remains unclear. Our previous studies demonstrated that the glycogen contents in the cyst walls decreased markedly when mice infected with metacestodes of *E granulosus* were treated with ig Meb and Alb[1]. Further investigations confirmed that Meb mainly inhibited exogenous glucose uptake[2], but had no apparent effect on glycolysis of the cyst[3]. Thus we determine alkaline phosphatase (AKP), acid phosphatase (ACP), and adenosine triphosphatase (ATPase) activities in cyst walls, which may be relevant to glucose transport. We also observed the effects of Meb, Alb, and Pra on these enzyme activities to reveal possible action mechanism.

MATERIALS AND METHODS

Parasites The cyst fluid containing protoscoleces was aspirated under aseptic condition from *E granulosus* cysts in naturally infected sheep in Xinjiang. Penicillin 500 IU · ml^{-1}, streptomycin 500 IU · ml^{-1}, and amphotericin 0.25 μg · ml^{-1} were added to the cyst fluid stored at 4℃. Before use, the protoscoleces were washed 5-8 times with Hanks' balanced salt solution (HBSS) and the parasites with a mean viability over 95%, as judged by parasitic activity and methylene blue stain, were used for inoculation.

Mice 190 NIH mice, ♀, weighing 20 ± *s* 2 g were inoculated ip with 2000 protoscoleces. After 12-14 months, the mice were divided randomly into 40 groups, each containing 4-5 mice, and treated ig with Meb 25-50 mg · kg^{-1} · d^{-1}, Alb 200 mg · kg^{-1} · d^{-1}, or Pra 500 mg · kg^{-1} · d^{-1} for 7-14 d, and untreated as control.

Preparation of cyst wall homogenate At 24 h after last medication of each drug treatment course,

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mice were killed by bleeding. Ten collapsed cysts without cyst fluid and 10 cysts full of fluid were harvested from the peritoneal cavity. The endocyst was then separated and cut with scissors after the cyst fluid was removed with filter paper. About 200 mg of the cyst wall were placed in a glass homogenizer containing 2 ml Tris–HCl buffer 50 mmol · L^{-1} at pH 7.4 and homogenized in ice bath. After centrifugation (100 $g \times 3$ min) at 4℃, the supernatant was kept for use.

Drugs and reagents Meb, Alb, and Pra provided by Shanghai Institute of Pharmaceutic Industry, Hangzhou Pharmaceutic Factory, and the Sixth Pharmaceutic Factory of Shanghai, respectively. *p*–Nitrophenyl phosphate disodium (PNPP), polyvinyl alcohol (PVA), ouabain, and adenosine triphosphate (ATP) were purchased from Sigma Chemical Co. *p*–Nitrophenol, malachite green, and other reagents were of AR grade.

AKP and ACP measurements AKP and ACP activities were determined according to the hydrolysis of *p*–nitrophenylphosphate by the enzymes and color reaction of the hydrolysate, *p*–nitrophenol in alkaline condition[4]. Some modifications of the method has been made, i.e. Alkaline or acid buffered substrate solution was prepared by mixing stock substrate solution (0.4 g disodium *p*–nitrophenylphosphate dissolved in 100 ml of HCl 1 mmol · L^{-1} 1 : 3 (vol / vol) with glycin–NaOH buffer 0.05 mol · L^{-1} at pH 10.0, or citric–NaOH 0.05 mol · L^{-1} at pH 4.8, respectively. The tubes containing 1.0 ml alkaline or acid buffered substrate solution were preincubated at 37℃ for 10 min and 400 μl or 200 μl of tissue homogenate was added, respectively. One hour after addition of tissues homogenate, NaOH 0.1 mol · L^{-1} 4 ml was added to each tube to cease the reaction. AKP and / or ACP activities in cyst wall tissue homogenate were expressed as nmol *p*–nitrophenol produced / (mg protein · h).

ATPase mesurement ATPase including (Na, K, Mg)–ATPase, (Na, K)–ATPase, and (Mg)–ATPase were assayed by a modified malachite green phosphate method[5,6]. ATPase activity of cyst tissue was expressed as μmol P_i produced · h^{-1} / mg protein.

Protein measurement Protein in the homogenate of cyst wall was determined by colorimetric method[7], using crystalline bovine serum albumin as the standard.

Statistics The *t* test was used.

RESULTS

AKP In *E granulosus* infected mice treated with ig Meb 25 or 50 mg · kg^{-1} · d^{-1} for 7–14 d, the AKP activities of the cyst walls of collapsed or full cysts were 2.0 ± 0.8–3.2 ± 1.8 (expressed by μmol P_i produced · h^{-1} / mg protein), which seemed to be slightly higher than those 2.0 ± 0.8–2.3 ± 1.9 of control group with an increase of 4.8–39.1% ($P > 0.05$). In mice treated with ig Alb 200 mg · kg^{-1} · d^{-1} or Pra 500 mg · kg^{-1} · d^{-1} for 14 d, most cysts were still filled with fluid. The increase in AKP activities in cyst walls induced by Alb was similar to that induced by Meb. However, in Pra group the AKP activity of 4.0 ± 3.7 was significantly higher than that of 2.3 ± 1.9 in the control group (Tab 1).

ACP In mice treated with ig Meb 25 mg · kg^{-1} · d^{-1} for 7 d, the ACP activities of the cyst wall of the collapsed cyst showed an inhibition rate of 21.9% ($P < 0.01$). When the treatment course was prolonged to 14 d, the ACP activities of the cyst walls of both collapsed and full cysts were significantly lowered than those of control, with inhibition rates of 22.4–34.3% ($P < 0.01$). Similar results were obtained from the group treated ig with Meb 50 mg · kg^{-1} · d^{-1} for 7 d. After the infected mice were treated with ig Alb 200 mg · kg^{-1} · d^{-1} or Pra 500 mg · kg^{-1} · d^{-1} for 14 d, the ACP activities of the cyst walls decreased by 30.5% and 29.8% ($P < 0.01$), respectively (Tab 2).

ATPase (Na,K,Mg)–ATPase, (Na,K)–ATPase, and (Mg)–ATPase activities of cyst walls of both collapsed and full cysts harbored in mice treated with ig Meb 25

Tab 1. Effects of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on alkaline phosphatase (AKP) activity (nmol *p*-nitrophenol produced · h^{-1} / mg protein) in *Echinococcus granulosus* cysts harbored in mice. $\bar{x} \pm s$. $^{*}P > 0.05$, $^{***}P < 0.01$ *vs* control.

Drug / $mg \cdot kg^{-1} \cdot d^{-1} \times d$	Cysts tested	Status of cyst	AKP activity	Increase %
Control	26	Full	2.3 ± 1.9	–
Meb 25×7	30	Collapsed	$2.5 \pm 2.2^{*}$	8.7
	19	Full	$3.2 \pm 1.8^{*}$	39.1
	49	Combined	$2.8 \pm 2.0^{*}$	21.7
Control	29	Full	2.1 ± 0.8	–
Meb 25×14	30	Collapsed	$2.2 \pm 1.3^{*}$	4.8
	22	Full	$2.3 \pm 0.7^{*}$	9.5
	52	Combined	$2.3 \pm 1.0^{*}$	9.5
Control	30	Full	2.0 ± 0.8	–
Meb 50×7	23	Collapsed	$2.0 \pm 0.8^{*}$	–
	14	Full	$2.6 \pm 2.0^{*}$	30.0
	37	Combined	$2.2 \pm 1.4^{*}$	10.0
Control	39	Full	2.3 ± 1.9	–
Alb 200×14	36	Full	$2.5 \pm 1.9^{*}$	8.7
Pra 500×14	28	Full	$4.0 \pm 3.7^{***}$	73.9

Tab 2. Effects of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on acid phosphatase (ACP) activity (nmol *p*-nitrophenol produced · h^{-1} / mg protein) in *Echinococcus granulosus* cysts harbored in mice. $\bar{x} \pm s$. $^{*}P > 0.05$, $^{***}P < 0.01$ *vs* control.

Drug / $mg \cdot kg^{-1} \cdot d^{-1} \times d$	Cysts tested	Status of cyst	ACP activity	Reduction %
Control	29	Full	13.7 ± 4.8	–
Meb 25×7	30	Collapsed	$10.7 \pm 4.5^{***}$	21.9
	24	Full	$11.7 \pm 4.5^{*}$	14.6
	54	Combined	$11.0 \pm 4.7^{***}$	19.7
Control	30	Full	13.4 ± 4.0	–
Meb 25×14	23	Collapsed	$8.8 \pm 2.6^{***}$	34.3
	16	Full	$10.4 \pm 2.0^{***}$	22.4
	39	Combined	$9.4 \pm 2.5^{***}$	29.9
Control	30	Full	11.7 ± 2.7	–
Meb 50×7	15	Collapsed	$8.4 \pm 1.8^{***}$	28.2
	20	Full	$9.0 \pm 3.1^{***}$	23.1
	35	Combined	$8.7 \pm 2.6^{***}$	25.6
Control	28	Full	13.1 ± 4.3	–
Alb 200×14	40	Full	$9.1 \pm 4.3^{***}$	30.5
Pra 500×14	28	Full	$9.2 \pm 3.5^{***}$	29.8

ng · kg^{-1} · d^{-1} for 7 d were similar to those of the control. When the same dosage was given for 14 d, the ATPase activities of the cyst walls of collapsed and full cysts were inhibited by 40–60% ($P < 0.01$). In mice treated g with Meb 50 mg · kg^{-1} · d^{-1} for 7 d, activities of the 3 ATPase were also inhibited. Meb caused inhibition rates of 60–80% for the Mg)–ATPase, while 46.2–61.5% for (Na, K, Mg)–ATPase and 25.0–50.0% for (Na, K)–ATPase. When mice were treated with ig Alb 200 mg · kg^{-1} · d^{-1} for 14 d, the 3 ATPase activities were apparently lower than those of control, but higher than those of Meb 50 mg · kg^{-1} · d^{-1} group except for Na, K)–ATPase activity. After mice were reated with ig Pra 500 mg · kg^{-1} · d^{-1} for 14 the 3 ATPase activities were inhibited ightly ($P > 0.05$) (Tab 3).

DISCUSSION

Our previous study showed that Meb could inhibit the intake of exogenous glucose of *E granulosus*[2]. Therefore, three kinds of enzyme related to the transport to glucose[8,9], ie. AKP, ACP, and ATPase have been determined. It has been proved that Meb, Alb, and Pra exhibited different therapeutic efficacies on cysts of *E granulosus* harbored in mice, ie. Meb > Alb > Pra[10]. In the present study, these 3 drugs showed similar inhibitory effects on ACP activities of the cyst walls, but not on AKP activities, ie. Pra could stimulate markedly the enzyme activity whereas Meb or Alb could not. The inconsistency between the therapeutic efficacies and effects on AKP and ACP activities induced by the 3 drugs suggested that AKP and

Tab 3. Effects of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on ATPase activities (μmol P_i produced · h^{-1} / mg protein) in *Echinococcus granulosus* cysts harbored in mice. $\bar{x} \pm s$. $^{*}P > 0.05$, $^{***}P < 0.01$ vs control.

Drug / mg · kg^{-1} · d^{-1} × d	Cysts tested	Status of cysts	(Na,K,Mg)–ATPase activity	Inhibition / %	(Na,K)–ATPase activity	Inhibition / %	(Mg)–ATPase activity	Inhibition / %
Control	53	Full	0.13 ± 0.06	–	0.08 ± 0.05	–	0.05 ± 0.05	–
Meb 25 × 7	20	Collapsed	0.13 ± 0.05*	–	0.06 ± 0.42*	25.0	0.07 ± 0 04*	–
	16	Full	0.12 ± 0.05*	7.7	0.07 ± 0.59*	12.5	0.05 ± 0.04*	–
	36	Combined	0.12 ± 0.05*	7.7	0.06 ± 0.05*	25.0	0.06 ± 0.04*	–
Meb 25 × 14	20	Collapsed	0.07 ± 0.04***	46.2	0.05 ± 0.03***	37.5	0.02 ± 0.02***	60.0
	17	Full	0.07 ± 0.04***	46.2	0.05 ± 0.03***	37.5	0.03 ± 0.02***	40.0
	37	Combined	0.07 ± 0.04***	46.2	0.05 ± 0.03***	37.5	0.03 ± 0.02***	40.0
Meb 50 × 7	23	Collapsed	0.05 ± 0.03***	61.5	0.04 ± 0.02***	50.0	0.02 ± 0.01***	60.0
	17	Full	0.07 ± 0.04***	46.2	0.06 ± 0.04*	25.0	0.01 ± 0.01***	80.0
	40	Combined	0.06 ± 0.03***	53.8	0.05 ± 0.03***	37.5	0.01 ± 0.01***	80.0
Control	39	Full	0.13 ± 0.06	–	0.10 ± 0.06	–	0.04 ± 0.03	–
Alb 200 × 14	40	Full	0.08 ± 0.05***	38.5	0.05 ± 0.05***	50.0	0.02 ± 0.02***	50.0
Pra 500 × 14	28	Full	0.11 ± 0.06*	15.4	0.09 ± 0.05*	10.0	0.04 ± 0.02*	–

ATPase activities were expressed as μmol · P_i produced · h^{-1} / mg protein.

ACP of the cyst wall might not be the targets attacked by the 3 drugs.

By contrast, the activities of 3 ATPase of the *E granulosus* cyst wall were inhibited significantly by Meb, and the inhibitory action exhibited earlier and more apparently when a higher dosage of Meb was given. However, in mice treated with ig Meb 50 mg · kg^{-1} · d^{-1} for 7 d, the (Na, K)–ATPase activity of cyst wall of full cysts was inhibited to a lesser extent, suggesting that the (Na, K)–ATPase was less affected among the ATPases by Meb. With lower efficacy than Meb, Alb could also inhibit these 3 ATPase activities, but to a lesser degree than Meb. Pra with less therapeutic efficacy against the metacestodes showed no apparent effect on these 3 ATPase activities. The consistency between the therapeutic efficacies and effects on these 3 ATPase activities suggested that ATPase, at least one of the three ATPases above–mentioned, might be vulnerable target for anti–hydatid drugs. Interestingly, in addition to the functions in the intake and transport of glucose as well as carbohydrate metabolism, ATPases may play an important role in osmoregulation and salt homeostasis[9], which may account for the appearance of many collapsed cysts in the peritoneal cavity of infected mice after Meb treatment. Further studies on the effect of Meb on these 3 ATPases as well as (Ca)–ATPase and other ATPases in *E granulosus* cysts might elucidate the mechanism of anti–hydatid drugs and identify a vulnerable chemotherapeutic target enabling the rational design of novel anti–hydatid drugs.

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甲苯达唑、阿苯达唑和吡喹酮对小鼠细粒棘球蚴碱性磷酸酶、酸性磷酸酶和 ATP 酶的影响

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提要 感染细粒棘球绦虫原头节 12–14 个月的小鼠 ig 甲苯达唑(Meb)，阿苯达唑(Alb)或吡喹酮(Pra)各 25–50，300 或 500 mg · kg^{-1} × 7–14 d 时，各组囊壁 ACP 活性均明显降低；Pra 组囊壁 AKP 活性明显升高，Meb 和 Alb 组的则否；Meb 和 Alb 明显抑制囊壁 3 种 ATPase 活性，但后者逊于前者，Pra 对 ATPase 则无作用.

关键词 棘球属；甲苯达唑；阿苯达唑；吡喹酮；酸性磷酸酶类；碱性磷酸酶类；腺苷三磷酸酶类

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物升降压作用的时反应量–效关系分析[1]

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ned dose–response relationship analysis of pressor hypotensive action of drugs[1]

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ABSTRACT The graded and timed dose–response relationship (TDRR) of pressor and hypotensive action by iv norepinephrine (NE) and sodium nitroprusside (SNP) respectively were studied in 13 ♂ rabbits. The arterial blood pressure was dose–dependently raised by NE 0.98 – 125 $\mu g \cdot kg^{-1}$ and lowered by SNP 7.81 – 500 $\mu g \cdot kg^{-1}$ (F test, $P<0.01$). The comparisons between dose groups showed that the latency and duration of both NE and SNP action were dose–dependent ($P<0.01$),