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Effect of mebendazole and praziquantel on glucosephosphate isomerase and glyceraldehydephosphate dehydrogenase in *Echinococcus granulosus* cyst wall harbored in mice

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KEY WORDS *Echinococcus*; glucosephosphate isomerase; glyceraldehydephosphate dehydrogenase, mebendazole; praziquantel

AIM: To study effects of antihydatid drugs on glucosephosphate isomerase (GPI) and glyceraldehydephosphate dehydrogenase (GAPDH) in *Echinococcus granulosus* cyst wall. **METHODS**: Mice infected with the parasite for 8 - 10 months were treated ig with mebendazole (Meb) or praziquantel (Pra). The activities of GPI and GAPDH in the cysts were measured by the formation of NADH or NADPH. **RESULTS**: GPI activity in the cyst wall was 197 ± 103 U, while that of GAPDH was 25 ± 13 U. When infected mice were treated ig with Meb 25 - 50 mg·kg^{-1}·d^{-1} for 7 - 14 d, no apparent effect on the GAPDH activity in the cyst was found. In mice treated ig with praziquantel (Pra) 500 mg·kg^{-1}·d^{-1} for 14 d, the GAPDH activity in the cyst wall was inhibited by 26.5 %. As to GPI activity only the group treated ig with Meb 25 mg·kg^{-1}·d^{-1} for 14 d showed 33.2 % inhibition of the enzyme in the collapsed cyst wall. **CONCLUSION**: GPI and GAPDH are not the major targets attacked by the antihydatid drug.

Our previous paper showed that in *Echinococcus granulosus* cysts the major pathways for glucose metabolism is glycolysis and that mebendazole (Meb) and albendazole exhibited marked inhibition of pyruvate kinase of the cyst wall, while praziquantel (Pra) had no effect, indicating the coincidence with the *in vitro* efficacy of the 3 drugs in treatment of infected mice[1]. In this study glyceraldehydephosphate dehydrogenase (GAPDH) and glucosephosphate isomerase (GPI) were determined.

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MATERIALS AND METHODS

Parasite Cyst fluid containing protoscoleces collected aseptically from sheep naturally infected with *E granulosus* was added benzylpenicillin 5 × 10^5 IU·L^{-1}, streptomycin 5 × 10^5 IU·L^{-1}, and amphotericin 0.25 mg·L^{-1}, and kept at 4 ℃. Before inoculation, the protoscoleces were washed with Hanks' balanced salt solution (HBSS) for 5 - 8 times.

Mice Kunming strain ♀ mice, weighing 20 ± *s* 2 g were each inoculated ip with 2000 protoscoleces. After 8 - 10 months groups of 3 - 5 mice were treated by intragastric gavage (ig) with Meb 25 - 50 mg·kg^{-1}·d^{-1} × 7 - 14 d, or Pra 500 mg·kg^{-1}·d^{-1} × 14 d.

Drugs and reagents Meb and Pra were the products of Shanghai Institute of Pharmaceutical Industry and Shanghai 6th pharmaceutical Factory, respectively. Meb and Pra were suspended in 1 % tragacanth at respective concentrations of 2.5 - 5 g·L^{-1} and 50 g·L^{-1}. Substrates for measurement of GAPDH and GPI activities, glyceraldehydephosphate (GAP) and fructose-6-phosphate were the products of Sigma. Coenzymes NAD (purity >90 %) and NADP were the products of Shanghai Dongfeng Biochemical Technique Co and Sigma Co, respectively. Other reagents were AR.

Preparation of cyst wall homogenate At 24 h after the last medication, collapsed cysts without fluid and full cysts filled with fluid were collected rapidly from the peritoneal cavity and placed in ice bath. After the endocysts were separated and the fluid was removed with filter paper, about 200 mg of the cyst wall were homogenized in 1.0 mL distilled water in ice bath. After centrifugation (1000 × *g*, 4 ℃, 1 h), the supernatant was stored at 4 ℃ for use.

Measurement of GAPDH The tube containing NAD and cyst wall homogenate was preincubated at 25 ℃ for 5 min and the absorbance at 340 nm was recorded. The substrate GAP was added and the enzyme activity was assayed by the formation of NADH[2].

Measurement of GPI The tube containing the substrate F6P and NADP was preincubated at 30 ℃ for 5 min and the cyst homogenate 0.2 mL was added. The absorbance at 340 nm was recorded in 10 s after addition of the homogenate. The enzyme activity was measured by the formation of

NADPH[12]

The protein in the supernatant of the cyst homogenate was measured colorimetrically[13].

Statistics The t test was used.

RESULTS

The GAPDH activity in the cyst wall was 16.6 – 33.8 U with an average of 25 ± 13 U (n = 106), while GPI activity was 142 – 295 U with an average of 197 ± 103 U (n = 105).

When infected mice were treated ig with Meb 25 mg·kg^{-1}·d^{-1} for 7 – 14 d, the GAPDH in both full and collapsed cyst walls were similar to that of the corresponding control. In mice treated ig with Meb 50 mg·kg^{-1}·d^{-1} for 7 d, the GAPDH activity was slightly increased ($P > 0.05$ *vs* the control). When Pra 500 mg·kg^{-1}·d^{-1}, 14 d was given to the infected mice, the GAPDH activity in the cyst wall was reduced *vs* the control (Tab 1).

Only the mice treated with Meb 25 mg·kg^{-1}·d^{-1}, 14 d showed an inhibition of GPI activity in the collapsed cyst wall (Tab 1).

DISCUSSION

Although there have been studied on glycolysis of *E granulosus* protoscoleces[4], no studies have been carried out on the glycolytic enzymes of *E granulosus* cyst wall. Although the presence of both GPI and GAPDH was confirmed in the protoscoces of *E granulosus* and in the cyst wall, they differ in their activities, suggesting that there are differences in function of the glycolytic pathway between the cyst wall and protoscoleces of *E granulosus*.

Our results showed that in the cyst wall of *E granulosus* higher enzyme activity was seen in GPI and less in GAPDH. Only the enzyme activity in collapsed cyst wall of Meb 25 mg·kg^{-1}·d^{-1}, 14 d group showed significant inhibition. Besides, Meb exhibited no apparent effect on GAPDH in the cyst wall, while Pra appeared to be effective on GAPDH, but which was not consistent with the efficacy of Pra. Therefore, it is seemingly probable that the GPI and GAPDH of the cyst wall of *E granulosus* might not be the target enzymes attacked by effective antihydatid drug.

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Tab 1. Effects of mebendazole (Meb) and praziquantel (Pra) on glyceraldehydephosphate dehydrogenase (GAPDH) and glucosephosphate isomerase (GPI) in cyst wall of *Echinococcus granulosus* harbored in mice.
Number of samples in parentheses. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Drug/ mg·kg^{-1}·d^{-1} · d	Cyst	GAPDH activity: Formation of NADH 1 μmol·min^{-1} /mg protein	GAPDH activity: Inhibition /%	GPI activity: Formation of NADPH 1 μmol·min^{-1} /mg protein	GPI activity: Inhibition /%
Control	Full	28 ± 15 (27)	–	169 ± 64 (27)	–
Meb 25 × 7	Full	24 ± 13 (27)^a	14.3	208 ± 100 (26)^a	–
	Collapsed	22 ± 9 (11)^a	21.4	151 ± 60 (11)^a	10.7
Control	Full	17 ± 7 (33)	–	142 ± 55 (33)	–
Meb 25 × 14	Full	19 ± 9 (33)^a	–	152 ± 73 (31)^a	–
	Collapsed	16 ± 7 (27)^a	5.9	109 ± 43 (29)^b	33.2
Control	Full	23 ± 9 (20)	–	200 ± 55 (20)	–
Meb 50 × 7	Full	29 ± 11 (20)^a	–	210 ± 70 (19)^a	–
	Collapsed	28 ± 10 (20)^a	–	225 ± 83 (20)^a	–
Control	Full	34 ± 14 926)	–	295 ± 133 (25)	–
Pra 500 × 14	Full	25 ± 10 (34)^b	26.5	304 ± 137 (32)^a	–

甲苯达唑 吡喹酮 细粒棘球蚴 GPI GAPDH

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甲苯达唑和吡喹酮对小鼠细粒棘球蚴囊壁的葡萄糖磷酸异构酶和甘油醛磷酸脱氢酶的影响

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关键词 棘球属；葡萄糖磷酸异构酶；甘油醛磷酸脱氢酶；甲苯达唑；吡喹酮

目的：测定抗包虫药物对细粒棘球蚴囊壁的葡萄糖磷酸异构酶（GPI）和甘油醛磷酸脱氢酶（GAPDH）的影响。方法：感染细粒棘球蚴囊达 8 - 10 个月的小鼠 ig 甲苯达唑（Meb）或吡喹酮（Pra）治疗，然后剖杀取囊，用生化方法测定 GPI 和 GAPDH 活力。结果：感染小鼠用 Meb 25 - 50 mg·kg^{-1}·d^{-1}治疗，连续 7 - 14 d，未见对 GAPDH 活力有明显影响。若用 Pra 500 mg·kg^{-1}·d^{-1} ig 14 d，囊壁的 GAPDH 活力被抑制 26.5 %。至于 GPI，仅 Meb 25 mg·kg^{-1}·d^{-1} × 14 d 组的癌囊示该酶活力被抑制 33.2 %。结论：GPI 和 GAPDH 不是有效的抗包虫药的主要作用靶。

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Antioxidative and chelating activities of phenylpropanoid glycosides from *Pedicularis striata*[1]

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KEY WORDS phenylpropanoid glycosides; verbascoside; isoverbascoside; *Pedicularis striata*; antioxidants; chelating agents

AIM: To study the antioxidative and iron chelating activities of phenylpropanoid glycosides (PPG) isolated from a Chinese herb *Pedicularis striata*. METHODS: Antioxidative effects of PPG on lipid peroxidation induced by $FeSO_4$-edetic acid in linoleic acid were measured by thiobarbituric acid method. Chelating activities of PPG for Fe^{2+} were tested by differential spectrum method. RESULTS: The reaction rates (A_{532} · min^{-1}) of lipid peroxidation were 0.0046 in the control, 0.0021 in verbascoside group, and 0.0008 in isoverbascoside group. The chelating activity of isoverbascoside was 2-fold stronger than that of verbascoside. Permethyl verbascoside showed neither antioxidative nor chelating activities. CONCLUSION: The inhibitory effects of PPG with phenolic hydroxy groups on lipid peroxidation are owing to their chelating properties. Under physiological condition PPG-Fe^{2+} chelates are sufficiently stable. Thus PPG are able to inhibit the Fe^{2+}-dependent lipid peroxidation *in vivo* through chelating Fe^{2+} and exhibit their therapeutic potential by the same mechanism *in vitro*.

Phenylpropanoid derivatives existing in plants were used as antibiotics, ultraviolet protectants, and insect repellents[1]. Phenylpropanoid glycosides (PPG) exhibited antibiotic[2], antiviral[3], antiplatelet aggregation[4] and inhibition of leutriene B_4 formation[5]. We found PPG possessed scavenging effects on superoxide[6], inhibition on lipid peroxidation[7], and protection against oxidative

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