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Effects of mebendazole, albendazole, and praziquantel on glutathione *S*-transferase and superoxide dismutase of *Echinococcus granulosus* cyst wall harbored in mice

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AIM: To explore the existence of glutathione *S*-transferase (GST) and superoxide dismutase (SOD) in *Echinococcus granulosus* cyst, and the effect of anthydatid drugs on these 2 enzymes, mice infected with *E granulosus* protoscoleces for 10 — 12 months were used. **METHODS**: The activity of GST was measured by conjugation rate of 1-chloro-2,4-dinitrobenzene with glutathione (reduced form), while the activity of SOD was measured by a modified autoxidation of 1, 2, 3-trihydroxybenzene method. **RESULTS**: Activities of both GST and SOD in the cyst wall were 12.3 ± 4.3 to 13.5 ± 4.8 μmol min^{-1}/mg protein and 4.4 ± 2.9 to 6.1 ± 1.4 U min^{-1}/mg protein, respectively. When infected mice were treated ig with mebendazole (Meb) 25 mg kg^{-1} d^{-1} for 14 d, the GST activity of both collapsed and full cyst walls were inhibitied by 30.1 % and 26.8 %, respectively. Whereas SOD activity of the cyst walls were activated by 105 %—163 %. Albendazole 300 mg kg^{-1} d^{-1} for 14 d and praziquantel 500 mg kg^{-1} d^{-1} for 14 d had no apparent effect on both GST and SOD of *E granulosus* cyst wall. **CONCLUSION**: The results suggested that the inhibition of GST activity in the cyst wall induced by Meb might damage the defence system of the parasite.

KEY WORDS *Echinococcus*; cysts; glutathione transferase; superoxide dismutase; mebendazole; albendazole; praziquantel

In studying the mode of action of anti-hydatid drugs, a major problem concerning whether the failure of chemotherapy is due to permeability barrier or detoxifying system of *Echinococcus granulosus* cyst remains to be solved. Despite the importance of the detoxifying system of *E granulosus* cyst, very little is known about this aspect. The purpose of this study was to explore the existence of glutathione *S*-transferase (GST) and superoxide dismutase (SOD) in the cyst and to determine the effects of mebendazole (Meb), albendazole (Alb), and praziquantel (Pra) on the GST and SOD in the cyst wall.

MATERIALS AND METHODS

Parasites Cyst fluid containing protoscoleces of *E granulosus* was collected aseptically from hydatid cysts harbored in naturally infected sheep in Xinjiang Uighur Autonomous Region. After adding penicillin 5×10^5 U L^{-1}, streptomycin 5×10^5 U L^{-1}, and amphotericin B 0.25 mg L^{-1}, the cyst fluid was stored at 4 C. The protoscoleces in the cyst fluid were used for inoculation[1].

Mice Kunming strain ♀, ♂ mice, weighing 20 ± *s* 2 g (n=170) were inoculated ip with 2000 protoscoleces. Mice were maintained on a rodent feed and water *ad lib*. Starting at 10—12 months after inoculation, groups of 3—4 mice each were treated ig with Meb 25—50 mg kg^{-1} d^{-1} × 7—14 d, Alb 300 mg kg^{-1} d^{-1} × 14 d, or Pra 500 mg kg^{-1} d^{-1} × 14 d.

Drugs and reagents Meb, Alb, and Pra were the products of Shanghai Institute of Pharmaceutical

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Industrial Research, Hangzhou Pharmaceutical Factory, and Shanghai 6th Pharmaceutical Factory, respectively. 1-Chloro-2, 4-dinitrobenzene (CDNB) and glutathione (reduced form) were the products of Sigma Chemical Co, and 1,2,3-trihydroxybenzene was the product of Zunyi 2nd Chemical Factory. Other reagents were of AR grade.

Preparation of cyst wall homogenate Each group of mice treated were killed by blood letting at 24 h after the last medication. Five to 8 cysts without fluid and 5—8 full cysts filled with fluid of each group were harvested rapidly from the peritoneal cavity and placed in ice baths. After the endocysts were separated and the fluid was removed by blotting with filter paper. The cyst wall 200 mg were homogenized in a glass homogenizer containing 1.5 mL phosphate buffer (0.1 mol L^{-1}, pH 6.5) in ice bath. The homogenate was centrifuged (1000 × *g* at 4 C for 20 min) and the supernatant was stored at 4 C before use.

GST measurement The activity of GST was measured by conjugation of GST with glutathione (reduced form)[2] with some modifications: the test tube containing CDNB and glutathione 0.1 mol L^{-1} in a volume of 3 mL was preincubated at 30 C for 15 min. Cyst wall homogenate supernatant (0.2 mL) was added and the absorbance at 340 nm was measured 10 s later. At 5 min after further incubation the absorbance was measured again. One unit of enzyme activity represented the conjugation of 1 μmol CDNB with glutathione min^{-1}/mg protein, ie, $GST = \Delta OD_{340} \times 3.2/5 \times 0.0096 \cdot$ mg protein. ΔOD_{340} was the increase in optical density at 340 nm 5 min after the start of reaction; 3.2 was the total volume of reaction system (mL); the reaction time was 5 min; 0.0096 was the extinction coefficient of CDNB 1 μmol.

SOD measurement The activity of SOD was measured by a modified 1, 2, 3-trihydroxybenzene method[3]. Four test tubes containing 2.7 mL each of phosphate buffer 0.1 mol L^{-1} (pH 8.2) were preincubated at 30 C for 15 min. Then, 0.2 mL of 1,2,3-trihydroxybenzene 6 mmol L^{-1} were added, to the remaining one, which served as a whereas the blank control was add HCl 10 mmol L^{-1} 0.2 mL. After 4 min the mean value of ΔOD_{420} between 3 test tubes and the blank control represented the autoxidation rate of 1,2,3-trihydroxybenzene (AS).

Meantime, cyst wall homogenate supernatant (0.2 mL) was added to the reaction system before adding 1,2,3-trihydroxybenzene, and the ΔOD_{420} between the supernatant tube and the blank control tube served as autoxidation rate after adding the enzyme (AT). One unit of SOD activity was calculated from inhibition of 1 % autoxidation rate of 1, 2, 3-trihydroxybenzene min^{-1}/mg protein, ie,

$(AS - AT)/AS \times 4$ (min) ÷ Protein (mg) × 100.

Protein measurement The protein in the cyst wall homogenate supernatant was assayed by the colorimetric method[4].

RESULTS

GST When infected mice were treated ig with Meb 25 mg kg^{-1} d^{-1} × 7 d, the GST activities of the cyst walls of collapsed and full cysts were 10.2 ± 2.6 (expressed by conjugation of 1 μmol CDNB with glutathione reduced form min^{-1}/mg protein) and 13.6 ± 5.8, respectively, being slightly lower or similar to that of the control. When the treatment course was lengthened to 14 d, the GST activities of both collapsed and full cyst walls were inhibited as compared to those of the control with inhibitory rates of 30.1 % and 26.8 %, respectively. Similar results were found in infected mice treated ig with Meb 50 mg kg^{-1} d^{-1} × 7 d, but the difference between the cyst walls of full cyst group and the control was not significant. In infected mice treated ig with Alb 300 mg kg^{-1} d^{-1} × 14 d or Pra 500 mg kg^{-1} d^{-1} × 14 d, the GST activities of cyst walls of full cyst were nearly unchanged (Tab 1).

SOD When infected mice were treated ig with Meb 25—50 mg kg^{-1} d^{-1} × 7—14 d, the SOD activities of cyst walls were higher than those of corresponding controls with an increase of 105 %—163 %. In mice treated ig with Alb 300 mg kg^{-1} d^{-1} × 14 d or Pra 500 mg kg^{-1} d^{-1} × 14 d, the SOD activities of the cyst walls were similar to that of the corresponding control (Tab 2).

Tab 1. Effects of mebendazole, albendazole, and praziquantel on glutathione *S*-transferase activity of the cyst wall harbored in mice. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.01$ *vs* control.

Drug/ mg kg^{-1} d^{-1} × d	Cyst tested	Status of cyst	GST activity/ Conjugation of 1 μmol CDNB with glutathione, min^{-1}/mg protein	Reduction/%
Control	20	Full	13.4±7.9	—
Meb 25 × 7	19	Collapased	10.2±2.6[a]	23.9
	30	Full	13.6±5.8[a]	—
Control	25	Full	12.3±4.3	—
Meb 25 × 14	20	Collapsed	8.6±2.8[b]	30.1
	26	Full	9.0±4.3[b]	26.8
Control	26	Full	13.4±4.3	—
Meb 50 × 7	11	Collapased	7.1±3.0[b]	47.0
	15	Full	10.9±4.0[a]	18.7
Control	31	Full	13.0±5.1	—
Alb 300 × 14	35	Full	12.5±5.4[a]	4.0
Control	27	Full	13.5±4.8	—
Pra 500 × 14	29	Full	12.3±6.1[a]	8.0

Tab 2. Effects of mebendazole, albendazole, and praziquantel on superoxide dismutase activity of the cyst wall (full cyst) harbored in mice. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.01$ *vs* control.

Drug/ mg kg^{-1} d^{-1} × d	Cysts tested	SOD activity/ Inhibition of 1 % autoxidation rate of 1,2,3-benzenetriol min^{-1}/mg protein	Increase/ %
Control	19	4.4±2.9	—
Meb 25 × 7	58	9.6±6.5[b]	118
Control	29	5.9±5.6	—
Meb 25 × 14	37	15.5±13.4[b]	163
Control	37	6.0±2.6	—
Meb 50 × 7	37	12.3±7.4[b]	105
Control	31	4.9±2.0	—
Alb 300 × 14	22	5.5±3.2[a]	11
Control	18	6.1±1.4	—
Pra 500 × 14	21	6.3±1.3[a]	3

DISCUSSION

The results of our present study demonstrated that both GST and SOD were present in the *E granulosus* cyst wall. It is worthy to be mentioned that the activities of both GST and SOD were relatively high, suggesting an effective protective mechanism against oxidant, immune or chemotherapeutic attacks[5]. Our study indicated that Pra exhibited less effect on the cyst of *E granulosus* revealing no apparent action on GST and SOD in the cyst wall. Interestingly, for 2 antihydatid benzimidazoles, Alb also exerted no effect on these 2 enzymes. In contrast, Meb showed significantly inhibitory effect on GST in the cyst wall. The above-mentioned results might accounting for, at least in part, difference in antihydatid mechanism of the 2 benzimidazole carbamates.

Brophy and Barrett[6] noted that GST is one of the major detoxification systems found in helminths because of lacking important cytochrome P-450-dependent detoxification reactions. Although the exact function of GST in the cyst wall is still unknown, the higher activity of this enzyme shows its importance in the cyst wall. Therefore, the inhibition of GST activity induced by Meb might damage the defensive system of the parasite.

The most important function of SOD is to eliminate the superoxide radical produced during the metabolism of the living organism. Therefore, SOD displays an important role in detoxication inside the organism. The interesting thing is that while the GST activity of the cyst was inhibited by Meb, the SOD activity, on contrast, was increased significantly to more than one-fold as compared to the controls. Further investigations are necessary to explain the mechanism of the observed activating effect of Meb on SOD activity of *E granulosus* cyst wall.

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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谷胱甘肽硫转移酶和超氧化物歧化酶的作用

R965.2

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目的：探讨了抗包虫药物对细粒棘球蚴囊壁谷胱甘肽硫转移酶(GST)和超氧化物歧化酶(SOD)的影响，用感染细粒棘球蚴原头节达10—12个月的小鼠作试验. **方法**：GST 用氯硝基苯与还原型谷胱甘肽结合法测定. SOD 用改进的邻苯三酚自氧化法测定. **结果**：囊壁 GST 和 SOD 的活力各为12.3±4.3—13.5±4.8 μmol min^{-1}/mg 蛋白质，和4.4±2.9—6.1±1.4 U min^{-1}/mg 蛋白质，感染鼠用甲苯达唑(Meb) 25 mg kg^{-1} d^{-1}治疗14 d 时，瘪囊与充盈囊壁的 GST 活力各被抑制30 %和26.8 %，而 SOD 活力则升高105 %—163 %. 阿苯达唑300 mg kg^{-1} d^{-1}和吡喹酮500 mg kg^{-1} d^{-1}，连给14 d，对上述2种酶无明显影响. **结论**：Meb 抑制囊壁的 GST 可能损害囊防御系统.

关键词 棘球属；囊；谷胱甘肽硫转移酶；超氧化物歧化酶；甲苯达唑；阿苯达唑；吡喹酮