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新灯盏素对血细胞与内皮细胞花生四烯酸代谢影响的差异

R973.2

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摘要 新灯盏素是从灯盏花中提取的 4′–羟基–7–*o*–葡萄糖醛酸甙的可溶性钠盐与钙盐. 该药抑制血小板 TXB_2 生成而不影响羟廿碳四烯酸的产量; 抑制内皮细胞 6–酮 $PGF_{1\alpha}$ 的生成; 对白细胞 TXB_2 生成无影响, 但明显加强钙离子载体刺激 LTB_4 生成的作用. 结果表明新灯盏素对血细胞与内皮细胞的花生四烯酸代谢有不同的影响.

关键词 黄酮类; 新灯盏素; 血小板; 白细胞; 血管内皮; 血栓素 B_2; 前列腺素 F 类; 羟廿碳四烯酸; 白细胞三烯 B 类

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Effects of mebendazole, albendazole, and praziquantel on succinate dehydrogenase, fumarate reductase, and malate dehydrogenase in *Echinococcus granulosus* cysts harbored in mice

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ABSTRACT *Echinococcus granulosus* cyst wall possesses high biochemical activities of malate dehydrogenase (MD) and fumarate reductase (FR), but low activity of succinate dehydrogenase (SD), suggesting that the cyst wall may utilize a partial reverse tricarboxylic acid cycle. When infected mice were given intragastrically with mebendazole, 25–50 mg·kg^{-1}·d^{-1}, albendazole 300 mg·kg^{-1}·d^{-1} or praziquantel 500 mg·kg^{-1}·d^{-1} for 7–14 d, no apparent effects on SD and FR activities of the cyst wall were found, while the MD activity was suppressed by all the 3 drugs, the inhibition rates being 34.6–61.6%, 59.8%, and 50.6%, respectively. The results suggested that MD may not be an important target for the antihydatidosis drugs.

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KEY WORDS *Echinococcus*; malate dehydrogenase; fumaric reductase; succinate dehydrogenase; mebendazole; albendazole; praziquantel

Although parasitic helminths derive their energy via carbohydrate metabolism, their metabolic pathways are usually quite different from the carbohydrate metabolism of their host[1]. One of the important differences is that they fix CO_2 and utilize a partial reverse tricarboxylic acid cycle, resulting in production of ATP[1]. Since the protoscoleces of *Echinococcus granulosus* exhibit the specific pathway in carbohydrate metabolism[2], it was of interest to study the 3 enzymes relevant to the subsequent metabolism after formation of oxaloacetic acid through the fixation of CO_2 into the phosphoenolpyruvate, ie, malate dehydrogenase (MD), fumarate reductase (FR) and succinate dehydrogenase (SD) in the cyst wall. Concurrently, the effects of mebendazole (Meb), albendazole (Alb) and praziquantel (Pra) on these enzymes were also studied.

MATERIALS AND METHODS

Parasites Cyst fluid containing protoscoleces of *E granulosus* was collected aseptically from sheep naturally infected with hydatid cysts from Xinjiang Uygur Antonomous Region. After adding penicillin and streptomycin 500 IU · ml^{-1} each and amphotericin B 0.25 $\mu g \cdot ml^{-1}$, the cyst fluid was kept at 4℃. The processing of the protoscoleces in the 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 12–14 months after infection, 3 groups of 3–5 mice each were treated with ig Meb 25–50, Alb 300, and Pra 500 $mg \cdot kg^{-1} \cdot d^{-1}$, respevtively for 7–14 d.

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 from the peritoneal cavity. The endocysts were separated and cut with scissors. After the cyst fluid was removed with filter paper, 200 mg of the endocyst wall were homogenized in a glass homogenizer containing ice cold KCl (180 $nmol \cdot L^{-1}$, prepared with Tris–HCl 10 $mmol \cdot L^{-1}$, pH 7.4) 1.5 ml in ice bath. After centrifugation (1000× *g*, 10 min) at 4℃, the supernatant was stored for use.

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. Disodium succinate, oxaloacetic acid, sodium fumarate, and Commassie brilliant blue G–250 were the products of Sigma. Nicotinamide adenine dinucleotide–reduced (NADH, disodium salt) was a biochemical reagent with a purity of over 70%. Potassium ferricyanide and other reagents were all of AR grade.

Succinate dehydrogenase measurement The enzyme activity was assayed by the reduction of potassium ferricyanide[4]. The testing tube containing substrate, disodium succinate and potassium ferricyanide was preincubated in water bath at 30℃ for 10 min, and 0.2 ml of the cyst homogenate was then added. Ten min later, the reaction was terminated by adding 15% trichloroacetic acid 1.0 ml. After centrifugation (1000× *g*, 10 min), the absorbance rate of the supernatant was measured at 420 nm. In the control tube, 15% trichloroacetic acid was added prior to the cyst homogenate. According to the difference of absorbance rates between the testing and control tubes, the enzyme activities were calculated. Thus, 1 unit of enzyme activity represented the reduction of 1 μmol of potassium ferricyanide per min per mg of protein, ie, $SD = \Delta OD_{420} \times 4.2 / 0.00103 \times$ mg protein× 10. In the formula, ΔOD_{420} was the difference value of absorbance between the testing and control tubes; 4.2 was the total volume of reaction system, 0.00103 was the absorbance coefficient per μmol of potassium ferricyanide; protein was measured in 0.2 ml of the cyst homogenate and expressed as mg and the reaction time was 10 (min).

Fumarate reductase measurement The enzyme activity was measured according to the consumption of NADH[5]. The testing tube containing NADH and the substrate, sodium fumarate was preincubated in water bath at 30℃ for 10 min, and the cyst homogenate 0.2 ml was then added. The decrease in absorbance rate produced in 15–30 s at 340 nm was recorded. One unit of enzyme activity represented consumption of 1 μmol NADH per min per mg of protein, ie. $FR = \Delta OD_{340} \times 3.2 / 0.0062 \times$ mg protein $\times 0.25$. In the formula, ΔOD_{34} was the decrease in absorbance at 340 nm in 15–30 s after start of reaction; 0.0062 was the extinction coefficient of 1 μmol of NADH; 3.2 was the total volume of reaction system; protein was measured in 0.2 ml of the cyst homogenate and expressed as mg and the reaction time was 0.25 (min).

Malate dehydrogenase measurment The enzyme activity was assayed by the consumption of NADH[2]. Prior to the start of assay, the testing tube containing NADH and the substrate oxaloacetic acid was preincubated in water bath at 30℃ for 10 min. The assay was initiated by the addition of the cyst homogenate 0.1 ml. The decrease in absorbance rate produced in 15–30 s at 340 nm was recorded. One unit of enzyme activity represented the consumption of 1 μmol of NADH per min by per mg of protein, ie. $MD = \Delta OD_{430} \times 3.1 / 0.0062 \times$ mg protein $\times 0.25$. In the formula, ΔOD_{340} was the decrease in absorbance at 340 nm in 15–30 s after reaction; 0.0062 was the extinction coefficient; protein was measured in 0.1 ml of the cyst homogenate and expressed by mg and the reaction time was 0.25 (min).

Protein measurement The protein in the cyst homogenate sample was assayed by the colorimetric method after the binding of the dye to protein[6].

RESULTS

Succinate dehydrogenase In infected mice treated with ig Meb 25 mg· kg^{-1}· d^{-1}× 14 d, the SD activity of the cyst wall was similar to that in the control group. When the dosage of Meb was increased to 50 mg· kg^{-1}· d^{-1}× 7 d, no apparent effect on the enzyme activity was seen, but prolongation of the treatment course to 14 d resulted in 20.6% suppression of SD activity. No significant difference in the enzyme activities between treated and control groups was noted. In groups of infected mice treated with ig Alb 300 or Pra 500 mg· kg^{-1}· d^{-1}× 14 d, the SD activity of the cyst wall remained similar to that in the control group (Tab 1).

Tab 1. Effects of mebendazole (Meb), albendazole (Alb) and praziquantel (Pra) on succinate dehydrogenase (SD), fumarate reductase (FR), and malate dehydrogenase (MD) activities 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	SD activity, reduction of 1 μmol $K_3Fe(CN)_6$· min^{-1} / mg protein	Inhibition rate, %	FR activity, consumption of 1 μmol NADH min^{-1} / mg protein	Inhibition rate, %	MD activity, consumption of 1 μmol NADH min^{-1} / mg protein	Inhibition rate, %
Control	30	10± 7	–	85± 16	–	769± 240	–
Meb 25× 14	30	11± 7*	–	91± 18*	–	295± 141***	61.6
Control	30	11± 5	–	85± 18	–	753± 289	–
Meb 50× 7	30	10± 4*	0.3	77± 22*	9.4	492± 177***	34.6
Control	15	13± 5	–	91± 31	–	927± 401	–
Meb 50× 14	25	10± 5*	20.6	84± 24*	7.7	361± 119***	61.1
Control	30	10± 8	–	87± 27	–	705± 359	–
Alb 30× 14	30	10± 8*	7.8	76± 36*	12.6	286± 157***	59.4
Control	30	9± 6	–	101± 35	–	986± 390	–
Pra 500× 14	30	11± 7*	–	114± 36*	–	487± 203***	50.6

Fumarate reductase When infected mice were treated with ig Meb 25 mg· kg^{-1}· d^{-1}× 14 d, no effect on FR activity of the cyst wall was seen *vs* the control group. In infected mice treated with ig Meb at 50 mg· kg^{-1}× 7–14 d, the FR activity of the cyst wall tended to decrease (7.7–9.4%, $P>0.05$ *vs* the control). After ig Alb 300 or Pra 500 mg· kg^{-1}· d^{-1} × 14 d, no apparent effect on FR activity of the cyst wall was identified (Tab 1).

Malate dehydrogenase In infected mice treated with ig Meb 25 mg· kg^{-1}· d^{-1}× 14 d, the MD activity of the cyst wall declined markedly with an inhibition rate of 61.6% *vs* the control group. With 50 mg· kg^{-1}· d^{-1}× 7 and 14 d, the respective inhibition rates of MD activity were 34.6% and 61.1% ($P<0.01$ *vs* the control). When infected mice were treated with ig Alb 300 or Pra 500 mg· kg^{-1}· d^{-1}× 14 d, the MD activity of the cyst wall also declined markedly with inhibition rates of 59.4% and 50.6%, respectively (Tab 1).

DISCUSSION

Some parasitic belminths including *E granulosus* protoscoleces may utilize a partially reverse tricarboxylic acid cycle[2]. In studies on carbohydrate metabolism of *E granulosus* cyst wall, the activities of the 3 enzymes related to the partial reverse tricarboxylic acid cycle have been delineated. The results showed that the cyst wall exhibited a high MD activtiy, which catalyzed the conversion of oxaloacetic acid to malate. Another important enzyme, FR, which catalyzed the conversion of fumarate to succinate, also exhibitied a high activity but to a lesser degree than that of MD. On the other hand, SD which catalyzes the oxidation of succinate to fumarate only exhibited a low activity, indicating that succinate might be a metabolite produced by the parasite and possibly be further metabolized to propionic acid or some volatile fatty acids.

When infected mice were treated with ig Meb, Alb or Pra, no apparent effect of the drugs on SD and FR activities was observed, while MD activity of the cyst wall was suppressed with inhibition rates of over 50% except for some individual case. Since Alb and Pra, which exhibitied less therapeutic efficacy than Meb, also showed similar inhibitory effect on MD activity of the cyst walls, MD might not be an important target to be attacked by antihydatidosis drug.

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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的琥珀酸脱氢酶、延胡索酸还原酶和苹果酸脱氢酶的影响

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摘要 细粒棘球蚴囊壁具有较强的苹果酸脱氢酶(MD)活力，次为延胡索酸还原酶(FR)，而琥珀酸脱氢酶(SD)的活力则甚低，提示囊壁组织可能有逆向利用部分三羧酸循环的功能。抗包虫药物甲苯达唑、阿苯达唑和吡喹酮对 FR 和 SD 均无明显影响，但对 MD 的抑制率各为 34.6–61.6%、59.4%和 50.6%，提示 MD 可能不是有效药物作用的重要靶部位。

关键词 棘球属；苹果酸脱氢酶；延胡索酸还原酶；琥珀酸脱氢酶类；甲苯达唑；阿苯达唑；吡喹酮