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In vitro Effects of Some Anthelminthics on the Malate Dehydrogenase and Lactate Dehydrogenase Enzyme Activities of *Taenia Saginata*

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Abstract: Malate Dehydrogenase (MDH) and Lactate Dehydrogenase (LDH) activities were demonstrated in the mitochondrial and cytosolic fractions of an intestinal cestode, *Taenia saginata*, and then the in vitro effects of three anthelminthics, albendazole, niclosamide, and piperazine on these enzymes were investigated. The Vmax and Km values of MDH were found to be 3.00 µmol (min mg protein)$^{-1}$ and 0.0166 mM respectively. In vitro addition of albendazole and niclosamide increased the Vmax value of MDH, whereas it decreased Km value of MDH ($p<0.05$). The Vmax and Km values of LDH were found to be 0.028 µmol (min mg protein)$^{-1}$ and 0.055 mM respectively. Albendazole and niclosamide solutions increased the Vmax value of LDH ($P<0.05$). Km value of LDH was reduced albendazole and niclosamide solutions ($P<0.05$). Piperazine affected neither MDH nor LDH activites. These results suggest that the activity of MDH and LDH in *T. saginata* are activated and the carbohydrate metabolism of this parasite is changed by albendazole and niclosamide.

Key Words: Malate Dehydrogenase, Lactate Dehydrogenase, *Taenia saginata*, albendazole, niclosamide, piperazine.

Introduction

Infections caused by helminths represent a major threat to the health of milions of people throughout the world. Many species of parasitic helminths are responsible for disease in humans. The range of anthelminthic drugs currently available provides a powerful armamentarium against most human helminthic infections. Niclosamide is generally regarded as a very effective agent for treating most infections by cestodes in animals and man (1). The anthelminthic mechanism of niclosamide is not completely understood (2). Albendazole is a broad–spectrum anthelminthic of the benzimidazole carbamate class, which is effective against larval and adult stages of cestodes and trematodes (3). Some workers have shown its effect by interaction with micro tubules of parasites; however, it has been suggested that anthelminthics work in different ways in different parasites (4). Piperazine, a heterocyclic organic base, has been widely used as an anthelminthic, particularly against *Ascaris lumbricoides* and *Enterobius vermicularis* (3). The anthelminthic activity of piperazine is reported to be dependent on its anticholinergic action (5).

Taeniidis are large tapeworms, common throughout the world. Two species, *Taenia saginata* and *T. solium*, are common parasites of man. In Turkey, *T. saginata* has become widespread, especially in south–east Anatolia, east Anatolia and in Sivas (6, 7). Little information is available on the nutrition and metabolism of *T. saginata* with regard to that of other cestodes (8). Glycogen is the main energy reserve and glycogen and glucose are degraded to phosphoenolpyruvate via the Emden–Meyerhof glycolytic pathway. There are two major pathways for the subsequent catabolism of phosphoenolpyruvate. First, phosphoenolpyruvate is reduced to malate, which enters the mitochondria. Second, it is transformed to pyruvate which is reduced to lactate by lactate dehydrogenase (9). Similar to that of vertebrates, the inner membrane of the mitochnodrion in helminths is impermeable to NADH, which is transferred from the cytoplasm to the mitochondrion in mammalian mitochondria via the malate/aspartate shuttle system. Malate dehydrogenase is the rate–limiting enzyme in the phosphoenolpyruvate metabolism and of this shuttle system. It is not known if this shuttle system is operative

in some helminths (9) The present studies were designed to delineate the kinetic properties of MDH and LDH in *T. saginata*, and to determine the effects of niclosamide, albendazole and piperazine on the activities of these enzymes.

Material and Methods

Materials

KH_2PO_4, K_2HPO_4, sucrose, EDTA Coomassie Brilliant Blue G250 and perchloric acid were obtained from Merck. Bovine serum albumin (BSA), pyruvate, oxaloacetate, and NADH were purchased from Sigma. Niclosamide, piperazine and albendazole were a gift from Atabay Drug Factory (İstanbul). Andazol was purchased from Biofarma. Albendazole did not dissolve in the solvents tested, and was therefore tested using an andazol suspension.

Methods

Proglottids of *T. saginata* expelled spontaneously were collected from patients with taeniasis. Proglottids of *T. saginata* were thoroughly rinsed in Ringer's solution and used within 1–2 h after removal from the host. Proglottids were weighed wet. Each gram of proglottid was homogenised in 10 mL of 0.24 M sucrose buffer containing 0.005 M EDTA (pH 7.4) solutions with a Braun Potter Elvehjen homogeniser with telfon piston at 0°C. The homogenate was centrifuged at 110 g for 10 min using a Beckman L5–70 centrifuge. This and further processes were performed at 4°C. The sediment was removed and the supernatant was then centrifuged at 6750 g for 30 min. The supernatant was collected and used as a source of LDH. The mitochondrial pellet was suspended in 5 mL mitochondrial media (10). The suspension was sonicated in an ice bath for 5–6 sec and used as a source of MDH. Protein concentrations of these mitochondrial and cytosolic fractions were determined by the method of Bradford using BSA as standard (11).

LDH activity was measured as the rate of oxidation of NADH at 340 nm and 25°C in a recording Shimadzu model UV–VIS spectrophotometer (12). In all cases, the volume of the reaction mixture was 3 mL, the light path 1 cm and the value of molar extinction coefficient $\varepsilon_{340\ nm}$=6.22 $cm^2\ \mu mol^{-1}$. The composition of the reaction mixture was 94 mM phosphate buffer (pH 7.5), 0.038 mM pyruvate, 0.2 mM NADH and 50 µL cytoplasmic fraction (corresponding to 12.8 mg mL^{-1} protein).

MDH activity was measured as the rate of oxidation of NADH at 340 nm and 25°C in a recording Sichmatsu model UV–VIS spectrophotometer (13). In all cases, the volume of the reaction mixture was 3 mL, the light path 1 cm and the value of molar extinction coefficient $\varepsilon_{340\ nm}$=6.22 $cm^2\ \mu mol^{-1}$. The composition of the reaction mixture was 94 mM phosphate buffer (pH 7.5), 0.25 mM oxaloacetate, 0.2 mM NADH and 50 µL of mitochondrial medium (corresponding to 0.44 mg mL^{-1} protein).

Michaelis–Menten kinetic parameters Vmax (maximum of apparent initial enzyme velocity) and Km (substrate affinity constant) values were determined by assaying the enzymes at different substrate concentrations and constructing a Lineweaver–Burk double reciprocal plot of substrate concentrations and enzyme velocity (14).

The method for determination of the effects of albendazole, niclosamide and piperazine on enzyme activity was similar to that for enzyme activity described above, except that different concentrations of anthelminthics (0.158 mM, 0.633 mM, 1.266 mM albendazole, 0.032 mM, 0.063 mM, 0.127 mM niclosamide, 3.0 mM, 12.0 mM, 22.0 mM piperazine) were used, and measurements were made following incubation at 25°C for 10 min. This period was chosen because greater incubation times did not give greater activation percentages. Data were analysed statistically with the Mann–Whitney u–test and correlatin analyses (15).

Results

Initial rates of NADH oxidation were obtained for various levels of oxaloacetate and pyruvate solutions. These data were plotted according to the method of Lineweaver –Burk, and Vmax and Km values were determined. The Vmax and Km values for MDH were 3.00 µmol $(min\ mg\ protein)^{-1}$ and 0.0166 mM, respectively. The Vmax and Km valueus for LDH were 0.028 µmol $(min\ mg\ protein)^{-1}$ and 0.055 mM, respectively.

As the results of the interaction of albendazole and niclosamide with MDH Lineweaver –Burk plots are shown in Figure 1 and Figure 2, respectively. Lineweaver –Burk plots showing the results of experiments investigating the effects of albendazole and niclosamide on LDH are shown in Figure 3 and Figure 4, respectively. Vmax and Km values for these enzymes are shown in Table 1 and Table 2.

At all concentrations tested, piperazine did not affect MDH or LDH activity.

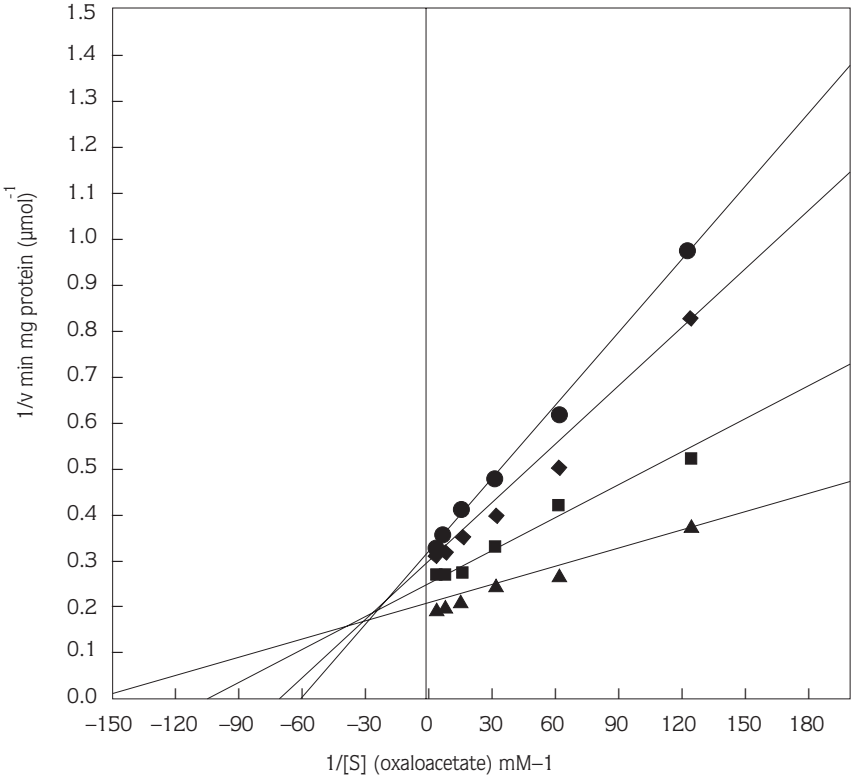

Figure 1. Lineweaver–Burk plot for MDH in *Taenia saginata* with varying amounts of albendazole. —●—: Enzyme; —◆—: 0.158 mM albendazole; —■—: 0.633 mM albendazole; —▲—: 1.266 mM albendazole.

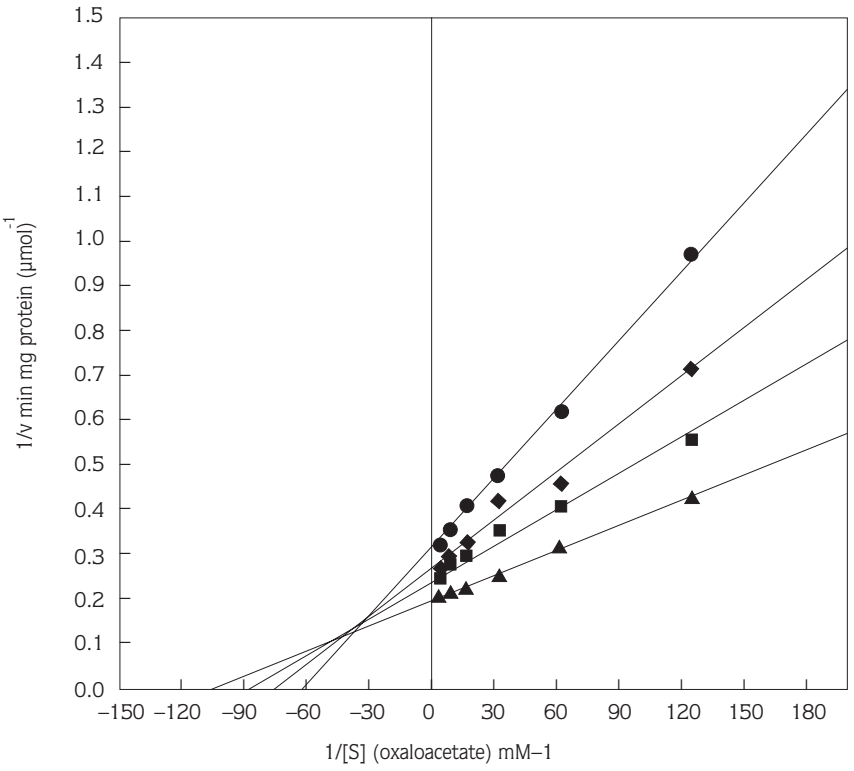

Figure 2. Lineweaver–Burk plot for MDH in *Taenia saginata* with varying amounts of niclosamide. —●—: Enzyme; —◆—: 0.032 mM niclosamide; —■—: 0.063 mM niclosamide; —▲—: 0.127 mM niclosamide.

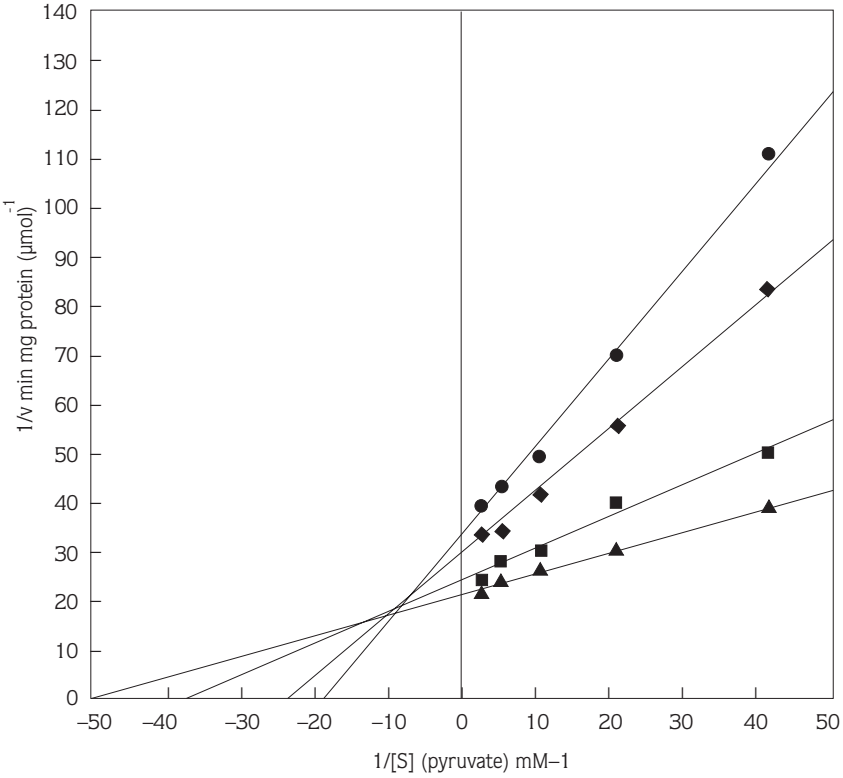

Figure 3. Lineweaver–Burk plot for LDH in *Taenia saginata* with varying amounts of albendazole. —●—: Enzyme; —◆—: 0.158 mM albendazole; —■—: 0.633 mM albendazole; —▲—: 1.266 mM albendazole.

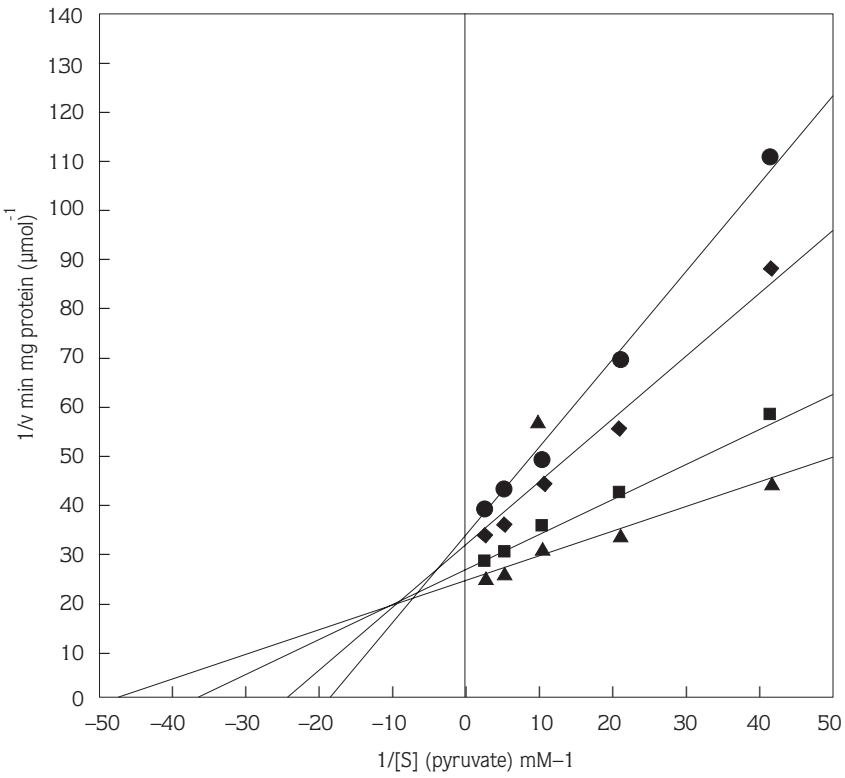

Figure 4. Lineweaver–Burk plot for LDH in *Taenia saginata* with varying amounts of niclosamide. —●—: Enzyme; —◆—: 0.032 mM niclosamide; —■—: 0.063 mM niclosamide; —▲—: 0.127 mM niclosamide.

Table 1. Effects of albendazole on MDH and LDH in *T. saginata*.

Concentrations of Albendazole/mM	MDH*		LDH*	
	Vmax/µmol (min mg protein)$^{-1}$	Km/mM (for oxaloacetate)	Vmax/µmol (min mg protein)$^{-1}$	Km/mM (for pyruvate)
0.000	3.00 ± 0.01	0.0166 ± 0.0003	0.028 ± 0.001	0.055 ± 0.002
0.158	3.30 ± 0.02	0.0138 ± 0.0003	0.030 ± 0.001	0.040 ± 0.003
0.633	3.46 ± 0.01	0.0095 ± 0.0002	0.042 ± 0.002	0.027 ± 0.003
1.266	5.00 ± 0.03	0.0066 ± 0.0001	0.050 ± 0.002	0.020 ± 0.002

* values are mean ± Standard error from five sets of experiments.

Table 2. Effects of niclosamide on MDH and LDH in *T. saginata*.

Concentrations of Niclosamide/mM	MDH*		LDH*	
	Vmax/µmol (min mg protein)$^{-1}$	Km/mM (for oxaloacetate)	Vmax/µmol (min mg protein)$^{-1}$	Km/mM (for pyruvate)
0.000	3.00 ± 0.01	0.0166 ± 0.0003	0.028 ± 0.001	0.055 ± 0.002
0.032	3.70 ± 0.03	0.0133 ± 0.0002	0.030 ± 0.001	0.042 ± 0.002
0.063	4.60 ± 0.02	0.0120 ± 0.0002	0.036 ± 0.003	0.032 ± 0.003
0.127	5.80 ± 0.01	0.0100 ± 0.0002	0.045 ± 0.003	0.022 ± 0.001

* values are mean ± Standard error from five sets of experiments.

Discussion

Parasitic infections constitute a significant health risk to humans, particularly in undeveloped and developing countries. For this reason, the relationships between parasites and chemotherapeutics are important. In this study, the effects of niclosamide, albendazole and piperazine on the activities of MDH and LDH in *T. saginata* were investigated.

In the experiments of various investigators, the MDH activity of parasitic helminths has been investigated. Zenka et al. found the cytoplasmic MDH activity of *Taenia crassiceps* larvae to be 0.1235 µmol (min mg protein)$^{-1}$ and the mitochondrial MDH activity of the same parasite's larvae to be 84 µmol (min mg protein)$^{-1}$ (16). In another study by Zenka et al., the Km value of cytoplasmic MDH of *T. crassiceps cysticercus* was found to be 7.8 x 10^{-5} M, and the Km value of *S. mansoni* was found to be 4 x 10^{-5} M (17).

In the present study, Vmax an Km values for MDH were found to be 3.00 µmol (min mg protein)$^{-1}$ and 0.0166 mM, respectively.

Tejada et. al., showed that MDH activity in *A. suum, F. hepatica* and *M. expansa* was inhibited by mebendazole, albendazole and parbendazole (18). However, it was suggested that anthelminthics work in different ways in different parasites. In the present study, there was a significant positive correlation between the Vmax value for MDH in *T. saginata* and anthelminthic concentration (r=0.95 for albendazole and r=0.99 for niclosamide) and there was a significant negative correlation between the Km value for MDH in *T. Saginata* and anthelminthic concentration (r=–0.97 for albendazole and r=–0.95 for niclosamide). That is to say, a significant activation of MDH was observed following exposure to albendazole and niclosamide adipate ($P<0.05$) (Table 1 and Table 2). Piperazine did not affect MDH activity.

In experiments by various investigators, the LDH activity of parasitic helminths has been investigated. It has been found to be 143 nmol (min mg protein)$^{-1}$ in adult *A. lumbricoides*, 353 nmol (min mg protein)$^{-1}$ in adult *H. diminuta*, 1365 nmol (min mg protein)$^{-1}$ in adult

Litomosoides carinii and 6.1 nmol (min mg protein)$^{-1}$ in adult *Ascaridia galli* (19). In the present study LDH activity of *T. saginata* was investigated. The Vmax and Km values of this enzyme were 0.028 µmol (min mg protein)$^{-1}$ and 0.055 mM, respectively.

In a study by Pampori et al. (20) niclosamide, praziquantel, and mebendazole were shown to increase lactate production. Sharma et al. showed that LDH in *A. galli* and *H. gallinae* was not affected by albendazole and levamisole (4). Statistical evaluation of the present results reveals a positive correlation between the Vmax value for LDH in *T. saginata* and anthelminthic concentration (r=0.98 for albendazole and r=0.98 for niclosamide), and a negative correlation was observed between the Km value for LDH in *T. saginata* and anthelminthic concentration (r=–0.92 for albendazole and r=–0.92 for niclosamide). That is to say, significant activation of LDH was observed following exposure to albendazole and niclosamide adipate statistically (P<0.05) (Table 1 and Table 2).

Sharma et al. (5) showed that the LDH activity of *A. galli* and *H. gallinae* was not affected by piperazine. Similarly, in the present study, LDH activity in *T. saginata* was not affected by piperazine.

According to these results, it was established that anthelminthics like niclosamide and albendazole affect LDH and MDH for *T. saginata*, and hence the carbohydrate metabolism of this parasite changes.

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