In vitro stressing factors altering the TCA cycle and morphology of Taenia crassiceps cysticerci

Marina Clare Vinaud1*, Lilian Cristina Morais de Andrade2, Patricia Fernandes Melo Alves3, Carolina Miguel Fraga4, José Clecildo Barreto Bezerra1, Ruy de Souza Lino Junior5

1Host-Parasite Relationship Studies Laboratory, Tropical Pathology and Public Health Institute, Federal University of Goias, Rua 235 S/N, Setor Universitário, Goiânia, Goiás, Brazil
2Host-Parasite Relationship Post-Graduation Programme, Tropical Pathology and Public Health Institute, Federal University of Goias, Rua 235 S/N, Setor Universitário, Goiânia, Goiás, Brazil
3Medicine School, Federal University of Goias, Rua 235 S/N, Setor Universitário, Goiânia, Goiás, Brazil
4Tropical Medicine and Public Health Post-Graduation Programme, Tropical Pathology and Public Health Institute, Federal University of Goias, Rua 235 S/N, Setor Universitário, Goiânia, Goiás, Brazil
5Experimental Pathology Laboratory, Tropical Pathology and Public Health Institute, Federal University of Goias, Rua 235 S/N, Setor Universitário, Goiânia, Goiás, Brazil

ARTICLE INFO

Objective: To determine the morphological and biochemical alterations of in vitro induced Taenia crassiceps cysticerci by the presence of glucose, insulin and praziquantel isolated and in association.

Methods: The cysticerci were cultured for 24 h in supplemented Roswell Park Memorial Institute culture medium and added to two different concentrations of glucose, insulin and praziquantel. The morphometrical analysis was performed through the ImageJ programme, and the biochemical one through high-performance liquid chromatography.

Results: The exposure to the stressing factors led to alterations in the morphology and decrease in the growth rate of the parasite.

Conclusions: The metabolic effects are related to a decrease in the tricarboxylic acid cycle and fatty acids oxidation metabolites due to the drug’s mode of action. Interestingly, the praziquantel, insulin and glucose association enhanced the drug’s mode of action with a greater decrease of the tricarboxylic acid cycle metabolites.

1. Introduction

Taenia crassiceps (T. crassiceps) cysticerci are a well known experimental model to Taenia solium cysticercosis due to their antigenic similarity, source of antigens for diagnosis purposes and facilities of laboratory maintenance and growth[1-4]. These cysticerci have been explored in several different aspects, such as morphology, morphological response to anthelmintic drugs, biochemical aspects and response to drugs[5-10]. As an experimental model, T. crassiceps may provide several answers to the metabolic reaction of the parasite when they are challenged with drugs or hostile environments which could not be obtained with Taenia solium cysticerci. Also, in spite of several studies regarding the control of porcine cysticercosis around the world, this zoonosis is still a public health problem with its increasing incidence[11-15].

The study of stressing factors such as insulin, glucose and anthelmintic drugs on T. crassiceps cysticerci has been performed separately in in vitro analysis of the morphology and biochemical aspects of T. crassiceps cysticerci. These factors are found mixed within the host and it is important to determine how they may interfere within the host-parasite relationship[5-8]. For instance, insulin is known to act as a mitogenic factor both in vitro and in vivo and is involved in the regulation of the fundamental processes within the cells such as metabolic pathways, reproduction and ageing[8,16]. Insulin and glucose receptors have been described in helminthes and...
played a major role in its adaptation to the environment regulating the glucose uptake and cell replication in those parasites[17,18].

Furthermore, the understanding of the parasite response to multiple stimulation may help to unfold the survival mechanisms used by the parasite to successfully inhabit tissues in the intermediary host and gut lumen in the definitive one[17]. Tissue parasites tend to prefer glucose as energy source due to its availability and abundance. Therefore, in order to increase its uptake, these parasites have developed insulin receptors to optimize the glucose uptake, development and growth in response to hormonal variations of the environment[17]. Due to the importance of glucose, as an energy source to cestodes, two glucose transporters have already been described presently in the tegument of both adults and larval stages[19].

The environment in which T. crassiceps cysticerci are found is determinant to which metabolic pathway cells will preferentially be used. For instance, the presences of anthelmintic drugs which block or impair the glucose uptake induce a preference to fatty acids oxidation and the use of alternative energy sources[9,20]. The presence of insulin acts as a mitogenic factor leading to an increase in the budding formation of the cysticerci[8]. On the other hand, anthelmintic drugs are widely used in cysticercosis treatment such as praziquantel which impair the glucose uptake altering the glycolysis, tricarboxylic acid (TCA) cycle and fatty acids oxidation metabolic pathways[10,20,21].

Several aspects of the metabolic response of T. crassiceps cysticerci to the presence of anti-helmintic drugs and other stressful factors have been described, leading to alterations in the uptake of glucose and metabolites production[9,20,21]. However, the combination of stressful factors may show the adaptation pathways used by the parasite to sustain its survival. Therefore, the aim of this study was to determine the morphological and biochemical alterations of in vitro induced T. crassiceps cysticerci by the presence of glucose, insulin, albendazole and praziquantel isolated and in association.

2. Materials and methods

2.1. Maintenance of the T. crassiceps biological cycle

The T. crassiceps (ORF strain) biological cycle is maintained in the animal facility of the Tropical Pathology and Public Health Institute from the Federal University of Goias as described previously[3,9].

The ethical principles for animal experimentation professed by the Brazilian Society of Laboratory Animal Sciences were followed and this study was authorized by the Committee for Ethical Research of the Federal University of Goias (registration number 008/09). The mice received daily care, acidified water and standard rations.

2.2. In vitro exposure of cysticerci to stressing factors

The culture of the cysticerci was performed according to the literature and previously established protocols[6,8]. Therefore, those cysticerci were removed from the peritoneal cavity of BALB/c mice with 60 days of infection, washed in saline buffer and 10 larval stage cysticerci were transferred to each well of six well culture plates with Roswell Park Memorial Institute culture medium with 200 mmol/L of L-glutamine, 10% fetal bovine serum, 1/1 000 (200 000 IU) of penicillin/estreptomicina, 1.0 mol/L of 2-hydroxyethyl, 50 mmol/L of 2-mercaptoethanol. The exposure time was 24 h, after which the cysticerci were photographed for the morphometry analysis or frozen in liquid nitrogen for the biochemical analysis.

The stressing factors to which the cysticerci were exposed were: glucose (56 and 120 mmol/L), insulin (Lantus®, 1.5 and 3.0 IU/mL) and praziquantel (Merck®, 0.03 and 0.06 μg/mL). All these factors were tested isolatedly and in association. The concentrations were determined based on the previous studies[6-8]. These substances were diluted in 0.25% dimethylsulphoxide (DMSO). The control groups consisted of cysticerci in vitro cultured with supplemented Roswell Park Memorial Institute culture medium (control group 1) added to 0.025% DMSO (control group 2).

2.3. Morphometrical analysis of cysticerci

Each well of the tested groups was photographed at 0 h and 24 h after the exposure to the stressing factors. The images were analyzed through the ImageJ (National Institutes of Health) program in which the circumference of each cysticercus was measured. The results were grouped as a mean per well at 0 h and compared to the mean measures of the same well at 24 h. Six wells for each test were performed to ensure at least six mean measures.

2.4. Biochemical analysis of Cysticerci

After 24 h of exposure to glucose, insulin and praziquantel, the cysticerci were removed from the culture medium. The culture medium was analyzed through high-performance liquid chromatography as described by Vinaud et al.[7] to determine the secretion/excretion (SE) of organic acids related to the energetic metabolism. The organic acids were identified according to the previously determined retention time and calibration. The organic acids analyzed were the ones which indicated the energetic metabolism, such as pyruvate, citrate, oxaloacetate, malate, fumarate, succinate, α-ketoglutarate, acetate, acetocetate, β-hydroxybutyrate and propionate[7,20].

2.5. Statistical analysis

The statistical analysis was performed by using the SigmaStat 3.5 program. Descriptive statistics were applied to determine the mean and standard deviation and to evaluate the differences between the groups analyses. The variables were tested for normal distribution and homogeneous variance. As they presented normal distribution, variance analysis was used. The morphometric analysis was made by using the t-test to make comparison between the mean measures of circumference of 0 h and 24 h of the same well, i.e., before and after treatment. The differences were considered significantly when P < 0.05. All experiments were performed in six replicates.
3. Results

This study determined the presence or absence of in vitro stressing factors. For instance, different concentrations of glucose, insulin and praziquantel may interfere in the external morphology and the energetic metabolism of T. crassiceps. It was important to highlight that the DMSO in the concentrations used to dilute the drugs did not interfere in any of the analyzed parameters.

3.1. Insulin and glucose, isolated or in association

The presence of insulin at the concentration of 3.0 IU induced a larger circumference in the larval stage cysticerci, and the circumference was measured 24 h after the exposure (P < 0.05) (Table 1). The treatment with glucose in both concentrations and with glucose associated to insulin showed an increase in the circumference of the parasites which was lower than the one observed in the control group, showing that the excess of glucose and insulin interfered in the in vitro growth rate of the cysticerci.

Table 1

Circumferences of T. crassiceps cysticerci before 0 h and after 24 h in vitro exposure to stressing factors. Mean ± SD.

| Group                | 0 h (mmol/L) | 24 h (mmol/L) | Difference (%) |
|----------------------|--------------|---------------|---------------|
| Control              | 1.58 ± 0.44  | 2.43 ± 0.61   | 53.56         |
| Control + DMSO       | 1.22 ± 0.16  | 1.43 ± 0.20   | 17.28         |
| G56                  | 1.99 ± 0.20  | 2.29 ± 0.01   | 14.93         |
| G120                 | 1.85 ± 0.19  | 2.04 ± 0.42   | 10.37         |
| I 1.5                | 2.06 ± 0.15  | 2.34 ± 0.42   | 13.19         |
| I 3.0                | 1.51 ± 0.07  | 1.83 ± 0.16±  | 21.28         |
| G56 + I 1.5          | 1.38 ± 0.20  | 1.62 ± 0.44   | 17.61         |
| G56 + I 3.0          | 1.37 ± 0.31  | 1.71 ± 0.59   | 24.91         |
| G120 + I 1.5         | 1.23 ± 0.07  | 1.43 ± 0.31   | 16.97         |
| G120 + I 3.0         | 1.40 ± 0.21  | 1.48 ± 0.31   | 5.36          |
| P 0.03               | 2.40 ± 0.84  | 1.97 ± 0.65   | -17.68        |
| P 0.03 + G56         | 2.48 ± 0.73  | 2.15 ± 0.58   | -13.49        |
| P 0.03 + G120        | 2.67 ± 0.42  | 1.97 ± 0.26±  | -26.40        |
| P 0.03 + I 1.5       | 2.57 ± 0.60  | 2.19 ± 0.40   | -14.74        |
| P 0.03 + I 3.0       | 2.25 ± 0.35  | 2.31 ± 0.76   | 2.56          |
| P 0.03 + G56 + I 1.5 | 2.01 ± 0.51  | 1.95 ± 0.58   | -3.28         |
| P 0.03 + G56 + I 3.0 | 1.70 ± 0.74  | 2.17 ± 0.95   | 27.37         |
| P 0.03 + G120 + I 1.5| 2.49 ± 0.57  | 2.05 ± 0.54   | -17.66        |
| P 0.03 + G120 + I 3.0| 2.64 ± 0.63  | 2.29 ± 0.52   | -13.21        |
| P 0.06               | 2.68 ± 0.95  | 2.56 ± 0.58   | -4.51         |
| P 0.06 + G56         | 2.13 ± 0.44  | 1.90 ± 0.38   | -10.83        |
| P 0.06 + G120        | 2.39 ± 1.16  | 1.83 ± 0.92   | -23.29        |
| P 0.06 + I 1.5       | 1.88 ± 0.40  | 2.29 ± 0.66   | 21.35         |
| P 0.06 + I 3.0       | 1.84 ± 0.64  | 2.04 ± 0.60   | 11.13         |
| P 0.06 + G56 + I 1.5 | 1.48 ± 0.22  | 1.41 ± 0.20   | -4.74         |
| P 0.06 + G56 + I 3.0 | 1.57 ± 0.34  | 1.45 ± 0.31   | -7.40         |
| P 0.06 + G120 + I 1.5| 1.79 ± 0.42  | 1.73 ± 0.49   | -3.00         |
| P 0.06 + G120 + I 3.0| 1.68 ± 0.59  | 1.57 ± 0.33   | -6.53         |

G56: Glucose 56 mmol/L; G120: Glucose 120 mmol/L; I 1.5: Insulin 1.5 IU; I 3.0: Insulin 3.0 IU; P 0.03: Praziquantel 0.03 µg/mL; P 0.06: Praziquantel 0.06 µg/mL; *< 0.05 (t-test).

As to the metabolic alterations due to the presence of insulin and glucose in the culture medium, it was possible to observe a two-fold (glucose 56 mmol/L), three-fold (glucose 120 mmol/L), five-fold (insulin 1.5 IU) and four-fold (insulin 3.0 IU) increase in the SE of succinate (P < 0.05) as compared to the control group (Table 2). Also, it was possible to detect a decrease in half of the SE of fumarate (P < 0.05) which indicated its consumption by the fumarate reductase enzyme to produce succinate. The decrease in the SE of fumarate accompanied the increase in the SE of succinate. Also the non-detection of propionate was explained by the greater SE of succinate.

Table 2

Concentration of organic acids secreted/excreted by T. crassiceps cysticerci in vitro exposed to different concentrations of glucose and insulin. Mean ± SD, mmol/L.

| Group                          | Malate  | Fumarate  | Succinate  |
|--------------------------------|---------|-----------|------------|
| Control + DMSO                 | 19.15 ± 3.63 | 2.95 ± 0.64 | 21.95 ± 3.93 |
| G56                            | 9.60 ± 2.40 | 1.28 ± 0.08 | 47.67 ± 12.39 |
| G120                           | 10.58 ± 1.90 | 1.50 ± 1.26 | 66.60 ± 22.31 |
| I 1.5                          | 10.56 ± 4.97 | 1.11 ± 0.41 | 110.59 ± 15.60 |
| I 3.0                          | 8.00 ± 1.00 | 0.80 ± 0.16 | 92.81 ± 17.86 |
| G56 + I 1.5                    | 7.80 ± 1.56 | 3.19 ± 2.94 | 90.39 ± 41.14 |
| G56 + I 3.0                    | 9.22 ± 0.19 | 1.40 ± 0.27 | 73.50 ± 11.01 |
| G120 + I 1.5                   | 9.18 ± 0.16 | ND         | 25.55 ± 18.60 |
| G120 + I 3.0                   | 7.37 ± 0.84 | 1.15 ± 0.16 | 70.62 ± 55.28 |

G56: Glucose 56 mmol/L; G120: Glucose 120 mmol/L; I 1.5: Insulin 1.5 IU; I 3.0: Insulin 3.0 IU; ND: Non-detected; *< 0.05 (ANOVA).

Another substrates of the TCA cycle, malate presented a decrease in its SE when the cysticerci were treated with high concentrations of glucose (120 mmol/L) and insulin (3.0 IU). Also in these groups, the succinate production was elevated.

3.2. Praziquantel associated to insulin and glucose

The treatment of the T. crassiceps cysticerci with praziquantel induced a decrease in the circumference of the cysticerci (P < 0.05) (Table 1).

The biochemical analyses showed that the partial reverse of the TCA cycle was affected by the association of praziquantel (0.03 µg/mL) and glucose (120 mmol/L), as it was not possible to detect neither fumarate nor succinate in those samples (Table 3). Furthermore, the use of alternative energy sources such as the fatty acids oxidation was also impaired, which was shown as a significant decrease in β-hydroxybutyrate concentrations and non-detection of acetoacetate. This effect was also observed in the group exposed to praziquantel (0.03 µg/mL), glucose (56 mmol/L) and insulin (1.5 IU). However, as the concentration of insulin increased (3.0 IU), the cysticerci were able to use the fatty acids oxidation pathway as the concentrations of β-hydroxybutyrate are closer to the ones found in the control group. Also the higher concentrations of insulin led to significantly higher concentrations of acetoacetate (P < 0.05) indicating that the presence of this hormone helped the cysticerci in the use of alternative energy sources, such as the fatty acids oxidation.

Also, it was possible to observe a decrease in the SE of malate and fumarate (P < 0.05) which led to an undetectable concentration of succinate when the cysticerci were exposed to praziquantel at 0.03 µg/mL with insulin 1.5 IU/mL and both concentrations of glucose.
This study evaluated the biochemical and morphological alterations induced in *T. crassiceps* cysticerci when exposed to praziquantel associated to insulin and glucose. The larval stage of these cysticerci was responsible for budding production and presented an enlargement in their overall size induced by the stimulating factors associated to insulin and glucose. The larval stage of these cysticerci exposed to insulin has also been described by Escobedo et al.[23].

The increase in size of *T. crassiceps* cysticerci in vitro exposed to insulin has also been described by Escobedo et al.[8]. Also, the influence of hormones produced by the host in the development and growth of parasites had already been reported previously[16,22,24,25]. These results indicated that the cysticerci cells responded to this condition similarly to human cells[26].

The effect of insulin in the growth rate of the cysticerci is related to the increase in the availability and intake of the preferable energy source which is glucose and its greater catabolism into the TCA cycle. As succinate is one of the end products of this cycle, it indicates that the most available energy production pathway is being used[7,27].

The correlation of SE of fumarate, succinate and propionate is explained as succinate is the precursor of the propionate production. If it is being secreted, it is not being used for propionate production in accordance to previous descriptions in *T. crassiceps* cysticerci[20].

The decrease of malate concentrations associated to the increase of succinate ones indicates that the excess of substrate for energy production may lead to an intensification of the partial inversion of the TCA cycle which these parasites have already performed[10].

The morphometric effect of praziquantel in the cysticerci circumference may be explained to the effects of praziquantel on the parasite’s tegument associated to the metabolic impairment observed due to the presence of the drug[7,28,29].

The use of alternative energy sources by *T. crassiceps* cysticerci exposed to praziquantel has been described previously[9,20]. The effect of the association of praziquantel and other stressful variables on the metabolism of the cysticerci has not yet been described on the literature. This study indicates that the mode of action of the drug may suffer influences by other substances normally present in the extracellular medium. The decrease in the SE of malate and fumarate when the cysticerci was exposed to praziquantel associated to insulin indicates that the drug’s mode of action was enhanced by the stressing environment provided by the excess of insulin and glucose, as these alterations were detected neither in the control group nor in the cysticerci exposed to insulin or glucose isolated. The decrease in this metabolism is in accordance to previous studies from our group[20].

The presence of insulin in the culture medium induced morphologic alterations in *T. crassiceps* cysticerci, while the association of praziquantel, glucose and insulin induced the shrinking of the cysticerci. The metabolic effects are related to a decrease in the partial reverse of the TCA cycle metabolites due to the drug’s mode of action. Interestingly, the praziquantel, insulin and glucose association enhanced the drug’s mode of action.

### 4. Discussion

This study evaluated the biochemical and morphological alterations induced in *T. crassiceps* cysticerci when exposed to praziquantel associated to insulin and glucose. The larval stage of these cysticerci was responsible for budding production and presented an enlargement in their overall size induced by the stimulating factors such as insulin, which acted as growth factor for several different cell types[22]. The increase in size of *T. crassiceps* cysticerci in vitro exposed to insulin has also been described by Escobedo et al.[8].

This may be explained as cestodes present an insulin receptor which is able to modulate the parasite’s development and differentiation[23]. Also, the influence of hormones produced by the host in the development and growth of parasites had already been reported previously[16,22,24,25]. These results indicated that the cysticerci cells responded to this condition similarly to human cells[26].

The effect of insulin in the growth rate of the cysticerci is related to the increase in the availability and intake of the preferable energy source which is glucose and its greater catabolism into the TCA cycle. As succinate is one of the end products of this cycle, it indicates that the most available energy production pathway is being used[7,27].

The correlation of SE of fumarate, succinate and propionate is explained as succinate is the precursor of the propionate production. If it is being secreted, it is not being used for propionate production in accordance to previous descriptions in *T. crassiceps* cysticerci[20].

The decrease of malate concentrations associated to the increase of succinate ones indicates that the excess of substrate for energy production may lead to an intensification of the partial inversion of the TCA cycle which these parasites have already performed[10].

The morphometric effect of praziquantel in the cysticerci circumference may be explained to the effects of praziquantel on the parasite’s tegument associated to the metabolic impairment observed due to the presence of the drug[7,28,29].

The use of alternative energy sources by *T. crassiceps* cysticerci exposed to praziquantel has been described previously[9,20]. The effect of the association of praziquantel and other stressful variables on the metabolism of the cysticerci has not yet been described on the literature. This study indicates that the mode of action of the drug may suffer influences by other substances normally present in the extracellular medium. The decrease in the SE of malate and fumarate when the cysticerci was exposed to praziquantel associated to insulin indicates that the drug’s mode of action was enhanced by the stressing environment provided by the excess of insulin and glucose, as these alterations were detected neither in the control group nor in the cysticerci exposed to insulin or glucose isolated. The decrease in this metabolism is in accordance to previous studies from our group[20].

The presence of insulin in the culture medium induced morphologic alterations in *T. crassiceps* cysticerci, while the association of praziquantel, glucose and insulin induced the shrinking of the cysticerci. The metabolic effects are related to a decrease in the partial reverse of the TCA cycle metabolites due to the drug’s mode of action. Interestingly, the praziquantel, insulin and glucose association enhanced the drug’s mode of action.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

This study was financed by National Council for Scientific and Technological Development (Grant No. 473499/2009-6). Also the
authors would like to thank Merck for the praziquantel donation.

References

[1] Vaz AJ, Nunes CM, Piazza RM, Livramento JA, Da Silva MV, Nakamura PM, et al. Immunoblot whith cerebrospinal fluid form patients with neurocysticercosis using antigen from cysticerci of Taenia solium and Taenia crassiceps. Am J Trop Med Hyg 1997; 57(3): 354-7.

[2] Willms K, Zurabian R. Taenia crassiceps: in vivo and in vitro models. Parasitology 2010; 137(3): 335-46.

[3] Matos-Silva H, Reciputi BP, Paula EC, Oliveira AL, Moura VB, Vinaud MC, et al. Experimental encephalitis caused by Taenia crassiceps cysticerci in mice. Arq Neuropsiquiatr 2012; 70(4): 287-92.

[4] Sciuuto E, Fragoso G, Hernández M, Rosas G, Martínez JJ, Fleury A, et al. Development of the S3Pvac vaccine against porcine Taenia solium cysticercosis: a historical review. J Parasitol 2013; 99(4): 686-92.

[5] Palomares F, Palencia G, Ambrosio JR, Ortiz A, Jung-Cook H. Evaluation of the efficacy of albendazole sulphoxide and praziquantel in combination on Taenia crassiceps: in vitro studies. J Antimicrob Chemother 2006; 57(3): 482-8.

[6] Palomares F, Palencia G, Pérez R, Gonzalez-Esquivel D, Castro N, Cook HJ. In vitro effects of albendazole sulfoxide praziquantel against Taenia solium and Taenia crassiceps cysts. Antimicrob Agents Chemother 2004; 48(6): 2302-4.

[7] Vinaud MC, de Souza Lino Junior R, Bezerra JC. Taenia crassiceps organic acids detect in cysticerci. Exp Parasitol 2007; 116(4): 335-9.

[8] Escobedo G, Romano MC, Morales-Montor J. Differential in vitro effects of insulin on Taenia crassiceps and Taenia solium cysticerci. J Helminthol 2009; 83(4): 403-12.

[9] Fraga CM, Costa TL, Bezerra JC, de Souza Lino Junior R, Vinaud MC. Fatty acids oxidation and alternative energy sources detected in Taenia crassiceps cysticerci after host treatment with anthelmintic drugs. Exp Parasitol 2012; 131(1): 111-5.

[10] de Almeida Leandro L, Fraga CM, de Souza Lino R Jr, Vinaud MC. Partial reverse of the TCA cycle is enhanced in Taenia crassiceps experimental neurocysticercosis after in vivo treatment with anthelmintic drugs. Parasitol Res 2014; 113(4): 1313-7.

[11] O’Neal SE, Moyano LM, Ayvar V, Gonzalez G, Diaz A, Rodriguez S, et al. Geographic correlation between tapeworm disease and heavily infected cysticercotic pigs. PLoS Negl Trop Dis 2012; 6(12): e1953.

[12] Komba EV, Kimbi EC, Ngowi HA, Kimera SI, Mlangwa JE, Lekule FP, et al. Prevalence of porcine cysticercosis and associated risk factors in smallholder pig production systems in Mbeya region, southern highlands of Tanzania. Vet Parasitol 2013; 198(3-4): 284-91.

[13] Liu L, Zhu HR, Yang GJ. [Current situation of endemic status, prevention and control of neglected zoonotic diseases in China]. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 2013; 25(3): 307-11. Chinese.

[14] Rasamololina-Andriamanivo H, Porphyre V, Jambou R. Control of cysticercosis in Madagascar: beware of the pitfalls. Trends Parasitol 2013; 29(11): 538-47.

[15] Jayashii CM, Gonzalez AE, Castillo Neyra R, Rodriguez S, Garcia HH, Lightowers MW, et al. Validity of the enzyme-linked immunoelectrotransfer blot (EITB) for naturally acquired porcine cysticercosis. Vet Parasitol 2014; 199(1-2): 42-9.

[16] Brehm K, Spiliotis M. The influence of host hormones and cytokines on Echinococcus multilocularis signaling and development. Parasitol Int 2008; 15(3): 286-90.

[17] You H, Gobert GN, Jones MK, Zhang W, McManus DP. Signalling pathways and the host-parasite relationship: putative targets for control interventions against schistosomiasis: signalling pathways and future anti-schistosome therapies. Bioessays 2011; 33(3): 203-14.

[18] Vanderstraete M, Gouignard N, Cailliau K, Morel M, Lancelot J, Bodart JF, et al. Dual targeting of insulin and venus kinase receptors of Schistosoma mansoni for novel anti-schistosome therapy. PLoS Negl Trop Dis 2013; 7(5): e2226.

[19] Rodriguez-Contraseras D, Skelly PJ, Landa A, Shoemaker CB, Laclette JP. Molecular and functional characterization and tissue localization of 2 glucose transporter homologues (TGTP1 and TGTP2) from the tapeworm Taenia solium. Parasitology 1998; 117(Pt 6): 579-88.

[20] Vinaud MC, Ferreira CS, Lino Junior Rde S, Bezerra JC. Taenia crassiceps: fatty acids oxidation and alternative energy source in in vitro cysticerci exposed to anthelmintic drugs. Exp Parasitol 2009; 122(3): 208-11.

[21] Fraga CM, Costa TL, Bezerra JC, de Souza Lino R Jr, Vinaud MC. Taenia crassiceps: host treatment alters glycolysis and tricarboxylic acid cycle in cysticerci. Exp Parasitol 2012; 130(2): 146-51.

[22] Hemer S, Konrad C, Spiliotis M, Kozioł U, Schaack D, Förster S, et al. Host insulin stimulates Echinococcus multilocularis insulin signaling pathways and larval development. BMC Biol 2014; 12: 5.

[23] Konrad C, Kroner A, Spiliotis M, Zavalà-Góngora R, Brehm K. Identification and molecular characterization of a gene encoding a member of the insulin receptor family in Echinococcus multilocularis. Int J Parasitol 2003; 33: 301-12.

[24] Escobedo G, Roberts CW, Carrero JC, Morales-Montor J. Paratube regulation by host hormones: an old mechanism of host exploitation? Trends Parasitol 2005; 21(12): 588-93.

[25] Bernin H, Lotter H. Sex bias in the outcome of human tropical infectious diseases: influence of steroid hormones. J Infect Dis 2014; 209(Suppl 3): S107-13.

[26] Kayegama S, Yokoo H, Tomita K, Kayegama-Yahara N, Uchimido R, Matsuda N, et al. High glucose-induced apoptosis in human coronary artery endothelial cells involves up-regulation of death receptors. Cardiovasc Diabetol 2011; 10: 73.

[27] Corbin I, Simcoff R, Novak M, Blackburn BJ. Metabolism of [3-(13)C]-pyruvate by cysticerci of Taenia crassiceps. Parasitol Res 1998; 84(6): 516-8.

[28] Chan JD, Zarowiecki M, Marchant JS. Ca2+ channels and praziquantel: a view from the world. Parasitol Int 2013; 62(6): 619-28.

[29] Cioli D, Pica-Mattoccia L, Basso A, Guidi A. Schistosomiasis control: praziquantel forever? Mol Biochem Parasitol 2014; 195(1): 23-9.