METHODS OF DIAGNOSIS AND TREATMENT

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COMPARISON OF ANTI-OPISTHORCHIASIS EFFECT OF PRAZIQUANTEL AND COMPLEXES OF ALBENDAZOLE WITH ARABINOGALACTAN

ABSTRACT

Opisthorchiasis is a helminthiasis affecting mainly the hepatobiliary system and pancreas; its most dramatic complication is malignization of the organs infected by the parasites. The causative agents of opisthorchiasis are two species of liver flukes, the trematodes belonging to the family Opisthorchiidae - Opisthorchis felineus and O. viverrini. The Chinese liver fluke, Clonorchis sinensis, also member of the family Opisthorchiidae, causes clonorchiasis, a disease very close in symptomatology.

Two thirds of world area of opisthorchiasis is located in the territory of Russia. The hyper-endemic region is world's largest opisthorchiasis foci in the Ob-Irtysh river basin. Incidence of opisthorchiasis in different localities of this region exceeds the all-Russian from 3 to 28 times.

The main drug for specific therapy of opisthorchiasis is praziquantel. This drug exhibited positive effects upon treatment of acute opisthorchiasis, though it was not sufficiently effective in cases of most widely spread chronicopisthorchiasis. Previously, we showed that the complexes of albendazol, the anthelminthic of a broad spectrum of activities, and arabinogalaktan possess anti-opisthorchiasis effect.

Aim of this study is the comparative assessment of efficiency of praziquantel and complexes of albendazole with arabinogalactan on O. felineus on model of experimental opisthorchiasis.

The experiments were carried out on five groups of golden hamsters Mesocricetus auratus infected with O. felineus and receiving various doses of praziquantel and complexes of albendazole with arabinogalactan. The group of the animals infected with O. felineus who weren't receiving anthelminthics has been used as control. The obtained data indicate that efficiency of complexes of albendazole with arabinogalactan on model of experimental opisthorchiasis on golden hamsters is similar to therapeutic efficiency of praziquantel.

Thus, results of this study confirm a possibility of the development of drugs for treatment of opisthorchiasis on the basis of intermolecular complexes of albendazole with arabinogalactan. Besides, as praziquantel and albendazole possess different mechanisms of action on helminths, it is possible to assume that combinatory action of a praziquantel and complexes of albendazole with arabinogalactan can be more effective on O. felineus than individual effects of these drugs.

Keywords: opisthorchiasis, Opisthorchis felineus, praziquantel, albendazole, intermolecular complexes of albendazole with arabinogalactan.

INTRODUCTION

Opisthorchiasis is a helminthiasis affecting mainly thehepatobiliary system and pancreas. Infection occurs when eating raw river fish infested with helminth larvae. Specificof the human disease is a long duration, frequent exacerbations, and possible induction of the primary liver and pancreatic cancers [1, 13]. The causative agents of opisthorchiasis are two species of liver flukes, the trematodes belonging to the family Opisthorchiidae - *Opisthorchis felineus* and *O. viverrini*. The Chinese liver fluke, *Clonorchis sinensis*, also member of the family Opisthorchiidae, causes clonorchiasis, a disease very close in symptomatology [6].

Opisthorchiasis and clonorchiasis are food borne trematodiasis the natural foci of which cover a considerable part of Europe and Asia. However, these parasitoses can be currently only arbitrary attributed to natural focal diseases. Many emigrants from Asia live in the areas non-endemic for opisthorchiasis and clonorchiasis, and the tourist exchange between various countries is ever increasing. Consequently, the patients suffering from liver fluke infection can be recorded far from the corresponding endemic regions [14].

According to different estimations, up to 40 million peopleare currently infected with the liver flukes belonging to the family Opisthorchiidae (*O. felineus, O. viverrini*, and *C. sinensis*) and up to 600–750 million people in Eurasian countries constitute the risk group[5, 7].

Two thirds of world area of opisthorchiasis is located in the territory of Russia. According to the state reports of 2007 and 2008 year "About a sanitary and epidemiologic situation in the Russian Federation" in the country about 40 thousand patients annually are registered. The hyper-endemic region is world's largest opisthorchiasis foci in the Ob-Irtysh river basin. Incidence of opisthorchiasis in different localities of this region exceeds the all-Russian from 3 to 28 times. According to the results of researches which are carried out by different groups of the Russian scientists, the prevalence of opisthorchiasis amongst rural population in endemic regions fluctuates from 10% and to 45%, which exceeds data of official statistics significantly [1-3]. Thus, causative agent of opisthorchiasis, O. felineus, can be counted as an adverse factor influencing a state of health of the population in subarctic regions of Siberia.

Taking into account a high abundance of opisthorchiasis in Russian Federation, developing of adequate therapeutic and prevention methods for this pathology is a topical problem. Since any vaccines for prevention of parasitoses are yet unavailable, chemotherapy plays the main role in treatment of invasions. The selection of pharmacological preparations for chemotherapy is determined by a number of characteristics, including a wide range of action, high efficiency and selectivity of the drug towards the parasite in combination with a low toxicity, and a minimum of side effects towards the final host, first and foremost, human. The first preparation maximally meeting these requirements was praziquantel. Today, it is the drug of choice and is widely used in the therapy of trematodiases of various etiologies, in particular, opisthorchiasis, clonorchiasis, and schistosomiasis [8].

Praziquantel is not a perfect drug in every respect, because it (1) is inefficient towards the eggs and immature

worms;(2) is not free from side effects; (3) is able to induce resistance development; (4) fails to prevent reinfection; and (5) is administered as a race mate rather than as a pure active enantiomer, which lessens its pharmacological characteristics. Besides, we have shown that efficiency of praziquantel for therapy of experimental O. felineus opisthorchiasis is not more than 80% [12].

Therefore, the development of novel anti-opisthorchiasis agents and/ or increase in efficiency of the existing anthelminthics is considered to be a challenging problem of modern medicine and pharmacology.

Albendazole is anthelminthic with broad spectrum of activity. This drug is efficient against many species of helminths, however its action on *O. felineus* is not so efficient than praziquantel action. Earlier we have carried out comparative assessment of anti-opisthorchiasis effect of officinal albendazole and supramolecular complexes of albendazole and polysaccharide arabinogalactan isolated from the wood of larches Larix sibirica and Larix gmelinii (ABZ-AG).It has been shown that at equal dosages anthelminthic activity of the ABZ-AG complexes is significantly higher, than activity of officinal albendazole [4].

Aim of this study is the comparative assessment of efficiency of praziquantel and complexes of albendazole with arabinogalactan on O. felineus on model of experimental opisthorchiasis.

MATERIALS AND METHODS

O. felineus metacercariae were collected from naturally infected fish (Leuciscus idus) caught in the Ob River near the city of Novosibirsk. The fish meat was digested by pepsin – HCl overnight at 37°C, metacercariae were washed and counted [1]. Golden hamsters (Mesocricetus auratus) were purchased from the stock of the Puschino Animal Facility (Russia). The animals were kept and treated according to protocols approved by the Committee on the Ethics of Animal Experiments of the Institute of Cytology and Genetics (Permit Number: 7 of 19.12.2011). Hamsters were infected per os with 50 viable active metacercariae two months before the experiment.

Praziquantel was used as the suspension in 7% Tween-80 and 3% Ethanol, ABZ-AG complexes were used as water suspensions. ABZ-AG complexes were synthesized in the Institute of Solid State Chemistry and Mechanochemistry of Siberian Branch of Russian Academy of Sciences. The studied compositions were administered to the animals per os. The hamsters were treated either once or daily for several days, depending on the experimental scheme used. The experimental groups contained 7 – 22 animals. The control group was formed of the hamsters who weren't receiving anthelminthics. Animals were euthanized in 21 days after the anthelminthics treatments. Adult worms of O. felineus were obtained from bile ducts of euthanized hamsters.

Two doses of praziquantel were used in the study: 75 mg/kg and 400 mg/kg of weight of an animal. The first dose (75 mg/kg) and a way of administration (3 times in 4-6 hours on 25 mg/kg) is taken from recommendations for opisthorchiasis therapy. The second dose (400 mg/kg, single administration) is chosen for an assessment of efficiency of praziquantel high dose, essential exceeding the recommended doses for therapy of opisthorchiasis.

For comparison of efficiency of praziquantel and the ABZ-AG complexes, we used the compositions of albendazole and arabinogalactan prepared in the ratio 1:10 [10]. It has been found in preliminary experiments, that arabinogalactan doesn't possess anthelminthic activity, therefore, when testing activity of composition of albendazole and arabinogalactan, only the mass of albendazole were considered. ABZ-AG complexes were used in doses 75 mg/kg, 150 mg/kg and 300 mg/kg. We have chosen these doses of albendazole in combination with arabinogalactan, since they have relatively high anthelminthic efficiency and do not have toxic effect on liver tissues of golden hamsters [5]. To reach dose 75 mg/kg, animals received 25 mg/kg within three days once a day. To reach doses 150 mg/kg and 300 mg/kg, animals received 50 mg/kg once a day until achievement of a final dose. The obtained results are presented in table 1.

RESULTS AND DISCUSSION

The data obtained in this study indicate that efficiency of ABZ-AG complexes on model of an experimental opisthorchiasis on golden hamsters is similar to therapeutic efficiency of praziquantel. So, efficiency of the maximum dose of ABZ-AG (300 mg/kg) above efficiency of the maximum dose of praziquantel (400 mg/kg). However efficiency of praziquantel dose 75 mg/kg, which is recommended for therapy of opisthorchiasis, surpasses efficiency of ABZ-AG doses 75 mg/kg and 150 mg/kg, which are similar to albendazole doses used in clinical and laboratory practice (table).

Application of albendazole against tissue parasites is limited by its extremely low water solubility and, respectively, low absorption and bioavailability (<5%). Earlier we have shown that inclusion of albendazole in complexes with arabinogalactan allows increasing repeatedly its water solubility [10]. It is also important to note that the ABZ-AG complexes possess smaller toxicity, than albendazole and praziquantel [4]. Obtained results confirm a possibility of the development of drugs for treatment of opisthorchiasis on the basis of intermolecular complexes of albendazole with arabinogalactan.

It is important to note that praziquantel and albendazole possess different mechanisms of action on helminths and damage different molecular targets. Praziquantel increases permeability of cell membranes for Ca2+ that leads to the generalized reduction of muscles passing into persistent paralysis which consequence is a death of helminths [9,12]. Besides, praziquantel causes a vacuolization and damage of an epithelium that significantly decreases efficiency of helminth protection from host immune system and increases helminth vulnerability by digestive enzymes.

The mechanism of albendazole action is connected with suppression of polymerization of β-tubulin and destruction of cytoplasmatic microtubules of helminths intestinal cells. In addition, albendazole impairs glucose utilization, suppresses ATP synthesis, blocks transportation of secretory granules and other organelles in muscle cells of helminths.

Thus, it is possible to assume that combinatory action of praziquantel and albendazole can be more effective than individual effects of these drugs. Testing of combinatory effect of praziquanteand the ABZ-AG complexes is perspective research in the context of development of novel anthelminthic drugs possessing increased efficiency and safety.

CONCLUSION

Therapeutic efficiency of complexes of albendazole with arabinogalactan on model of experimental opisthorchiasis on golden hamsters is similar to therapeutic efficiency of praziquantel. Thus, results of this study confirm a possibility of the development of drugs for treatment of opisthorchiasis on the basis of intermolecular complexes of albendazole

Comparison of anti-opisthorchiasis effect of praziquantel and the ABZ-AG complexes various doses

Anthelmin-thic drugs	Dose per animal (mg/kg)	Number of animals	Numberof *O. felineus*adults per animal, ± SD	Mortality of *O. felin-eus*adults, %
Praziquantel	75	17	10 ± 4	70
Praziquantel	400	7	6 ± 4	81
ABZ-AG	75*	19	14 ± 5	60
ABZ-AG	150*	20	12 ± 5	64
ABZ-AG	300*	20	2 ± 2	94
-	0	22	34 ± 12	0

* dose of albendazole

with arabinogalactan. Besides, as praziquantel and albendazole possess different mechanisms of action on helminths, it is possible to assume that combinatory action of a praziquantel and complexes of albendazole with arabinogalactancan are more effective on *O. felineus*than individual effects of these drugs.

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References

1. Brazhnikova N.A. Tolkaeva M.V. Rak pecheni, zhelchnyh putej i podzheludochnoj zhelezy pri hronicheskom opistorhoze [Cancer of liver, biliary tracts and pancreas at chronic opisthorchiasis]. Bjulleten' sibirskoj mediciny [Bull Sib Med.] – 2002. - № 2. - p. 71-77.

2. Il'inskih E. N. Novickij V. V. Il'inskih N. N. [i dr.]. Invazii Opisthorchis felineus (Rivolta, 1884) i Metorchis bilis (Braun, 1890) u cheloveka v razlichnyh regionah Ob'-Irtyshskogo rechnogo bassejna [Opisthorchis felineus (Rivolta, 1884) and Metorchis bilis (Braun, 1890) infections in population of some regions of the Ob river basin]. Parazitologija [Med Parazitol (Mosk)]. - 2007. - V. 41 (1). - p. 55 - 64.

3. Zubov N.A., Belikov E.S., Zajceva L.N. [i dr.]. Opistorhoz v Tobol'ske po dannym autopsii za 1950-1987 gg.[Opisthorchiasis in Tobolsk based on 1950-1987 autopsy data]. Med Parazitol (Mosk). – 1989. - № 5. – P. 10-3.

4. A Physicochemical and Pharmacological Study of the Newly Synthesized Complex of Albendazole and the Polysaccharide Arabinogalactan from Larch Wood / Y.S. Chistyachenko , E.S. Meteleva, M.Y. Pakharukova [et al.] // Current Drug Delivery. – 201. - 12(5):477-90.

5. Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of interleukin-6 / B. Sripa, E. Mairiang, B. Thinkhamrop [et al.] // Hepatology. – 2009. - 50(4):1273-1281.

6. King S. Trematodes of the family Opisthorchiidae: a minireview / S. King, T. Scholz // Korean Journal of Parasitology. – 2001. - 39(3):209-221.

7. Liver fluke induces cholangiocarcinoma / B. Sripa, S. Kaewkes, P. Sithithaworn [et al.] // PLOS Medicine. – 2007. - 4(7):e201.

8. Mordvinov V.A. The Digenea parasite *Opisthorchis felineus*: a target for the discovery and development of novel drugs / V.A. Mordvinov, D.P.Furman // Infectious Disorders - Drug Targets. – 2010. - 10(5):385-401.

9. Olliaro P. The little we know about the pharmacokinetics and pharmacodynamics of praziquantel (racemate and R-enantiomer) / P. Olliaro, P. Delgado-Romero, J. Keiser // Journal of Antimicrobial Chemotherapy. – 2014. - 69(4): 863-70.

10. Physicochemical properties and anti-opisthorchosis effect of mechanochemically synthesized supramolecular complexes of Albendazole with the polysaccharide Arabinogalactan from Larix sibirica and Larix gmelinii / Y.S. Chistyachenko, M.V. Khvostov, A.I. Belousov [et al.] // Doklady Biological Sciences. – 2014. - 456 (1):212-4.

11. Sripa B. Localization of parasite antigens and inflammatory responses in experimental opisthorchiasis / B. Sripa, S. Kaewkes // International Journal for Parasitology. – 2000. - 30(6):735-740.

12. The first comprehensive study of praziquantel effects in vivo and in vitro on European liver fluke *Opisthorchis felineus* (Trematoda) / M.Y. Pakharukova, A.G. Shilov, D.S. Pirozhkova [et al.] // International Journal of Antimicrobial Agents. – 2015. - 46(1):94-100.

13. Torgerson P.R. World Health Organization Estimates of the Global and Regional Disease Burden of 11 Foodborne Parasitic Diseases, 2010: A Data Synthesis / P.R. Torgerson, B. Devleesschauwer, N. Praet // PLOS Medicine. – 2015. - 12(12):e1001920.

14. Tselepatiotis E. A case of Opisthorchis felineus infestation in a pilot from Greece / E. Tselepatiotis, S. Mantadakis, S. Papoulis // Infection. – 2003. - 31(6):430-432.

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