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A Morphological Study on the Effect of Liposomally Administered Albendazole on the *Trichinella spiralis* Muscle Stage in Mice

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The aim of the present morphological study is to establish the effect of the liposomally encapsulated and separately applied albendazol during the muscle stage of Trichinella infection. The therapy was applied once a week at doses of 24 mg/kg body weight for the liposome encapsulated albendazol, the separately applied albendazol and the control groups. The corresponding medications were injected intraperitoneally once a week. The treatment was started on day 35-38 after the infection when the larvae are localized in the muscle tissue (muscle stage of Trichinellosis). The efficiency of the conducted therapy is measured as follows: liposome-encapsulated albendazol shows a statistically significant efficiency of 69 percent for the 24 mg/kg dose while albendazol applied separately indicates a statistical significance of 36 percent for the 24 mg/kg dose. The pathomorphological impact obtained as a result from the action of the antihelminthics shows that in the different groups there are differences only as to the numbers of the living and dead larvae. According to pathomorphological changes of the capsules, the parasites and the spaces around them are identical both for the liposome-encapsulated and the separately applied albendazol. The muscle samples for the histological study were removed from the thoracic musculature of the experimental animals.

Key words: pathomorphology, liposomes, *Trichinella spiralis*, albendazole.

Introduction

Trichinellosis is a parasitic disease spread throughout the world. It is caused by various representatives of the Trichinella genus after consumption of infected meat or meat products insufficiently cooked.

The modern therapy of Trichinellosis is being carried out by means of antihelminthics from the benzamidazole group with mebendazole, albendazole, flubendazole, etc. [4]. The intestinal phase of the ailment is prone to treatment but unfortunately it is diagnosed rather late when the parasite is already encapsulated in the muscle cells. At that stage, the treatment requires high doses of the preparation which in most cases leads to undesired side effects [7].

Recently it became possible to solve some of the problems associated with the usage of the therapeutic agents through a system of carriers in the organism. These systems

optimize the action of the medicament and direct it to the target parts of the organism in order to overcome the cell barriers.

Liposomes as the most promising carriers are biodegradable, easy-to-prepare and also diverse in terms of composition, size and other structural parameters defining the various putative mechanisms of interaction of the liposomes with the biological entities [10].

For the first time, liposomes were received and used by the team of A. Bangham [2]. Originally, they were used for modeling ionic transport via the cell membrane due to their structural similarity with the lipid bilayer membrane of the cell. Later on, Gregoriadis et al. have found application of the different types of liposomes for improving the therapy of various diseases and their diagnostics [10]. Liposomal encapsulation of the antihelminthics helps the therapy, decreases the resistance and creates the so-called depot-effect (the corresponding medicines are introduced once per week).

The purpose of current study is to compare morphologically the action of the liposome-encapsulated and free-applied albendazol in the muscular stage of Trichinellosis caused by *Tr. spiralis*.

Materials and Methods

Experimental animals and concept of experiment

White mice weighing about 30 g on average were infected per os with about 100 *Trichinella* larvae of the *Tr. spiralis* species. On day 35 after the infection with the well expressed muscle stage of the disease the corresponding experimental groups were formed – one for the free applied albendazole and the other for the liposome-encapsulated albendazole respectively. Each group numbered 30 mice. Albendazole at a dose of 24 mg/kg body weight was used i.p. introduced in the course of nine weeks. A control group was injected with saline.

Biotechnological methods: Liposomal encapsulation of albendazole

The technology encompasses three stages: The production of multilamellar liposomes of the Banghams type [2]. For this purpose, the corresponding amounts of albendazole (pharmaceutically pure substances Cypla, India) were dissolved in a chloroform solution of the lipid, evaporated on a vacuum evaporator until the final elimination of the solvent and the lipid film such obtained was hydrated by the addition of the water liposomal phase 150 ml saline. A thermal cycle of freezing (in liquid nitrogen) and defrosting of the obtained liposomal suspension was carried out and subjected to extrusion through 100 nm of polycarbonate membranes by an extruder Emulsiflex C5 of the Avestin firm. Thus obtained liposomal medicament containing albendazole was kept in the presence of an inert gas at – 25 °C and used for treating the experimental animals.

Preparation of non-liposomal albendazole

Immediately prior to the experiment the needed amounts of albendazole were dissolved in saline with an ensuing intermittent ultrasonic processing twice for 20 sec with a 1-minute pause between both sonifications.

Pathomorhological methods

Histological studies on thoracic musculature from control and experimental animals were carried out. Material was removed from the experimental animals in the ninth week after the beginning of the therapy with the corresponding preparation.

Fixation and embedding

The material was fixed in the medium of Serra for 45 min up to 1 hour. After the fixation, the samples were dehydrated in a series of alcohols with growing concentrations (70%, 80%, 96% and 100%). Impregnation in cedar oil was performed for 12-18 h followed by embedding in paraffin. The material was cut out on a microtome and sections of a 6 μ thickness were prepared and then mounted on slides. Then staining was performed with hematoxylin – eosin with the sections first deparaffinized in xylol, whereafter dehydrated in a series of alcohols, washed in water and stained with the hematoxylin of Maier for 5 to 7 min and counterstained with 1% water solution of eosin for 1-2 min and embedded in Canadian balm. As a result of the staining the nuclei were colored in blue and the cytoplasm of the cells in pink.

Results and Discussion

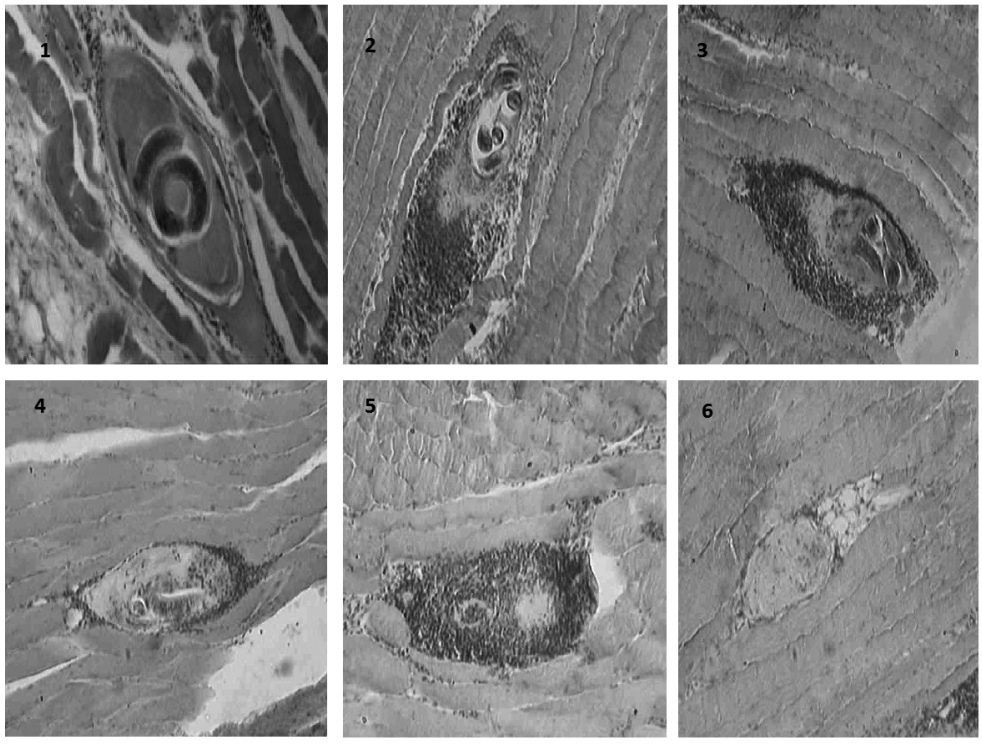
The following results were obtained upon comparing the therapeutic activity of liposomal albendazol and free (non-encapsulated in liposomes) albendazol on a murine experimental model. The exact efficacy of the preparations is 69% for the dose of 24 mg/kg body weight in the case of the liposome-encapsulated antihelminthics, while for the free applied albendazol the efficiency is 36% per dose of 24 mg/kg body weight, respectively.

The conducted morphological research definitely confirms the destructive action of the antihelminthic medicines over the Trichinella larvae encapsulated in the muscle. The obtained results are shown in **Figs. 1-6**.

The pathomorphological pattern in the separate experimental groups shows that differences are found only in the numbers of the live and dead larvae. The samples for the histological studies were taken from the pectoral musculature of the experimental animals. This is in accordance with the observations of other authors who have found that the larvae of the *Tr. spiralis* species have a predilection to the tonic muscles with good blood supply – the diaphragm, pectoral musculature, the masseter muscles, tongue and intercostals musculature [1, 6].

A structure typical for the muscle phase of trichinellosis is observed in the tissues of the control animals: a well-shaped capsule of connective tissue around an intact Trichinella larva which is lemon-shaped and with a thick matrix (**Fig. 1**).

Inflammatory cells are not found in the surrounding muscular tissue. After mebendazole treatment a multitude of pathomorphological alterations are established affecting the capsules, the parasite itself, and the spaces around them. A strong inflammatory reaction is observed in the infected zones expressed in an augmentation of the macrophage and polymorphonuclear numbers (**Figs. 2, 3**). The reaction of inflammation is localized around and inside the Trichinella capsules. This is due to progressive destruction of the capsule wall and the following resorption which allows the inflammatory cells to penetrate into it (**Figs. 3, 4)** The matrix is strongly reduced and modified as the capsule is filled with cell detritis (**Fig. 4).** Parallelly to this process, the parasite larvae inside the capsule are also subject to changes. Initially, the cuticle is injured which is expressed in the damage of its integrity. Gradually, the parasite larvae die out and destroyed capsules containing residual parasitic material are observed (**Figs. 3, 4**). The process is terminated by a complete destruction of the Trichinella larvae and their total lysis (**Fig. 6**). In most cases the striation of miofibres adjacent to the capsules is lost (**Fig. 3**). The application of the liposome-encapsulated medicament with albendazol over a seven-day interval in the course of 9 weeks has led to the killing of the Trichi-



Figs. 1-6. Major morphological changes in the chest musculature of mice infected with *Tr. spiralis* following albendazole treatment. Hematoxylin-eosin staining: 1. – A control group – well formed capsule containing an undamaged larva; 2. – A capsule with lytic changes and an abundant intra – and pericapsular infiltration with inflammatory cells; 3. – A total resorption of the capsule wall infiltrated by a great number of macrophages and polymorphonuclears; 4. – A capsule with strongly attenuated matrix filled with cell detritis; 5. – Totally destroyed larvae in capsules infiltrated with inflammatory cells; 6. – A total lysis of a capsule and a larva, 10×

nella larvae – 69% at the dose of 24 mg/kg (**Fig. 6**). Such a result was not recorded in any of the controls. In all groups treated with the liposome-encapsulated antihelminthic a progressive development of the pathomorphological changes in the encapsulated larvae was observed ending with destruction of the trichinellae. According to Despommier [5], the infective larva of *Tr. spiralis* causes a redifferentiation of the muscle fibres expressed in an augmentation of the nuclei and the mitochondria numbers. The nurse cells maintain the biochemically compatible environment which is needed for the development and the continuous existence of the parasite allowing it to assimilate nutrients and to remove waste products. In that respect the development and encapsulation of the Trichinella larvae does not cause an inflammatory process in the entire tissue only with a local character. The immune response of the tissues of the host against the parasites usually is very weak and it is reinforced by the negative chemotaxis of the leucocytes and platelets, secretory or excretory products of the parasite and the immunosuppressive action of the larvae. The application of albendazole changes the character and the intensity of the cellular inflammatory reaction [8]. Albendazole is considered to be one of the most efficient antihelminthics but it has a low solubility in water solutions. Therefore after an oral application of the medicine only a small part of it reaches the parasite

while the majority of the substance remains in the intestines and is disposed from the body undigested [9]. Consequently, increasing doses of the benzimidazole preparations is acceptable especially in the treatment of the muscle stage of trichinelosis but is accompanied by the risk of side effects for the host. According to Bogan and Mariner [3] the higher activity of the benzimidazole preparations leads to greater toxicity. Such disadvantage can be resolved by the encapsulation of much lower concentrations of the medicament in a suitable carrier such as the liposome. In this way the biological activity of the medicine is definitely enhanced [8].

The results obtained by us confirm the higher efficiency of the liposome-encapsulated albendazole compared to that applied in the free state.

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