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Therapeutic Activity of Albendazole on a Murine Experimental Model of the Muscle Stage of Trichinellosis

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A comparative study on the anti-Trichinella efficiency of liposomal Albendazole (LA) and free (unincorporated in liposomes) Albendazole (A) has been carried out on an experimental murine model of the muscle stage of trichinellosis, i.e. when parasite larvae are encapsulated into the muscle tissue and routine therapy has proven inadequate. The results showed that (LA) yields a 69% efficiency compared to a slight 36% for (A) applied at equal doses.

Key words: liposomes, Trichinellosis, Albendazole, fitting analysis.

Introduction

Trichinellosis is one of the most detrimental parasitic ailments in the animals and the human caused by representatives of the Trichinella genus (Nematoda class – roundworms).

The epidemic outbursts in their character-massiveness and unpredictability resemble the ones of many infectious diseases (dysentery, typhoid, tularemia) the complications being very perilous both in its acute and chronic forms. In the literature known to us to the present moment no successful treatment of the muscle stage has ever been reported [2, 3, 4]. That is why our investigations were aimed at the treatment of this phase of the disease.

Based on the liposomal theory for transferring therapeutic substances to damaged organs and cells [1] the mechanism of the improved therapeutic activity of (LA) as compared with (A) is explained by the "delivery" of Albendazole in the muscle cell in close proximity to the parasite. Thus, the larvae are found at a constant concentration of the anthelmintic which is detrimental to them while at routine application a higher concentration of the drug is reported in the patient's blood while in situ concentration is insufficient and dwindling very fast with time. The period of demi-elirainationfor most drugs is several hours. A paradox is observed when the parasite is attacked by an insufficiently high concentration of the anthelmintic which hasn't capacity to destroy it and the concentration in the target cell rapidly decreases being at the same time comparatively high which leads to toxic effects for the healthy tissues. After the liposome comes

in contact with the cell either through adsorption or fusion it supplies the intracellularly active therapeutic substance (Albendazole in our case). Its release gradually started from the liposome either via the action of the intracellular enzymes (lipases) found in the liposomes or by diffusion (it passes through the liposome and cell lipid membranes). This deposition of the active therapeutic substances in situ in the affected organs and cells provides the increased therapeutic activity as well as for the typical of the liposomal preparations depot-effect, i.e. their retarded and long-term action. This allows for the preparations to be introduced only once or twice a week. The depot-effect of the liposomal action is explained by the slow degradation of millions of liposomal vesicles and the slow diffusion through the bi-layer lipid membrane, especially in the case of the multilamellar liposomes used in our experiments.

Materials and Methods

Animal Experiments

White mice, weighing around 30 g, were infected per os with 100 Trichinella larvae of *T. spiralis* species. On day 35 following the infection, when the muscle stage is well-expressed, five experimental groups with 30 mice each were formed.

Group 1 – controls, injected with saline i.p. once a week;

Group 2 – experimental, injected with Albendazole dose of 24 mg/kg i.p. once a week;

Group 3 – experimental, injected with Albendazole encapsulated in liposomes at a dose of 24 mg/kg i.p. once a week.

Three mice of each group were sacrificed each week. The number of live *Trichinella larvae* has been recorded in the diaphragms of the mice preliminarily processed by the compressor method and the effect of application of the preparation has been assessed after the diaphragms have been separately digested in artificial stomach juice and the number of live and dead *Trichinella larvae* has been registered.

Preparation of liposome Encapsulated Albendazole (LM)

Albendazole substance of pharmaceutical grade (Cipla, India) was encapsulated in the lipid phase of extrusion type of liposomes. Briefly, freeze-thawed multilamellar liposomes were subjected to high pressure of argon gas jet homogenization with subsequent in line extrusion through l00 nm pore size polycarbonate membrane implemented by Emulsi-Flex C5 apparatus (Avestin, Canada). Lipid phase consists of 20 mg/ml total egg yolk lipids. Sodium saline (150 mM NaCl) dissolved in double distilled and sterile water was used as an aqeous phase of the liposomes. The resulted liposome sterile suspension was used for treatment of the experimental animals.

Preparation of Non-liposome Encapsulated (free) Albendazole

Albendazole substance of the same quality as above was dissolved in sodium saline using intermittent sonification (2 × 20 s with 1 min pause between). Bath type sonifier B220, 125 W (Branson, USA) was used.

Results and Discussion

The experimental results of the investigation are presented in **Fig. 1**. The number of live *Trichinella larvae* in the target organ - the diaphragm (data are placed on the ordinate axis) served as criteria for anti-Trichinella activity during the recording

period (abscissa). The interpretation of the results is carried out in two aspects - an intuitive-visual one which readily shows the advantage of LA as compared to A and a mathematical one which through a detailed statistcal analysis proves the significance of the obtained data.

Figure 1 shows the diminution of the live *Trichinella larvae* number under the action of A and LA in dose (24 mg/kg bw). A linear decrease of the parasite numbers is registered until their final extermination is accomplished by week 9 to 10.

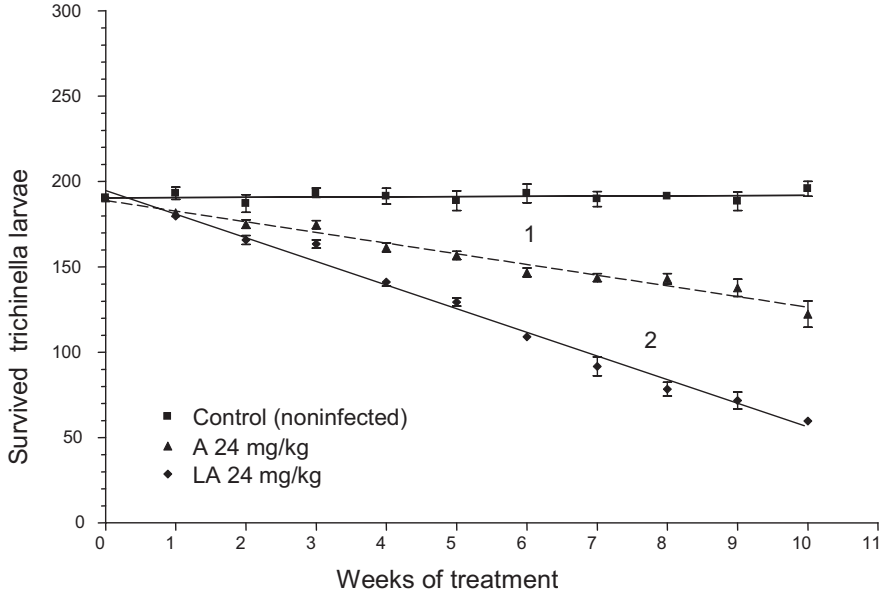

Fig. 1. Treatment of experimental muscle phase of trichinellosis with liposomized Albendazole (LA) and free Albendazole (A). Treatment started 35 days after the infection

The results obtained from the mice treated with liposome-free Albendazole and liposomal Albendazole are presented in **Fig. 1**. It is quite clear that after the application of free Albendazole (A) the larvae number decreases much more slightly, and the most important – they do not reach zero level despite continuing the treatment. After about 4 to 5 weeks we observed an inefficiency of the therapy, without tendency to reach the zero level (unlike the case with LA). At the dose of 24 mg/kg A the efficacy is 36%.

The results interpretation shows that LA at a dose of 24 mg/kg leads to a complete annihilation of *Trichinella larvae* compared to A at the same dose and there is an upward trend which proves an impossibile annihilation of *Trichinella larvae* encapsulated in the muscle cell by free Albendazole. At the dose of 24 mg/kg the efficacy of liposomal Albendazole (LA) is 69%.

This fact explains the unsatisfactory results obtained in the practice of treating muscle trichinellosis [7, 6]. The applied analysis clearly demonstrates the advantages of liposomal Albendazole compared to free one but does not provide strict proofs for the significance of data which imposed the undertaking of a detailed statistical analysis which provoked the above-presented conclusions.

All experimental data have been pretested for correspondence to the normal Gauss distribution. The data were also checked for presence of extreme values by Grubb on line test (these are usually very low or very high values compared to others left unconsidered). The Curve Expert version 1.36 (Hans Development, USA) for preliminary automatic fit with 36 models and interpolation of the viral load with time was used. The Prism version 2.01 [5] for further detailed analysis of the best fitting linear regression line and estimation of the best fit parameters and calculation of the theoretical end point time necessary for obtaining different viral loads was used. The Prism version was also implemented for construction of the graphs. For data obtained from the therapy with LA a linear equation was formulated

$$y = a + bx, \quad (1)$$

and for those obtained from the treatment with free A the equation

$$y = a \exp(bx) + c \quad (2)$$

is exponential with a residual coefficient c differing from the aero with a biological value, meaning that it defines the number of the live larvae left in spite of the treatment with the preparation and demonstrates the impossibility for a radical extermination of the parasite.

From the analysis it has been established that the parameters showing a degree of correspondence between the experimental data (goodness of fit) and the data of the theoretical linear curve have a big importance and also prove that the liposomal preparation leads to a significant extermination of the larvae for a short period of treatment (about 10 weeks).

Linear-regression analysis of the control for LA shows that it is absolutely horizontal and related to the abscissa (period of treatment), i.e. there is no loss of larvae upon incubation with saline which means that the anti-Trichinella effect is solely due to the preparation rather than other factors.

From the analyses is clear that liposomal encapsulation of Albendazole leads to a considerably improved therapeutic efficiency compared to the one displayed by free, routinely applied Albendazole. It is expressed not only in the quantitative augmentation of the larvocidal effect but also in the cardinal alteration of the model (equation) of the mechanism of action of LA compared to A. The latter kills the larvae after an exponential dependency (equation 2) with lack of efficiency (plateau) despite the continuation of drug administration. The mathematical model LA shows the effect as a linear regression with a progressive diminishing of larvae number accomplishing their total annihilation in the end.

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