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## Parasitological and histological study in experimental Trichinellosis of mice treated with TFX-Thymomodulin

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### Introduction

The preliminary experimental studies and clinical observation by KOCIĘCKA et al. (3, 4) demonstrated that immunomodulating preparation Thymus Factor X (TFX, Polfa) given in early and late phase of muscular invasion, helps partly to destroy *Trichinella spiralis* larvae in muscle tissue, promotes cellular reaction and improve general condition. In patients with grave course of trichinellosis the treatment has lead to a favourable outcome of the process of the disease.

Thymus Factor X was isolated from the extract of calf thymus. It is a mixture of polypeptides and peptides and consists of 25 fractions that have different immunological potentials. According to authors GIELDANOWSKI et al. (1), JASZCZ et al. (2), ŚLOPEK et al. (9) TFX is a preparation that immunoreconstructs the number and function of T-lymphocytes and regulates the immunological response. Immunogenic action of TFX is noted exclusively in cellular reaction and is manifested by positive skin reaction as well as in migration inhibition tests. Acting on the bone marrow, TFX increases granulopoiesis and erythropoiesis as well as the number of trombocytes. In experimental studies TFX affects reparatory processes, e. g. induces regeneration of hepatocytes, intensifies granulation and connective tissue cicatrization.

TFX-Thymomodulin (Thymoorgan GmbH Pharmazie CoKG, Viennenburg; Licence: Polfa Pharmaceutical Works in Jelenia Góra, Poland) is composed of lyophilized substance of Thymus Factor X à 10 mg to be dissolved in 1 ml of normal saline.

The aim of the study was to evaluate effect of *TFX-Thymomodulin* on the course of intestinal and muscular invasion of *Trichinella spiralis* in mice. Since intestinal invasion is the essential phase, which decides on further development of muscular invasion, in present study TFX-Thymomodulin was applied:

- a) in early period of intestinal invasion;
- b) in later period of intestinal invasion characterized by elimination of adult forms from the intestine and by settlement of *Trichinella* larvae in muscle tissue.

## Material and Methods

The experiments were performed on 73 mice of BALB/c strain, each 8.5 - 14.0 grams of weight, three months old, infected per o. s. with *T. spiralis* (strain of Laboratory, Poznań) larvae in high doses of 700 larvae per animal.

TFX-Thymomodulin was applied in two periods: from the 5<sup>th</sup> to the 18<sup>th</sup> day post invasion (group I, 29 animals) and in the later period, from the 16<sup>th</sup> to the 28<sup>th</sup> day post invasion (group II, 15 animals). The control group (III) consisted of 29 untreated animals. TFX-Thymomodulin preparation was administered subcutaneously in doses of 40 mg per 1 kg body weight. The dose, according to ŠLOPEK et al. (9) is non toxic and well tolerated.

The effect of TFX-Thymomodulin on the course of intestinal invasion was evaluated on days 6, 12, 18 and 24 post infection (p. i.). The effect of TFX-Thymomodulin on the course of muscular invasion was evaluated on days 18, 24 and 58 post invasion, i. e. 1, 7 and 41 days after the drug administration.

Intensity of intestinal invasion was measured by incubating the intestine in 0.9% NaCl for 2 h at 37° C and evaluating the number of adults of *Trichinella* (male and female). The intensity of muscle invasion was measured by digesting the mouse carcasses in an artificial gastric juice and calculating the larvae content per 1 gram of the muscle. In addition, the femoral and diaphragm muscles were examined trichinoscopically. Samples of the muscle tissue were examined histologically; they were fixed in 10% neutral formalin and Carnoy liquid and then stained with hematoxylin-eosin (HE) and Van Gieson methods.

The criteria for evaluation of TFX-Thymomodulin activity were as follows:

- a) animal survival and general condition;
- b) dynamics of intestinal invasion;
- c) intensity of muscle invasion;
- d) dynamics and character of histological changes in the intestine and in muscle tissue.

## Results

All animals untreated and treated survived the period of observation. However, the general condition of the treated mice improved. The animals gained weight, they ate more and became more lively and active.

### Parasitological examination

Table 1 presents the numbers of adults of *Trichinella spiralis* in the intestine in the first experimental group (I) in relation to the control group (III).

On the 6<sup>th</sup>, 12<sup>th</sup>, 18<sup>th</sup> and 24<sup>th</sup> day after infection mice of the treated group were found to contain less mature intestinal form of *Trichinella* (by 22.1%, 15.8%, 62.7% and 30.9%, respectively) than control mice did. Parasitological studies of muscles on 18<sup>th</sup>, 24<sup>th</sup> and 58<sup>th</sup> day of invasion (i. e. 1, 7, 41 days after therapy) demonstrated less numerous larvae (by 55.7%, 45.4% and 37.4%, respectively) in the treated group as compared to the control group (Tab. 2).

In the group of animals (II) treated in the later period of infection (16 - 28 days of invasion), parasitological examination of muscle on 58<sup>th</sup> day post infection (i. e. 30 days after the therapy) demonstrated difference in number of *T. spiralis* larvae (23.0% reduction of larvae number) as compared with the control group (Tab. 3). In animals treated in early period of invasion (group I) and examined on 58<sup>th</sup> day post invasion 37.4% reduction of larvae number was found as compared to the control group (Tab. 3).

TABLE 1

**Number of *Trichinella spiralis* adults in animals treated with TFX-Thymomodulin in early period of invasion (group I) and in control group (III)**

days of invasion (d.p.i.)	days of drug administration (d.a.) and post administration (d.p.a.)	group I treated 5 - 18 d.p.i.	group III control not treated	difference between groups in %
6	1 d.a.	275.5 ± 38.6	353.0 ± 34.6	22.1
12	8 d.a.	228.8 ± 11.7	271.0 ± 26.3	15.5
18	1 d.p.a.	35.0 ± 12.0	94.0 ± 13.8	62.8
24	7 d.p.a.	16.8 ± 10.5	24.3 ± 10.5	30.9

TABLE 2

***Trichinella spiralis* larvae recovered in muscle tissue in group of mice (I) treated in early period of invasion (5 - 18 d.p.i.) and in control group (III) of animals**

days of invasion	days post administration TFX-Thymomodulin	average number/g of larvae group I	average number/g of larvae group III	difference between groups in n %
18	1	163.3 ± 18.3	368.0 ± 18.5	205.0 55.7
24	7	4100.0 ± 141.4	7520.0 ± 128.8	3420.0 45.4
58	41	3000.0 ± 1817.0	4795.0 ± 1354.0	1795.0 37.4

TABLE 3

**Differences of intensity muscle invasion in 3 groups of animals examined in late period of invasion**

Groups of animals period of drug administration	I 5 - 18 d.p.i.	II 16 - 28 d.p.i.	III control
days of examination: post invasion post therapy	58 40	58 30	58 —
average number of larvae per 1 gram/muscle	3000.0 ± 1917.0	3690.0 ± 1394.0	4785.0 ± 1354.0
differences in % between treated and control groups	37.4	23.0	—

## Histological examination

Characteristic features in the intestine and in the muscle tissue were as follows.

### 1. Intestine of animals (group I) treated in early period of invasion

- a) *T. spiralis* adults — not numerous or absent on 6<sup>th</sup> day p. i.
- b) Cellular infiltration — of low intensity but with presence of numerous eosinophils and globular leukocytes.
- c) Numerous mast cells on 6<sup>th</sup> day p. i.
- d) Focal fibrosis (initial process) presents on 12<sup>th</sup> day p. i.

### 2. Muscle tissue of animals (group I) treated in early period of invasion

- a) Basophilic transformation of few muscle cells — of low intensity and appearing late.
- b) *T. spiralis* larvae not numerous, not encapsulated or with very thin-wall capsules on 24<sup>th</sup> day p. i.
- c) Cellular infiltrates — of low intensity, with presence of numerous fibroblasts at early period of the invasion (from 18<sup>th</sup> day after infection).
- d) Small foci of fibrosis (initial stage) in vicinity of capsules on 24<sup>th</sup> day p. i.

### 3. Muscle tissue of animals (group II) treated in later period of invasion

- a) Presence of basophilic transformation of muscle cells.
- b) Well encapsulated larvae with thick-wall capsule.
- c) Intense cellular infiltrates and presence of small foci of fibrosis.

Figures 1, 2 and 3 demonstrate histological changes in muscle tissue.

## Discussion

In our experiment we observed two main parasitological phenomena. Firstly, involving progressive elimination of adult forms of *Trichinella* from the intestine, and secondly, lower intensity of invasion in muscle tissue of animals treated with TFX-Thymomodulin as compared with the control group.

Considering the selective influence of TFX-Thymomodulin as a preparation of the immunoregulating organ on T-lymphocytes, on their maturation and function, one may expect that pathomechanism of drug action involves the cell-dependent immunity response. The prevalence of mast cells and eosinophils in the wall of the intestine in the early stage of invasion point to the role of the immunomechanism. T-cells play an important role in both the host anti-parasitic response and the pathogenesis of tissue lesions. Results of experimental studies in mice infected with *T. spiralis* described by LUFT and KIM (6) have documented at the acute stage of the invasion an immunosuppression resulting in part from the intestinal phase-induced T-cell hypofunction. The defect has provided grounds for application of TFX-Thymomodulin in the early stage of intestinal phase in presented experiments. It would be of interest to examine participation and activity of interleukins, particularly IL1, IL2, IL4 and IL5 which might explain the modulating effect of TFX-Thymomodulin on the course of intestinal invasion and early course of muscle invasion and the respective pathomorphology (prevalence of mast cells, eosinophils and early appearance of fibroblasts in cellular infiltrates).

Regretably, it seems impossible to exclude direct influence of the preparation on the parasite, i. e. on spermatogenesis, oogenesis or on the development of the stichosome which is the essential source of the antigen. No such studies have been performed till

now. Nevertheless, it can be suggested that application of TFX-Thymomodulin results in reproduction of less valuable second generation of *Trichinella* — the newborn larvae of which are unable to anchor in muscle cells characterized by scanty or even absent basophilic transformation. The phenomenon may additionally explain the slowly progression of less intense muscle invasion, noted particularly late after completion of the therapy. Explanation of the absence or scanty character of basophilic transformation of muscle cells in animals treated in early period of intestinal invasion require further parasitological, immunological and histochemical examinations.

The pathomechanism of destruction of larvae in mice treated in the later period of the invasion has involved cumulation of cellular infiltrates in vicinity of encapsulated larvae, composed of mononuclear cells, eosinophils and numerous fibroblasts. Such pattern has been observed in experimental animals as well as in humans treated at the late stage of trichinellosis with thiabendazole or mebendazole by LAPSZEWICZ et al. (7), KOCIĘCKA et al. (3), DE NOLLIN et al. (8). In such cases deterioration of clinical status has occasionally been observed and some authors (3) have found intensified electro-myographic alterations. In our experiments the general conditions of the treated mice improved and all animals survived treatment despite very high infecting dose of *Trichinella*.

Early appearance of fibroblasts after drug administration is an interesting finding which explains the proliferation of the connective tissue visible before all in later period in the spots where *Trichinella* larvae were subjected to destruction. KOCIĘCKA et al. (4, 5) have observed the phenomenon earlier in experimental animals as well as in biopsies of muscles of TFX (Polfa) treated patients with trichinellosis.

## Conclusions

Parasitological study of TFX-Thymomodulin treated mice at the early stage (5 - 18 days p. i.) of intestinal invasion of *Trichinella spiralis* documented a more rapid elimination of adult forms from the intestine and a decreased intensity of muscular invasion.

Pathomorphological studies of muscular tissue in animals treated at early stage of intestinal invasion showed that basophilic transformation of muscle cells was less frequent, cell infiltrates were less intense, and contained fibroblasts earlier in compared to the control group; *Trichinella* larvae in the treated animals encapsulated slower and their capsule was thinner than in the control group.

In mice treated at the stage of progressing muscular invasion (16 - 28 days p. i.) parasitological studies performed in the 58<sup>th</sup> day post invasion demonstrated lower intensity of invasion compared to the control group; pathomorphological examination documented destruction of encapsulated *Trichinella* larvae, presence of rich cellular infiltrates of focal fibrosis around the larvae.

## Summary

The aim of the study was to evaluate the effect of TFX-Thymomodulin (Thymoorgan GmbH Pharmazie, Vienenburg) on the course of *Trichinella spiralis* invasion in mice. The results of the study indicates that the preparation induced progressive elimination of adult forms of *Trichinella* from the intestine and helped to destroy part of the *Trichinella* larvae in muscle tissue.

## Key words

*Trichinella spiralis*, TFX-Thymomodulin, intestinal invasion, muscle invasion, parasitological evaluation, histological changes.

## Zusammenfassung

### Parasitologische und histologische Untersuchung experimenteller Trichinellose von Mäusen, die mit TFX-Thymomodulin behandelt wurden

Zweck des Versuches war die Beurteilung der Wirkung von TFX-Thymomodulin (Thymoorgan GmbH Pharmazie, Viennenburg) auf den Verlauf der *Trichinella spiralis*-Invasion von Mäusen.

Die Versuchsergebnisse weisen darauf hin, daß das Mittel eine fortschreitende Beseitigung der adulten Trichinellen aus dem Darm und die Abtötung eines Teiles der Muskeltrichinen bewirkte.

## Schlüsselwörter

*Trichinella spiralis*, TFX-Thymomodulin, Darminvasion, Muskelinvasion, parasitologische Beurteilung, histologische Veränderungen.

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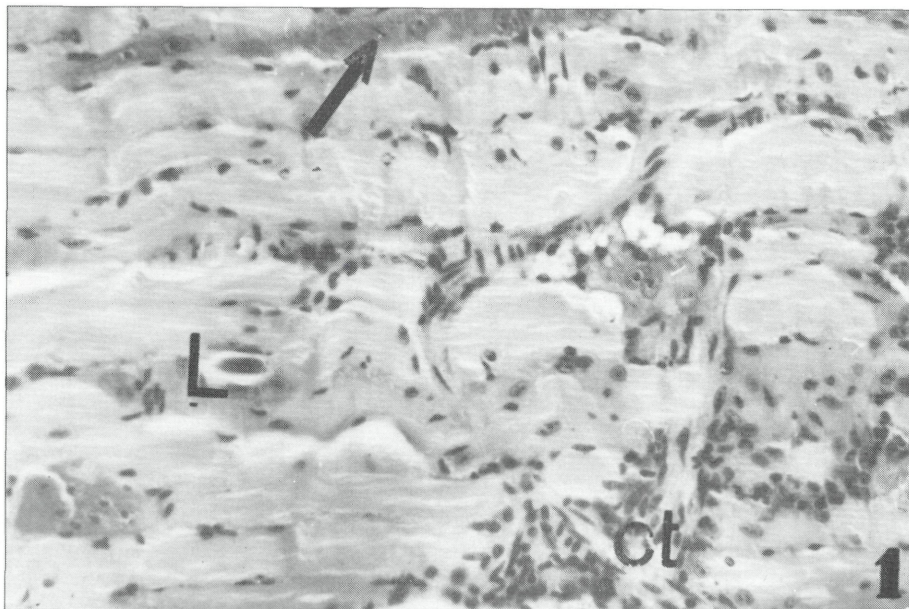


Fig. 1: Histopathological pattern of muscle tissue in animals (group I) examined on 18<sup>th</sup> day post invasion and on 14<sup>th</sup> day of TFX-Thymomodulin administration. Single muscle cell with basophilic transformation (arrow). Seen not intense cellular cumulation not encapsulated larva of *T. spiralis* (L) and initial proliferation of connective tissue (CT). (HE staining, magnification 10 × 3.2)

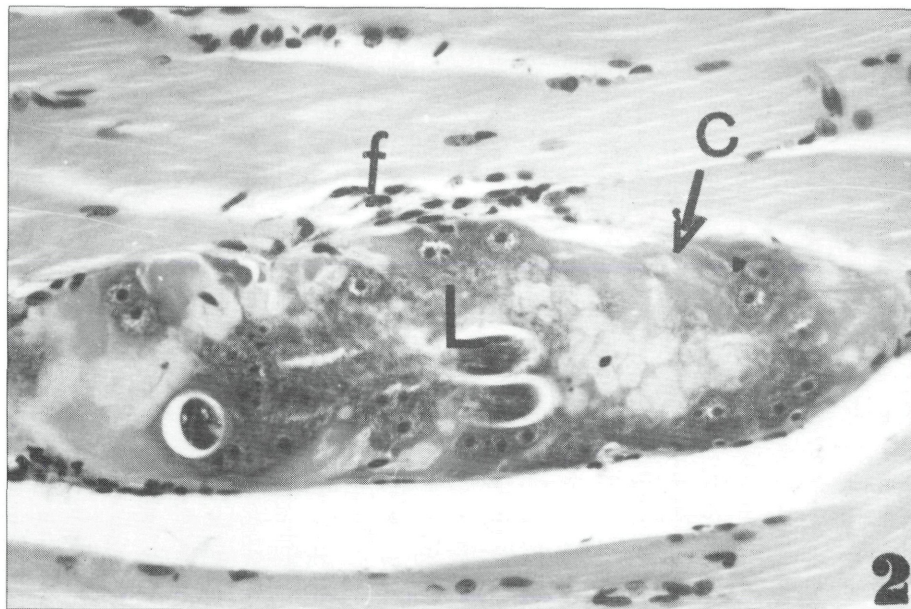


Fig. 2: Histopathological pattern of muscle tissue of animals (group I) examined on 24<sup>th</sup> day after infection and 6<sup>th</sup> day after the therapy. Encapsulated larva of *T. spiralis* with very thin-wall (C) capsule; very scanty character of infiltrates with presence of fibroblasts (F). (HE staining, magnification 40 × 3.2)



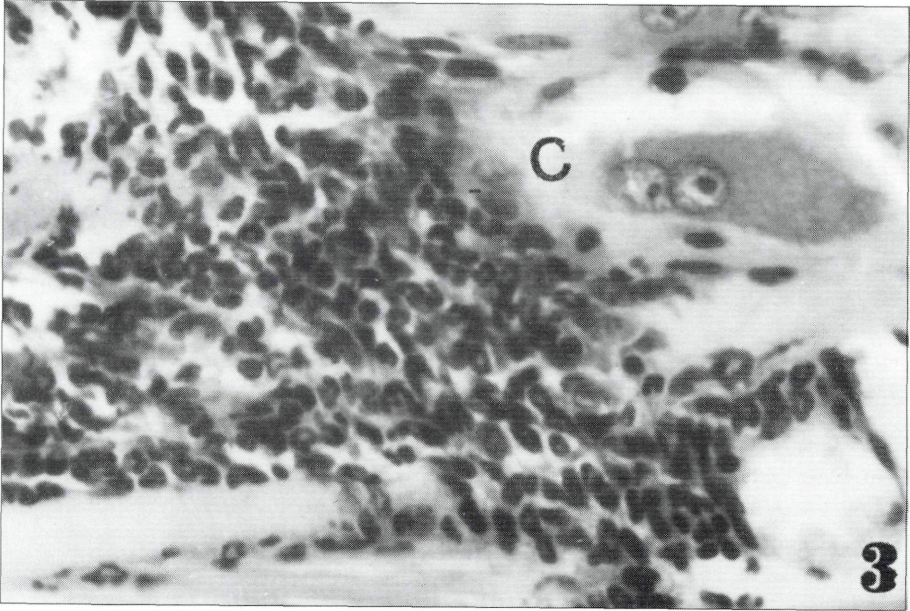


Fig. 3: Histopathological pattern of muscle tissue of mice treated in later period of invasion (group II) and examined on 58<sup>th</sup> day post invasion and 30 days after the therapy. Encapsulated *T. spiralis* larva with very thick-wall of capsule (C). Note intense cellular infiltration of the capsule.  
(HE staining, magnification 100 × 3.2)

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