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Effect of aminoguanidine and albendazole on inducible nitric oxide synthase (iNOS) activity in *T. spiralis*-infected mice muscles

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Abstract: The aim of this study was to provide evidence for the expression of iNOS in the cells of inflammatory infiltrates around larvae in skeletal muscles of *T. spiralis* infected mice. The BALB/c mice (n=8) divided into subgroups, received either aminoguanidine (AMG) - a specific iNOS inhibitor or albendazole (ALB) - an antiparasitic drug of choice in trichinellosis treatment. Control animals (n=2 in each subgroup) were either uninfected and treated or uninfected and untreated. Frozen sections of hind leg muscles from mice sacrificed at various time intervals after infection were cut and subjected to immunohistochemistry, using monoclonal anti-iNOS antibody. The ALB-treated mice revealed stronger iNOS staining in the infiltrating cells around larvae than the infected and untreated animals. On the contrary, in the AMG-treated animals, the infiltrating cells did not show any specific iNOS reaction. These data confirm the specificity of iNOS staining in the cellular infiltrates around *T. spiralis* larvae and shed some light on the role of nitric oxide during ALB treatment in experimental trichinellosis.

Key words: Aminoguanidine - Albendazole - iNOS - Trichnellosis - Muscle

Introduction

The importance of research on immunodependence of chemotherapy has been highlighted already in the 1990s [1, 3, 4, 5, 6]. It has been found that there is a correlation between a successful therapy and the immunological status of the host in some parasitic infections, including trichinellosis [3]. According to our biochemical study the albendazole may potentiate its inhibitory influence on parasite's tubulin (which is considered as its primary target point) stimulating nitrogen-free radical-based host defense mechanism in host muscle tissue [1]. Recently, in spectroscopic measurements of glutathione transferase (GST) activity [4] we have also shown the stimulating effect of albendazole (ALB), a popular benzimidazole used in the treatment of human trichinellosis, on this enzyme of glutathione metabolism in murine trichinellosis. Levamisole, an anthelmintic, which is considered an immunomodulatory antiparasitic agent, has no influence on iNOS activity, but despite its different mode of action in comparison with ALB, it intensifies the activity of GST in experimental trichinellosis.

Our recent molecular studies on the iNOS mRNA as well as on expression and localization of iNOS protein have shown that mRNA measured at the muscular phase of experimental trichinellosis is expressed starting from the 21st day after infection (dpi) [2] and simultaneously positive staining for iNOS shown by immunohistochemistry (anti-iNOS monoclonal antibodies) occurs in the majority of the infiltrating mononuclear cells around encapsulated larvae. The host cells showing specific reaction for NOS in the cytoplasm seem to correspond to tissue macrophages as evidenced by hematoxylin-eosin staining and anti-CD68 reactivity [2]. The sensitivity of iNOS to the inhibitor, aminoguanidine (AMG) which is considered to be iNOS-specific in experimental trichinellosis has not been studied so far. Therefore, the aim of the present study was to assess by immunohistochemistry the sensitivity of iNOS to albendazole (ALB), a well known drug of choice in the treatment of human trichinellosis and aminoguanidine (AMG) - a structural analogue of L-arginine and a specific iNOS inhibitor.

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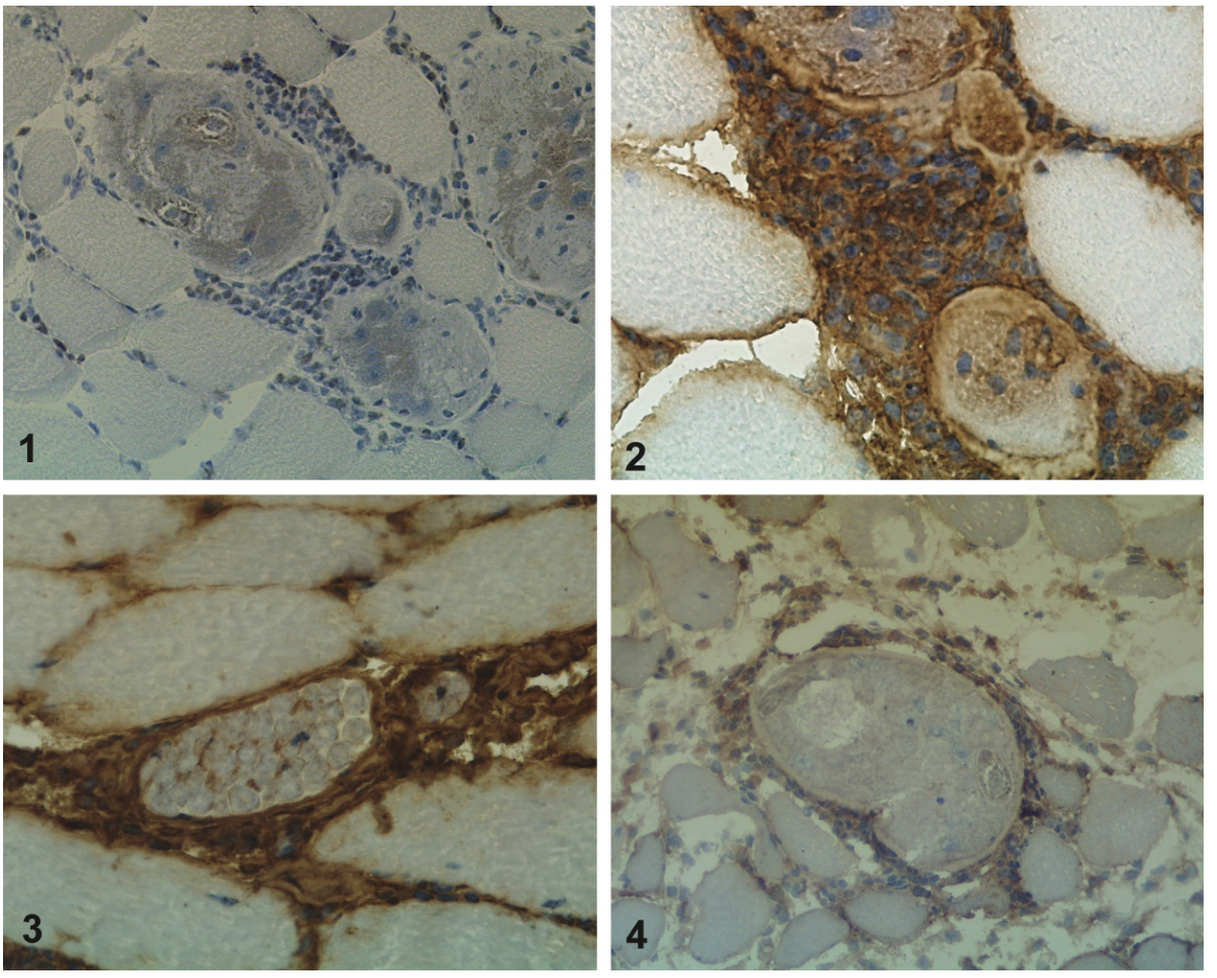

Fig. 1. Encapsulated *T. spiralis larvae*; infiltrates in infected, untreated mouse on 44dpi; control: anti-iNOS MoAb replaced by normal mouse serum. No positive reaction in infiltrating cells. × 400. **Fig. 2.** Encapsulated *T. spiralis* larvae; cell infiltrates in muscles of infected, untreated mouse on 44 dpi. Anti-iNOS primary antibody. Distinct positive reaction in cells surrounding larvae. × 400. **Fig. 3.** Tissue section and reaction as in Figure 2 in mice treated with albendazole (ALB). The immunostaining for iNOS is much stronger than that in Figure 2. × 400. **Fig. 4.** Encapsulated *T. spiralis* larvae; infiltrates in muscles of infected mouse treated with iNOS-specific inhibitor aminoguanidine (AMG) on 44 dpi. Anti iNOS primary antibody. No immunostaining can be seen in cells surrounding larvae. × 400.

Materials and methods

The examined tissues were skeletal muscles collected from mice (strain BALB/c) previously infected *per os* with 450 *T. spiralis* larvae/mouse.

AMG - iNOS inhibitor was administered in a total dose of 50 mg/kg b.w. to mice (n=3) 9 times on the following days post infection (dpi): 2, 4, 7, 9, 11, 14, 16, 18 and 22. The other group of 3 mice was treated with ALB administered twice on dpi 4 and 11 in a total dose of 50 mg/kg b.w.

The imunohistochemical procedure was performed as described previously [2] on muscle tissue (hind legs) samples collected on dpi 21 from infected treated and untreated mice. In short, cryostat sections of hind leg muscles were incubated with anti-iNOS monoclonal antibody (MoAb), followed by DAKO ARK Animal Research kit consisting of biotinylated anti-mouse Ig, peroxidase-labeled streptavidin and diaminobenzidine (DAB) as a chromogen. In the control reactions, primary antibodies were replaced by normal mouse serum and/or PBS, or after primary anti-iNOS MoAb biotinylated anti-mouse Ig was omitted. Besides, the reaction was carried out on muscle sections from AMG- and ALB-untreated mice in parallel to the treated ones.

Results and discussion

In the control reactions no positive staining of infiltrating cells surrounding *T. spiralis* larvae could be seen (Fig. 1). In the AMG- and ALB-untreated mice (Fig. 2), iNOS activity was evident in almost all mononuclear cells surrounding the encapsulated larvae. As presented in Figure 3, an even stronger iNOS immunostaining could be seen in almost all mononuclear cells surrounding encapsulated *T. spiralis* larvae in infected mice muscles after ALB treatment. In contrast, the samples of muscles collected from mice after the administration of

AMG showed almost total inhibition of iNOS-specific staining in the infiltrating cells (Fig. 4).

Thus, the treatment of mice with ALB resulted in the stimulation of iNOS protein expression, while AMG showed an inhibitory effect. These results confirm our previous biochemical data.

The relationship between a successful antiparasitic chemotherapy and the immunological status of the host in the case of *T. spiralis* infection was already postulated in the 1990s and still may be found in the literature concerning helminthoses. The compromising influence of antischistosomal drugs of differentiated mode of action (hycanthone, oxamiquine and praziquantel) on immunosupressed animals observed by Falon *et al.* [5] has been explained as disruption of the integrity of parasite surface membranes (which exposes the antigens to the attack of the host antibody) common to all drugs. According to Mukhopadhyay and Ravindran [6], filarial antibodies react with diethylcarbamazine citrate (DEC) and therefore they may potentiate the microfilaricidal activity of this drug.

In *T. spiralis* infection, like in other parasitic infestations, cyclosporin A, a potent immunosuppressant, has an antiparasitic effect due to the inhibition of larvae development *in utero* of *T. spiralis* females, which results in a weak establishment of muscle larvae following infection [3]. Our recent papers reporting the stimulatory effect of ALB on enzymes involved in oxygen and nitrogen free radical-based host defense (GST and iNOS, respectively) in trichinellosis [2, 4] were the first publications considering the involvement of free radical-based host defense in the chemotherapy of trichinellosis.

The results obtained using the immunohistochemical method in this study speak in favor of the stimulating action of ALB on iNOS (thus confirming our previous results) on the one hand, and the inhibitory influence of specific iNOS inhibitor - AMG, on the other. Our latest observation provides additional evidence for the participation of the inducible form of NOS in the inflammatory reaction around the encapsulated *T. spiralis* larvae in mice muscles.

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