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Evaluation of recombinant CXCL8$_{(3–73)}$K11R/G31P in muscle fibrosis and *Trichinella* larvae encapsulation in a murine model of trichinellosis

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ABSTRACT

Trichinella spiralis (***T. spiralis***) larvae in raw or inadequately cooked meat can cause chronic infections in a wide range of hosts including humans. During the development inside the skeletal muscles, ***T. spiralis*** larvae infect muscle cells accompanying with the infiltration of host inflammatory cells, eventually create a new type of cell known as nurse cell developing a surrounding vascular network to support the larvae development. Controlling of host inflammatory responses and angiogenesis influences both the nurse cell differentiation and the parasite larvae development. CXCL8 is a chemokine that acts on G-protein coupled receptors, of which activation contributes to fibrosis and angiogenesis. CXCL8$_{(3–73)}$K11R/G31P (G31P) has been reported as a CXCL8 analogue. The aim of this study is to investigate the effect of G31P in inflammatory responses and the development of ***T. spiralis*** larvae in muscle tissues of mice infected with ***T. spiralis***. The level of inflammatory factors and the morphology of ***T. spiralis*** larvae in infected tissues were investigated through *ELISA* and electron-microscopy analysis. G31P up-regulated IFN-γ and down-regulated CXCL8 level, and impaired the encapsulation of ***T. spiralis*** larvae *in vivo*. The results showed that G31P influenced the development of *T. spiralis* larvae in muscle tissues.

1. Introduction

Trichinella infection is considered as an emerging/re-emerging disease and has been reported in 66 countries around the world [3,15]. A wide range of hosts including humans acquire *Trichinella* infection by ingesting raw or inadequately cooked meat of pigs or other animals containing *Trichinella* larvae [7]. *Trichinella spiralis* (*T. spiralis*) larvae develop inside the enterocytes of small intestine, after mating, the newborn larvae are released into circulation and some enter striated muscles to initiate chronic infection [5]. During the development inside the skeletal muscles, *T. spiralis* larvae infect the muscle cells accompanying with the infiltration of inflammatory cells, eventually create a new type of cell known as nurse cell [11,16]. The chronic inflammatory process inside the nurse cells is sustained by the host neutrophils, eosinophil, macrophages and Treg cells, leading to destruction of muscle fibers [1]. Eosinophils have been reported to protect muscle *T. spiralis* larvae through preventing the development of nitric oxide synthase which is produced by neutrophils and macrophages [6]. Angiogenesis plays a prominent role in nurse cell formation, during which infected myotubes develop a surrounding vascular network to support the larvae development [4]. Consequently, controlling of inflammatory responses and angiogenesis in *T. spiralis*-infected host tissues may influence both the nurse cell differentiation and the parasite larvae development.

CXCL8 (interleukin-8) is a chemokine that acts on G-protein coupled receptors (GPCR) known as CXCR1 and CXCR2, which mainly perform in recruitment and activation of neutrophils [14]. CXCL8 can be released by a wide range of cells including T lymphocytes, macrophages, fibroblasts, epithelial cells and keratinocytes. The two known CXCL8 receptors, CXCR1 and CXCR2, are expressed on a variety of leukocytes, including neutrophils, monocytes, $CD8^+$ T cells, basophils, natural killer cells, while cells other than leukocytes also express CXCR1 and CXCR2, including epithelial, endothelial, fibroblasts, and keratinocytes. Activation of the receptors in these cells contributes to fibrosis and angiogenesis [17]. Murine CXCL8 family is not strictly comparable to human IL-8, however, both murine CXCR1 and CXCR2 homolog have been noted. CXCL8$_{(3–73)}$K11R/G31P (G31P) has been reported as a CXCL8 analogue and selective CXCR1 and CXCR2 antagonist, which contains Arg and Pro substitutions for Lys11 and Gly31 respectively of human CXCL8$_{(3–72)}$. G31P has an affinity to CXCR1 and CXCR2 that is 2–3 orders of magnitude higher than that of CXCL8 and CXCR1/CXCR2, and inhibited by 50% the chemotactic responses of neutrophils, as well as blocks both CXCR1 and CXCR2-mediated responses involved in the neutrophil-induced inflammatory response [12]. G31P has been demonstrated that it decreases ischemia/reperfusion-induced injury following superior mesenteric artery occlusion in rat, inhibits human cancer growth and metastasis in a mouse model by decreasing

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angiogenesis [13]. Thus, on the basis of above information, we hypothesized that inhibition of CXCR1 and CXCR2 by G31P in a murine model of trichinellosis might decrease the angiogenesis and the muscle fibrosis surrounding the parasite larvae, further influence the encapsulation of the parasite larvae.

In this study, we aimed to investigate the effect of G31P in muscle fibrosis and encapsulation of *T. spiralis* larvae. Inflammatory factors in *T. spiralis*-infected mice were examined and the ultra-structural morphology of muscle cells and *T. spiralis* larvae were analyzed by electron-microscopy. This study is to report the potential role of G31P in *T. spiralis* larvae development and primarily investigate the correlation of nurse cell formation with the inflammation responses mediated by chemokines.

2. Materials and methods

2.1. Animals

6–8 weeks old female BALB/c mice were obtained from Experimental Animal Center, Dalian Medical University. Infective *T. spiralis* larvae were obtained from Department of Parasitology, Zhengzhou University. The G31P purification was carried out as previously described [9]. Mice were randomly divided into G31P treatment group ($n = 12$, infected mice were treated with G31P), model control group ($n = 12$, infected mice were treated with saline) and negative control group ($n = 6$, normal mice were treated with saline). Rearing conditions of three groups were same in the process of the experiments. Each mouse in treatment and model control group was orally fed with 100 infective *T. spiralis* larvae. 10 days after infection, the mice in G31P treatment group were subcutaneously injected with 500 μg/kg G31P in every 48 h for consecutive 14 days. The mice in model control group and negative control group were subcutaneously injected with the same amount of saline in every 48 h for consecutive 14 days. After 24 days, the blood was collected from retro orbital vasculature and incubated at 37 °C for 10 min, centrifuged at 3000*g* for 10 min. The mice were sacrificed and aseptically dissected. Diaphragm tissues were collected. The animal treatment was approved by the Animal Center Committee, Dalian Medical University.

2.2. Hematoxylin-eosin (HE) Staining

Briefly, the tissues were fixed, embedded in paraffin and processed in 3 μm sections. Sections were baked at 60 °C for 30 min. The samples were gradually placed in xylene dewax for 10 min and in alcohol for dehydration for 5 min, then stained in hematoxylin for 5 min, dichroic with hydrochloric acid and ethanol, and stained with eosin. Sliced tissue was dehydrated and mounted with neutral gum. The numbers of encapsulated *T. spiralis* were counted under microscope. The results were analyzed by double blinded experiment.

2.3. Electron microscopy analysis

Diaphragm tissues were fixed in 2.5% glutaraldehyde and cacodylate buffer for 2 h. All samples were subsequently washed in PBS and post-fixed in 1% osmium tetroxide for 1 h, dehydrated in ethanol and embedded in epon resin. The areas were selected from 0.5 μm toluidine blue-stained sections, and stained with uranyl acetate and lead citrate, observed through transmission electron microscope (JEM-2000 EX, Japan).

2.4. ELISA

The levels of CXCL8 and IFN-γ in sera were detected by ELISA (Shanghai Westang Bio-Tech Co, Shanghai, China). Serum samples were incubated at 37 °C for 30 min. After five washes, horseradish peroxidase (HRP)-Conjugate reagent was added into samples and incubated at 37 °C for 30 min. The samples were read at 490 nm in a microplate reader (Assay Biotechnology Company, San Francisco, USA).

2.5. Statistical analyses

The results were analyzed by one-way analysis of variance and Student's *t*-test. *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. G31P regulated CXCL8, IFN-γ level in sera of T. spiralis-infected mice and decreased the numbers of encapsulated T. spiralis larvae

The level of CXCL8 in sera of mice of different groups was assessed by ELISA. As shown in Fig. 1, G31P reduced the level of CXCL8 in mice in G31P treatment group compared with the mice in the model control group (Fig. 1A). The lower level of IFN-γ was shown in sera of mice in model control group compared with the mice in negative control group. G31P up-regulated the IFN-γ level in the mice of G31P treatment group compared with the mice in model control group (Fig. 1B). After HE staining, the numbers of encapsulated *T. spiralis* larvae were counted under light microscope. After G31P treatment, the numbers of encapsulated *T. spiralis* larvae were decreased (Fig. 1C).

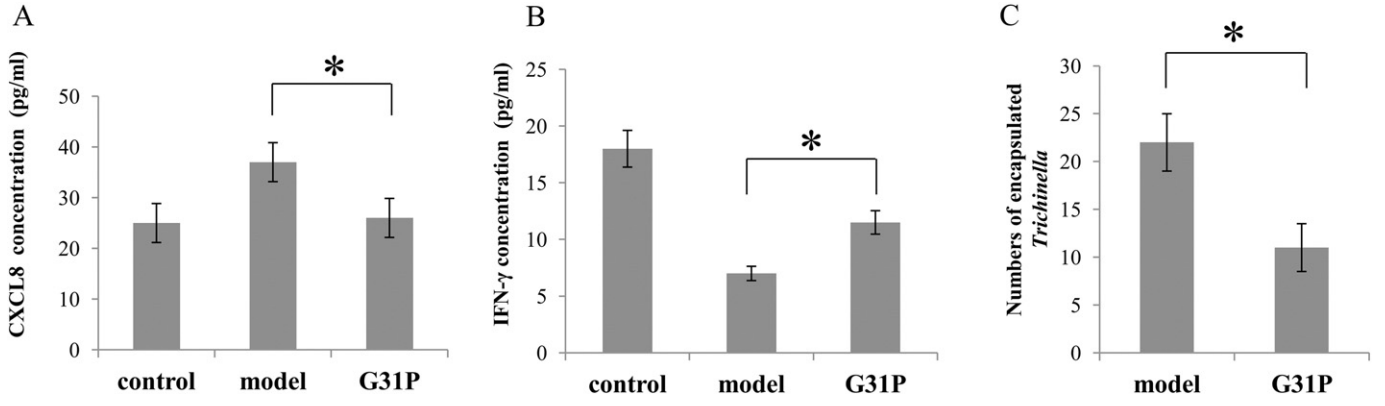

Fig. 1. (A): G31P suppressed Trichinella-induced CXCL8 level. *P < 0.05 VS model control group. (B): G31P up-regulated the level of IFN-γ in sera of mice infected with Trichinella. *P < 0.01 VS model control group. (C): The numbers of encapsulated Trichinella were reduced after G31P treatment at 24 days post infection in 4 arbitrarily selected fields in a double blinded manner . *P < 0.01 VS model control group.