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The temporary dynamics of inflammation-related genes expression under tuftsin analog Selank action

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ABSTRACT

Previous studies have shown that synthetic tuftsin analogue Selank and its fragments cause a number of alterations in the expression of certain genes involved in inflammation in mouse spleen. In this work we studied the effect of Selank and its short fragment Gly-Pro on the temporary dynamics of *C3*, *Casp1*, *Il2rg*, and *Xcr1* genes expression in mouse spleen after single intraperitoneal injection (100 μg/kg) of peptides using real-time PCR method. We found a significant 3-fold decrease in the *C3* mRNA level just 30 min after Selank injection and similar alteration this gene mRNA level after Gly-Pro administration. A wave-like alteration in the *Casp1* mRNA level was observed after Selank injection. We found a significant alteration in the mRNA level of the *Il2rg* gene at early time points after Selank and Gly-Pro administration and an almost equal reduction in the *Xcr1* mRNA level 90 min after the administration of Selank and its fragment. Our results showed that, Selank and its short fragment Gly-Pro influence the expression of genes that mediate different types of immune responses, thereby maintaining the balance of the immune system. It should be noted that in most cases, there was a coincidence in the expression profiles of the studied genes after Selank and Gly-Pro administration. This might indicate an active contribution of the dipeptide to the final effect of Selank.

1. Introduction

In recent years, one of the main directions of drug development has been the use of compounds based on endogenous regulatory peptides, which, on the one hand, exhibit a directional effect on the organism, and, on the other hand, have minimal side effects. Although synthetic regulatory peptides have a wide spectrum of clinical effects, the precise mechanism of their action remains unclear.

Selank is a member of the synthetic regulatory peptide family. It was designed and produced at the Institute of Molecular Genetics, Russian Academy of Sciences, in cooperation with the V.V. Zakusov Research Institute of Pharmacology, Russian Academy of Medical Sciences. This peptide is a synthetic analog of the tuftsin molecule (the short Thr-Lys-Pro-Arg fragment of the human immunoglobulin G heavy chain), which was elongated at the C terminus via the addition of three natural L-amino acids (Pro-Gly-Pro) to improve its metabolic stability and yield a relatively longer duration.

Recent studies have shown that Selank has pronounced anxiolytic and nootropic activities, as well as a marked antiviral activity against the influenza A virus (H_3N_2), the herpes simplex virus (HSV-1 and HSV-2), the cytomegalovirus (CMV), and other viruses (Andreeva et al., 2010a; Ershov et al., 2009; Uchakina et al., 2008). Moreover, the individual fragments of regulatory peptides have their own specific physiological effect (Ashmarin et al., 2005), and Selank is no exception. Some Selank fragments have their own biological activity, which may differ significantly from the action of

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the original peptide (Andreeva et al., 2010b). However, the mechanisms that underlie the wide range of Selank biological effects remain unknown.

Our previous studies have shown that administration of a single intranasal dose or a course of Selank cause an alteration in the expression of many genes in different rat brain regions. A pronounced change in the expression of the genes involved in inflammatory processes in the brain was observed. In particular, the use of both Selank administration schemes led to a significant change in the expression of the *Cx3cr1* gene, which ensures local inflammation in the brain via the regulation of microglial activation and migration (Kolomin et al., 2010, 2013).

Subsequently, the influence of Selank and its two fragments (Arg-Pro-Gly-Pro and Gly-Pro) on the expression of genes involved in inflammatory processes was investigated using a mouse model. It was shown that the mRNA levels of 35 genes encoding chemokines, cytokines, and their receptors, as well as of other genes involved in the immune response, were altered 6 and 24 h after a single intraperitoneal injection of the peptides in the murine spleen. Changes in expression of the largest number of genes were observed with the dipeptide Gly-Pro 6 h after its administration. The most pronounced changes in mRNA levels after the administration of each of the peptides were observed for the *Bcl6*, *C3*, *Casp1*, *Il2rg*, and *Xcr1* genes (Kolomin et al., 2011a).

A detailed study of the expression profiles of some of the individual genes that responded most strongly to Selank and its fragments will allow the elucidation of the molecular genetic mechanisms of peptide immunomodulatory action. Large-scale research of the expression profiles of the *Bcl6* gene, its target, and corepressor genes after treatment with Selank and its fragments has been performed previously (Kolomin et al., 2011b). In this study, we analyzed the temporal dynamics of the expression of the *C3*, *Casp1*, *Il2rg*, and *Xcr1* genes in the murine spleen after Selank and Gly-Pro administration.

2. Materials and methods

2.1. Chemicals

Dry preparations of Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) and Gly-Pro were dissolved to a concentration of 20 μg/ml in saline solution.

2.2. Animal models

The male white mongrel mice ($n = 75$; average weight, 20 g) used in our experiment were kept under a 12 h light/dark cycle with free access to water and food. The animals were divided into 15 groups: five "control" groups ($n = 25$), five groups with Selank injection ($n = 25$) and five groups with Gly-Pro injection ($n = 25$). Each experimental group was divided into five subgroups ($n = 5$ per subgroup), according to the time points (30 min, 90 min, 3 h, 6 h, and 24 h). All groups were handled twice a day every day for 10 days. All animals were treated at the middle of the light phase of the diurnal cycle and had free access to water and food. After this period of preparation, all "control" groups were treated with saline solution (single intraperitoneal injections), and the experimental groups were treated with Selank or Gly-Pro solution (100 μg/kg; single intraperitoneal injections). Animals were decapitated at 30 min, 90 min, 3 h, 6 h, and 24 h after the treatment (all mice of one "control" and one "experimental" group per time point).

The animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23), which was revised in 1996.

2.3. Sample preparation

Mouse spleens were removed immediately, dissected, and kept at −70 °C in tubes pretreated with a 0.1% diethylpyrocarbonate (DEPC) water solution until needed for the isolation of mRNA.

2.4. RNA isolation and reverse transcription

Total RNA was extracted from the mouse spleen using the RNeasy® Mini Kit (Qiagen, Germany) and treated with RNase-free DNase I (Thermo Scientific, Lithuania). First-strand cDNAs were synthesized individually for each rat using the RevertAidTM H Minus First-Strand cDNA Synthesis Kit (Thermo Scientific, Lithuania). Subsequently, individual samples of cDNAs were combined into one for each group.

2.5. Real-time quantitative RT-PCR

The analysis of the expression of the genes under study (*C3*, *Casp1*, *Il2rg*, and *Xcr1*), as well as of reference genes (*Actb*, *Gapdh*, *Hprt1*, *Hsp90ab1*, and *Rpl13a*), in the murine spleen was performed using the real-time PCR method in a StepOnePlusTM Real-Time qPCR System (Live Technologies, USA) using the SYBR® Green 1 dye (Syntol, Russia) and RT^2 qPCR Primer Assay SYBR® Green primers (Qiagen, Germany) (Table 1). Thermal cycling was carried out as follows: (1) 95 °C for 600 s, followed by (2) 40 cycles of 15 s at 95 °C and 60 s at 72 °C. All reactions were repeated three times for the cDNAs in each experimental and control group for each time point using specific gene primers.

2.6. Statistical analyses

Statistical data analysis was performed using the Relative Expression Software Tool-384 version 2 (REST-384) (Pfaffl, 2001; Pfaffl et al., 2002). The threshold reaction cycle (Ct) values obtained for the genes under study were normalized to the Ct values of the reference genes.

3. Results

We have studied, using an animal model, the temporal dynamics of the expression of the *C3*, *Casp1*, *Il2rg*, and *Xcr1* genes under the action of Selank and its Gly-Pro fragment.

Our results are shown in 1A–B and 2A–B. Fig. 1A shows a significant 3-fold decrease in the *C3* mRNA level just 30 min after Selank injection. A similar alteration of the *C3* mRNA level was observed after Gly-Pro administration: 5.8- and 3.7-fold decreases in the expression of this gene were observed 30 min and 90 min after the injection of the dipeptide, respectively. Unlike Selank, Gly-Pro evoked a significant 2-fold increase in the *C3* mRNA level 3 h after its injection.

A wave-like alteration in the *Casp1* mRNA level was observed after Selank injection (Fig. 1B): a significant increase in the expression of the gene (2.4-fold) was noted 90 min and 6 h after the injection of the peptide. The administration of Gly-Pro led to a decrease in the level of the *Casp1* mRNA 30 min (5-fold) and 6 h (2.6-fold) after injection.

A statistically significant alteration in the mRNA level of the *Il2rg* gene was observed at early time points after Selank and Gly-Pro administration (Fig. 2A). For instance, as early as 30 min after the injection of the two peptides, the expression of the *Il2rg* gene decreased 5.9- and 14.3-fold, respectively; 90 min after their injection, the expression of this gene decreased 2.4- and 2.6-fold, respectively. Three hours after the administration of the peptides, a multidirectional alteration in the level of the *Il2rg* mRNA was