Article

Changes in Expression of Chemokines, Cytokines and their Receptors under the Action of Selank and its Fragments

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Abstract: Currently, pharmacological preparations based on endogenous regulatory peptides are being actively studied as the most promising class of drugs that are practically devoid of side effects. This class of drugs includes a synthetic analogue of tuftsin - Selank. Selank, on the one hand, has an anxiolytic and nootropic effect, and on the other hand, it has pronounced antiviral properties. During the study of the immunomodulatory effect of Selank, we proved that both the whole peptide and its individual fragments can cause significant changes in the expression of genes of chemokines, cytokines, and their receptors in the mouse spleen 6 and 24 hours after a single injection. We also showed that a change in the mRNA level of most of the considered genes is observed after the introduction of Gly-Pro, previously proposed as a minimal fragment of Selank with antiviral activity - pharmacophore.

Keywords: Chemokine Gene, Single Intraperitoneal Injection, Relative Expression Soft Ware Tool.

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1. Introduction

Selank is a regulatory peptide, a synthetic analog of taftsin (a short fragment of Thr-Lys-Pro-Arg of the heavy chain of human immunoglobulin G), extended with a C-terminal part of the three Pro-Gly-Pro peptide to increase metabolic stability and increase the duration of action of the final peptide [1, 2]. Selank has a pronounced anxiolytic and nootropic effect and is highly effective in the treatment of anxiety and anxiety-asthenic disorders, leads to the activation and optimization of mnestic and cognitive functions of the brain, learning and memory processes[3, 4].

Along with the functions described above, Selank also has a pronounced immunotropic activity and is able to induce the secretion of interferons. The antiviral effect of Selank was found against influenza virus A/Aichi2/68 (strain H3N2) both in vivo and in vitro in experimental models [5]. In previous studies aimed at searching for genes that change their expression under the influence of Selank, we have shown that the introduction of Selank causes a transcriptomic response in rat hippocampus and spleen cells. At the same time, the most significant change in the mRNA level was noted for the Cx3cr1 gene in the rat spleen after a single injection of the drug [6].

Previous studies have shown that glyprolines are able to exert their own physiological effect, and synthetic peptides containing them can combine different physiological properties of their structural units [7].

In this regard, for a more detailed study of the immunomodulatory activity of Selank, we evaluated changes in the expression of chemokine and cytokine genes in the mouse spleen under the influence of both the whole Selank peptide Thr-Lys-Pro-Arg-Pro-Gly-Pro and three of its fragments: Gly-Pro (GP), Arg-Pro-Gly-Pro (RPGP), and taftsin Thr-Lys-Pro-Arg, 6 and 24 h after a single injection of the peptides.

2. Patients and Methods

A mouse was chosen as an experimental animal, since this species is the most suitable model object for immunological studies, and the spleen is one of the main organs of the immune system. In the experiment, male outbred mice were used, which were divided into 10 groups (10 individuals each with an average weight of 20 g). Of these, two control groups for two time points (6 and 24 h) and eight experimental groups, one for each peptide and corresponding time point. Animals from the control groups received saline intraperitoneally once. Animals from the experimental groups received a single intraperitoneal injection of Selank or its fragments (at a rate of 100 mcg/kg

of body weight). Animals from the control and experimental groups were decapitated after 6 or 24 h in accordance with their belonging to the selected time point, the spleen was immediately removed and frozen at –70°C. Total RNA was isolated from spleen tissues using the RNeasy® Mini Kit (Qiagen, Israel). On its basis, the first cDNA strand was synthesized using the RevertAid™ H Minus First Strand cDNA Synthesis Kit ("Fermentas", Lithuania).

25 genes from four groups were selected for analysis: chemokine genes (Ccl3, Ccl7, Ccl9, Ccl11, Ccl17, Ccl19, Ccl20, Cxcl10, Cxcl12, Cxcl15), chemokine receptor genes (Ccr2, Ccr4, Xcr1), cytokine genes (Il10, Il16, Il1f8, Il20, Ifng, Itgam, Itgb2, Scye1) and cytokine receptor genes (Il1r2, Il2rg, Il5ra, Il13ra1). These genes are involved in the cascade of immune responses that occur during inflammatory processes. Three housekeeping genes were selected as reference genes: Actb, Hprt1, and Hsp90ab1. Analysis of the effect of Selank and its three fragments on the expression of these genes in the mouse spleen was performed using real time PCR on an Mx3000P™ Real Time QPCR System (Stratagene Equipment, USA) using the SYBR Green I kit (Sintol, Russia) and commercial primers RT2 qPCR Primer Assay SYBR®Green (SABioscience, USA). The obtained values of reaction cycle thresholds (Ct) were normalized with respect to Ct of housekeeping genes and statistically processed using the Relative Expression Software Tool 384, version 2 [8].

3. Results

Quantitative evaluation performed during the study showed that the response of the largest number of genes upon administration of Selank is observed 24 h after the administration of the peptide (Table 1). The administration of GP, on the contrary, leads to a significant change in the level of mRNA of the largest number of genes 6 h after injection. Changes in the mRNA level of the largest number of chemokine genes were observed 6 h after the administration of GP and tuftsin, and also a day after the administration of Selank. A similar significant decrease in the mRNA level of the Cxcl12 gene was noted, by an average of 1.3 times 6 h after the administration of GP, RPGP, and tuftsin. There were no significant changes in the expression of this gene after the administration of Selank. The genes of chemokine receptors Ccr2 and Ccr4 were characterized by a significant increase in the expression level only after GP administration. A day after the administration of each of the peptides, a decrease in the level of mRNA of the Xcr1 gene encoding the receptor for the activator of the chemotactic activity of lymphocytes was observed. Six hours after the administration of GP, RPGR, and tuftsin, a significant decrease in the level of Itgam gene mRNA was observed, on average, by 50 times, and a twofold decrease in the level of mRNA of this gene a day after the administration of GP. A significant increase in the expression of the gene encoding gamma interferon (Ifng) by 1.2 times was also noted 6 h after administration of tuftsin and Selank. For the Il1r2 gene, a significant decrease in the mRNA level (by 14 times) was observed 6 h after the administration of Selank, and then an increase in the mRNA level of this gene by 4.5 times a day after the administration of the peptide. Also, a decrease in the level of expression of this gene by 11 times 6 hours after the administration of GP and its growth by 3 times a day after the administration of tuftsin was noted. The Il2rg gene, which encodes a common subunit of receptors for various interleukins, was characterized by a drop in the mRNA level 6 h after administration of each of the peptides.

4. Discussion

The results obtained showed that the administration of Selank and each of its fragments considered in this study has a significant effect on changes in the mRNA level of genes for chemokines, cytokines, and their receptors, and activation of some of them is observed even a day after a single administration of peptides. This suggests that Selank is involved in the regulation of inflammatory processes and is capable of inducing targeted changes in the expression of genes involved in the body's immune response.

5. Conclusions

A change in the mRNA level of a greater number of selected genes is observed after the introduction of GP, and the activation of most of them is noted as early as 6 h after the introduction of this peptide. Thus, we can assume that the minimal fragment of Selank that plays the role of a pharmacophore is the dipeptide Gly -Pro. To reveal a more detailed picture of the effect of Selank and its fragments on the expression of genes involved in inflammation processes, we will further study the temporal dynamics of changes in the mRNA level, as well as the dynamics of protein expression of the studied genes after the introduction of peptides. This study was supported by the programs of the Russian Academy of Sciences "Molecular and Cellular Biology", "Fundamental Sciences for Medicine"; State contracts No. 02 740 11 5084, P419; support programs for leading scientific schools (NSh 8418.2010.4, NSh 3438.2010.4).
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Table 1. Relative change in the mRNA level of genes of chemokines, cytokines and their receptors in the mouse spleen after 6 and 24 hours after the introduction of Selank or its fragments (the table shows only statistically significant results)
Note. The base expression level is 1. * p < 0.05; ** p < 0.01.

	Gene	Name of gene	Selank		GP		RPGP		Taftsin	
			6h	24h	6h	24h	6h	24h	6h	24h
chemokines	Ccl3	CC-chemokine ligand 3			1.42*				1.30**	
	Ccl7	CC-chemokine ligand 7		0.87**						
	Ccl9	CC-chemokine ligand 9							0.72*	
	Ccl11	CC-chemokine ligand 11				0.84*				
	Ccl17	CC-chemokine ligand 17	1.13**		1.63**					
	Ccl19	CC-chemokine ligand 19		0.64*						
	Ccl20	CC-chemokine ligand 20			1.38*			0.37**		
	Cxcl10	CXC-chemokine ligand 10			0.73**		0.79*			
	Cxcl12	CXC-chemokine ligand 12			0.81**		0.82**		0.87**	
	Cxcl15	CXC-chemokine ligand 15		0.74**						0.80**
chemokine receptors	Ccr2	CC-chemokine receptor 2			1.53*					
	Ccr4	CC-chemokine receptor 4			1.17*					
	Xcr1	XC-chemokine receptor 1		0.86*		0.80**		0.75**	0.81**	0.88**
cytokines	Il10	Interleukin 10			1.22*					0.69*
	Il16	Interleukin 16					0.77*			
	Il1f8	Interleukin 1-8		1.40**						
	Il20	Interleukin 20		0.76**		0.77*				
	Ifng	Gamma interferon	1.23*						1.25*	
	Itgam	Integrin alpha M			0.02**	0.47**	0.02**		0.02**	
	Itgb2	Integrin beta 2				0.81**		0.64**		
	Scye1	Small inflammatory cytokine E1			1.32*					
cytokine receptors	Il1r2	Interleukin 1 receptor type 2	0.08*	4.46**	0.09**					3.09*
	Il2rg	Interleukin 2 receptor gamma chain	0.57*		0.43**		0.63**		0.51**	
	Il5ra	Interleukin 5 receptor alpha		0.83*						
	Il13ra1	Interleukin 13 receptor alpha-1		0.82*						0.78*

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ashmarin IP, Samonina GE, Lyapina LA, Kamenskii AA, Levitskaya NG, Grivennikov IA, Dolotov OV, Andreeva LA, Myasoedov NF. Natural and hybrid ("chimeric") stable regulatory glyproline peptides. Pathophysiology. 2005; 11(4):179-185.
2. Ashmarin IP. Glyprolines in Regulatory Tripeptides. Neurochemical Journal. 2007; 1(3):173-175.
3. Zozulia AA, Neznamov GG, Siuniakov TS, Kost NV, Gabaeva MV, Sokolov OIu, Serebriakova EV, Siranchieva OA, Andriushenko AV, Telesheva ES, Siuniakov SA, Smulevich AB, Miasoedov NF, Seredenin SB. Efficacy and possible mechanisms of action of a new peptide anxiolytic selank in the therapy of generalized anxiety disorders and neurasthenia. Zh Nevrol Psikhiatr Im S S Korsakova. 2008; 108(4):38-48 (in Russian).
4. Kozlovskii II, Danchev ND. Optimizing action of synthetic peptide Selank on active avoidance conditioning test in rats. Zh Vyssh Nerv Deiat Im I P Pavlova. 2002; 52(5):579-84 (in Russian).
5. Ershov FI, Uchakin PN, Uchakina ON, Mezentseva MV, Alekseeva LA, Miasoedov NF. Antiviral activity of immunomodulator Selank in experimental influenza infection. Vopr Virusol. 2009; 54(5):19-24 (in Russian).
6. Kolomin TA, Shadrina MI, Agniullin YV, Shram SI, Slominskii PA, Limborska SA, Myasoedov NF. Transkriptomny`j otvet kletok gippokampa i selezenki kry`sy` na odnokratnoe i kursovoe vvedenie peptida selanka. Doklady Akademii Nauk. 2010; 430(1): 127–29 (in Russian).
7. Andreeva LA, Nagaev IY, Mezentseva MV, Shapoval IM, Podchernyaeva RY, Shcherbenko VE, Potapova LA, Russu LI, Ershov FI, Myasoedov NF. Antiviral properties of structural fragments of the peptide Selank. Doklady Akademii Nauk. 2010; 431(3):414-418.
8. Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper--Excel-based tool using pair-wise correlations. Biotechnol Lett. 2004; 26(6):509-15.
9. Kolomin TA, Shadrina MI, Slominskii PA, Limborska SA, Myasoedov NF. Izmenenie ekspressii genov hemokinov, citokinov i ih receptorov pod dejstviem selanka i ego fragmentov. Genetika. 2011; 47(5):711-714 (in Russian).