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EFFECT OF GLYCOSYLATED POLYPEPTIDES COMPLEX ISOLATED FROM SEA URCHIN ON THE ACTIVITY OF CYCLOXYGENAZE IZOPHORES AND 5-LIPOXYGENASE

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Abstract

Objective. To study the effect of the complex of glycosylated polypeptides (CGP) isolated from the intestines of sea urchins of the *S. droebachiensis* species on the activity of two isoforms of cyclooxygenase (COX-1 and COG-2), 5-lipoxygenase (5-LOX) enzyme in in vitro tests.

Methods. The activity of the complex of glycosylated polypeptides at final concentrations of 200, 100, 50, 25, 12.5 and 6.25 μg/ml for COX-1 / -2 and 5-LOX enzymes was studied using commercial biochemical test kits (Cayman Chemicals, USA). As a positive control, a selective COX-1 SC-560 inhibitor at a concentration of 0.0035-3.5 μg/ml and a selective COX-2 niflumic acid inhibitor at a concentration of 0.0028-2.8 μg/ml were used.

Results. In vitro tests revealed that the effective concentration (IC50) of the glycosylated peptide complex selectively inhibiting COX-2 activity was 67±3.1 μg/ml. No effects on the activity of the 5-lipoxygenase enzyme were revealed.

Conclusion. The findings make it possible to draw a conclusion that only one isoform of cyclooxygenase – COX-2 is the target of the anti-inflammatory effect of the studied complex of glycosylated peptides isolated from sea urchins.

Keywords: complex of glycosylated polypeptides, sea urchins of the *S. droebachiensis* species, cyclooxygenase

ВЛИЯНИЕ КОМПЛЕКСА ГЛИКОЗИЛИРОВАННЫХ ПОЛИПЕПТИДОВ, ВЫДЕЛЕННЫХ ИЗ МОРСКИХ ЕЖЕЙ, НА АКТИВНОСТЬ ИЗОФОРМ ЦИКЛООКСИГЕНАЗЫ И 5-ЛИПООКСИГЕНАЗУ

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Резюме

Цель. Изучение влияния комплекса гликозилированных полипептидов (КГП), выделенного из внутренностей морских ежей вида *S. droebachiensis,* на активность двух изоформ циклооксигеназы (ЦОГ-1 и ЦОГ-2), фермента 5-липооксигеназы (5-ЛОГ) в тестах *in vitro*.

Методика. Активность комплекса гликозилированных полипептидов в конечных концентрациях 200, 100, 50, 25, 12,5 и 6,25 мкг/мл в отношении ферментов ЦОГ-1/-2 и 5-ЛОГ изучали с использованием коммерческих биохимических тест-наборов (Cayman Chemicals, USA). В качестве позитивных контролей использовали селективный ингибитор ЦОГ-1 SC-560 в концентрациях 0,0035-3,5 мкг/мл и селективный ингибитор ЦОГ-2 нифлумовую кислоту в концентрациях 0,0028-2,8 мкг/мл.

Результаты. В тестах in vitro показано, что эффективная концентрация (IC50) комплекса гликозилированных полипептидов, селективно ингибирующая активность ЦОГ-2, составила 67±3,1 мкг/мл. Влияния на активность фермента 5-липооксигеназы не выявлено.

Заключение. Полученные результаты позволяют сделать вывод о том, что мишенью противовоспалительного действия изучаемого комплекса гликозилированных полипептидов, выделенного из морских ежей, является только одна изоформа циклооксигеназы – ЦОГ-2.

Ключевые слова: комплекс гликозилированных полипептидов, морские ежи вида *S. droebachiensis*, циклооксигеназа

Introduction

Studies aimed at analyzing biologically active substances from marine organisms revealed their antioxidant, antimicrobial, immunomodulating, antiviral, regenerative, lipid-lowering and antidiabetic activities. At present, seven preparations from marine hydrobionts are registered in the world pharmaceutical market. In the Russian Federation, the following preparations are registered and used - Histochrome for Ophthalmology, Histochrome for Cardiology, and Collagenase. The preparations were developed by the Pacific Institute of Biochemistry of the Far Eastern Branch of the Russian Academy of Sciences [6].

It was revealed that peptides obtained from hydrolysates of proteins of marine organisms, for example, mussels, sea eel, microalgae, sea urchins, possess an anti-inflammatory effect [10]. The complexity of studying the anti-inflammatory activity of certain substances is due to the fact that inflammation is a multivalent, dynamic process with various alternative and crossed paths existing both at the level of intracellular interactions of the signaling cascades and at the level of regulation of the production of inflammatory mediators [7, 8]. Thus, the urgency of creating new drugs that can regulate the functional activity of molecules involved in inflammation, with minimal manifestations of side effects is obvious.

Since one of the most probable studied mechanisms of drugs anti-inflammatory action is the depression in the production of prostaglandins from arachidonic acid by inhibiting cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) enzymes [7], it is reasonable to study a similar mechanism of action in a complex of glycosylated polypeptides isolated from hydrobionts. This will make it possible to obtain innovative chemical compounds (molecules) for later creation of new drugs [1, 2].

The aim of the study was to assess the effect of a complex of glycosylated polypeptides isolated from S. droebachiensis sea urchins on the activity of two cyclooxygenase isoforms (COX-1 and COX-2) and the 5-LOX enzyme.

Methods

In the experiment, male Wistar rats weighing 200 ± 15.0, obtained from the "Rappolovo"laboratory animal bank of the Russian Academy of Medical Sciences, were studied. The rats were divided into groups of 5 and 6 individuals and kept on the bedding (the floor area per animal - 440 and 367 cm2, respectively). For feeding, the PK-120-1 mixed fodder (GOST R 50258-92) was used in accordance with the norms approved by the Order of the Ministry of Health of the USSR of 12.08.1977 No. 755. The animals were given water ad libitum in accordance with SanPiN 2.1.4.1074-01 "Drinking water. Hygienic requirements for water quality of centralized drinking water supply systems. Quality control". The animals were kept under controlled environmental conditions (19-25 ° C, air humidity 30-70%), NH3 ≤ 10 mg / m3, CO□ ≤ 0.15% vol. The light regime amounted to 12 hours of light and 12 hours of darkness. The air exchange regime ensured the change of about 15 room volumes per hour. The temperature and humidity of the air were recorded daily. Before the beginning of the study, the animals were divided into groups by the modified block randomization method.

The studies were carried out in accordance with the guidelines and normative documents, the rules of laboratory practice of preclinical research in the Russian Federation (GOST R 53434-2009) and Directive 2010/63 / EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes.

The test substance is a complex of glycosylated polypeptides (CGP) isolated in the laboratory of the St. Petersburg Institute of Pharmacy, from the entrails of green sea urchins of the S. droebachiensis species. Gas-liquid chromatography with mass spectrometry (GLC-MS) and high-performance liquid chromatography (HPLC-ELSD) showed that the substance contains 10-15% of peptides, 35-45% of amino acids, 4-8% of phospholipids, trace elements, and sugars [4].

The effect of CGP at final concentrations of 200, 100, 50, 25, 12.5 and 6.25 μg / ml on the activity of COX-1 and COX-2 was assessed using the "COX inhibitor screening assay kit" (Cayman Chemicals, USA) in accordance with the manufacturer's instructions. As positive controls, the selective COX-1 inhibitor SC-560 (Cayman Chemicals, USA) was used at concentrations of 0.0035-3.5 μg / ml and the selective COX-2 inhibitor niflumic acid (Cayman Chemicals, USA) - at concentrations of 0.0028 -2.8 μg / ml. The enzyme activity was evaluated by the concentration of the final product of the prostaglandin F2α (PGF2α) reaction with the enzyme immunoassay.

The optical density of the samples was measured on the xMark plate spectrophotometer (BioRad, USA) at the wavelength of 405 nm. The concentration of prostaglandin produced during the catalytic reaction involving COX-1 / -2 was calculated with the calibration curve constructed according to the optical density of different concentrations of the standard. The values characterizing the control samples were combined into the control group; the mean value was determined and taken as 100%. The concentration values of the other groups were expressed as the percentage of the control (% inhibition).

Inhibition percentage was calculated according to the following formula:

$$100 - \frac{C_{sample}}{C_{control}} \times 100$$, with C – reaction product concentration

Analysis of the effect of CGP on the enzymatic activity of 5-LOX was carried out at final concentrations of 1000, 100, 10, 1, 0.1 and 0.01 μg / ml using the commercial kit "Lipoxygenase Inhibitor Screening Assay Kit" (Cayman Chemical, USA) and purified 5-LOX of potatoes (Cayman Chemical, USA). The 5-LOX-NDGA non-selective inhibitor (Cayman Chemicals, USA) was used as the comparison preparation in accordance with the test system manufacturer's data at concentrations of 0.5-15 μg / ml. The amount of hydroperoxides released during the lipoxygenation reaction was evaluated with the test system.

The incubation time of the NDGA non-selective inhibitor / the studied preparation with 5-LOX (enzyme) and linolenic acid (substrate) was 10 min. Enzymatic catalysis was stopped by applying the chromogen in 5 minutes, after which the photometric measurement was made on the xMark (BioRad, USA) plate spectrophotometer at the wavelength of 490 nm.

The activity of the 5-LOX enzyme was calculated by the value of the optical density, according to the following formula:

$$\frac{A490/\min}{9{,}47 mM^{-1}} \times \frac{0{,}21 ml}{0{,}09 ml} \times 1000 = mmol / \min / ml$$

, with: A490/min – optical density at the wavelength of 490 nm; 9,47 mM^{-1} – extinction coefficient; 0,21 ml – total sample volume; 0,09 мл – volume of the control.

The inhibition process was calculated according to the following formula:

$$100 - \frac{EA_{sample}}{EA_{control}} \times 100,$$ with EA – enzyme activity

Statistical analysis of the data was carried out using the Statistics 10.0 (StatSoft, USA) software. The one-way ANOVA test was used to statistically evaluate the indicators obeying the normal distribution law, followed by the inter-group post hoc comparison using the Tukey's post hoc test. The nonparametric Kruskel-Wallis criterion was used for data not obeying the normal distribution law (scores). The differences were determined at 0.05 significance level.

Results and discussion

The study revealed that the calculated effective concentration (IC50, 50% of inhibitory activity) for the SC-560 selective inhibitor was 0.1 μM (35 ng / ml). The CGP substance at all the tested concentrations had no significant effect on the COX-1 activity (ANOVA, $p > 0.05$), but inhibited the activity of the COX-2 enzyme in a dose-dependent manner.

The curve characterizing the dependence of the amount of the PGF2α prostaglandin produced during the catalytic reaction with the participation of the COX-2 enzyme on the concentration of CGP was sigmoid-like, reaching the plateau at 80% at a concentration of 50-200 μg / ml (Fig. 1).

The calculated effective concentration (IC50) for the standard selective COX-2 niflumic acid inhibitor was 0.03 μg / ml, while for the complex of glycosylated peptides it was 67 ± 3.1 μg / ml.

It was suggested that in addition to inhibiting the cyclooxygenase enzyme, a possible mechanism of the anti-inflammatory action of the studied complex could be the inhibition of the 5-lipoxygenase enzyme. The design of the study, aimed at comparing the 5-lipoxygenase inhibitory concentrations of the studied substance (CGP) and the NDGA lipoxygenase non-selective inhibitor, is presented in Table. 1.

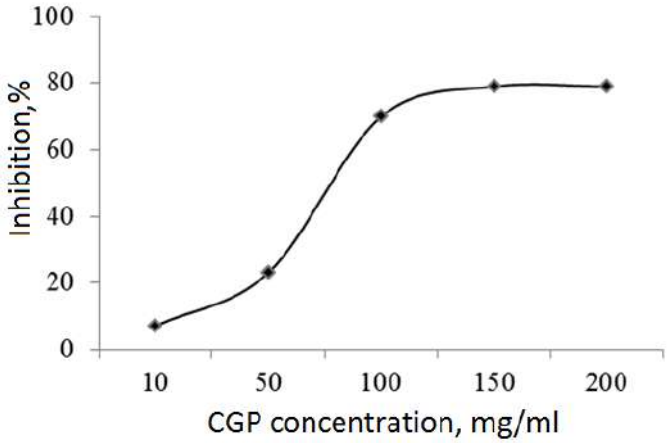

Рис. 1. Dependence of the amount of the PGF2α prostaglandin, produced during the catalytic reaction with the participation of the COX-2 enzyme on the concentration of the complex of glycosylated polypeptides (CGP) in μg / ml isolated from the *S. Droebachiensis* sea urchins

Table 1. Results of the evaluation of the interaction of the glycosylated peptides complex substance and the NDGA 5-lipoxygenase non-selective inhibitor with the 5-lipoxygenase enzyme.

Sample	5-LOX enzyme activity (mmol/min/ml)	5-LPX inhibition % (from the control)
Control (distilled water)	10,1±0,4 (n=3)	-
NDGA, 15 μg / ml (50 μ)	0±0 (n=3)	100±1
NDGA, 10 μg / ml (33μ)	0±0 (n=3)	100±2
NDGA, 5 μg / ml (17 μ)	1,2±0,6* (n=3)	88±3
NDGA, 3 μg / ml (10 μ)	4,8±0,2* (n=3)	53±1
NDGA, 1 μg / ml (3 μ)	7,0±0,3* (n=3)	31±2
NDGA, 0,5 μg / ml (2 μ)	9,1±0,1* (n=3)	10±1
CGP, 1000 μg / ml	10,0±0,1 (n=3)	1±1
CGP, 100 μg / ml	10,0±0,3 (n=3)	1±2
CGP, 10 μg / ml	9,9±0,2 (n=3)	3±1
CGP, 1 μg / ml	10,6±0,2 (n=3)	-5±1
CGP, 0,1 μg / ml	10,1±0,1 (n=3)	0±1
CGP, 0,01 μg / ml	10,5±0,1 (n=3)	5±1

Note: CGP – complex of glycosylated polypeptides; NDGA – non-selective 5-LOX inhibitor, used in the appropriate test-system; * – statistically relevant differences compared to the control, Tukey test, $p < 0,05$

The findings revealed that the NDGA 5-lipoxygenase nonselective inhibitor reduced the activity of the enzyme. The calculated effective IC50 concentration was 5.3 μM (1.6 μg / ml). The glycosylated polypeptides complex in the studied concentrations showed no inhibitory activity relating to the 5-lipoxygenase enzyme.

Experimental studies of the pharmacological activity of peptides isolated from sea urchins are of particular interest, since representatives of this group of hydrobionts contain a very wide range of pharmacologically active peptides. Chloroform and methanol extracts obtained from the intestine and gonads of the *Tripneustes gratilla* sea urchins possess a pronounced antibacterial activity against *Salmonella typhi, E. coli, Shigella sonnei and P. aeruginosa* [3]. The peptide, encoded CEN1 HC, isolated from the *Strongylocentrotus droebachiensis* green sea urchin, showed antistaphylococcal activity, including the antistaphylococcal activity on the methicillin-resistant strains of staphylococcus [5]. Along with the antibacterial action, anti-inflammatory activity was demonstrated, which was confirmed in models of infected wounds in vitro and in vivo.

A number of consequent studies focused primarily on identifying and confirming the anti-inflammatory activity. In particular, water extract of the gonads of the Heliocidaris erythrogramma sea urchins at a dose of 50 mg / kg exhibited an anti-inflammatory effect on the model of adjuvant arthritis in rats, reducing the paw edema by 55% compared to the control group. The same extract at a concentration of 0.5 mg / ml

inhibited the activity of cyclooxygenase-1 (COX-1) by more than 45%, which exceeded the inhibitory activity of indomethacin at the same concentration by 1.4 times [9].

The selective inhibitory effect of the hydrobiont glycosylated polypeptides complex on COX-2 (the enzyme required for the synthesis of cyclic endoperoxides such as PGG2, PGH2) is likely to violate the known "arachidonic acid cascade", realized during the formation of biologically active substances from cell membrane phospholipids, taking part in the inflammatory process. The result of the COX-2 blockade is a decrease in the biosynthesis of prostanoids. The decrease in the activity of prostaglandinsynthetase resulting from the inhibition of cyclooxygenase is completed by a decrease in the biosynthesis of prostaglandins of the PGE2 subtype, as well as the achievement of anti-inflammatory, analgesic and antipyretic effects, which will be further confirmed in the experimental models in vivo.

Absence of inhibition of the 5-lipoxygenase activity, affecting the production of leukotrienes, unfortunately limits the immunomodulatory effect of glycosylated polypeptides in allergic and inflammatory reactions. However, the immunosuppressive activity can be excluded only after studying the ability of the substance to bind to leukotriene receptors (TBT4) and block them, similar to that of zafirlukast.

Conclusion

It is common knowledge that COX-2 inhibition causes the anti-inflammatory effect of non-steroidal anti-inflammatory drugs (NSAIDs), whereas suppression of the COX-1 enzyme and subsequent blockade of the cytoprotective prostaglandins (PG) synthesis, in particular PGI_2, PGE_2 and A2 thrombocon, cause side effects, among which the most severe are erosion and ulcers of the gastrointestinal tract, hepatic and renal insufficiency, impaired blood clotting and bronchospasm. In the current study, the selective inhibitory effect of the glycosylated polypeptides complex on the COX-2 enzyme at an effective concentration of 67 ± 3.1 μg / ml, comparable to aspirin and ibuprofen, was established. However, due to the selective action, it is obviously devoid of the ability to cause side effects characteristic of classical nonsteroidal anti-inflammatory drugs.

Toll-like receptors (TLR) responsible for antigen recognition, activation of antigen-presenting cells, inflammatory mediators synthesis and the development of an immunological response, as well as the P38 mitogen-activated protein kinase providing post-transcriptional regulation of inflammatory proteins synthesis can be considered the application point for the implementation of the anti-inflammatory effect of the studied complex. Data on the studies of the ability of hydrobionts glycosylated polypeptides complex to suppress Toll-like receptors (TLR) and the P38 mitogen-activated protein kinase, will be provided in subsequent publications.

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