Матриксные металлопротеиназы-1, -13 и их тканевой ингибитор 1-го типа при эндокринной офтальмопатии

© Е.С. Таскина*, С.В. Харинцева

ФГБОУ ВО «Читинская государственная медицинская академия», Чита, Россия

Эффективная регенерация поврежденных при эндокринной офтальмопатии (ЭОП) мягких тканей орбиты требует координированного ремоделирования внеклеточного матрикса. Матриксные металлопротеиназы (MMPs) играют решающую роль в гомеостазе синтеза и деградации компонентов внеклеточного матрикса при различных физиологических и патологических состояниях. Их протеолитическая активность ингибируется тканевыми ингибиторами (TIMP). Биохимия фиброгенеза экстраокулярных мышц и ретробульбарной клетчатки при ЭОП изучена недостаточно.

Цель исследования — раскрытие некоторых биохимических механизмов фиброгенеза экстраокулярных мышц и ретробульбарной клетчатки при ЭОП.

Материал и методы. Под наблюдением находились 65 человек (130 глаз) в возрасте 43 (35–50) лет. Из них сформированы три группы: 32 пациента с ЭОП средней степени тяжести (основная группа), 18 пациентов с аутоиммунной патологией щитовидной железы без ЭОП (группа сравнения) и 15 здоровых лиц (контроль). Диагноз был выставлен на основании клинических, лабораторных и инструментальных данных. Проводилось комплексное офтальмологическое обследование; в крови определяли концентрации матриксных металлопротеиназ-1, -13 (ММР-1, -13), тканевого ингибитора металлопротеиназ-1 (TIMP-1), сульфатированных гликозаминогликанов (sGAG) и антител к рецептору тиреотропного гормона (АТ к рТТГ). Статистическая обработка данных осуществлялась в программе Statistica 10.0.

Результаты. Уровень ММР-13 был повышен у всех пациентов с ЭОП (p<0,05). В активную фазу ЭОП зафиксировано увеличение концентрации ММР-13 в 3,5 раза (p<0,001) и TIMP-1 в 1,17 раза (p>0,05) по сравнению с контролем. После пульс-терапии глюкокортикостероидами (ГКС) происходило снижение уровня ММР-13 на 48,6% (p<0,001), TIMP-1 на 2,7% (p<0,001) и АТ к рТТГ на 93% (p<0,001) по сравнению с активной ЭОП, но значения данных показателей превышали референсные границы контрольной группы (p>0,05). В неактивную фазу ЭОП, несмотря на повышенные показатели ММР-13, уровень TIMP-1 снижался до референсных значений контроля (p=0,533). Значимых различий по уровню ММР-1 в группах исследования не зафиксировано (p=0,865).

Заключение. Выявлен дисбаланс между продукцией ММР-13 и TIMP-1 в разные фазы активности ЭОП. Активная ЭОП характеризуется повышением уровней ММР-13 и TIMP-1 в сыворотке крови. Дисрегуляция ремоделирования межклеточного матрикса, возможно, лежит в основе развития фиброза экстраокулярных мышц и ретробульбарной клетчатки при ЭОП.

Ключевые слова: эндокринная офтальмопатия, патогенез, фиброз, матриксные металлопротеиназы, тканевой ингибитор металлопротеиназ, гликозаминогликаны.

Matrix metalloproteinases-1, -13 and their tissue inhibitor-1 in endocrine ophthalmopathy

Elizaveta S. Taskina*, Svetlana V. Kharintseva

Chita State Medical Academy, Chita, Russia

Effective regeneration of damaged soft orbital tissues in Graves' ophthalmopathy (GO) requires coordinated remodeling of the extracellular matrix. Matrix metalloproteinases (MMPs) play an important role in the synthesis and degradation homeostasis of extracellular matrix components in various physiological and pathological conditions. Their proteolytic activity is inhibited by tissue inhibitors of metalloproteinases (TIMP). The biochemical processes taking place in extraocular muscles and retrobulbar tissue fibrogenesis in GO are not fully understood.

Aims — to assess some biochemical mechanisms of extraocular muscles and retrobulbar tissue fibrogenesis in GO patients.

Material and methods. The study included 65 people (130 eyes) at the age of 43 (35–50) years. Three groups of subjects were formed: 32 patients with a moderate GO severity (clinical group), 18 patients with autoimmune thyroid pathology without GO (comparison group), and 15 healthy persons (control). The diagnosis was based on clinical, laboratory, and instrumental data. A comprehensive ophthalmologic examination and blood sampling for determination of MMP-1, -13, TIMP-1, sulfated glycosaminoglycans (sGAG) and antibodies to thyroid-stimulating hormone receptor (TSHRAbs) were conducted. The data were statistically processed using the program Statistica 10.0.

Results. An elevated level of MMP-13, observed in all GO patients (p<0.05). For the active phase of GOP, the comparison with the control group showed a 3.5-fold increase in MMP-13 (p<0.001) and 1.17-fold rise in TIMP-1 (p>0.05). Pulse glucocorticoid therapy reduced MMP-13 by 48.6% (p<0.001), TIMP-1 by 2.7% (p<0.001), and TSHRAbs — by 93% (p<0.001) compared with active GO, but these indicators were higher than the reference limits of control (p>0.05). In inactive GO, despite increased MMP-13, TIMP-1 decreased to the reference values (p=0.533). There were no significant differences in MMP-1 in groups of subjects (p=0.865).

Conclusions. We have found imbalance between MMP-13 and TIMP-1 production in different activity phases of GO. Active GO is characterized by an increase in serum MMP-13 and TIMP-1. Dysregulation of intercellular matrix remodeling, possibly, underlies the development of extraocular muscles and retrobulbar tissue fibrosis in GO.

Keywords: Graves' ophthalmopathy, pathogenesis, fibrosis, matrix metalloproteinases, tissue inhibitor of metalloproteinases, glycosaminoglycans.

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Background

Endocrine ophthalmopathy (GO) is a chronic orbital disease, often occurring against the background of thyroid dysfunction, and characterized by autoimmune inflammation of extraocular muscles and/or retrobulbar tissue with the possibility of their subsequent fibrosis [1, 2]. The result of GO can be characterized by a limiting of the range of movement of the eyeball, and the formation of persistent diplopia, strabismus and exophthalmos [1]. At present, the biochemical processes of fibrogenesis in the intercellular matrix of extraocular muscles and retrobulbar tissue in GO are insufficiently studied.

Activated orbital fibroblasts play a key role in the pathogenesis of GO [3, 4]. Under the influence of antibodies to the thyroid-stimulating hormone receptor (TSHRAbs), and various proinflammatory cytokines and growth factors, these cells secrete excess intercellular matrix components, including collagen, fibronectin, elastin and glycosaminoglycans [5, 6]. Hypersecretion of glycosaminoglycans leads to the binding of water molecules and the formation of soft retrobulbar edema, and the development of clinical manifestations characteristic of GO [4, 6].

It is known that matrix metalloproteinases (MMPs) play a decisive role in the process of remodeling the components of the extracellular matrix and the development of fibrosis in various inflammatory diseases [7]. MMPs are extracellular zinc-dependent proteolytic enzymes belonging to the cathepsin group [7]. More than 20 enzymes have been described, which, depending on their properties and substrate specificity, are divided into collagenases, stromelysins, gelatinases, matrilysins, and membrane-linked MMPs [8]. MMPs synthesis is regulated mainly at the transcription level, and their proteolytic activity is controlled through the activation of proenzymes and the interaction with tissue inhibitors of metalloproteinases -1 and -2 (TIMPs-1, -2) [7, 8]. MMPs are secreted by normal and transformed fibroblasts, epithelial cells, phagocytes, and lymphocytes [7].

Fibrosis formation is characterized by changes in the homeostasis of synthesis and degradation of collagen. MMP-1 (interstitial collagenase-1) and MMP-13 (collagenase-3) break down collagen types 1-3, 7, and 10 [8]. Therefore, changes in the concentration and activity of MMPs may play an important role in the change in the metabolism of collagen of the extracellular matrix and initiate the development of fibrosis. The problem of MMP-1, -13 and TIMP-1 imbalance influence on soft retrobulbar tissue fibrogenesis in GO remains current.

AIM

To discover some of the biochemical mechanisms of fibrogenesis of extraocular muscles and retrobulbar tissue in Graves' ophthalmopathy (GO).

Methods

Research Design

The study was an observational case control study with parallel groups: clinical and laboratory activity scores of the main group (patients with GO) were compared with those of the comparison group (autoimmune thyroid pathology without GO) and the control group (healthy individuals). During the study, the core group was divided into two subgroups:

- patients with GO in an active phase;
- patients with inactive GO in the stage of extraocular muscle fibrosis and retrobulbar tissue.

In the subgroup of patients with GO in an active phase, a prospective observational study was carried out to assess the effect of glucocorticosteroids (GCS) pulse therapy on clinical and laboratory activity scores in GO.

Compliance criteria

Patients with a clinically and medically confirmed autoimmune thyroid pathology and/or a diagnosis of moderate GO were included in the study. The diagnosis was verified based on the consultation of an endocrinologist and ophthalmologist, thyroid ultrasound, CT scan of the orbits (in cases of GO), as well as a determination of levels of TTG and thyroxin (T4).

The control group included individuals who considered themselves to be essentially healthy with a normal psychosomatic disposition, who were not taking medications at the time of the study, and who were examined by relevant specialists in the course of clinical examinations.

Criteria for exclusion from the study: orbital diseases of other etiologies; severe somatic pathology impeding further study; cancer; systemic autoimmune diseases; infectious diseases; diabetes mellitus; pregnancy and lactation.

Terms and conditions

The study was conducted at the State University of Health "Regional Clinical Hospital No. 1", Department of Ophthalmology, and in the biochemistry laboratory of the Research Institute of Molecular Medicine of the Chita State Medical Academy.

Duration of the study

The study included patients hospitalized in the endocrinology unit of the State University of Health "Regional Clinical Hospital No. 1", or referred to the outpatient clinic of the Chita State Medical Academy in the period of 2016 to 2018.

Patients with inactive GOs, as well as patients in the comparison and control groups, were examined. Venous blood samples were taken for single determinations of MMP-1, MMP-13, TIMP-1 concentrations, and sulfated glycosaminoglycans (sGAG) and TSHRAbs in serum. A subgroup of patients with active GOs had two planned visits, including clinical and laboratory assessments be-

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fore and immediately after GCS pulse therapy, in two-month intervals.

Description of medical intervention

Complex ophthalmologic examinations included collection of complaints and anamnestic data, examination of the visual organ, exophthalmometry, visometry, autorefractometry, tonometry, bio microscopy, indirect ophthalmoscopy, and perimetry. Pachymetry, A-scan of eyeballs and optical coherence tomography of optic nerve discs were performed according to indications.

The material for the study was the patient's venous blood serum. Blood was taken at the same time of day (8:00 a.m.), on an empty stomach, in a sitting position.

The main outcome of the study

The main datapoints of the study were the concentration of MMP-1, MMP-13 and TIMP-1 in blood serum and their connection with clinical manifestations of GO, as well as with changes from glucocorticosteroids (GCS) pulse therapy.

Additional outcomes of the study

The presence and strength of correlation links between sGAG concentration and MMP-1, MMP-13, TIMP-1 and TSHRAbs level in blood serum in patients with GO were determined, as well as the possibility of using sGAG concentration as an additional laboratory criterion for GO activity and severity.

Analysis in subgroups

In the course of the study, 3 groups of participants were formed:

• *the main group* included patients with moderate GOs in different phases of activity

• *the comparison group* – patients with autoimmune thyroid pathology without GO;

• *the control group* – healthy individuals, with comparable age and gender parameters.

Depending on the activity phase of autoimmune inflammation in the orbit, the patients in the main group were divided into 2 subgroups:

• Subgroup 1 – active GO;

• 2nd subgroup – inactive GO in the stage of ocular motor muscle and retrobulbar fibrosis.

The criteria for the distribution of patients from the main group to the subgroups of the study were the measures of the severity and the progression of activity of GO on the CAS scale (Clinical Activity Score), as well as the condition of soft retrobulbar tissues as seen in computed tomography of orbits (proptosis, thickness of ocular motor muscles, X-ray density of retrobulbar fibers and ocular motor muscles).

Methods of recording outcomes

The severity of GOs were classified according to NOSPECS classifications, as recommended by the European Graves Orbitopathy Research Group (EUGOGO), which included mild, moderate and severe forms of GO. Disease activity was evaluated using the CAS point scale, indicating inactive (CAS<3) and active (CAS⩾3) GO phases [1, 9].

The serum levels of MMP-1, MMP-13 and TIMP-1 were determined by solid-phase enzyme-linked immunoassay using Cloud-Clone Corp. (USA), sensitivity: 0.059 ng/ml for MMP-1 and TIMP-1 and 12.1 ng/ml for MMP-13. To determine the level of sGAG in the serum we used reagents of EURO Diagnostica AD (Sweden), measuring range: 1.4-400 μg/ml. TSGHRAbs levels were determined by competitive enzyme immunoassay using reagents from MEDIPAN GMBH (Germany). Normal limits for TSHRAbs: ⩽ 1.5 mEd/l.

Ethical review

The study was approved by the Local Ethics Committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation (Minutes No. 81 of 28.10.2016).

Statistical analysis

Statistica 10.0 software was used for statistical analysis ("StatSoft", USA). Before the analysis began, the variation series were tested for normal distribution using criteria of Kolmogorov-Smirnov and Shapiro-Wilk tests. Because the signs in the study groups had a different distribution than normal, the median and interquartile range were calculated for each indicator (Me [Q25; Q75]). The Mann-Whitney U-criterion was used to evaluate the statistical significance of the differences in the numerical data of the two independent groups; Wilcoxon T-criterion was used to compare the two dependent groups; and Kruskal-Wallis H-criterion was used in more than two samples. The statistical significance of differences in clinical and laboratory parameters was analyzed using Pearson's Chi-square ($\chi 2$) and Yates correction criterion. The ratio of chances and risks of the active form of EOP development with an increase in the concentrations of biochemical parameters in serum was calculated. The Spearman Rank Correlation Coefficient was calculated to assess the relationship between the indicators. The critical level of significance in testing statistical hypotheses in this study was assumed to be $p<0.05$.

Results

Objects (participants) of the study

All the patients included in the study were separated into either a comparison group, consisting of those patients with autoimmune thyroid pathology without GO (n=18), or the main group, consisting of those patients

with medium severity of GO of different activity phases (n= 32).

Depending on the activity phase of GO, patients in the main group were divided into 2 subgroups. Subgroup 1 was represented by 15 patients with active GO with a median age of 46 [35-52] years, with a less-than-6-months history of the disease and signs of swelling and infiltration of the soft retrobulbar tissues, as seen in computer tomography of the orbits. This subgroup was divided into group A, which consisted of 15 patients with active GO before GCS pulse therapy, and group B – 15 patients with inactive GO immediately after the course of GCS pulse therapy (total loading dose 6-8 mg of methylprednisolone). Subgroup 2 consisted of 17 patients with a median age of 46 [35-51] years with inactive GO in the stage of ocular motor fibrosis and retrobulbar fibrosis, and anamnesis of the disease for more than 18 months. **(Table 1)**.

Main findings of the study

The level of TSHRAbs was significantly higher ($p<0.05$) for all phases of GO activity than in the comparison and control groups **(Table 2)**. In 100% of patients with active phase GO (CAS⩾3), the TSHRAbs titer was higher or equal to 1.5 MEU/l. In 15% of patients with inactive phase GO (CAS<3) the results of the TSHRAbs determination were false positive ($\chi^2=33.03$; $p<0.001$). A direct strong correlation link ($r=0.77$) was revealed between the TSHRAbs titer, higher or equal to 1.5 MEU/l and GO activity ($p<0.001$) **(Table 3)**.

No significant differences in the levels of SMR-1 were recorded in the study groups ($p=0.865$). However, in the active phase of GO, a 3.5-fold ($p<0.001$) increase in the concentration of MMP-13, and a 1.17-fold ($p>0.05$) increase in TIMP-1 was revealed in comparison with the control group **(Table 2)**. At the same time, these indicators had weak direct correlation relations ($r=0.33$; $p<0.001$) **(Table 3)**.

After GCS pulse therapy there was a 48.6% ($p<0.001$) decrease in MMP-13, and a 93% ($p<0.001$) decrease in TSHRAbs, in comparison with the active phase of GO, but the values of these parameters exceeded the reference boundaries of the control group ($p>0.05$). TIMP-1 levels in patients with active GO before and after treatment did not differ significantly ($p>0.05$) (Table 2).

At the same time, the level of MMR-13 in inactive GO in the fibrosis stage remained 2 times higher than in the control group and did not differ from its level in patients after pulse GCS therapy ($p=0.6778$). In patients with inactive GO in the fibrosis stage, the TIMP-1 level returned to the reference control group boundaries ($p=0.255$) (Table 2).

Direct links of mean force ($r=0.65$) between the content of MMP-13 and TSHRAbs ($p<0.001$) were revealed, as well as weak links ($r=0.35$) between the content of TIMP-1 and TSHRAbs in the study groups ($p<0.001$) **(Table 3)**.

The number of patients with active phase GO and levels of MMP-13>60 ng/ml and TIMP-1>105 ng/ml was 88%, and with inactive phase GO and levels of MMP-13 ⩽ 60 ng/ml and TIMP-1 ⩽ 105 ng/ml – 93% ($\chi2=63.^{07}$; $p<0.001$). A direct strong correlation ($r=0.8$; $p<0.001$) between MMP-13>60 ng/ml and TIMP-1>105 ng/ml and GO activity (CAS ⩾3) was revealed. A direct correlation ($r=0.76$; $p<0.001$) between MMP-13>60 ng/ml and TIMP-1>105 ng/ml and TSHRAbs⩾1.5mEd/l was also found. **(Table 3)**.

Patients with concentrations of MMP-13>60 ng/ml and TIMP-1>105 ng/ml had a relative risk of active GO of 18.3 (95% CI, 4.65-72.13). The ratio of chances of developing the active phase of the disease was 105 (95% CI, 15.9-690.8).

Additional research results

In all patients with GO, regardless of the activity phase, the sGAG concentration in serum was significantly higher than in the control group ($p<0.001$). There was also an increase in the sGAG level in the comparison group by a factor of 1.7 compared to control ($p<0.05$) **(Table 2)**.

No significant differences in sGAG levels in serum before and after GCS pulse therapy were recorded ($p>0.05$) (Table 2).

The increase in the sGAG level is directly and weakly related to the increase in the concentration of MMP-13 ($r=0.43$; $p<0.001$) and TSHRAbs titre ($r=0.43$, $p<0.05$). No dependencies were found between sGAG and TIMP-1 levels ($r=0.20$; $p=0.074$) **(Table 3)**.

Adverse events

No adverse events were observed in this study.

Discussion

Summary of the main result of the study

We revealed an imbalance in the production of MMP-13 and TIMP-1 in different phases of GO activity. The MMR-13 level was elevated in all patients with GO ($p<0.05$), but the maximum value was recorded in the active phase ($p<0.001$). The TIMP-1 content in blood serum in the active phase of GO also increased ($p<0.05$). However, in inactive GO in the stage of soft retrobulbar tissue fibrosis, despite the increased parameters of MMP-13, the level of TIMP-1 decreased to the reference values of the control group ($p=0.533$).

The result of the study was identification of an additional laboratory diagnostic marker: concentration of MMR-13>60 ng/ml and TIMP-1>105 ng/ml in blood serum characterizes active GO of medium severity.

The study groups did not differ significantly in terms of MMR-1 level ($p=0.865$).

One of the possible mechanisms of the profibrotic influence of GCS pulse therapy on soft retrobulbar tissues in GO is the imbalance between MMP-13 and TIMP-1,

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Table 1. **Length and activity of endocrine ophthalmopathy, (Me [Q25; Q75])**

Indicator	Core group		
	Active GO		Inactive GO (n=17)
	Group A before the GCS treatment (n=15)	Group B afterGCS treatment (n=15)	
GO experience, months	3 [3; 5]	5 [5; 6]	24 [19; 25]
GO activity on CAS scale (0-7)[1], points	4 [4; 5]	2 [2; 3]	1 [0; 2]

Note. Here and in Table 2.3: n – number of observations in groups; Me [Q25; Q75]1 – median (interquartile sweep in the form of 0.25 and 0.75 quartiles);
[1]CAS (Clinical Activity Score) – scale of clinical activity of endocrine ophthalmopathy.

Table 2. **Dynamics of antibodies to thyroid hormone receptor, sulfated glycosaminoglycans, matrix metalloproteinases-1, -13 and tissue inhibitor-1 in groups (Me [Q25; Q75])**

Indicator	Control (n=15)	Comparison Group (n=18)	Основная группа		
			активная ЭОП		Inactive EOP (n=15)
			Before GCS treatment (n=15)	After GCS treatment (n=15)	
TSHRAbs concentration, mEd/l	0,01 [0; 0,27]	0,1 [0; 0,3] p=0,5387	16,9 [7,78; 24,4] p<0,001 p_1<0,001	1,16 [0,16; 6,33] p<0,05 p_1<0,05 p_2<0,001	0,51 [0,25; 0,82] p<0,001 p_1<0,001 p_3<0,001 p_4=0,597 p_5<0,001
sGAG concentration, μg/ml	11,74 [7,2; 15,5]	19,7 [13,3; 25,2] p<0,05	25,6 [21,5; 33,3] p<0,001 p_1<0,05	30,77 [16,3; 32,05] p<0,001 p_1<0,05 p_2=0,609	29,06 [22,35; 35,04] p<0,001 p_1<0,05 p_3=0,597 p_4=0,558 p_5<0,05
Concentration MMP-1, ng/ml	2,89 [2,58; 3,64]	2,94 [2,78; 3,4] p=0,745	3,02 [2,79; 3,62] p=0,619 p_1=0,492	2,8 [2,57; 3,65] p=0,648 p_1=0,448 p_2=0,57	2,93 [2,45; 3,29] p=0,558 p_1=0,338 p_3=0,206 p_4=0,879 p_5=0,865
Concentration MMP-13, ng/ml	21,3 [17,6; 27,5]	24,8 [17,6; 29,7] p=0,856	73,7 [61,6; 92,4] p<0,001 p_1<0,001	37,9 [20,9; 52,8] p<0,05 p_1<0,001 p_2<0,001	42,9 [18,7; 50,6] p<0,05 p_1<0,05 p_3<0,001 p_4=0,6778 p_5<0,001
Concentration TIMP-1, ng/ml	101,03 [93,5; 111,03]	108,9 [102,4; 119,32] p=0,071	118,35 [106,15; 165,7] p<0,05 p_1<0,05	115,1 [109,05; 134,6] p<0,05 p_1<0,05 p_2=0,733	103,6 [94,1; 112,04] p=0,533 p_1=0,255 p_3<0,05 p_4<0,05 p_5<0,05

Note. p – statistical significance in comparison with control; p_1 – statistical significance in comparison with comparison group; p_2 – statistical significance in comparison group with active GO before and after GCS pulse therapy; p_3 – statistical significance between active GO before GCS pulse therapy and inactive GO in the stage of fibrosis; p_4 – statistical significance between active GO after GCS pulse therapy and inactive GO; p_5 – statistical significance in comparison of all samples.

Table 3. **Results of correlation analysis of clinical and laboratory parameters of endocrine ophthalmopathy**

Corrective signs	Correlation indicators	
	Correlation coefficient, r	Level of significance, p
Concentration of TSHRAbs ⩾ 1.5 mEd/l and CAS ⩾ 3	0,77	<0,001
Concentration of TSHRAbs and MMR-13	0,65	<0,001
Concentration of TSHRAbs and TIMP-1	0,35	<0,001
Concentration of TSHRAbs and sGAG	0,43	<0,05
Concentration of MMR-13 and TIMP-1	0,33	<0,05
Concentration of MMR-13 > 60 ng/ml and CAS ⩾ 3	0,76	<0,001
Concentration of TIMP-1 > 105 ng/ml and CAS ⩾ 3	0,49	<0,001
Concentration of MMR-13 > 60 ng/ml, TIMP-1 > 105 ng/ml and CAS ⩾ 3	0,80	<0,001
Concentration of MMR-13 > 60 ng/ml, TIMP-1 > 105 ng/ml and TSHRAbs⩾ 1.5 mEd/l	0,76	<0,001
Concentration of sGAG and MMR-13	0,37	<0,001

at which the content of TIMP-1 in serum remains increased against the background of suppression of excessive expression of MMP-13. Moreover, after GCS pulse therapy, the increased concentration of sGAG in the serum remained for a long time, which may indicate the continued destruction of the extracellular matrix and activation of orbital fibroblasts.

Discussion of the main research result

Orbital fibroblasts play an important role in the process of remodeling and development of soft retrobulbar tissue fibrosis in GO [4]. Activated, they excessively synthesize the components of the intercellular matrix, in particular glycosaminoglycans, which are linear polyanionic heteropolysaccharides, including chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate and hyaluronic acid [10]. Chains of these macromolecules – except for hyaluronic acid – are covalently bound to the nucleus proteins, forming proteoglycans, which are elements of the cell membrane, and intracellular granules, as well as the main substance of connective tissue forming the intercellular matrix [10].

GO autoimmune inflammation in the orbit leads to the increase of extracellular matrix catabolism with an increase in the concentration of the above-mentioned macromolecules in blood serum, and their increased excretion in urine in the form of oligosaccharides [10]. Some authors consider the excretion of sGAG in urine as a marker of GO infiltration process activity in the orbit [11]. It is known that the level of sGAG in serum characterizes the state of proteoglycan metabolism, and allows us to indirectly judge the degree of connective tissue destruction in various diseases [12]. According to our data, the sGAG level in serum is not a specific criterion of GO activity, because it is elevated in all phases of disease activity. However, this indicator can be used to assess the degree of destruction of the intercellular matrix in the orbit and the efficacy of GO treatment.

MMPs are key enzymes of the metabolism of connective tissue components participating in various physiological and pathological processes of morphogenesis, resorption and remodeling of tissues requiring migration, adhesion and differentiation of cells [7, 8]. MMPS synthesis is controlled by cytokines (interleukin-1β, tumor necrosis factor α, interleukin 6) and growth factors (fibroblast growth factor, epidermal growth factor, platelet growth factor) [8]. Heparin, glucocorticosteroids, estrogens, and progesterone are capable of suppressing excess MMP secretion [8].

MMP activation is one of the leading mechanisms of the development of diseases with connective tissue dysplasia [13]. In addition, MMPs are considered as serum fibrosis markers. For example, chronic hepatitis transforming into cirrhosis is characterized by an increase in MMP activity and cholestasis [13]. The content of MMPs and their tissue inhibitors is determined by different forms of IHD to create a model of left ventricular remodeling [14].

It has recently been shown that TIMP-1 and TIMP-2 act as growth factors, stimulating fibroblast growth and excessive collagen synthesis [15, 16]. Moreover, the inhibitory effect of TIMP on the processes of collagen and glycosaminoglycans proteolysis may slow down the utilization of damaged components of the extracellular matrix and inhibit tissue fibrosis [14].

Our data on the imbalance of MMP-13 and TIMP-1 in GO are comparable with the results of other studies. J. Myśliwiec et al. did not find any changes in the concentration of MMP-2, but recorded a significant increase in the level of MMP-9 in patients with active GO; after GCS therapy, this indicator decreased [17]. TIMP-1 levels remained high before and after treatment. H. Kim and colleagues noted that GCS do not inhibit the production of TIMP-1 induced by interleukin 1β in EOP [18].

However, other authors have shown that overexpression of MMR-1 and MMR-13 stimulates the migration and differentiation of myoblasts in the process of muscle tissue repair in cases of injury [19, 20]. Therefore, a significant increase in the level of MMR-13 in active GO may be necessary for the regeneration of extraocular muscles after the damaging effects of oxidative stress and autoimmune inflammation.

Thus, chronic autoimmune inflammation in GO leads to increased destruction of soft retrobulbar tissues. The need to dispose of destroyed and newly synthesized components of connective tissue may lead to an increase in the level of MMR-13 in the active phase of the disease. However, excessive degradation of collagen under the action of MMPs may become a trigger of unregulated synthesis of intercellular matrix components by orbital fibroblasts, with subsequent development of fibrosis. Therefore, raising the TIMP-1 level in the acute phase of inflammation in the orbit may be a compensatory reaction. After pulse therapy, there is an imbalance in the "enzyme-tissue inhibitor" system, since GCs are unable to inhibit TIMP-1 production in GO [18]. Thus, against the background of the increased level of TIMP-1, there is a marked decrease in the content of MMP-13. A high level of TIMP-1 can slow down the utilization of damaged components of the extracellular matrix and, in addition, may activate orbital fibroblasts [14-16]. These disturbances of intercellular matrix remodeling processes after GCS pulse therapy may trigger fibrogenesis of extraocular muscles and retrobulbar tissue in GO. In the inactive phase of GO, in the fibrosis stage, the concentration of MMP-13 remains moderately high, while the content of TIMP-1 reaches the reference values of the control group. This phase is dominated by destructive processes leading to chronic activation of orbital fibroblasts and synthesis of extracellular matrix components. Such an assumption is confirmed by the results of the present study, which showed the preservation of a high level of sGAG in patients with inactive phase GO in the fibrosis stage.

Limitations of research

The study of serum concentrations of MMP-1, MMP-13, TIMP-1 and sGAG was limited only to patients with moderate GO severity. Studying the dynamics of these parameters in patients with different degrees of GO severity will allow researchers to more accurately determine the threshold concentration of these biochemical parameters in serum, characterizing active autoimmune inflammation in the orbit.

Conclusion

In GO there is an imbalance in the «matrix metalloproteinases – tissue inhibitor of metalloproteinases» system in different phases of autoimmune inflammation activity.

Active GO is characterized by a 3.5-fold increase in MMP-13 content and a 1.17-fold increase in TIMP-1 ($p<0.05$). These indicators directly correlate with the TSHRAbs titre and CAS scale of GO activity ($p<0.001$). Diagnostic criterion for GO activity includes concentrations of MMP-13 and TIMP-1 in the blood serum. The active phase of GO is characterized by a level of MMP-13>60 ng/ml and TIMP-1>105 ng/ml.

In addition to the pronounced immunosuppressive action (93% reduction of TSHRAbs), GCS pulse therapy may have a profibrotic effect on soft retrobulbar tissues, which is manifested by the imbalance between MMP-13 and TIMP-1. The high level of TIMP-1 can slow down the disposal of destroyed components of the extracellular matrix, and stimulate orbital fibroblasts to proliferate and produce such components. Moreover, the level of sGAG in blood serum, indirectly characterizing the degree of connective tissue destruction and the synthetic function of orbital fibroblasts, did not decrease after GCS pulse therapy. These biochemical parameters can be used for patients with active GOs to control treatment and select individual dosages of GCS.

In patients with inactive GO in the fibrosis stage, the levels of MMP-13 remained 2 times higher than in the control group, and did not differ from the levels in patients after GCS pulse therapy. The concentration of TIMP-1 in this group has normalized.

More information

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.
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ИНФОРМАЦИЯ ОБ АВТОРАХ
***Таскина Елизавета Сергеевна** [Elizaveta S. Taskina, MD]; адрес: 672000, Россия, Чита, ул. Горького, 39а [address: 39a Gorkiy street, Chita, Russia, 672000]; ORCID: https://orcid.org/0000-0002-6223-8888; eLibrary SPIN: 5687-2122; e-mail: taskins@yandex.ru
Харинцева Светлана Владимировна, д.м.н., профессор [Svetlana V. Kharintseva, MD, PhD, Professor]; ORCID: https://orcid.org/0000-0002-8899-5465; eLibrary SPIN: 6788-2110; e-mail: s.v.19.28@mail.ru

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ИНФОРМАЦИЯ ОБ АВТОРАХ
*__Таскина Елизавета Сергеевна__ [Elizaveta S. Taskina, MD]; адрес: 672000, Россия, Чита, ул. Горького, 39а [address: 39a Gorkiy street, Chita, Russia, 672000]; ORCID: https://orcid.org/0000-0002-6223-8888; eLibrary SPIN: 5687-2122; e-mail: taskins@yandex.ru
Харинцева Светлана Владимировна, д.м.н., профессор [Svetlana V. Kharintseva, MD, PhD, Professor]; ORCID: https://orcid.org/0000-0002-8899-5465; eLibrary SPIN: 6788-2110; e-mail: s.v.19.28@mail.ru

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