STEROID RESISTANCE AND ANNEXIN A1 LEVEL IN PATIENTS WITH ACANTHOLYTIC PEMPHIGUS

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Systemic glucocorticosteroids due to their powerful anti-inflammatory and immunosuppressive properties are widely used in the treatment of severe chronic autoimmune diseases. Systemic glucocorticosteroids are the basic therapy for acantholytic pemphigus that must be taken continually. Therapy regimens of systemic glucocorticosteroids in acantholytic pemphigus depend on the sensitivity of patients to hormonal drugs. Recently, there have been observed an increasing number of patients with acantholytic pemphigus who have reduced sensitivity to systemic glucocorticosteroids. The scientific literature describes theories of the development of steroid resistance: they are diverse and still insufficiently studied. Therefore, further investigation of new pathogenetic links of steroid resistance, including the presence of annexin A1 (annexin A1, ANXA1, lipocortin-1) in patients with acantholytic pemphigus receiving long-term therapy with systemic glucocorticosteroids, in relation to the dose of hormonal drugs and the course of the disease, is extremely relevant and timely. The aim: to determine the level of annexin A1 in patients with acantholytic pemphigus during treatment depending on the dose of systemic glucocorticosteroids and steroid sensitivity. Materials and methods: 33 patients with acantholytic pemphigus were under observation at the Department of Dermatology, Institute of Dermatology and Venereology of the National Academy of Medical Sciences of Ukraine, Kharkiv. The level of ANXA1 in blood serum was studied by enzyme-linked immunosorbent assay (ELISA). The study was conducted in patients with acantholytic pemphigus before treatment, at the beginning of treatment and over the course of maintenance therapy, taking into account the daily dose of systemic glucocorticosteroids. Evaluation of sensitivity to systemic glucocorticosteroids in patients with acantholytic pemphigus was assessed by a specially developed 12-point scale of the index of resistance to systemic glucocorticosteroids. Discussion. According to the results of the study, a significant increase in the level of ANXA1 was observed in patients with acantholytic pemphigus at the beginning of therapy and under the maintenance therapy with systemic glucocorticosteroids. In untreated patients, ANXA1 levels were equal to those in the control group. This is confirmed by the theory of glucocorticosteroid sensitivity of this mediator. An increase in ANXA1 level at the beginning of therapy with systemic glucocorticosteroids was determined: in steroid-sensitive group at an average daily dose of 45.63±5.49 mg, the level of ANXA1 was on average 0.82±0.09 ng/mL; in steroid-resistant group, with an average daily dose of systemic glucocorticosteroids of 59.57±7.94 mg/d, ANXA1 was 1.18±0.13 ng/mL. With a decrease in the daily dose of systemic glucocorticosteroids to 17.25±4.14 mg/d, the ANXA1 level in patients of steroid-sensitive group had the values of the control group 0.46±0.11 ng/mL and 0.48±0.05 ng/mL, respectively, while in patients of steroid-resistant group the ANXA1 level was 0.68±0.11 ng/mL at an average daily dose of corticosteroids of 27.23±2.94 mg/d. Conclusion. ANXA1 level depends on the dose of systemic glucocorticosteroids. All patients who did not receive systemic glucocorticosteroids had the level of ANXA1 equal to the control group. All patients with acantholytic pemphigus at the beginning of therapy had a significant increase in ANXA1 levels and their decrease when a maintenance dose was reached. The average daily maintenance dose of systemic glucocorticosteroids is 1.57 times higher in the group of steroid-resistant patients.

Key words: acantholytic pemphigus, annexin A1 (annexin A1, ANXA1, lipocortin-1), glucocorticosteroids, steroid-sensitive, steroid-resistant condition.

Introduction

The central link of autoimmune diseases including acantholytic pemphigus (AP) is an imbalance of the human immune system, resulting in the perception of own tissues as foreign ones and their further damage due to the immune response [1]. The mechanisms of the immune system disorder are still not well studied and, as a result, the existing therapeutic tactics are limited to symptomatic and / or aggressive therapy, which contributes to the development of serious side effects.

Today, therapeutic tactics are aimed at suppressing the immune response. The most effective means are glucocorticosteroids (GCS) used for patients with AP. However, there is a certain category of patients that does not have a positive response to GCS and present therapeutic difficulties thus prompting the search for alternative treatments. The condition of such patients is assessed as steroid-resistant (SR). In order to understand the how the lack of sensitivity to GCS develops, it is essential to investigate the molecular mechanisms of GCS interaction at the cellular level and their dis-
ruption.
Thus, GCSs have pronounced anti-inflammatory and immunosuppressive properties due to their genomic and non-genomic mechanisms. Genomic mechanisms are mediated by active replication of specific genes encoding anti-inflammatory proteins [2].

Scientific studies carried by R. Hannon and co-authors (R. Hannon et al. 2003) described the resistance to the GCS treatment in aberrant inflammation in ANXA1-deficient rats and provided the first evidence of the physiological significance of this protein [3]. In this study, increased inflammatory response, severe autoimmune disease and severe allergic reaction were observed in ANXA1-deficient rats [4].

One of the mediators of counteracting inflammation and restoring homeostasis is the protein Annexin A1 (annexin A1, ANXA1, lipocortin-1), which is synthesized in immune cells under the influence of GCS [5]. This protein is encoded in humans by the ANXA1 gene and mediates various immunosuppressive, anti-inflammatory, anti-allergic effects of SCS and promotes tissue regeneration. It belongs to the annexin superfamily, is known as a potent endogenous anti-inflammatory mediator, which is well described in acute and chronic inflammation; its role in the activation of the pro-resolution phase receptor, FPR2 is currently used for therapeutic purposes [6].

Data on the level of ANXA1 in the blood serum in patients with AP, who receive long-term therapy with systemic glucocorticosteroids (SGCS), are described in the scientific literature, so it is important to determine the level of ANXA1 in patients with AP during treatment to predict the SR condition.

The aim
The aim of this study is to determine the level of annexin A1 in patients with AP during treatment depending on the dose of systemic glucocorticosteroids and steroid sensitivity.

Materials and methods
The study involved 33 patients aged from 29 to 73 years with AP, who took treatment at the Department of Dermatology, Institute of Dermatology and Venereology of the National Academy of Medical Sciences of Ukraine, Kharkiv. The patients received the SGCS therapy in the recalculation of prednisolone. The control group consisted of 20 healthy individuals comparable by age and sex.

Assessment of sensitivity to SGCS in patients with AP was determined by a specially developed 12-point scale of the index of resistance to SGCS (IR to SGCS). The following signs were determined: absence of therapeutic effect in the first 2 weeks of the therapy with high doses of SGCS was estimated as 3 points; duration of AP up to a year was estimated at 1 point; 2-5 years – at 2 points; more than 5 years – at 3 points; frequency of relapses during the year: no recurrence was assessed at 0 point; 1 recurrence was estimated at 1 point; up to 2 recurrences per year – at 2 points; more than 2 recurrences – at 3 points; prevalence of the process at recurrence: mucous membranes was assessed at 1 point; skin – at 1 point; mucous membranes and skin – at 3 points. Patients with 0 – 4 points were referred to the group with mild course; those who had 5-8 points made up the group with moderate severity; those who had 9 – 12 points formed the group with severe clinical course.

According to the IR to SGCS, patients were divided into 2 groups. Group I included 10 steroid-sensitive (SS) patients (30.3%), the index ranged from 3.6±0.3 to 6.3±2.2 points and averaged 4.4±0.7 points. Group II included 23 (69.7%) steroid-resistant (SR) patients, whose IR to SGCS was within the range from 7.5±0.6 points to 9.2±0.4 points, with an average of 8.2±0.4 points.

The concentration of ANXA1 mediator in the blood serum of patients with AP was measured by enzyme-linked immunosorbent assay (ELISA) [7]. The measurement was carried out to all patients with AP before the therapy started, at the beginning of therapy, and over the course of maintenance SGCS doses; the patients of SS and SR groups underwent the measurement at the beginning of the treatment and when reaching maintenance doses of SGCS. Doses of SGCS in terms of prednisolone ranged from 145 to 17.5 mg/d.

Annexin A1 levels were studied in 33 patients with AP, before and during the hormone therapy (14, 28 days). All patients were divided into 3 groups, taking into account the dose of SGCS: group I included 7 patients who did not receive hormone therapy before hospitalization; group II included 29 patients whose daily dose was <30 mg/d; group III included 30 patients whose daily dose was >30 mg/d. Control group IV involved 20 healthy individuals (Table 1).

The data were processed statistically using Statistica 12.6. Mean values and errors of the mean (M ± SD) were used, and the data were described using the median (Me) and lower and upper quartiles (Q1-Q3). For multiple comparisons between groups, the nonparametric Craskell-Wallis test was used, and post hoc comparisons were performed using the Mann-Whitney U test. Differences were considered significant at p <0.05 [8]. Pearson's correlation coefficient was applied for the correlation analysis between ANXA1 level and SGCS dose, the strength of the correlation was determined by the Cheldock scale [9]. The probability of changes in variations during treatment was determined by nonparametric Wilcoxon t-test.

Results and discussion
The diagnosis of AP was established according to standard clinical criteria and confirmed by cytological and laboratory tests. The dose of SGCS in patients with AP receiving injections and/or oral form was recalculated to the equivalent of prednisolone.
The study showed that in all patients with AP (33 people), the level of ANXA1 at the beginning of treatment with SGCS (63.45±5.48 mg/d) was equal to 1.01±0.11 ng/mL, at the maintenance dose of SGCS (25.99±0.71 mg/d) it was equal to 0.76±0.09 ng/mL that significantly exceeded the values in control group, 0.48±0.05 ng/mL.

The results of the study of ANXA1 level depending on the dose of SGCS are shown in Table 1.

As seen from the Table 1, the level of ANXA1 in patients who did not receive SGX therapy before hospitalization did not significantly differ from that in the control group, 0.48±0.11 ng/mL vs. 0.48±0.05 ng/mL. The difference between these indicators according to Mann-Whitney U-criterion is not statistically significant (p>0.05).

In patients of group II, whose daily dose on average did not exceed 30 mg/d (25.99±0.71 mg/d), ANXA1 level was 0.76±0.09 ng/mL. The level of ANXA1 in patients of group III with a dose of SGCS >30 mg/d (63.45±5.48 mg/d) was 1.01±0.11 ng/mL. According to the Mann-Whitney U-criterion, these data are significantly higher than those of the control group, respectively (p<0.01), as shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>N groups</th>
<th>Dose of SGCS (mg/d)</th>
<th>n</th>
<th>ANXA1 level, ng/mL</th>
<th>Me</th>
<th>Q1-Q3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>7</td>
<td>0.48±0.11</td>
<td>0.43</td>
<td>0.21-0.7</td>
<td>p = 0.007</td>
</tr>
<tr>
<td>II</td>
<td>&lt;30</td>
<td>29</td>
<td>0.76±0.09</td>
<td>0.54</td>
<td>0.38-1.1</td>
<td>p1&gt;0.05</td>
</tr>
<tr>
<td>III</td>
<td>&gt;30</td>
<td>30</td>
<td>1.01±0.11</td>
<td>0.86</td>
<td>0.44-1.5</td>
<td>p2&lt;0.05</td>
</tr>
<tr>
<td>IV</td>
<td>control</td>
<td>20</td>
<td>0.48±0.05</td>
<td>0.48</td>
<td>0.31-0.6</td>
<td>p3&lt;0.01</td>
</tr>
</tbody>
</table>

Note: p1 - significance of differences between 0 mg/d dose and control; p2 - significance of differences between <30 mg/d dose and control; p3 - significance of differences between >30 mg/d dose and control.

Thus, the SGCS administration contributes to a significant increase in the level of ANXA1 (according to the Cruiscal-Wallis test p=0.007) depending on the dose of the drug (Fig. 1).

Pearson's correlation coefficient between ANXA1 level and SGCS dose was 0.43 (middle strength correlation according to Cheldock's scale).

To determine the dependence of ANXA1 level in patients with AP on the state of SR, patients were divided into two groups. The basis for the division was clinical and anamnestic signs, IR to the SGCS. The first group involved 10 SS people (30.3%), whose IR to the SGCS was on average 4.4±0.7 points. In patients of the second group, including 23 SR people (69.7%), the average value of IR to the SGCS was 8.2±0.4 points. It should be noted that the number of SR patients was twice as much as SS (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>%</th>
<th>IR to SGCS (points) Me±SD</th>
<th>SGCS Initiation of therapy ANXA1 level (ng/mL) Me±SD</th>
<th>Dose of SGCS (mg/d) Me±SD</th>
<th>SGCS Maintenance therapy ANXA1 level (ng/mL) Me±SD</th>
<th>Dose of SGCS (mg/d) Me±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>30.3%</td>
<td>4.4±0.7</td>
<td>0.82±0.09</td>
<td>45.63±5.49</td>
<td>0.46±0.11</td>
<td>17.25±4.14</td>
</tr>
<tr>
<td>SR</td>
<td>23</td>
<td>69.7%</td>
<td>8.2±0.4</td>
<td>1.18±0.13</td>
<td>59.57±7.94</td>
<td>0.68±0.11</td>
<td>27.23±2.94</td>
</tr>
</tbody>
</table>

Figure 1. Comparison of ANXA1 level depending on the dose of SGCS.
According to the results of studies of ANXA1 level in patients with AP at the beginning of therapy: SS group, which received an average dose of SGCS 45.63±5.49 mg/d in complex treatment, the average value of ANXA1 was 0.82±0.09 ng/mL; in patients of SR group, which received SGCS in an average dose of 59.57±7.94 mg/d – 1.18±0.13 ng/mL (Fig. 2).

The Pearson's correlation coefficient between the dose of SGCS and the level of ANXA1 in the SS group at the beginning of therapy was -0.51 (middle negative correlation according to the Cheldock scale), in the SR group 0.38 (middle positive correlation according to the Cheldock scale). Thus, the level of ANXA1 in the blood serum of patients with AP of the SS group had significantly lower values than in patients of the SR group.

In patients of the SS group, who received maintenance doses of SGCS 17.25±4.14 mg/d, the level of ANXA1 during treatment significantly decreased to the level of control values (0.48±0.05 ng/mL) and amounted to 0.46±0.11 ng/mL; in patients of the SR group, in which the maintenance dose of SGCS was 27.23±2.94 mg/d, the level of ANXA1 significantly decreased from 1.18±0.13 to 0.68±0.11 ng/mL, but did not reach the level of the same as in the SS and control groups (Fig. 3).
The study shows a significant decrease in the level of ANXA1 in patients of SS and SR groups under a decrease in the SGCS dose. Pearson's correlation coefficient between the SGCS dose and the ANXA1 level in the SS group on maintenance therapy was -0.23 (low negative correlation on the Cheldock scale), in the SR group 0.13 (low positive correlation on the Cheldock scale).

According to the results obtained, it has been found that the level of ANXA1 in patients of the SS group, during the therapy with a reduced SGCS dose to a maintenance dose, significantly decreased by 2.03 times (according to Wilcoxon t-criterion, p≤0.01). In the SR group, during treatment to the maintenance dose, the level of ANXA1 decreased significantly by 2.76 times (p≤0.01) (Fig. 4).

Thus, there has been found a significant decrease in the ANXA1 level with a decrease in the SGCS dose in patients of both groups. In patients of the SS group, who received a maintenance dose of SGCS 17.25±4.14 mg/d, the level of ANXA1 was equal to the level of the control group 0.46±0.11 ng/mL and 0.48±0.05 ng/mL, respectively. In the SR group of patients with AP, the level of ANXA1 on maintenance therapy, which was equal to 27.23±2.94 mg/d, decreased to 0.68±0.11 ng/mL, but did not reach the level of the control group.

Also, there was a significant difference in the mean values of maintenance doses of SGCS between the SS and SR groups of patients with AP. In patients of the SS group, the average daily dose of SGCS was 17.25±4.14 mg, in patients of the SR group, it was 27.23±2.94 mg. Consequently, SR group patients received a maintenance daily dose of SGCS 1.57 times higher than in the SR group (according to Mann-Whitney U-test p<0.05).

**Conclusions**

This study indicates that the ANXA1 level depends on the dose of exogenous GCS. In patients receiving SGCS in an average dose of 63.45±5.48 mg/d at the beginning of therapy, the level of ANXA1 is 1.01±0.11 ng/mL; in an average maintenance dose of 25.99±0.71 mg/d the level of ANXA1 is 0.76±0.09 ng/mL, but does not reach the control group. SGCS significantly stimulate the synthesis of ANXA1 and are in direct interdependence, the level of ANXA1 depends on the dose of external GCS, and the dose of SGCS on steroid sensitivity.

It has been found that patients with AP with a steroid-resistant state of the disease have a higher level of ANXA1, which over the treatment decreases significantly by 2.76 times, but does not reach the level of the control group, which required further treatment with correction of hormone therapy regimens and the appointment of cytostatic drugs.

There is a significant difference in the...
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maintenance average daily dose of SGCS at patients with AP in the SS and SR groups, which according to the Mann-Whitney U-test (p<0.05) is 1.57 times higher in the SR patients.

References

Reферат
СТЕРОЙДНА РЕЗИСТЕНТНІСТЬ ТА РІВЕНЬ АНЕКСИНУ А1 У ХВОРИХ НА АКАНТОЛІТИЧНИЙ ПЕМФІГУС

Абдулла А. Е.

Ключове слова: акантолітичний пемфігус, анексин A1 (annexin A1, ANXA1, ліпокортин 1) у хворих на акантолітичний пемфігус, які отримують довготривало, майже пожиттєво. Схеми терапії системних глюкокортикостероїдів при акантолітичному пемфігусу є базисною терапією, що використовується довготривало, майже пожиттєво. Схеми терапії системних глюкокортикостероїдів при акантолітичному пемфігусу завдяки своїм потужним протизапальним та імуносупресивним властивостям широко застосовуються в терапії тяжких хронічних автоімунних захворювань. При лікуванні акантолітичного пемфігусу системні глюкокортикостероїди є базисною терапією, і використовується довготривала, майже пожиттєва терапія високими, деякі автори рекомендують навіть високими дозами глюкокортикостероїдів.

Системні глюкокортикостероїди завдяки своїм потужним протизапальним та імуносупресивним властивостям широко застосовуються в терапії тяжких хронічних автоімунних захворювань. При лікуванні акантолітичного пемфігусу системні глюкокортикостероїди є базисною терапією, і використовується довготривала, майже пожиттєва терапія високими, деякі автори рекомендують навіть високими дозами глюкокортикостероїдів. Оцінка чутливості до системних глюкокортикостероїдів.