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# THE ROLE OF ANTIBACTERIAL THERAPY IN THE TREATMENT OF ATOPIC DERMATITIS IN CHILDREN

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#### Summary

**Background.** Atopic dermatitis is one of the most common chronic inflammatory skin diseases. In atopic dermatitis, the diversity of the normal microflora decreases, so that the number of S. aureus prevails and the number of bacteria with antistaphylococcal activity decreases. The aim of this study was to evaluate the effectiveness of mupirocin 2% cream in the treatment of atopic dermatitis in children as an additional therapy, and the effect of the rs4696480 polymorphism in the TLR2 gene on the effectiveness of the treatment.

Materials and methods. The study included patients with atopic dermatitis (n = 37), aged 1-18 years (7.9±4.9) from the Department of Allergy at Kyiv City Children Clinical Hospital №2. All children included in the study with atopic dermatitis had a positive S. aureus culture. Patients were randomized into two groups: a group receiving mupirocin 2% cream on the affected skin areas 2 times a day 10 days (group A) and a control group (group B). Children of the control group received only symptomatic therapy. The SCORAD score and CDLQI (children's dermatology life quality index) questionnaire score was recorded before and after the treatment, side effects were recorded during the study. Skin swab cultures were taken before and after treatment. Genotyping for TLR2 rs4696480 was performed by using Real-time PCR.

This study was approved by the ethical committee of the O.Bogomolets National Medical University; all patients/parents of the children gave an informed consent to participate.

Statistical processing of the obtained data was carried out using the statistical package IBM SPSS Statistics Base (version 22) and the software EZR version 1.32 (graphical interface of the R environment (version 2.13.0). The difference in the effectiveness of therapy between the two subgroups and the influence of genotype on the effectiveness of therapy was determined using the Student criterion for parametric data (T) and Wilcoxon W-test for nonparametric data (W). The dynamics of indicators in each group before and after treatment were evaluated using the Wilcoxon T-test (T-W). Eeffectiveness of S. aureus eradication was estimated using the odds ratio (OR) with a 95% confidence interval (CI). Results were considered statistically significant at the level of p < 0.05.

**Results.** In both groups of children, improvement was observed in 10 days after the treatment. In group A, the improvement in  $\Delta SCORAD$  was found to be 13.8 (T-W=190.0, p<0.001). In group B, there was also an improvement:  $\Delta SCORAD$  8.5 (T-W=153.0, p<0.001). The difference in  $\Delta SCORAD$  scores between groups A and B was statistically significant (T=2.70, p=0.011). The decrease in CDLQI score after treatment was 31.3% in group A (T-W=190.0, p<0.001), and 18.3% in group B (T-W=171.0, p<0.001). The difference between these two groups was not statistically significant (W=334.0, p=0.409).

After the treatment, the skin culture showed that in group A, 57.9% of patients were S. aureus-negative, in group B, only 22.2% of children had a negative culture (OR = 5.50, CI 1.32-22.86).

We compared  $\Delta SCORAD$  depending on the genotype of the rs4696480 polymorphism in TLR2 gene and found no difference in the two subgroups: In the subgroup with the AA genotype,  $\Delta SCORAD$  was found to be 12.6±3.7; in the subgroup with the TT genotype – 14.4±5.1 (T=0.84, p=0.413).

**Conclusions.** Our results demonstrate the effectiveness of the use of topical mupirocin in the treatment of atopic dermatitis as an additional therapy.

Key words: Mupirocin; Atopic Dermatitis; Staphylococcus Aureus; Children.

### **Background**

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases. In AD, the diversity of the normal microflora decreases, so that the number of S. aureus prevails and the number of bacteria with antistaphylococcal activity decreases [1,2,3]. Staphylococcus aureus (S. aureus) tends to form molecules that have the potential to cause inflammation and contribute to further disruption of immune dysregulation. S. aureus is often shed from the skin of AD patients during exacerbations. The prevalence of S. aureus colonization of atopic skin is  $\geq 70\%$  in patients with active disease compared to 10-20% of healthy carriers in the background population [4,5]. S. aureus colonization contributes to the exacerbation of the disease and is directly correlated with the severity of the disease. S. aureus may also be a contributing factor in the pathogenesis

of AD, as it contributes to the deterioration of skin barrier function and increases inflammation.

Proteins such as adhesion factor B and fibronectinbinding proteins promote the adhesion of S. aureus to the stratum corneum. Staphylococcal protein A can activate pro-inflammatory nuclear factor kappa B (NF- $\kappa$ B) signalling through direct involvement of tumour necrosis factor receptor 1 (TNFR1) [6,7]. Lipoprotein and lipoteichoic acid induce TSLP in human keratinocytes via Toll-like receptors (TLR) 2 and 6, and phenol-soluble modulins also induce proinflammatory cytokines in human keratinocytes [6,7].

#### The aim

The aim of this study was to evaluate the effectiveness of mupirocin 2% cream in the treatment of AD in children as an additional therapy, and the effect of the rs4696480 polymorphism in the TLR2

gene on the effectiveness of the treatment.

#### Materials and methods

The study included patients with AD (n = 37), aged 1-18 years (7.9±4.9) from the Department of Allergy at Kyiv City Children Clinical Hospital №2. This study was approved by the ethical committee of the O.Bogomolets National Medical University; all patients/parents of the children gave an informed consent to participate.

The diagnosis of AD was established according to the criteria of Hanifin & Rajka, by the patient's history. Clinical parameters of patients included age,

gender, age of onset and severity of eczema, total IgE. The severity of AD was assessed using SCORing Atopic Dermatitis (SCORAD) index.

The inclusion criteria were the duration of AD for more than 1 year; degree of severity on the SCORAD scales of 10-60 points, positive S. aureus culture from skin. Exclusion criteria were as follows: Treatment with systemic corticosteroids within the past 4 weeks, treatment with topical or systemic antibacterial drugs of any other dermatological condition within the past 4 weeks, severe systemic disease, or malignancy. Table 1 presents demographic, clinical and serological data.

Table 1
Demographic and clinical characteristics of patients at the beginning of the study

Parameters	Group A	Group B
Patients, n	19	18
Age, years, Me [QI;QIII]	8 [5;13]	6 [3;9]
Boys\girls	10/9	10/9
AD duration, years, Me [QI;QIII]	7 [4;12]	5,5 [3;8]
SCORAD	55 [30;60]	56,5 [30;64]
CDLQI	10 [6;13]	11,5 [8;13]
Total IgE, IU∖ml	312 [67;832]	211 [56;306]

SCORAD - SCORing for Atopic Dermatitis, CDLQI - children's dermatology life quality index.

Patients were randomized into two groups: a group receiving mupirocin 2% cream (group A) and a control group (group B). Group A patients were treated with mupirocin 2% cream (1 g of cream contains 20 mg of mupirocin) on the affected skin areas 2 times a day and the necessary symptomatic agents. Children of the control group received only symptomatic therapy. The treatment period was 10 days. The SCORAD score and CDLQI (children's dermatology life quality index) questionnaire score was recorded before and after the treatment, side effects were recorded during the study.

Skin culture

Skin swabs were taken by wiping the skin with a sterile cotton swab for 30 seconds on the flexural (antecubital fossa) surface of the hand in the affected area of skin in 37 children with AD before and after the treatment. Blood agar and yolk-salt agar were inoculated from the swab. The plates with the material were incubated in a thermostat at 37 ° C for 48 hours. For further study, colonies were selected, which, according to the results of bacterioscopy, were formed by Gr + cocci. These colonies were then tested for catalase. Subsequent identification of catalasepositive colonies was performed on a Vitek2compact bacteriological analyser. Subjects were classified as carriers if the cultures were positive, while those with culture found to be negative were classified as non-carriers.

DNA extraction

Buccal epithelium was taken by using buccal brushes, followed by freezing of the samples and their storage at -20°C. DNA for genotyping was extracted from the samples by using NeoPrep 100 DNA (Neogen, Ukraine) according to the manufacturer's protocol. The concentration of total DNA was determined by using a NanoDrop spectrophotometer

ND1000 (NanoDrop Technologies Inc., USA). *qPCR Genotyping* 

Amplification reactions were performed by using a 7500 Fast Real-time PCR System ("Applied Biosystems", USA) in a final reaction volume of 20 µl, which contained 2X TaqMan Universal Master Mix ("Thermo Scientific", USA), assay C\_27994607\_10 and the template DNA. The thermal cycling conditions involved a denaturation step at 95°C for the duration of 20 s, followed by 40 cycles of amplification at 95°C for 3 s and at 60°C for 30 s. Analysis of the data was carried out with 7500 Fast Real-Time PCR Software.

Depending on the severity of AD, patients received symptomatic therapy – topical betamethasone dipropionate cream. Children in group B received treatment only in the form of topical application of an emollient and betamethasone dipropionate cream (1 g of cream contains 0.64 mg of betamethasone dipropionate) on the affected skin areas for 7–14 days.

Statistical analysis

Statistical processing of the obtained data was carried out using the statistical package IBM SPSS Statistics Base (version 22) and the software EZR version 1.32 (graphical interface of the R environment (version 2.13.0). The difference in the effectiveness of therapy between the two subgroups and the influence of genotype on the effectiveness of therapy was determined using the Student criterion for parametric data (T) and Wilcoxon W-test for nonparametric data (W). The dynamics of indicators in each group before and after treatment were evaluated using the Wilcoxon T-test (T-W). Eeffectiveness of S. aureus eradication was estimated using the odds ratio (OR) with a 95% confidence interval (CI). Results were considered statistically significant at the level of p < 0.05.

## Results and discussion

All participants completed the study. Both groups were compared by age, gender, duration and severity of the AD, and the level of total IgE (p > 0.05; table 1).

In both groups of children, improvement was observed in 10 days after the treatment (p<0.001). In group A, the improvement in  $\Delta SCORAD$  was found to be 13.8 (T-W=190.0, p<0.001). In group B, there was also an improvement:  $\Delta SCORAD$  was 8.5 (T-W=153.0, p<0.001) (Fig. 1). The difference in  $\Delta SCORAD$  scores between groups A and B was statistically significant (T=2.70, p=0.011). Therefore, children receiving mupirocin 2% cream in addition to therapy had significantly better dynamics of the severity score. The decrease in CDLQI score after treatment was 31.3% in group A (T-W=190.0,

p<0.001), and 18.3% in group B (T-W=171.0, p<0.001). Although group A showed a more significant decrease in score CDLQI, the difference between these two groups was not statistically significant (W=334.0, p=0.409) (Fig. 2).

Patients with AD who had a positive S. aureus culture were included in the study. After the treatment, the skin culture showed that in group A, 11 patients (57.9%) were S. aureus-negative, and 8 patients still had positive S. aureus culture. In group B, only 4 (22.2%) children had a negative culture, and 14 were still positive (77.8 %). Therefore, eradication of S. aureus was significant in the treatment group (OR= 5.50, CI 1.32-22.86), p=0.02, although the rather high culture-positive rate after treatment is noteworthy.

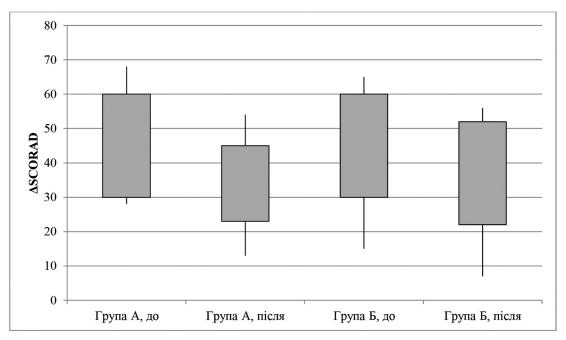


Fig. 1. Dynamics of the SCORAD score in groups A and B SCORAD – SCORing for Atopic Dermatitis

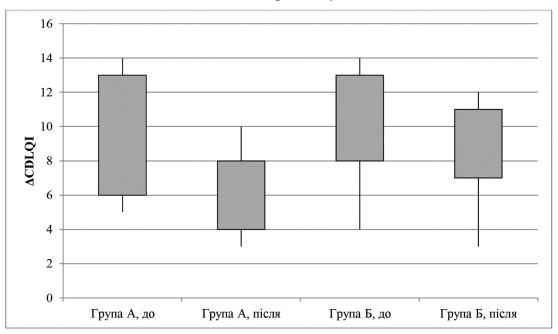


Fig. 2. Dynamics of the CDLQI score in groups A and B CDLQI – children's dermatology life quality index

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The analysis of the distribution of the rs4696480 polymorphism in TLR2 gene among children in group A showed that 12 children (63.2%) had the AA genotype, 11 (36.8%) had the TT variant, and there were no heterozygotes in this subgroup. We

compared  $\Delta SCORAD$  depending on the genotype and found no difference in the two subgroups: In the subgroup with the AA genotype,  $\Delta SCORAD$  was found to be 12.6±3.7; in the subgroup with the TT genotype – 14.4±5.1 (T=0.84, p=0.413) (see Fig. 3).

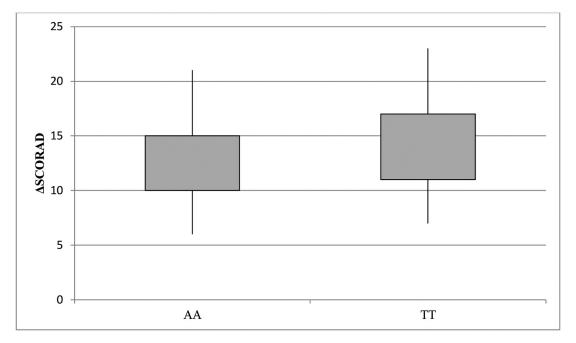


Fig. 3. ΔSCORAD in subgroups of children depending on the rs4696480 polymorphism in TLR2 SCORAD – SCORing for Atopic Dermatitis

No serious systemic side effects or adverse events from treatment were reported during the study.

S. aureus is a major burden for individuals with moderate-to-severe AD and a known inducer of disease exacerbation. Antibacterial drugs, such as mupirocin, can reduce the bacterial antigen load on the skin in AD [8,4]. In this study, we demonstrated the effectiveness of the use of mupirocin in a group of children with AD, who had a positive culture of S. aureus on the affected skin. We found a statistically significant difference in the effectiveness of treatment in the group of children, who received additional mupirocin cream, compared to the group of children, who received only standard therapy. Colonization of S. aureus also decreased in the treatment group. A study examining the resistance of S. aureus to various antibacterial drugs demonstrated sufficient resistance to ampicillin – 58.5%, lincomycin – 37.5% and erythromycin - 31.0%, but not to mupirocin (17.5%) and fusiic acid (15.5%) [9]. Recent studies reveal increased resistance to mupirocin [10]. MRSA (methicillin-resistant S. aureus) was more frequently resistant than methicillin-susceptible S. aureus to the other antibiotics, including ciprofloxacin [41% (7/17) vs 2.6% (10/384); p<0.0001], erythromycin [34% (8/23) vs 22% (114/507); p=0.17], clindamycin [26% (6/23) vs 16.9% (85/504); p=0.39], andmupirocin [14.3% (3/21) vs 6.7% (31/462); p=0.18] [11]. Resistance to mupirocin is likely to be the cause of the insufficiently high eradication rate.

Liu, Y. et al. classified AD patients as S. aureuspredominant group and S. aureus-non-dominant group and showed that the 2-week treatment with mupirocin leads to cutaneous microbial diversity normalization in S. aureus-predominant group of patients [12]. Yet, the authors noted that, as a result, the decreased cutaneous microbial diversity was observed, while the cutaneous lesions were recovered. Since Pseudomonas fluorescens is the source of mupirocin [13], to which other Pseudomonas species are resistant [14], mupirocin may relatively or absolutely select for these pathogens. The authors conclude that application of antibiotics in these patients may worsen their cutaneous microbial dysbiosis, and, in case of very young patients, may affect normal immunologic maturation [15].

The role of rs4696480 polymorphism in TLR2 in the development of AD was previously studied by us. We found that the polymorphism rs4696480 contributed to a more severe AD phenotype, the development of food sensitization and sensitization to house dust mites [16], susceptibility to carriage and S. aureus infection. Therefore, we hypothesized that the minor genotype may in some way affect the effectiveness of the treatment: The deterioration of signal transmission from the pathogen may also lead to a poor response to therapy. In this research, the patients in group A, to a certain extent, gave the same response to the antibacterial drug, and the response did not depend on the genotype. But it should be noted that the group was quite limited in size.

#### **Conclusions**

Our results demonstrate the effectiveness of the use of mupirocin in the treatment of AD as an additional therapy: Patients with AD, who received mupirocin cream 2% had significantly better dynamics of the SCORAD indicator than children, who received only the traditional therapy.

# Prospects for further research

Despite the demonstrated good response to the use of mupirocin, antibacterial therapy has a number of drawbacks: There is still a risk of S. aureus autoinfection in AD patients from nasal carriage; the use of antibacterial therapy will continuously

contribute to the development of antibiotic resistance, and may negatively affect the commensal flora of the skin. Given these adverse effects, the search for new interventions for the disrupted microbiome of AD-affected skin is worth continuing.

**Conflict of interest.** The author declares no conflict of interest.

**Sources of funding.** This study had no source of funding.

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## РОЛЬ АНТИБАКТЕРІАЛЬНОЇ ТЕРАПІЇ В ЛІКУВАННІ АТОПІЧНОГО ДЕРМАТИТУ У ДІТЕЙ

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#### Резюме

Вступ. Атопічний дерматит – одне з найпоширеніших хронічних запальних захворювань шкіри. При атопічному дерматиті зменшується різноманітність нормальної мікрофлори, внаслідок чого переважає S. aureus і зменшується кількість бактерій з антистафілококовою активністю. Метою даного дослідження будо опінити ефективність крему мупіропину 2% при дікуванні атопічного дерматиту у дітей як додаткової терапії та вплив поліморфізму rs4696480 гена TLR2 на ефективність лікування.

**Матеріали та методи.** У дослідження були включені хворі на атопічний дерматит (n = 37) віком 1-18 років (7.9±4.9) з алергологічного відділення Київської міської дитячої клінічної лікарні №2. Усі включені в дослідження діти з атопічним дерматитом мали позитивний посів на S. aureus. Пацієнти були рандомізовані на дві групи: група, яка отримувала мупіроцин 2% крем на уражені ділянки шкіри 2 рази на день 10 днів (група А) і контрольна група (група Б). Діти контрольної групи отримували лише симптоматичну терапію. Оцінка SCORAD та CDLQI (індекс якості життя дітей в дерматології) реєструвалися до і після лікування, побічні ефекти реєструвалися під час дослідження. Шкірні мазки брали до і після лікування. Генотипування TLR2 rs4696480 проводили за допомогою ПЛР у реальному часі.

Дослідження схвалено етичним комітетом НМУ імені О. Богомольця; всі пацієнти/батьки дітей дали інформовану згоду на участь. Статистичну обробку отриманих даних проводили за допомогою статистичного пакету IBM SPSS Statistics Base (версія 22) та програмного забезпечення EZR версії 1.32 (графічний інтерфейс середовища R (версія 2.13.0). Різниця в ефективності терапії між двох підгруп та вплив генотипу на ефективність терапії визначали за допомогою критерію Стьюдента для параметричних даних (T) та W-тесту Вілкоксона для непараметричних даних (W). Динаміку показників у кожній групі до та після лікування оцінювали за допомогою Т-тесту Вілкоксона (T-W). Ефективність ерадикації S. aureus оцінювали за відношенням шансів (OR) з 95% довірчим інтервалом (CI). Результати вважалися статистично значущими на рівні р < 0,05.

Результати. В обох групах дітей покращення спостерігалося через 10 днів після лікування. У групі А покращення ∆SCORAD склало 13.8 балів (T-W=190.0, p<0.001). У групі Б також відбулося покращення: ∆SCORAD становило 8.5 балів (T-W=153.0, p<0.001). Різниця в показниках ΔSCORAD між групами А та В була статистично значущою (T=2.70, p=0.011). Зниження показника CDLQI після лікування становило 31,3% у групі A (T-W=190.0, p<0.001), і 18.3% у групі В (T-W=171.0, p<0.001). Різниця між цими двома групами не була статистично значущою (W=334.0, p=0.409).

Після лікування шкірний посів показав, що в групі А 57.9% пацієнтів були S. aureus-негативними, у групі В тільки 22.2% дітей мали негативний посів (OR= 5.50, CI 1.3222,86).

Ми порівняли ΔSCORAD залежно від генотипу поліморфізму rs4696480 в гені TLR2 і не виявили відмінностей v двох підгрупах: у підгрупі з генотипом AA ΔSCORAD було 12.6±3.7 балів; у підгрупі з генотипом TT – 14.4±5.1 (T=0.84, p=0.413).

Висновки. Наші результати демонструють ефективність використання топічного мупіроцину в лікуванні атопічного дерматиту як додаткової терапії.

Ключові слова: мупіроцин; атопічний дерматит; Staphylococcus aureus; діти.

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