UDC 616.5-002.2

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Detection and treatment of skin infection with *Staphylococcus aureus* in children with atopic dermatitis

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Modern Pediatrics. Ukraine. (2023). 2(130): 74-78. doi 10.15574/SP.2023.130.74

For citation: Mozyrska OV. (2023). Detection and treatment of skin infection with Staphylococcus aureus in children with atopic dermatitis. Modern Pediatrics. Ukraine. 2(130): 74-78. doi 10.15574/SP.2023.130.74.

Introduction. Atopic dermatitis is a common chronic relapsing inflammatory multifactorial skin disease. *Staphylococcus aureus* skin colonization increases during disease outbreaks and correlates with the severity of skin symptoms.
Purpose — to evaluate the effectiveness of thyrothricin gel as an adjuvant therapy in the treatment of atopic dermatitis and the influence of polymorphisms of pattern recognition receptors on the achieving a therapeutic effect.
Materials and methods. This study included patients with atopic dermatitis (n=37) aged 2–18 years from the Allergy Department of the Kyiv City Children's Clinical Hospital No. 2. Patients were devided into two groups: a group that received thyrothricin gel on the affected skin areas 2 times a day for 7 days (the Group A) and a control group (the Group B) that received only basic symptomatic therapy. SCORAD (SCORing AD) and CDLQI (Children's Quality of Life Index in Dermatology) scores were recorded before and after treatment, side effects were recorded during the study. Skin swabs were taken in the Group A before and after treatment. Genotyping of polymorphisms rs4696480 in the TLR2 gene, rs7309123 in the Dectin-1 gene were performed using polymerase chain reaction.
Results. In both groups of children, improvement was observed in 7 days after treatment ($p<0.001$). In the Group A, the improvement in ΔSCORAD was 16.6±5.9 points, in the Group B — 8.1±4.5 points. The difference in ΔSCORAD scores between the Groups A and B was statistically significant ($W=225.0$, $p<0.001$). The decrease in CDLQI score after treatment was 3.8±1.8 points in the Group A and 2.3±1.25 points in the Group B. A significant decrease in CDLQI score was demonstrated in the intervention group than in the control group after treatment ($W=275.5$, $p=0.004$). After treatment, a bacteriological examination of the skin demonstrated the eradication of *Staphylococcus aureus:* in the Group A, 94.4% of patients were *Staphylococcus aureus*-negative. Polymorphisms rs4696480 in the TLR2 gene and rs7309123 in the Dectin-1 gene did not affect the speed of achieving the therapeutic effect.
Conclusions. The addition of thyrothricin to standard therapy for atopic dermatitis in children with *Staphylococcus aureus* infection of affected skin may provide significant clinical benefit in SCORAD and CDLQI scores.
The research was carried out in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the Local Ethics Committee of the participating institution. The informed consent of the patients was obtained for conducting the studies.
No conflict of interests was declared by the author.
Keywords: atopic dermatitis, *Staphylococcus aureus*, single nucleotide polymorphism, thyrothricin.

Виявлення та лікування інфікування золотистим стафілококом шкіри в дітей з атопічним дерматитом

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Вступ. Атопічний дерматит — це поширене хронічне рецидивуюче запальне мультифакторне захворювання шкіри. Колонізація шкіри *Staphylococcus aureus* зростає під час спалахів захворювання та корелює з тяжкістю шкірних симптомів.
Мета — оцінити ефективність тиротрицину гелю як ад'ювантної терапії в лікуванні атопічного дерматиту; виявити вплив поліморфізмів рецепторів розпізнавання патернів на швидкість досягнення терапевтичного ефекту.
Матеріали та методи. Обстежено хворих на атопічний дерматит (n=37) віком 2–18 років з алергологічного відділення Київської міської дитячої клінічної лікарні № 2. Пацієнти поділені на дві групи: група, яка отримувала тиротрицин гель на уражені ділянки шкіри 2 рази на добу протягом 7 діб (група А); контрольна група (група Б), що отримувала тільки базову симптоматичну терапію. Оцінки за шкалами SCORAD та CDLQI (індекс якості життя дітей у дерматології) зареєстровано до і після лікування, побічні ефекти зареєстровано під час дослідження. Дослідження наявності *Staphylococcus aureus* на шкірі проведено в групі А до і після лікування. Генотипування поліморфізмів rs4696480 у гені TLR2, rs7309123 у гені Dectin-1 визначено за допомогою полімеразної ланцюгової реакції.
Результати. В обох групах дітей спостерігалося поліпшення через 7 діб після лікування ($p<0,001$). У групі А поліпшення ΔSCORAD становило 16,6±5,9 бала, у групі Б — 8,1±4,5 бала. Різниця в балах ΔSCORAD між групами А і В була статистично значущою ($W=225,0$, $p<0,001$). Зниження оцінки за CDLQI після лікування у групі А становило 3,8±1,8 бала, у групі В — 2,3±1,25 бала. Виявлено значуще зниження бала CDLQI в групі втручання порівняно з контрольною групою після лікування ($W=275,5$, $p=0,004$). Бактеріологічне дослідження шкіри після лікування показало ерадикацію *Staphylococcus aureus:* у групі А 94,4% хворих були *Staphylococcus aureus*-негативними. Поліморфізми rs4696480 у гені TLR2 та rs7309123 у гені Dectin-1 не впливали на швидкість досягнення терапевтичного ефекту.
Висновки. Додавання тиротрицину до стандартної терапії атопічного дерматиту в дітей з інфікуванням ураженої шкіри *Staphylococcus aureus* може забезпечити значну клінічну ефективність за показниками шкали SCORAD та балами за опитувальником якості життя CDLQI.
Дослідження виконано відповідно до принципів Гельсінської декларації. Протокол дослідження ухвалено Локальним етичним комітетом зазначеної в роботі установи. На проведення досліджень отримано інформовану згоду батьків, дітей.
Автор заявляє про відсутність конфлікту інтересів.
Ключові слова: атопічний дерматит, *Staphylococcus aureus,* однонуклеотидний поліморфізм, тиротрицин.

Introduction

Atopic dermatitis (AD) is a common chronic relapsing inflammatory multifactorial skin disease characterized by intense itching. AD affects up to 20% of children and 1–3% of adults [10,11]. *Staphylococcus aureus* (*S. aureus*) is often found in the affected areas in AD. *S. aureus* skin colonization increases during disease outbreaks and correlates with the severity of skin symptoms in children with AD [8]. In addition, E.L. Simpson et al. demonstrated that AD patients who were colonized with *S. aureus* had higher levels of biomarkers of type 2 inflammation (higher blood eosinophils and serum total IgE, CCL17, and periostin) and showed greater allergen sensitization than patients with AD, whose skin was not colonized by *S. aureus* [6].

Thyrothricin, an antimicrobial peptide combination produced by *Bacillus brevis*, composed of gramicidins and tyrocidins, has broad antimicrobial activity against gram-positive bacteria and some yeast *in vitro*. The polypeptide and its components have been used therapeutically for approximately 60 years for topical treatment of infected skin and infected oropharyngeal mucosa [7].

According to our data, studies on the effectiveness of thyrothricin as an additional therapy in the treatment of AD complicated by secondary *S. aureus* infection are limited and poorly described. One small study demonstrated the safety and therapeutic efficacy of thyrothricin gel in the treatment of bacterial complications of AD in 30 children [2].

Pattern recognition receptors play a major role in the development of infectious lesions on atopic skin, in particular. We decided to test the influence of pattern recognition receptor polymorphisms (rs4696480 in the TLR2 gene, rs7309123 in the Dectin-1 gene) on the speed of achieving the therapeutic effect.

The purpose of the study — to evaluate the effectiveness of tyrothricin as an adjuvant therapy in the treatment of AD and the influence of polymorphisms of pattern recognition receptors on the effectiveness of treatment.

Materials and methods of the research

The study included patients with AD (n=37); aged 2–18 years, median 6 (3;10) from the allergy department of the Kyiv City Children's Clinical Hospital No. 2. This study was approved by the ethics commission of Bogomolets National Medical University, all patients/parents of sick children gave informed consent to participate. The diagnosis of AD was established according to the Hanifin & Rajka criteria.

Clinical parameters of patients included age, sex, age of disease onset and severity of AD, total IgE. The severity of AD was assessed by the SCORing atopic dermatitis index (SCORAD scale).

SCORAD severity assessment and *S. aureus* skin sampling were performed at the same visit.

The inclusion criteria were the duration of AD for more than 1 year; degree of severity according to the SCORAD scale 10–60 points, for the intervention group — a positive *S. aureus* skin culture. *Exclusion criteria* were: treatment with systemic corticosteroids within the past 4 weeks, treatment with topical or systemic antibacterial drugs for any other dermatological disease within the past 4 weeks, severe systemic disease, or malignancy.

Patients were divided into two groups: a group that received thyrothricin gel (the Group A, n=18) and a control group (the Group B, n=19). The Group A patients underwent a bacteriological examination of the skin before and after treatment. Bacteriological skin cultures were not performed on the Group B patients, as they did not receive antibacterial treatment. The Group A patients were treated with thyrothricin (1 g of gel contains 1 mg of thyrothricin) and the necessary symptomatic agents (emollients and betamethasone dipropionate cream (1 g of cream contains 0.64 mg of betamethasone dipropionate) twice a day), the duration of treatment was 1 week. Children in the control group received only basic symptomatic therapy — topical betamethasone dipropionate cream and emollients.

Before and after the treatment, the severity was assessed according to the SCORAD scale and the CDLQI (children's quality of life index in dermatology) questionnaire score, and side effects were recorded during the study.

Bacteriological research

In 18 children with AD from the Group A, swabs were taken from the skin by wiping with a sterile cotton swab for 5 seconds in the area of the ulnar fossa, on the affected area of the skin. Blood and yolk-salt agar were inoculated from the smear. The tablets with the material were incubated in a thermostat at 37°C for 48 hours. For further research, colonies of Gr + cocci were selected and tested for catalase. Further identification of catalase-positive colonies was performed on a Vitek2compact bacteriological analyzer.

Table

Demographic and clinical characteristics of patients

Parameters	Group A	Group B
Patients, n	18	19
Age, years, Me (QI;QIII)	6.5 (3;13)	6 (4;10)
Boys/Girls	10/8	10/9
Duration of AD, Me (QI;QIII)	7±5.1	6.9±4.8
SCORAD	30 (17;40)	30 (20;45)
CDLQI	8 (8;13)	12 (6;13)
Serum IgE, IU/ml	111 (56;451.4)	306 (111;832)
Dynamics of the average score ΔSCORAD, %	16.6±5.9	8.1±4.5
Dynamics of the average score ΔCDLQI, %	3.8±1.8	2.3±1.25

Notes: SCORAD — SCORing for Atopic Dermatitis; CDLQI — children's dermatology life quality index; Me (QI; QIII) — median (quartiles I; III); AD — atopic dermatitis; IgE — immunoglobulin E.

DNA extraction

The buccal epithelium was collected with buccal brushes, followed by freezing of the samples and storage at -20ºC. DNA for genotyping was isolated from samples using NeoPrep 100 DNA (Neogen, Ukraine) according to the manufacturer's protocol.

Genotyping

Amplification reactions were performed 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 (Applied Biosystems, USA), assay C__27994607_10, and template DNA. Thermal cycling conditions included a denaturation step at 95°C for 20 s, followed by 40 cycles of amplification at 95°C for 3 s and 60°C for 30 s. Data analysis was performed using 7500 Fast Real-Time PCR software. The primer sequences of the rs4696480 polymorphism in TLR2 were as follows:

for TLR2-F 5' AACAGAAATTTATCCATTCATGGTT 3', Rev TLR2-R 5' AGCAGTTTATTGTGAGAATGAGTTT 3';

for rs7309123 Dectin-1 (https://www.ncbi.nlm.nih.gov/SNP/): CP1:GTAGAAGTATACG TGTTGAAATAATAGATTACGP1:GTAGAA GTATACGTGTTGAAATAATAGATTAG C/G P2:ACCTTTCACATATCTTCCGGTCATC

Statistical analysis

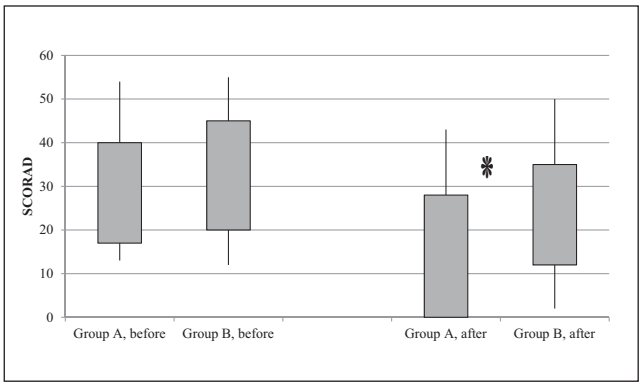
Statistical processing was performed using EZR software version 1.32 (R graphical interface (version 2.13.0)). Quantitative data for each of the studied groups are presented in the form of median – Me (QI; QIII). SNPAnalyzer (web-based software) was used to examine Hardy–Weinberg equilibrium. The difference in treatment efficacy between the two groups and the effect of genotype on treatment efficacy were determined using the Student's t-test for parametric data (T) and the Wilcoxon W-test for non-parametric data (W). The dynamics of indicators in each group before and after treatment were evaluated using the Wilcoxon T-test (T-W). The results were considered statistically significant at the $p<0.05$ level.

Results of the research

All participants completed the study. Both groups did not differ in age, sex, duration and severity of AD, level of total IgE ($p>0.05$; Table).

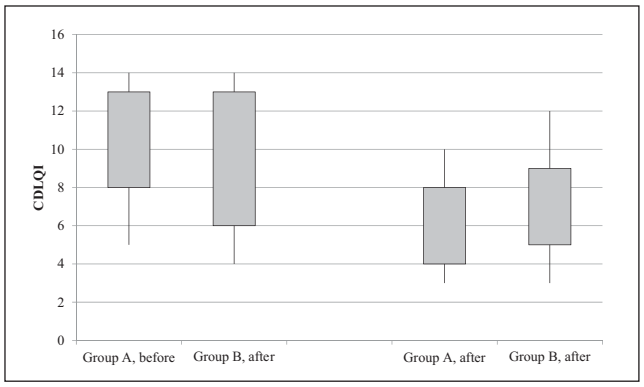
In both groups of children, improvement was observed 7 days after treatment ($p<0.001$). In the Group A, the improvement in ΔSCORAD was 16.6±5.9 points (T-W=171.0, $p<0.001$). The Group B also improved: ΔSCORAD was 8.1±4.5 (T-W=171.0, $p<0.001$) (Fig. 1). The difference in ΔSCORAD scores between the Groups A and B was statistically significant (W=225.0, $p<0.001$). Thus, in children who received thyrothricin gel in addition to therapy, the dynamics of the degree of severity was significantly better. The decrease in the CDLQI score after treatment was 3.8±1.8 points in the Group A (T-W=171.0, $p<0.001$) and 2.3±1.25 points in the Group B (T-W=190.0, $p<0.001$). A significant decrease in the CDLQI score was demonstrated in the intervention group than in the control group after treatment (W=275.5, p=0.004) (Fig. 2, Table 1).

After treatment, a skin culture study demonstrated the eradication of *S. aureus*: in the Group A 17 (94.4%) patients were *S. aureus*-



Note: SCORAD — SCORing for Atopic Dermatitis.

Fig. 1. Comparison of SCORAD indicators in the studied subgroups before and after treatment (W=225.0, p<0.001)



Note: CDLQI — children's dermatology life quality index.

Fig. 2. Comparison of CDLQI indicators in the studied subgroups before and after treatment (W=275.5, p=0.004)

negative, 1 (5.6%) patient had a positive culture for *S. aureus.*

In order to determine the category of AD patients who may benefit most from the addition of an antibacterial drug to standard therapy, we investigated whether pattern recognition receptor gene polymorphisms influenced treatment efficacy. Analysis of the distribution of the rs4696480 polymorphism of the TLR2 gene among children of the Group A showed that 4 (63.2%) children had the AA genotype, 14 (36.8%) children had the AT and TT variant. We compared ΔSCORAD depending on the genotype and found no differences in the two subgroups: in the subgroup with the AA genotype, ΔSCORAD was 15.8±4.1 points; in the subgroup with genotype AA and TT — 16.8±6.4 points(T=0.84, p=0.413).

When studying the distribution of the rs7309123 polymorphism in the Dectin-1 gene among children of the Group A, it was found that 3 (63.2%) children had the GG genotype, 11 (36.8%) children had the CC+C\G genotype. We compared ΔSCORAD depending on the genotype and found no differences in the two subgroups: in the subgroup with the GG genotype ΔSCORAD was 14.8±2.5 points; in the subgroup with the CC+C\G genotype — 17±6.5 points (T=0.66, p=0.519). Thus, the therapeutic effect of tyrothricin as an additional therapy for AD did not depend on the genotype of pattern recognition receptor polymorphisms.

No adverse drug reactions or treatment side effects were reported during the study.

Thereby, our study demonstrated clinical efficacy in terms of SCORAD and CDLQI scores. It was demonstrated that in the Group A the improvement of ΔSCORAD was 16.6±5.9 points (T-W=171.0, p<0.001), in the Group B — ΔSCORAD was 8.1±4.5 points (T-W=171.0, p<0.001). Although there was improvement in skin condition in both groups, the difference in ΔSCORAD scores between the Groups A and B was statistically significant (W=225.0, p<0.001). The difference in CDLQI scores after treatment was also statistically significant (W=275.5, p=0.004). In addition to clinical effectiveness, the bacteriological effectiveness of the drug was also demonstrated: in the Group A, 17 (94.4%) patients were *S. aureus*-negative as a result of treatment, 1 (5.6%) patient had a positive culture for *S. aureus.* Literature data on the effectiveness of thyrothricin as an adjunctive therapy for AD are very limited. There are data that have demonstrated the safety of this drug with regard to the development of resistance to it by *S. aureus*: a ten-year experience of using tyrothricin for the local treatment of infected skin does not pose a great risk of acquiring resistance of initially sensitive gram-positive bacteria and yeast, even in the case of *S. aureus*, including methicillin-resistant strains [7]. Described studies on the study of other antibacterial measures and drugs — chlorine baths [3,4,5] antibiotics [1], bacteriophage endolysin [9], show contradictory results.

Thus, it can be concluded that the effectiveness of thyrothricin as an adjuvant therapy for AD lies in the eradication of *S. aureus*, an important pathogenetic factor of AD.

Conclusions

Therefore, this study showed that the addition of thyrothricin to the standard therapy of AD in children with *Staphylococcus aureus* infection of affected skin could provide significant clinical efficacy in terms of SCORAD and CDLQI scores. The antibacterial and antifungal activity of thyrothricin combined with the lack of risk of develop-

ing resistance makes the antimicrobial peptide an important addition to the pathogenetic treatment of infected skin lesions in children with AD.

Acknowledgments. The author expresses her gratitude to the patients and their parents, the Institute of Physiology named after O.O. Bogomolets National Academy of Sciences of Ukraine, Department of Molecular Physiology.

Sources of funding. This study was funded from the state budget. This work was carried out within the framework of the Research and Development Program of the Department of Pediatrics No. 2 of the National Medical University named after O.O. Bogomolets of the Ministry of Health of Ukraine «Peculiarities of the clinical course and treatment of asthma in children with overweight and obesity» (2020–2022). State registration code — 0120U100804.

No conflict of interests was declared by the authors.

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Стаття надійшла до редакції 11.01.2023 р., прийнята до друку 13.03.2023 р.