

Topical tacrolimus vs medium-dose ultraviolet A1 phototherapy in the treatment of atopic dermatitis – a preliminary study in relation to parameters of the epidermal barrier function and high-frequency ultrasonography

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Abstract. – OBJECTIVE: Atopic dermatitis (AD) is a chronic, relapsing skin disorder, which is characterized by intense pruritus, skin dryness and concomitant epidermal barrier dysfunction. The basic therapy involves the application of anti-inflammatory topical drugs like; glucocorticosteroids and calcineurin inhibitors. Phototherapy in AD is regarded as an additional form of treatment. The latest invention, ultraviolet A1-UVA1 phototherapy (340-400 nm), was introduced to the treatment of AD by Krutmann et al in 1992. It appears that the main mode of action of UVA1 phototherapy in AD is through activation of apoptosis of T lymphocytes. Additionally, new studies show that UVA1 can also inhibit the activity of calcineurin phosphatase, similarly to calcineurin inhibitors such as cyclosporin A or tacrolimus. The aim of this study is to, for the first time, compare the efficacy of medium dose UVA1 phototherapy and tacrolimus ointment in patients with moderate-severe AD.

PATIENTS AND METHODS: This study involved 20 AD patients. Half of the patients were treated with UVA1 phototherapy, while another 10 participants were treated with the application of tacrolimus ointment. The severity of the disease progress was assessed on the basis of EASI score (Eczema Area Severity Index). Moreover, the clinical condition of patients was assessed using non-invasive techniques such as measurement of transepidermal water loss – TEWL and skin capacitance, as well as high-frequency ultrasonography (20 MHz).

RESULTS: This study described above confirmed the beneficial influence of both therapies on the course of moderate-severe AD. Tacrolimus induced a greater reduction in TEWL, while phototherapy caused the reduction of subepidermal low echogenic band-SLEB within sites affected with pathological lesions.

CONCLUSIONS: Both tacrolimus and phototherapy treatment seemed to significantly reduce EASI.

Key Words:

Atopic dermatitis, Treatment, Tacrolimus, UVA1 phototherapy.

Abbreviations

UVR = ultraviolet radiation; UVA/B, broad-band UVA/B (290-400 nm); BB UVB = broad-band UVB (290-320 nm); BB UVA = broad-band UVA (320-400 nm); NB UVB = narrow-band UVB (311 nm); PUVA = psoralen plus UVA; UVA1 = ultraviolet A1 (340-400 nm); HD UVA1 = high dose UVA1 (> 60 J/cm²); MD UVA1 = medium dose UVA1 (30-60 J/cm²); LD UVA1 = low dose UVA1 (10-30 J/cm²).

Introduction

Atopic dermatitis (AD) is a chronic, relapsing skin disorder, which is characterized by intense pruritus, skin dryness and concomitant epidermal barrier dysfunction forms^{1,2}. During the period of exacerbation, a wide range of different therapies are used. The basic therapy involves the application of anti-inflammatory topical drugs, like glucocorticosteroids and novel calcineurin inhibitors. Phototherapy in AD is regarded as an additional form of treatment or a second line of treatment. All forms of phototherapy – NB UVB, PUVA – as well as the novel form of UVA1 phototherapy, are recommended according to the

current guidelines^{3,4}. The latest UVA1 phototherapy (340-400 nm) was introduced to the treatment of AD by Krutmann et al in 1992⁵. Since then, the beneficial effect of UVA1 phototherapy has been presented in various studies in which the doses administered were low, medium as well as high⁶⁻¹⁰. The factor that determines the mechanism of action in AD seem to be the apoptosis of T lymphocytes. Additionally, new studies have showed that UVA1 can also inhibit the activity of calcineurin phosphatase, similarly to a group of calcineurin inhibitors such as cyclosporin A or tacrolimus^{11,13}. Until now, the effect of UVA1 phototherapy has been directly related to standard forms of phototherapy, like PUVA and UVB. The aim of this study was to compare for the first time the efficacy of medium dose of UVA1 phototherapy and tacrolimus ointment in patients with moderate-severe AD. Moreover, the clinical condition of patients was evaluated through measurement of trans epidermal water loss – TEWL and skin capacitance. The effect of treatment was additionally visualized by means of high-frequency ultrasonography (20 MHz). It is acknowledged that even the normal-looking skin in AD presents with the subclinical inflammation, which can be detected on the basis of the measurement of TEWL. The normal-appearing skin shows increase in TEWL and presents with persistent immunologic changes even after skin lesions have subsided^{13,14}. For this reason, it is advisable and sensible to maintain constant skin care and treatment even in the periods of remission. This is the so-called proactive approach and it may be performed, among others, with tacrolimus.

Patients and Methods

Patients

The study included 20 AD patients aged 8-60 years (mean age 27,15 years). There were 10 females and 10 males. In all patients, the diagnosis of the disease was based on Hanifin and Rajka's diagnostic criteria.

All subjects provided written informed consent and the Institutional Review Board approved the study.

Clinical Evaluation

The severity of the disease process was assessed twice (before and after the treatment) on the basis of EASI score (Eczema Area Severity

Index), which evaluates 4 clinical parameters: erythema, induration/papulation, excoriation, and lichenification on a 0-3 scale within 4 defined body regions (head/neck, upper extremities, trunk, lower extremities) by the same blinded physician¹⁵.

Instrumental Measurements

TEWL was determined by means of Tewameter TM 300 (Courage-Khazaka, Köln, Germany) according to the guidelines of the standardization group of the European Society of Contact Dermatitis¹⁶. At least 20 measurements given as a mean value and expressed in SI units (g/m²/h) were performed. TEWL values within the normal range were established at 0-25 g/m²/h.

Skin hydration (corneometry) was determined as the electrical capacitance with the use of Corneometr CM 825 (Courage-Khazaka, Köln, Germany)¹⁷. The proper value of SC hydration was accepted as higher than 40 u. Five measurements given as a mean value in arbitrary units (range: 0-130) were obtained in accordance with the guidelines.

The evaluation of the following criteria: the width of the subepidermal low echogenic band-SLEB in mm was performed with the use of the high frequency ultrasound scanner (Dermascan C ver. 3, Cortex Technology, Hadsund, Denmark) that operates at a frequency of 20 MHz, with a resolution of 60 µm × 200 µm (axial × lateral) and approximately 15-mm penetration, the ultrasonic parameter that reflects the degree of skin inflammation. In accordance with the general principles, the behavior of the ultrasonic wave in the tissue results in the formation of echoes of different amplitudes. The micro-processor evaluates the intensity of reflection echoes, which can be visualized as a color-coded two-dimensional B-mode image (standardized code of 255 levels). The average amplitude of the echoes in a defined area of the image is known as echogenicity, which may be objectively measured with the computer-assisted image analysis. Hypoechogenicity is defined as an intensity < 30 pixels. In the A-mode, interfaces are presented as well-defined peaks. A typical gain curve was 25-70 dB. The velocity of ultrasound in the skin was set at 1.580 m/s¹⁸.

The defined two USG parameters were examined using the software built into the USG system. SLEB was detected in A-mode scanning by measuring the vertical distance between the lower edge of the entry echo and the posterior mar-

gin of the hypoechoic zone (A-mode scans). All the scans were performed by the same trained observer¹⁹.

Each of the instrumental measurements was performed within the affected skin of the antecubital fossa before the treatment and after its completion. The same order for the biophysical test was always preserved: TEWL, skin hydration, and HF-USG. All the tests were performed in the same room conditions (temperature 20-22°C, humidity 20-40%) after 15-30 minutes of adaptation led by the same trained physician.

Treatment Protocol

Due to the exacerbation of the disease, the patients were offered two possible forms of therapy. The group treated with UVA1 phototherapy included 10 patients (33 years on average, 5 females and 5 males), who were able to travel 5 times a week for 4 consecutive weeks to the clinic. Another 10 participants, (aged 24 years on average, 5 females and 5 males) were treated with the use of tacrolimus ointment (0.1% in adult patients, 0.03% in children over 2 years of age) 2 times daily for 4 weeks. Only adult patients (over 18 years of age) were qualified for irradiation sessions. The irradiated patients presented with the phototype II according to Fitzpatrick.

The exclusion criteria included: pregnancy, breastfeeding, hypersensitivity to light, the use of possibly phototoxic and photoallergic medicines, skin neoplasm or severe systemic disease in the patient's medical history, the use of the topical therapy within 2 weeks prior to the study, and the phototherapy or systemic therapy within 4 weeks prior to the study. During the therapy, all patients were allowed to use only emollients.

The group treated with UVA1 phototherapy: the irradiations were performed with the use of the UVA1 irradiation couch (GP-24H, Cosmedico, Medical Systems, Stuttgart, Germany), equipped with 24 high-pressure fluorescent lamps that emit radiation within UVA1 (350-400 nm) with the intensity of 115 mW/cm². The medium dose of UVA1-MD UVA1 was used (the average 60 J/cm², the median 60 J/cm²; the average number of irradiations was 18.5, the median was 20, 5 times a week for 4 weeks.

After the treatment had been finished, the patients were referred to the Outpatient Clinic, where the time of remission to the next exacerbation of skin lesions was assessed. They were examined again after 1, 3, 6 and 12 months after completing the therapy.

Statistical Analysis

Non-parametric tests were used for the statistical analysis.

Wilcoxon signed-rank test was used for the before-after analysis, Mann-Whitney test was used for the comparison of f1 vs t2 groups, and in order to establish the relationship between the variables, Spearman's rank correlation coefficient test was employed. The statistic relevance level was established at $p < 0.05$.

The mean and the median was used as the measurement of the central tendency, while the distribution of the variables was assessed by means of the standard deviation and the definition of the minimum and the maximum. The calculations were performed by means of the CSS-statistica software.

Results

The tested groups did not show any statistically significant differences as far as the age, sex or the severity of the clinical condition before the therapy was initiated (Table I).

Both methods of treatment seemed to improve the clinical condition, referred to as EASI. The statistically relevant decrease in EASI was obtained both in the group treated with tacrolimus ointment (EASI before, the mean: 40.45, EASI after, the mean: 27.88) $p = 0.005$, and in the group treated with UVA1 phototherapy (EASI before, the mean: 38.01, EASI after, the mean: 24.49) $p = 0.005$.

After the comparison of both methods, no statistically significant differences were established between them, as far as EASI is concerned (Figure 1).

Additionally, the beneficial effect of the therapy on the course of AD was confirmed by means of non-invasive diagnostic techniques of the skin. Comparing the TEWL parameters before and af-

Table I. Characteristics of the study group (no = 20).

	Tacrolimus	UVA1 phototherapy
No of patients	10	10
Age: mean	24.53	33
Sex:		
Female, no, (%)	5, (50%)	5, (50%)
Male, no, (%)	5, (50%)	5, (50%)
EASI before treatment:		
Mean $\pm$ SD;	40.45 $\pm$ 7.00	38.01 $\pm$ 15.10
Median	39.50	33.60

ter the treatment of the affected areas of the skin, the group treated with tacrolimus ointment presented a statistically significant reduction in TEWL (TEWL before, the mean: 31.27; TEWL after, the mean: 21.70) $p = 0.005$. Nevertheless, after UVA1 therapy, there was a statistically relevant reduction in SLEB within the area of the affected skin before and after the treatment (SLEB before, the mean: 0.29; SLEB after, the mean: 0.11) $p = 0.007$.

The last comparison concerned the duration of remission after the use of the aforementioned methods of treatment, expressed in weeks. In the group treated with phototherapy, the average duration of remission was 6.6 weeks. While in the tacrolimus treated group the patients after the maximum 20-day-long course of treatment twice a week used tacrolimus as the so-called proactive therapy twice a week, which made it possible to prolong the remission period to 4 weeks on average. The recurrence of lesions was defined as the increase in EASI by 50% or more in relation to the effect observed immediately after the treatment.

The most frequently observed adverse reactions during UVA1 phototherapy included: the occurrence of tan of a various degree in all the irradiated patients, the feeling of warmth and burning during the irradiation session, and the intensification of pruritus at the first stage of irradiations. Transient erythema or effusion,

most probably connected with the use of high temperature, were observed more seldom. The group of patients treated with topical tacrolimus related only the feeling of burning on the first applications.

Discussion

Phototherapy in the Treatment of Atopic Dermatitis

The majority of patients with atopic dermatitis observe the beneficial influence of natural sunlight on the course of the disease (heliotherapy, climatotherapy, balneophototherapy). However, approximately 10% of patients experience the exacerbation of skin lesions after the exposure to sunlight. The irritation may be caused by the intensification of skin dryness as a result of the exposure to natural sunlight as well as phototherapy from artificial sources. The warming effect, usually not well tolerated by patients, is also important²⁰.

The Mechanism of Action of UVA1 Phototherapy in AD

The lamps emitting longwave UVA1 radiation within 340-400 nm were constructed in 1981²¹ and this is when the research on the biological effect of UVA1 radiation on human skin was commenced. In 1992, for the first time Krutmann

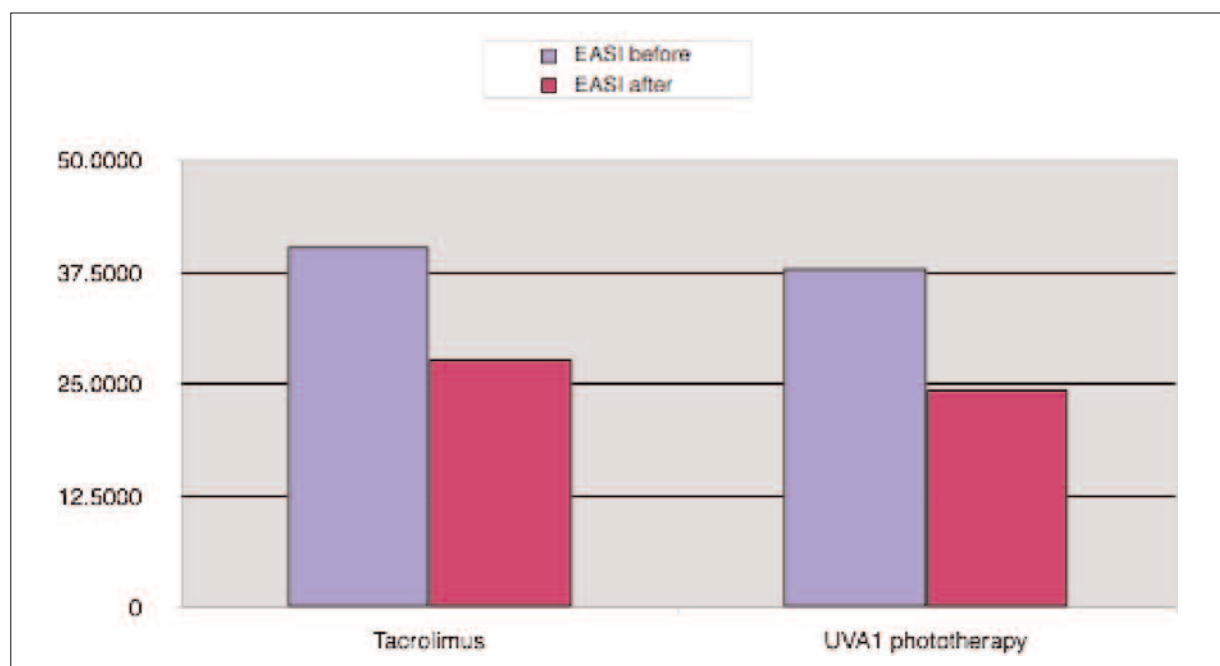


Figure 1. EASI before and after the treatment.

et al⁷ reported very satisfactory effects of the treatment of atopic dermatitis with high doses of UVA1. Since that time, the indications for this type of therapy have been constantly broadened.

Due to the wavelength, UVA1 radiation penetrates deeper into the dermis, reaches the reticular layer and has immunomodulatory properties. The mechanism of its action has not yet been fully recognized. It was initially thought that UVA1 is able to generate free oxygen radicals – ROS, and in this manner it indirectly influences DNA. Nowadays it is known that similarly to UVB, UVA1 also triggers the formation of mutations within DNA¹². Unlike UVB, a unique feature of UVA1 is the phenomenon of the so-called immediate apoptosis, associated to the activity of ROS²². The ability to induce apoptosis of T and B lymphocytes may probably account for the effectiveness of this method in the treatment of atopic dermatitis. Moreover, after irradiation, the number of Langerhans cells, mast cells and basophils is reduced in AD patients, followed by decreased histamine release^{12,23}. In recent years, there has been research published that confirms both in vitro and in vivo that UVA1 phototherapy inhibits calcineurin, acting similarly to cyclosporine A and tacrolimus. Calcineurin – a calcium-dependent phosphatase – is a molecule that participates in signal transmission, playing the main part in the control of the immunological response. It is the main therapeutic aim in transplant rejection as well as in many inflammatory skin disorders. UVA1 phototherapy, by means of oxidation of Met and Cys residues, induces conformational changes of this protein¹³. What is more, UVA1 phototherapy has an immunomodulatory influence on the network of pro-inflammatory cytokines that are significant in AD pathogenesis. After a cycle of UVA1 irradiations, the expression of cytokines IL 5, 13, 31 in skin sections has been greatly reduced²⁴.

Research on the Use of UVA1 Irradiation in AD

In the pioneering research², patients with exacerbations in the course of AD were treated with high doses of UVA1 130J/cm, 5 times a week for 15 consecutive days. The effectiveness of the therapy was assessed by means of monitoring the clinical condition using the SCORAD scale, and measuring the level of the cationic eosinophil protein that had been significantly reduced⁶. The next stage involved the comparison of the efficacy of phototherapy with a high dose of UVA1

with the hitherto prevailing standard treatment with broadband UVA/B and steroids, which revealed considerable superiority of UVA1. Researchers⁷ confirmed the distinctly better effect of monotherapy with high doses of UVA1- HD UVA1 in comparison to external glucocorticosteroids (fluocortolone) and broadband UVA/B. Next, the efficacy of medium doses was also confirmed. In this study, half of the patient's skin was irradiated 5 times a week with a high dose, while the other half was irradiated with a medium dose. No significant differences were found after 3 weeks of treatment, while the remission period was 4 weeks on average¹⁰. Afterwards, medium and low doses were compared analogically, revealing the activity of medium, and not low, doses²⁵. Von Kobyletzki et al²⁶ tested a new UVA1 apparatus that leveled the thermal effect of the so-called cold-light UVA1 and the traditional UVA1 and UVA/B for three weeks of treatment, which proved its superiority. MD UVA1 was effective in AD exacerbations, and the result was visible for approximately 1 month, with the relapse of lesions after 3 months.

Moreover, another compared issue²⁷ was the effect of irradiation with medium doses 5 times a week in 15 procedures versus 20 procedures, which proved that the increased number of irradiations prolonged the remission period from 4 to 6 weeks on average. Further comparative research projects²⁸ showed that narrowband UVB 311nm is as effective as medium doses of UVA1 provided during 6 weeks (18 irradiations) in patients with moderate-severe AD.

Recently, there has been a study published¹¹ that has compared the efficacy of medium doses of UVA1 and PUVA photochemotherapy with the use of 5-methoxypsoralen, proving the superiority of photochemotherapy. The randomized and blind crossover trial involved 23 AD patients, and the clinical condition was estimated by means of the SCORAD scale. The patients were treated with a medium dose UVA1 50-70J/cm² for 15 times (5 times a week during 3 weeks), and next, in the case of subsequent exacerbation, 5-MOP and UVA 15 times (3 times a week, during 5 weeks) or the opposite. There was a significantly greater reduction in SCORAD after PUVA, as well as longer, 12-week-long remission periods, as compared to 4 weeks after irradiation with MD UVA1. Up till now, there have been no comparative studies of high doses of UVA1 or medium doses in 20 irradiations and PUVA/PUVA bath or topical tacrolimus (Table II).

Apart from efficacy, it seems significant to compare those methods as related to their safety. The apparent advantage of UVA1 irradiations is that the use of psoralen is unnecessary, and the risk of phototoxic reactions is lower in comparison with PUVA phototherapy. On the other hand, disadvantages include limited accessibility and the high cost of the apparatus. What is more, the distant adverse effects of UVA1 irradiations are not known, as compared to the precisely defined recommendations of not exceeding 200 irradiation sessions by means of PUVA, which, if exceeded, may increase the risk of non-melanoma skin neoplasm²⁹⁻³¹.

The treatment strategy of AD exacerbations is mostly based on topical glucocorticosteroids and calcineurin inhibitors, with better benefit/risk ratio in the long-term treatment for the latter group of drugs. The action of tacrolimus involves the inhibition of the phosphatase action of calcineurin, which leads to the suppression of interleukin production by T-cells. While it has been demonstrated that the prolonged application of glucocorticosteroids can damage the structure and function of the epidermal barrier, the beneficial effect of calcineurin inhibitors on TEWL has recently been shown³². The epidermal barrier of the skin affected with atopic dermatitis was evaluated in many previous reports, and it is well known that it is characterized by the increased TEWL and decreased water content³³⁻³⁵. The results presented herein are in concordance to the literature data. For the first time, the additional measurement of TEWL and skin hydration were employed in order to evaluate the effectiveness of UVA1 phototherapy. Despite the harmful effect that the UV irradiation has on the skin, the possibility to irritate it, as well as its undeniably drying properties, the beneficial and rebuilding effect on the function of the stratum corneum by reducing inflammation was observed³⁶. Similarly,

the improvement in the epidermal barrier function was observed after tacrolimus therapy.

According to our previous studies, we used high-frequency ultrasonography, as an objective method, to assess the results of both studied therapies^{14,18}. SLEB as an indicator of skin inflammation in AD decreased significantly after phototherapy, which was related to the improvement of the condition of the skin. We also observed a decrease in SLEB during tacrolimus therapy, however, it was not significant. Due to its noninvasiveness, HF-USG seems to be especially useful for the evaluation of the clinical state of children suffering from AD.

Conclusions

This study has confirmed the beneficial influence of both treatments on the course of the moderate-severe AD. Both tacrolimus and phototherapy seemed to significantly reduce EASI. Tacrolimus had a greater influence on the reduction of TEWL, while phototherapy reduced SLEB within the affected areas.

Tacrolimus is a standard remedy in the topical treatment of AD. At the preliminary stage of the more intensive treatment, it is applied on the affected areas twice a day, and later, as a part of the so-called proactive therapy, it is applied on the apparently unaffected skin, in the sites where the lesions occur most frequently (antecubital and popliteal fossae). In our country, however, the cost of such therapy remains high and therefore calcineurin inhibitors are recommended to be applied only on particularly sensitive areas, such as the face and the neck, where glucocorticosteroids are used reluctantly due to their well-known side effects. On the other hand, phototherapy seems to be the complementary method in the topical and systemic treatment of atopic dermatitis, particu-

Table II. The summary of the studies of UVA1 efficacy.

Conclusions		References
1.	HD UVA1 > UVA/B or topical fluorocortolone	Krutmann J et al, 1998 (7)
2.	HD UVA1 = MD UVA1 (15 irradiations, remission 4 weeks)	Tzaneva S et al, 2001 (10)
3.	MD UVA1 > LD UVA1	Kovalzick L et al, 1995 (26)
4.	MD UVA1 (20 irradiations, remission 6 weeks) > MD UVA1 (15 irradiations, remission 4 weeks)	Poldermann MCA et al, 2005 (27)
5.	MD UVA1 = NB UVB (18 irradiations)	Gambichler T et al, 2009 (28)
6.	PUVA (duration 5 weeks; remission 12 weeks) > MD UVA1 (duration 3 weeks; 15 irradiations, remission 4 weeks)	Tzaneva S et al, 2010 (11)

larly when the skin lesions are exacerbated and widespread. According to the latest guidelines, it is considered a method of second choice, used mainly in adult patients, more rarely in children below 12 years of age, with the moderate (NB UVB 211 nm, UVA1) and severe (PUVA) course of AD. In general, phototherapy should not be recommended (except for UVA1) in the period of exacerbations. It may be combined with external medications, i.e. glucocorticosteroids or emollients. The combination with tacrolimus or pimecrolimus, as well as with widely used cyclosporine A, should be avoided^{3,4,5}. It should be noted, though, that this therapy affects the whole skin, also apparently unaffected places. However, its limitations include its availability only in the specialist phototherapy centers and expensive operation. The treatment with phototherapy requires patients to travel and is very time-consuming.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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