VILNIUS UNIVERSITY

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PHOTOTHERAPY IN THE ANIMAL MODELS OF SYSTEMIC SCLEROSIS

Summary of doctoral dissertation

Biomedical sciences, medicine (06 B)

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VILNIAUS UNIVERSITETAS

DIANA KARPEC

FOTOTERAPIJOS POVEIKIS SISTEMINĖS SKLEROZĖS GYVŪNŲ MODELIUOSE

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To my husband Laurynas

ABBREVIATIONS

Bleo – bleomycin

DAB – diaminobenzidine

H&E – hematoxylin and eosin

IHC – immunohistochemistry

LED – light emitting diode

MMP-1 – matrix-metalloproteinase-1

MMP-3 – matrix-metalloproteinase-3

NaCl – sodium chloride

PT – phototherapy

SD – standard deviation

SSc – systemic sclerosis

UVA – ultraviolet A

UVA1 – ultraviolet A1

w – week

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1. INTRODUCTION

1.1. Background

Systemic sclerosis (SSc) is a rare, chronic, autoimmune disease of unknown etiology. The pathogenesis of SSc includes vasculopathy, progressive fibrosis and alterations in the immune system [1]. Despite the current existing treatment with glucocorticoids and cytostatic drugs, the management of the disease remains a challenge. Patients with SSc exhibit a long-standing, established dermal fibrosis with ongoing pathogenic mechanisms [2]. Because of this, an effective antifibrotic treatment should target both the degradation of excessive extracellular matrix components and stop any further accumulation of collagen.

Over the last few decades, phototherapy has been implied as a new possibility for the treatment of many skin diseases (atopic dermatitis, urticaria pigmentosa, psoriasis, prurigo, etc.) [3, 4]. There are no randomized, double blind, placebo-controlled studies concerning the ultraviolet A1 (UVA1) phototherapy in dermal fibrosis [5]. A few clinical studies using the broadband UVA1 (340-400 nm) or even full spectrum UVA (320-400 nm) for the treatment of localized scleroderma and systemic sclerosis were performed [6-8]. It is known that the longer the wavelength used, the greater the depth of penetration by ultraviolet (UV) waves is observed [9]. The concentration of radiation energy on the superficial layers of the skin raises some concerns associated with carcinogenesis [10]. Whereas the key molecules that absorb UVA1 radiation, leading to the biological effects of phototherapy, are present in the deeper layers of the dermis [11]. The United States Food and Drug Administration approved the UVA1 (340-400 nm) phototherapy device (*Daavlin*©) with the peak of 365-375 nm for the treatment for psoriasis, vitiligo and atopic dermatitis [12]. The wide spectral waveband of UVA1 could react with the different chromophores in the skin, leading to different effects on the immune system of skin [13]. The special-purpose medical device “IMC-Light” has been constructed during the doctoral studies. The equipment consists of UVA1 light emitting diodes (LEDs), which emit the UV rays with a peak wavelength of 365 ± 5 nm. In accordance with the physical properties of UVA1 and the optical features of the skin, the narrowband UVA1 would influence collagen metabolism with a reduced chance of side effects, such as erythema and carcinogenesis [14]. None of the previously conducted studies evaluated the

safety and effectiveness of narrowband UVA1 on dermal fibrosis. Clinical trials of SSc are difficult to carry out due to the severe conditions of the patients, the variety of symptoms, the small patients' cohort size and any probable disease-related complications. Thus, animal models are essential tools in investigating in-depth the search of new therapeutic modalities [15]. The aim of the present study was to evaluate the efficacy and safety of narrowband (365 ± 5 nm) UVA1 for the dermal fibrosis treatment using the bleomycin-induced systemic sclerosis model in DBA/2 mice.

1.2. The Aim of the Study

To evaluate the efficacy and safety of narrowband (365 ± 5 nm) ultraviolet A1 (UVA1) for the dermal fibrosis treatment using the animal models of systemic sclerosis.

1.3. The Objectives of the Study

1. To assess the effect of high and medium doses of narrowband UVA1 on the dermal thickness, mast cells infiltration and expression of active caspase-3 in the established, bleomycin-induced animal model of fibrosis.
2. To evaluate the impact of high and medium doses of narrowband UVA1 on the carcinogenesis in the established, bleomycin-induced animal model of fibrosis.
3. To assess the effect of high dose of narrowband UVA1 on the progression of fibrosis and regression of fibrotic changes in the established, bleomycin-induced animal model of fibrosis when bleomycin is applied during the whole experiment.

1.4. Defended Statements

1. The narrowband UVA1 phototherapy reduces dermal thickness, stops the progression of fibrosis and induces the regression of existing fibrotic changes in the established, bleomycin-induced animal model of fibrosis.
2. The narrowband UVA1 phototherapy does not cause an increased risk of carcinogenesis in the established, bleomycin-induced animal model of fibrosis.

1.5. The Scientific Novelty of the Study and Implementation in Clinical Practice

The results of the present study have revealed for the first time that narrowband UVA1 phototherapy (365 ± 5 nm) not only stops the progression of dermal fibrosis, but also induces

the regression of already existing fibrotic changes in the established, bleomycin-induced mouse model of scleroderma. Furthermore, the cumulative doses of 1200 J/cm^2 and 600 J/cm^2 of narrowband UVA1 do not cause an increased expression of p53 nor Ki-67 proteins, resulting in a low carcinogenetic risk of phototherapy.

The special-purpose medical device “IMC-Light” has been constructed during the course of doctoral studies. The Lithuanian State Health Care Accreditation Agency under the Ministry of Health has authorized the use of this special-purpose medical device in the clinical study, which will be held at the Santaros Clinic of Vilnius University Hospital (permission No. S8-5-15). Accreditation data and authorization are registered in the European Database on Medical Devices EUDAMED (CIV-ID): CIV-15-10-013898. In the near future, the efficacy of narrowband UVA1 phototherapy for patients with systemic sclerosis will be evaluated. The use of this non-invasive method in day-to-day clinical practice would be an important step in addressing the complexities of systemic sclerosis treatment.

2. METHODS

2.1. Animals

Eighty-four 6-weeks-old female mice (each weighing 17-20 g) of the DBA/2 strain were involved in the study and kept under standard housing conditions with unlimited food and water access. The use of animals in this study was in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The study was approved by the Lithuanian Laboratory Animal Use Ethical Committee under the State Food and Veterinary Service (No G2-15 and G2-42).

2.2. Bleomycin-induced Mouse Model of Scleroderma

Skin fibrosis was induced by subcutaneous injections of bleomycin (Bleo) (*Bleomycin sulfate* from *Streptomyces verticillus*, Sigma Aldrich, Buchs, Switzerland) [16]. At first, the hair from the mouse back was removed using depilation cream (*Veet*®, Reckit Benckiser, England) and a square area of 1 cm² was marked. The injection site included 5 points – every corner and the center of the marked square. Bleomycin was dissolved in 0.9% of sodium chloride (NaCl, *Sanitas*, Lithuania) at a concentration of 0.5 mg/ml and a subcutaneous injection of 100 µl was performed every other day for 3 or 8 weeks. Other healthy mice received 100 µl of sodium chloride injections at the matched area on the back and served as the control.

2.3. Phototherapy

The special-purpose medical device "IMC-Light" was used as a phototherapy source in the study (Fig. No. 1). The equipment consisted of 20 built-in UVA1 LEDs (UVLED-365-330 – surface mounted devices, *Roithner LaserTechnik*, Austria) emitting 365 ± 5 nm radiation. LEDs were laid out to ensure the uniform power density of 21 mW/cm² at the surface distant 4 cm from the device. Phototherapy was performed in a black metal box with separate cages for each mouse. Mice were irradiated 3 times per week for 5 weeks. The applied doses per session were 80 J/cm^2 for a high and 40 J/cm^2 for a medium dose of UVA1. Thus, the average cumulative doses over five weeks were 1200 J/cm² for high and 600 J/cm² for medium dose treatments.

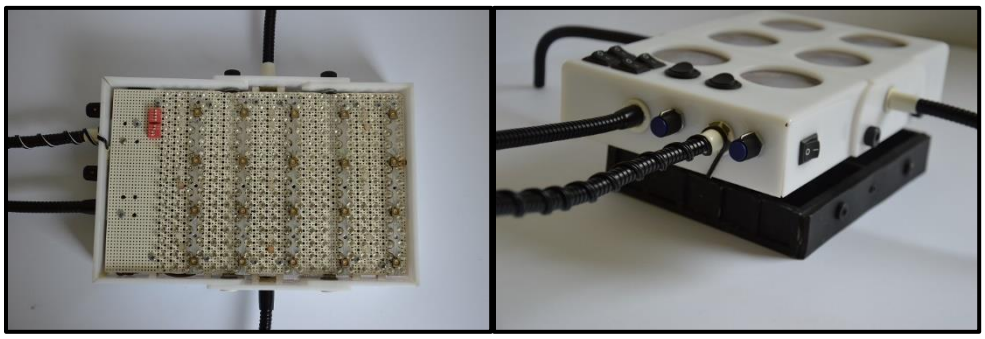

Fig. No. 1. Special-purpose medical device “IMC-Light”

Phototherapy Course for the Established, Bleomycin-Induced Mouse Model of Scleroderma

Forty-two DBA/2 line mice were randomly assigned to 6 groups (seven mice in each)

- Group I (Healthy) – healthy animals;
- Group II (Bleo) – control group with bleomycin-induced scleroderma;
- Group III (BleoHighPT) – mice with an established scleroderma treated with a high dose of UVA1;
- Group IV (BleoMediumPT) – mice with an established scleroderma treated with a medium dose of UVA1;
- Group V (HealthyHighPT) – healthy mice treated with a high dose of UVA1;
- Group VI (HealthyMediumPT) – healthy mice treated with a medium dose of UVA1.

All mice were sacrificed at the end of experiment on week 8. A graphical illustration of the study design is summarized in Fig. No. 2.

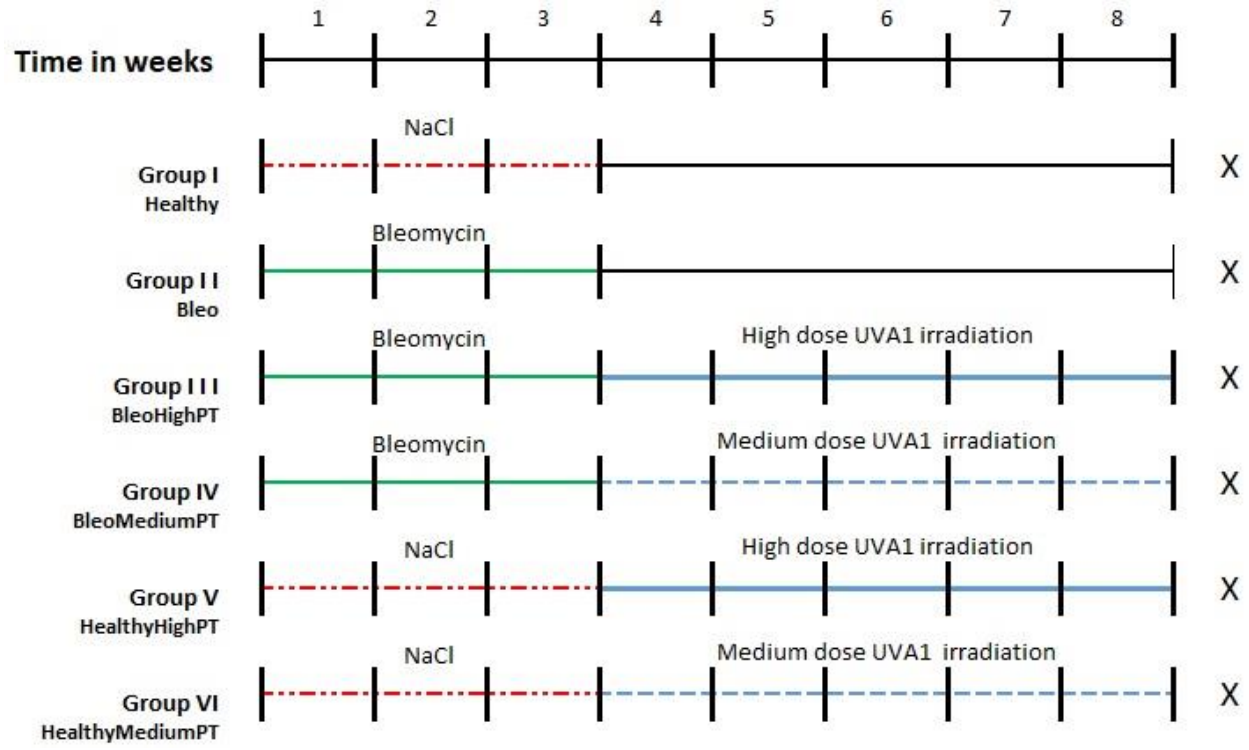

Fig. No. 2. Design of the study. NaCl – sodium chloride; bleo – bleomycin; PT – phototherapy; high dose of UVA1 – 80 J/cm² (3 times per week for 5 weeks); medium dose of UVA1 – 40 J/cm² (3 times per week for 5 weeks); X – sacrifice of mice

Phototherapy Course for the Established, Bleomycin-Induced Mouse Model of Scleroderma, when Bleomycin is Applied During the Whole Experiment

Forty-two DBA/2 line mice were randomly divided into the following 6 groups (7 animals in each):

- Group I (Healthy 3w) – Mice injected with NaCl for 3 weeks;
- Group II (Bleo 3w) – Mice injected with bleomycin for 3 weeks;
- Group III (Healthy 8w) – Mice injected with NaCl for 8 weeks;
- Group IV (Bleo 3w + NaCl 5w) – Mice injected with bleomycin for 3 weeks and then with NaCl for 5 weeks;
- Group V (Bleo 8w) – Mice injected with bleomycin for 8 weeks;

- Group VI (Bleo 8w + UVA1) – Mice injected with bleomycin for 8 weeks and in parallel treated with an average cumulative dose of 1200 J/cm^2 of UVA1 for the last 5 weeks (the treatment group).

Mice from I, II groups were sacrificed after 3 weeks and mice from III, IV, V, VI groups – after 8 weeks. A graphical illustration of the study design is summarized in Fig. No. 3.

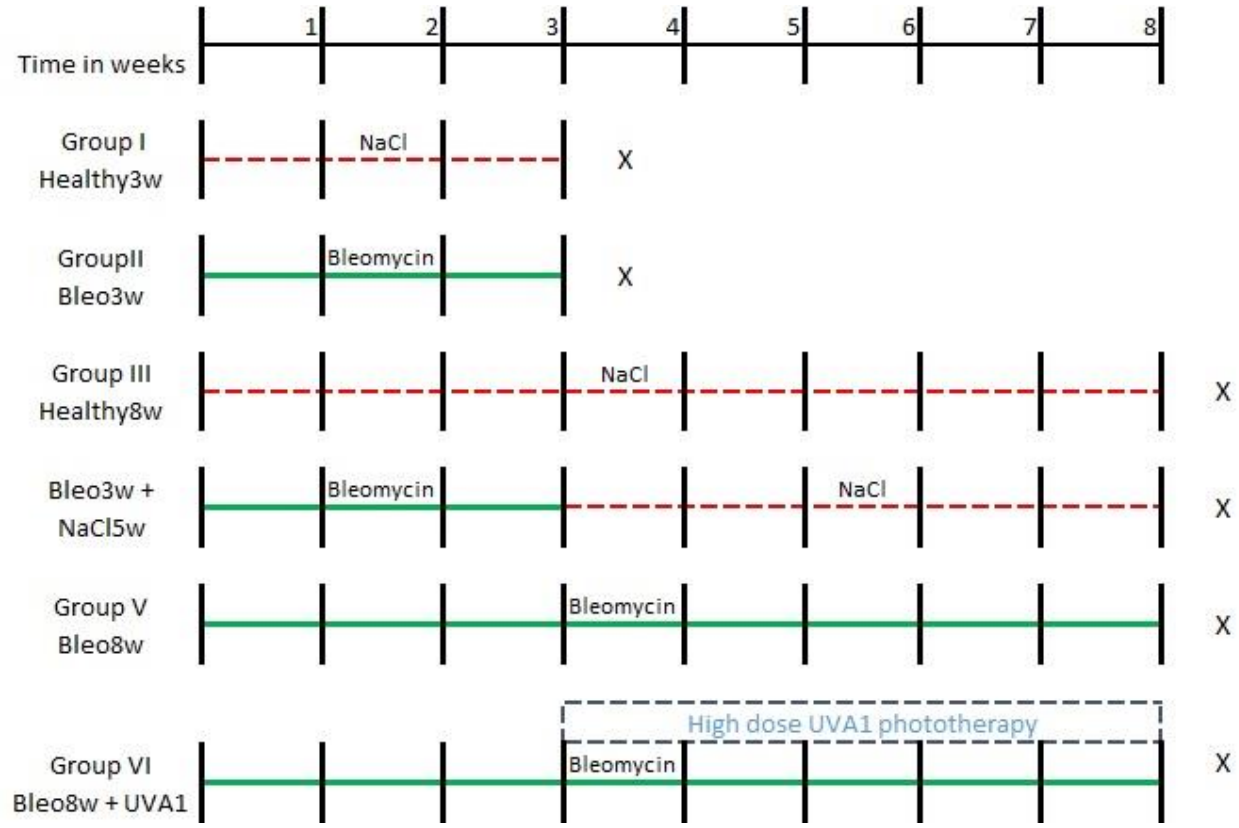

Fig. No. 3. Design of the study. NaCl –sodium chloride; bleo – bleomycin; w – week; UVA1 –ultraviolet A1; X – sacrifice of mice

2.4. Histological and Immunohistochemical Analyses

The skin samples analysis in the established, bleomycin-induced mouse model of scleroderma consisted of the following elements:

- A histological dermal thickness analysis;
- A histological analysis of mast cells infiltration;
- An immunohistochemical analysis of active caspase-3 for the evaluation of apoptosis;
- An immunohistochemical analysis of p53 and Ki-67 proteins for the evaluation of carcinogenesis.

The skin samples analysis in the established, bleomycin-induced mouse model of scleroderma, when bleomycin is applied during the whole experiment, consisted of the following elements:

- A histological dermal thickness analysis;
- An immunohistochemical analysis of matrix metalloproteinase-1 (MMP-1) and matrix metalloproteinase-3 (MMP-3);
- An immunohistochemical analysis of collagen type I and collagen type III.

Histological Dermal Thickness Analysis

Skin samples were stained with hematoxylin and eosin (H&E) in accordance with the standard protocol. The histological preparations were photographed using an Olympus BX51 microscope (*Olympus Corporation*, Inc.,Tokyo, Japan) with a Nikon DXM 1200 camera (*Nikon Instruments, Inc.*, New York, USA). Dermal thickness was evaluated by measuring the distance (μm) between the epidermal-dermal junction and the dermal-subcutaneous fat junction at four different points of skin sections stained with H&E. The quantitative analysis was made using NIS ELEMENTS software v.BR 2.30 (*Nikon instruments*, Netherlands).

Histological Analysis of Mast Cells Infiltration

Skin samples were stained with toluidine blue for the analysis of mast cells. Stained histological sections were photographed using a Nikon "Eclipse TE2000" microscope (*Nikon*, Tokyo, Japan) with a Nikon DS-Fi2 high-definition color camera head (*Nikon*, Tokyo, Japan). Violet (red-purple) color cells containing metachromatic cytoplasmic granules were counted in four random grids under a magnification of x 200. The mean number was calculated. The mast cell density was expressed as the average of mast cell count/high power field. The quantitative analysis was made using NIS ELEMENTS software v.BR 2.30 (*Nikon instruments*, Netherlands).

Immunohistochemical Analysis

Immunohistochemical staining was performed manually by using DAKO Envision + System-Peroxidase Kit (Glostrup, Denmark) following the protocols of the manufacturer. The dilutions and positive control groups of the used primary antibodies are summarized in Table No. 1. Stained sections were photographed using a Nikon "Eclipse TE2000" microscope (*Nikon*, Tokyo, Japan) with a Nikon DS-Fi2 high-definition color camera head (*Nikon*, Tokyo, Japan).

The quantification of p53 and Ki-67 proteins was performed with open-source software *ImageJ® Fiji* (National Institutes of Health, Maryland, US) along with the *Immunoratio* (version 1.0c, Institute of Biomedical Technology, University of Tampere, Findland) plug-in, available at http://153.1.200.58:8080/immunoratio/ [17]. The *Immunoratio* plug-in calculated the percentage of the positively stained nuclear area by using a color deconvolution algorithm [18] in 4 fields for each specimen. The results were represented as the mean percentage (%) of positive nuclei (brown pixels) to negative nuclei (blue pixels) ± standard deviation. Immunoreactivity to active caspase-3, MMP-1, MMP-3, collagen type I and collagen type III was characterized by brown cytoplasmic staining and evaluated individually following image segmentation. *ImageJ® Fiji* software (National Institutes of Health, Maryland, US) was used for morphometric analysis [17]. The digital images were processed by the color deconvolution (H&E DAB-diaminobenzidine) technique [18]. The mean percentage staining of the

demarcated area (positive pixels were equal to the areas of diaminobenzidine staining) related to the total area of each field (total pixels) was evaluated. This technique was performed in 4 fields for each specimen. Results were represented as the mean percentage (%) of stained area ± standard deviation.

Table No. 1. Primary antibodies for immunohistochemical analysis

Antibody (description)	Manufacturer (catalog number)	Dilution	Positive control tissue
Anti-caspase 3 antibody (rabbit polyclonal to active caspase 3)	*Abcam* Cambridge, United Kingdom (ab44976)	1:500	Human tonsil tissue
Anti-Ki67 antibody (mouse anti-Human Ki-67 antibody [Clone Sp6])	*Spring Bioscience*, Pleasenton, US (M3064)	1:200	Human tonsil tissue
Anti-p53 antibody (rabbit polyclonal to p53)	*Abcam* Cambridge, United Kingdom (ab31333)	1:200	Human breast adenocarcinoma tissue
Anti-MMP1 (matrix metalloproteinase-1; rabbit polyclonal)	*Thermo Fisher Scientific*, Illinois, United States (PA5-27210)	1:600	Human breast adenocarcinoma tissue
Anti-MMP3 (matrix metalloproteinsase-3; rabbit monoclonal)	*Abcam*, Cambridge, United Kingdom (ab52915)	1:100	Human gastric adenocarcinoma tissue
Anti-Collagen Type I (rabbit polyclonal)	*Abcam*, Cambridge, United Kingdom (ab34710)	1:300	Human salivary gland tissue
Anti-Collagen Type III (rabbit polyclonal)	*Thermo Fisher Scientific*, Illinois, United States (PA1-85323)	1:400	Mouse placenta tissue

2.5. Statistics

SPSS Statistics software (v. SPSS16) system package for Windows software was used for statistical analysis. Continuous data were expressed as mean ± standard deviation. Continuous variables were compared by a non-parametric Mann-Whitney U test. Statistical significance was expressed by a P-value ≤ 0.05.

3. RESULTS

3.1. Narrowband UVA1 Phototherapy in the Established, Bleomycin-Induced Mouse Model of Scleroderma

First, we evaluated the dermal thickness differences in all the groups of experiment. The mean dermal thickness of mice with bleomycin-induced scleroderma (group II) was 598.95 ± 55.74 μm, whereas it was only 179.34 ± 25.30 μm in the group of healthy mice ($P \leq 0.05$). After narrowband UVA1 phototherapy on mice with scleroderma (groups III and IV), the histopathological skin examination showed both the thinning of skin layers and the reduced number of irregular fibroblasts with less cellular infiltration between the collagen bundles (Fig. No. 4). The dermal thickness of mice with scleroderma, treated with high and medium dose of UVA1, was lower (group III – 272.94 ± 113.20 μm; group IV – 393.95 ± 125.87 μm; $P \leq 0.05$) in comparison to the thickness of non-irradiated animals (group II – 598,95 ± 55.74 μm). Thus, the skin thickness reduction by 54% and 34% after irradiation with the cumulative doses of 1200 J/cm^2 and 600 J/cm^2, respectively, was observed. There were no statistically significant differences in the mean dermal thickness between the healthy mice (group I – 179.34 ± 25.30 μm) and the UVA1-treated healthy mice (group V – 208.71 ± 48.99 μm; group VI – 175.51 ± 32.83 μm).

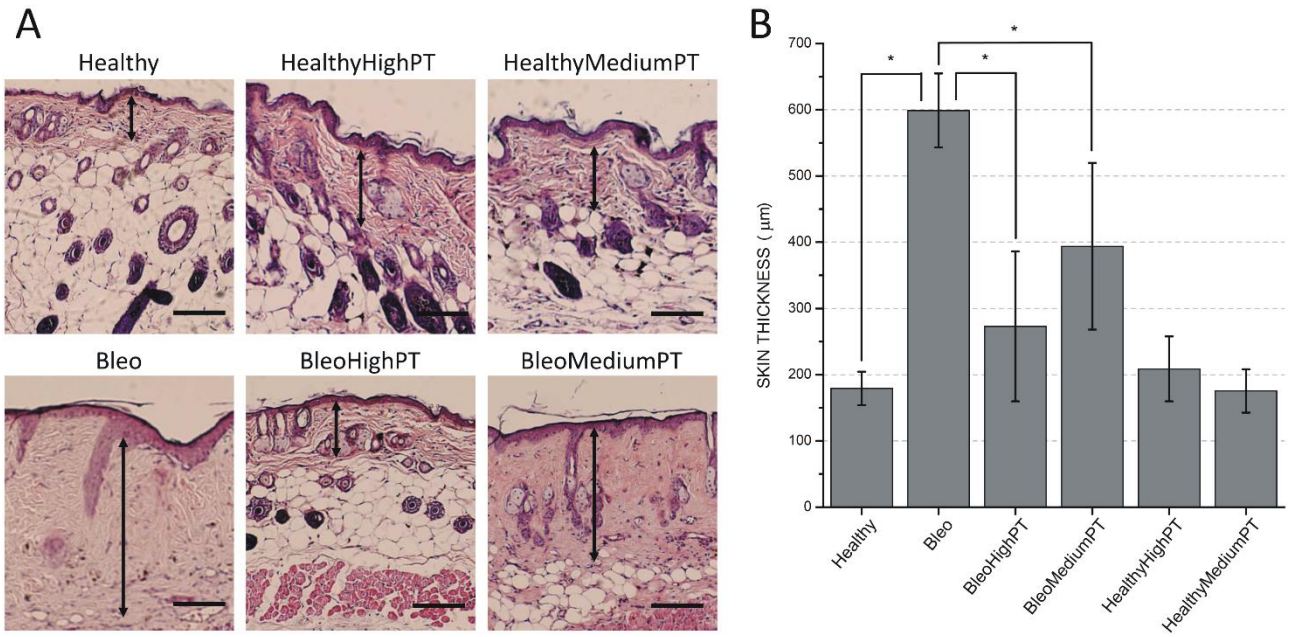

Fig. No. 4. The evaluation of dermal thickness. a) H&E-stained sections of the skin; magnification x 20; scale bar – 100 µm. The arrow indicates the length between epidermal-dermal and dermal-subcutaneous fat layers; b) Quantification of the dermal thickness (µm).The graph shows the mean (±SD) results obtained from the 7 mice of each group. *P ≤ 0.05; H&E – hematoxylin and eosin; bleo – mice with bleomycin-induced scleroderma group; healthy – healthy mice control group; highPT – high-dose phototherapy; mediumPT – medium-dose phototherapy; SD – standard deviation

Mast cells might be the link between the inflammation and fibrosis in the pathogenesis of SSc [19]. A histological examination after a toluidine blue stain (pH 2.0) highlighted the mast cells, which were distinguished by a violet/red-purple color (Fig. No. 5). The dermal mast cells density was significantly higher in mice with scleroderma as compared to that in the healthy mice group (23.29 ± 3.09 and 6.71 ± 1.98, respectively; P ≤ 0.05). After irradiation with high and medium dose of narrowband UVA1 on mice with scleroderma, the density of mast cells was reduced to 10.71 ± 1.80 and 12.57 ± 2.23, respectively (P ≤ 0.05). The infiltration of mast cells did not differ between non-irradiated and UVA1-treated healthy mice groups (group I – 6.71 ± 1.98; groups V and VI: 5.86 ± 1.07 and 6.57 ± 1.13, respectively).

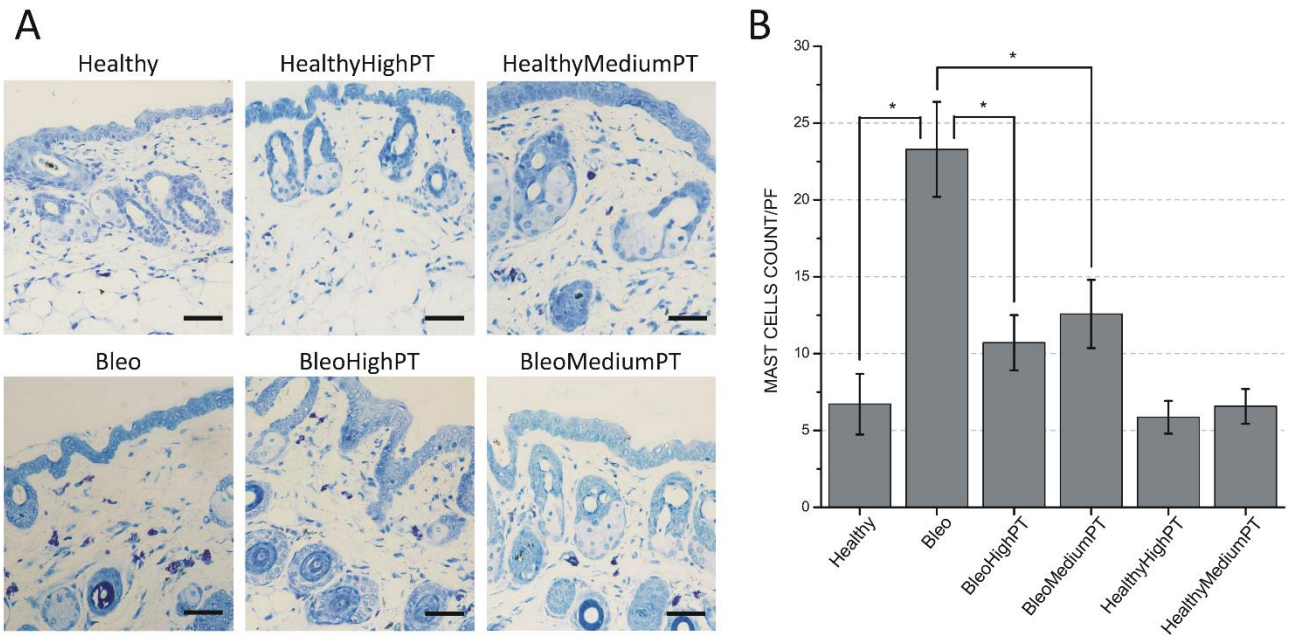

Fig. No. 5. The infiltration of mast cells. a) Histopathological views representing infiltration with dermal mast cells; toluidine blue staining; magnification x 20; scale bar – 50 µm; b) Count of mast cells. The mean number of cells counted under magnification of a x 200 high power field in 4 random grids. The graph shows the mean (±SD) results obtained from the 7 mice of each group. *P < 0.05; bleo – mice with bleomycin-induced scleroderma group; healthy – healthy mice control group; highPT – high-dose phototherapy; mediumPT – medium-dose phototherapy; SD – standard deviation

An impaired apoptosis process is suggested to be involved in the pathogenesis of SSc [20]. Caspase-3 is the main executor of apoptosis and can be activated via both intrinsic and extrinsic apoptotic signaling pathways [21]. The mice with scleroderma exhibited a significantly higher level of active caspase-3 expression as compared with that in the healthy control group (9.87 ± 1.57% and 4.26 ± 1.45% respectively; $P \leq 0.05$). After irradiation with cumulative doses of 1200 J/cm^2 and 600 J/cm^2 of UVA1 on mice with scleroderma, the expression of active caspase-3 was significantly ($P \leq 0.05$) higher (17.24 ± 3.30% and 17.10 ± 4.43%, respectively) as compared with that in the control II group (9.87 ± 1.57%). The expression profile of active caspase-3 in the skin did not differ between healthy and UVA1-treated healthy mice groups (group I – 4.26 ± 1.45 %; group V and group VI: 3.99 ± 1.21% and 4.17 ± 1.18%, respectively) (Fig. No. 6).

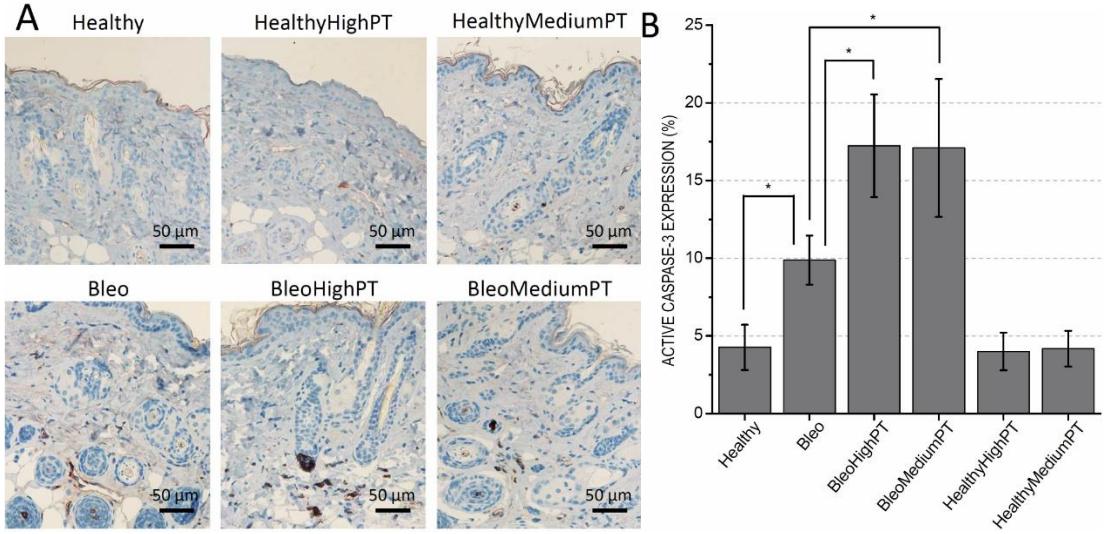

Fig. No. 6. Active caspase-3 immunohistochemistry (IHC). a) Representative images of active caspase-3 in all the groups of the experiment; IHC staining; x 20 magnification; scale bar – 50 µm; b) Percentage of active caspase-3 expression; x 20 magnification. The graph shows the mean (±SD) results obtained from the 7 mice of each group. *P ≤ 0.05; bleo – mice with bleomycin-induced scleroderma group; healthy – healthy mice control group; highPT – high-dose phototherapy; mediumPT – medium-dose phototherapy; SD – standard deviation

TP53 or p53 is a tumor suppressor gene and its mutations are involved in the development of cancer [22]. After 3 weeks of bleomycin injections, the mice with scleroderma exhibited a significantly higher level of p53 expression as compared with that in the healthy control group (26.69 ± 6.03% and 4.57 ± 3.26%, respectively, P ≤ 0.05). The percentage of p53 positive cells after high and medium dose of UVA1 treatment in bleomycin-induced scleroderma (groups III and IV) were 28.25 ± 6.16% and 27.02 ± 5.57%, respectively (Fig. No. 7). However, these expressions did not significantly differ from the control group II, which had received bleomycin without phototherapy (P > 0.05). After treatment with a high and medium dose of UVA1 in healthy mice (groups V and VI), the percentage of p53 positive cells in the skin were 5.73 ± 3.40% and 5.43 ± 1.86%, respectively. The protein expression in skin samples did not differ between healthy (group I) and UVA1-treated healthy mice (groups V and VI).

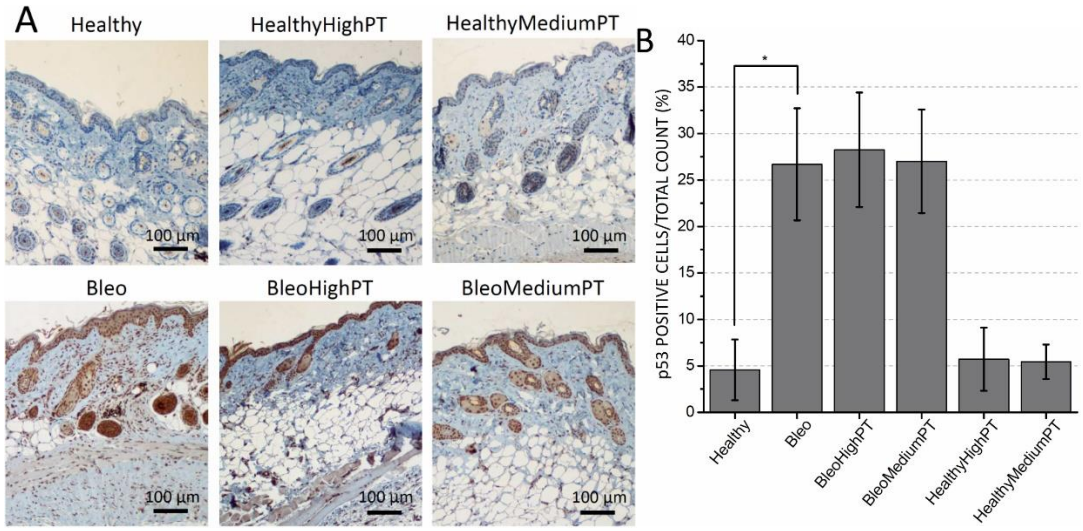

Fig. No. 7. p53 immunohistochemistry (IHC) a) Representative images of p53 nuclear expression in all the groups of the experiment; IHC staining; x 10 magnification; scale bar – 100 µm; b) Percentage of p53 positive cells to total cell population; x 20 magnification. The graph shows the mean (±SD) results obtained from the 7 mice of each group; bleo – mice with bleomycin-induced scleroderma group; healthy – healthy mice control group; highPT – high-dose phototherapy; mediumPT – medium-dose phototherapy; SD – standard deviation

The Ki-67 antigen is a high molecular weight protein and a marker of proliferation. An increased epidermal Ki-67 expression is a sign of hyperproliferation and altered cells differentiation, which might play a role in the cutaneous carcinogenesis [23]. The nuclear expression of Ki-67 in proliferating keratinocytes is stained in a brown color in the immunoperoxidase histochemical reaction (Fig. No. 8). In the healthy skin (group I), the percentage of Ki-67 positive cells was 50.43 ± 2.64%. The number of these positive cells was reduced to 36.14 ± 3.02% in the skin of the control group II after bleomycin injections, reflecting the reduced proliferation of keratinocytes ($P \leq 0.05$). The percentage of the Ki-67 positive cells after high and medium dose of UVA1 treatment in bleomycin-induced scleroderma (groups III and IV) were 35.43 ± 3.26% and 37.29 ± 2.81%, respectively. The results did not differ from the control group II that received bleomycin without phototherapy procedures. The effect of a high and medium dose of UVA1 on the Ki-67 expression in the skin of healthy mice (groups V and VI: 50.14 ± 1.95% and 49.43 ± 1.40%, respectively) did

not differ when compared to the expression of protein in the skin of healthy mice without phototherapy (group I – 50.43 ± 2.64%).

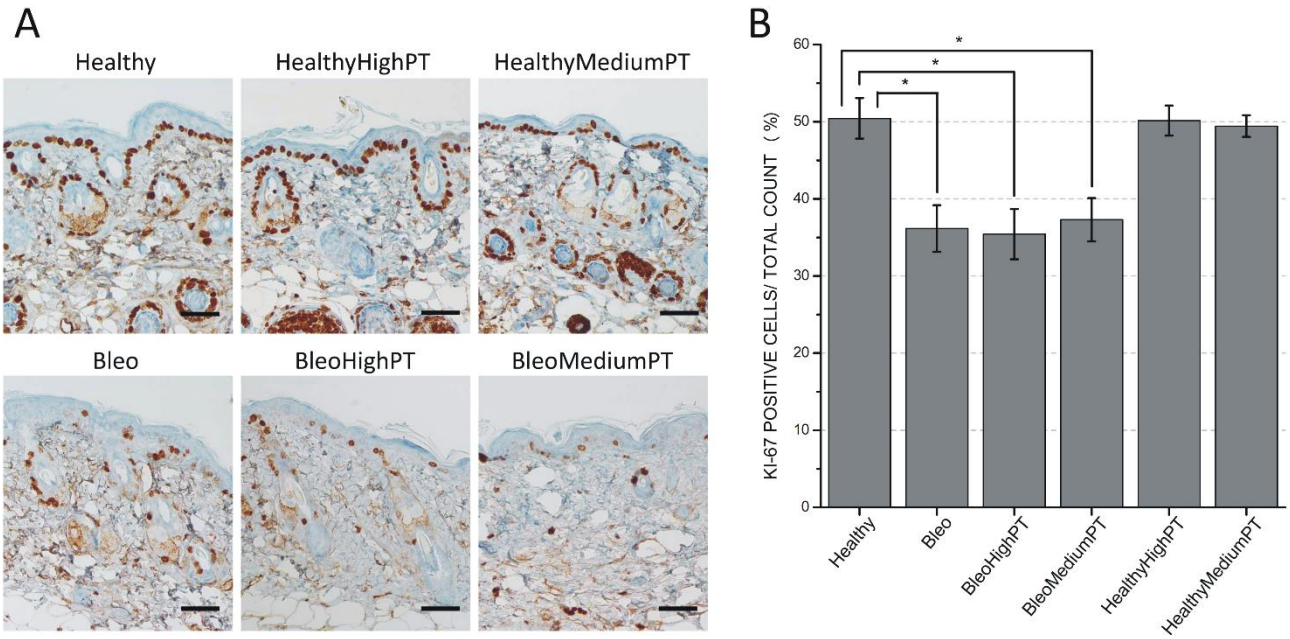

Fig. No. 8. Ki-67 immunohistochemistry (IHC). a) Representative images of nuclear expression of Ki-67 in all the groups of the experiment; IHC staining; x 20 magnification; scale bar – 50 µm; b) Percentage of Ki-67-positive cells to total cell population; x 20 magnification. The graph shows the mean (±SD) results obtained from the 7 mice of each group. *P ≤ 0.05; bleo – mice with bleomycin-induced scleroderma group; healthy – healthy mice control group; highPT – high-dose phototherapy; mediumPT – medium-dose phototherapy; SD – standard deviation

3.2. Narrowband UVA1 Phototherapy in the Established, Bleomycin-Induced Mouse Model of Scleroderma, when Bleomycin is Applied During the Whole Experiment

First, we assessed the dermal thickness differences in the healthy and mice with scleroderma groups. After bleomycin injections that spanned a period of 3 and 8 weeks, the dermal thicknesses were significantly (P = 0.002) higher (group II – 433.69 ± 54.37 µm and group V – 497.43 ± 57.83 µm, respectively) as compared to that of the healthy controls (group I – 166.04 ± 25.29 µm and group III – 178.18 ± 42.35 µm, respectively). The 3 weeks of bleomycin injections and the following 5 weeks of NaCl did not cause any spontaneous regression of dermal fibrosis (group IV – 443.87 ± 41.77 µm; group II – 433.69 ± 54.38 µm;

P = 0.482). Dermal thickness in mice injected with bleomycin for 8 weeks and irradiated with UVA1 for the last 5 weeks was significantly lower than that in mice challenged only with bleomycin for 8 weeks (group VI – 253.96 ± 31.83 µm and group V – 497.43 ± 57.83 µm, respectively; P = 0.002). Furthermore, treatment with 1200 J/cm^2 of UVA1 in parallel with profibrotic stimulus of bleomycin resulted in a lower dermal thickness as compared with pre-existing fibrotic changes in the group IV observed after 3 weeks of bleomycin injections (group VI – 253.96 ± 31.83 µm and group IV – 443.87 ± 41.77 µm; P = 0.002) (Fig. No. 9).

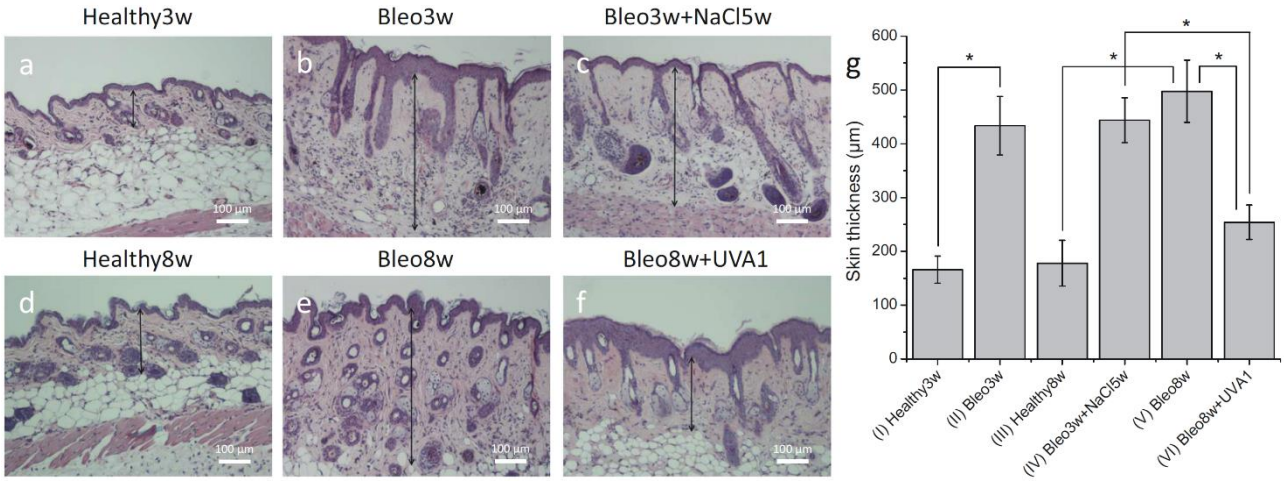

Fig. No. 9. Evaluation of dermal thickness. Left panel (a – f): representative images of H&E-stained sections of the skin; original magnification x 20; scale bar – 100 µm. The arrow indicates the length between epidermal-dermal and dermal-subcutaneous fat layers. a) Healthy 3w – mice injected with NaCl for 3 weeks; b) Bleo 3w – mice injected with bleomycin for 3 weeks; c) Bleo 3w + NaCl 5w – mice injected with bleomycin for 3 weeks and then with NaCl for 5 weeks; d) Healthy 8w – mice injected with NaCl for 8 weeks; e) Bleo 8w – mice injected with bleomycin for 8 weeks; f) Bleo 8w + UVA1 – mice injected with bleomycin for 8 weeks and in parallel treated with an average cumulative dose of 1200 J/cm^2 of UVA1 for the last 5 weeks (the treatment group). Right panel (g): quantification of the dermal thickness (µm). The graph shows the mean (±SD) results obtained from the 7 mice of each group. *P ≤ 0.05; SD – standard deviation; NaCl – sodium chloride; bleo – bleomycin; w – week; UVA1 – narrowband ultraviolet A1

To evaluate the impact of UVA1 on collagen metabolism in pre-established, bleomycin-induced dermal fibrosis, the expressions of MMP-1, MMP-3, collagen type I and collagen type III immunohistochemically in all the groups of the experiment were assessed.

After 3 and 8 weeks of bleomycin injections, the mice with scleroderma exhibited significantly lower levels of MMP-1 and MMP-3 than those assessed in the healthy control groups (both $P \leq 0.05$). The expressions of MMP-1 (15.31 ± 2.37%) and MMP-3 (23.58 ± 3.04%) in the skin of mice treated with bleomycin for 8 weeks and irradiated with UVA1 for the last 5 weeks were significantly ($P = 0.002$) higher as compared to the expressions in the skin of non-irradiated mice after bleomycin injections during 3 or 8 weeks (group IV: MMP-1 – 4.40 ± 1.26%; MMP-3 – 5.42 ± 1.34%; group V: MMP-1 – 2.66 ± 0.59%; MMP-3 – 4.55 ± 1.85%) (Fig. No. 10 and Fig. No. 11).

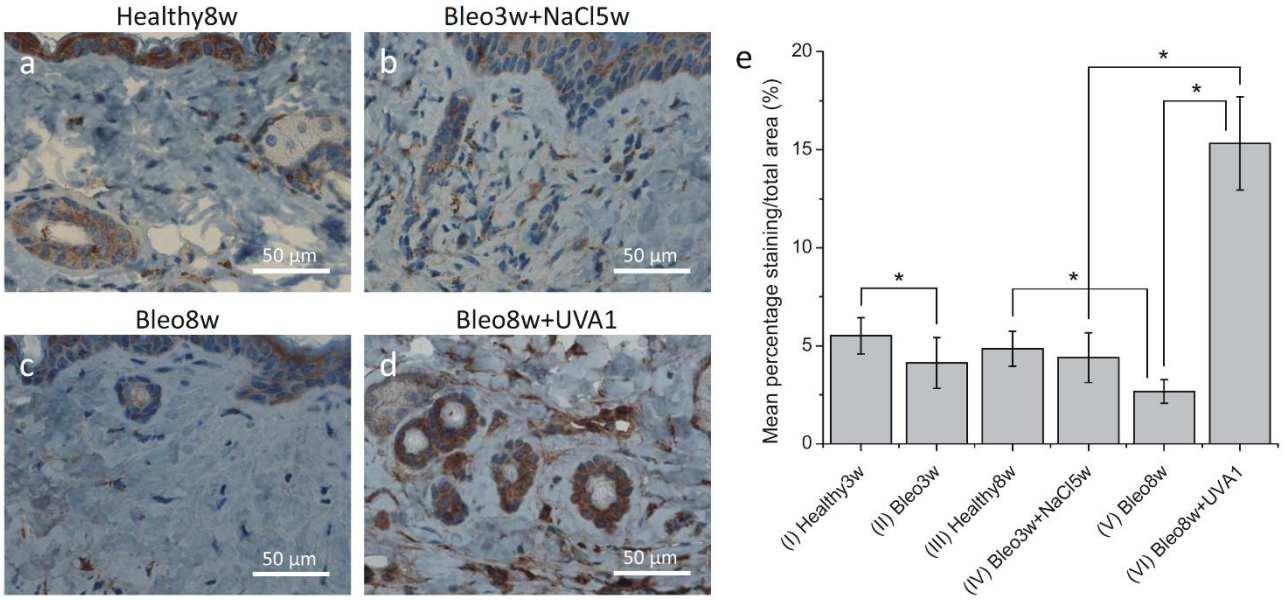

Fig. No. 10. Matrix-metalloproteinase-1 (MMP-1) immunohistochemistry (IHC). Left panel (a – d) – representative images of IHC staining; positive staining of MMP-1 appears as brown color; original magnification x 40; scale bar – 50 µm; a) Healthy 8w – mice injected with NaCl for 8 weeks; b) Bleo 3w + NaCl 5w – mice injected with bleomycin for 3 weeks and then with NaCl for 5 weeks; c) Bleo 8w – mice injected with bleomycin for 8 weeks; d) Bleo 8w + UVA1 – mice injected with bleomycin for 8 weeks and in parallel treated with an average cumulative dose of 1200 J/cm^2 of UVA1 for the last 5 weeks (the treatment group). Right panel (e) – The mean percentage staining of MMP-1/high-power field (%). The graph shows the mean (±SD) results of 4 fields obtained from the 7 mice of each group. *$P \leq 0.05$; SD – standard deviation; NaCl – sodium chloride; bleo – bleomycin; w – week; UVA1 – narrowband ultraviolet A1

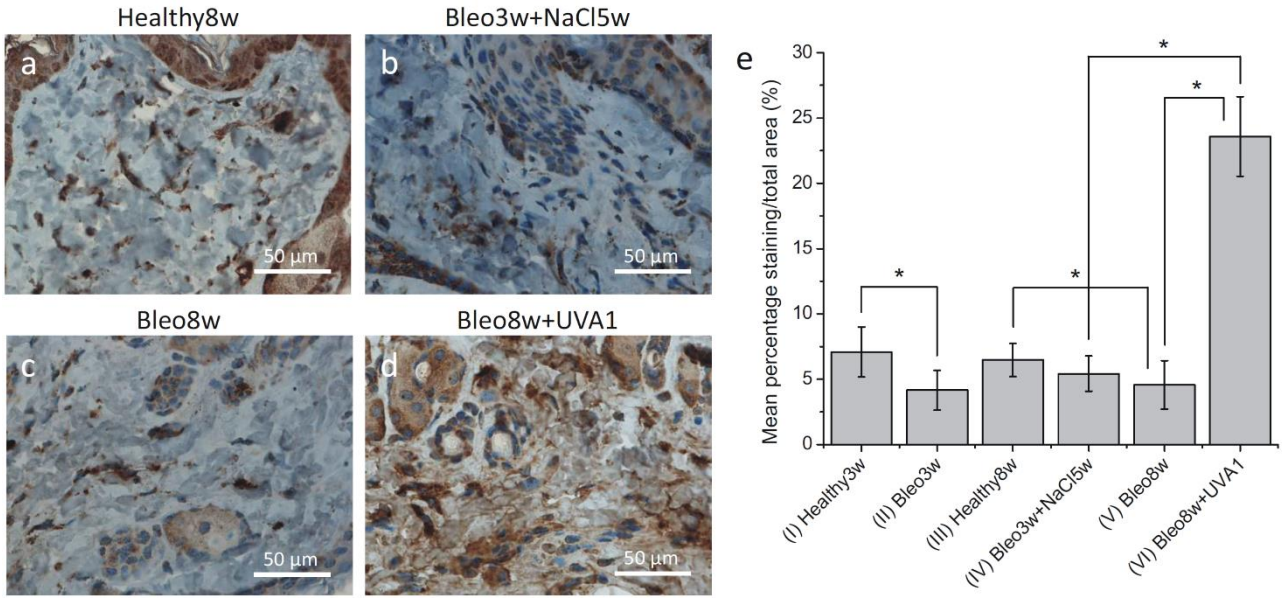

Fig. No. 11. Matrix-metalloproteinase-3 (MMP-3) immunohistochemistry (IHC). Left panel (a – d) – representative images of IHC staining; positive staining of MMP-1 appears as brown color; original magnification x 400; scale bar – 50 µm; a) Healthy 8w – mice injected with NaCl for 8 weeks; b) Bleo 3w + NaCl 5w – mice injected with bleomycin for 3 weeks and then with NaCl for 5 weeks; c) Bleo 8w – mice injected with bleomycin for 8 weeks; d) Bleo 8w + UVA1 – mice injected with bleomycin for 8 weeks and in parallel treated with an average cumulative dose of 1200 J/cm^2 of UVA1 for the last 5 weeks (the treatment group). Right panel (e) – The mean percentage staining of MMP-3/high-power field (%). The graph shows the mean (±SD) results of 4 fields obtained from the 7 mice of each group. *P ≤ 0.05; SD – standard deviation; NaCl – sodium chloride; bleo – bleomycin; w – week; UVA1 – narrowband ultraviolet A1

The immunohistochemical staining for collagen type I and type IIII demonstrated the immunopositivity of the proteins in all dermal layers (Fig. No. 12 and Fig. No. 13). The expressions of collagen type I (72.33 ± 3.48%) and type III (62.15 ± 8.37%) were significantly (P = 0.002) elevated in the skin of mice with scleroderma after 8 weeks of bleomycin injections (group V) as compared to those in the skin of healthy mice (group III: collagen type I and type III – 51.24 ± 7.91% and 39.95 ± 4.47%, respectively). The mean percentages of collagen type I (59.65 ± 5.33%) and type III (44.41 ± 6.27%) were significantly (P = 0.002) lower in the skin of mice with prolonged injections of bleomycin after UVA1 phototherapy

(group VI) as compared to that in the skin of non-irradiated mice with scleroderma (groups IV and V).

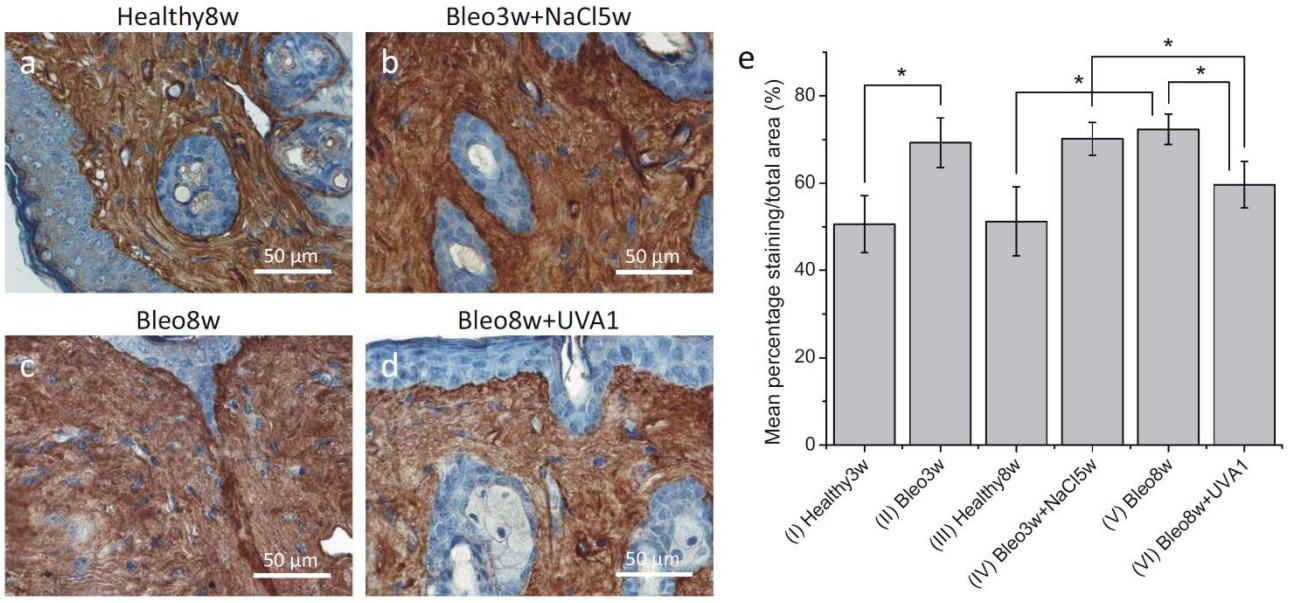

Fig. No. 12. Collagen type I immunohistochemistry (IHC). Left panel (a – d) – representative images of IHC staining; positive staining of collagen type I appears as brown color; original magnification x 40; scale bar – 50 µm; a) Healthy 8w – mice injected with NaCl for 8 weeks; b) Bleo 3w + NaCl 5w – mice injected with bleomycin for 3 weeks and then with NaCl for 5 weeks; c) Bleo 8w – mice injected with bleomycin for 8 weeks; d) Bleo 8w + UVA1 – mice injected with bleomycin for 8 weeks and in parallel treated with an average cumulative dose of 1200 J/cm^2 of UVA1 for the last 5 weeks (the treatment group). Right panel (e) – The mean percentage staining of collagen type I/high-power field (%). The graph shows the mean (±SD) results of 4 fields obtained from the 7 mice of each group. *P ≤ 0.05; SD – standard deviation; NaCl – sodium chloride; bleo – bleomycin; w – week; UVA1 – narrowband ultraviolet A1

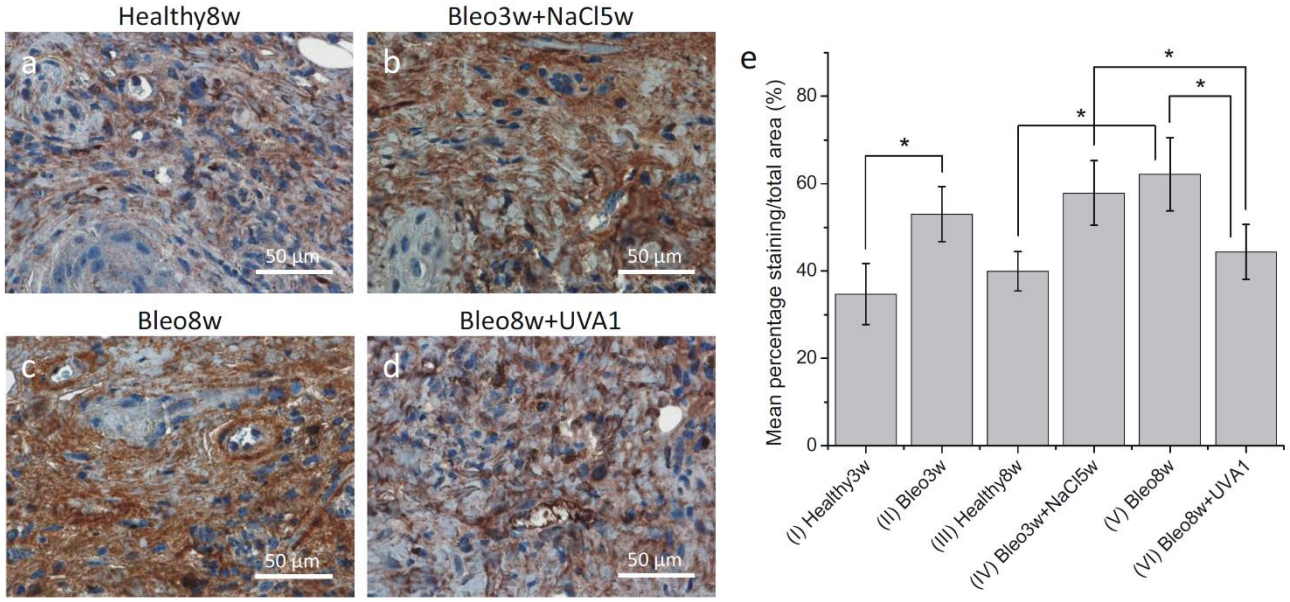

Fig. No. 13. Collagen type III immunohistochemistry (IHC). Left panel (a – d) – representative images of IHC staining; positive staining of collagen type III appears as brown color; original magnification x 40; scale bar – 50 µm; a) Healthy 8w – mice injected with NaCl for 8 weeks; b) Bleo 3w + NaCl 5w – mice injected with bleomycin for 3 weeks and then with NaCl for 5 weeks; c) Bleo 8w – mice injected with bleomycin for 8 weeks; d) Bleo 8w + UVA1 – mice injected with bleomycin for 8 weeks and in parallel treated with an average cumulative dose of 1200 J/cm^2 of UVA1 for the last 5 weeks (the treatment group). Right panel (e) – The mean percentage staining of collagen type III/high-power field (%). The graph shows the mean (±SD) results of 4 fields obtained from the 7 mice of each group. *P ≤ 0.05; SD – standard deviation; NaCl – sodium chloride; bleo – bleomycin; w – week; UVA1 – narrowband ultraviolet A1

4. DISCUSSION

The aim of the present study was to evaluate the efficacy and safety of narrowband (365 ± 5 nm) ultraviolet A1 (UVA1) for the dermal fibrosis treatment in the bleomycin-induced mouse model of scleroderma. A histological analysis was performed for the evaluation of the dermal thickness and mast cells density. The expressions active caspase-3, p53, Ki-67, MMP-1, MMP-3, collagen types I and III were assessed by immunohistochemical analyses.

In the first phase of the study we successfully induced an experimental model of systemic sclerosis in DBA/2 mice by repeated injections of bleomycin for 3 weeks. The phototherapy course with high and medium doses of narrowband UVA1 was started after the last injection of bleomycin. The histological analysis revealed that cumulative doses of 1200 J/cm^2 and 600 J/cm^2 of narrowband UVA1 effectively reduced the dermal thickness in a bleomycin-induced mouse model of scleroderma, and the impact was dose-dependent. Thus, dermal thickness was reduced by 54% in the high-dose and by 34 % in the medium-dose UVA1 groups after the phototherapy as compared to the skin thickness of the control groups without irradiation.

The impact of narrowband UVA1 on mast cells density was evaluated in the healthy and mice with scleroderma skin. Abundant dermal mast cells as well as its' degranulation in mice with scleroderma group indicate the important role of mast cells in the pathogenesis of systemic sclerosis by promoting both inflammation and fibrosis [19, 24]. We observed the decrease of dermal mast cells infiltration in mice with scleroderma after phototherapy. It proves the direct effect of narrowband UVA1 on the mast cells, which are one of the pathogenic factors in systemic sclerosis, especially in the early phase of the disease. The induction of apoptosis is confirmed by the increased expression of active caspase-3 in the deep dermal layers after UVA1 phototherapy. UVA1-induced apoptosis of inflammatory cells is considered as one of the mechanisms of action of phototherapy [25, 26]. On the contrary, a histological analysis of skin samples in UVA1-treated healthy mice did not show any changes in the count of mast cells. This phenomenon can be explained as the resistance of the normal mast cells to UVA1-induced apoptosis [27, 28].

The second objective of the study was to evaluate the safety of narrowband UVA1 phototherapy for the dermal fibrosis treatment in the bleomycin-induced scleroderma model. As described by Khodaeiani et al., the high prevalence of the p53 protein and Ki-67 antigen immunoreactivity in malignant skin lesions support their pathogenic role in the cutaneous carcinogenesis [29]. Our results did not show any significant increase of neither p53 nor Ki-67 proteins after medium and high doses of UVA1 phototherapy as compared with the groups of non-treated mice. However, it would be reasonable to exclude the carcinogenic risk in future research by using a gene expression analysis.

Patients with SSc exhibit a long-standing, established dermal fibrosis with ongoing pathogenic mechanisms. Because of this, effective antifibrotic treatment should target both the degradation of excessive extracellular matrix components and stop any further accumulation of collagen. Thus, the objective of the second phase of the study was to evaluate the impact of high-dose of 365 ± 5 nm UVA1 on the dermal fibrosis in the established, modified model of scleroderma. So, we initiated the phototherapy procedures after 3 weeks of bleomycin injections and continued the irradiation every second day for 5 weeks maintaining a prolonged administration of bleomycin. The cumulative doses of 1200 J/cm^2 of UVA1 diminished the dermal thickness by 49 % and 43 % in comparison with the control groups, which were challenged only with bleomycin for 8 and 3 weeks, respectively. After the phototherapy course, dermal thickness was relatively lower than the pre-existing fibrotic changes.

An excessive accumulation of extracellular matrix is a consequence of imbalance between the activity of MMP-1, MMP-3 and the synthesis of collagen types I and III. An augmented deposition of collagens in the skin and internal organs leads to the development of progressive fibrosis which is extremely dangerous for the maintenance of normal functions [30]. Besides a broad range of the other substrates, MMP-1 (collagenase-1) degrades the extracellular fibers comprised from types I and III collagen, while MMP-3 (stromelysin-1) is responsible for collagen type III degradation [31, 32]. Hence, the reduced activity of matrix-metalloproteinases results in a lesser extent of matrix breakdown and plays a role in SSs pathogenesis. In our study, the high-dose of UVA1 induced the 5.8- and 5.2-fold increase in

MMP-1 and MMP-3 expressions, whereas after phototherapy the expressions of type I and type III collagen decreased by 1.2- and 1.4-fold in the pre-established, bleomycin-induced scleroderma model as compared to that in non-irradiated mice. Reduced collagen types I and III expressions after UVA1 therapy might be associated with the induction of a higher activity of collagen-degrading enzymes, or else, with an impaired *de novo* synthesis of collagen [33]. After narrowband UVA1 phototherapy, the expressions of collagen type I and III were relatively lower than the levels before the irradiation despite the prolonged injections of bleomycin. Overall, the cumulative doses of 1200 J/cm^2 of 365 ± 5 nm UVA1 not only prevented the progression of fibrosis, but also induced the regression of existing fibrotic changes in the established, bleomycin induced animal model of fibrosis, when bleomycin was applied during the whole experiment.

5. CONCLUSIONS

1. The cumulative doses of 1200 J/cm^2 and 600 J/cm^2 of narrowband UVA1 significantly diminish the dermal thickness in the established, bleomycin-induced animal model of fibrosis.
2. The cumulative doses of 1200 J/cm^2 and 600 J/cm^2 of 365 ± 5 nm UVA1 reduce the mast cells infiltration and expression of active caspase-3 in scleroderma skin using the established, bleomycin-induced animal model of fibrosis.
3. 365 ± 5 nm UVA1 phototherapy with cumulative doses of 1200 J/cm^2 and 600 J/cm^2 does not cause any increased expression of p53 and Ki-67 proteins in the scleroderma skin using a bleomycin-induced animal model of fibrosis.
4. The narrowband UVA1 phototherapy with cumulative doses of 1200 J/cm^2 and 600 J/cm^2 reduce the accumulation of collagen types I and III as well as increase the expression of matrix metalloproteinase-1 and matrix metalloproteinase-3 in the established, bleomycin-induced animal model of fibrosis, when bleomycin is applied during the whole experiment.
5. The cumulative doses of 1200 J/cm^2 of narrowband UVA1 not only stop the progression of fibrosis, but also induce the regression of existing fibrotic changes in

the established, bleomycin-induced animal model of fibrosis when bleomycin is applied during the whole experiment.

6. SANTRAUKA (SUMMARY IN LITHUANIAN)

Įvadas

Sisteminė sklerozė (SS) – viena iš sunkiausių ir fatališkiausių autoimuninių reumatinių ligų, nuo kurios dažniausiai nukenčia gana jauni, darbingo amžiaus žmonės [1]. Nepaisant sisteminio gydymo, pacientų kasdieninės veiklos funkcijos išlieka apribotos dėl galūnių, ypač rankų, progresuojančio odos pažeidimo. Ankstyvas odos fibrozės gydymas yra būtinas ne tik dėl paciento diskomforto pojūčio, bet ir dėl vėlyvų komplikacijų: judesių amplitudės sumažėjimo, kontraktūrų, negyjančių žaizdų bei kalcinatų susidarymo. Todėl ne tik sisteminis, bet ir lokalus gydymas turi būti nepavėluotas ir kompleksinis. Tačiau neinvazinio, nemedikamentinio gydymo galimybės stabdyti odos fibrozės progresavimą yra ribotos.

Per paskutinius du dešimtmečius fototerapija ultravioletiniais spinduliais (UV) tapo viena iš odos ligų, tokių kaip atopinis dermatitas, psoriazė, dilgėlinė, niežulys, neinvazinio gydymo galimybių [3, 4]. Fototerapijos vieta gydant SS nėra aiškiai apibrėžta. Tyrimais parodyta, kad ultravioletiniai A1 (UVA1) 340–400 nm bangos ilgio spinduliai pasižymi uždegimą slopinančiu ir imunomoduliaciniu poveikiu [4]. Su lokalia skleroderma ar SS sergančiais pacientais atlikta keliolika studijų, iš kurių tik trys kontrolinės, ir jose įvertintas UVA1 klinikinis poveikis [6-8]. Pateiktose studijose buvo naudojamas platus UVA1 spinduliuotės spektras (340–400 nm), kartais apimantis tiek ultravioletinę A2 (bangos ilgis < 340 nm), tiek regimosios spinduliuotės sritis (bangos ilgis > 400 nm).

Plati spektrinė UVA1 sritis reaguoja su daugeliu chromoforų odoje, taip sukeldama skirtingus imuninius atsakus [13]. Tad plataus UVA1 spinduliuotės spektro naudojimas gali būti susijęs su didesne šalutinių reakcijų, tokių kaip eritema ar piktybinių navikų išsivystymas, rizika, silpnesniu terapiniu poveikiu ir mažesne spinduliuotės skvarba į audinius. Šio tyrimo metu buvo iškelta hipotezė, kad siauro spektro UVA1 spinduliuotė gali būti veiksminga ir saugi fototerapijos rūšis odos fibrozei gydyti. Įvertinus fizikines UVA1 ir optines odos savybes, siaurajuostės 365 ± 5 nm UVA1 spinduliuotės fotonai, pasiekdami tikrosios odos sluoksnius, tiesiogiai ir selektyviai veiktų kolageno ir elastino sankaupas su mažesniu šalutinių reakcijų

pasireiškimo dažniu. Iki šiol nėra atlikta tyrimų, kuriais būtų įvertintas siaurajuostės UVA1 spinduliuotės poveikis odos fibrozei.

Tyrimai su gyvūnais leidžia įvertinti naujo gydymo metodo veiksmingumą ir saugumą bei gyvo organizmo atsaką į fototerapiją [15]. Šiems tikslams pasirinktas eksperimentinis bleomicinu sukeltas SS modelis, naudojant DBA/2 linijos peles. Moksliniame darbe buvo tirtas 365 ± 5 nm UVA1 antifibrozinis efektyvumas, fototerapijos įtaka fibrozinių pokyčių regresijai ir fibrozės progresavimui bei karcinogenezės išsivystymui bleomicinu sukeltuose SS pelių modeliuose.

Mokslinio darbo tikslas

Ištirti siauro spektro (365 ± 5 nm) ultravioletinės A1 (UVA1) spinduliuotės poveikį fibrozės procesui ir saugumą sisteminės sklerozės (SS) eksperimentiniuose gyvūnų modeliuose.

Mokslinio darbo uždaviniai

1. Įvertinti siaurajuostės UVA1 spinduliuotės didelių ir vidutinių dozių poveikį sklerodermines odos storiui, putliųjų ląstelių infiltracijai ir aktyvios kaspazės-3 raiškai, taikant bleomicinu sukeltą susiformavusios fibrozės gyvūnų modelį.
2. Ištirti siaurajuostės UVA1 spinduliuotės didelių ir vidutinių dozių poveikį karcinogenezės išsivystymui, naudojant bleomicinu sukeltą susiformavusios fibrozės gyvūnų modelį.
3. Įvertinti siaurajuostės UVA1 spinduliuotės didelių dozių poveikį fibrozinių pokyčių išsivystymui ir fibrozės regresijai, taikant bleomicinu sukeltą susiformavusios fibrozės gyvūnų modelį, kai bleomicinas skiriamas visą eksperimento laikotarpį.

Mokslinio darbo naujumas

- Šiame moksliniame darbe pirmą kartą buvo įrodyta, kad siauro spektro UVA1 (365 ± 5 nm) spinduliuotė ne tik sustabdo odos fibrozės progresavimą, bet ir sukelia jau

buvusių fibrozinių pokyčių regresiją bleomicinu sukeltame susiformavusios fibrozės gyvūnų modelyje.

- Gauti rezultatai rodo, kad siaurajuostės UVA1 didelių ir vidutinių (atitinkamai suminės 1 200 J/cm^2 ir 600 J/cm^2) dozių fototerapija nesukelia padidėjusios karcinogenezės rizikos sisteminės sklerozės gyvūnų modelyje.
- Sukurtas specialiosios paskirties medicinos prietaisas „IMC-Light“, kuris bus naudojamas klinikiniame sistemine skleroze sergančių pacientų tyrime (prietaisas yra įregistruotas Europos medicininių prietaisų duomenų banke).

Ginamieji teiginiai

1. Siaurajuostė UVA1 fototerapija sumažina sklerodermines odos storį, sustabdo fibrozės progresavimą ir sukelia fibrozinių pokyčių regresiją bleomicinu sukeltame susiformavusios fibrozės gyvūnų modelyje.
2. Siaurajuostė UVA1 spinduliuotė nesukelia padidėjusios karcinogenezės rizikos bleomicinu sukeltame SS gyvūnų modelyje.

Tyrimo medžiaga ir metodai

Gyvūnai

Gavus Valstybinės maisto ir veterinarijos tarnybos (VMVT) leidimus atlikti bandymus su gyvūnais (Nr. G2-15 ir G2-42), eksperimentams buvo pasirinktos 84-ios DBA/2 linijos 6–8 savaičių pelių patelės. DBA/2 linijos pelės buvo pasirinktos dėl didelio jautrumo bleomicinu (BLM) sukeltam fibrozės modeliui [34]. Viso eksperimento metu gyvūnai buvo laikomi numatyta tvarka, remiantis Europos Sąjungos direktyva ir kitais norminiais bandomųjų gyvūnų naudojimo eksperimentams aktais.

Eksperimentinis pelių sisteminės sklerozės modelis

Odos fibrozė buvo sukelta vietinėmis BLM (bleomicino sulfatas iš *Streptomyces verticillus*, vaistinė forma – milteliai; gamintojas – *Sigma-Aldrich Chemie GmbH*, Steinheim, Vokietija) injekcijomis į apibrėžtą 1 cm^2 pelių nugaros plotą. Iš pradžių nuo pažymėto ploto depiliaciniu kremu (Veet®, Anglija) buvo pašalinti plaukai. BLM tirpintas 0,9 % natrio chlorido (NaCl)

tirpale ir injekcijoms naudotas 0,5 mg/ml koncentracijos BLM [16]. Poodinės 100 µl BLM injekcijos buvo leistos į penkis taškus pažymėtoje nugaros srityje kas antrą dieną (5 pav). Injekcijų kurso trukmė – 3 arba 8 savaitės. Kontrolinių grupių gyvūnams skirtos poodinės 100 µl 0,9 % NaCl injekcijos.

Fototerapija

Bendradarbiaujant su Vilniaus universiteto Fizikos fakulteto Kvantinės elektronikos katedros mokslininkais, Valstybiniame mokslinių tyrimų institute Inovatyvios medicinos centre sukurtas specialiosios paskirties medicinos prietaisas „IMC-Light“. Fototerapijos prietaisas sudarytas iš 20 šviesos diodų (angl. *Light emitting diode* – LED), skleidžiančių UVA1 spinduliuotę, kurios bangos ilgis – 365 ± 5 nm, o galios tankis – 21 mW/cm^2. Fototerapijos kursas – 15 procedūrų (3 kartai per savaitę, trukmė – 5 savaitės). Vienos procedūros metu skiriama vidutinė – 40 J/cm^2 arba didelė – 80 J/cm^2 UVA1 dozė (suminės dozės – 600 J/cm^2 arba 1 200 J/cm^2).

Fototerapijos kurso schema bleomicinu sukeltos susiformavusios fibrozės pelių modelyje

DBA/2 linijos pelės suskirstytos į 6 grupes (po 7 gyvūnus kiekvienoje):

- I grupė (Sveikos) – sveikų pelių grupė;
- II grupė (BLM) – pelės, kurioms BLM sukelta sisteminė sklerozė;
- III grupė (BLM+1200UVA1) – pelės, kurioms BLM sukelta sisteminė sklerozė, gydytos didelėmis, 1 200 J/cm^2, UVA1 dozėmis;
- IV grupė (BLM+600UVA1) – pelės, kurioms BLM sukelta sisteminė sklerozė, gydytos vidutinėmis, 600 J/cm^2, UVA1 dozėmis;
- V grupė (Sveikos+1200UVA1) – sveikos pelės, gydytos didelėmis, 1 200 J/cm^2, UVA1 dozėmis;
- VI grupė (Sveikos+600UVA1) – sveikos pelės, gydytos vidutinėmis, 600 J/cm^2, UVA1 dozėmis.

Fototerapijos kurso schema bleomicinu sukeltos susiformavusios fibrozės pelių modelyje, kai bleomicinas skiriamas visą eksperimento laikotarpį

DBA/2 linijos pelės suskirstytos į 6 grupes (po 7 gyvūnus kiekvienoje):

- I grupė (Sveikos_3sav) – trijų savaičių sveikų pelių kontrolinė grupė;
- II grupė (BLM_3sav) – pelių, kurioms BLM sukelta sisteminė sklerozė, grupė, kai bleomicinas buvo skirtas tris savaites;
- III grupė (Sveikos_8sav) – aštuonių savaičių sveikų pelių kontrolinė grupė;
- IV grupė (BLM_3sav+NaCl_5sav) – pelės, kurioms BLM sukelta sisteminė sklerozė, kai BLM buvo leidžiamas tris savaites, paskui kitas penkias savaites – natrio chlorido tirpalas;
- V grupė (BLM_8 sav) – aštuonių savaičių pelių, kurioms BLM sukelta sisteminė sklerozė, grupė, kai bleomicinas buvo skirtas aštuonias savaites;
- VI grupė (BLM_8sav+1200UVA1) – pelės su BLM sukelta sisteminė skleroze, kurios paskutines penkias eksperimento savaites buvo gydytos didelėmis, 1200 J/cm^2, siaurajuostės UVA1 dozėmis, kartu veikiant BLM visas aštuonias savaites.

Histologinė ir imunohistocheminė analizė

- Tyrimai bleomicinu sukeltos susiformavusios fibrozės modelyje:

- Histologinė odos storio ir putliųjų ląstelių infiltracijos analizė.
- Imunohistocheminė aktyvios kaspazės-3 raiškos analizė (apoptozės įvertinimas).
- Imunohistocheminė p53 ir Ki-67 baltymų raiškos analizė (karcinogenezės rizikos įvertinimas).

- Tyrimai bleomicinu sukeltos susiformavusios fibrozės modelyje, kai bleomicinas skiriamas visą eksperimento laikotarpį:

- Histologinė odos storio analizė.
- Kolageną skaidančių fermentų (MMP-1 ir MMP-3) ir kolageno I ir III tipų sankaupų imunohistocheminė analizė.

Statistinė analizė

Rezultatų statistinė analizė buvo atlikta naudojant SPSS v. 16.0 programą. Duomenys išreikšti kaip vidurkis ± standartinis nuokrypis (SD). Tolydiesiems kintamiesiems (odos storis, putliųjų ląstelių infiltracija, kaspazės-3, p53 baltymo, Ki-67 baltymo, MMP-1, MMP-3, I ir III tipų kolageno raiškos) palyginti naudotas Mano ir Vitnio testas. Duomenys laikyti statistiškai reikšmingais, kai $P \leq 0,05$.

Rezultatų aptarimas

Šie tyrimai buvo atlikti tikintis netolimoje ateityje siaurajuostę UVA1 pritaikyti sistemine skleroze sergantiems pacientams gydyti. Siekiant įvertinti, ar šis gydymo metodas yra veiksmingas ir saugus, šiame moksliniame darbe buvo tirtas siaurajuostės UVA1 (365 ± 5 nm) spinduliuotės poveikis bleomicinu sukeltos susiformavusios fibrozės ir bleomicinu sukeltos susiformavusios fibrozės, kai bleomicinas skiriamas visą eksperimento laikotarpį, gyvūnų modeliuose. Atlikus histologinius ir imunohistocheminius tyrimus, buvo įvertintas siaurajuostės UVA1 poveikis odos storiui, putliųjų ląstelių infiltracijai, aktyvios kaspazės-3, p53 ir Ki-67 baltymų raiškai, kolageno I ir III tipo kiekiui bei juos skaidančių fermentų MMP-1 ir MMP-3 raiškoms.

Pirmame tyrimo etape DBA/2 linijos pelėms poodinėmis bleomicino injekcijomis buvo sukelta sisteminė sklerozė. Paskui gyvūnams buvo skirtas penkių savaičių siaurajuostės UVA1 fototerapijos kursas, sukaupiant dideles (suminė dozė – 1 200 J/cm^2) arba vidutines (suminė dozė – 600 J/cm^2) spinduliuotės dozes. Nustatyta, kad didelių ir vidutinių dozių UVA1 fototerapijos kursas reikšmingai sumažina sklerodermines odos storį, o antifibrozinis efektas yra tiesiogiai priklausomas nuo dozės. Histologinės analizės rezultatai parodė, kad 365 ± 5 nm UVA1 neturi įtakos sveikos odos storiui ir struktūrai.

Siekiant įvertinti fototerapijos veikimo mechanizmo grandis, buvo tirtas UVA1 poveikis uždegiminių ląstelių infiltracijai. Yra žinoma, kad putliosios ląstelės dalyvauja sisteminės sklerozės patogenezėje ir yra vienos iš fibrogenezę skatinančių faktorių [19]. Po didelių ir vidutinių UVA1 fototerapijos dozių buvo stebėtas sumažėjęs putliųjų ląstelių sankaupų kiekis sklerodermineje pelių odoje. Tad 365 ± 5 nm UVA1 sukėlė patogenetinių

uždegiminių ląstelių apoptozę, tai patvirtina ir padidėjęs aktyvios kaspazės-3 raiškos kiekis po fototerapijos kurso pelių, kurioms buvo skleroderma, odoje. Svarbu paminėti, kad siaurajuostė UVA1 spinduliuotė neturėjo įtakos sveikų pelių odos ląstelių kiekiui ir jų žūčiai. Skleroderminiai fibroblastai, esantys giliuosiuose odos sluoksniuose yra atsparūs apoptozei, dėl to išlieka aktyvūs ir toliau gausiai gamina tarpląstelinio matrikso komponentus [20]. Aktyvios kaspazės-3 raiškos nustatymas giliuosiuose odos sluoksniuose yra čia vykstančios apoptozės žymuo [35]. Tai leidžia daryti prielaidą, kad suplonėjęs odos storis gali būti susijęs su sumažėjusiu putliųjų ląstelių, fibroblastų skaičiumi ar pačių ląstelių aktyvumu dėl siaurajuostės UVA1 sąlygotos apoptozės. Tačiau šių tyrimų duomenimis dermos sluoksnyje tam tikros rūšies ląstelių apoptozė po UVA1 fototerapijos negali būti paaiškinta, todėl reikia tolesnių tyrimų *in vitro,* pavyzdžiui, naudojant skleroderminius fibroblastus.

Siekiant nustatyti, ar siaurajuostė UVA1 fototerapija nesukelia padidėjusios piktybinių procesų rizikos, buvo įvertinta karcinogenezę skatinančių p53 ir Ki-67 baltymų raiška sveikoje ir skleroderminėje gyvūnų odoje. Imunohistocheminės analizės duomenys parodė, kad didelių ir vidutinių dozių 365 ± 5 nm UVA1 fototerapija nesukėlė reikšmingai padidėjusios p53 ir Ki-67 baltymų raiškos nei sveikų, nei pelių, kurioms buvo skleroderma, grupių odoje. Be abejo, norint visiškai atmesti UVA1 sąlygojamą karcinogenezę, imunohistocheminės reakcijos rezultatams patvirtinti būtų tikslinga atlikti genų ekspresijos analizę [29].

Kadangi pacientams, sergantiems sistemine skleroze, būdingi įsisenėję fibroziniai pokyčiai su besitęsiančiu fibrozę skatinančiu patogenetiniu mechanizmu, veiksmingas antifibrozinis gydymas turėtų ne tik skatinti kolageno degradaciją, bet ir sustabdyti tolesnį tarpląstelinio užpildo kaupimąsi. Tad antrame mokslinio darbo etape buvo atlikti tyrimai, siekiant įvertinti siaurajuostės UVA1 didelių dozių poveikį susiformavusiai fibrozei, naudojant bleomicinu sukeltą susiformavusios fibrozės modelį, tęsiant bleomicino injekcijas. Histologinės analizės duomenys parodė, kad po didelių dozių 365 ± 5 nm UVA1 fototerapijos, kartu skiriant bleomiciną visą eksperimento laikotarpį, pelių su skleroderma oda suplonėjo net labiau nei prieš gydymą buvę fibroziniai pokyčiai.

Fibrozės patogenezėje svarbiausios proteazės yra MMP-1 (kolagenazė-1) ir MMP-3 (stromelizinas-1), kurių susilpnėjęs aktyvumas ir sumažėjęs kiekis lemia padidėjusį I ir III tipo kolageno kaupimąsi [31, 32]. Siekiant įvertinti siaurajuostės UVA1 poveikį kolageno metabolizmui, buvo atlikti tyrimai su pirminiais antikūnais prieš MMP-1, MMP-3, kolageno I ir III tipus. Nustatyta, kad pelių, veiktų bleomicinu aštuonias savaites ir paskutines penkias eksperimento savaites gydytų didelėmis siaurajuostės UVA1 spinduliuotės dozėmis, odoje buvo reikšmingai padidėjusi MMP-1 ir MMP-3 raiška, palyginti su grupe pelių, kurioms skirtas tik bleomicinas visas aštuonias savaites. Gauti rezultatai taip pat patvirtino, kad suminės 1 200 J/cm^2 UVA1 fototerapijos dozės statistiškai reikšmingai sumažina kolageno I bei III tipo kiekius sklerodermineje odoje, nepaisant bleomicino skyrimo per visą eksperimento laikotarpį. Imunohistocheminės analizės duomenys atspindi, kad tiek padidėjusi kolageną skaidančių fermentų raiška, tiek susilpnėjusi kolageno sintezė lemia kolageno sankaupų sumažėjimą po fototerapijos.

Šiame moksliniame darbe buvo patvirtinti ginamieji teiginiai, kad siaurajuostė 365 ± 5 nm UVA1 fototerapija sumažina sklerodermines odos storį, sustabdo fibrozės progresavimą ir sukelia fibrozinių pokyčių regresiją bleomicinu sukeltame susiformavusios fibrozės gyvūnų modelyje, nesukeliant padidėjusios karcinogenezės rizikos.

Išvados

1. Suminės 1 200 J/cm² ir 600 J/cm² siaurajuostės UVA1 spinduliuotės dozės statistiškai reikšmingai sumažina odos storį bleomicinu sukeltame susiformavusios fibrozės gyvūnų modelyje.
2. 365 ± 5 nm UVA1 fototerapija didelėmis (1 200 J/cm^2) ir vidutinėmis (600 J/cm^2) dozėmis sumažina putliųjų ląstelių infiltraciją ir padidina aktyvios kaspazės-3 raišką sklerodermineje odoje, naudojant bleomicinu sukeltą susiformavusios fibrozės gyvūnų modelį.
3. Taikant 365 ± 5 nm UVA1 fototerapiją suminėmis 1200 J/cm^2 ir 600 J/cm^2 dozėmis neaptikta karcinogenezę lemiančių p53 ir Ki-67 baltymų raiškos gyvūnų, kuriems bleomicinu sukelta SS, odoje.

4. 365 ± 5 nm UVA1 fototerapija suminėmis 1 200 J/cm^2 dozėmis sumažina I ir III tipų kolageno sankaupas ir padidina matrikso metaloproteinazės-1 ir matrikso metaloproteinazės-3 raišką gyvūnų odoje, naudojant bleomicinu sukeltą susiformavusios fibrozės modelį, kai bleomicinas skiriamas visą eksperimento laikotarpį.
5. Suminės 1 200 J/cm^2 siaurajuostės UVA1 fototerapijos dozės ne tik sustabdo fibrozės progresavimą, bet ir sukelia jau buvusių fibrozinių pokyčių regresiją bleomicinu sukeltos susiformavusios fibrozės gyvūnų modelyje, bleomiciną skiriant visą eksperimento laikotarpį.

Praktinė nauda

Pasaulyje autoimuninėms ligoms gydyti kasmet yra patvirtinami nauji veiksmingi gydymo metodai ar medikamentai, tačiau SS gydymas kol kas yra neišspręstas uždavinys tiek klinicistams, tiek mokslininkams. Pacientų, sergančių SS, sisteminio ar lokalaus gydymo galimybės yra ribotos. Šio mokslinio darbo rezultatai parodė, kad 1 200 J/cm² ir 600 J/cm² siaurajuostės UVA1 spinduliuotės dozės statistiškai reikšmingai sumažina odos storį bleomicinu sukeltos susiformavusios fibrozės modelyje, nesukeliant padidėjusios karcinogenezę lemiančių baltymų raiškos. Be to, didelių dozių, 1 200 J/cm^2, 365 ± 5 nm UVA1 fototerapija ne tik sustabdo fibrozės progresavimą, bet ir sukelia jau buvusių fibrozinių pokyčių regresiją susiformavusios fibrozės modelyje, kai bleomicinas skiriamas visą eksperimento laikotarpį. Tad ši fototerapijos rūšis gali būti viena iš neinvazinio gydymo galimybių odos fibrozei mažinti.

Netolimoje ateityje planuojama atlikti klinikinį sistemine skleroze sergančių pacientų tyrimą, siekiant gydyti odos fibrozę, taikant 365 ± 5 nm UVA1 spinduliuotę. Valstybinė akreditavimo sveikatos priežiūros veiklai tarnyba prie Sveikatos apsaugos ministerijos suteikė leidimą naudoti specialiosios paskirties medicinos prietaisą „IMC-Light“ klinikiniame tyrime, vyksiančiame Vilniaus universiteto ligoninės Santaros klinikose. Akreditacijos duomenys ir suteiktas leidimas (D2-11748 - (5.19) (Nr. S8-5)) yra įregistruoti Europos medicininių

prietaisų duomenų banke EUDAMED; klinikinio tyrimo identifikacijos Nr. (CIV-ID): CIV-15 - 10-013898.

Šio neinvazinio metodo pritaikymas kasdieninėje klinikinėje praktikoje būtų svarbus žingsnis sprendžiant sudėtingas SS gydymo problemas. O dėl savo gerų techninių parametrų ir saugumo, siaurajuostės UVA1 spinduliuotės prietaisas gali pakeisti ir plataus UVA1 spektro prietaisus, skirtus atopiniam dermatitui, psoriazei ir kitoms odos ligoms gydyti.

7. LIST OF PUBLICATIONS

Publications listed in the Thomson Reuters Web of Science database:

D. Karpec, R. Rudys, L. Leonaviciene, Z. Mackiewicz, R. Bradunaite, G. Kirdaite, A. Venalis. The impact of high-dose narrowband ultraviolet A1 on dermal thickness, collagen and matrix-metalloproteinases in animal model of scleroderma. Journal of Photochemistry and Photobiology B: Biology. 2017, 173:448-455.

Accepted:

D. Karpec, R. Rudys, L. Leonaviciene, Z. Mackiewicz, R. Bradunaite, G. Kirdaite, A. Venalis. The safety and efficacy of light emitting diodes-based ultraviolet A1 phototherapy in bleomycin-induced scleroderma in mice. Advances in Medical Science. 2018, 63.

Other peer-reviewed publications:

1. D. Karpec, L. Leonavičienė, R. Bradūnaitė, A. Venalis, G. Kirdaitė. Animal Models Of Scleroderma And Their Application Facilities. Laboratory Medicine. 2014, t16, Nr. 4, p. 187 – 193.
2. D. Karpec, R. Rudys, L. Leonavičienė, Z. Mackiewicz, R. Bradūnaitė, G. Kirdaitė, A. Venalis. The impact of narrowband ultraviolet A1 on active caspase-3 expression in scleroderma animal model. Laboratory Medicine. 2017, t19, Nr. 3(75), p. 151 – 157.

Presentations in international conferences:

1. D. Karpec, R. Rudys, Z. Mackiewicz, L. Leonaviciene, R. Bradunaite, R. Rugiene, G. Kirdaite, A. Venalis. Narrowband 365 nm ultraviolet A1 light therapy in bleomycin induced scleroderma animal model. 4-th Systemic Sclerosis Congress, 18 – 20 February, 2016; Lisbon, Portugal.
2. D.Karpec, R.Rudys, L.Leonaviciene, Z.Mackiewicz, R.Bradunaite, G. Kirdaite, R.Rugiene, A.Venalis. The effect of narrowband ultraviolet A1 light on bleomycin-induced mouse model of scleroderma. American College of Rheumatology, 12 – 16 November, 2016; Washington DC, United States.
3. D. Karpec, R. Rudys, Z. Mackiewicz, L. Leonaviciene, R. Bradunaite, R. Rugiene, G. Kirdaite, A. Venalis. The impact of narrowband ultraviolet A1 on proliferation and apoptosis markers in animal model of scleroderma. European League Against Rheumatism annual congress, 14 – 18 June, 2017; Madrid, Spain.
4. D. Karpec, R. Rudys, Z. Mackiewicz, L. Leonaviciene, R. Bradunaite, R. Rugiene, G. Kirdaite, A. Venalis. The impact of high-dose narrowband ultraviolet A1 on dermal thickness in pre-established, bleomycin-induced mouse model of scleroderma. Baltic Rheumatology Conference, 21 – 22 September, 2017; Vilnius, Lithuania.
5. D. Karpec, R. Rudys, Z. Mackiewicz, L. Leonaviciene, R. Bradunaite, R. Rugiene, G. Kirdaite, A. Venalis. The impact of high-dose narrowband ultraviolet A1 on dermal fibrosis in pre-established, bleomycin-induced mouse model of scleroderma. 19th Asia Pacific League of Associations for Rheumatology Congress, 16 – 20 October, 2017; Dubai, United Arab Emirates.

Scholarships

1. Laureate of the Resarch Council of Lithuania scholarship (2017).
2. Laureate of the World Federation of Scientists National Scholarship Program (2017).

8. REFERENCES

1. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007; 117(3): 557-67.
2. Katsumoto TR, Whitfield ML, Connolly MK. The pathogenesis of systemic sclerosis. Annu Rev Pathol 2011; 6: 509-37.
3. Kerr AC, Ferguson J, Attili SK, Beattie PE, Coleman AJ, Dawe RS *et al*. Ultraviolet A1 phototherapy: a British Photodermatology Group workshop report. Clin Exp Dermatol 2012; 37(3): 219-26.
4. Gambichler T, Terras S, Kreuter A. Treatment regimens, protocols, dosage, and indications for UVA1 phototherapy: facts and controversies. Clin Dermatol 2013; 31(4): 438-54.
5. Hassani J, Feldman SR. Phototherapy in Scleroderma. Dermatol Ther (Heidelb) 2016; 6(4): 519-53.
6. de Rie MA, Enomoto DN, de Vries HJ, Bos JD. Evaluation of medium-dose UVA1 phototherapy in localized scleroderma with the cutometer and fast Fourier transform method. Dermatology 2003; 207(3): 298-301.
7. Durand F, Staumont D, Bonnevalle A, Hachulla E, Hatron PY, Thomas P. Ultraviolet A1 phototherapy for treatment of acrosclerosis in systemic sclerosis: controlled study with half-side comparison analysis. Photodermatol Photoimmunol Photomed 2007; 23(6): 215-21.
8. Kreuter A, Hyun J, Stucker M, Sommer A, Altmeyer P, Gambichler T. A randomized controlled study of low-dose UVA1, medium-dose UVA1, and narrowband UVB phototherapy in the treatment of localized scleroderma. J Am Acad Dermatol 2006; 54(3): 440-7.
9. Mahmoud BH, Hexsel CL, Hamzavi IH, Lim HW. Effects of visible light on the skin. Photochem Photobiol 2008; 84(2): 450-62.
10. Moura Valejo Coelho M, Matos TR, Apetato M. The dark side of the light: mechanisms of photocarcinogenesis. Clin Dermatol 2016; 34(5): 563-70.
11. Matsumura Y, Ananthaswamy HN. Short-term and long-term cellular and molecular events following UV irradiation of skin: implications for molecular medicine. Expert Rev Mol Med 2002; 4(26): 1-22.
12. Connolly KL, Griffith JL, McEvoy M, Lim HW. Ultraviolet A1 phototherapy beyond morphea: experience in 83 patients. Photodermatol Photoimmunol Photomed 2015; 31(6): 289-95.
13. Matthews YJ, Halliday GM, Phan TA, Damian DL. Wavelength dependency for UVA-induced suppression of recall immunity in humans. J Dermatol Sci 2010; 59(3): 192-7.
14. Svobodova A, Vostalova J. Solar radiation induced skin damage: review of protective and preventive options. Int J Radiat Biol 2010; 86(12): 999-1030.
15. Beyer C, Schett G, Distler O, Distler JH. Animal models of systemic sclerosis: prospects and limitations. Arthritis Rheum 2010; 62(10): 2831-44.
16. Yamamoto T, Takagawa S, Katayama I, Yamazaki K, Hamazaki Y, Shinkai H *et al*. Animal model of sclerotic skin. I: Local injections of bleomycin induce sclerotic skin mimicking scleroderma. J Invest Dermatol 1999; 112(4): 456-62.
17. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T *et al*. Fiji: an open-source platform for biological-image analysis. Nat Methods 2012; 9(7): 676-82.
18. Brianezi G, Grandi F, Bagatin E, Enokihara MM, Miot HA. Dermal type I collagen assessment by digital image analysis. An Bras Dermatol 2015; 90(5): 723-7.

19. Hugle T. Beyond allergy: the role of mast cells in fibrosis. Swiss Med Wkly 2014; 144: w13999.
20. Santiago B, Galindo M, Rivero M, Pablos JL. Decreased susceptibility to Fas-induced apoptosis of systemic sclerosis dermal fibroblasts. Arthritis Rheum 2001; 44(7): 1667-76.
21. Boatright KM, Salvesen GS. Mechanisms of caspase activation. Curr Opin Cell Biol 2003; 15(6): 725-31.
22. Ansarin H, Daliri M, Soltani-Arabshahi R. Expression of p53 in aggressive and non-aggressive histologic variants of basal cell carcinoma. Eur J Dermatol 2006; 16(5): 543-7.
23. van Diest PJ, Brugal G, Baak JP. Proliferation markers in tumours: interpretation and clinical value. J Clin Pathol 1998; 51(10): 716-24.
24. Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nat Rev Immunol 2008; 8(6): 478-86.
25. Godar DE. UVA1 radiation triggers two different final apoptotic pathways. J Invest Dermatol 1999; 112(1): 3-12.
26. Chabaud S, Moulin VJ. Apoptosis modulation as a promising target for treatment of systemic sclerosis. Int J Rheumatol 2011; 2011:13.
27. Guhl S, Hartmann K, Tapkenhinrichs S, Smorodchenko A, Grutzkau A, Henz BM *et al*. Ultraviolet irradiation induces apoptosis in human immature, but not in skin mast cells. J Invest Dermatol 2003; 121(4): 837-44.
28. Mikita N, Kanazawa N, Yoshimasu T, Ikeda T, Li HJ, Yamamoto Y *et al*. The protective effects of ultraviolet A1 irradiation on spontaneous lupus erythematosus-like skin lesions in MRL/lpr mice. Clin Dev Immunol 2009; 2009:9.
29. Khodaeiani E, Fakhrjou A, Amirnia M, Babaei-Nezhad S, Taghvamanesh F, Razzagh-Karimi E *et al*. Immunohistochemical evaluation of p53 and Ki67 expression in skin epithelial tumors. Indian J Dermatol 2013; 58(3): 181-7.
30. Ho YY, Lagares D, Tager AM, Kapoor M. Fibrosis--a lethal component of systemic sclerosis. Nat Rev Rheumatol 2014; 10(7): 390-402.
31. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. J Biol Chem 1999; 274(31): 21491-4.
32. Kahari VM, Saarialho-Kere U. Matrix metalloproteinases in skin. Exp Dermatol 1997; 6(5): 199-213.
33. Mempel M, Schmidt T, Boeck K, Brockow K, Stachowitz S, Fesq H *et al*. Changes in collagen I and collagen III metabolism in patients with generalized atopic eczema undergoing medium-dose ultraviolet A1 phototherapy. Br J Dermatol 2000; 142(3): 473-80.
34. Yamamoto T, Kuroda M, Nishioka K. Animal model of sclerotic skin. III: Histopathological comparison of bleomycin-induced scleroderma in various mice strains. Arch Dermatol Res 2000; 292(11): 535-41.
35. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW *et al*. Human ICE/CED-3 protease nomenclature. Cell 1996; 87(2): 171.

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Darbo patirtis

Darbovietės pavadinimas	Data	Pareigos
Vilniaus universiteto ligoninė Santaros klinikos, reumatologijos centras	Nuo 2013 m.	Gydytoja reumatologė
Baltijos ir Amerikos klinika	Nuo 2013 m.	Gydytoja reumatologė
Valstybinis mokslinių tyrimų institutas Inovatyvios medicinos centras	Nuo 2013 m.	Jaunesnioji mokslo darbuotoja

Išsilavinimas

Mokymo įstaiga	Baigimo data	Specialybė, laipsnis	Mokymo sritis
Vilniaus universiteto Medicinos fakultetas	Nuo 2013 m.		Doktorantūros studijos
Vilniaus universiteto Medicinos fakultetas	2013 m.	Gydytoja reumatologė	Reumatologijos rezidentūros studijos
Lietuvos sveikatos mokslų universiteto Medicinos fakultetas	2009 m.	Medicinos gydytojo kvalifikacija	Medicinos krypties studijos
Lietuvos sveikatos mokslų universiteto Medicinos fakultetas	2008 m.	Gydytojo kvalifikacija	Medicinos krypties studijos
Kauno jėzuitų gimnazija	2002 m.	Vidurinis išsilavinimas	

Studijos ir stažuotės užsienyje

2004–2005 m. Medicinos krypties studijos Madrido Komplutensės (Complutense) universitete, Madridas, Ispanija (Erasmus programa).

2010 m. lapkritis–2011 m. vasaris. Reumatologijos rezidentūros studijos, Florencijos universiteto ligoninė, Florencija, Italija (Erasmus programa).

2012 m. vasaris–2012 m. balandis. Rotary fondo „Tarptautiniai mainai“ (angl. *Rotary International Group Study Exchange*) praktika ligoninėje, Niukaslas (Newcastle), Australija.

Dabartinė narystė profesinėse, mokslinėse organizacijose:

Organizacija	**Pareigos**
Lietuvos reumatologų asociacija	Narė
Europos antireumatinės lygos jaunųjų reumatologų tinklas (EMEUNET, angl. *Emerging European League Against Rheumatism network*)	Darbo grupės narė 2013–2017 m. Lietuvos atstovė

Kalbos

Anglų k. – puikiai; ispanų k. – labai gerai; rusų k. – pradmenys.

10. CURRICULUM VITAE

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Professional Experience

Institution	Date	Position
Vilnius University Hospital Santaros klinikos	2013–present	Rheumatologist
Baltic American Clinic	2013–present	Rheumatologist
State Research Institute Centre for Innovative Medicine	2013–present	Junior research fellow

Education

Institution	Graduating year	Degree	Field of study
Vilnius University Faculty of Medicine	2013–present		Doctoral studies
Vilnius University Faculty of Medicine	2013	Rheumatologist	Rheumatology residency training
Lithuanian University of Health Science Faculty of Medicine	2009	Doctor of medicine (MD)	Medicine
Lithuanian University of Health Science Faculty of Medicine	2008	Medical qualification	Medicine
Kaunas Jesuit Gymnasium	2002	Secondary Education	

Professional Training and Internships

2004–2005 – Medical qualification studies at University Complutense of Madrid, Madrid, Spain (Erasmus Socrates program);

November 2010–January 2011 – Rheumatology residency training program at Florence University Hospital, Florence, Italy (Erasmus Socrates program);

February 2012–April 2012 – Rotary International Group study exchange program, Newcastle, Australia.

Activities

Organization	Position
Lithuanian Rheumatology Association	Member
Emerging EULAR Network	Member 2013 – 2017 country liaison

Languages

Lithuanian – native; English, Spanish – fluent; Russian – basics.