

SERUM LEVEL OF A SOLUBLE FORM OF ENDOGLIN (CD105) IS DECREASED AFTER GOECKERMAN'S THERAPY OF PSORIASIS

David Pohl¹, Ctirad Andrys¹, Lenka Borská², Zdeněk Fiala³, Květa Hamaková⁴, Karel Ettler⁴, Jan Krejsek¹

Charles University in Prague, Faculty of Medicine and University Hospital Hradec Králové, Czech Republic: Department of Clinical Immunology and Allergy¹, Department of Pathological Physiology², Department of Hygiene and Preventive Medicine³, Department of Dermatology and Venereology⁴

Summary: *Background.* Goeckerman's therapy (GT) of psoriasis is based on daily application of pharmacy grade coal tar on affected skin with subsequent exposure to UV light. Disturbances in angiogenic activity are characteristic for the immunopathogenesis of psoriasis. The aim of study was to evaluate the influence of GT of psoriasis on proinflammatory and angiogenic activities expressed as changes in levels of endoglin (CD105). *Methods.* Serum levels of a soluble form of endoglin were measured in peripheral blood samples of 38 patients with psoriasis before and after therapy. Sixty three otherwise healthy blood donors serve as a control group. The efficacy of GT was expressed as changes in Psoriasis Area and Severity Index (PASI). *Results.* PASI score was significantly diminished by GT ($p < 0.001$). Serum levels of soluble CD105 were significantly diminished after GT. The serum level of soluble CD105 dropped from 7.85 ± 2.26 ng/ml before therapy to 7.01 ± 1.71 ng/ml after therapy ($p = 0.0002$). Compared to serum levels of soluble CD105 in healthy blood donors, serum levels of soluble CD105 in patients before GT were significantly higher ($p < 0.001$) and remained elevated after therapy ($p < 0.001$). Angiogenic activity expressed as serum endoglin is diminished in patients with psoriasis treated by GT.

Key words: Psoriasis; Goeckerman's therapy; Angiogenesis; Endoglin; CD105

Introduction

The original Goeckerman's method of therapy (GT) of psoriasis, which is based on daily application of pharmacy grade coal tar to affected skin with subsequent exposure of the body surface to UV light, was introduced by William Goeckerman in 1925 (10). This therapy is still exploited worldwide with minimal modifications (12).

Psoriasis is one of the most frequent skin diseases affecting approximately 2.5 % of the world's population. Psoriasis could be characterized from rather narrow point of view as a T-cell driven hyperproliferative keratinocyte disease (19). Regardless the fact, the most important role in psoriasis pathogenesis is given to specific immunity; there are numerous abnormalities in innate immunity as well. The presence of high levels of antimicrobial peptides in lesions was found. Two major families of skin antimicrobial peptides, defensins and cathelicidins, are present in psoriatic lesions. Especially, beta-defensin-2 (HBD-2), the first antimicrobial peptide discovered from human skin, is abundant in psoriatic plaques.

The typical feature of psoriasis is epidermal hyperproliferation, which is closely linked to very high angiogenic activity. This angiogenic activity is firmly established in psoriatic lesions (13). Highly sophisticated process of an-

giogenesis is carefully regulated by numerous intimate cell-to-cell contacts and humoral interactions involving cytokines, growth and differentiation factors, matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases and various products of blood coagulation and fibrinolysis (5). The proangiogenic factors and inhibitory activities are tightly regulated and are in balance under physiological conditions (8). Neoangiogenesis is also an integral part of the immunopathogenesis of chronic inflammatory diseases such as rheumatoid arthritis and retinopathy (13,16). Endoglin (CD105) is a proliferation-associated and hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells. It is a transmembrane accessory receptor for transforming growth factor- β (TGF- β) (11). Endoglin is essential during angiogenesis. Tangible evidence of its proangiogenic role comes from knockout studies in which CD105 null mice die in utero as a result of impaired angiogenesis (9). CD105 is able to be shed into the circulation, with elevated levels detected in patients with various types of cancer (21) or immunopathological disorders such as systemic sclerosis and rheumatoid arthritis (25).

Our research is principally focused on investigation of the impact of polyaromatic hydrocarbons present in coal tar used in Goeckerman's therapy on immune system. Psoriasis is serving as a clinical model for us. Several param-

ters of inflammation and angiogenesis have already been determined and published by our group (4,1).

We found the enhanced presence of a soluble form of proangiogenic molecule endoglin in peripheral blood of patients with psoriasis. The inhibitory effect of Goeckerman's therapy on this proangiogenic factor was described in our study. Regarding to our experience, soluble endoglin seems to be a promising marker which could add relevant information to our research effort.

Methods

Altogether, 38 adult patients with chronic plaque form of psoriasis were enrolled to this study. The study was approved by the Ethics Committee of the University Hospital in Hradec Kralove. Informed written consent was obtained from each patient. Our study group consisted of 18 females and 20 males (average age: 43.4 years, range: 18–76 years). Sixty three otherwise healthy blood donors (31 female and 32 male, average age: 34.4 years, range: 19–56 years) served as a control group.

In the course of GT coal tar ointment with 5 % pharmaceutical grade coal tar (Pix Lithanthracis) was overnight applied on affected skin (10–75 % of total body surface) and removed in the morning by olive oil and water bath. Subsequently, patients were irradiated with UV light daily (source of UV radiation: filtered mercury lamp Chirana 397, Chirana, Czech Republic). The light beam density (dose) was controlled by Sola – Scope 2000 spectrometer (Solatell, UK).

The average intensity of UV radiation in distance of 100 cm in front of UV lamp was $134.45 \mu\text{W}/\text{cm}^2$ (wavelength 280–315 nm) and $245.60 \mu\text{W}/\text{cm}^2$ (wavelength 315.5–400 nm). The daily doses of irradiation ranged from $0.8868 \text{ mJ}/\text{cm}^2$ (exposure 1 minute) to $17.736 \text{ mJ}/\text{cm}^2$ (20 minutes of irradiation). The duration of UV irradiation was individual, limited by skin erythema development (range 1–15 minutes). The average duration of therapy was 24 days (range: 12–30 days). The efficacy of Goeckerman's therapy was followed using PASI score (7). GT is ceased when 50 % decrease of PASI is achieved.

The only patients treated for the first time with GT therapy were enrolled to our study. No further medications regarding psoriasis or influencing inflammatory reaction were administered to our patients. Patients who are enrolled to this study are followed by dermatologist. Personal and family history was taken. Patients were examined physically. Basic biochemical and hematological parameters including leukocytes and differential cell count were determined. Special emphasis was done regarding exposure to harmful chemical substances, smoking and exposure to polycyclic aromatic hydrocarbons in food and indoor environment (coal burning). No patients suffering from psoriatic arthritis were enrolled to our study as this form is not indicated to GT. Psoriatic arthritis was excluded by the rheumatological consultant based on results of clinical, radiographic

and serological examinations. No apparent diseases affecting vascular system were found in our patients.

Peripheral blood samples were collected before the start of GT and immediately after termination of treatment and evaluation of PASI score. Samples were allowed to clot and serum was obtained after sample centrifugation. Serum samples were stored frozen at -20°C . Repeated thawing and freezing of samples was avoided. Soluble form of endoglin in serum was assessed by sandwich ELISA using diagnostic kit manufactured by R&D Systems (USA). The standard range of this kit is from 0.15 to 10 ng/ml and the minimum detectable dose is 0.007 ng/ml. Samples (50 μl) were examined neat in duplicates.

Statistical analysis of data was performed using MedCalc (Belgium) statistical software. Normal distribution of data was assessed. Student's paired and unpaired t-tests or Wilcoxon's test were used. Probability level less than 0.05 was considered as significant.

Results

All results are expressed as an average \pm 1SD. Disease severity was significantly positively affected by Goeckerman's therapy. Good clinical response was achieved in all patients. Pre-therapy PASI scores of 19.22 ± 7.49 dropped to 8.85 ± 6.29 after therapy ($p < 0.001$). No correlations between serum levels of both endoglin levels before (correlation coefficient 0.0191, $p > 0.05$) and after therapy (correlation coefficient -0.01216, $p > 0.05$) to disease activity expressed as PASI score were found. Serum levels of soluble endoglin were significantly diminished after Goeckerman's therapy. The serum level of soluble endoglin dropped from $7.85 \pm 2.26 \text{ ng/ml}$ before therapy to $7.01 \pm 1.71 \text{ ng/ml}$ after therapy ($p = 0.0002$, power of test: 0.984) (Fig. 1).

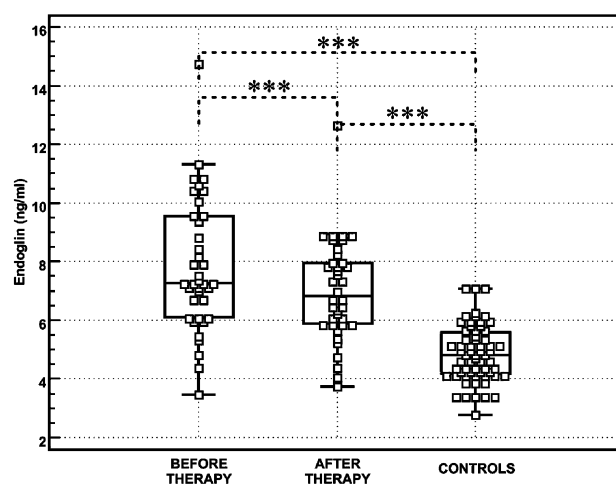


Fig. 1: The differences in the serum levels of soluble endoglin molecule (ng/ml) in patients with psoriasis before and after Goeckerman's therapy and comparison with healthy controls. A standard box-and-whisker plot (median, quartiles and range) was used.

The control group consisted of 63 healthy blood donors (32 male and 31 female). Serum levels of soluble CD105 measured in control group were 4.88 ± 0.95 ng/ml. No differences were found between males and females ($p = 0.7022$). In comparison with serum levels of soluble endoglin in healthy blood donors, serum levels of soluble endoglin in patients before Goeckerman's therapy were relatively high ($p < 0.001$) and remained elevated after therapy ($p < 0.001$).

Discussion

It is well established that Goeckerman's therapy of psoriasis (GT), which is based on the combination of anti-inflammatory and immunomodulatory effects of poorly characterized aliphatic and aromatic hydrocarbons present in pharmaceutical grade coal tar and well documented immunosuppressive and immunomodulatory activities of UV irradiation, is highly efficient in the treatment of psoriasis. GT induces a good clinical response followed by a long term remission in a majority of patients (12).

This complex therapeutical regimen has numerous targets. The abnormal hyperproliferative capacity of epidermal cells is known to be diminished by this therapy (19). We found in our previous study significant decrease of serum levels of proangiogenic factors such as vascular endothelial growth factor VEGF and basic fibroblast growth factor bFGF during Goeckerman's therapy (2). Our present project addressed whether or not Goeckerman's therapy influences the serum level of proangiogenic protein endoglin in patients with psoriasis.

Recently, it was published by Arbiser et al. (3) that the carbazole in coal tar is the active antiangiogenic compound. In addition to its antiangiogenic activity, carbazole inhibited the production of IL-15 which is thought to contribute to the immunopathogenesis of psoriasis by the recruitment of mononuclear cells.

Endoglin (CD105) is a coreceptor for transforming growth factor- β and modulates TGF- β signalling by interacting with TGF- β receptors (11). The importance of endoglin in vascular morphogenesis is well established (15). Endoglin is predominantly expressed on endothelial cells. It is upregulated under inflammatory conditions as well as in skin lesions where endothelial cell proliferation occurs. Elevated expression of endoglin determined by immunohistochemical method in psoriatic plaques has already been described by van de Kerkhof et al. (23) and Rulo HFC et al. (18). Higher expression of endoglin was detected in psoriatic skin lesions whereas in uninvolved skin the expression of endoglin was low, than found in normal healthy skin. Similar results were reached using electron microscopic detection of endoglin in psoriatic lesions (24). Beside the membrane form, endoglin can occur in a soluble form, which is shed by membrane-type metalloproteinases into the circulation (22). Elevated levels of soluble CD105 are detected in various types of tumors, and in patients with various inflammatory diseases (25).

Information regarding the role of endoglin in angiogenesis is with a few exceptions based on investigation of membrane expressed molecule CD105. Data referred to the role of soluble form of endoglin are much sparse. Majority of them is derived from studies focused on neoangiogenesis associated with cancer (20). In the context of these studies, soluble form of endoglin is generally recognized as proangiogenic molecule. However, the unequivocal conclusion supporting this suggestion is still lacking (21, 14). Ultraviolet B irradiation (UVB) is an integral component of Goeckerman's therapy. Therapeutic benefit of GT is based on the synergistic effect of coal tar and UV irradiation. UV light with low intensity is sensitising skin thus enabling better penetration of immunomodulatory compounds from coal tar ointment into the skin. However, it has been reported that UVB could enhance cutaneous angiogenesis in an exposed skin. Several angiogenic factors, including VEGF and bFGF have been found to be upregulated after experimental UVB irradiation in the skin (6). Nevertheless, the intensity of UVB irradiation in the course of GT is relatively low probably with limiting proangiogenic potential. Our data describing significantly diminished serum levels of proangiogenic factor endoglin in patients with psoriasis after finishing GT is further supporting our opinion.

Angiogenesis is mandatory to support endometrial growth after menstruation and to provide a vascularized, receptive endometrium for implantation and placentation (17). Unfortunately, the data relevant to the menstruation cycle are lacking in our investigated group of patients.

Conclusion

In conclusion, we found that the serum level of endoglin (soluble CD105) which is increased in patients with psoriasis is significantly decreased after Goeckerman's therapy. We propose serum level endoglin as a promising marker of proangiogenic activity in patients suffering from psoriasis.

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Corresponding author:

Dr. Ctirad Andrys, Department of Clinical Immunology and Allergy, Charles University in Prague, Faculty of Medicine in Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic; e-mail: andrys@lfhk.cuni.cz
