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Cytogenetic and Immunological Changes after Dermal Exposure to Polycyclic Aromatic Hydrocarbons and UV Radiation

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Summary

Goeckerman's therapy (GT), which combines exposure to coal tar (polycyclic aromatic hydrocarbons – PAHs) and UV radiation (UV) is often used as the first option for treatment of psoriasis. However, PAHs and UV represent mutagenic, carcinogenic and immunotoxic agents. Therefore GT can represent a health risk for the patients. The group under observation consisted of thirty patients undergoing GT. Before and after the treatment, blood samples were collected and chromosomal aberrations and selected immunological markers were determined. The relationships between chromosomal aberrations and immunological markers and the extent (duration) of exposure to GT were evaluated. The Psoriasis Area and Severity Index (PASI) score confirmed the high efficacy of GT. However, significantly elevated levels of chromosomal aberrations of peripheral lymphocytes were also found after the therapy ($p<0.001$). The levels of chromosomal abnormalities correlated to the extent and the total duration of exposure to PAHs ($r = 0.682$, $p<0.01$ and $r = 0.605$, $p<0.05$). After the therapy, significantly decreased levels of IgE, IgM isotypes of immunoglobulin, α_2-macroglobulin and transferrin together with β_2-microglobulin were found. From the immunological markers listed above only the decreased level of α_2-macroglobulin correlated to the extent of exposure to PAHs ($r = -0.568$, $p<0.05$). No correlation was found between chromosomal aberrations, significantly changed immunological markers and the duration of UV exposure. Our study revealed that GT has a significant impact on both genetic and immunological parameters of psoriatic patients. The results indicate that GT could increase genotoxic risk and modulates immunity of treated patients.

Key words
Psoriasis • UV-radiation • Polycyclic aromatic hydrocarbons • Genotoxicity • Immunotoxicity

Introduction

Psoriasis is one of the most frequent skin diseases in the Czech Republic. Patients with psoriasis represent about 7 % of all patients hospitalized with dermatosis (Novotný 2002). The pathogenesis of this

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disease remains enigmatic. It is an inflammatory skin disease in which abnormal individual immune reactivity plays an important role (Gudjonsson *et al.* 2004, Krejsek and Kopecký 2004). The genetic predisposition, e.g. certain HLA (Human Leukocyte Antigens) haplotype (HLA-Cw*0602) in association with environmental stimuli are involved in the immunopathogenesis of psoriasis (Kundakci *et al.* 2002). The environmental stimuli are largely unknown but microbial infections, especially exposures to streptococcal antigens, are responsible for the induction of immunopathological reactivity that is targeted predominantly, but not exclusively, to the skin (Telfer *et al.* 1994). For example, at least 10 % of patients suffering from psoriasis develop arthritis (Espinoza *et al.* 1992). Heavy granulocytic infiltration with scattered T cells is the hallmark of inflammatory reaction in the skin. Intradermal microabscesses are seen in the most severe cases. A significant increase in the proliferation activity of keratinocytes is another typical feature of psoriasis. It is caused by abnormal stimuli provided to keratinocytes by T cells (Valdimarsson *et al.* 1986).

There is no contemporary treatment available for psoriasis. The only therapeutic approaches are focused on alleviation of inflammation. Goeckerman's therapy (GT) combines exposure to therapeutic coal tar (tar with high portion of polycyclic aromatic hydrocarbons – PAHs) and UV radiation (UV). GT is often used as the first option for treatment of psoriasis (Benáková 2001, Lebwohl and Ali 2001, Lüllmann *et al.* 2002, Thami and Sarkar 2002, Borská *et al.* 2004a). However, PAHs and UV represent potentially mutagenic, carcinogenic and immunotoxic agents and thus GT can represent certain health risks for patients (Luster and Rosenthal 1993, Fiala *et al.* 2000, 2004). In the present study, selected cytogenetic and immunological markers in patients undergoing GT are evaluated. Primarily, the work was focused on the possible relationships between changes of selected cytogenetic and immunological markers and the extent (and/or duration) of GT.

Methods

The observed group consisted of 30 patients (17 male, 13 female) with psoriasis who were undergoing Goeckerman's therapy. The average age of the patients was 28 years, 48 % of them were smokers. Patients with psoriasis were carefully evaluated clinically. Severity of the disease was estimated and expressed as a PASI score (Psoriasis Area and Severity Index) (Ettler 1995). PASI scores were monitored before and after GT. The average duration of Goeckerman's therapy was 24 days. All patients filled a questionnaire about their personal and medical history, occupational anamnesis and non-occupational activities. The Ethics Committee of the Medical Faculty of Charles University, University Hospital and Purkyně Military Medical Academy in Hradec Králové reviewed and approved this study.

The therapeutic coal tar ointment (containing 3-5 % of therapeutic coal tar) was applied daily to 10-75 % of the patient's body surface. The UV radiation (UV-B and UV-A) was applied daily to the whole body surface. Duration of UV exposure ranged between 1-15 min. Flow densities of UV-B radiation (24.14 $\mu W/cm^2$) and UV-A radiation (70.31 $\mu W/cm^2$) were analyzed by spectroradiometer Sola-Scope 2000 (Solatell Ltd., United Kingdom).

Blood samples were collected before the therapy and after the last application of coal tar ointment and UV exposure. Selected parameters of cell-mediated immunity (lymphocyte subpopulations CD3, CD4, CD8, HLA-DR, CD45 RA, CD45 RO) and chromosomal aberrations of peripheral lymphocytes were determined in heparin-treated blood. Blood serum was used to estimate the parameters of humoral immunity (IgM isotypes of immunoglobulin, IgG, IgA, IgE, β_2-microglobulin, α_2-macroglobulin and transferrin, C3 complement, orosomucoid, prealbumin, haptoglobin, neopterin).

The number of chromosomal aberrations in peripheral lymphocytes was determined by a standard method (AHEM 2000). The number of cells with chromosomal abnormalities was analyzed microscopically in lymphocytes stimulated to proliferate by phytohemaglutinin.

The levels of serum proteins (α_2-macroglobulin, IgG, IgA, IgM, C3 complement, orosomucoid, prealbumin, haptoglobin and transferrin) were determined using rate nephelometry (Beckman, USA). IgE and β_2-microglobulin were measured by chemiluminescent immunoassay (DPC, USA). Levels of neopterin were evaluated using the ELISA technique (Brahms, Germany). The immunophenotyping analysis of lymphocyte subpopulations (CD3, CD4, CD8, HLA-DR, CD45 RA, CD45 RO) was performed on a flow cytometer Coulter Epics XL (Coulter-Beckman, USA). All immunological analyses were performed by using standard methods at the Institute of Clinical Immunology and Allergology, University Hospital in Hradec Králové.

Table 1. Chromosomal aberrations in peripheral lymphocytes.

Type of chromosomal aberrations	Level of chromosomal aberrations (%) Before GT (n = 30)	After GT (n = 30)	Statistical significance
ABC	0.97 ± 0.85	2.36 ± 1.37	$p < 0.001$
SAC	0.58 ± 0.77	1.29 ± 1.123	$p < 0.01$
NAC	0.39 ± 0.54	1.07 ± 0.85	$p < 0.001$
ATA	0.34 ± 0.53	0.53 ± 0.81	$p < 0.05$

Data are mean ± SD; n = number of patients; ABC = total number of aberrant cells; SAC = structurally aberrant cells; NAC = numerically aberrant cells; ATA = another type of aberration.

Table 2. Significant immunological changes in blood and serum.

Parameters	Before GT (n = 30)	After GT (n = 30)	Statistical significance
IgE (IU/ml)	1.67 ± 0.59	1.62 ± 0.62	$p < 0.05$
IgM (g/l)	1.30 ± 0.53	1.24 ± 0.48	$p < 0.05$
β_2M (mg/l)	1.61 ± 0.37	1.55 ± 0.33	$p < 0.05$
α_2M (g/l)	1.91 ± 0.60	1.86 ± 0.56	$p < 0.01$
TRf (g/l)	2.46 ± 0.37	2.36 ±0.45	$p < 0.01$

Data are mean ± SD; n = number of patients; IgE = immunoglobulin E; IgM = isotypes of immunoglobulin M; β_2M = β_2-macroglobulin; α_2M = α_2-macroglobulin; TRf = transferrin.

The differences between average values of parameters were evaluated by Student's t-test. Relationships between chromosomal aberrations, significantly changed immunological markers and the basic characteristics of GT were evaluated by Pearson's correlation coefficient. The statistical significance was determined on a probability level of less than 0.05.

Results

The PASI score before and after GT was 25.84 ± 11.54 and 5.75 ± 4.77, respectively. The results showed significant improvement in the clinical state of psoriatic patients treated by GT ($p<0.001$).

Chromosomal abnormalities in peripheral lymphocytes of psoriatic patients before and after GT are shown in Table 1. The results showed a significant elevation of the total number of aberrant cells, structurally aberrant cells, numerically aberrant cells and cells aberrant from other aspects after the therapy.

Significant immunological changes in psoriatic patients before and after GT are shown in Table 2. After the therapy significantly decreased levels of IgE, IgM isotypes of immunoglobulin, β_2-microglobulin, α_2-macroglobulin and transferrin were found. The changes of other immunological markers (IgG, IgA, C3 complement, orosomucoid, prealbumin, haptoglobin and lymphocyte subpopulations – CD3, CD4, CD8, HLA-DR, CD45 RA, CD45 RO) were not significant.

We found a correlation between the level of chromosomal aberrations, α_2-macroglobulin and the basic characteristics of GT. The results are summarized in Table 3. A correlation was found between the level of chromosomal aberrations and the extent of exposure to PAHs and also between the level of chromosomal aberrations and the total duration of GT. From the significantly changed immunological parameters only the level of α_2-macroglobulin correlated to the extent of exposure to PAHs. No correlation was found between the level of chromosomal aberrations and the duration of UV exposure or between α_2-macroglobulin and the duration of UV exposure or the total duration of GT.

Table 3. Relationships between chromosomal aberrations, α_2-macroglobulin levels and basic characteristics of GT.

Indicators	Chromosomal aberrations	α_2-macroglobulin
Extent of PAHs exposure	n = 30 r = 0.682 p < 0.01	n = 30 r = –0.568 p < 0.05
Time of UV exposure	n = 30 r = 0.337 NS	n = 30 r = –0.448 NS
Total duration of GT	n = 30 r = 0.605 p < 0.05	n = 30 r = –0.257 NS

Extent of PAHs exposure = area of exposed body surface (%); Time of UV exposure = total time of whole body exposure (min); chromosomal aberrations = total number of aberrant cells; n = number of patients; r = Pearson's correlation coefficient; NS = non-significant

Discussion

The extent and intensity of potentially adverse effects induced in patients treated by GT is very difficult to evaluate or predict by methods that are now available. In addition, therapeutical coal tars are poorly characterized. The individual variability of patients, including unique immunological patterns, also contributes to the substantial variability of results obtained by different authors (Novotný 2002). These results are very often contradictory and do not enable to optimize a therapeutic approach for patients suffering from psoriasis (Prodanovich *et al.* 2000, Warin 2001). The doubts about the safety of GT lead, in some countries, to its termination despite the fact that this therapy is effective in a majority of patients, is readily available and has a high cost-benefit ratio in comparison with other therapeutic modalities.

The genotoxic effect of GT was monitored in 30 patients with psoriasis by the assessment of cytogenetic changes in peripheral blood lymphocytes when using standard methods. Representative cell samples (100 cells in mitosis) were analyzed in each patient before and after GT. The total number of aberrant cells together with the number of structurally and numerically aberrant cells was evaluated. A significant increase in both the total number of aberrant cells ($p<0.001$) and the number of numerically aberrant cells ($p<0.001$) was found after GT. The number of observed patients was sufficient to assess the overall risk for the whole group which was statistically significant ($p<0.001$). A significant positive correlation between the number of aberrant cells and extent of PAHs exposure, measured as the area of exposed body surface ($p<0.01$), was found in our study. The reference level of chromosomal aberrations for adults in the Czech population (n = 30) has been declared in the range from 0 to 1.74 % (AHEM 2000). In our study, the final value of aberrant cells after the therapy was 2.36 % which was found to be significantly higher ($p<0.001$) when compared to the reference level (Table 1). In conclusion, our study revealed a higher risk of genotoxicity induced by GT in patients suffering from psoriasis. Our results are in good concordance with other studies (Clonfero *et al.* 1990, Gardošová *et al.* 1997, Borská *et al.* 2004b). PAHs seem to be predominantly responsible for genotoxicity, but it has to be stressed that both PAHs in coal tar and UV radiation could also be involved in the final genotoxic effect. It seems likely that their effects are mutually potentiated. To distinguish between them, an experimental animal model should be used. The contribution of PAHs to this effect is probably more important because a positive correlation ($r = 0.682$, $p<0.01$) between the extent of PAHs exposure and relative number of chromosomally aberrant cells was found. A positive correlation ($p<0.05$) was also found between the presence of aberrant cells and the duration of GT. It will be necessary to follow patients treated with GT for longer period. The majority of patients is relapsing and thus repeatedly treated. Long-term

genotoxic and immunomodulatory effects are very likely to be involved. Without any doubt, GT of psoriasis influences the immune system because both components of GT, i.e. exposure to PAHs and exposure to UV, are generally immunosuppressive (Fiala *et al.* 2000).

A significant decrease was found in the serum level of β_2-microglobulin after GT. Molecular β_2-microglobulin is a glycoprotein that is noncovalently associated with the α-chain of HLA I molecules. It is stabilizing for this complex, which is necessary for the physiological role of HLA I molecules, presenting antigenic peptides to $CD8^+$ cytotoxic T cells. This glycoprotein is easily shed from the surface of cells. It is assumed that the serum level of β_2-microglobulin reflects the activity of the immune system. The decrease of serum concentration of β_2-microglobulin after GT is probably caused by effective antiinflammatory action of this therapy that is documented by positive clinical response in our patients. It can only be speculated whether the decrease of soluble β_2-microglobulin is also associated with a decreased expression of HLA I molecules on the cell surface. If this is true, the lower HLA I expression could result in less extensive antigenic stimulation of $CD8^+$ T cells in psoriatic lesions because the stimulation of $CD8^+$ T cells is dependent on the HLA I molecule. It is well documented that at least 80 % of T cells in chronic lesional epidermis are $CD8^+$ positive T cells. Most of the epidermal $CD8^+$ T cells are closely associated with keratinocytes (Valdimarsson *et al.* 1995).

Alpha$_2$-macroglobulin, another significantly decreased immunological parameter, is the representative of a heterogeneous group of plasma proteins with an overall capacity to down-modulate activity of proteolytic enzymes. The production and release of various proteinases is increased during inflammatory response, including immunopathological inflammation such as psoriasis (Orem *et al.* 1997, Chodorowska *et al.* 2001). The decrease in the serum level of α_2-macroglobulin very likely reflects the anti-inflammatory effect of GT. It is in good accordance with the results of Chodorowska *et al*. (2004), which showed a significantly increased serum level of α_2-macroglobulin in patients with active psoriasis. It was also found that α_2-macroglobulin was the only immunological parameter, which was significantly associated with the extent of exposure to PAHs in our study. This correlation was negative. In agreement with others (Rocha-Pereira *et al.* 2004) it could be extrapolated from our results that the serum level of α_2-macroglobulin is reflecting the activity of disease. We suggest that the serum level of α_2-macroglobulin could serve as a surrogate marker of exposure to genotoxic and immunotoxic PAHs.

The serum concentration of immunoglobulin IgM, which is also recognized as a positive reactant of inflammation, was significantly decreased after GT of psoriasis. It is very likely associated with diminished activity of immunopathological inflammation in treated patients.

It is not easy to interpret the significant decrease in the level of transferrin. In spite of the fact that transferrin is a typical negative marker of inflammation, its serum level was significantly decreased after GT. However, Rocha-Pereira *et al.* (2004) found that the level of transferrin and other iron-binding plasma proteins such as lactoferrin and ceruloplasmin were higher in active psoriasis than in inactive psoriasis. The positive clinical effect that was achieved in our psoriatic patients treated by GT can be associated with a decrease in serum levels of transferrin. The iron-binding plasma proteins are participating in endogenous antioxidant systems promoting the detoxification of reactive oxygen species. They avoid the development of the Fenton reaction leading to generation of hydroxyl radicals, the more deleterious oxygen metabolites from hydrogen peroxide. Reactive oxygen species have been shown to mediate inflammatory processes and to be involved in oxidative reactions such as lipid peroxidation and protein oxidation. They may greatly increase the inflammatory process, but they may also contribute to tissue damage in psoriatic patients (Rocha-Pereira *et al.* 2004).

There is TH1 skewing in TH1/TH2 rate in psoriatic patients with increased production of interferon γ and other TH1 cytokines. These cytokines influence other cells locally to secrete numerous proteins including chemokines (IL-8), GM-CSF (granulocyte macrophage-colony stimulating factor) and epidermal growth factor (EGF). These factors regulate the migration of new inflammatory cells into the skin and increase the activity of these cells and keratinocytes, resulting in psoriatic plaque (Mehlis and Gordon 2003). The production of IgE class of immunoglobulins is clearly in an opposite sense under the regulatory control of TH2 T cells. The serum level of total IgE immunoglobulins in our psoriatic patients was significantly decreased after GT. The successful allergen immunotherapy for people who suffered from IgE mediated allergy is followed by the decrease of TH2 driven (IL-4) cytokines with concurrent increase of both TH1 driven (interferon γ) and

Treg (IL-10) cytokines (Krčmová and Hanzálková 2001). In contrast to allergen immunotherapy the GT is not antigenically targeted. The changes in the serum level of IgE could be attributed to the response to streptococcal antigens as was shown in patients with chronic sinusitis/nasal polyposis (Tripathi *et al.* 2004). It could be promising to monitor the presence of IgE class specific antibodies against streptococcal and staphylococcal antigens in patients suffering from psoriasis and their changes during GT treatment.

Our study revealed that GT has a significant impact on both genetic and immunological parameters of psoriatic patients. The results indicate that increased genotoxic risk and disturbances of immunity could endanger the patients treated by GT. Further research is necessary to evaluate the level of discussed health risks.

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References

AHEM: Methods of biological monitoring of genotoxic effects of environmental factors. Cytogenetic analyses of peripheral lymphocytes. Actualization of valid standard method. (in Czech). *Acta Hyg Epidem Microbiol* **5 (Suppl)**: S22, 2000.

BENÁKOVÁ N: Pharmacotherapy of psoriasis. (in Czech.) *Remedia* **11**: 25-34, 2001.

BORSKÁ L, FIALA Z, HAMÁKOVÁ K, ANDRÝS C, KREJSEK J, VOKURKOVÁ D, ŠMEJKALOVÁ J, KREMLÁČEK J: Selected cytogenetic and immunological changes after dermal exposure to polycyclic aromatic hydrocarbons (PAHs). *Physiol Res* **53**: 48P, 2004a.

BORSKÁ L, FIALA Z, ŠMEJKALOVÁ J, HAMÁKOVÁ K, KREMLÁČEK J: Possible genotoxic risk of combined exposure to pharmaceutical coal tar and UV-B radiation. *Cent Eur J Public Health* **12 (**Suppl): S14-S15, 2004b.

CHODOROWSKA G, JUSZKIEWICZ-BOROWIEC M, CZELEJ D, WOJNOWSKA D, KOWAL M: Activity of tumor necrosis factor-alfa (TNF-alpha) and selected acute phase proteins in plasma of psoriatic patients receiving local treatment. *Ann Univ Mariae Curie Sklodowska* **56**: 165-169, 2001.

CHODOROWSKA G, WOJNOWSKA D, JUSZKIEWICZ-BOROWIEC M: C-reactive protein and alpha$_2$-macroglobulin plasma activity medium-severe and severe psoriasis. *J Eur Acad Dermatol Venereol* **18**: 180-183, 2004.

CLONFERO E, JONGENEELEN F, ZORDAN M, LEVIS AG: Biological monitoring of human exposure to coal tar. Urinary mutagenicity assays and analytical determination of polycyclic aromatic hydrocarbon metabolites in urine. *IARC Sci Publ* **104**: 215-222, 1990.

ESPINOZA LR, CUELLAR ML, SILVEIRA LH: Psoriatic arthritis. *Curr Opin Rheumatol* **4**: 470-478, 1992.

ETTLER K: Clinical evaluation of psoriasis and atopic eczema expressed as indexes. (in Czech) *Čs Dermatol* **70**: 45-47, 1995.

FIALA Z, BORSKÁ L, VYSKOČIL A, MAŠÍN V, SRB V: Polycyclic aromatic hydrocarbons II. Toxic effects. (in Czech) *Acta Med (Hradec Králové)* **43** (Suppl): S37-S61, 2000.

FIALA Z, BORSKÁ L, HAMÁKOVÁ K, KRAJÁK V: Excretion of selected urinary metabolites after dermal exposure to polycyclic aromatic hydrocarbons (PAHs). *Physiol Res* **53**: 50P, 2004.

GARDOŠOVÁ D, MEZENCEVOVÁ V, KALINA I, HEJJ F, KOPKA M, PETROVIČOVÁ J: Mutagenic effects of pharmaceutical coal tar in psoriatic patients (in Slovak). *Bratisl Lek Listy* **98**: 146-149, 1997.

GUDJONSSON JE, JOHNSTON A, SIGMUNDSDOTTIR H, VALDIMARSSON H: Immunopathogenic mechanisms in psoriasis. *Clin Exp Immunol* **135**: 1-8, 2004.

KRČMOVÁ I, HANZÁLKOVÁ Y: Is the immunomodulating effect of allergen immunotherapy allergen specific? *Allergy* **56**: 89, 2001.

KREJSEK J, KOPECKÝ O: *Clinic Immunology* (in Czech). Nucleus, Hradec Králové, 2004, 985 p.

KUNDAKCI N, OSKAY T, ÖLMEZ Ü, TUTKAK H, GURGEY E: Association of psoriasis vulgaris with HLA class I and class II antigens in the Turkish population, according to the age at onset. *Int J Dermatol* **41**: 345-348, 2002.

LEBWOHL M, ALI S: Treatment of psoriasis. Part 1. Topical therapy and phototherapy. *J Am Acad Dermatol* **45**: 487-498, 2001.

LUSTER MI, ROSENTHAL GJ: Chemical agents and the immune response. *Environ Health Perspect* **101**: 219-226, 1993.

LÜLLMANN H, MOHR K, WEHLING M: *Pharmacology and Toxicology*. (in Czech) Grada, Prague, 2002, 696 p.

MEHLIS SL, GORDON KB: The immunology of psoriasis and biologic immunotherapy. *J Am Acad Dermatol* **49**: 44-50, 2003.

NOVOTNÝ F: *Psoriasis. Actual views* (in Czech). Grada, Prague, 2002, 144 p.

OREM A, DEGER O, CIMSIT G, BAHADIR S: Plasma polymorphonuclear leukocyte elastase levels and its relation to disease activity in psoriasis. *Clin Chim Acta* **264**: 49-56, 1997.

PRODANOVICH S, KIRSNER RS, TAYLOR JR: Treatment of patients hospitalized for psoriasis. *Dermatol Clin* **18**: 425-435, 2000.

ROCHA-PEREIRA P, SANTOS-SILVA A, REBELO I, FIGUEIREDO A, QUINTANILHA A, TEIXEIRA F: The inflammatory response in mild and in severe psoriasis. *Br J Dermatol* **150**: 917-928, 2004.

TELFER NR, CHALMERS RJ, WHALE K, COLMAN G: The role of streptococcal infection in the initiation of guttate psoriasis. *Arch Dermatol* **128**: 39-42, 1994.

THAMI GP, SARKAR R: Coal tar: past, present and future. *Clin Exp Dermatol* **27**: 99-103, 2002.

TRIPATHI A, CONLEY DB, GRAMMER LC, DITTO AM, LOWERY MM, SEIBERLING KA, YARNOLD PA, ZEIFER B, KERN RC: Immunoglobulin E to staphylococcal and streptococcal toxins patients with chronic sinusitis/nasal polyposis. *Laryngoscope* **114**: 1822-1826, 2004.

VALDIMARSSON H, BAKER BS, JONSDOTTIR I, POWLES A, FRY L: Psoriasis: a disease of abnormal proliferation induced by T lymphocytes. *Immunol Today* **7**: 256-259, 1986.

VALDIMARSSON H, BAKER, BS, JONSDOTTIR I, POWLES A, FRY L: Psoriasis: a T cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol Today* **16**: 1445-1449, 1995.

WARIN AP: Dermatology day care treatment centres. *Clin Exp Dermatol* **26**: 351-355, 2001.

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