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Vitiligo patients present lower plasma levels of α-melanotropin immunoreactivities

Robert Pichler [a,*], Konstantin Sfetsos [b], Birgit Badics [c], Sabrina Gutenbrunner [d], Josef Auböck [b]

[a] Institute of Nuclear Medicine, Wagner-Jauregg Hospital, Wagner-Jauregg Weg 15, A-4021 Linz, Austria
[b] Department of Dermatology, General Hospital Linz, Linz, Austria
[c] Institute of Laboratory Medicine, Wagner-Jauregg Hospital, Linz, Austria
[d] Institute of Laboratory Medicine, General Hospital Linz, Linz, Austria

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Abstract

Vitiligo is a depigmenting disorder characterized by the development of white patches with evidence in favour of an autoimmune mechanism. We investigated the role of melanotropins and the plasma levels of α-melanotropin and ACTH-like immunoreactivities in 40 vitiligo patients with the aim of detecting a possible influence of neuropeptide regulation of immunity. Twenty-one patients had active and 19 had stable vitiligo disease, 16 persons presented with an additional autoimmune thyroid disease.

Median α-MSH levels in vitiligo patients were 6.4 pmol/l [5.2;11.3] and significantly lower than in control persons with 11.4 pmol/l [8.6;13.4]. Median ACTH levels of the affected patient group were 17 pg/ml [10.5;28] and appeared statistically higher than 12 pg/ml [7;17] measured in the control group. Measured morning cortisol levels in both groups were not significantly different.

Reduced cutaneous α-MSH immunoreactivities have been related to the development of autoimmune-induced depigmenting disorders. Our data present lower α-MSH plasma levels in vitiligo patients which may be associated with the development of vitiligo depigmentation and may indicate a condition of impaired peripheral tolerance in this autoimmune disorder.

Keywords: Vitiligo; Melanotropins; Autoimmunity

1. Introduction

Vitiligo is a depigmenting disorder characterized by the development of white patches in various distributions, which are due to the loss of melanocytes from the epidermis. Strong evidence in favour of an autoimmune mechanism has been obtained (Ongenae et al., 2003). The role of humoral immunity as well as the involvement of cellular immunity in the pathogenesis of vitiligo has been investigated (Ongenae et al., 2003; van den Wijngaard et al., 2001).

Melanotropins are involved in tanning by stimulating melanocytes via the activation of the melanocortin-1 receptor to melanin production. Its main site of production is the pituitary gland, but α-MSH and related ACTH peptides are produced at other sites, including the skin (Pichler et al., 2004). Melanotropins exert both paracrine and autocrine functions in human skin, but the neuropeptide α-MSH is also involved in host defense

Abbreviations: ACTH, adrenocorticotropic hormone; α-MSH, alpha-melanocyte-stimulating hormone; NF-κ-B, nuclear-factor-kappa-B; POMC, pro-opiomelanocortin.

* Corresponding author. Tel.: +43 732 6921 36100; fax: +43 732 6921 46112.
E-mail address: Robert.Pichler@gespag.at (R. Pichler).

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and acts as a modulator of cutaneous inflammation (Catania et al., 2000; Luger et al., 2000). There is strong evidence that α-MSH influence a variety of immunomodulating and anti-inflammatory activities, mainly by affecting functions of monocytes, dendritic cells and endothelial cells. The activation of the transcription factor NF-κ-B appears to be a crucial event in immune and inflammatory responses. Data suggest that α-MSH appears to function as a general inhibitor of NF-κ-B activation (Luger et al., 2000).

The presence of the POMC derived peptides ACTH and α-MSH in human skin has been proven (Slominski et al., 2000). The local melanotropins are involved in tanning via both the autocrine and paracrine processes (Imokawa, 2004; Thody, 1999). Additionally, α-MSH has the potential to suppress T cell-mediated inflammation and to regulate lymphokine production by effector T cells (Taylor et al., 2000). We investigated the plasma levels of α-melanotropin and ACTH-like immunoreactivities in vitiligo patients with the aim of detecting a possible influence of neuropeptide regulation of immunity.

2. Subjects and methods

2.1. Patients

We collected morning blood samples from 32 women and 8 men throughout January to December 2002 at the General Hospital Linz. Mean age was 52 ± 18 years, 21 patients had active and 19 had stable vitiligo disease. All patients were suffering from non-segmental vitiligo at different stages of the disease. Sixteen persons presented with an additional autoimmune thyroid disease (11 with Hashimoto's thyroiditis and five with Graves'disease, all with levels of fT4 and fT3 in the normal range with or without medication). Three had pernicious anemia and there was one patient with insulin dependent autoimmune diabetes mellitus and one patient with autoimmune hepatitis. To evaluate a possible involvement of the melanotropins in vitiligo disease we measured ACTH, α-MSH and serum cortisol. We compared the patient data to that from matched (number, age, gender) normal persons. Data of CMV infection parameters in the same patient cohort has been presented elsewhere (Pichler et al., 2005). The study has been approved by the local ethic committee.

2.2. Laboratory methods

α-MSH was measured in human plasma by the EURIA-α-MSH radioimmunoassay provided by EURO-DIAGNOSTICA AB, Ideon, Malmö, Sweden. It is analysed by the competitive assay using an antiserum to an α-MSH-albumin conjugate. α-MSH in standards and samples compete with 125-I-labelled α-MSH in binding to the antibodies, which binds in a reverse proportion to the concentration of α-MSH. In order to increase the sensitivity of the assay, a sequential assay with delayed additions of 125-I- α-MSH is performed. Antibody-bound 125-I-α-MSH is separated from the free fraction using the double antibody polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured. The antiserum in this kit is directed to the C-terminal part of the α-MSH molecule and shows no cross-reactivity with ACTH.

Blood was collected in 3 ml ampoules containing EDTA and Trasylol. The specimen were centrifuged and the plasma was stored at −20 °C until testing.

Performance of the test was done in accordance with the manufacturer's guidelines. Hundred microliters of plasma were required for each testing tube, all samples, standard and controls were tested in duplicates or underwent repeated testing.

The antibody is specific for α-MSH and des-actyl-α-MSH. The crossreactivity to des-amino-α-MSH, ACTH 1-13, ACTH 1-24, ACTH 1-39, β-MSH and γ-MSH is <0.002%. At a level of about 35 pmol/l, the coefficient of variation is 4.7% for intra-assay and 8.4% for inter-assay.

ACTH was measured by the IMMULITE 2000 ACTH test (EURO/DPC, Glyn Rhonwy, Llanberis, Gwynedd, UK) in EDTA plasma which is a sequential, immunometric assay. Preparation, set-up, dilutions, adjustment, assay and quality control procedures were performed according to the operator's manual. At 40 pg/ml, the coefficient of variation is 6.8% for intra assay and 8.2% for inter-assay. Cross-reactivity with α-MSH is 0.1% or less.

Cortisol was measured by the IMMULITE 2000 Cortisol (EURO/DPC) competitive immunoassay in serum according to the manufacturer's guidelines. Precision (coefficient of variation) at a level of roughly 10 μg/dl is 5.2% for intra-assay and 6.8% for inter-assay.

2.3. Statistics

Values are presented as mean ± standard deviation or as median with quartiles in brackets. Adjustment to normal distribution was tested by the Kolmogorov–Smirnov test. Ordinal variables or metric variables with uneven distribution were analyzed with the Mann–Whitney U test. Regression analysis was used to investigate relations between two parameters of one group. The correlation between two variables reflects the degree to which the variables are related. For measure of correlation the Pearson product moment correlation was applied. For all tests a p value < 0.05 was considered significant.