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ORIGINAL ARTICLE

Detection and distribution of endogenous steroids in human stratum corneum

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ABSTRACT

Objectives: The objective of the study was to investigate the presence and distribution of endogenous steroids in human stratum corneum (SC) with respect to sex, age, anatomical site, and depth into SC, using a noninvasive sampling technique and a sensitive analytic method for quantitation.
Materials and methods: Corneocytes in the SC samples removed by sequential tape stripping from the forearm, forehead, and back sites were processed and analyzed using a validated liquid chromatography–tandem mass spectrometry method for the quantitation of hydrocortisone, cortisone, and testosterone.
Results: In the 32 volunteers surveyed, testosterone was only detected at the forearm site in a single volunteer. Both hydrocortisone and cortisone were detected in 5–7 individuals out of 16 from both the age 20–35 years and age 50–65 years groups. Maximal amounts of hydrocortisone and cortisone found in SC of forehead, forearm, and back were 0.37 ng/cm^2, 0.96 ng/cm^2, and 0.49 ng/cm^2; and 0.20 ng/cm^2, 0.12 ng/cm^2, and 0.06 ng/cm^2, respectively, and were either higher than or comparable to those reported in human hair in terms of concentration by SC weight. In the population with either hydrocortisone or cortisone detected, no significant differences relating to sex, age groups, and anatomical sites were observed for the amount of hydrocortisone and cortisone in the SC. However, significantly higher amount of cortisone was found in the surface layers of SC than deeper layers in the age 50–65 years group.
Conclusion: The results demonstrate that, with the achievable sensitivity of current analytical technology, physiological concentrations of endogenous steroids, such as hydrocortisone and cortisone, can be found in the SC of some individuals.

Introduction

Skin is the largest human organ, accounting for approximately 12–15% of body weight and consists of a complex layered structure, which forms a barrier between the body and the outside environment. It is structured in three layers: epidermis, dermis, and subcutaneous layer. The stratum corneum (SC) is the outermost layer of the epidermis with a thickness of approximately 10–20 μm, which is the rate-limiting barrier to percutaneous absorption and protects the body against the outer environment.[1,2]

Hormones influence the development and function of human skin, which also produces and releases hormones. Other than the adrenal gland, ovary, and testis, skin has been well recognized as the site of steroid hormone formation and metabolism. The skin and sebaceous glands are capable of synthesizing cholesterol *de novo* from acetate, and express genes coding and activity for enzymes obligatory for steroidogenesis; for example, the cytochromes P450scc, P450c17, and P450c21 convert cholesterol to androgens and other steroids.[3] Especially in postmenopausal women, almost all active sex steroids are made in target tissues by an intracrine mechanism. Human skin also expresses elements of the central hypothalamic–pituitary–adrenal axis including pro-opiomelanocortin, corticotropin-releasing hormone,

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the corticotropin-releasing hormone receptor-1, key enzymes of corticosteroid synthesis and synthesizes glucocorticoids.[4,5] These hormones exhibit a wide range of biological activities on the skin. For example, glucocorticoids induce hair growth, stimulate sebocyte proliferation, and regulate keratinocyte differentiation.[6] Androgens stimulate proliferation of sebocytes and dermal papilla cells, while estrogens improve collagen content and quality, increase skin thickness and enhance vascularization. Several studies have reported the production of cortisol in cultured human epidermal melanocytes and dermal fibroblasts,[7,8] and in histocultured human hair follicles.[9] Physiological concentrations of different endogenous steroids have also been found in hair samples.[10–12] Given that SC is the terminal differentiational layer of the skin, steroids produced in the local tissue or diffused from systemic circulation should eventually reach SC.

To the best of our knowledge, there are no existing literature data concerning endogenous steroid content of the human SC. In this study we employed a sensitive method applying liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) to quantitate the endogenous steroids in human SC harvested by tape-stripping technique,[13,14] and to investigate their distribution with respect to sex, age, anatomical site, and depth into the SC.

Materials and methods

Chemicals and reagents

Hydrocortisone and cortisone were supplied by Sigma Chemical Co. (St. Louis, MO, USA), whereas testosterone was purchased from Fluka (Buchs, Switzerland). LC–MS grade methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile (Lab-Scan, Rongmueng, Pathumwan Bangkok, Thailand), formic acid (Riedel-deHaën, Seelze, Germany) and water (Alps Chem Co., Taipei, Taiwan) were of LC grade. For extraction of steroids, dichloromethane was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Sodium hydroxide (Showa, Tokyo, Japan) and hydrochloric acid (Riedel-deHaën) were also used.

Participants

Sixteen Chinese males and 16 Chinese females were recruited in this study. All individuals had no known skin disorders that needed topical corticosteroid treatment at the time of the study, and were not taking any medication for at least 1 month prior to the study. After the volunteers signed the informed consent form approved by the Institutional Review Board for Human Research, National Cheng Kung University Hospital, they were categorized into two groups according to their ages: Group 1 (age 20–35 years), and Group 2 (age 50–65 years). Each group included 8 males and 8 females, coded as Nos. 1–16, respectively.

Collection of SC samples

Forehead, ventral forearm, and back were selected as the test sites. Participants were instructed not to apply any soap or cosmetics to those sites for at least 12 hours prior to the study. On the day of the study, the skin sites were cleaned with tissues and marked with a marker pen and the tapes were consistently applied to the same area. Booktape (Scotch 845; 3M Co., St Paul, MN, USA) was used in the study, provided on a roll and cut into pieces of 2 cm × 2 cm to give a surface area of 4 cm.[2] The tape was pressed onto the skin site at the forehead, forearm, and back with five constant-pressure strokes and was removed with a forceps in one swift movement. The procedure was repeated 20 times and the tape-strips were divided into five, seven, and eight strips per glass tube on each site. Two volunteers were selected to investigate the effect of sample collection time on the amounts of endogenous steroids detected in the tape strips. The second and third collection of SC samples was conducted at approximately 1-month intervals between each collection at the same three sites using the above-described procedure. Throughout the entire study, tape stripping of the SC was performed by the same operator, which typically removed approximately 70–80% of the SC.

Determination of SC protein content

The protein content of SC removed by 20 tape strippings from three individuals at different skin sites was evaluated to validate tape-stripping techniques. The assay was based upon the Bradford dye reaction (BioRad Laboratories, Hercules, CA, USA).[1,15] Each sample was incubated with 2 mL of 1N NaOH and shaken in an incubator shaker overnight. After neutralization with 2 mL of 1N HCl and centrifugation, 200 μL of the supernatant was pipetted into a plastic tube, to which 600 μL deionized water and 200 μL of the dye reagent were added. After 5 minutes, the optical density of the sample was measured at 595 nm on a spectrometer (Varian Cary 50; Mulgrave, Victoria, Australia). A standard curve was prepared with lyophilized bovine serum albumin in concentrations ranging from 5 μg/mL to 30 μg/mL.

Sample extraction

An aliquot of 0.3 mL/tape of reporter lysis buffer (Promega Corporation, Madison, WI, USA) was added to each glass tube containing five, seven, or eight strips, and gently rotated overnight. The solution was extracted twice with dichloromethane/water (1/1, v/v). The organic layer was collected and filtered through 0.22 μm syringe filter (Chrom Tech, Apple Valley, MN, USA). The sample solution was evaporated to dryness and stored at −20°C until assayed. On the day of analysis, the residues were re-suspended in 100 μL of methanol, and 40 μL of each sample was then injected into the LC–MS/MS. Endogenous steroid content in the SC expressed in ng/cm^2 was calculated as follows: ng/cm^2 = concentration obtained from calibration curve in μg/mL / 4 cm^2 × 0.1 mL × 1000.

LC–MS/MS

We have adapted the LC–MS/MS method from Hauser et al.[16] An Alliance 2695 separation system from Waters Co. (Milford, MA, USA) was used. Separation was achieved at 20°C on a Cosmosil 5C18-AR II column (4.6 mm × 150 mm, 5 μm, Nacalai Tesque Inc., Tokyo, Japan). Eluent A was composed of water/acetonitrile (95/5, v/v) and eluent B of water/acetonitrile (5/95, v/v), both containing 0.1% formic acid. Elution was performed at a flow rate of 0.5 mL/min starting from 30% B (0–2 minutes), linearly increased to 70% B (2–10 minutes), and returned to 30% B (10–15 minutes). The analysis time for a sample was 15 minutes. Sample analyses were carried out on a Quattro Premier XE tandem mass spectrometry equipped with an electrospray ionization interface (Waters Co.). Nitrogen was used as desolvation and cone gas, with flow rates of 700 L/hour and 50 L/hour, respectively. Source and desolvation temperatures were set at 120°C and 350°C, respectively. The electric potential applied on the capillary was 3.0 kV, and the sample cone voltage was set individually for each compound. Argon was used as collision gas at a pressure of 1.0×10^{-4} mbar and a collision energy setting adapted for each compound. Steroids were detected using multiple reaction monitoring (MRM) of the two most abundant product ions per compound. Dwell time for each transition was 25 ms. Data processing and quantitation were performed by the MassLynx 4.1 (Waters Co.).