Immunolocalization of 5α-Reductase Isozymes in Acne Lesions and Normal Skin

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Background: Dihydrotestosterone mediates androgen-dependent diseases, such as acne, hirsutism, and androgenetic alopecia. This hormone is produced from testosterone by the 5α-reductase enzyme. There are 2 isozymes of 5α-reductase (types 1 and 2) that differ in their localization within the body and even within the skin. Activity of the type 1 isozyme predominates in sebaceous glands, where it may be involved in regulation of sebum production. Since specific inhibition of 5α-reductase type 1 may represent a novel therapeutic approach to acne, it is important to define the localization of these isozymes in normal sebaceous follicles and acne lesions.

Observations: Skin biopsy specimens were obtained from the backs of 11 subjects: 8 with acne and 3 without acne. Sections of normal follicles, open comedones, closed comedones, and inflammatory lesions were incubated with antibodies to types 1 and 2 5α-reductase. In all samples, the type 1 antibody localized specifically to sebaceous glands, and the type 2 antibody localized to the companion layer of the hair follicle (the innermost layer of the outer root sheath) and granular layer of the epidermis. Localization of the type 2 isozyme was also noted within the walls of open and closed comedones and in endothelial cells from sections of inflammatory lesions.

Conclusions: The immunolocalization of 5α-reductase isozymes in normal sebaceous follicles and acne follicles is similar to the pattern described in terminal hair follicles and corresponds with the findings of biochemical studies that have demonstrated predominance of type 1 activity in sebaceous glands. The function of type 2 5α-reductase in comedones or endothelial cells in inflammatory lesions is unknown.

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The enzyme 5α-reductase converts testosterone to dihydrotestosterone. It may play an important role in androgen-mediated conditions of the skin, such as acne, hirsutism, and androgenetic alopecia. There are 2 forms of 5α-reductase that differ in their tissue localization and biochemical characteristics.1 The type 2 isozyme predominates in prostate tissue and has been localized to certain regions of terminal hair follicles.2-4 It is inhibited by the drug finasteride. In contrast, the type 1 isozyme localizes predominantly within sebaceous glands.4-6 Higher levels of type 1 5α-reductase activity are detected in glands derived from acne-prone areas of skin, such as the face, compared with glands from other areas, such as the legs or arms.5

The specific localization and activity of the type 1 isozyme in sebaceous glands makes this isozyme a potential therapeutic target in the treatment of acne. Inhibition of the type 1 isozyme may represent a means of blocking the local production of dihydrotestosterone within sebaceous glands. This inhibition in turn may reduce sebum production and improve acne, provided that sebum production is mediated largely by dihydrotestosterone and not by testosterone.

The distribution of 5α-reductase isozymes within sebaceous follicles and acne lesions has not been described. Since inhibitors of type 1 5α-reductase may be useful in the treatment of acne, it becomes important to identify the specific sites within sebaceous follicles, where these agents may act. The purpose of this study was to determine if the distribution of 5α-reductase isozymes in normal sebaceous follicles and acne lesions differs from that previously reported in normal terminal hair follicles.

The therapeutic armamentarium for the treatment of acne includes keratolytics, benzoyl peroxide, retinoids, antibiotics, and hormonal therapies, such as estrogens, oral contraceptives, glucocortico-
SUBJECTS, MATERIALS, AND METHODS

SUBJECTS AND SAMPLE PROCESSING

After approval from the institutional review board of the Pennsylvania State University College of Medicine, Hershey, a total of 8 patients with acne lesions on the back and 3 subjects without acne were recruited for this study. Informed consent was obtained, and each subject underwent a 5-mm punch biopsy of back skin containing an acne lesion or normal skin. Biopsy sites were closed with a single interrupted suture. Skin samples were embedded in tissue-freezing medium, frozen over liquid nitrogen, and stored at −80°C until the time of sectioning.

MONOCLONAL ANTIBODIES TO TYPES 1 AND 2 5α-REDUCTASE

Mouse monoclonal antibodies to types 1 and 2 5α-reductase used in this study were previously described and characterized. Briefly, monoclonal antibodies were derived from Balb/c mice that were immunized with a recombinant peptide corresponding to the N-terminal half of the either the human type 1 5α-reductase or the human type 2 5α-reductase. Antibody specificity was confirmed by reaction with mouse kidney cells (COS) transfected with type 1 or type 2 complementary DNA.

IMMUNOHISTOCHEMICAL ANALYSIS

Eight-micron-thick frozen sections of skin samples were cut and mounted on coated glass slides. The sections were fixed, pretreated, blocked, and incubated with either type 1 or type 2 antibody, as previously described. Bound antibodies were detected by immunoperoxidase microscopy using the avidin-biotin complex method. Peroxidase reaction product was developed with a glucose oxidase–diaminobenzidine–nickel method. Sections were counterstained with hematoxylin-eosin. Negative controls were run using nonimmune mouse IgG in place of the primary antibody. As a control for inflammatory acne lesions, sections of skin containing dermatitis were incubated with types 1 and 2 antibodies. Also, blocking experiments were run in which the antibodies were preincubated with the relevant synthetic peptide or an irrelevant control peptide to confirm antibody specificity.

OBSERVATIONS

5α-REDUCTASE LOCALIZATION IN SEBACEOUS FOLLCLES FROM NORMAL SKIN

In the 3 subjects with clinically normal skin, the majority of follicles contained a mild degree of hyperkeratosis. Antibodies to type 1 5α-reductase specifically localized to the sebaceous glands of sebaceous follicles from normal skin (Figure 1A). Reactivity was most pronounced at the periphery of the gland but was noted throughout the sebaceous gland in most sections. No localization within hair follicles was noted.

Antibodies to type 2 5α-reductase localized to the companion layer (innermost layer of the outer root sheath) of sebaceous follicles and sebaceous ducts (Figure 1B). Immunoreactivity was also noted in the granular layer of the epidermis and in the sheath of myelinated cutaneous nerves (not shown). No staining within sebaceous glands was noted with type 2 antibody.

With both types 1 and 2 antibodies, immunoreactivity was blocked when primary antibodies were preincubated with their corresponding peptides type but not when antibodies were incubated with irrelevant peptides (data not shown).

5α-REDUCTASE LOCALIZATION IN ACNE LESIONS

The pattern of immunoreactivity in acne lesions was similar to that observed in normal sebaceous follicles. The type 1 antibody localized to sebaceous glands in normal sebaceous follicles (Figure 1). Immunolocalization of the type 2 antibody was noted within the sebaceous follicle and sebaceous duct but not within the sebaceous gland (Figure 1). In sections of closed and open comedones, type 1 immunoreactivity was noted only in remnants of sebaceous glands and not within follicles (Figure 2). The type 2 antibody localized within the walls of both open and closed comedones and was noted to extend into keratinized cells contained within the lumen (Figure 3). Immunoreactivity was not noted in follicles or any other epidermal or dermal structures (Figure 4). Immunoreactivity was most pronounced within basal sebocytes and least evident in highly differentiated sebocytes in proximity to sebaceous ducts (Figure 4). Localization of the type 2 antibody was noted within the granular layer of the epidermis, companion layer of the follicle, sebaceous duct, and vascular endothelial cells of inflammatory lesions (Figure 5).

Type 2 immunoreactivity was not noted in endothelial cells in control sections of dermatitic skin (Figure 6).

steroids, and spironolactone. Apart from isotretinoin and hormonal therapies, there are few agents that can effectively reduce sebum production. Since sebum production is regulated by dihydrotestosterone, inhibitors of type 1 5α-reductase may form a new therapeutic class of agents designed to treat acne. For this reason, it becomes important to understand where and how the various iso-enzymes of 5α-reductase act in the skin.

The immunolocalization of 5α-reductase isozymes has been reported in skin from the scalp, lips, scrotum, and pubic region, but not in regions of skin affected by acne. These studies were performed using various polyclonal antibodies raised in rabbits and mouse monoclonal antibodies. In each of these studies, immunoreactivity with the type 1 antibody was noted in sebaceous glands, and reactivity with the type 2 antibody was noted in hair follicles. Localization of type 1 antibody has also been reported in the epidermis, sweat gland duct, dermal papilla, outer root sheath of hair follicles, endothelial cells, and myelin sheath of cutaneous nerves. Immunoreactivity in sweat glands and in the cuticle of the hair follicle and weak reactivity in the basal layer of sebaceous glands have also been reported with type 2 antibody. The apparent discrepancies among the different studies may in part be explained by differences in methodology or by different antibody recognition sequences.
The pattern of immunolocalization of 5α-reductase isozymes within normal sebaceous follicles and acne lesions in the present study was similar to that observed in terminal hair follicles from the scalp using the same mouse monoclonal antibodies. Immunolocalization of type 1 5α-reductase within sebaceous glands in acne skin specimens corresponds to the findings of biochemical studies that have demonstrated a predominance of type 1 activity in sebaceous glands of normal subjects and subjects with acne. Furthermore, studies have shown that oral administration of a type 1 5α-reductase inhibitor results in a dose-dependent suppression of serum and sebum levels of dihydrotestosterone, without affecting levels of dihydrotestosterone found in semen. These data suggest that inhibition of type 1 5α-reductase may represent an alternative means of reducing sebum production if this process is regulated by dihydrotestosterone that is produced locally within the sebaceous gland.

Questions have been raised as to whether finasteride, an inhibitor of type 2 5α-reductase, is beneficial in the treatment of acne. It is, however, the type 1 isozyme that clearly predominates within sebaceous glands. If sebum production is significantly regulated by locally produced dihydrotestosterone, it would be unlikely that finasteride would influence sebum production. In fact, it has been shown that there was no decrease in sebum production in men treated with 5 mg of finasteride daily. These data are further supported by the observation that although oral administration of 5 mg of finasteride daily for 2 weeks resulted in a substantial reduction in serum and semen dihydrotestosterone levels, only a modest reduction in sebum dihydrotestosterone levels was noted. Approximately 70% to 80% of serum dihydrotestosterone in men is produced by the type 2 isozyme, and 20% to 30% of circulating dihydrotestosterone is produced by the type 1 isozyme. Alternatively, if serum levels of dihydrotestosterone are more important than sebum dihydrotestosterone levels in regulating sebum production, dual inhibition of types 1 and 2 5α-reductase would...
be most effective in lowering serum dihydrotestosterone levels.

Although the findings of immunolocalization and biochemical studies are in agreement regarding the predominance of type 1 5α-reductase in sebaceous glands, differences in the patterns of isozyme expression in hair follicles were noted depending on the methods used. In the present study and a previous immunolocalization study of normal terminal scalp follicles, contiguous type 2 reactivity was noted extending from the granular layer of the epidermis through the infrainfundibulum, infundibulum, sebaceous duct, and lower follicle. No type 1 localization in follicles was noted. However, in vitro biochemical studies in skin samples from subjects with and without acne have demonstrated that activity of type 1 5α-reductase predominates in the follicular infrainfundibulum. The reasons for this discrepancy are not apparent. One hypothesis is that type 1 enzyme activity may
be favored over type 2 activity in an in vitro biochemical system in which enzyme cofactors are supplied in excess. Alternatively, it is possible that epitopes of the type 1 antibody might be masked in tissue and therefore not apparent in tissue sections.

An interesting observation is the localization of the type 2 isozyme of 5alpha-reductase in the walls of both open and closed comedones and in endothelial cells surrounding inflammatory acne lesions. This localization parallels that seen in similar regions of normal follicles. The effect of 5alpha-reductase activity on comedones is not known. It has been suggested that androgens may play a role in the follicular hyperkeratinization seen in acne.12-14 Although a cause-and-effect relationship between androgens and follicular hyperkeratinization certainly cannot be drawn from immunohistochemical findings, localization of 5alpha-reductase within the comedonal wall provides a rationale for research in this area.

The significance of type 2 5alpha-reductase activity in vascular endothelial cells contained within the inflammatory infiltrate of acne lesions is also unknown. In studies of gingival fibroblasts, increasing concentrations of dihydrotestosterone inhibited the expression of interleukin 6.15 Cytokines are known to play a role in inflammatory acne. Perhaps in inflamed lesions, activity of type 2 5alpha-reductase may be stimulated in endothelial cells to increase local production of dihydrotestosterone and hence down-regulate cytokine expression. Additional studies are clearly needed to elucidate the role that 5alpha-reductase may play in sebum production, comedogenesis, and inflammatory acne.

In terms of clinical relevance, these data clearly support the finding of type 1 5alpha-reductase in human sebaceous glands in both normal skin and inflammatory acne lesions. If specific inhibition of type 1 5alpha-reductase decreases sebum production, then drugs of this class may be beneficial in the treatment of acne.

References