Spontaneous Comedones on the Skin of Hairless Descendants of Mexican Hairless Dogs

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Abstract: In the first experiment, the skin sebum and humidity, perspiration ability of sweat glands, and histology of spontaneous comedones were examined in hairless descendants of Mexican hairless dogs. The skin of females showed lower humidity than that of males. Some animals with a large number of comedones exhibited remarkably high skin sebum scores. The comedones were distributed throughout the dorsal skin, and a cluster of lesions was found mainly in the limbs and prepuces. The sweat glands showed no perspiration in the sudorific test. Histologically, both infant and adult animals had lesions of micro- and/or "blackhead" comedones. Plugged follicles containing abundant keratic substances associated well-developed sebaceous glands. Spontaneous comedones in the skin of hairless dogs were grossly and histologically similar to the acne vulgaris observed in human beings. The skin of some adult animals showed a large number of protrusive comedones which were solid cystic structures containing organized substances. In the second experiment, three kinds of antiacne agents (sulfur and camphor, sulfur and resorcinol, and ibuprofen piconol) were applied daily to the test sites for one month. These antiacne agents caused prominent extrusion of keratin plugs from follicular sites. The results suggest that the hairless dogs are a predictive model for evaluating the efficacy of antiacne agents proposed for acne treatment.

Key words: antiacne agents, comedones, hairless dogs, skin humidity, skin sebum

Introduction

The flanks and dorsum of Mexican hairless dogs (MHD) have been observed to contain pigmented keratin follicular plugs which share some clinical and histological similarities to the comedones seen in human beings [2, 15, 28]. As previously reported [9], we have established a colony of experimental hairless dogs

derived from MHD. During a series of breeding trials, these hairless dogs still had so-called "blackhead" comedones on the skin surface.

It is assumed that there is a definite causal relationship between the sebum content and the development of human comedones. In addition, it is hypothesized that certain constituents of sebum may directly elicit human comedones by exciting hyperkeratinization of

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the follicular epithelium [1, 11]. Nevertheless, because of the lack of suitable animal models showing spontaneous comedones except for rhino mice [13], experimental evaluations of antiacne treatment rely on chemically induced comedones (a rabbit ear model) [11, 12, 17] and/or especially well-developed sebaceous glands in laboratory animals such as Syrian hamsters [7, 21, 22], rats and mice [3], and *Suncus murinus* [8, 14, 19].

In the present study, we first examined the skin sebum and humidity, perspiring ability of sweat glands, and distribution and histologic nature of comedones in hairless dogs. Secondly, in an endeavor to evaluate the usefulness of hairless dogs for acne investigations, a study was conducted to examine preparations currently employed to treat clinical acne in human beings.

Materials and Methods

Experiment 1

Dogs: Thirty-one hairless hybrids (F1, BCF1, BCF2, BCF3, and BCF4) of both sexes were selected from our colony crossed male MHD descendants and female Beagles (Table 1). Their size and weight varied from 0.5 to 18 kg and their age from 3 weeks old to 8 years old. The dogs were individually housed in stainless steel cages (90 × 90 × 90 cm) in an animal room environmentally controlled at $25 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ relative humidity with 10 to 15 exchanges of 100% fresh air/h and a 12-hr light (7AM to 7PM) and 12-hr dark (7PM to 7AM) cycle. They were fed a commercial dry dog food (Labo D Standard, Nihon Nosan Kogyo Co., Ltd.) and water *ad libitum*.

Skin sebum and humidity: The Corneometer CM 420 (Courage + Khazaka electronic GmbH Co., Ltd.), based

on the principle of constant dielectric measurement, was used to assess the hydrating activity of the skin surface [4, 5]. The values are given without denomination as AE (German "Anzeige-einheiten"). The mean value of 3 recordings obtained at intervals of 5 seconds was used in assessing skin hydration.

The sebum level was determined by using a Sebumeter SM 810 PC (Courage + Khazaka electronic GmbH Co., Ltd.), an instrument that measures the quantity of sebum superficially present on the skin [16, 24, 26]. The Sebumeter shows the sebum level in $\mu\text{g}/\text{cm}^2$. The determination of the sebum was repeated 3 times for each dog, and then its mean value was calculated.

Sudorific test [23, 25]: The aforementioned hairless dogs (Table 1) were used. Two % tincture of iodine was applied to the skin over the dorsum of hairless dogs under room temperature at 40°C . After making the surface of the treated skin dry, a soluble starch-olive oil suspension (50 g soluble starch/100 g olive oil) was applied to the same sites. In 1 to 2 min, the perspiration reaction was indicated by the development of deep violet spots which corresponded with the openings of sweat glands.

Histological examination of the skin: Tissue specimens were obtained from the comedo lesions on the skin over the dorsum of each dog with a 6-mm biopsy punch (Nagatoishi Co., Ltd., Tokyo) under local anesthesia with 0.5% procaine in an annulus surrounding the 6-mm biopsy site. The skin specimens were fixed in 10% neutral buffered formalin, and 4- μm paraffin sections were stained with hematoxylin and eosin (HE) and toluidine blue (TB) and by Fontana-Masson's (FM) method.

Distribution of comedones: The distribution of comedones was observed on the skin of the whole body, and the result was represented diagrammatically.

Experiment 2

Dogs: Three 5-year-old male F1 hairless hybrids (a male MHD × female Beagles) and two 4-year-old female BCF1 hairless hybrids (male F1 hairless dogs × female Beagles) were used.

Procedures: Antiacne agents were as follows: 1. Sulfur and camphor lotion ® (6.0% sulfur, 0.5% dl-camphor, Maruishi Seiyaku Co., Ltd.), 2. Clearacil ® (3.0% sulfur, 2.0% resorcinol, Proctor and Gamble

Table 1. Dogs examined

	Males	Females
F1	4	1
BCF1	4	7
BCF2	3	1
BCF3	3	3
BCF4	4	1
Total	18	13

Health Care Co., Ltd.) and 3. Staderm ointment ® (5.0% ibuprophen piconol, Torii Yakuhin Co., Ltd.). Test sites (3 × 3 cm) with grossly evident acne lesions were selected on the skin over the dorsum and cervix. Antiacne agents were applied with 4 $\mu\text{l}/\text{cm}^2$, gently rubbed over the test sites. The test sites were treated once a day for a month. After a month of the treatment, the treated sites were compared with the untreated lesions of comedones. In our other studies, we demonstrated that the histological findings of the untreated skin did not differ from those of the skin treated with each vehicle for 2 to 3 months.

Histological examination of the skin: Before the treatment and after completion of the experimental course, biopsy specimens were taken with a 6-mm punch for histological evaluation. Each dog was pretreated with medetomidine which has sedative effects (Domitol ®, Meiji Seiyaku Co., Ltd.). The tissue specimens were examined in the aforementioned manner.

Statistical analysis: All values were expressed as the mean $\pm$ SD, and statistical analysis was performed by Student's *t*-test.

Results

Experiment 1

Skin sebum and humidity: There were no significant differences in skin humidity between each generation, but the skin of females had lower values than that of males (Fig. 1). On the other hand, there were no marked differences in the skin sebum content between males and females (Fig. 2), but the skin sebum showed apparent individual differences and some hairless dogs had a score over 150. Skin with a score over 50 had a large number of comedones. The distribution of comedones is shown diagrammatically in Fig. 3. The large "black-head" type comedones developed on the dorsal aspect of the trunk, and they partially congregated in the skin of limbs and prepuces.

Sudorific test: The skin of hairless dogs showed no sign of perspiration (Fig. 4). Iodo-starch reaction was not detected in their skin surface.

Histological findings of the skin: The skin of the 3-week-old infant hairless dogs contained microcomedones. In sebaceous follicles, the follicular ducts had thick and short structures and were obstructed

by hyperkeratotic plugs. Formation of comedones seemed to be associated with excess keratin plugging of the follicular infundibula and narrow follicular ostia. All follicular ducts including the infrainfundibulum showed signs of hyperkeratosis associated with well-

Fig. 1. Skin humidity in hairless dogs (●: Male, ○: Female). Bars represent the standard deviation from the mean.

Fig. 2. Sebum content in hairless dogs (●: Male, ○: Female). Bars represent the standard deviation from the mean.

developed sebaceous glands. Hair follicles included abundant contents and resulted in so-called "retention hyperkeratosis". No inflammatory reaction was observed in any portion of the lesions (Fig. 5).

In 5-year-old hairless dogs, the skin contained follic-

Fig. 3. The distribution of comedones on the skin of hairless dogs.

Fig. 4. Sudorific test in a hairless dog. No sweat secretion is detected.

ular structures filled with black keratogenous materials, much like the "blackheads" observed in human acne (Fig. 6). Histologically, these plugged follicles seemed to be equivalent to invaginations of the epidermis, and they had large cystic structures (dermal cysts) with sebaceous glands and clusters of nevus-like cells at their bases. The follicles expanded prominently and their contents consisted largely of keratic substances. The comedones had dilated, cystic structures lined by thinned and flattened epithelial cells. There was no inflammatory reaction. The skin of some adult hairless dogs (over 3 years old) had a large number protrusive lesions (Fig. 7). These lesions were aged comedones, and histologically they were solid cystic structures containing organized substances (Fig. 8).

Fig. 5. Dorsal skin of a 3-week-old hairless dog. Microcomedones showing follicular hyperkeratosis and proliferation of sebaceous glands. HE, $\times 350$.

Fig. 6. Dorsal skin of a 5-year-old hairless dog. Enlarged comedones with retention of keratin and sebum. HE, $\times 55$.

Fig. 7. Lumbar skin of a 5-year-old hairless dog. Gross appearance of aged comedo lesions.

Experiment 2

Three kinds of antiacne agents were applied daily to the test sites for a month. The test sites treated with antiacne agents (sulfur and resorcinol) developed gross excess removal of surface lipids with dirt. As a result, the stratum corneum was desquamated and the skin surface became rough and showed signs of a mild erythematous reaction. During the experiments, none of the antiacne agents decreased the number of comedones, but these antiacne agents caused marked extrusion of keratin plugs from follicular sites.

Histological findings in the skin: There were no changes in the size of hair follicles, but keratinized plugs which occluded follicular ostia of comedones were opened (Fig. 9). Such changes were common to the skin treated with 3 kinds of antiacne agents. The treatment with antiacne agents (sulfur and camphor, and sulfur and resorcinol) produced exfoliation of keratin layers.

Fig. 8. Microscopic photograph of the aged comedo shown in Fig. 7. The comedo contains a large quantity of organized substances. HE, $\times 45$.

Fig. 9. A comedo treated with antiacne agents. The head of the comedo is open. HE, $\times 90$.

Discussion

Many difficulties have been encountered in measuring the water and the sebum contents on the skin surface *in vivo* [20]. Newly simplified instruments (Corneometer and Sebumeter) have recently been used to assess the hydration and/or sebum in the field of dermatology and cosmetic science [4, 5, 16, 24, 26]. In this study, we determined the sebum and humidity on the skin surface of hairless dogs with Corneometer and Sebumeter. The results of skin humidity readings revealed that the skin of hairless dogs is equivalent to the dry type skin of human beings. This skin feature of hairless dogs seems to be associated with no perspiration on their skin. As Yankell *et al.* [28] pointed out, the skin care of hairless dogs requires that adequate attention be paid to maintenance of room temperature and humidity.

The oily type skin of hairless dogs which had a score over 50, contained numerous comedones. This finding revealed that the quantity of sebum secretion in the epidermis was closely associated with the development of comedones. Bedord *et al.* [2] reported that the lipids

obtained from the acne lesions of hairless hybrids (MHD $\times$ Beagles) were primarily epidermal in origin and that sebaceous gland contribution was minimal in plugged follicles. This result also showed that the oily type skin of hairless dogs is predisposed to the occurrence of comedones.

Sudorific tests demonstrated that there was no perspiration on the skin surface over the dorsum of hairless dogs. This showed that the sweat glands distributed on the trunk of hairless dogs are identical to the apocrine glands detected on the dorsal skin of hairless dogs.

Histological examinations showed that the skin of 3-week-old hairless pups contained some microcomedones. It has been recognized that the activity of sebaceous glands of human beings is enhanced at the 31st to 40th day after birth, and that human neonates at this stage are prone to develop acne-like lesions and/or seborrheic dermatitis [27]. The histopathologic features of comedones found in the skin of hairless pups resembled those of acne neonatorum. The lesion showed hyperkeratosis of hair follicles and enhanced secretion of sebaceous glands and it contained a large amount of keratic substances and sebum. These findings were in accordance with those of acne vulgaris of human beings. In the rabbit ear model, the histological structure of this pilosebaceous unit differs anatomically from that of human skin. Chemically induced comedones of the rabbit ear exhibit many erythematous indurated lesions, accompanied by diffuse infiltration of mononuclear and polymorphonuclear cells around the hair follicles [12, 17]. The formation of human acne is related to enhanced sebum secretion, depending on the following endocrine factors: testosterone derived from the testes, and dehydroepiandrosterone and androstenedione synthesized in the ovaries and adrenal glands [1]. Franz *et al.* [7] reported that the sebaceous gland of the hamster flank organ was apparently more sensitive to antiandrogens than the human sebaceous gland. In the comedones of hairless dogs, we could not elucidate this point.

When aged comedones were palpated, their contents were gradually absorbed and organized. These lesions were characteristic of the skin found in hairless dogs. It is known that eruptive vellus hair cysts are infundibular cysts resulting from abnormal development of follicles [6]. These cystic lesions tend to originate frequently in the skin of the frontal chest and four limbs

and contain cheese-like substances including a large number of vellus hairs. The comedones of hairless dogs may have some associations with abnormal development of hairs.

All the 3 antiacne agents showed efficacy in preventing or alleviating keratin invagination. These antiacne agents seemed to open follicular plugs of comedones and to facilitate the extrusion of keratin substances from follicular sites. It is well known that sulfur agents cause some desquamation of the stratum corneum and remove excess sebum from the skin surface. These sulfur agents have long been used as antiacne agents [18], but they have some adverse effects such as drying, flaking and tension of treated sites. In the present study, these adverse effects were also detected on the treated sites of hairless dogs. Non-steroid antiinflammatory agents did not have such adverse effects and they were recognized to be effective drugs for treatment of comedones.

The mechanism for initiation of these comedones is presumed to be as follows: in the course of the development of hairs, the vellus hairs fail to differentiate into terminal hairs. Follicular ducts are occluded by sebum derived from the epidermis, accompanied by hyperplasia of sebaceous glands and hyperkeratosis of follicular epithelium. Following these changes, fragmentation of hairs and retention of hyperplastic substances seem to be provoked. Such follicular changes may have some association with the formation of comedones. In addition, it is assumed that melanin granules derived from hyperkeratotic substances and follicular epithelium have the appearance of "black-head comedones". These results revealed that hairless dogs developed spontaneous comedones in the skin over the trunk, and that they were a predictive model for evaluating both the efficacy and potential adverse effects of antiacne agents proposed for acne treatment.

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