Plantar Hyperkeratosis: A Study of Callosities and Normal Plantar Skin

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Although callosities of the plantar skin are common and often disabling, little is known of their pathology or the reasons for their persistence. In this study plantar epidermal structure and cell renewal were investigated in patients with callosities and normal, age-, sex- and site-matched control subjects. Tritiated thymidine autoradiographic labeling indices were increased in the calluses but the dansyl chloride fluorescence clearance time was prolonged, reflecting the increased thickness of the stratum corneum. The number of corneocytes that could be removed from the surface of callosities by a standardized stimulus was considerably increased compared to controls but after adhesive tape stripping no such increase was observed. The density of corneocytes as measured on Percoll gradients was decreased in corneocytes from callus compared to normal plantar skin, and their volume was increased. These observations suggest that there are differences in epidermal differentiation due to an increased rate of epidermal cell production in plantar skin affected by callosity.

It is curious that the ability to stand, walk, and run efficiently depends on the proper function of a tiny proportion of the skin's surface. Healthy plantar skin is vital to our well-being. If it is blistered, fissured, or severely calloused the resulting discomfort may completely incapacitate the individual concerned.

Little is known of the pathology or pathophysiology of plantar callosities and the majority of the few reported studies have centered on the use of this material as a convenient source of horn for investigations of the biomechanics of stratum corneum or studies of its biochemical composition [1-5]. During normal keratinization, viable epidermal cells differentiate into horn cells which are lost from the surface of the stratum corneum during desquamation. In some conditions there is a failure of the normal loss of binding forces between corneocytes near the surface, and a build-up of these cornified cells, leading to hyperkeratosis. However, the details of the alterations in the epidermis and stratum corneum that take place and lead to hyperkeratosis are unknown.

Thickening of the stratum corneum after injury may be regarded as a purely physiologic process in which the thickening is a protective response to the mechanical trauma. If the traumatic stimulus is removed, the stratum corneum reverts to normal. In contrast, in plantar callosities, the hyperkeratosis tends to persist even when the mechanical trauma has been removed. The persistent hyperkeratosis may be painful, even disabling, and is resistant to present-day treatments [6].

The investigations reported here have concentrated on documentation of the differences in the epidermis and stratum corneum between callosities and normal plantar skin in normal age- and sex-matched control subjects.

MATERIALS AND METHODS

Patients

Twenty-six patients (6 male, 20 female, aged 22-68 years) attending the Chiropody outpatients clinic at the University Hospital of Wales, who all had callosities (as distinct from corns or calvarus) of the mid region of the sole of the foot, were studied. In addition, the same regions of the soles of 26 age- and sex-matched normal volunteer subjects were studied. Both patients and controls had given their informed consent.

Materials

Dansyl chloride was purchased from Sigma Chemical Co. Ltd., (Poole, U.K.). Percoll and density marker beads were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Azone was kindly donated by Nelson Research (Irvine, California). [3H]Thymidine (sp act 25 Ci/mmol) was purchased from Amersham International PLC (Ameresham, U.K.). Eagle's minimal essential medium was purchased from Wellcome Research Ltd. (Beckenham, U.K.). All other reagents were of Analar grade and were purchased from BDH Chemicals Ltd. (Poole, U.K.).

Collection of Corneocytes

Individual corneocytes were obtained by gently abrading the skin surface with a rotating perspex paddle set at a constant pressure (torque 2 g cm) using an electrically driven desquamator (Cutex Ltd., U.K.) in the presence of phosphate buffer pH 7.4 containing 0.1% Triton X-100 [7]. The corneocyte suspension was collected after a 10-s application of the device.

The number of cells released from the skin were counted using a modified Fuchs Rosenthal hemacytometer and the number of cells expressed per cm² of skin surface area.

Measurement of corneocyte area: A few drops of the corneocyte suspensions were smeared onto glass slides and air dried. Three slides prepared from each area were studied. The surface area of individual cells was determined using a Nikon 102 microscope with a projection side arm. Seventy corneocytes were measured from each site. Calculation of the mean corneocyte area was determined using a digitizer tablet linked to an HP98 computer.

Measurement of corneocyte modal volume: Corneocyte volumes were measured using a Coulter Counter (model ZBI) with an aperture of 100 μm and a size frequency module (channelyzer) which was calibrated using latex beads (diameter 13.1 μm).

Measurement of corneocyte density: Buoyant corneocyte densities were determined using isosmotic Percoll. Stock isosmotic Percoll in saline was diluted (9.5:0.5, vol:vol) with 0.15 M NaCl and corneocyte suspensions (90 μl) were layered onto 10 ml of 95% Percoll. Tubes containing density marker beads were run in parallel and all tubes were centrifuged at 8000 g for 15 min at 4°C. Corneocyte densities were determined from a curve of density against sedimentation distance obtained from gradients containing marker beads.
Measurement of Stratum Corneum Turnover Time

The method used was a modification of the method of Jansen et al [8] employing dansyl chloride (6%) incorporated with 2% Azone (a penetration enhancer) [9] in cetomacrogol base. The skin fluorescence was measured using the comparator method of Marks et al [10]. The dansyl chloride preparation was placed under occlusion on the mid region of the sole in 8 individuals with calluses on this area and 8 normal volunteers. The preparation was renewed 8 times over a 48-h period. Preliminary studies had shown that unless this procedure was adopted, complete penetration of the callus by the dansyl chloride was not attained. This procedure had also been found in previous studies not to affect the titrated thymidine autoradiographic index of epidermis in normal forearm skin.

Determination of Epidermal Labeling Index and Stratum Corneum Thickness

Four millimeter-diameter trephine biopsies were obtained from 4 individuals with calluses and 5 normal, healthy, age- and sex-matched volunteers without calluses, who had given their informed consent. The biopsies were divided into two. Half was placed in Tissue Tek medium, frozen in hexane, and later sectioned in a motorized cryostat. The sections were then either fixed and stained with hematoxylin and eosin or processed for determination of stratum corneum thickness using a modification of the method of Christophers and Kligman [11]. Briefly, cryostat sections (7 μm) were air dried, washed in 70% alcohol for 5 min, stained for 2 min with methylene blue (0.5%), washed, and mounted in Sorensen Walbum's buffer pH 11.5. Slides were viewed under a light microscope and the number of corneocyte layers within the stratum corneum counted. An estimate of the nucleated suprabasal epidermal cell population was made in vertical hematoxylin and eosin-stained sections. The number of nuclei immediately below a 10 μm length of granular cell layer was determined at regular intervals across each section. At least 8–12 fields per section were measured.

The remaining half of the biopsy was incubated with tritiated thymidine and prepared for autoradiography [12]. The sections were stained through the emulsion with hematoxylin and eosin, and the number of labeled basal and suprabasal cells counted and expressed as a percentage of the total number of basal cells.

RESULTS

A comparison of normal plantar stratum corneum and callus is presented in Table I. Corneocyte area was compared in 22 age- and sex-matched individuals with and without plantar hyperkeratosis. No difference was found in the surface area of corneocytes in the two groups.

Corneocyte modal volume was larger in calli, 1526 μm³ compared to 935 μm³ for controls. The mean density of normal plantar corneocytes was 1.1369 g/cm³ while that from corneocytes from calli was 1.1240 g/cm³. These differences, although small, were statistically significant (p < 0.001) using the unpaired Student's t-test. (Normal forearm corneocytes had previously been found to have a mean buoyant density of 1.1006 g/cm³). The stratum corneum of plantar hyperkeratosis was much thicker (349 cell layers) than normal plantar skin (123 layers).

The number of cells released during stimulated desquamation was increased in the group with calli (118 ± 56 X 10⁶) compared to the control group (16.8 ± 14 X 10⁶). The number of corneocytes released from an adjoining site before and after 15 adhesive tape stripings was also measured in both groups. There was no difference in the numbers released after adhesive tape stripping (10 ± 6.1 X 10⁶) compared to the prestripping value in the control group (16.8 ± 14 X 10⁶), whereas in the patient group the number of corneocytes released during stimulated desquamation (118 ± 56 X 10⁶) was significantly decreased after adhesive tape stripping (18 ± 8.4 X 10⁶) and was reduced to levels found in the control subjects.

Autoradiographic labeling indices (LI) were increased in individuals with plantar hyperkeratosis producing a mean LI of 11.4 ± 1.5 (n = 4) compared to normal plantar skin with a mean LI of 6.26 ± 0.6 (n = 5). The results from areas of callus were significantly different from normal plantar skin using the unpaired Student's t-test (p < 0.001).

The renewal time of the stratum corneum, using dansyl chloride, suggested that the rate of loss of fluorescence in the two groups was similar until most of the fluorescence was lost, at approximately day 16 for the normal group. The loss from calli showed a slower rate of disappearance of fluorescence until day 26 (Fig 1).

Histologic Findings

All normal plantar skin examined showed a similar morphology. The epidermis was thickened compared to trunk and limb skin and was 12–20 viable epidermal cells thick. The suprabasal nucleated cell population was larger in calli (mean 25.5 ± 3.1 cells/10 μm) compared to normal plantar skin (mean 12.16 ± 2.6 cells/10 μm). There was accentuation of the granular cell layer which was often 4 cell layers thick. The rete pattern was exaggerated compared to skin of other sites. Calli demonstrated considerably thickened epidermis which varied among specimens and was more irregular in profile along the lengths of the sections (Fig 2). There were no notable dermal features in any of the samples examined.

DISCUSSION

The present studies have illustrated that differences exist within the epidermis of plantar calli as compared to the epidermis of normal plantar skin. Stimulated desquamation of the stratum corneum released a greater quantity of corneocytes from calli, whereas when the stratum corneum was stripped 15 times with adhesive tape the number of corneocytes released during stimulated desquamation decreased to a level

Table 1. Comparison of normal plantar stratum corneum and calli

<table>
<thead>
<tr>
<th></th>
<th>Normal forearm</th>
<th>Normal plantar</th>
<th>Callus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneocyte area (μm²) (n = 11)</td>
<td>987 ± 63</td>
<td>806 ± 61</td>
<td>770 ± 58</td>
</tr>
<tr>
<td>Corneocyte volume (μm³) (n = 11)</td>
<td>1418 ± 174</td>
<td>935 ± 61</td>
<td>1526 ± 356*</td>
</tr>
<tr>
<td>Corneocyte density (g/cm³) (n = 5)</td>
<td>1.006 ± 0.0036</td>
<td>1.1369 ± 0.002</td>
<td>1.1240 ± 0.0027*</td>
</tr>
<tr>
<td>Number of corneocyte layers (n = 5)</td>
<td>17 ± 1.67</td>
<td>123 ± 12</td>
<td>349 ± 67*</td>
</tr>
</tbody>
</table>

* Significantly different from normal plantar skin (Student's t-test, p < 0.001).
seen in normal plantar skin, which released the same number of corneocytes after this maneuver. This suggests that the corneocytes in the superficial layers of the stratum corneum of calli are less tightly bound together than in normal plantar skin but that this change is confined to the superficial stratum corneum. Normal forearm skin shows a dramatic desquamation with depth [13]. However, normal stratum corneum is 16–20 cells thick as opposed to the 123 layers in normal plantar skin. Changes in intracorneal binding are a complex function of maturation and it is difficult to speculate on the reasons for the changes observed without further information on the biochemical alterations in callused tissue. Clearly the point at which intracorneal cohesion changes in different body sites is influenced by regional differences in thickness of the stratum corneum.

Corneocyte surface area was essentially similar in the two groups studied but the mean volume of corneocytes from callus was increased, indicating that corneocytes from callus are thicker (or rounder) than in normal plantar skin. The density of stratum corneum corneocytes obtained using Percoll gradients produced values similar to those obtained by Weigand et al [14], using cadaver lumbar corneocytes on gradients of sucrose. Plantar corneocytes have a slightly higher density than callus corneocytes, which may indicate that the corneocytes obtained from calli are less well differentiated than normal plantar corneocytes. Normally the density of epidermal cells increases as a function of maturation [15]. The corneocyte volume measurements also support this suggestion.

Forearm corneocytes are less dense than either of the above groups and illustrate that all “normal” body areas are not the same and that an abnormality of corneocytes present in one area should be compared only to normal skin of an equivalent or adjoining site.

The number of cells within the stratum corneum was found to be 2–3 times increased in callosities compared to normal plantar skin. The suprabasal nucleated cell population was found to be twice as large in calli as compared to normal plantar skin. Assuming a steady state, the increase in suprabasal nucleated cell population and stratum corneum cell layers suggests an increase in transit time through the epidermis. The thymidine autoradiographic LI was also found to be increased in calli, suggesting that the epidermis has a higher rate of cell division than normal plantar skin and that the resulting cells do not stay within the epidermis for a sufficient time to mature and differentiate fully.

The rate of renewal of the whole stratum corneum was found to be longer than normal in callus. Normal plantar stratum corneum had a renewal time of 16 days. Callosity cell layers were 2–3 times thicker than normal stratum corneum. Theoretical turnover rate for calli would be 32–48 days if the rate of entry of cells from the viable epidermis remained constant. Experimentally, however, there was a renewal time of 26 days for calli. The calculated rate of loss of stratum corneum cell layers from normal plantar skin is 7.75 layers per day and from callus 13.4 layers per day, i.e., an increase of 1.73 times. This increase in the rate of loss of stratum corneum in callus compared to normal is similar to the increase in LI, i.e., 11.4 compared to 6.26—an increase of 1.82 times.

Mackenzie [16] proposed that the cells of the stratum corneum were less well differentiated after mechanical stimulation due to an increased rate of epidermal cell production and were bound together more strongly. The stratum corneum of friction-stimulated mouse ears was thicker but contained fewer corneocyte layers than controls [16]. This suggested that stimulated ears contained larger corneocytes than did controls. Our findings indicate that callosities too show an increased rate of epidermal cell production and that this may indicate that there is increased traffic of cells. In addition the corneocytes from callosities were less dense and of larger volume, suggesting that they are not fully differentiated.

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REFERENCES

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