Skin Morphology of the Clawn Miniature Pig

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Abstract: Skin morphology of the Clawn miniature pig (CMP) was investigated at the axilla, medial thigh, back and loin. The mean thickness of the epidermis (excluding the corneal layer), the mean number of layers of keratinocytes comprising the epidermis and the mean height of keratinocytes were assessed morphometrically. When observed under a light microscope, the skin of the CMP resembled human skin. Morphometrically, skin from the back and loin of the CMP most resembles human skin. Electron microscopic observations revealed sparse but typical Birbeck granules in the epidermal Langerhans cells of the CMP. The results of the present study indicate that CMP skin is potentially useful as a model for human skin.

Key words: Clawn miniature pig, Langerhans cell, skin morphology

The skin of miniature pigs is considered to be a good model for human skin because it is morphologically, physiologically and pharmacologically similar [6, 11]. Detailed investigations of skin morphology have been performed for the Yucatan miniature pig, and light and electron microscopy studies have revealed a high level of similarity to human skin [3, 4]. In the Göttingen miniature pig, allergic contact dermatitis (ACD) was induced by treatment with 2,4-dinitrofluorobenzene as a hapten, and the pathological and immunopathological changes of the miniature pig ACD were very similar to those that occur in human ACD [14]. However, neither Yucatan nor Göttingen pigs are major laboratory miniature pigs in Japan, whereas the Clawn miniature pig (CMP) is. The CMP was developed in 1978 at Kagoshima University with the aim of establishing an inbred miniature pig for biomedical research, especially transplantation research, and now CMPs are maintained and supplied by the Clawn Institute, Japan Farm [10]. Like the skin of other miniature pigs, CMP skin is expected to be a good model for human skin, however no dermatological investigations have been carried out to date and no morphological data on CMP skin are currently available. Therefore, in the present study, we histologically and morphometrically investigated the skin of the CMP.

We used male and female CMPs that were bred and maintained at the Japan Farm Clawn Institute.
(Kagoshima, Japan) (n=3 for each sex, two males and one female aged 17 months, and one male and two females aged 34 months). All pigs were sacrificed by exsanguination while under xylazine and ketamine-induced deep anesthesia. After sacrifice, skin samples were immediately collected using an 8-mm biopsy punch from each animal at the following sites: axilla, medial thigh, back and loin. Skin samples were fixed in 10% neutral buffered formalin and routinely embedded in paraffin, and 3- and 6-µm thick sections were cut at 30-µm intervals and stained with hematoxylin-eosin (HE) and periodic acid Schiff (PAS). Commercial paraffin sections of adult human skin were purchased from BioChain Institute Inc. (Hayward, CA, USA).

Morphometric analysis was conducted in accordance with the methods described by Marks [7]. Quantitations were performed for a minimum of 20 points per section using the 6-µm-thick HE stained sections (3 sections per animal), and the following parameters were evaluated: 1) mean thickness of epidermis (excluding the corneal layer), 2) mean number of layers of keratinocytes comprising the epidermis, and 3) mean height of keratinocytes. All quantitative results are expressed as the mean ± standard error (SE). Differences between males and females were analyzed using the non-parametric Wilcoxon test (P<0.05). Differences between more than two groups were analyzed using the Tukey-Kramer HSD test (P<0.05). The statistical analyses were performed using JMP 5.1 software for Windows (SAS Institute Inc., Cary, NC, USA).

For electron microscopy, skin samples were cut into small pieces, then fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (CB, pH 7.4) containing 2% sucrose. After being washed in CB, specimens were postfixed in 1% OsO4 in CB, then dehydrated through a graded ethanol series. After being routinely embedded in Spurr’s resin (Polysciences Inc., Warrington, PA, USA), ultrathin sections stained with uranyl acetate and modified Sato’s lead [1] were observed using a transmission electron microscope (H-7000KU, Hitachi, Tokyo, Japan).

Light micrographs of CMP and human skin are shown in Fig. 1. In the CMP, corneal, granular, spinous and basal layers were clearly observed in the epidermis (Fig. 1A). A basement membrane and many well-developed projections were seen at the dermal surface (dermal papillae). Similar findings were obtained for all skin sites examined in the CMP, and these findings resembled those of human skin (Fig. 1B).

Morphometric data for the CMP epidermis are shown in Table 1 and Fig. 2. The thickness of the epidermis (excluding the corneal layer) differed among different skin sites and between male and female pigs (Fig. 2A). The epidermis was significantly thicker at the loin than at the medial thigh in males. In females, the epidermis was significantly thicker on the back and at the loin than at the axilla and medial thigh. Males had a significantly thicker epidermis than females at the medial thigh. There was no difference in the number of layers of keratinocytes between skin sites, but there was a
difference between males and females at the medial thigh (males had a significantly greater number of layers of keratinocytes) (Fig. 2B). In males, the height of the keratinocytes was significantly greater on the back than at the medial thigh (Fig. 2C). In females, the height of keratinocytes was significantly greater on the back and at the loin than at the axilla and medial thigh. There was no difference between males and females with respect to the height of keratinocytes at corresponding sites. There were significantly more layers of keratinocytes in the axillae of 17-month-old CMPs than in 34-month-old CMPs (17 months: 4.13 ± 0.02; 34 months: 3.55 ± 0.25). No other parameters at any site differed significantly between pigs of different ages.

Electron microscopy studies were performed to observe the fine structure of the epidermal Langerhans cells (LC) in the CMP. The epidermal LC were observed as light cells sporadically located from the basal to spinous layers, with irregular-shaped nuclei and electron-lucent cytoplasm lacking tonofilaments, desmosomes and melanosomes (Figs. 3 and 4). A very small number of rod- or racquet-shaped Birbeck granules, which are typical in human LC [12], were observed in the LC of the 34-month-old CMPs (Fig. 4) but not in the 17-month-old CMPs.

It is well known that the skin of densely haired mammals such as rodents, dogs, cats and monkeys has distinctive morphological differences from human skin, whereas the skin of domestic or miniature pigs morphologically resembles that of humans [4, 6, 8]. In the present study, morphological similarities between CMP skin and human skin were revealed by light microscopy. Furthermore, in the present study we assessed CMP skin morphometrically in accordance with the method of Marks [7], who assessed the skin of humans aged 15–92 years at the upper arm, thigh and abdomen. Marks found that the number of layers of keratinocytes ranged from 3.03 to 4.64, and that the height of the

Table 1. Morphometric results for Clawn miniature pig skin

<table>
<thead>
<tr>
<th>Site</th>
<th>Male (Mean ± SE)</th>
<th>Female (Mean ± SE)</th>
<th>Male (Mean ± SE)</th>
<th>Female (Mean ± SE)</th>
<th>Male (Mean ± SE)</th>
<th>Female (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axilla</td>
<td>28.9 ± 2.6</td>
<td>34.1 ± 3.8</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.4</td>
<td>8.8 ± 1.1</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>Medial thigh</td>
<td>19.8 ± 1.6</td>
<td>27.5 ± 2.3</td>
<td>4.2 ± 0.2</td>
<td>3.3 ± 0.1</td>
<td>6.6 ± 0.2</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td>Back</td>
<td>43.1 ± 1.1</td>
<td>41.7 ± 3.8</td>
<td>4.2 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>10.0 ± 0.4</td>
<td>10.4 ± 0.1</td>
</tr>
<tr>
<td>Loin</td>
<td>43.4 ± 5.1</td>
<td>42.3 ± 2.7</td>
<td>4.6 ± 0.1</td>
<td>4.0 ± 0.3</td>
<td>9.2 ± 0.5</td>
<td>10.7 ± 0.5</td>
</tr>
</tbody>
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1)Excluding the corneal layer. 2) Mean ± SE.
keratinocytes ranged from 9.73 to 12.90 \( \mu \text{m} \). With respect to these parameters, CMP skin from the back and loin was most similar to human skin. Keratinocytes in CMP skin from the axilla and medial thigh were smaller than keratinocytes in human skin; however, the observers, skin locations and the tissue preparation protocols differed between the present study and that of Marks [7].

Epidermal LC play an important role in the immune system of the skin as antigen-presenting cells. Electron microscopy is used to identify LC, and light cells that are distributed sporadically from the basal to the spinous layers in CMP skin can be identified as LC on the basis of their ultrastructural features (their lack of tonofilaments, desmosomes and melanosomes). Birbeck granules are unique cytoplasmic granules with a rod or racket shape that are present in large numbers in human LC [12]. In the present study, typical Birbeck granules were observed in the LC of the 34-month-old CMPs, whereas in 17-month-old CMPs, there were very few granules and no typical granules. These ultrastructural features of the CMP LC are similar to those in other strains of pig. Typical Birbeck granules were found to be absent in the Yucatan miniature pig at one month of age [4]. Typical Birbeck granules have been found in skin from the ear of a white crossbred farm pig at six months of age, but in smaller numbers than in humans [13]. The role of the LC Birbeck granules in the skin’s immune system is not completely understood. Although it has been suggested that endocytosis of antigens or trafficking between the endosomal recycling compartment and the plasma membrane are the principal roles of LC [5, 12], a recent study using langerin (a potent inducer of Birbeck granules) knock-out mouse suggested that formation of Birbeck granules is dispensable for a number of LC functions [2]. Thus, further studies are needed to clarify the functional relevance of the ultrastructural differences between CMP and human LC.

Age-dependent differences were found in CMP skin. There were fewer layers of keratinocytes in the axilla at 34 months than at 17 months of age. Similar aging-related changes were found by Marks in a previous study [7], and in humans, the number of layers of keratinocytes and the height of keratinocytes decreased in an age-related manner at the upper arm, thigh and abdomen. In the CMP LC, typical Birbeck granules were observed at 34 months of age, but not at 17 months of age. This finding suggests that Birbeck granules develop in the CMP skin as the pig ages, which supports the hypothesis of Monteiro-Riviere [9]. However, to better understand the age-dependent changes that oc-
In conclusion, the present study demonstrates that the skin of the CMP histologically and morphometrically resembles human skin. However, further studies are necessary to determine whether CMP skin will be useful as a model for human skin.

**References**