Experimental study of decubitus ulcer formation in the rabbit ear lobe

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Abstract—An animal model of decubitus ulcer was created with the use of ear lobes of Japanese white rabbits. When the strength of the cyclic compressions and the duration of the cycles of compression and release are adjusted, the model successfully reproduced the four grades used to characterize decubitus ulcer. Compressions were recorded with video microscopy to continuously monitor the changes in tissue blood flow, in both the compressed and surrounding regions. This model is unique insofar as the blood-flow characteristics are clearly visible before, during, and after compression. Because long-term observation is possible in a living-body model, our study can easily be extended.

Key words: animal model, decubitus ulcer, reproducibility.

INTRODUCTION

Decubitus ulcers detract from the quality of life of patients with motor dysfunction (including patients with spinal cord injuries) in the postacute period. It is estimated that, in Japan, medical expenditure increases as the number of patients with decubitus ulcers increase. Further research is required on the prevention of ulcer formation, the promotion of wound healing, and the prevention of ulcer recurrence.

Many factors contribute to the formation of decubitus ulcers. These include load concentration on a single point, shear stress, ischemia, changes in body composition, infection, and fever. Animal experiments and clinical experience have confirmed the importance of some of these factors in the etiology of decubitus ulcers.

We have examined early research dating back to the 1950s that used animal experiments to elucidate the etiology of decubitus ulcers. The research that stands out was undertaken by Kosiak and Lindan who showed quantitatively that the decubitus ulcer is the product of compression and its duration [1–3]. Their reports are unclear as to whether a quantitative relationship exists between the severity of the ulcer and the strength and duration of the causative compression. These reports also focused only on the occurrence of ulcers and did not involve a live model that would allow observation of the processes of decubitus ulcer aggravation and healing over a long period. In a report by Peirce et al. [4], in which ischemic sites were created on the backs of rats with the use of 50 mmHg compression, the occurrence of ischemic sites was quantitatively reproducible. However, the model did not allow full analysis of the spread of the decubitus ulcer through all the layers of the skin and into the subcutaneous tissue.

Abbreviations: IAET = International Association for Enterostomal Therapy, NPUAP = National Pressure Ulcer Advisory Panel.

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To better understand decubitus ulcers, which display a complicated pathology with a varied etiology, investigators must conduct research with animal models that ensure the reproducibility of the severity of the ulcers and allow their observation over long periods. Few studies have been conducted hitherto that satisfy these conditions. Therefore, the aim of the present study was to develop an animal model that reproduces decubitus ulcer formation in the ear lobes of rabbits. The innovations of this animal model were based on the following two considerations. First, we wished to maintain close observation of the condition in vivo over an extended period. Second, we aimed to reproduce the four types defined in the classification of decubitus ulcer in a reproducible model, because these are important for any analysis that purports to clarify the mechanism of decubitus ulcer formation.

METHODS

Materials

Adult Japanese white rabbits weighing approximately 3 kg were used as experimental animals, constituting 46 rabbit ear lobes. Rabbit ear lobes were used because they are composed of epidermis, dermis, soft tissue, and cartilage, and these structures closely resemble the human sacrum. Moreover, one can clearly view the blood-flow characteristics over the entire ear lobe using permeating light. Rabbits were housed under standard laboratory conditions (12 h light, 12 h dark cycles, with lights on at 8:00 am; 23 °C), and maintained on standard laboratory food and water ad libitum. All procedures were conducted in accordance with the Health Guidelines on Animal Welfare of Japan.

Experimental System

The experimental system (Figure 1) consisted of a pressure-control unit and a measurement and observation unit. The duration of compression, the interval between compression and release, and the degree of compression were controlled independently. An area measuring 10 mm in diameter on each ear lobe of the rabbit was treated with a different degree of compression during the experiments.

The compression device consisted of two pieces of transparent acrylic 10 mm in diameter and was operated by air pressure. We controlled the duration and degree of compression by regulating the air pressure.

Procedures

The arterial-venous network of the rabbit ear lobe branches into three main vessels, with arterial-venous communication between parallel arteries and veins. During the experiments, the arterial-venous connection located at the tip of the rabbit ear lobe was compressed without anesthesia.

Anesthesia was not used, to exclude any effects of anesthetic drug on hemostasis. We increased compression gradually using air pressure, and the rabbits did not thrash about as if in pain, even without anesthesia. Even at pressures of 300 mmHg, the rabbits did not thrash about, indicating that we were not applying unbearable stress. We observed that many rabbits fell asleep during the application of 300 mmHg compression. Therefore, the above procedure did not appear to have caused pain or extreme distress to the experimental animals.

A preliminary study to evaluate the interruption of blood flow was performed to identify the degree of compression that causes decubitus ulcers. The present experiment was conducted subsequently.

The degrees of compression applied were 50 mmHg, 100 mmHg (weak compression), and 300 mmHg (strong compression). Compression was applied according to the following four protocols, established based on the compression (C) and release (R) periods. Each group, comprising 10 rabbit ear lobes, was treated with a different protocol as shown in the following:

- A (C: 6 h × 3, R: 12 h × 3, the sum of experiment time [sum] was 54 h).
• B (C: 6 h × 3, R: 6 h × 3, sum: 36 h).
• C (C: 12 h × 3, R: 12 h × 3, sum: 72 h).
• D (C: 12 h × 3, R: 6 h × 3; sum: 54 h).

The maximum duration of compression totalled 36 h (compression for 12 h, repeated three times), and the minimum period totalled 18 h (compression for 6 h, repeated three times).

Blood flow in the compressed areas in the right ear lobes of the rabbits was observed with the use of permeating light. Blood flow and hemorrhage were photographed in the compressed and surrounding areas before and after compression. All observations and recordings were performed before and during compression and during the compression release periods and were continued for 30 d after the total experimental period was completed.

Classifications of Decubitus Ulcers in Rabbit Ear Lobes

The classification of decubitus ulcers induced experimentally in the ear lobes of rabbits is defined as follows:

• Grade I: Reversible flare (redness). Erythema is not resolved within 30 min of pressure release. Epidermis remains intact.
• Grade II: Persistent flare (redness), edema, and partial-thickness skin loss involving the epidermis.
• Grade III: Full-thickness skin loss involving damage to or necrosis of subcutaneous tissue. The ulcer is superficial and presents clinically as a shallow crater.
• Grade IV: Full-thickness skin loss with extensive destruction of the skin, tissue necrosis, or damage to muscle and bone. Necrosis and perforation are visible on the rabbit ear lobes.

These classifications were compared with the classification definitions of Shea, IAET (the International Association for Enterostomal Therapy), and the National Pressure Ulcer Advisory Panel (NPUAP) [5–7]. The greatest difference was in the definition of Grade I. Redness and erythema are features of the initial stage of decubitus ulcer formation under all classifications, but the clinically important point occurs when the reversible redness, that is, the reactive hyperemia response, has disappeared. Once past this point, a decubitus ulcer begins to enter the process of aggravation. The classification used in the present study defines this point in the same way as IAET and NPUAP in their work on human subjects. This view is not consistent with the Shea classification. Grade I is defined by Shea as irreversible degeneration, including invasion into the epidermis. We have defined Grades II to IV in the same way as the other three classifications of Shea, IAET, and NPUAP.

Definitions of Healing Ulcers and Severe Healing Ulcers

Two types of pressure patterns were defined, (+) healing ulcers and (++) severe healing ulcers so that we could observe the process of ulcer aggravation over time in our animal model. In (–) Grade I, there is no wound on local skin tissue after the total experimental period. No ulcer forms. In (+) Grades II and III, a decubitus ulcer forms but subsequently heals. Necrosis and skin separation are observed. Finally, in (++) Grades III and IV, the experimental area develops flare, edema, and ulceration that progress to necrosis and perforation without healing.

RESULTS

A preliminary study examining hemostasis was performed to identify the degree of compression that causes a decubitus ulcer. The rabbit ear lobes were compressed at 0, 50, 100, 150, 200, and 300 mmHg for 10 min. Blood flow at the compressed area was interrupted when compressions of 100 mmHg or more were applied (Figure 2).

Table 1 shows the relationship between ulcer formation in rabbit ear lobes and the compression protocol, in terms of the degree of compression, the duration of compression, and the interval between compression and release. In rabbit ear lobes to which we applied 50 mmHg compression using Protocols B and D, no Grade I response was observed and no ulcer formed (–).

In rabbit ear lobes to which we applied 100 mmHg compression using Protocol B, a Grade I response was observed but no Grade II response. No ulcer formed (–). In rabbit ear lobes to which 100 mmHg compression was applied using Protocol D, a Grade II response was observed. Decubitus ulcer formation was observed (+).

In rabbit ear lobes to which we applied 300 mmHg compression using Protocol B, a Grade III response was observed. Nine out of ten ears showed the same result. However, 10 days after release of the compression, the skin lesions that exhibited flare, discoloration, edema, and local necrosis had healed (+).

In rabbit ear lobes to which we applied 300 mmHg of compression using Protocol D, progressive decubitus ulcer...
formation was observed (++). The ulcer surfaces did not heal, and perforation was visible. Grade IV responses were observed. Nine out of ten ears showed the same result.

With the latter compression protocol, time-dependent changes were observed. Immediately after the first release from compression, reversible flare occurred at the compressed area, similar to that of Grade I. After the second release from compression, flare and swelling occurred in the compressed and surrounding areas, as in Grade II. Some lesions were composed of white semitransparent ischemic tissue, suggesting that the flare phenomenon, discoloration, and redness had disappeared because of the development of microcirculation and the cessation of blood flow to the compressed area. Approximately 30 h after the end of the experimental period, ulcer formation was observed in the white semitransparent areas, as described for Grade III. Approximately 24 d after the end of the experimental protocol, necrosis and perforation were visible on the rabbit ear lobes and the ulcers had healed completely (Figure 3).

On the basis of the study results (Table 1), we created a 5 × 5 incidence distribution table (Table 2) to determine the extent of the reproducibility achieved between the classification of the decubitus ulcer as defined in this study and the study protocol, and we analyzed the data statistically. To estimate similarity, we calculated the matching coefficient. The results indicate that the matching coefficient was high (0.90) and reproducibility was high. Therefore, this study is considered reproducible.

**DISCUSSION**

The pathology of decubitus ulcer varies and can include healing within a few days of serious ulcer formation, especially in the elderly and people with spinal cord

### Table 1.

Results of decubitus ulcer formation and compression protocol.

<table>
<thead>
<tr>
<th>Degrees of Compression</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mmHg (–)</td>
<td>(–)</td>
<td>(–)</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>&lt; Grade I</td>
<td>&lt; Grade I</td>
<td>&lt; Grade I</td>
<td>&lt; Grade I</td>
<td></td>
</tr>
<tr>
<td>6/6</td>
<td>6/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mmHg (Weak Type)</td>
<td>(–)</td>
<td>(–)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Grade I</td>
<td>Grade I</td>
<td>Grade II</td>
<td>Grade II</td>
<td></td>
</tr>
<tr>
<td>9/10</td>
<td>8/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 mmHg (Strong Type)</td>
<td>(+)</td>
<td>(+)</td>
<td>(++)</td>
<td>(++)</td>
</tr>
<tr>
<td>Grade III</td>
<td>Grade III</td>
<td>Grade IV</td>
<td>Grade IV</td>
<td></td>
</tr>
<tr>
<td>9/10</td>
<td>9/10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(–) Grade I: No wound was on local skin tissue after total experimental period. No ulcer formed.

(+) Grades II and III: A decubitus ulcer formed but subsequently healed. Necrosis and skin separation were observed.

(++) Grades III and IV: Experimental area developed flare, edema, and ulceration that progress to necrosis and perforation without healing.
Figure 3.
Development of experimentally decubitus ulcers on rabbit ear lobes. (a) (−) Grade I: Local skin tissue had no wound after the total experimental period. No ulcer formed. (b) (+) Grades II and III: A decubitus ulcer formed but subsequently healed. Necrosis and skin separation were observed. (c) (++) Grades III and IV: Experimental area developed flare, edema, and ulceration that progressed to necrosis and perforation without healing.
injuries and chronic malnutrition or circulatory dysfunction. The etiology of decubitus ulcers has been defined differently by various researchers. Charcot proposed the nerve theory [8], Munro thought that the etiology of decubitus ulcers is a reflection of the autonomic nervous system [9], and Brown-Séquard cited compression and sweating as pathogenic factors [10]. Thus, the mechanism of decubitus ulcer formation has not yet been clarified. To understand decubitus ulcers, investigators must undertake research using animal models that can reproduce the severity of the ulcers and can be observed over long periods.

Because rabbit ear lobes are composed of epidermis, dermis, soft tissue, and cartilage, we consider that these structures closely resemble the human sacrum. However, an important difference between human skin tissue and the rabbit ear is that there is no feces or urine around the rabbit ear. For this reason, our results only pertain to rabbit ears and cannot be quantitatively applied to human skin. These results should nevertheless contribute to research on the prevention of decubitus ulcers in humans because (1) the occurrence of highly reproducible specific-grade decubitus ulcers, which depend on compression strength and time conditions, should be quantitatively applicable to human tissue and (2) a highly reproducible animal model constitutes a valuable tool with which to study factors affecting the occurrence of decubitus ulcers.

Our experimental results have clarified that each different grade of decubitus ulcer can be reproduced. The matching coefficient was high at 0.90, indicating that the reproducibility of the experiments was high. All instances where results were inconsistent involved classifications of low grades, and results did not show symmetry. Accordingly, if the compression intensities used in these experiments were increased slightly (for example, by prolonging the duration of the compression or increasing the intensity of the compression), most likely the results would show greater symmetry for severity.

The matching coefficient calculated here is sufficiently high at 0.90, but if the compression intensity were raised only slightly, the matching coefficient should increase further, resulting in a better reproducible model of decubitus ulcers.

We have shown that the development of an experimentally induced decubitus ulcer depends on both the duration of the compression and the degree of pressure applied. This is consistent with the results of Kosiak, who applied a pressure of 275 mmHg to the backs of dogs for a 7 h compression period [1,2].

The method of applying compressions was the greatest difference between our protocol and that of Kosiak. In Kosiak’s experiments, the compression was applied only once, whereas compression was applied repetitively in our experiments. This experimental protocol was chosen after duplicate experiments in which 300 mmHg compressions were applied continuously for 24 h. No ulcers formed; only necrosis caused by ischemia was observed. For this reason, a repeated-compression protocol was used for our model.

In our study, Grades I, II, III, and IV were observed. The complete process of severe healing decubitus ulcer development was evident in rabbit ear lobes. We infer that severe healing decubitus ulcers are induced by repeated compressions rather than by continuous compression. Possibly, a “no reflow” phenomenon or a decrease in blood reflow is involved in the exacerbation of decubitus ulcers.

No Grade I ulcers were induced in rabbit ear lobes by 50 mmHg compressions, because blood flow was maintained during 50 mmHg compressions. This is an important observation for estimating the critical value for external compression.

Burton and Yamada measured pulse waves plethysmographically and reported that blood flow in human capillary vessels stops with 70 mmHg compression [11]. Landis reported, using a microinjection method, that human upper-limb arterial pressure is 32 mmHg, inner-capillary pressure is 20 mmHg, and inner-venous pressure is 12 mmHg [12,13]. McLennan measured inner-capillary pressure plethysmographically in the human forearm and found it to be 16 mmHg to 23 mmHg [14].

Table 2. A 5 × 5 incidence distribution table.

<table>
<thead>
<tr>
<th>Classification</th>
<th>B/50 &amp; D/50</th>
<th>B/100</th>
<th>D/100</th>
<th>B/300</th>
<th>D/300</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Ulcers</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Grade I</td>
<td>0</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Grade II</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Grade III</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Grade IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>52</td>
</tr>
</tbody>
</table>
There have been no reports of arteriole or venous pressure. Based on the results of our experiment, a compression of 100 mmHg was sufficient to stop capillary flow in the venous bed. However, no Grade III decubitus ulcer formed in those ears, except for a flare phenomenon, as defined for Grade II.

CONCLUSIONS

We created an animal model of decubitus ulcer using the ear lobes of Japanese white rabbits. With this model, we can experimentally reproduce the characteristic four grades of decubitus ulcer classification by adjusting the degree and duration of compression and the interval between compression and release. Moreover, because long-term observation is possible in a living-body model, our study can be easily extended. Future studies, focusing on the formation of ischemic areas and the aggravation of decubitus ulcers, will continue this line of investigation.

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REFERENCES


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