Temperature Modulation of Pressure Ulcer Formation: Using a Swine Model

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Paul A. Iaizzo, PhD

Abstract and Introduction

Abstract

Developing reliable animal models as a means to study the etiology, prevention, and/or treatment of pressure ulcers is not a simple task. Numerous considerations need to be evaluated for appropriateness, such as similarity of the cutaneous layers to those of humans, reproducibility of injuries, the effects of administered anesthetic or analgesic agents, the locations of the created lesions, the typical rates of healing (controls), and/or the overall health status of the animals. The author's laboratory previously developed one such model: a porcine model to aid in investigations of pressure ulcer formation, healing, and prevention. The author and colleagues specifically studied the relationships between temperature, pressure, and time in the formation of cutaneous and/or deep tissue injuries. To do so, an apparatus and procedure were created to apply 12 metal discs (each with a diameter of 51mm) to the dorsal aspect of the swine. At equal pressures ranging between 10-150mmHg, four discs were applied for 1 to 10 hour periods, while their temperatures were servo-controlled between 25-45°C. The severity of resultant tissue injuries correlated with increases in applied parameters. Briefly, no damage was observed in the superficial or deep tissues underlying the sites of the 25°C pressure discs even with 10 hours of applied pressure. Only deep tissue damage resulted from the application of the 35°C discs for five hours, and the application of higher temperatures for shorter durations caused both cutaneous and subdermal damage. In addition, degrees of healing could be easily monitored in such animals for months and was typically uniform relative to the degree of induced damage. This animal model of temperature-modulated pressure ulcers has the potential for significant use in all major areas of this field, i.e., wound formation, healing, and prevention. The use of this approach on transgenic individuals or those with induced disease would also be of great interest.

Introduction

There remains a need to optimize various animal models to investigate cutaneous and/or pressure-related ulcers. Singer and McClain recently noted that significant medical advances are dependent on the performance of fundamental basic and clinical research, and a key component of a comprehensive strategy is the development and validation of standardized experimental animal models.[1] To this end, the author's laboratory sought to develop a comprehensive swine model to evaluate and reproducibly create cutaneous and/or deep tissue injuries that were the result of imposed pressures to the skin at various temperatures for given durations.[2-7] As with many wound models involving cutaneous tissues, domestic swine were employed in the author and colleagues' approach due to the fact that pigs' skin resembles that of humans both structurally and functionally.[1,2,8-10]

Although extensive literature exists concerning pressure ulcers, no clear consensus is apparent for the etiology of such wounds, and this is likely dependent on multiple factors.[11-17] To illustrate the prevailing uncertainties, previously reported animal studies show the progression of a pressure ulcer may occur in either direction, i.e., from muscle upward[12-14] or from the upper dermis downward.[15] The application of elevated pressure (approximately > 90mmHg) over time (several hours) on a bony prominence is believed to be the primary factor in the causation of these ulcers.[6,10] Other ancillary factors include the magnitude of shear, friction, and/or moisture.[14] However, it has been shown that pressure thresholds for wound causation (defined by other models) are routinely surpassed clinically without apparent damage to tissue, e.g., in paraplegic patients without other complications as reported by Patterson and Fisher.[16] On the other hand, other patients may experience tissue damage before such determined thresholds are reached; hence, supplementary physiological factors, which include age, nutrition, psychologic factors, sensory loss, mobility, and/or temperature, warrant consideration.[18,19] It is noteworthy that the role of temperature in the causation of pressure ulcers has not been well explored. With such variations, it is a challenge to develop a reliable model for such responses. Yet, the author's laboratory was able to do so using creative engineering approaches. The following is a brief summary of protocols, instrumentation, and methodologies employed for producing reproducible wounds and a description of several potential means for their assessment that were part of the author's series of investigations. The reproducibility and consistency of the created wounds were established by histological evaluation of the application sites.

Methodologies Developed and Employed

Pressure-Temperature Applicator

Devices capable of applying uniform and controlled pressure and temperature to preselected cutaneous sites were developed.[2] Briefly, a set of four pressure-temperature applicators was mounted on a support fixture, which allowed for independent positioning of each applicator to adapt to the contour of the animal (Figure 1a). Twelve pressure-temperature applicators were typically used in each experiment (i.e., three independent support fixtures). Each individual pressure-temperature applicator consisted of a brass disc, 51mm in diameter and 10mm thick, a Kapton-encased heating element, and an 8mm-thick polystyrene disc to thermally guard the heater. To facilitate arbitrary modulation of the applied temperature at levels both below and above normal skin temperature, passages were created in each brass disc to accommodate inflow and outflow of cooling water. As seen in Figure 1, the four applicators of a given set were connected to a platform, which accommodated deadweights (not shown), the magnitude of which could be selected to attain a desired loading (i.e., pressure) at the down-facing surfaces of the interface discs. The deadweights were centered and held in place by a shaft attached to the platform. During each experiment, the temperature of each applicator was held constant by a microprocessor control unit. Among the 12 applicators, temperatures ranging between 25 and 50°C were employed in each experiment (normal swine skin temperatures in the location of the discs are ~35°C). Typically, in each experiment, at least three applicators were controlled at the same temperature; when applied to the animal, the temperature at each location of application could be randomly selected.[2-7]

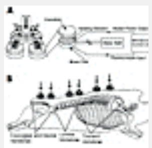

Figure 1. (click image to zoom) (A) Pictured is a cluster of four discs whose function is to apply pressure and temperature to preselected wound sites. The temperature modulation was obtained using a microprocessor-controlled unit. Cooling was provided by a water bath and heating by electrical resistance wire, and temperatures were maintained within ± 0.5°C. (B) Shown is the typical location of the disc application and subsequent assessment sites; care is taken to ensure that they are placed over bony prominences. Figure 1A is adapted with permission from the American Congress of Rehabilitation Medicine and the American Academy of Physical Medicine and Rehabilitation. Kokate JY, Leland KJ, Held AM, et al. Temperature-modulated pressure ulcers: A porcine model. *Arch Phys Med Rehab* 1995;76:666-73. Figure 1B is reprinted from Kokate JY, Leland KJ, Sparrow EM, Iaizzo PA. Critical thresholds for pressure ulcer formation in a porcine model. *WOUNDS* 1997;9(4):111-21.

The weights were chosen to yield application pressures between the minimum of 10mmHg up to 100mmHg with the latter approximating pressure applied to a standard bed by a human.[16] When desired, a pressure transducer could be mounted on the side of one of the discs as a means to verify the deadweight loading.

Typical Pre-Operative Protocol

All such investigations received prior approval from the Animal Care Committee at the University of Minnesota. They were performed on three- to four-month-old mongrel swine for an experimental period of up to 28 days. Animals initially weighed between 30-40kg. A large, flat, up-facing back surface (dorsal) area enabled the attainment of uniform pressures at the 12 applicator sites. Upon arrival, the animals were housed in an area with appropriate facilities and staff to administer a prescribed diet and regimen of care. After a two-day acclimatization period, the swine were fasted overnight and then anesthetized using intramuscular Telazol (12-15mg/kg-1) to facilitate the subsequent placement of a cannula in an ear vein. With the cannula in place, lactated Ringer's solution and thiopental (2-3mg/kg-1) were administered, enabling the placement of an indwelling catheter in the external jugular vein to dispense antibiotics, analgesics, and anesthetics.[2] The next day, the animals were initially anesthetized using intravenous thiopental (2-3mg/kg-1), given atropine (0.03mg/kg-1), and intubated. The lungs were mechanically ventilated at a rate of 10-15 breaths/min-1 with an inspired oxygen fraction of 0.5 and 1.5-2% isoflurane. Tidal volumes were adjusted to maintain an end-tidal partial arterial pressure of carbon dioxide between 35-45mmHg. Ringer's solution was administered for fluid maintenance at 10mL/kg-1/hr-1.

Disc Application Protocol

The pressure-temperature applicators were placed anterior to posterior along the prone swine's back (coccygeal and sacral, lumbar, and thoracic vertebrae, respectively) as shown in Figure 1B. The applied pressures and temperatures were maintained constant for planned periods of time. Core temperatures were monitored using rectal probes (Monatherm model 6510, Mallinckrodt Medical, Inc., St. Louis, Missouri) and adjusted to and maintained at either 38 or 35°C using convective air warming/cooling (Bair Hugger Therapy, Augustine Medical, Inc., Eden Prairie, Minnesota). Heart rate was monitored continuously (model #1020, Spacelabs, Inc., Chattsworth, California) and blood pressure determined every five minutes using an automated blood pressure cuff (Dinamap 847XT, Critikon, Inc., Tampa, Florida). In addition, cutaneous forearm and hoof temperatures were recorded via Monatherm skin probes as a means to estimate the degree of vasodilation.

Post-Application Protocol

At the end of the application periods, the anesthesia was discontinued and the mechanical ventilation turned off. Once the animals were spontaneously breathing, they were transported to a post-surgical care area where they were given intravenous injections (via the aforementioned indwelling jugular catheter), i.e., for analgesia. Buprenorphine hydrochloride (0.001-0.002mL/kg-1) was provided, and in case of infection, antibiotics were administered as needed. Typically, starting with the first day following the removal of the applicators and every three days thereafter for a four-week period, wound characterization measurements were made at all applicator sites and at relevant control areas. Blood pressure, heart rate, and core temperature were also monitored at these times. Thiopental was administered by a constant infusion to minimize error in measurements due to fluctuations in depth of anesthesia.

A qualitative ranking of erythema and edema was performed by qualified blinded observers. A ranking scale of 0-4 was used in both categories, with zero indicating no visible manifestation and four denoting the most severe condition.

In some studies,[3] local subsurface tissue perfusion was recorded at the central location and at the periphery (closest to the spine) of each applicator site and each control site with a laser Doppler blood perfusion monitor (Laserflo, Vasamedics, Inc., St. Paul, Minnesota). The probe's sensors were positioned at the skin's surface to measure blood perfusion data (at an approximated depth of 1mm beneath the surface); the blood perfusion monitor measured blood flow in units of mL/min/100g of tissue.

In several studies, local wound temperatures were sensed optically by an infrared microscanner (model D501, Exergen Corporation, Newton, Massachusetts) or by an infrared camera system.[2,4,7]

When histological samples were obtained, the biopsies extended through the subcutaneous fat into the underlying muscle, providing a basis for assessing the reproducibility and consistency of the tissue under each pressure applicator disc. The samples were removed using a double-bladed scalpel with an interblade distance of 2-3mm. Such samples were typically obtained from the periwound or disc applicator border, i.e., from approximately 10-12mm of tissue outside the applicator site to 10-12mm within the applicator region. The average depth of these samples was 25mm, which extended into the underlying skeletal muscle. Next, the specimens were fixed in formalin and embedded in paraffin using an automated tissue-processing unit; 5-μm sections were stained with hematoxylin and eosin. After the animal was euthanized, incisions were made to a depth of 8-10cm below the applicator sites down to the bone. Damage to deep tissue was visually assessed, recorded, and compared with the histological samples. The degree of damage within a tissue layer for a given sample was evaluated by a blinded, trained observer. A scaling system (Table 1) was used to provide statistical analyses of responses.[3]

Discussion

The author's laboratory published a series of studies in which the author and colleagues utilized the swine as a model for pressure ulcer research.[2-7] The choice of the swine model is related to the swine's accepted similarities in skin properties and comparable cardiovascular systems with humans.[1,8-10] Furthermore, the possibility of inducing conditions, such as radiation impairment, paraplegia, diabetic state, etc., in both domestic and miniature swine allows for modulation of these parameters to perhaps better mimic clinical situations in humans. Numerous groups of other investigators have extensively used the swine and mini-pigs for wounding studies, with the latter group of animals allowing for the study of a more mature or an aging population.[20]

Studies have illustrated variable pressure applications. One study showed deep tissue pressures as high as 275mmHg and surface pressures less than 50mmHg.[21] While sophisticated pressure distribution systems have been used previously,[11] uneven distribution among different layers in those studies was not investigated. In the present model, pressure discs were placed carefully to reduce the variability of applied pressure at the surface. In classifying wounds by extent of injury, sites over bony prominences were identified after visual and histological assessment.

The first manuscript in a series of published studies by the author and colleagues described in greater detail the methodologies employed involving temperature-modulated pressure ulcers.[2] In addition, the examination of histological data and visual assessment of incisions below wound sites indicate that those under the discs servo-controlled at 35°C caused deep tissue injury specifically over bony prominences (Figure 2). In contrast, no such damage was apparent under any of the 25°C sites, which could lead to the conjecture that lower temperatures could be protective and clinically significant for pressure ulcer prevention (Figure 2). In a follow-up study, the author and colleagues investigated these potential protective effects in greater depth. Specifically, the study attempted to determine 1) the effects of the duration of applied pressure and applied temperature on wound formation and 2) the threshold temperature below which focalized cooling would minimize the potential for wound formation.[3] In the first set of experiments, the standard 12 metal discs were applied on the dorsal aspect of the swine at an equal pressure of 100mmHg for a 2-, 5-, or 10-hour period while servo-controlling the disc temperatures at either 25, 35, 40, or 45°C (three discs at each temperature). In the second set of experiments, the 12 metal discs were applied at 100mmHg for 10 hours at temperatures of 25, 27.5, 30, and 32.5°C. The severity of resultant tissue injuries, determined histologically, correlated with increases in applied temperature and duration. Minimal damage was observed in both the superficial and deep tissues underlying the sites of the 25°C applicator discs even after the 10-hour application period. It was concluded that cutaneous and subdermal tissue damage can be reduced by focal cooling at temperatures less than 30°C.

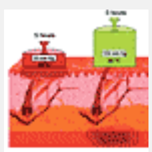

Figure 2. (click image to zoom) Shown here is an illustrative representation of typical difference in relative tissue damage observed at several combinations of pressure, temperature, and duration. The four combinations chosen represent relatively dramatic responses that were verified in the histological samples. The application of a disc for five hours at a temperature of 50°C and a pressure of 10mmHg caused damage restricted to the epidermal and upper dermal layers. The application of a disc for five hours at a temperature of 35°C and a pressure of 100mmHg caused damage restricted to the deep tissue layers. In contrast, the application of a disc for five hours at a temperature of 45°C and a pressure of 100mmHg caused damage throughout all four tissue layers. Typically, tissue damage does not occur even after the application of a disc for 10 hours at a pressure of 100mmHg if the interface temperature causes focal cooling; however, it should be noted that some shear damage may be observed in the subcutaneous fat layer

after such long application durations. Because such responses were highly reproducible, one could employ the author's approach to affect various tissue layers selectively. Reprinted from Kokate JY, Leland KJ, Sparrow EM, Iaizzo PA. Critical thresholds for pressure ulcer formation in a porcine model. *WOUNDS* 1997;9(4):111-21.

In a subsequent report, the author and colleagues compiled the resultant injury scores relative to the disc application parameter and developed a predictive mathematical representation of potential damage at each of the four tissue layers investigated throughout: epidermal, dermal, subcutaneous fat, and muscle layers.[6] In other words, the overall objective of this study was to define critical thresholds of applied pressure, applied temperature, and duration of application necessary for the formation of pressure ulcers or cutaneous burns. In all, 70 different disc application experiments that employed differing magnitudes of the aforementioned parameters were analyzed. A standardized histological scaling method (Table 1) provided a basis to determine tissue damage at different levels.[3] A positive correlation was found between the severity of resultant tissue injuries and increases in applied temperature, applied pressure, and/or duration of application (Figure 2). Three-dimensional plots of the pressure, temperature, and duration data were developed to visualize the critical thresholds for damage within each tissue layer (Figure 3). More specifically, an algebraic fit of the experimental data provided equations that could then be used to predict wound formation for any combination of these parameters. It was concluded that since porcine models have historically correlated well with humans due to physiological similarities and careful consideration of any confounding variables encountered in clinical practice (e.g., poor nutrition, impaired circulation, etc.), these critical thresholds and the resultant models should have widespread utility for healthcare providers (e.g., in the formulation of patient turning schedules), engineers concerned with new product design and development (e.g., for support surfaces that could reduce the incidence of pressure ulcer formation), and basic scientists.[6]

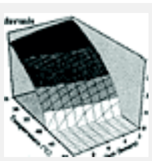

Figure 3. (click image to zoom) Shown here are three-dimensional representations of the thresholds for tissue damage caused by the various combinations of pressure, temperature, and duration of application. An algebraic best-fit was used to generate these graphs which provide a threshold for each individual tissue layer (epidermal, dermal, subcutaneous fat, and skeletal muscle). In each graph, combinations of applied pressure, applied temperature, and duration of application that fall beneath the plotted surface are considered not to cause injury, whereas

those combinations that fall above the surface indicate the combination of parameters that would allow the tissue to be prone to injury (Damage Score =1 taken as threshold). The shading in each graph was used to emphasize pressure ranges in 100mmHg steps. Modified from Kokate JY, Leland KJ, Sparrow EM, Iaizzo PA. Critical thresholds for pressure ulcer formation in a porcine model. *WOUNDS* 1997;9(4):111-21.

Today, there remains a lack of commonly employed clinical methods to quantitate events in the wound, and this need is often cited as one of the primary factors limiting progress in wound healing research.[22] In the author and colleagues' initial wound model report, analyses of laser Doppler fluximetry were deemed more successful than local temperature measurements or visual ranking for the assessment of wounds.[2] However, importantly, the author and colleagues also noted that the indicated perfusion was highly responsive to fluctuations in depth of anesthesia and/or variations in blood pressures and heart rates. Yet, laser Doppler fluximetry did not detect deep tissue injury.

In a complementary study, the author's lab explored reactive hyperemia as a noninvasive means to characterize wound severity relative to the authors' porcine model.[4] This study explored two methods for monitoring the extent of the reactive hyperemic reflex in newly formed pressure injuries. First, color image analyses were used to measure the hues of erythemic tissues. It was observed that the specific hues of the ischemic regions provided insights as to the depth and severity of the wound. Second, infrared imaging techniques, coupled with computer image processing, were used to detect differences in skin temperature, and both approaches correlate with the severity of injuries as determined histologically. Furthermore, it is generally considered that an accurate diagnosis of burns and pressure ulcers in their early stages could be made by computerized image processing.[4]

In the next study in the series by the author's group, the noninvasive evaluation of wounds using an inexpensive color imaging system was described.[5] For this study, the authors developed a system, both hardware and software, to quantify color changes visible at the skin surface and correlated these observations to histologically identified changes to assess the utility of using video signals to evaluate the severity of wounds at various post-formation stages. The authors noted that the difference in calibrated hues between injured and noninjured skin provided a repeatable differentiation of wound severity for situations when the time of injury was known.[5]

In the final published report by the author's lab employing this swine model, the utility of infrared thermography to indirectly assess severity and depth of pressure injuries of dermal tissue was again evaluated.[7] Two new approaches were considered: 1) evaluation of wounds at thermal equilibrium and 2) evaluation of the changes that occur after focal cooling of the affected area. In this situation, the focal cooling provoked a compensatory response that provided a sensitive test of tissue function. A series of trials was performed on the known standardized pressure injuries that were created by the authors' approach, where the infrared measurements were compared with injury assessments based on visual necropsy and histological examination. In general, the authors observed that deep tissue injuries may be easily distinguished from shallow wounds by their responses to focal cooling. The authors concluded that this method may be of particular clinical utility when one is attempting to detect abscessed areas of skeletal muscle that are concealed by a healthy epidermal bridge.[7]

The author and colleagues have continued to use this animal model of temperature-modulated pressure ulcers to study wound formation, healing, and prevention. For example, in one unpublished report, the author and colleagues investigated the effects of "focal cooling" on pre-existing pressure ulcers (Pathak M, MS thesis, University of Minnesota). It was proposed that by cooling areas of tissue that were subjected to high pressures and, thus, ischemia, a decreased metabolic rate of the tissue may be achieved and effectively increase tissue viability over time. Initially, discs were applied to a group of animals to produce known levels of selective tissue injuries, i.e., by the initial application of discs at 100mmHg for five hours at temperatures that were controlled at 25, 35, and 40°C. A second day application of 25°C was then used to examine the effects of cooling on tissue damage incurred by the Day 1 application. The results of the study indicated that focal cooling tended to decrease tissue pressure damage in pre-existing ulcers. The author and colleagues concluded that this study provided critical data needed for ongoing device development within the author's lab to implement this preventative and therapeutic approach through examination of temperature profiles at various tissue depths, noting that a temperature decrease of 4-5°C in the deepest tissue layer (muscle) will provide sufficient protection for all layers.

Conclusion

A reliable swine model for the development and assessment of temperature-modulated tissue damage was successfully devised. This model facilitates easy, independent modulations of pressures, temperatures, and durations to allow for the creation of specific wound types within various tissue layers. These techniques can be used to induce injuries classically defined as pressure ulcers and burns. For the potential noninvasive assessments of wound formation and subsequent healing, laser Doppler fluximetry and infrared imaging were useful, but qualitative visual ranking and local temperature were not beneficial. The application of 100mmHg metal discs for five hours at 35°C caused deep tissue damage, at 40°C caused dermal and deep tissue damage, and at 45°C caused full-thickness cutaneous and deep tissue injury. In contrast, the application of discs at 25°C resulted in the absence of damage; hence, injuries were deemed preventable by temperature modulation.

Acknowledgements

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Reprint Address

Paul A. Iaizzo, PhD, Experimental Surgical Services, Department of Surgery, MMC 107, University of Minnesota 420 Delaware St. S.E., Minneapolis, MN 55455; Phone: 612-624-7912; Fax: 612-624-2002; E-mail: iaizz001@umn.edu.