An animal model and computer-controlled surface pressure delivery system for the production of pressure ulcers

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Abstract—Pressure ulcers continue to be a major health care problem. This paper describes an animal model and surface pressure delivery system for the production of experimentally derived pressure ulcers. A method for inducing dermal pressure lesions on the fuzzy rat was developed using a computer-controlled displacement column which produced a constant tissue interface pressure. The pressure column consists of a force transducer located between two 0.5-in (1.27-cm) diameter metal cylinders. The desired cutaneous pressure is maintained by a computer-controlled miniature stepper motor which displaces the column with the aid of interactive software. The force transducer signal is converted from analog to digital form, amplified, and recorded. Blood perfusion is monitored using a laser Doppler flowmeter (located in the tip of the column) during the application of pressure. The application of 145 mmHg pressure for 5 consecutive 6-hr sessions resulted in a greater than 90% incidence of pressure ulcers. The implications of our model and contributions of earlier animal models are discussed. This model provides a tightly controlled and measured environment making possible the scientific study of ulcer development and the evaluation of potential preventative or curative compounds.

Key words: animal model, decubitus ulcers, fuzzy rat, pressure ulcers.

INTRODUCTION

Pressure ulcers (bed sores, decubitus ulcers) continue to be a major health care problem affecting a large segment of the population which includes individuals with spinal cord injury, patients with neurological disease, those with immobility, and the aged (1). A multitude of etiologic, pathomechanical, and pathophysiologic mechanisms are associated with the development of pressure ulcers, including pre-existing neuropathology, immobility, shear, friction, malnutrition, maceration, ischemia, and pressure (1). Unrelieved pressure is generally considered the most important of these factors (1). However, the amount of pressure and critical threshold of reduced blood flow leading to ischemia and pressure ulcer formation is unknown (2). In 1930, Landis (3), using micro-injection to study capillary blood pressure, found an average pressure of 32 mm Hg in the arteriolar limb, 22 mm Hg in the mid-capillary bed and 12 mm Hg on the venous side. A number of authors using various
methods of measurement have documented pressures at specific anatomical locations at risk for pressure ulcers in humans (4–8). The widely held perception that pressure at the surface of the skin or interface pressure, in excess of 32 mm Hg will lead to closure of capillary beds and result in tissue ischemia remains unestablished in laboratory studies (2). Many clinicians have assumed that pressures below 32 mm Hg are “safe” (2). Although pressure is known to be a primary risk factor for the development of pressure ulcers, the relationship between the magnitude of pressure, the duration of pressure, and tissue perfusion in the development of pressure ulcers is unknown. Evaluation of the generality of the possible link between pressure and tissue perfusion is critical, especially considering that the susceptibility of tissue and muscle varies according to the tissue characteristics at the particular anatomical location (9,10). Additionally, tissue resiliency may be influenced by other characteristics of the patient such as age, diet, and various immunological factors (11). However, pressure as a primary risk factor for the development of pressure ulcers requires more intense study. The following critical questions are relevant to clinical practice and gaps in our research knowledge base: 1) how much pressure, and over what period of time, typically results in ulceration, and 2) what tissues are at greatest risk (2). Because of multiple variables found in the human environment, pressure ulcer research is extremely difficult to conduct in the clinical setting (2). To answer these questions it is necessary to develop an animal model in which variables that contribute to the development of pressure ulcers can be monitored and quantified.

Animal models have been used to study the etiology of pressure ulcers. Groth (12) applied varying degrees of pressure to the posterior ischii of rabbits for varying periods of time. He reached several conclusions: 1) that pressure ulcers simulating those in humans can be produced experimentally; 2) that the larger the muscle mass, the greater the ability to withstand pressure; and 3) that the effective force may be greater below the surface, especially at sites overlying a bony prominence, which might account for the greater destruction of tissue at the base of the inverted cone so often seen clinically. In 1953, Husain (13) reported microscopic changes in rat muscle subjected to a pressure of 100 mm Hg for as little as 1 hour. Husain emphasized the importance of time in the development of tissue anoxia and demonstrated that relatively light pressure over an extended period of time causes more intense tissue necrosis than higher pressure over a shorter period of time. His findings were similar to those reported by Groth (12) and Kosiak (5). Kosiak conducted a classic experimental pressure ulcer study in dogs, which focused primarily on the epidermis. Pressure ranged from 60 to 550 mm Hg for periods of from 1 to 12 hours. Microscopic examination of tissue after 1 hour of pressure application (60 mm Hg) showed significant histological lesions, including inflammatory cell infiltration, extravasation, and hyaline degeneration. Tissues subjected to higher pressures for longer periods of time also showed significant lesions including muscular degeneration and venous thrombosis. In either case, tissue ischemia resulted in irreversible cellular changes and ultimately in necrosis and ulceration. He concluded that low pressure of longer duration was as injurious to tissues as intense pressure of short duration, and that prolonged pressure was the direct and primary cause of pressure ulcers. Daniel, et al. (14) investigated the effect of spinal cord transection on pressure ulcers in paraplegic pigs. Pressure was applied by an electromechanical pressure applicator controlled by a computer. Following tissue atrophy, pressure was applied to the paraplegic animals for varying durations of time. This experimental model produced pressure ulcers extending down to the bone. The resulting tissue damage was assessed and graded, and a pressure-duration curve for paraplegic animals was established. Daniel (14) agreed with Harman (15) and others (16,17) that muscle is extremely sensitive to ischemic damage and that degeneration begins in the muscle as early as 4 hours after the application of pressure. He did not embrace the theory that pressure-induced ischemia was a primary cause of pressure ulcers, but did suggest that an important cause was the inability of the tissue to respond to external loads because of tissue wasting associated with paraplegia, repeated trauma, and infections. Nola and Vistnes (16) documented significant areas of muscle necrosis in rats when pressure was applied to a transposed muscle flap over bone. The model used inverted plastic syringes, driven by compressed air, as pistons to deliver force to the greater trochanter. Force was measured using a pressure transducer system. Dinsdale (18) analyzed the role of pressure and friction in the production of pressure ulcers in normal and paralyzed pigs by utilizing air-driven pistons. Pressure of 160–1120 mm Hg was mechanically applied with and without friction for 3 hrs. The resulting ulcerations extended into the dermis and were present after 24 hrs. Dinsdale concluded that friction contributed to the pathogenesis of pressure ulcers, and he cautioned against blindly accepting the pressure-ischemia relationship as the only cause for ulceration. He also concluded that, al-
though constant pressure applied continuously over a given time period caused irreversible tissue damage, only minimal damage resulted if pressure was intermittently relieved. Ferguson-Pell, et al. recently described a system designed to measure hyperemia in the clinical setting (19, 20) which shares many of the characteristics of our pressure delivery system. Their system is a complex pneumatic skin indentation system suitable for short-term clinical studies. “Blood content” and “blood oxygenation” in control patients and patients with spinal cord injuries were measured using a tissue reflectance spectrophotometer. The authors concluded that the reactive hyperemia response was not substantially different between the two groups of patients.

Contributions and Critique of Previous Models

Although our particular approach is relatively new, we obtained considerable guidance from preceding models. Groth (12), as early as 1942, utilized a balanced beam device to apply pressure to the posterior ischii of rabbits for varying periods of time and pressure via two 15 mm circular discs. Force was applied perpendicular to the skin surface to study pressure ulcer etiology. Skin breakdown did not occur in this model and the applied pressure was not continuously monitored.

In 1953, Husain (13) studied pressure effects by means of a plethysmograph or pressure cuff and reported on microscopic changes in rat muscle. In 1959, a classic study by Kosiak (5) subjected 16 dogs to accurately controlled pressure created by inverted air driven piston syringes. He demonstrated that the magnitude of pressure is an important determining factor in the development of pressure ulcers. This relationship to pressure may be the result of ischemia with respect to the capillary closing pressure, but these variables were not directly assessed. As with the Husain study, the pressure delivery using air-driven syringe pistons had the shortcoming of “metastable” pressure delivery, and no determination of the actual cutaneous pressure.

Although in some cases the pig may be as appropriate as the rat for the study of pressure ulcers, their added expense is prohibitive, and they are not suited for large experimental studies. A major reason we selected the rat as our animal model of choice is that more is known about the pharmacological effects on absorption, distribution, and metabolism of drugs in rats than in any other species.

The recently developed bellows indentation system of Ferguson-Pell et al. (20) was designed to study short-term interventions such as hyperemia in the clinical environment; however, because of its large size this system is not readily adaptable for use in a study of small animals.

This paper describes a new model that incorporates many features for studying the determinants of and potential treatments for experimentally derived pressure ulcers. Although the studies described above provide systems with varying degrees of appropriateness for pressure ulcer study, none of these systems combine the features of reliability, servo control of pressure delivery, simultaneous monitoring of physiological endpoints, cost effectiveness, compactness, accuracy, flexibility, and the ability to handle large numbers of animals simultaneously, all of which are inherent in our system. We describe here a rodent model and pressure delivery system that results in the reliable production of experimental pressure ulcers in a controlled environment.

METHODS

Subjects

Male or female fuzzy rats (21) (Harlan Sprague-Dawley, Inc., Indianapolis, IN), each weighing 150–350 g, can be used to characterize pressure-induced damage to cutaneum and subcutaneous tissue. In the experiments reported here we used rats weighing 200–300 g. Hypotrichotic fuzzy rats were selected to minimize the effect of hair on skin and subcutaneous tissue pressure and to eliminate the need for artificially preparing the skin by shaving or by other methods that could introduce artifact. Rats were housed in groups in a cycle of 12 hrs of light and 12 hrs of dark; food and water were available ad libitum. During the series of daily experiments described below, the rats were individually housed under the same conditions—under Institutional Animal Care and Use Committee (IACUC) protocol 90-0051M.

Anesthesia

Each rat was anesthetized by intraperitoneal (i.p.) delivery of a xylazine and ketamine combination at a dose of 14.41 mg/kg Rompun (Miles, Inc., Shawnee Mission, KS) combined with 79.95 mg/kg Ketaset (Fort Dodge Laboratories, Inc., Fort Dodge, IA). At appropriate intervals, supplemental doses were administered to maintain a stable plane of anesthesia over the 6-hr pressure session. At the end of the pressure session, each animal received an i.p. injection of physiologic 2.5 percent dextrose and 0.45 percent saline to maintain adequate hydration.
Procedure

The integration of the various components of the cutaneous pressure delivery system is illustrated in Figure 1a. After induction of anesthesia, the animal was placed in a custom-made saddle and restraining device. The base of this device is constructed of durable 9-lb Ethafoam (Dupont, Foam Design, Inc., Lexington, KY) designed to ideally expose the hip areas for delivery of pressure to the skin. Curved Plexiglas braces stabilize the animal when pressure is applied by the pressure columns. During each experiment, the core body temperature of the rat was monitored by a thermistor probe (model 44033, Yellow Springs Instruments, Yellow Springs, OH) inserted rectally, and maintained at 35–36° C by a heat blanket (model T200, Gaymar T Pump, Orchard Park, NY) incorporated into the walls of the restraining device. The base is attached to an adjustable track that provides flexibility when the pressure column is positioned so that the contact surface of the column is parallel to the skin surface over the greater trochanter. This positioning of the column is accomplished by a swivel joint that moves in three dimensions (see Figure 1b). When the column is properly positioned over the skin surface, the positioning swivel joint is tightened. After the first pressure session was completed, the pressure indentations on the skin were marked with an indelible marker to insure proper placement of the pressure column in subsequent sessions. During each daily session, pressure is applied for 6 hrs at a pressure of 145 mm Hg, the components of which are 250 g force over the 13 mm diameter surface area at the end of the pressure column. Pressure was measured at the skin surface and applied over the trochanter region. Unless indicated otherwise, pressure was applied for 5 consecutive daily sessions of 6 hrs duration each. This magnitude of pressure was selected on the basis of a review of the pressure ulcer literature and on the results of preliminary experiments.

Apparatus

Tissue interface pressure is produced by using a closed-loop system that regulates the applied force (see Figures 1a and 1b). In addition, pressure-induced reduction of cutaneous blood perfusion is monitored by using a fiber optic laser Doppler flowmeter (Figure 1c). Pressure is applied to the skin by a metal column consisting of two stainless steel cylinders and a force transducer. The central portion of the column is fitted with a force transducer to measure the applied force. The force signal is also used by the computer to precisely maintain

and deliver constant cutaneous pressure within a range of ±5 g of force (±2.9 mm Hg). At the tip of the second stainless steel cylinder is a shape indenter which is labeled "textured surface" in Figure 1b. This is the material that makes contact between the pressure column and the skin surface. Each of these components is discussed in detail below.
Laser Unit

Figure 1c.
Detailed schematic diagram of laser Doppler flowmeter. The laser produces light with a wavelength of 780 nm. After penetrating the skin, the light beam is reflected back into the fiber optic cable. The beam splitter then separates the incident beam from the reflected signal beam. The preprocessing module subtracts the incident frequency from the reflected frequency to yield a value for the laser Doppler shift which is recorded by the strip chart recorder and saved on disk by the data collection computer.

Textured Surface

The textured surface or shape indenter is made from Safety-Walk® Tread (3 M Company, Cat.#7739, St. Paul, MN). This medium duty friction tape contains a pressure sensitive adhesive on one side (attached permanently to the pressure column) and on the other side contains a rubber-like, resilient material with a stippled surface that comes into contact with the skin.

Force Transducer

A 5-lb capacity single axis force transducer (load cell, model ALD-Mini-UTC-5, A. L. Design, Inc., Buffalo, NY) provides cutaneous surface pressure data to the computer. The force transducer is threaded on both ends, allowing it to be placed between two stainless steel cylinders, each 0.5 in (1.27 cm) in diameter. The force transducer and the two metal cylinders compose the pressure column (Figure 1b). The force transducer load cell requires 10 V excitation and delivers a rated output of 10 mV. The force transducer output is observed on a modified strip chart recorder (Servogor 124, Norma Goerz Instruments, Elk Grove Village, IL) for real-time hard copy output. The amplified signal from the chart recorder is sent to the analog to digital (A/D) converter (A/D card model number PCL-711, Laboratory Technology, Marlboro, MA) of a personal computer (stepper motor control computer) for feedback control of the tissue interface force. The strip chart recorder can also display flow measurements from the Perimed laser Doppler flowmeter (Periflux by Perimed, Piscataway, NJ) as described below. After A/D conversion (A/D card model DT2801, Data Translation, Marlboro, MA), the amplified force and flowmeter signals are recorded on a second personal computer with the aid of Perisoft software and saved to disk for future analysis.

The pressure column is calibrated using customized software (SMC8 version 1.3, Center for Biomedical Engineering, University of Kentucky, Lexington, KY). The pressure column is first inverted and zeroed with a weight. Known weights of up to 200 g are then placed on the flat surface of the metal column and the A/D counts are measured to establish the linear calibration for each transducer. The software then fits a linear relationship between counts and grams. When the column is turned right side up, the weight of the lower half of the column is zeroed, and the linear change in voltage per change in force as measured during the calibration procedure accurately indicates the force change. Calibration can also be achieved by having the column push against an appropriate scale.

Although this particular force transducer cell is designed to be used in a vertical position, we have demonstrated that it will function accurately when positioned in various angles so that we can best approximate perpendicular pressure to the tissue surface during the procedure. A 3-axis force cell is planned for future experiments in which shear force components are to be measured.

Stepper Motors

Delivery and maintenance of specified cutaneous pressure is accomplished by using highly reliable stepper motors to displace the metal column. The miniature stepper motors (model K92100, Phillips Technologies, Airpax Mechatronics Group, Charlotte, NC) were factory modified to act as bi-directional linear actuators. The motor shaft is a screw and the rotor is threaded on a low friction ball bearing in the motor casing. This makes it possible for the screw shaft to be moved in a bi-directional linear fashion as opposed to the more common clockwise and counterclockwise rotation without linear movement. A stepper motor driver chip (model SA1042V, Motorola, Phoenix, AZ) directly controls the firing sequence of each motor coil. Each 12-V direct current bipolar driver chip controls one stepper motor and receives two input signals via the printer port of the computer. The most significant bit of the computer signal controls the direction of the motor, and the least significant bit provides the step pulse. The motor driver chip accepts three input signals, contains a state controller, and provides two output signals.
The three driver input signals are “full/half step,” “direction,” and “clock.” The “full/half step” option is set at half step to give the motor a resolution of 0.0005 in (0.00127 cm) of linear movement per step. The chip achieves the half step option by activating at least two of the four motor coils per step. One coil “pushes” and another coil “pulls” to prevent a full rotation of the threaded nut. The half step allows better resolution but sacrifices speed and draws more current than the full step option. The “direction” input level is set by an open collector buffer chip (model 7407, Texas Instruments, Newark Electronics, Chicago, IL) and serves the function of keeping the direction in a definite logic state while permitting transistor-to-transistor logic (TTL) compatibility with the computer (22). The computer signal for a step is connected to the clock input pin. Stepping is achieved on the rising edge of the clock pulse. The state controller converts the digital input signal from the computer into a linear motor output step. This output is provided in the two states of full or half step. The activity and control of the moment-to-moment activity of the motor and the changes in the status of the motor (moving up, down, or not changing) are monitored in a feedback loop using an A/D board and then viewed on the monitor by using customized computer software.

**Interactive Software**

The cutaneous pressure is maintained by a customized interactive software system (Center for Biomedical Engineering, Lexington, KY) that utilizes the signal from the force transducer to control the stepper motors. The interactive software provides the user with the choices of manual motor control or automatic tracking. In the automatic tracking mode, the user initially enters the desired target cutaneous pressure and the system automatically maintains the pressure within the user defined range. When pressure falls outside the established target range, the computer will signal the stepper motors to move the number of steps required to return to the target range. The system automatically adjusts to minor changes in rat body movement. The number of steps estimated by the computer is a function of the difference between the current pressure and the target pressure. The computer system is capable of responding to a change in pressure as small as 0.25 g (0.15 mm Hg). The cutaneous pressure range is set so that pressure variations secondary to body movements from the cyclical pattern of normal respiration do not trigger motor stepping adjustments.

**Determination of Pressure-Induced Blood Perfusion Changes**

Alterations in cutaneous blood perfusion are monitored using the Periflux Laser Doppler Flowmeter system. With this system, a fiber optic probe emits laser generated monochromatic light (780 nm) that is scattered and absorbed by the tissue being studied. This fiber optic probe is located in the center of the distal portion of the metal pressure column to monitor changes in relative cutaneous blood flow before, during, and after application of cutaneous pressure. Light reflected off blood cells moving through the scattered laser light will experience a change in wavelength known as the Doppler shift and will travel back to the “master unit” through a second fiber optic cable. The velocity and number of moving blood cells in the skin is related to the proportion of Doppler-shifted light to non-Doppler-shifted light. The Doppler shift recorded by the master unit consists of the frequency difference between Doppler- and non-Doppler-shifted light.

The laser Doppler flowmeter is used to measure mean velocity of blood cells in a sample site of tissue, the number of blood cells moving in the sample site and blood cell perfusion (flux) which is the product of mean velocity and the number of blood cells. The sample depth is approximately 1 mm below the surface of the skin. Because of the sensitivity of the laser light to penetration, absorption, and reflection to tissue and blood parameters, the values obtained from the flowmeter are used for relative comparisons from controls in the same animal and not as absolute values.

The basic unit of measurement by the flowmeter is the perfusion unit (PU) or flux. One PU is equal to an analog output of 10 mV. The PU or flux does not represent a standardized value, such as the absolute number of cells moving through a given volume of tissue over a specific time. Rather, the PU is an arbitrary value relative to a specific application and depends on such factors as the wavelength of laser light, type of probe, temperature of subject, systemic blood pressure, posture, and biological zero perfusion value (23). Because of Brownian motion, a true zero reading for tissue perfusion measurements cannot be attained; however, by using a latex control suspension it is possible to compensate for much of the variation caused by this phenomenon. The corrected value is referred to as the biological zero. In the present system, the combined movement and concentration of blood cells through the cutaneous microvasculature (flux, PU) is determined for the central region under the pressure column (Figure 2).
The relationship between increasing pressure and decreasing blood flow. The upper chart shows pressure versus time while the lower chart shows blood flow versus time. Pressure increases from 5 to 250 g while blood flow as measured by the laser Doppler flowmeter decreases from its initial value to biological zero. A typical hyperemic response (A) occurs when pressure is released.

The mode of delivery of the pressure is depicted in Figure 3; a line drawing of the hip of the rat that shows where cutaneous pressure is applied. Animals are routinely placed in the base of the device and the angle orientation of the pressure column is directed at the same location of the hip (greater trochanter) each time, as previously described. Pressure ulcers form in the trochanter region of the rat above the bony prominence, which is the area covered by the pressure column during the procedure.

Reliability of Measurements

On a daily basis, anesthetized subjects are placed in the device and pressure is applied, an initial zero baseline reading of the force cell having already been determined. The force cell output is recorded on the strip chart recorder. The feedback regulation of the stepper motors is accomplished by using the motor controlled PC system. In addition, a strip chart recorder and computer-based storage system are used to document the maintenance of cutaneous pressure throughout the 6-hr session (Figure 1a).

Prior to the evaluation of the impact of pressure on cutaneous ulcer development, a series of control studies was conducted to characterize the reliability and accuracy of the feedback system. Figure 4 demonstrates the maintenance of reliable pressure by using an inert substance (foam rubber) as a model to validate the feedback control of the stepper motors. Figure 5 demonstrates that reliable pressure can be maintained by the computer-controlled system over an extended period of time (2 hrs). Similar plots were achieved (not shown) for periods in excess of the typical 6-hr pressure session. To control for various physical compliance/elasticity aspects of normal skin and underlying tissue, the system was tested by using a recently euthanized rat (data not shown). Despite slight, momentary shifts, steady pressure (145 mm Hg ±2.9 mm Hg) can be maintained by the computer system for extended periods of time. This result demonstrates that the computer-controlled system can reliably alter the pressure column via the stepper motor to maintain the target pressure within preset limits.

After the initial characterization using a non-living model system (Figure 4), an anesthetized rat was placed
into the restraining device and the characterization of pressure induced perfusion changes in the cutaneous compartment below the pressure column was determined. Figure 2 presents a 4 min expanded scale on the x axis, showing the moment-to-moment movement in the force tracing when pressure brought tissue perfusion to "biological zero" (i.e., no flow as demonstrated by the laser Doppler flowmeter) and is a typical perfusion response to pressure. The initial baseline perfusion when no pressure is applied demonstrates a high rate of cutaneous blood flow. As the pressure on the skin is increased by the movement of the column, as controlled by the stepper motor, there is a progressive reduction in perfusion blood flow to an apparent level of zero. This level of ischemia is maintained with a pressure of 145 mm Hg until the pressure is released, resulting in a hyperemic response (see "A" in Figure 2).

Minor changes in the pressure delivery occur as a result of movements associated with the animal's respiratory cycle (see Figure 5) and are ignored by presetting the range of acceptable values. When force values become too large, the feedback mechanism of the system returns the pressure to within the preset limits as described in the methods section. It is important to note that this particular design of a feedback control stepper motor system is able to reliably maintain cutaneous pressure and minimize blood flow under the pressure column for the entire 6-hr session.

Physiological Measurements

After two fuzzy rats were anesthetized, the left carotid arteries were cannulated in order to allow for the intermittent measurement of mean arterial blood pressure, and heart rate (24). The animals were not subjected to pressure sessions.

RESULTS

After repeated exposure to daily pressure for 6-hr periods (5 consecutive daily sessions), rats develop macroscopic lesions associated with cutaneous ulceration. The pressure ulcers form directly under the area covered by the pressure column. As shown in Figure 6, there is a marked change in the appearance of postpressure skin as compared to normal skin in the rat. Stage 2 pressure ulcers show microscopic evidence of infarction with infiltration of white cells along the margin of the necrotic tissue (Figure 7a) and necrosis of tissue with the appearance of underlying emerging liquefactive necrosis. There
is evidence of edema and margination of white cells at the level of arteriolar supply of the muscularis underlying the skin. A common early and/or mild lesion was necrosis of the panniculus carnosus muscle and the superficial adipose tissue (Figure 7b). Thrombi were occasionally noted (Figure 7c). At this stage in the development of the lesion, it appears that the primary site of lesion development is in the subcutaneous muscle layer. To date, we have successfully used this system on more than 300 rats to evaluate hypotheses on the origin and treatment of pressure ulcers. Many of these rats were used in refining the histopathology of experimental ulcers, or in flow studies. Many were used in studies verifying the model and in exploring the models' utility in testing various drug interventions.

In order to further validate the model system, a single experiment was designed to elucidate the relationship between blood flow and pressure in the fuzzy rat. This experiment generated the flow versus pressure curve shown in Figure 8. Tissue perfusion blood flow decreased nonlinearly with increasing skin pressure and approached zero flow at 35–40 mm Hg, which is approximately equal to human capillary pressure. Tissue perfusion was completely cut off at 80 mm of Hg. A best fit curve analysis of
single i.p. injection of anesthesia was given. During the period in which the rats remained unconscious, mean blood pressure values ranged from 112–124 mm Hg, while average heart rate varied from 440–492 beats per minute. During the recovery period, mean blood pressure increased slightly to a range of 132–140 mm Hg, and the average heart rate varied from 424–508 beats per minute. At 24-hrs postinjection, the mean blood pressure reading for both animals was 120 mm of Hg, and the average heart rate had settled into a range of 360–376 beats per minute.

**DISCUSSION**

This project has developed a reliable animal model and surface pressure delivery system for the study of pressure ulcers in the laboratory environment. The animal model described in this paper differs from models in previous studies, in that it uses a computer-controlled system capable of making perfusion measurements during the application of precisely controlled tissue pressure, thus allowing experimental control and monitoring of some of the crucial variables central to the study of pressure ulcer development. These critical variables include pressure, temporal blood flow, interval histopathology, and biochemistry. Furthermore, the model has utility for the study of pharmacologic intervention.

Our use of 250 g force or 145 mm of Hg is almost 2 times higher than the amount of force required to completely cut off tissue perfusion (80 mm Hg) as measured by laser Doppler flowmeter. This application of 250 g force for 5 consecutive daily sessions of 6-hrs duration each, consistently results in the formation of experimental pressure sores (greater than a 90 percent incidence).

The selection of 145 mm Hg and the duration time of 6 hrs per session was based on a review of the pressure ulcer literature and the results of the pressure versus flow experiment (Figure 8). The model is extremely flexible, in that pressure and/or duration may be varied, as demonstrated by Figure 8. We are currently investigating various pressure and time combinations in order to further elucidate the model and to clarify the relationship between pressure and time in the etiology of experimental pressure ulcers.

The mean arterial blood pressure readings in fuzzy rats of 120 mm Hg at 24-hrs postanesthesia injection are within the normal range of 115–121 mm Hg reported for the conscious rat (24). After 24 hrs, the animals have had time to recover from both anesthesia and the cannulization...
Table 1. Mean blood pressure and heart rate measurements in the fuzzy rat.

<table>
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<tr>
<th>Time (HR)</th>
<th>Rat 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rat 2&lt;sup&gt;c&lt;/sup&gt;</th>
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<td></td>
<td>Mean Blood Pressure&lt;sup&gt;d&lt;/sup&gt; (mm Hg)</td>
<td>Heart Rate&lt;sup&gt;e&lt;/sup&gt; (BPM)</td>
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<td>24</td>
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<td>376</td>
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<sup>a</sup>Hours after the i.p. injection of the anesthesia mixture; <sup>b</sup>Male fuzzy rat weighing 360 grams; <sup>c</sup>Female fuzzy rat weighing 244 grams; <sup>d</sup>Mean arterial blood pressure is defined as the value of 2 times the diastolic pressure plus the systolic pressure divided by 3; <sup>e</sup>BPM equals beats per minute.

procedure. As one might expect, the mean blood pressure and heart rate values show less variation under anesthesia. The mean blood pressure values during anesthesia and the conscious state were similar. The force used to generate experimental pressure ulcers in this study is only slightly greater than the mean arterial pressure of the anesthetized or conscious fuzzy rat. Externally applied pressure as a pressure ulcer causative factor may be significant largely in its relationship to the internal blood pressure of the animal. The relationship between externally applied pressure and internal blood pressure as revealed in this study, and its subsequent impact on the generation of pressure ulcers is well within theoretical expectations.

**Advantages of the Fuzzy Rat**

The use of the fuzzy rat provides several advantages. This rat is hypotrichotic (21) and therefore does not require depilation or prior treatment of the skin which might otherwise result in artifacts during the formation of pressure ulcers. The rat is inexpensive in both initial cost and maintenance and therefore highly efficient from a cost standpoint and well-suited for large experimental trials. In addition, more is known about the pharmacological effects, the absorption, distribution, and metabolism of drugs with the rat than with any other species, including the human.

**The Long-Term Utility of This Model**

The model presented in this paper permits the investigator to continuously monitor the exact pressure delivered to the skin. The force transducer provides moment-to-moment data on the pressure delivered and the laser Doppler flowmeter provides concurrent information on tissue perfusion. In this regard, it is possible to determine pressure-flow relationships in the development of pressure lesions and to test hypotheses relating to this interaction. With respect to evaluating potential therapeutic approaches (e.g., drugs), the ability to monitor relative changes in blood flow to tissues during pressure delivery makes it possible to control for drug-induced changes in blood flow to tissue as a potential mechanism of protection. In addition to tissue surface pressure and tissue perfusion, interval histopathology and biochemistry are among the critical variables central to the study of pressure ulcer development.

By using our model, the pathophysiologic continuum observed in the development of a pressure ulcer can be monitored and evaluated. Components of this continuum and their influence can be studied separately and in concert. Additionally, the development of this model will allow us to test pharmacologic compounds and mechanisms that have a potential use in the prevention and treatment of pressure ulcers, including anti-inflammatory drugs, angiogenic agents, growth factors, fibrogenic drugs, antioxidant compounds, free radical scavengers, and specialized drug delivery systems.

Although this model allows for manipulation and control of the critical variables in the laboratory environment regarding the pathophysiology of pressure ulcers, it lacks the natural history and the elements found in the human environment. However, we will be able to test cer-
tain hypotheses in order to gain a more fundamental approach to study the complex process involved in the development of pressure ulcers. The model mimics the human environment in several key respects; thus, the results of our research may be generalized to the human condition. The development of histopathological changes (lesions) following pressure application has been the focus of several studies utilizing different models of pressure sore development. Husain (13) reported microscopic changes in rat muscle subjected to pressure for periods of 1–10 hrs. Keane (17) reported that muscle is more susceptible to pressure damage than skin in the immobilized patient. Using the model described in this paper, we have confirmed these results (25) and presented data suggesting that these lesions may be initiated by postischemic free radical production (26) analogous to what has been reported in brain and cardiac tissues following ischemia-reperfusion injury (27). As indicated in the histopathology figures, some of this damage may be initiated by neutrophil-mediated activation and infiltration of the tissue. In this process, activated neutrophils generate reactive oxygen species and injure cells. Although Koziak’s study (5) gave evidence that lesions first developed in the dermis and then eroded downward, our results are more in agreement with the findings of Husain (13) and Keane (17), that lesions originate in the muscle, and that muscle may be more sensitive to ischemia than skin. Our findings show that muscle is very sensitive to pressure injury and first to show injury; however, the lesion that becomes an ulcer may develop at all depths simultaneously. We also have a second, invasive, laser Doppler flow probe to measure muscle blood perfusion, before, during, and after the application of tissue pressure to further evaluate this point. Although these observations suggest a role of free radical and white cell mediated damage, mechanistic studies remain to be conducted.

CONCLUSIONS

This novel multisystem approach in the scientific study of pressure ulcers will serve as a basis for a better understanding of the molecular biology and pathophysiology leading to the development of pressure ulcers in a controlled environment. Although other animal/human models with various types of pressure surface delivery devices (5,12,16,17,28) are available, some with computer and/or closed loop feedback control (14,20), we believe that the model system presented here, is the smallest, most efficient and cost effective system for large scale studies of pressure ulcer development in a laboratory controlled environment.

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