AN EXPERIMENTAL PRESSURE SORE MODEL FOR FUNCTIONAL ELECTRICAL STIMULATION: CONTINUOUS PRESSURE APPLICATION ON MONOPLEGIC PIGS

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ABSTRACT

The effects of unilateral radiculectomies followed by steady pressure application on pressure sore development was examined and the influence and the critical importance of the resultant soft tissue necrosis was analyzed. A technique resulting in partial laminectomies and radiculectomies was developed in adult mini-pigs. Following tissue atrophy, pressure was continuously applied to the monoplegic animals. Pressure application was employed after one percutaneous cancellous bone screw was used to anchor a spring-loaded indentor for compression of the soft tissues between the trochanter and the skin surface. Steady pressure adjusted to 800 mmHg was maintained fro one to three days. Debridement of necrotic material to viable bleeding tissues completed the development of the pressure sore. This paper presents the results of studies using this pressure model on the initial wound areas, volume and wound tolerance after the denervation of mini-pig hind limbs.

KEY WORDS: FES; Pressure Sore Model, Radiculectomies.

INTRODUCTION

A number of existing studies indicate that local surface electrical stimulation may be beneficial in the promotion of ischemic tissue healing and the prevention of pressure sores. Both direct and pulsating currents have been effective, suggesting different mechanisms for the interaction between tissue healing and electrical energy. The objective of this paper is to describe a new model for the creation of a stage 3 or greater chronic pressure ulcer for the study of the effect of electrical stimulaton on tissue healing (1). A controlled environment trochanteric pressure sore was created on the denervated atrophic unilateral hind limb of male Hanford mini-pigs. One percutaneous cancellous bone screw was used to anchor a spring loaded plastic indentor for

compression of the soft tissues between the trochanter and the skin surface. Steady pressure adjusted to 800 mmHg was maintained for one to three days. Each day the tissues were inspected and the pressure reapplied until the desired stage of ulcer was observed. Debridement of necrotic material to viable bleeding tissues completed the development of the pressure sore.

MATERIALS AND METHODS

The mini-pig was selected as the experimental animal because of the simlarity of its soft tissue coverage to man. Ten adult pigs were used in this tudy. Five were assigned to the control group, initial body weight 15.6±4.5 kg and five to the direct current (DC) stimulation group with initial body weight of 16.6±8 kg. There was no significance difference in the body weight of the two groups of animals. The pigs were isolated and acclimated to the laboratory environment for at least 5 days prior to initiation of the study. All surgical and experimental procedures were carried out subject to the approval of the institutional Animal Research Committee and in accordance with the *NIH Guidelines for the Care and Use of Laboratory Animals.*

Surgical Procedure

1. Denervation Surgery: All surgeries were done under sterile operating room conditions to remove the motor and sensory supply to the right hind limb. General anesthesia was induced and the backs of all pigs were scrubbed with Betadine and draped with sterile sheets.

A skin incision was made in the midline of the back from L1 to S1 level. The supraspinous ligament and the periosteum were incised over the tips of spinous processes from l1 to S1. The lateral muscle masses of the right side were elevated from the vertebrae and hemostasis was secured by packing. Self-retaining retractors were inserted. The muscle mass over the laminae and the spinous processes were retracted and curetted laterally to the facets. The partial removal of the laminae was done from spinal level l2 to L7 to visualize the dura dn the nerve roots on the right side. The nerve roots of the right side from L2 to S1 were tied by 2-0 silk sutures 5 mm from the dura and cut 2 mm distally from the tied sutures. Bleeding from the ventro-lateral venous plexus to the dural sac was treated by gentle suction and packing with fibrin foam. After the complete right L2 to S1 rhizotomy, irrigation was done and the supraspinous ligament, sucutaneous tissues and skin were sutured by 3-0 Vicryl sutures. post-operatively, aspirin for pain relief and Ampicillin for infection control were given for seven days. A fiberglass cast (Scotchcast Plus, 3M) was applied on the insensitive right foot to prevent the pig from self-injury.

2. Creation of Pressure Sores: Seven days after the denervation procedure, a newly developed continuous pressure applicator was placed on the atrophied limb to create a pressure sore on the lateral side of the thigh over the trochanter. An 8 mm skin incision was made on the shaft of the femur 2 cm proximal to the trochanter. A drill guide was inserted through the incision and centered on the femur. A hole extending through both cortices was made with a 5 mm diameter drill bit. A self-tapping cancellous screw, used to anchor the pressure indentor device, was inserted into the femur. A modified locking collar was used to compress a calibrated spring against a 30 mm

diameter plastic indentor disk placed on the skin (Fig. 1). Pressure was adjusted up to 800 mmHg. Neither hemostatic agents nor sutures were required to control bleeding. To immobilize the limb and prevent disturbance of the pressure indentor, a right hip spica cast was applied immediately post-operatively. The animal was allowed to recover from anesthesia and given prophylactic antibiotics. Routine pain medication was not necessary because the limb had already been denervated. The pressure applicator was inspected daily and adjusted to 800 mmHg.

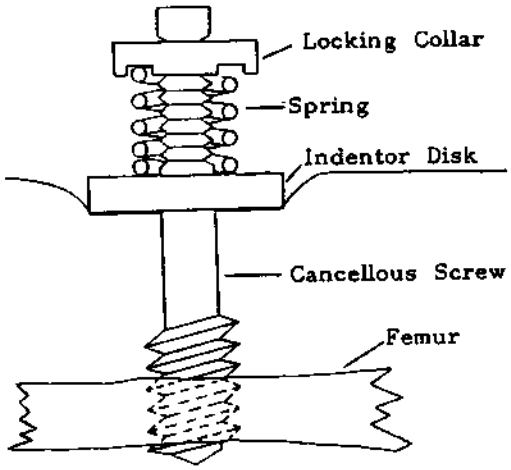

Figure 1. Spring loaded pressure applicator anchored through the femur

One to three days following the application of pressure, each pig underwent general anesthesia again and was prepared for debridement surgery. The decubitus site was debrided of all necrotic skin, subcutaneous tissue and muscle to viable bleeding tissue, usually to the depth of the trochaner. Four millimeter punch biopsies were taken from the periphery of the wound and from the opposite trochanter as control samples for histologic and biochemical assays. The wound cavity was photographed, packed with saline-soaked gauze, and the animal was returned to the paddock for recovery. Subsequent daily treatment included a change of wound dressing, cleansing with saline and 0.25 % acetic acid and administration of oral antibiotic. This procedure resulted in wound closure and healing without complication and the formation of healthy granulation tissue rapidly filling the wound cavity.

Electrical stimulation of the wound on each pig began the day after wound debridement. The wound on each animal was cared for daily following the standard hospital care for decubitus ulcers as described above. Two self-adhering Encore Plus 1.25 inch diameter electrodes (Uni-Patch Inc., Wakasha, MN) were placed on healthy skin opposite each other on the wound periphery. The stmulating direct current was supplied from an isolated source developed in this laboratory. The treatment perod continued for three to four weeks, with the treated wound stimulated for 2 hours ecah day for five days each week until termination. Testing and biopsies of the healing wounds were carried out at seven-day intervals.

The stimulation protocol used in the project was adapted from the clinical protocol employed at the Edward Kardelj University, Ljubljana, Yugoslavia (2).

Constant DC stimulation was applied on the healthy skin with electrodes distal and proximal to the wound and 3 cm away from the edge of the wound. It is not known if the current polarity plays some role in healing effects, but to unify the protocol, the cathode was applied distally. The appliied current amplitude was less than 1 mA, and the maximal current density between the electrodes was less than 80 $\mu A/cm^2$, depending on the size and depth of the wound. In all animals, skin irritation was not observed at any sites below the electrodes at this stimulation amplitude.

RESULTS

The denevation surgery required an average of 2-1/2 hours. There were no disturbanes in bladder and bowel function in any of the animals. The bone screw implant and placement of the pressure indentor device required approximately fifteen minutes. After full recovery from anesthesia, weak hip extension was the only voluntary movement observed in the denervated right limb. Any sensory deficit was evaluated with forceps to map the insensitive regions and any motor deficit was examined by manual muscle test. The sensory deficit observed in all animals was loss of sensation in dermatomes L3 to L7 on the right side. The borders (areas L2 and S1), however, were observed to be slightly sensitive. Although the right leg paralysis and cast did not appear to hinder their daily activity, most animals could not walk on their three intact limbs until 10 to 14 days after the denervation surgery. Muscle atrophy in the denervated limb was obvious within seven days after denervation. The spica casts did not impede the animals' ability to eat and drink. Their mobility, however, was compromised during the pressure application period. Indentor inspection and adjustment to maintain 800 mmHg was easily performed without sedation or anesthesia. Due to ischemia, the skin beneath the indentor was pale during the pressure application period.

Debridement surgeries averaged fifteen minutes and after the removal of the pressure application, the area was pale, depressed and had the odor of necrotic tissue. No bleeding was observed from the damaged tissue. All damaged tissue was removed until bleeding was seen. Nine wounds out of ten were extended down to the bone, establishing a Grade 4 woun (Table 1).

Pig Number	Pressure	Duration	Pressure Sore (grade)*
Control Group			
89-1	800 mmHg	72 hours	4
89-3	800 mmHg	48 hours	4
89-5	800 mmHg	48 hours	4
89-7	800 mmHg	48 hours	4
89-9	800 mmHg	48 hours	4
Stimulated Group			
89-2	800 mmHg	24 hours	3
89-4	800 mmHg	48 hours	4
89-6	800 mmHg	48 hours	4
89-8	800 mmHg	48 hours	4
89-10	800 mmHg	48 hours	4

* Following the Grading Schedule of the International Association for Enterostomal Therapy Grading.
If wound involves necrotic tissue, grading cannot be confirmed until wound base is visible.

Table 1. The results of pressure sore formation

Pressure sore wounds were created by the application of tissue compression using a 3 cm diameter plastic indentor anchored to the trochanter by a percutaneous cancellous bone screw. The uniformity of all wounds was determined by measurements of their initial volume and surface area.

Variations in the wound size between the control group and the stimulated group of animals was minimized by the uniform procedure, the use of identical pressure indentros and the body weight of the experimental animals. The initial and final body weights of the animals are shown in Table 2. Although the tendency of the animals was to gain weight, there was no significant difference found in the body weights of the control and experimental groups. The similar size of the animals suggested similar tissue thickness and wound depth in the trochanteric area of the animas. The increasing body weight, however, indicated a possible change in the depth of tissues at the periphery of the wounds toward the end of the healing period.

Pig Number	Initial Wt. (kg)	Final Wt. (kg)
Control Group		
89-1	12.0	12.9
89-3	9.5	14.2
89-5	19.0	18.2
89-7	19.5	23.0
89-9	18.0	23.2
Mean ± S.D.	15.6 ± 4.5*	18.3 ± 4.8*
Stimulated Group		
89-2	12.0	14.4
89-4	11.0	15.8
89-6	22.0	25.4
89-8	19.0	25.0
89-10	19.0	22.8
Mean ± S.D.	16.6 ± 4.8*	20.7 ± 5.2*

* No significant difference at $p < 0.05$ by the paired comparison or the difference in two means t-tests.

Table 2. Change in body weight of control and experimental animals

The initial wound area was measured in triplicate from a black and white photograph of the wound and from tiplicate markings of the wound perimeter on a transparent overlay. The photographed wound area was measured by tracing the wound at the border of the epidermis on a digitizing tablet wigh Sigma Scan attachment on an IBM PC/AT. A disk of known area, included in each photograph, was also traced for calibration of the measuring system. Wound area from the transparent overlay was measured by "counting the squares" on a millimeter grid within the marked wound perimeter. Among different observers, these methods of wound area measurements

correlated well and yielded highly reproducible results without statistically significant differences.

The initial wound areas, shown in Table 3, were recorded approximately three days after the removal of the indentor. During the intervening time, daily wound care, debridement and stimulation were provided in the ususal manner. The use of the constant 706 mm area indentor produced relatively uniform surface area wounds, but these were enlarged by debridement to an average slightly exceeding 1000 mm for both the control and the experimental groups of animals. Comparison of the mean areas of wound surface showed no significant difference between these groups.

Pig Number	Area (mm^2)	Age of Wound (Days)
Control Group		
89-1	1031	0
89-3	1544	1
89-5	1248	3
89-7	801	8
89-9	864	1
Mean ± S.D.	1098 ± 304	2.6
Stimulated Group		
89-2	924	4
89-4	1735	3
89-6	834	0
89-8	939	7
89-10	818	3
Mean ± S.D.	1050 ± 387	3.4

Table 3. Initial recorded wound areas

The volume of wounds was also measured by filling the tissue defect with sterile isotonic saline to the level of intact skin surface while the wound surface was maintained horizontally. The volume of saline used was reported as the wound volume. The mean initial wound volumes, after debridement on the day of removal of the indentor, were 12.2±3.3 ml for the controls and 12.0±5.1 ml for the experimental wounds. These values are statistically nonsignificant at a p value of 0.05.

CONCLUSION

The literature describes the use of various animal models for studying spinal cord related injuries, however these models use spinal-transected animals (3). There are no reports cited on the use of the monoplegic animal model. The benefits of this monoplegic pig model involving radiculectomies from the spinous levels l2 to S1 are

related to the minimal animal maintenance required. Bowel or bladder disfunction and other major complications are minimized because of the animals' ability to maintain a relatively normal level of activity. These advantages permit both acute and chronic experimentation.

We have succeeded in creating Grade 4 pressure sores in all but one case by applying a continuous constant pressure to the skin surface with the pressure applicator device described. The benefits of the device are: (1) the application of direct pressure between the bone and skin surface; (2) control and regulation of the applied pressure; and (3) the management of continuous pressure for several hours to days.

This reproducible pressure sore model in the trochanteric area of the flaccid monoplegic hind limb of the mini-pig represents a basic new tool for determination of the factors that govern the process of pressure sore wound healing.

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