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Effect of Shock Waves on the Healing of Partial-Thickness Wounds in Piglets

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During the last 20 years, the role of various physical factors in wound healing has been widely studied and recognized. With the use of shock waves for the treatment of urolithiasis, a new mechanical medium has been introduced into medicine. The influence of shock waves on the reepithelialization of partial-thickness wounds was studied in four Yorkshire piglets by a quantitative morphometric method. Wounds were inflicted either in intact skin (three pigs) or in skin irradiated with 1500 rads to achieve delayed healing. A significant enhancement in normal or delayed healing was found with low-dose treatment (10 SW at 14 kV). High-dose application of shock waves (100 SW at 18 kV) resulted in inhibition of the rate of reepithelialization of the wounds. Shock waves of intermediate energies were without effect. The stimulating effect of low-energy shock waves coincides with significantly increased vascularization of the upper dermis and thicker layer of the newly formed epithelial cells covering the wound. © 1990 Academic Press, Inc.

INTRODUCTION

During the last 25 years, it has been shown that the healing process can be promoted by physical influences. Most of the physical factors were tested in biological systems involving bone growth or skin wound healing. These factors included electrical stimulation [19], electromagnetic field [3–5], piezo electricity [13, 15], ultrasound [10, 17, 18, 20] or mechanical influences such as intermittent tension [1, 2], effects of immobilization, and continuous passive motion [21] and micromovement [14, 16]. Although the actual mechanism(s) involved in the stimulation of wound repair by the above listed factors are not understood, several hypotheses were suggested. Cell proliferation, the activation of cells which enhanced deposition of structural macromolecules by these cells, and also the faster remodeling of the treated tissue may explain these effects. These hypotheses include the effect of the transduced physical energy on the structural and functional changes of molecules at the plasma membrane level (cAMP, cGMP, ATPase, etc.). By a second messenger mechanism they promote cell mitosis and enhance cell activity to synthesize structural macromolecules. It can well be an induced effect of physical forces on the mediators or activators of cell activity, such as release of serotonin or histamine by mast cells.

A new mechanical factor—shock waves—was introduced into medicine [6, 7, 11, 12] for the treatment of urolithiasis: extracorporeal shock wave lithotripsy (ESWL). A shock wave is a single-pressure impulse which can be created by a high-voltage spark discharge under water causing explosive evaporation of water. Shock waves can be focused by a semiellipsoid and therefore concentrated on rather small regions. Because of similar acoustical characteristics, shock waves can be transmitted into a human body.

The ability to control the intensity and number of shock waves and thus the actual effect of the shock waves on the tissue structures allows us to test the working hypothesis that shock waves (similar to other physical factors) at low energy levels will stimulate the cells of the wound and consequently will enhance the wound healing; whereas with shock waves of high energy, we expected inhibition of cells due to cell destruction and necrosis.

The purpose of this study was to evaluate the influence of different energy inputs of shock waves on the healing of split-thickness wounds in a standardized model in piglets [8]. This model quantitates the degree of reepithelialization of the wound by morphometric evaluation of epithelial coverage of the wound area.

EXPERIMENTAL DESIGN

Four Yorkshire female pigs with a body weight of 9–11 kg were used in two separate experiments.

Experiment I: Healing in Intact Pigs

Split-thickness wounds (1 × 1-cm area, 0.3–0.5 mm deep in pigs 1–3) were inflicted with an electrokeratome (Storz). Eight to twenty-two wounds were inflicted in each pig, totaling 143 wounds in the entire study. The wounds

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were covered with waterproof Op-Site adhesive film. With the Dornier experimental lithotriptor XL1, shock waves were applied at 14- or 18-kV generator voltage and 10 to 1000 shock waves (SW) per treatment. The experimental design is evident from Table 1. Before their exposure to shock waves, pigs were anesthetized with halothane and placed in a 38 ± 0.5°C water bath using a specially designed restrainer. Two to four wounds per pig remained untreated and served as control wounds. To achieve equal shock wave application to the complete wound area, the wounds were treated in the blast path 0.7 cm above the focus point F2 [6, 7]. This was established by treating normal skin with 1000 SW: the resulting reddened area was the same size as the wounds.

After treatment, the Op-Site occluded wounds were rinsed with a penicillin–streptomycin solution to avoid infection due to possible leakage of water onto the wound.

Experiment 2: Healing in Pigs with Irradiated Skin

To develop a model for delayed healing, one additional Yorkshire piglet, female, 9 kg body wt was used. The dorsal skin surface, left and right side, 7 × 10-cm area below the ribs, was irradiated with a single fraction of 1500 rads by electron beam with computerized dosimetry control produced by a Varian Clinac-18 linear accelerator. The treatment dose was approximately 450 cGy/min. The spine and surrounding skin were protected from radiation by a lead shield frame. Two days after irradiation, four partial-thickness wounds, 2 × 2 cm and 400 μm deep, were excised. Only one side of the back in the pig was treated with shock waves 36 hr after inflicting the wounds. The pig was sacrificed after 54 hr and the wounds were fixed in formalin. Four random cuts of each wound were stained with hematoxylin–eosin. The magnitude of epithelialization of the wound was counted in four sections in relation to the whole length of the wound [8]. The percentages of epithelialized wound area in individual treatment groups were compared with Duncan's multiple range test [9].

RESULTS

Reepithelialization of Partial-Thickness Wounds Inflicted in Intact Skin

Our previous study showed that after 54 hr the normal rate of reepithelialization in split-thickness wounds varied from 50 to 70% depending on the age, weight, and nourishment of the animal and the density of hair follicles [8]. In this experiment (Table 1), the control wounds inflicted in three piglets reepithelized by 56 to 67% of the original wound area. Wounds treated with 100 SW at 14 kV and 10 SW at 18 kV had similar rates of epithelialization as nontreated control wounds. With increased numbers of SW (500 and 1000 SW at 14 kV, 100 SW at 18 kV) healing was significantly inhibited ($P < 0.05$), while with low-dose treatment (10 SW at 14 kV) reepithelialization was significantly enhanced ($P < 0.05$) in two of three independent experiments. The reaction of the epithelial cells of the skin wound followed a dose–response curve, as documented in Fig. 1 based on combined data from Table 1. The variability of the results is shown in Table 1, which also indicates the number of individual measurements per treatment.

During morphologic evaluation of the sections, we noted a major distinction among various shock wave treatments. In wounds treated with low doses, the upper dermis, directly beneath the newly formed epithelial layer, showed increased numbers of dilated microvessels filled with erythrocytes and with increased numbers of macrophages in perivascular spaces. Also, the newly formed epithelial layer was four to five cells thick, almost twice as thick as in control wounds or wounds treated with medium-energy shock waves. Quantitation of the number of microvessels per six 100-fold magnification fields, as well as the thick-

TABLE 1

Effect of Shock Waves at Five Energies on the Epithelialization Rate of Partial-Thickness Wounds in Piglets

Treatment (voltage, number of pulses)	Pig 1		Pig 2		Pig 3	
	N[a]	Epithelialization[b]	N	Epithelialization	N	Epithelialization
Control	14	55.7 ± 10.1 A[c]	6	66.0 ± 17.9 A	7	67.3 ± 9.5 A
14 kV, 10	11	82.5 ± 11.7 B	7	89.1 ± 15.2 B	6	81.0 ± 21.3 A
14 kV, 100	13	55.9 ± 9.8 A	7	67.6 ± 15.5 A	8	60.5 ± 18.6 B
14 kV, 500–1000[d]	7	56.6 ± 10.5 A	5	52.2 ± 7.9 A	4	41.8 ± 3.9 C
18 kV, 10	16	61.8 ± 16.7 A	7	48.0 ± 27.3 A	4	65.8 ± 10.4 A, B
18 kV, 100	15	41.4 ± 10.0 C	6	39.7 ± 16.6 C		

[a] Number of evaluated wound sections.
[b] Variability is given as $X \pm$ SD.
[c] Letters refer to statistically significant differences within 95% confidence limits, calculated by Duncan's multiple-range test. Values followed by different letters are significantly different.
[d] 500 pulses in pigs 1 and 3, 1000 pulses in pig 2.

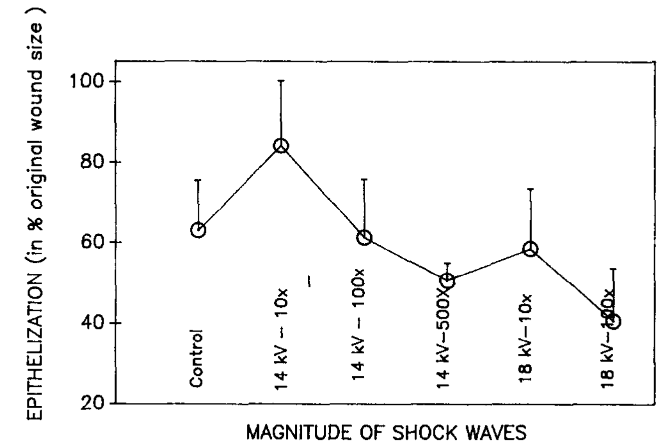

FIG. 1. Effect of shock waves of various energies on epithelialization of partial-thickness wounds in pigs.

ness of the epithelial layer in the three tested groups, is shown in Table 2. There are a significantly larger number of microvessels and a significantly thicker newly formed epithelial layer in wounds treated with low-energy/low-frequency shock waves.

Effect of Shock Waves on Epithelial Healing of Wounds Inflicted in Irradiated Skin

In our previous research, 6-month-old Yucatan miniature pigs were inflicted with partial-thickness and deep wounds. We found that after irradiation of the dorsal aspect of their skin with 1500 rads, the wounds healed 30 to 50% slower than control, nonirradiated skin wounds (Chvapil *et al.,* unpublished results). In this study, irradiation of the skin of Yorkshire piglets, approximately 6 weeks old, induced a delay in reepithelialization of control wounds by 20% when compared to data of Experiment 1 (Table 1).

The results shown in Table 3 document the significant increase in the rate of reepithelialization of wounds subjected to low-energy shock waves. Thus, the rate of healing was increased by 50%, based on the analysis of 16 sections made of two wounds exposed to shock waves and compared to the same number of analyzed sections of wounds exposed to radiation only.

DISCUSSION

Our experiment proved that the application of shock waves can influence the healing of split-thickness wounds in a dose-dependent fashion. Even though higher-energy shock waves (in number or generated voltage) result in inhibition, we were able to stimulate reepithelialization with low-energy treatment either in wounds inflicted in intact animals or in wounds in which healing had been delayed by exposure to radiation. Intermediate energies ranging from 100 SW at 14 kV to 10 SW at 18 kV were ineffective. This form of mechanical stimulus is similar to other physical factors studied, and even commercially used, to promote the healing process. While this study deals with the healing of partial-thickness skin wounds, another study revealed the promotion of healing of experimental fractures in rats by low-energy shock waves (Haupt, Chvapil, and Gerety, unpublished results, 1987). Thus, we are inclined to believe that shock waves, administered at certain energy levels and during a specific time of the healing process, can promote the repair process, cell kinetics, or other phenomena involved in wound healing. As a possible mechanism, we suggest that shock waves induce hyperemia. This is supported by the larger number of capillaries or dilated microvessels observed in wounds exposed to low doses. Another possible mechanism involves changes in cell membrane fluidity. Both hypotheses have been suggested as mechanisms by which other mechanical factors influence healing [3–5, 13–21].

There is no doubt that various mechanical–physical stimuli (tension, pressure, vibrations, electrical field, electromagnetic field, ultrasound) promote wound healing

TABLE 2

Effect of Shock Waves on a Number of Microvessels and Epidermal Thickness of Partial-Thickness Wounds in Yorkshire Piglets[a]

Treatment (voltage, number of pulses)	Number of microvessels in optical field[b]	Epidermal thickness[c]
Control	4.8 ± 1.9 A[d]	37 ± 12 A
14 kV, 10	9.2 ± 1.9 B	54 ± 11 B
14 kV, 500 or 1000	5.1 ± 2.1 A	29 ± 7 A

[a] Data are based on measurements from slides derived from wounds in pigs 2 and 3 (see Table 1).

[b] Measured at 100× magnification field in upper dermis adjacent to the newly formed epithelial layer.

[c] Measured by videomicrometer setup, with readout from TV screen projection in micrometers; $X \pm$ SD.

[d] Statistical significance by Duncan's multiple-range test is shown by letters. Values followed by different letters are significantly different at $P < 0.05$.

TABLE 3

Effect of Shock Waves on the Reepithelialization Rate of a Split-Thickness Wound in a Piglet[a]

Treatment	% Skin wound reepithelialized ($X \pm$ SD)		Number of measurements
Control, untreated	53.0 ± 14.6	$P < 0.05$	16
Lithotripsy at 36 hr, 14 kV, 200 shocks	77.2 ± 18.7		16

[a] Data refer to 9-kg Yorkshire piglets, exposed to 1500 rad radiation. Two days later, eight shallow wounds, 2.2 × 2.2 × 0.4 cm, were inflicted and exposed to shock waves 36 hr later.

under certain conditions. It remains to be shown which of these methods is most effective and convenient for clinical use.

Finally, we stress the methodological problems involved with this study. It is necessary to use extremely small, young pigs because of the size of the water bath in which the animals are immersed during shock wave administration. Thus, piglets of 8–9 kg body wt (corresponding to 3–4 weeks) were used. These animals are fragile and difficult to handle. Their skin is thinner, and we encountered difficulty in producing wounds of uniform depth around the lower part of the torso, as the thorax cannot be exposed to shock waves because of possible lung damage.

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