Therapeutic Ultrasound and Wound Closure: Lack of Healing Effect on X-Ray Irradiated Wounds in Murine Skin

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Objective: To evaluate the efficacy of ultrasonography as a therapeutic agent in wound healing.

Design: Randomized, controlled trial.

Setting: University animal laboratory.

Animals: Male BALB/c mice randomly allocated to 5 groups.

Interventions: In group 1, mice were left untreated; in groups 2 through 5, a well-defined area on the dorsum was exposed to 20Gy x-ray irradiation. Seventy-two hours postirradiation, all mice were anesthetized by inhalation (isoflurane anesthetic) and a 7 x 7mm area wound made on the dorsum. All wounds were videotaped alongside a marker scale 3 times weekly until closure was complete. Mice in groups 4 and 5 were treated with pulsed therapeutic ultrasound for 5 minutes, 3 times weekly at 1 and 3MHz, respectively (intensity, 0.5W/cm²); mice in group 3 received placebo ultrasound. Subsequently, the area of each wound was measured from video by using an image analysis system.

Main Outcome Measure: Wound closure as a fraction of day zero.

Results: Irradiation caused a significant (p < .01) delay in the rate of wound closure by day 11. However, neither placebo ultrasound nor treatment at 1 or 3MHz affected the closure rate.

Conclusion: These findings provide little evidence that 1 or 3MHz ultrasound applied to a radiation-impaired wound stimulates wound closure in mice.

Key Words: Mice, inbred BALB c; Rehabilitation; Skin; Therapeutic ultrasound; Wound healing.

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Therapeutic ultrasonic applications have been widely used over the past 50 years to treat various conditions, including bursitis, Dupuytren’s contracture, soft-tissue injuries, and herpes zoster. Reported effects include decreased pain, increased circulation, and increased tissue mobility. Since the 1960s, the benefits and effects of ultrasound in the promotion of wound healing have been investigated by many investigators. Although several studies have investigated its therapeutic benefits on pressure sores and venous ulcers, the clinical use of ultrasound for wound healing is still under investigation because many investigators report conflicting findings. In a recent review of the literature, Johannsen and al found only 14 studies on the effects of ultrasound in the treatment of chronic leg ulcers, and proposed that further research should be undertaken to determine its effects and to evaluate any dose-response relationship.

Current literature presents with conflicting findings. Early experiments reporting positive results included placebo controls, but no randomization, and some long exposure times. Current research has shown a significant uptake of calcium ions by using an ultrasound intensity of 0.5W/cm² at a frequency of 1MHz, which suggests changes in cell membrane permeability with subsequent effects on intracellular communication. It has also been shown that insonation (.75MHz, 0.5W/cm²) can effectively stimulate fibroblasts, possibly by influencing macrophage activity. From animal studies in rats, researchers have found that ultrasound at an intensity of 0.1W/cm² (at .75 and 3.0MHz) accelerates the inflammatory phase of repair. Several investigators have reported significant increases in rates of wound healing in rats and rabbits at intensities of 0.1 and 0.5W/cm², respectively; however, these groups did not specify the ultrasound frequency, making comparison with other studies difficult. Nevertheless, this reported accelerated repair is in agreement with findings from several other studies suggesting that low-dose ultrasound of approximately 0.5W/cm² pulsed with a frequency of 1 MHz promotes wound healing.
of experiments using a chronic wound model in animals would permit more rigorous examination of therapeutic ultrasound applied under controlled conditions. A wound that models impaired healing can be induced by (1) inoculating a bacterium or virus into the wound, (2) creating a diabetc state, or (3) irradiating the wound with x-rays.28-31 X-ray irradiation impairs healing by decreasing new collagen formation and contraction.30 The resultant delay in the healing process provides a suitable model for testing new wound healing agents in an impaired healing environment.30,31 In work conducted at the present study’s institution, Lowe et al32 reported that a single dose of 20Gy x-ray irradiation before wounding delays wound healing by approximately 7 days. Radiation has both acute and delayed effects on an organism’s ability to heal.33 It induces acute degenerative changes in basement membranes and increases vascular permeability.33 Changes in vasculature may include stasis and occlusion, edema of vascular walls, and thrombosis; radiation may also reduce neovascularization.33,34 Progressive loss of vessels and fibrous tissue replacement may eventually occur, whereas fibroblasts may be altered permanently—either the fibroblasts do not produce sufficient collagen to keep up the demands of the wound, or the collagen that is produced does not mature quickly enough.35,36 Late radiation effects attributable to fibroblast injury include atrophy, contraction, and fibrosis.35-38 The delayed healing allows procedures such as ultrasound therapy to be tested.

Our aim in the present study was to investigate the effects on wound closure of pulsed ultrasound at 1 and 3MHz at an intensity of 0.5W/cm² in a murine model to provide quantitative data to guide further research and use of therapeutic ultrasound in the clinical setting.

METHODS

The current investigation, which adhered to the University of Ulster’s regulations on animal experimentation, included male BALB/c mice, age-matched at 12 weeks old (n = 60; mean weight, 28.31g). Animals were supplied with food and water ad libitum, and housed individually to prevent cross-tampering with wounds. The animals were randomly assigned to 1 of 5 experimental groups (n = 12, each group). Group 1 was the nonirradiated control; group 2, the irradiated control. Groups 3, 4, and 5 all received x-ray irradiation and ultrasound treatment: group 3, placebo ultrasound; group 4, ultrasound at 1MHz; and group 5, ultrasound at 3MHz.

The hair on the dorsum of the mice was shaved; all animals were then placed individually into custom-made lead jigs, which allowed a 4cm² area of dorsal skin to be exposed. The area was marked with indelible ink and the mice exposed to 20Gy x-ray irradiation by using a Siemens Stabilipan x-ray machine.30 Seventy-two hours after irradiation, hair on the dorsal surface was reshaved (where required) and the skin cleaned with 70% alcohol. Mice in all groups were then anesthetized by inhalation by using isoflurane anesthetic,39 and a 7 × 7mm area of skin was removed from within the area previously exposed to x-rays. An equivalent area was removed from mice in group 1.

In groups 4 and 5, mice received ultrasound therapy 3 times weekly by using a stationary head, Sonopuls 590 device. The parameters of this unit were: group 4, 1MHz, pulsed 1:4, pulse duration 4ms at 48Hz, 0.5W/cm² intensity; and group 5, 3MHz, pulsed 1:4, pulse duration 4ms at 48Hz, 0.5W/cm² intensity. Group 3 received sham ultrasound therapy at equivalent time periods. During each treatment session, ultrasound therapy was administered for 5 minutes by using a stationary head and standard coupling gel.40 All wounds were videotaped adjacent to a marker scale 3 times weekly until closure was complete. Wound areas were then calculated by means of an image analysis system. To allow for variation between the initial size of the wound on each animal, data were expressed as the fractional change in wound area for each mouse. The results were then analyzed blind by using repeated-measures and 1-factor analysis of variance at 95% confidence levels, with post hoc Fisher tests where appropriate.31,32

RESULTS

In the present murine model, the overall rate of wound closure was not accelerated after therapeutic ultrasound treatment. Figure 1 shows wound closure (areas expressed as a fraction-of-day-0 values) for the nonirradiated controls (group 1) and the irradiated controls (group 2). Although both groups had a similar pattern of wound closure, by day 11 postwounding group 2 had a statistically significant (p < .01) delay in wound closure when compared with group 1. Such statistically significant differences continued until day 23. After day 23, the effects of x-ray irradiation on wound closure were statistically nonsignificant; however, the wounds in group 1 reached com-
The overall effect is dermal atrophy, contraction, and epithelial cell necrosis, which precipitates microvascular atrophy.

Wound healing is characterized by fibroblast injury and endothelial damage, and releasing free radicals that can cause endothelial damage, and releasing free radicals that can affect the onset of the proliferative phase. The effect of radiation on the rate of wound healing has also been shown to be dose dependent. Marx et al. used a dose of 60Gy in rabbits and reported fibrosis and hypovascularity 6 months postirradiation. Several investigators reported that a wound healing deficit can be caused by a single 18 to 20Gy dose to the skin surface before wounding. Such irradiation lets researchers evaluate in a laboratory the effects of various treatment methods on the rate of compromised or delayed wound healing.

Our present results contrast with most of the previous research on ultrasound. Young and Dyson reported that application of ultrasound (75MHz, 0.1W/cm²) caused an early reduction in wound size. Many investigators agree that the application of ultrasound shortens the inflammatory phase of repair, and can affect the onset of the proliferative phase. However, the lack of stimulatory effects in the present investigation supports the findings of Shamberger et al. who reported no increase in wound breaking strength in rats treated with ultrasound at .05 and .15W/cm² with a frequency of 5MHz. Interestingly, Shamberger—who used a stationary head technique as in the present study, but with a higher frequency—suggested that a stationary treatment head is necessary to determine accurately actual energy exposure within the wound. In contrast, Byl et al. suggested that the movement of the ultrasound head acts as a further mechanical stimulus to collagen deposition. These arguments notwithstanding, the small wound area in the present investigation was completely covered by the treatment head in all ultrasound treatment groups; a moving application would have been impractical and unnecessary.

Byl proposed that the treatment head should be moved when applying intensities over 1.5W/cm² and found increased wound breaking strength when ultrasound was applied at an intensity of 0.5W/cm² and a frequency of 1MHz. It was previously suggested that unless the treatment head is moved, standing waves form, arresting the local movement of blood, causing endothelial damage, and releasing free radicals that indicate transient cavitation, all of which may result in a detrimental effect on the rate of wound healing. Although this reaction may explain the present findings, we found no such inhibition of healing in the present study when we compared irradiated control and placebo groups.

In the present study, effects on wound healing were determined by the rate of wound closure. Measuring wound breaking strength may have allowed comparison with the study by Byl, in which the investigators used similar parameters, and wound breaking strength is an important area for future research. It may also be beneficial in further studies to examine the wounds histologically to detect differences between treatment groups and controls. This approach would allow researchers to investigate cellular content, granulation tissue formation, and collagen deposition for different physical parameters. In a study by Young and Dyson, a significantly higher level of fibroblasts occurred in rat wounds after sonation of 0.1W/cm² intensity at .75 and 3MHz. The cells were found to be aligned in a manner conducive to efficient wound contraction, and the overall conclusion was a resultant acceleration in the inflammatory phase of repair and an earlier onset of wound contraction. Because histologic examination was not performed in the present investigation, some differences in groups may have been overlooked.

In the search for an improved experimental model more relevant to wounds encountered clinically, the use of mice or other studies using x-ray irradiation as a method for producing a chronic wound model.
other loose-skinned animals may not be the optimum choice. Because these animals heal by wound contraction, in contrast to humans who heal by reepithelialization, conclusions made from studies on loose-skinned animals may not always be directly relevant to humans. Nevertheless, these studies can be useful as precursors to further research, such as those investigating the most beneficial physical parameters and mechanisms of action. Pigs were used in a study by Byl, et al because porcine skin physiology is similar to that of humans; the investigators reported that wound strength and rate of closure improved after ultrasound therapy (0.5W/cm² for the first 2 days; 1.5W/cm² for the subsequent days). Thus, pig skin may be a more suitable wound healing model for investigating the effects of ultrasound therapy.

Results from the present murine model may also be limited by the difficulty of extrapolating for application in humans. Considering the relative size of mice compared with the average human patient, dosage and area of treatment used in the present study were higher than would be used in clinical practice. The present findings are not necessarily indicative of potential effects in humans, particularly in cases of wounds from causes such as diabetic ulceration and infection. Nevertheless, the present protocol provides an animal model of radiation-impaired healing, which is a significant problem in oncology and radiation medicine. Whether a similar porcine model of healing would be more sensitive to the putative effects of therapies such as ultrasound remains to be established. The porcine model is an important area for future investigation, particularly because of its clinical relevance and the ethical constraints of undertaking controlled research in a patient population.

CONCLUSION

The present murine study provides no evidence to support the claimed stimulatory effects of therapeutic ultrasound on the rate of wound closure at 1 and 3MHz in the irradiation-impaired healing model. Nevertheless, because previous researchers proposed that the application of ultrasound therapy leads to an earlier proliferative phase of repair through a stimulatory effect on inflammatory mediators, further research is required to determine the effects of different doses of ultrasound therapy when applied for different durations at different stages in the repair process.

The chronic wound is a significant problem for health professionals throughout the world. A careful understanding of the repair process in compromised or delayed wound healing, and the effects of various treatments are required to make a positive intervention in this area. Although the present study does not provide evidence to support the claimed effects of therapeutic ultrasound, further work to establish its efficacy remains a priority.

References


Suppliers
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