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ORIGINAL ARTICLE

THE EFFECTS OF ULTRASOUND THERAPY ON SKIN SCARS OF RABBITS

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Abstract

Objective-The aim of this study was to determine the effects of ultrasound therapy on cell counts and the depth of healing of the rabbit's skin scar.

Method-Under general anesthesia and sterile conditions, four full-thickness incisions were made on the back of seven rabbits. The first, second, third and fourth wounds were selected for ultrasound therapy, placebo ultrasound, application of lubricant gel and betadine, respectively. Ultrasound therapy program (1 MHz, pulsed 2:8, 0.5 Watt/cm^2 and 4 minutes duration) and other treatments were performed daily from day zero to day 30. On day 30, the rabbits were sacrificed by ether and tissue samples were prepared and stained with hematoxylin and eosin (H & E). The fibroblasts, macrophages, neutrophils and endothelial cells were counted and the depth of the scars and new epidermis was measured with special eye pieces. Finally, data was analyzed with the analysis of variance method.

Results-The highest number of fibroblasts, macrophages and neutrophils belonged to the placebo group, the highest number of endothelial cells and depth of scars were present in the gel group, while the highest mean new epidermis depth belonged to the ultrasound therapy group. However, none of above-mentioned results were statistically significant.

Conclusion-According to this study, ultrasound therapy has no beneficial effect on the incisional wound scar tissue of rabbit's skin.

Keywords • Ultrasound therapy • cell count • skin scar • rabbit

Introduction

The healing of dermal incisional wounds is a dynamic and complicated process which is accomplished by the activity of a collection of cells that pursue a unified goal. The process of healing has been divided into the phases of inflammation, cellular multiplication and collagen synthesis (granulation tissue), wound renewal and collagen maturation (scar tissue).[1] These phases overlap and in fact, in the maturation phase, new epidermis, fibroblasts and vessels are produced. Renewal of the extracellular substance structure begins slightly after the appearance of these structures and continues to do so for a few more months.[2]

The major part of the research on wound healing has been focused on accelerating the healing process so that the normal strength is attained and hypertrophic and colloid scar formation is prevented.

Studies show that when compared to inflammation, defects in the healing process has resulted in a higher rate of mortality.[3]

Ultrasound therapy is one of the modalities of physical medicine which is used by specialists for pain management and for increasing blood flow and mobility.[4-6]

Studies about the effect of ultrasound therapy on wound repair show that during the phase of inflammation, ultrasound may decrease edema, increase mast cell degranulation[4,8] , and increase the blood supply and vascularization, which indirectly increases the transport of oxygen and macrophages to the wound site.[9,10] In the phase of collagen deposition and structural renewal, the scar tissue is stimulated.[4,8,11] However, most laboratory studies are related to the inflammatory and cellular division phases of the healing and unfortunately, few studies have been published about the effects

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of ultrasound therapy on scar tissue.

In 1979, after treatment of episiotomy scars with ultrasound therapy, Fieldhouse announced that ultrasound therapy decreases pain and leads to stronger tissue repair.[12] In 1999, in a review article, Ter-Haar announced that the scar exposed to ultrasound may be stronger and more elastic than normal scar tissues.[13] However, a study conducted by the authors did not support the benefical effect of ultrasound therapy on the tensile strength of scar tissue.[14]

Untreated scars may lead to difficulty in movement, impairment of social behavior, distorted self-esteem and therefore diminished daily activity.[15] With regard to the few controlled studies performed on the effect of ultrasound therapy on scar tissue formation and the phase of structural renewal along with the controversies in the field[4-6], the present study was designed. In this study, the impact of 30 days of ultrasound therapy on the skin wound of rabbits was assessed. The main variables measured comprised cell counts of fibroblasts, macrophages, neutrophils and vascular endothelial cells, along with calculation and assessment of new epidermis and scar tissue thickness.

Materials and Methods

In this study, seven male rabbits of the Dutch species, each weighing 1.5 kg and about five months, which were all bred in the Pasteur Institute of Iran were used. They were kept in a single animal house at temperatures between 20-23˚ C with an alternating equal 12-hour light and darkness periods.

In order to produce a skin wound, the hair on the back of the rabbits was shaved and the skin in the area was disinfected with povidone iodine (Iran Drug Production Co.). On the day of the incision, ketamine hydrochloride or Calypsol (Gedeon Richter Co. Hungary), diazepam (Chemidarou, Iran) and pentazocine (Iran Drug Production Co.) were administered intramuscularly. The dose of ketamine, diazepam and pentazocine was 35 mg/kg, 3 mg/kg and 0.4 mg/kg, respectively. A few minutes after disinfection with povidone iodine, 4 longitudinal incisions each approximately 3 cm in length were made in the following order: two incisions below the inferior angle of the right and left scapulae and two further incisions above the right and left iliac crests. While making these incisions, caution was made not to damage or excise the superficial cutaneous veins and to inflict the least possible vascular damage. Incisions were made with scalpel No. 24. Incisions included the whole skin thickness and cutaneous muscular layer (panniculus carnosus). In order to hold the two wound edges 2 mm apart, two separate stitches were made with the curved silk reverse cutting 3.0 needle (SUPA Industries, Iran) for each wound. The day the incisions were produced, was quoted as day zero. One incision was considered for the ultrasound therapy group and the other incisions for the control group as follows: two caudal incisions were randomly chosen for the ultrasound group and the silent (placebo) ultrasound group respectively and two cephalic incisions were considered for Betadine application and jelly impregnation respectively. In the experimental group, the ultrasound therapy program was applied immediately after the incision was given and was repeated on a daily basis for 30 days. During these sessions, the animals were kept immobile via a container. Transesophageal echocardiography lubricant (Nik Iran Co., Tehran) was applied on the incision as well as on the surrounding skin. Ultrasound therapy was delivered by the Sonoplus Enraf Nonius device using 1 MHz, pulsed 2:8, 0.5 Watt/cm^2 for 4 minutes. Ultrasound waves were applied to the area by to and fro-movements. Prior to the study, the device was calibrated by the Sepehran Importing and Servicing Company in Tehran. The ultrasound-therapy program was executed in the control group exactly similar to the case group, except that the device was off. In the lubricant receiving group, jelly was applied on the incision and its surrounding skin once a day. After 4 minutes, it was wiped off with sterile gauze. In the Betadine group, Betadine was poured on the site.

After the different therapies had been applied on the incisions, the wound surfaces were covered by sterile paraffin and cotton gauze. The animals' bodies were covered by tough canvas cloth, thus supporting the bandages and preventing infection. During the study, the rabbits were kept in clean solitary cages with free access to water and food including lettuce and carrots. On day 30, the rabbits were sacrificed by ether inhalation in a closed chamber. Specimens were taken from the scar tissues and surrounding skin and then suspended in formaline neutral buffer fixating solution.

After tissue processing and blocking with paraffin wax, 6 μm slices were prepared, using a

German microtome with a non-detachable Leitz blade and subsequently stained with hematoxylin and eosin. The eye piece of the MIC 0078-19 scale with 400 squares Euromex microscopes (Holland) was mounted on a light microscope to perform cell counts. Fibroblasts, macrophages, neutrophils and endothelial vascular cells of scar tissues were counted. The area of the surface examined for each cell type in each specimen was 625,000 μm^2 at the magnification of 400X. The eye piece MIC 1143, HWF 10X manufactured by the above-mentioned company was used to calculate the thickness of each specimen. Using 40X magnification on the light microscope, the thickness of five different points lying at equal distances from each other were measured and the mean value was divided by 40. The measurements obtained were converted to millimeters. Using the 400X magnification on the light microscope, the thickness of 5 different points on the new epidermis were calculated and the mean value was determined in micrometers. ANOVA was applied and $p<0.05$ was considered as significant.

Results

On the second day, a crust was formed on the incision and the stitches were removed on the 10th day. One of the rabbits died before obtaining specimens due to unknown reason. Table1 shows the mean and standard deviation of the number of fibroblasts, macrophages, neutrophils and vascular endothelial cells in the groups under study. The highest mean of the first three cells (fibroblasts, macrophages and neutrophils) was seen in the placebo group and the highest endothelial cell number was observed in the lubricant group. No significant difference existed between the above-mentioned variables in any of the study groups. Table 2 shows the mean and standard deviation of scar tissue and new epidermis thickness in all four groups. Scar and new epidermis thickness were highest in the lubricant and the ultrasound therapy groups, respectively. None of the differences between the various groups were significant.

Table 2. Skin thickness in four different groups as mean±SD.

Group	Scar (mm)	New epidermis (μm)
Ultrasound therapy	2.3±0.8	69.4±19.3
Placebo	2.4±0.6	67.2±13.9
Lubricant	2.8±0.4	69.2±23.7
Betadine	2.4±0.9	65±13.7

Discussion

Ultrasound therapy, as used in this study, results in a reduction in the number of fibroblasts, vascular endothelial cells and scar tissue thickness as compared to the placebo, lubricant and other groups, respectively. In addition, the number of neutrophils decreased as compared to the other groups (with the exception of the Betadine group) and the number of macrophages increased in all other groups (with the exception of the placebo group). None of the above changes were statistically significant. These changes indicate the absence of positive effects of ultrasound therapy in our study on the cellular components and tissue thickness of the wounds as applied in this study. If ultrasound had positive results on the healing process, we should have found tissue having significantly less fibroblasts in the test group in comparison to the controls.[16] The repair tissue had failed to show the characteristics of improved wound healing during the maturation phase of scar tissue (i.e. significant reduction in the number of

Table1. Number of fibroblast, macrophages, neutrophils and vascular endothelial cells in different groups under study (data shown as mean±SD).

Group	Number of fibroblasts	Number of macrophages	Number of neutrophils	Number of endothelial cells
Ultrasound therapy	198.6±42.4	3.2±0.8	0.2±0.3	2.1±1.6
Placebo	220.8±44.4	3.3±1.9	1±1.3	1.1±0.8
Lubricating gel	154.9±38.8	1.2±0.7	0.7±1	2.2±1.7
Betadine	191.5±37.9	1.3±0.1	0.1±1	0.4±0.4

cells and tissue thickness) as compared to the other groups under study. Table 1 shows that the number of macrophages, neutrophils and vascular endothelial cells were less than the number of fibroblasts in all four groups. Regarding that the specimens under study were in the structural renewal phase in which inflammation has subsided, this aspect seems normal. The results of the present study may in part be due to probable adverse effects which are attributed to ultrasound.[17,18]

In 1995, De Deyie and Kirch-Volders used ultrasound waves with a frequency of 1 MHz and pulse-mode for human fibroblasts in culture medium for periods of zero, 30, 60 and 90 seconds. Time related reduction in the number of improved cells showed a four-fold increase in the mitosis index of cells receiving ultrasound therapy and an eight-fold increase in the chromosomal aberration and absence of mitosis spindles.

It has been speculated that under the above-mentioned study conditions, the cells had received a higher dose of ultrasound, as compared to clinical conditions.[17]

Miller, et al in 1995 showed that *in vitro* ultrasound could povoke DNA damage through a phenomenon called cavitation.[18] It could act directly on cells or indirectly via the production of H_2O_2 or other audio-chemical effects. Researchers believe that it is hard to determine the potential cons and pros of cavitation in clinical ultrasound therapy considering its safety and therapeutic effects.

Regarding the results of our study, we conclude that ultrasound therapy, used as described above, probably has no curative effect on the scar tissue resulting from skin incisional wounds.

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