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ORIGINAL ARTICLE

Effect of continuous and pulsed therapeutic ultrasound in the appearance of local recurrence of mammary cancer in rats

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Summary

Purpose: *Ultrasound (US) therapy is an electrothermotherapeutic modality that uses US energy to provoke physical and chemical alterations. US therapy has been widely used in physical therapy. However, in clinical practice, it is contraindicated in cancer patients due to the possibility of exacerbating tumor growth.*

Methods: *Sixty-eight female Sprague-Dawley rats bred in UNIFAE vivarium were studied. At 50 days of age, 7,12-dimetylbenz(a)anthracene (7,12-DMBA) was administered to 35 rats by gastric gavage to induce mammary cancer. After 90 days the mammary glands of the rats belonging to the group with mammary cancer induction and stimulated by US were removed. Animals received either continuous or pulsed US. US waves were generated at a frequency of 1 MHz during 10 days, with an intensity dose of 0.5 W in the continuous group, and 0.9 W (duty cycle: 20%) in the pulsed group.*

Results: *Among the rats treated with continuous US, 44.4% developed local recurrence, while among the rats treated with pulsed US, 22.2% had local tumor recurrence ($p<0.05$). No evidence of distant metastases was shown in any of the rats studied.*

Conclusion: *The use of continuous and pulsed therapeutic US promoted the development of local recurrence of mammary cancer in female Sprague-Dawley rats in the postoperative period.*

Key words: breast cancer, breast cancer recurrence, therapeutic ultrasound, physical therapy

Introduction

Mortality from breast cancer among women is high. In Brazil, breast cancer is the second most frequent neoplasm in females and accounts for about 49,240 new cancer cases [1].

Considering that the median survival rate in these women is expected to be 17.5 years [2], it has become imperative to improve the rehabilitation techniques to provide these women with a better physical and mental quality of life. According to the American Cancer Society there are currently 1-2 million breast cancer survivors in the United States. About 15-20% of these women live each day with some level of upper limb discomfort or incapacity. It has been estimated that 120,000-600,000 patients suffer from some postoperative complication [3].

Physical rehabilitation, performed by means of physical therapy, plays a fundamental role in restoring upper limb function, preventing the formation of scar tissue adhesions and lymphatic dysfunction such as lymphedema. Rehabilitation programs for women undergoing breast cancer operations emerged from the need to minimize postoperative complications. These programs are based on guidance, exercises and functional rehabilitation [4], but proposals concerning the use of electrothermotherapy are lacking.

US therapy uses US energy that provokes physical and chemical alterations. Owing to the mechanical effect generated, a micromassage effect can be observed, resulting in an increased cell metabolism, blood flow and oxygen supply. Thermal effects may produce an increase in collagen tissue extensibility, pain reduction and muscle spasm [5,6].

In view of this concept, Ethics Committees have

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refused studies focused on cancer patients because of the probable damage generated. Two animal studies may be found in the literature, with the induction of rhabdomyosarcoma cells injected into mice, observing a significant increase in the volume and weight of animals exposed to continuous US therapy for 2 weeks [7]. This increase was found to be more discreet in pulsed ultrasound therapy [8].

Breast cancer patients could benefit from US use, due to the musculoskeletal alterations experienced in the postoperative period of breast cancer, as well as tissue complications, or its use could be ruled out in cancer patients. Another issue is the type of tumor response resulting from US therapy in a patient whose cancer had already been surgically excised. There are no studies correlating the use of US therapy with changes in local recurrence rates or the appearance of regional or distant metastases.

Methods

Animals and US application

Sixty-eight female Sprague-Dawley rats were studied. The animals were bred in UNIFAE vivarium. All rats were allowed *ad libitum* access to food (Purina chow for rodents) and water. The rodents were maintained on a 12-h light: 12-h dark cycle, with temperature at 22 ± 2° C.

Twelve groups of animals were used and are displayed in Table 1.

At 50 days of age, 7,12-DMBA was administered to 35 rats. After another 90 days, the mammary glands of rats with cancer induction were removed and after another 3 weeks 49 rats were subjected to US. The US target was the scar of the removed mammary glands. US was performed at working acoustic frequency of 1 MHz (15% range of peak intensity and beam non-uniformity < 6). The machine was precalibrated using a radiation pressure balance.

The US application was performed once a day for 10 consecutive days, except on weekends. The 49 rats were divided into 2 main groups: the first one included animals that received continuous US and the second one animals that received pulsed US. In the group stimulated by continuous US, the dose used was 0.5 W of irradiation intensity applied to an effective radiation area (ERA) head, for 9 min. In the group stimulated by pulsed US the dose used was 0.9 W (duty cycle: 20%) of irradiation intensity applied to an effective radiation area (ERA) head, also for 9 min. This dose was determined through a study by Sicard-Rosenbaum et al. [7]. Only US conversion from 3 MHz to 1 MHz was carried out for use in this study.

Table 1. Groups of animals

	With mammary cancer induction	*Without mammary cancer induction*
With mammary glands removed		
Continuous US	9	7
Pulsed US	9	4
Without US	3	3
Without mammary glands removed		
Continuous US	4	6
Pulsed US	4	6
Without US	6	7

US: ultrasound

Tumor induction

Mammary cancer was induced by a single dose of 20 mg of 7,12-DMBA diluted in soy oil (1 ml), and administered to 35 rats by gastric gavage [9].

Surgery

The rats were anesthetized with xylazine (5-13 mg/kg), ketamine (40-87 mg/kg) and atropine. Then, 8 pairs of rat mammary glands were removed after US sessions or after 90 days of mammary cancer growth. Antibiotic therapy was used during surgery (pentabiotic and terramycin).

After these procedures, the rats were euthanized and their organs were autopsied and the presence of microscopic and macroscopic metastases was evaluated. The viscera were surgically removed *en bloc*, in addition to 2 femoral bones, the vertebral column, brain, and the surgical scar. All slides were reviewed by two pathologists experienced in breast cancer pathology.

The Mann-Whitney U test was used for testing significant differences.

Ethical aspects

This project was approved by the UNIFAE Ethics Committee for Humans and Animals (report 22/2008 - Centro Universitário das Faculdades Associadas de Ensino - São João da Boa Vista, São Paulo, Brazil).

Results

Among the rat groups undergoing mammary cancer induction, surgery and application of continuous US, 4 (44.4%) of the 9 rats studied developed local recurrence at the excision site postoperatively. Among the rats from the group undergoing mammary cancer induction, surgery and application of pulsed US, 2 (22.2%) of the 9 rats studied developed local recurrence at the excision site postoperatively ($p<0.05$). Regarding the appearance of postoperative local recurrence, no macro or microscopic presence of tumor cells was verified in any of the rats evaluated with surgery and without US application (Table 2).

Local recurrences exhibited a more aggressive histopathological appearance, including cytologic atypia, evident nucleoli, nuclear pleomorphism and mitotic figures. Lobular mixed adenocarcinomas and more aggressive tubular adenocarcinomas were found, with solid areas and malignant mixed tumors (typical of carcinoma

Table 2. Local recurrence in the excision site after mammary gland removal and after US application

	Local recurrence		
	Continuous US N (%)	*Pulsed US N (%)*	*p-value*
Without mammary cancer induction	0 (0)	0 (0)	<0.05
With mammary cancer induction	4 (44)	2 (22)	

US: ultrasound

and sarcoma; Figure 1), and also signs of cancerization in the stroma. No distant metastases were observed in any of the rats studied, regardless of the group they belonged.

Macroscopically, tumors that had not been surgically excised showed growth in both groups receiving and not receiving US application. Thus, it was possible to observe that tumor cell proliferation remained active and independent of US action.

Discussion

The use of US in surgical scars in postoperative breast cancer patients would have a theoretical indication due to the well-known effects of promoting tissue healing accompanied by collagen tissue extensibility [10,11]. US also promotes tissue repair and more uniform wound healing with no fibrous tissue points [12,13].

The use of US in the presence of tumors or in tumor-suspect areas, e.g. in local, regional and distant recurrences, is contraindicated in clinical practice due to the risk of tumor cell growth [7,8]. However, studies evaluating this effect on surgically excised tumor sites have not been found.

A postoperative breast cancer patient, despite being adequately treated, still has a chance of disease recurrence. In a 20-year follow-up, Fisher et al. [14] reported a 14.3% local recurrence rate in patients following breast-conserving surgery and radiotherapy, observing that 73.2% of these events occurred in the first 5 years after surgery, 18.2% between 5 and 10 years and 8.6% after 10 years since surgery. In contrast, the incidence of local recurrence was 39.2% in 20 years among patients undergoing breast-conserving surgery without radiotherapy, and 39.7% of these recurrences occurred up to 5 years after surgery, 29.5% between 5 and 10 years, and 30.8% after 10 years since surgery. Veronesi et al. [15] observed local recurrence in 8.8% of patients after breast-conserving surgery and 2.3% after mastectomy in 20 years of follow-up.

Considering the disease-free period, 49% of patients undergoing mastectomy, 45% of patients after breast-conserving surgery and 46% of patients after breast-conserving surgery plus radiotherapy [14] had no distant metastasis in a 20-year follow-up. At 25 years of follow-up, the disease-free survival rate in patients undergoing radical mastectomy was 46%, 38% for those undergoing modified radical mastectomy plus radiotherapy and 43% for those undergoing modified radical mastectomy [16].

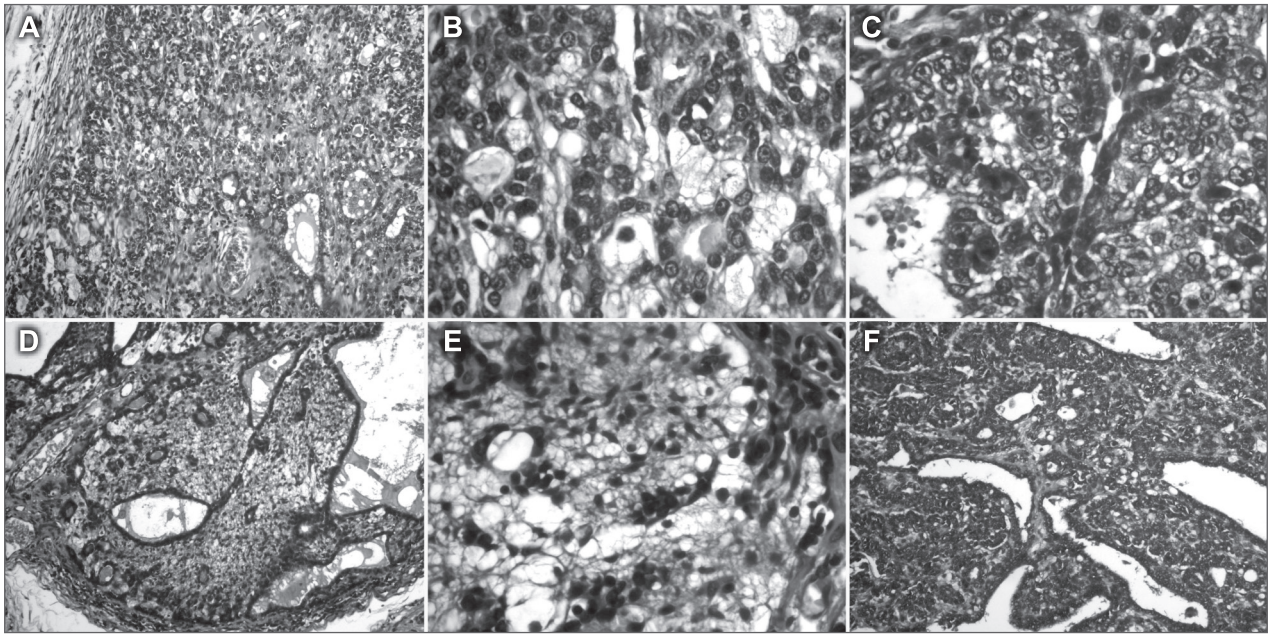

Figure 1. Serial sections of a case of mixed tubular and lobular adenocarcinoma with solid areas (H&E stain; **A:** ×100, **B:** ×400, **C:** ×400) and serial sections of a case of malignant mixed tumors (H&E stain; **D:** ×100, **E:** ×400, **F:** ×100).

Observing the data shown above, the superior quadrant ipsilateral to surgery for breast cancer removal should be considered a tumor-suspect area during an indeterminate period. Therefore, therapeutic US should not be used in that area. This study showed 44.4% of local recurrence rate in rats receiving continuous US sessions and 22.2% of local recurrence in rats receiving pulsed US sessions.

Conclusion

The use of continuous and pulsed therapeutic US promoted the development of local tumor recurrence in female Sprague-Dawley rats in the postoperative period.

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