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Topical superoxide dismutase reduces post-irradiation breast cancer fibrosis

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Abstract

Fibrosis following breast radiotherapy for mammary cancer is a frequent undesired effect with objective (esthetic) and subjective (pain) consequences. Forty-four patients with clinical radiofibrosis following conservative treatment of breast cancer were evaluated for the local antifibrosis effect of copper zinc superoxide dismutase [SOD(Cu/Zn)]. Extracted SOD(Cu/Zn) in a concentration of 3,600 units/mg was applied as ointment to the fibrotic affected area, b.i.d. for 90 days, in a total dose of 40 mg. The radiofibrosis intensity was scored on the basis of clinical criteria (pain and the fibrosis area) before and after SOD(Cu/Zn) treatment. SOD(Cu/Zn) was found to be effective in reducing radiation induced fibrosis by a lowering pain score in 36/39 patients and a decrease of the fibrotic area size in half cases, after 6 months. The intensity and changes of breast fibrosis were assessed also by mammography and, for the topographical distribution of subcutaneous temperature, by infrared thermography. Mammography density suggested decreased fibrosis in one third of patients. Thermography showed that fibrosis was accompanied by two zones clinically indistinctive: a central area with maximum thermal activity, called "Maximal Thermic zone" (MTZ) and a peripheral area with less thermal activity but higher than in the surrounding normal tissue, "Transitional Thermic Zone" (TTZ). Both MTZ and TTZ were significantly decreased in 36/44 patients after SOD(Cu/Zn) treatment. Clinical changes persisted all along the study. Treatment was well tolerated except for one case of local allergic reaction, and no important side effects. Molecular mechanisms involved are discussed. Further studies are running to confirm and explain these results.

Keywords: breast irradiation - free radicals - SOD (Cu/Zn) - superoxyde dismutase - radiofibrosis

Introduction

Fibrosis, which may cause patient discomfort (pain) and impair cosmetic, is a known late effect of breast irradiation in patients with breast cancer, treated without mastectomy. Dose, fractionation and duration of irradiation are the major factors of fibrotic growth [1-4]. In addition, fibrosis may be related to the size of the breast [5, 6], preexisting collagen diseases [7], concomitant or sequential administration of chemotherapy or estrogen blockers like Tamoxifen [8, 9], and the intrinsic radiosensitivity

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of the connective tissue, which genetically varies from patient to patient [10].

One of the main mechanisms responsible for fibrosis after tissue injuries is the release of free radicals, such as superoxide (O_2) and hydroxyl (OH^-) [11-13]. Because of their strong instability, these free radicals bind to adjacent structures and cause injuries to the connective tissue and vascular network. Superoxide dismutase (SOD) is an intracellular scavenging enzyme, first discovered by Mc Cord and Fridovich [12]. Together with other enzymes of the peroxidase pathway, SOD scavenges the superoxide, hydroxyl, and other oxygenated free radicals [14, 15]. In physiological conditions, the production of free radicals [16] and the action of antiradicals' enzymes is balanced. Following tissue injuries, either pathological or caused by agents such as radiation therapy, an excess production of free radicals is observed [17, 18]. Hence, SOD could be used to prevent the production of free radicals and its consequences on fibrosis.

Attempts have been made to use SOD as a radioprotector in patients receiving radiotherapy, with controversial results [19-21]. In addition, SOD was used to treat radiation-induced fibrosis [22, 23]. Preliminary studies [24] had suggested that a significant reduction of long term radiation-induced breast fibrosis could be obtained with SOD.

The present prospective study was aimed to measure by a semi-quantitative way the effects of SOD on radiation-induced breast fibrosis. The results of an immunohistochemical study performed simultaneously on the same group of patients were published elsewhere [25].

Material and methods

Patients and tumour characteristics

During 12 months, 44 patients with palpable skin fibrosis following breast irradiation from Curie Institute were enrolled in a prospective study to evaluate the benefit of topical SOD (Cu/Zn) treatment. The mean age at diagnosis of breast cancer was 58,6 years (44-79).

The initial tumour stages TNM were T1 (10 patients), T2 (20 patients), T3 (12 patients) and T4 (2 patients). None had metastatic disease. Patients with active signs of breast inflamation were excluded.

Fourteen patients were treated with radiotherapy alone, 15 patients with wide local excision and irradiation, 12 patients with adjuvant systemic therapy and irradiation, 2 patients with adjuvant systemic therapy, local excision and irradiation, finally one patient underwent a mastectomy followed by chest wall irradiation.

The radiation dose at mid-thickness of the breast was 55 Gy (52-60) for the 43 patients who had a breast conservative treatment. Thirty six patients received an irradiation boost to the tumour site. The median tumour dose was 73 Gy (70-80). Forty one patients received a loco-regional nodal irradiation at doses ranging from 45 to 65 Gy. All patients consented to participate to this study.

Clinical evaluation

The median time to evaluation of fibrosis following completion of radiotherapy was 84 months (7-261). *Fibrosis* was evaluated in all (44/44) patients by a clinical score. The fibrotic area in the breast and its lymph-node bearing area was measured clinically and scored as follows, according to the ratio between the breast size and the fibrotic area (Fig. 1) 1=mild, 2=moderate, 3=marked, 4=severe.

Pain was evaluated in all (44/44) patients by a self-reported clinical score. The intensity and frequency of pain in the fibrotic areas scored as follows (Fig. 2): 0=no pain, 1=mild, 2=moderate, 3=severe.

Mammography was performed bilaterally in 36/44 patients in order to score the radiofibrotic area. Eight patients who had a mammography 6 months before the study did not have a new radiography in order to limit the number of examinations. The X-ray density of fibrosis was scored as follows (Fig. 3): 0=no fibrosis, 1=moderate fibrosis, 2=marked fibrosis and 3=severe fibrosis.

Telethermography was evaluated in all (44/44) patients. The thermogram of fibrosis allowed to individualize two hyperthermic zones with significant differential threshold criteria fixed at 1°C level in comparison with symmetrical reference zones: a central area with maximum thermal activity which was called "Maximal Thermic Zone" (MTZ) and a peripheral area with less thermal activity but more than in the surrounding normal tissue which was called "Transitional Thermic Zone" (TTZ). The initial MTZ and TTZ sizes were measured by mean of a specially designed grid applied to the thermogram film. The same grid was applied to all following thermograms. Data were expressed as multiple of one surface unit (Fig. 4).

SOD Treatment

SOD (Cu/Zn) was administered as ointment. The consistency was a mixture of SOD (Biogenza, Lemania, Switzerland), polyethylene glycol, PEG 600 5%, (Sigma Chemical, Milwaukee, USA), and benzylic alcohol (0.1%) as preservative. The ointment's concentration was 3,600 units (IU) SOD(Cu/Zn)/mg. The biological activity of SOD (Cu/Zn) was tested and the bacteriological assay was carried out in the bacteriological Laboratory of Institute Curie at the time of preparation of the ointment and during the storage. Treatment consisted of local application of SOD (Cu/Zn) on the fibrotic palpable area only. Each patient was treated with applications of 800 IU of SOD (Cu/Zn) b.i.d. during 90 days, to a total dose of 144,000 IU (40 mg) SOD (Cu/Zn).

Assessment of the response

Observations were done before SOD(Cu/Zn) treatment and assessment was performed at time T0, at the beginning of the study, and at time T6, 6 months later. The effect of SOD(Cu/Zn) on the breast radiofibrosis was evaluated according to the clinical criteria:

-changes in the size of the fibrotic area.

-decrease of pain.

Telethermography and mammography were tested to develop an accurate and reliable method to quantify late cutaneous or sub-cutaneous fibrosis:

-Reduction of density of mammographic imaging.

-Variation of thermogram. The thermographic evaluation was based on the expression of the individual changes into the two hyperthermic areas. The comparison with the opposite breast may evaluate thermal abnormality in standard conditions without influence of biological variations. The results were expressed in percentage of surface variations and determined as follows: Δ0=0-20% (no change), Δ1=20-50% (mild change), Δ2=50-80% (marked change) and Δ3=80% (sub-total recovery).

Results

Tolerance: SOD (Cu/Zn) treatment was well tolerated in all patients but one who presented an erythematous skin reaction, which rapidly disappeared after discontinuation.

Clinical and paraclinical response

Breast fibrosis (Fig. 1)

The major indication for treatment was breast fibrosis with or without deformation, but associated with discomfort or pain. The areas affected by serious fibrosis and treated by SOD (Cu/Zn) were the breast and the sternal areas in all patients, but one

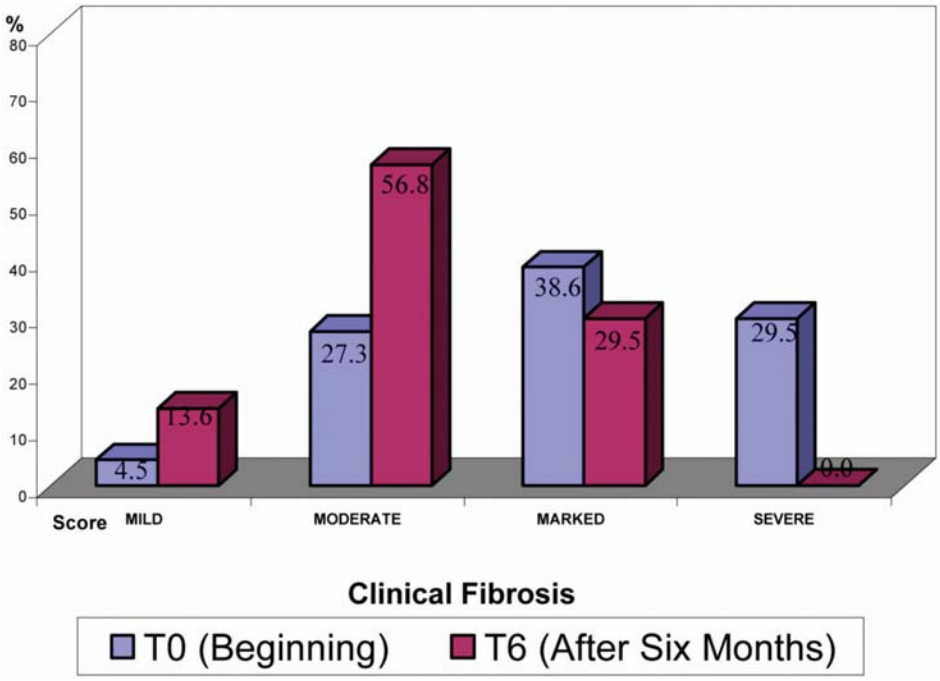

Fig. 1 Evaluation of breast fibrosis before and after SOD treatment. At T0 fibrosis scoring was as follows: score 1=2/44 patients, score 2=12/44 patients, score 3=17/44 patients, score 4=13/44 patients. At T6 fibrosis was as follows: score 1 =6/44 patients, score 2=25/44 patients, score 3=13/44 patients, score 4=no patients (T0=beginning, T6=6 months later). All severe patients (score 4) at T0, decreased the clinical fibrosis score after SOD treatment.

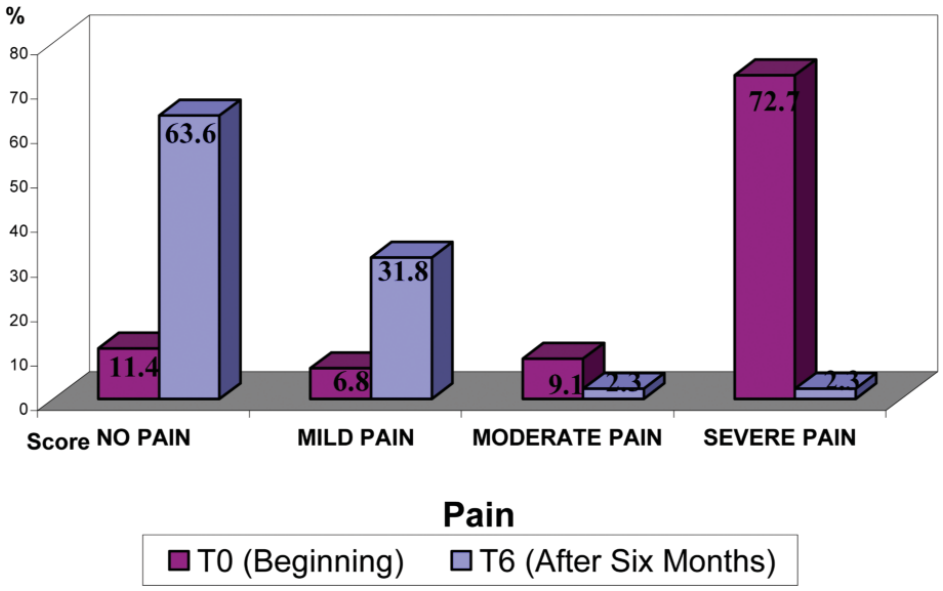

Fig. 2 Evaluation of breast pain before and after SOD treatment: score 0 =5/44 patients, score 1=3/44 patients, score 2=4/44 patients, score 3=32/44 patients. After six months of treatment: score 0=28/44 patients, score 1=14/44, score 2=1/44 patients, score 3=1/44 patients. (T0=beginning, T6=6 months later).

where the chest was irradiated. Before treatment, at T0, fibrosis scoring was as follows: score 1=2/44 patients, score 2=12/44 patients, score 3=17/44 patients, score 4=13/44 patients. At T6 fibrosis was as follows: score 1=6/44 patients, score 2=25/44 patients, score 3=13/44 patients, score 4=no patients (T0=beginning, T6=6 months later).

Fifty percent of patients showed a decrease of the fibrotic skin area size: one point score for 18/44 patients (41%) and two points score for 4/44 (9%). No patients had a progression of the fibrotic area. The difference was statistically significant (c^2: 41.78, $p<0.0001$).

Pain

Local pain was present in 39/44 patients and SOD treatment decreased pain in 36/39 of patients (92%). We observed at T0: score 0=5/44 patients, score 1=3/44 patients, score 2=4/44 patients, score 3=32/44 patients. After six months of treatment: score 0=28/44 patients, score 1=14/44, score 2=1/44 patients, score 3=1/44 patients (Fig. 2).

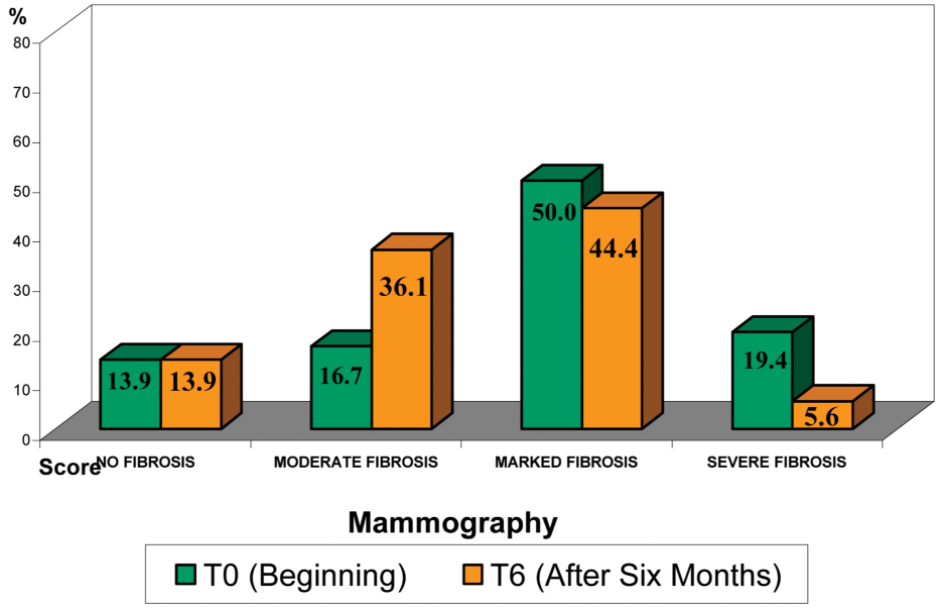

Fig. 3 Mammography evaluation before and after SOD treatment. Score 0 = 5/36 patients, score 1 = 6/36 patients, score 2 patients =18/36, score 3=7/36 patients. Re-evaluated at T6: score 0=5/36 patients, score 1=13/36 patients, score 2=16/36 patients, and score 3=2/36 patients. (T0=beginning, T6=6 months later).

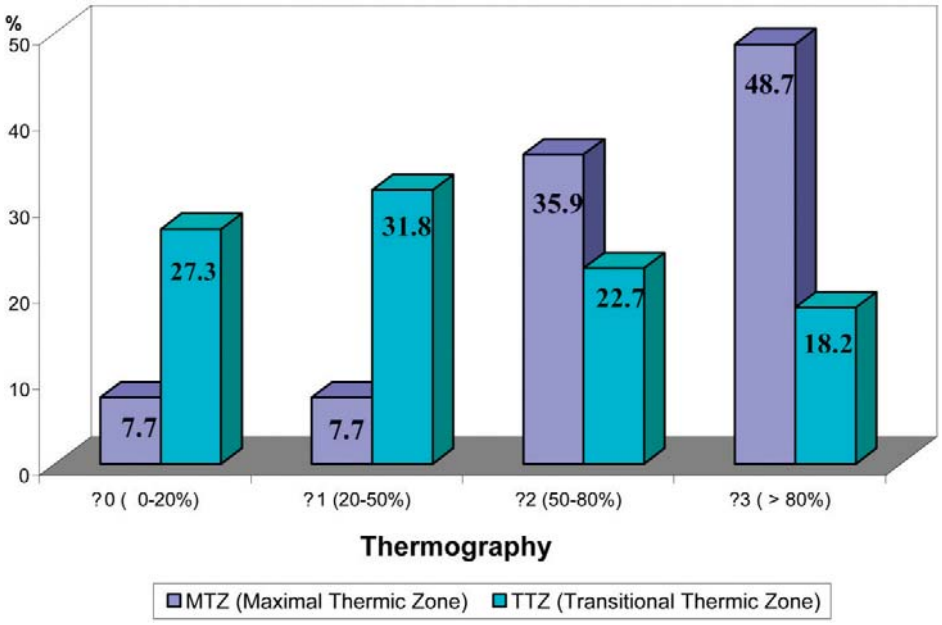

Fig. 4 Thermography evaluation before and after SOD treatment, expressed by Maximal Thermic Zone (MTZ) and Transitional Thermic Zone (TTZ), by the percentage of variation of each area. For MTZ, found a Δ0 (decreasing 0-20%) for 3/39 patients (8%), Δ1 (decreasing 20-50%) for 3/39 patients (8%), Δ2 (decreasing 50-80%) for 14/39 patients (35%) and Δ3 (decreasing more 80%) for 19/39 patients (49%). For TTZ, we observed: Δ0 for 12/44 patients (27%), Δ1 for 14/44 (32%), Δ2 for 10/44 (23%0, and Δ3 for 8/44 (18%).

Twenty-three patients (64%) presented a complete pain relief, and 13 patients (36%) a partial reduction in pain. Three patients (8%) had no changes. No patient showed increasing pain. The difference was statistically significant (χ^2: 997.5, $\alpha < 0.001$).

Mammography

The mammography density score showed changes after SOD treatment. We observed at T0: score 0 = 5/36 patients, score 1 = 6/36 patients, score 2 patients =18/36, score 3=7/36 patients. Re-evaluated at T6: score 0=5/36 patients, score 1=13/36 patients, score 2=16/36 patients, and score 3=2/36 patients (Fig. 3). We observed that score 0 had no modification, -one third of patients who presented initially score 2 and score 3 decreased of one unit score (7/36 patients in score 2 and 5/36 patients in score 3), -no case had an increasing score. Altogether, 67% of patients had no modified score (5/36 patients in score 0, 6/36 patients in score 1, 11/36 patients in score 2 and 2/36 patients in score 3). The difference was statistically significant (χ^2: 16.5, $\alpha < 0.001$).

Thermography (fig 4)

The thermogram at T0 and T6 showed variation in the fibrotic area in the two hyperthermal zones: MTZ in 36/39 patients, 5/44 patients without MTZ, and TTZ in 32/44 patients. We measured the percentage of variation of each area. For MTZ, we evaluated: Δ0 (decreasing 0-20%) for 3/39 patients (8%), Δ1 (decreasing 20-50%) for 3/39 patients (8%), Δ2 (decreasing 50-80%) for 14/39 patients (35%) and Δ3 (decreasing more 80%) for 19/39 patients (49%). For TTZ, we respectively observed: Δ0 for 12/44 patients (27%), Δ1 for 14/44 (32%), Δ2 for 10/44 (23%0, and Δ3 for 8/44 (18%). The systematic observation of the normal contralateral breast showed a good reproductibility of the method. No thermal variability upon 1^0C was observed in symmetrical area by all patients of this trial.

Discussion

Reactive oxygen metabolites including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) are involved in radiofibrosis. Therefore, protective enzymes like catalase and SOD, which decreases post irradiation, might be useful in preventing and/or treating these effects. Production of these active oxygen species is higher in breast cancer patients than in normal persons, which suggests a role for the oxidative stress in carcinogenesis. However, basal SOD is increased in breast cancer patients before radiation therapy as compared to controls [26], and decreases after radiotherapy [27]. Genetic variants of SOD (SOD2)

have been associated with increased breast cancer risk, but this association was not confirmed by others [28]. Another antioxidant enzyme, manganese superoxide dismutase (MnSOD) exhibit tumor suppressor activity in breast cancer cells by up-regulating a tumor suppressor gene, maspin, *via* increasing the maspin mRNA stability [29].

Orgotein is an anti-inflammatory superoxide dismutase agent successfully used in treating radiation-induced adverse effects administered either topically or parenterally [30]. *In vitro* and *in vivo* studies have demonstrated the radioprotective effect of the percutaneous administration of SOD (Cu/Zn) [31, 32], but the results of controlled clinical studies are controversial concerning its efficacy as radioprotector [7, 11, 12, 14].

A toxicity study of SOD (Cu/Zn) in humans demonstrated that it is safe and did not find any significant acute or chronic toxicity [1]. In the present study the treatment was well tolerated in all but one patient who developed an episode of local reaction. There were no significant or serious side effects. Local allergic reaction has also been reported by others [31]. Previous studies [2;18] did not show any risk in using SOD for treatment of breast or bladder radiofibrosis. In addition, SOD did not influence the tumoral response to radiotherapy. The effect of SOD (Cu/Zn) [18] was also studied in patients suffering from radiation cystitis. Local administration of SOD (Cu/Zn) into fibrosis areas of the urinary bladder in 20 patients achieved a favourable therapeutic effect. In addition to the subjective amelioration, the cystoscopy showed a reduction of mucosa oedema, necrosis and bleeding tendency. Similar results have been reported by Frick et al. [22].

The molecular bases of superoxide dismutase in radiofibrosis are not well understood. Irradiated fibroblasts present a brief lifespan, a reduced proliferative capacity associated with reduced levels of endogenous catalase or superoxide dismutase [33].

The effect of SOD (Cu/Zn) on chronic well-established radiofibrosis is neither well known nor recognized and only a few studies of the problem have been published [16].

A French study [24] focused on clinical effects of encapsulated injected liposomal SOD(Cu/Zn) of biological origin on 34 patients with measurable radiofibrosis. Twenty patients had breast radiofibrosis, 7 head and neck radiofibrosis and 7, radiofibroses in various other sites. The SOD (Cu/Zn) was administered intramuscularly at 2 or 5 mg (3,600 IU /mg) per injection, once a week during 6 weeks. In addition, an amelioration was achieved by 15/20 (75%) patients suffering from "functional problems". These effects remained stable during 5 to 25 months of follow up and except for one episode of fever, the treatment was well tolerated. The increase of SOD half life from approximately 25 minutes to several days was possible due to high MW PEG used for preparation. This procedure decreased immunogenity while retaining the enzymatic activity [34, 35].

The effect of topical SOD (Cu/Zn) on breast radiofibrosis has been studied in Institute Curie since 1989. Forty-two other patients in a published study [23] evaluated the clinical and physiological effects of SOD (Cu/Zn) on breast radiofibrosis. This ointment form was chosen because of its percutaneous administration. The amount given was strandardised at 800 IU b.i.d and not related to the fibrotic area size, in order to avoid variations in administred dose along the study. It is easy to apply and to achieve homogeneous concentration specifically on the fibrosis and painful area. Intramuscular injection was excluded to decrease the risk of allergic reaction and this way of administration did not permit a preferential concentration into the fibrosis area.

In the present study we tested effects of SOD (Cu/Zn) upon subjective and objective parameters. The efficiency of SOD in the treatment chronic breast radiofibrosis and the results are compatible with reports of other teams. The efficacy of SOD (Cu/Zn) is supported by the changes in the size of the radiofibrosis as determined by the physical examination. Clinical evaluation of topographical limits of fibrosis area was difficult to appreciate due to the variation according to physician. In our study only one physician took the measurement of the radiofibrosis. In the majority of patients a significant improvement was achieved in pain. Other parameters were tested to find a reproducible test to evaluate the radiofibrosis.

Mammography

Although the mammography is not in routine the best method to explore the breast fibrosis, neverthe-

less the mammogram is the imaging reference for breast examination. This method showed a statistically significant change of mammographic imaging density after 6 months of SOD (Cu/Zn) treatment. All the mammograms were done by the same team, but only patients presenting a real fibrosis showed a true decreasing in their score.

Thermography

Thermography provided topographical thermal emission regarding physiological or abnormal hyperthermic process like superficial hypervascularization or inflammatory reaction. Radiofibrosis causes physiopathological changes, one of them being the local tissue temperature. Previously this technique was intended in particular for mapping cutaneous fibrosis after accidental exposition. In our study we observed the topographical modifications of local thermal emission by telethermography using infrared video camera. This thermographic study showed that in fact fibrosis was composed of two zones, not clinically obvious, TTZ (Transitional Thermic Zone) and MTZ (Maximal Thermic Zone).

The MTZ showed a sharper change than TTZ. MTZ is probably due to the inflammatory process. TTZ showed a significant modification probably due to decreasing sclerosis of the tissue. The decreasing of this area after SOD treatment seemed to be an approach for objective measure of SOD effects on radiofibrosis.

The good reproducibility on the reference (opposite) breast supported the reality of the modifications on the irradiated breast. The technique is easy to perform without morbidity. The examination of the patient is as easy and quick as taking a picture. So we consider that the thermography is a good parameter to evaluate radiofibrosis.

Conclusions

Results of clinical tests (fibrosis size and pain) showed statistical changes after SOD (Cu/Zn) application. More than 92% of patients felt a greater degree of local comfort. New parameters, mainly thermography supported a great interest in the appreciation of breast fibrosis. Thermography was a safe reproductive method to test inflammation associated with radiofibrosis. It seemed more accurate and more reproducible than clinical examination. It was not examiner-dependent as for clinical examination. Finally SOD (Cu/Zn) performs a major effect to reduce breast radiation-induced fibrosis as well with clinical parameters as with objective methods tested in our study (thermography and mammography).

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