Topical Ozonated Autohaemotherapy for the Treatment of Skin Lesions

Proposal of a New Method: Concept, Technique and Initial Clinical Results

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SUMMARY - *Chronic skin lesions represent a major socio-economic problem and, in spite of several recent approaches with improved dressings, the healing process remains slow and painful. Previous studies based on a brief and moderate ozonation of human platelets showed a striking release of growth factors together with an effective and mild disinfectant activity. We propose a new method consisting in the controlled ozonation of a small volume of the patient's blood with its immediate application on skin ulcers of considerable size in both chronic limb arterial ischaemia and venous stasis. The ozonated blood, kept in situ for one day and subsequently renewed at least twice, leads to such a marked improvement as to allow rapid successful implantation of a skin graft. Besides describing the technical details, we report clinical data obtained in patients with ulcers undergoing venous stasis and arterial ischaemia. This new experimental method has been compared with conventional dressings. The reasons explaining the rapid improvements are discussed.*

Introduction

The problem of infectious skin lesions, namely chronic ulcers in diabetic and/or atherosclerotic patients and in patients with venous stasis, is becoming critically important because of an increased ageing of the population, patient discomfort and the huge economic cost of treatments. During the last decade, orthodox medicine has made remarkable progress in clarifying the local role of oxygenation, pH, humidity and temperature in favouring the healing process. Martin[1] schematically represented the theoretical sequence of wound healing of 3rd-4th degree lesions in three successive stages: phase I includes the inflammation stage following either an accidental trauma or diabetes, local ischaemia, or a burn, most often complicated by a localized infection due to a variety of bacteria. During this phase, neutrophils and macrophages are present and its duration depends on the underlying pathology and age of the patient. Phase II corresponds to the intermediate stage, normally lasting from 14 to 18 days during which the synthesis of extracellular matrix (fibronectin, collagen III/I, hyaluronic acid and chondroitin sulphate) is accompanied by an active proliferation of fibroblasts and keratinocytes. The *restitutio ad integrum*, i.e. phase III, includes the final healing and scar tissue remodelling.

In young and normal people, a skin lesion is likely to heal in about three weeks often without any special medical attention. However, the problem is becoming far more complex in elderly patients, particularly if disabled or undernourished, or obese, with hypoproteinemia and possibly with diabetes, heart, renal or vascular insufficiency. Once the cutaneous lesion is established, it becomes necessary to administer the best available therapy, as otherwise the ulcer will deteriorate and become chronic. Recent literature [2-8] has emphasized the need to avoid or reduce infection, to improve the local metabolism and to correct any metabolic deficiencies. There is a general consensus to avoid harsh antimicrobial agents because although they kill bacteria, they also damage the repairing cells. Recently the application of growth factors has elicited interest for accelerating healing [9], but has been disappointing because the persistence of an infection inhibits their activity [10,11].

During the last decade, blandly ozonated olive oil has been used as a topical treatment with excellent results because the slow local release of ozone

has a potent bactericidal activity without causing any cell damage [12-16]. Moreover, by activating cell proliferation the simultaneous vasodilatation and local release of oxygen accelerate the healing process. Although this recent approach is hardly known and appreciated by orthodox medicine, it is very effective in small ulcers using simultaneous parenteral administration of the patient's ozonated blood [12,16]. This is not always possible and we were compelled to devise a topical method able to achieve a local disinfection, improved metabolism and stimulation of the proliferation due to the release of growth factors by the activation of platelets during the oxygenation and ozonation process.

We to present the methodology, the results obtained so far and suggest that this new form of topical application may become very advantageous in critical patients.

Materials and Methods

All patients were informed of the purpose of the study and signed their informed consent.

The study was approved by the Barbantini Clinic's internal ethical committee complying with the ethical rules for human experimentation stated in the 1975 Declaration of Helsinki.

Ozone generation and measurement

Ozone was generated from medical-grade oxygen (O_2) using an electrical corona arc discharge by the O_3 generator (Ozonline Model E-80, Medica Bologna, Italy), which allows the gas flow rate and O_3 concentration to be controlled in real time by photometric determination, as recommended by the Standardisation Committee of the International O_3 Association.

The ozone flow-rate was kept constant at 3 L/min in all experiments. Polypropylene syringes (ozone-resistant) were used throughout the reaction procedure to ensure containment of O_3 and consistency in concentrations.

Collection of human blood and plasma samples

Blood samples were taken from the patients by venipuncture in the morning using heparin (20 U/ml blood) as an anticoagulant. Each blood sample of 3.0 ml was immediately treated with the gas mixture composed of a volume of 9.0 ml of a gas mixture of O_2 (~96%) and O_3 (~4%), at an ozone concentration of 60 mcg /ml corresponding to a total ozone dose of 540.0 mcg.

The blood/gas volume ratio is always of 1 ml blood/3 ml gas. The gas withdrawn in a 10 ml syringe was introduced into a second syringe of 20 ml containing the blood sample via a multidirectional stopcock.

We previously determined [19] that a rapid rotation of the syringe along its longitudinal axis (about 80 cycles/min) for one min achieves a complete mixing of the liquid-gas phases with minimal foaming and that, within this period of time, ozone reacts completely with substrates, implying that cell samples receiving ozone react with the ozone dose totally.

The pO_2 reached a value of about 400 mm Hg while the blood pCO_2 and pH values did not change. O_3 is a very reactive gas so that extremely rapid and precise handling is required to obtain reproducible results. The final gas pressure remained at normal atmospheric pressure.

Patients and clinical application of the donor's ozonated blood

A total of 32 patients were included in this preliminary protocol.

Group 1 patients (n=8) had limb arterial ischaemia and received the topical ozonated autohemotherapy treatment, while Group 2 (n=8), also with peripheral arteropathy, received a standard treatment and served as a control group. Group 3 (n=8) had venous stasis and received the topical ozonated treatment while Group 4 (n=8) was treated with conventional dressings.

The demographic characteristics of the patients are reported in table 1.

Table 1 Anthropometric and demographic data of patients

	Arterial ischaemia + topical OAHT	*Controls Arterial ischaemia*	*Venous stasis+ Topical OAHT*	*Control venous*
Age (years)	64.5±14.5	62.1±12.4	63.5±11.2	64.4±16.1
Sex (M:F)	5:3	6:2	5:3	4:4
BMI (kg/m2)	27.4±5	26.6±4.5	27.1±4	27±6.1

BMI= Body mass index.

Topical treatment of patients

If necessary, we performed surgical debridement followed by a thorough rinsing of the ulcer with freshly ozonated water. Three ml of ozonated blood, prepared as previously mentioned, were applied onto the ulcer and then the ulcer was immediately covered with polyurethane nonadhesive film for 24 hours. The next day, the film was removed and the ulcer washed with saline. The autologous blood ozonated mixture treatment was repeated at the same ozone dose. Groups 1 and 3 received three consecutive topical blood ozonated treatments.

Wound cleansing was also achieved by surgical excision of necrotic tissue in the control patients (Groups 2 and 4), followed by saline washing and completed by using hydrocolloid or semi occlusive dressing and vacuum therapy.

Methods for evaluating the progress of the skin ulcers

A computer image analysis (MICROLAB Mimix- Software, Padua, Italy) was used to measure the wound area. Briefly, a good quality standardized video image of the wound was made, digitized, and stored in the computer. The wound margins were traced on the screen using the computer mouse and the wound area in square centimetres was calculated by the computer. We posed the initial ulcerated area as 100% and then we calculated the percentage reduction of the area after the ulcer treatments of all groups. Each wound was visually estimated before and after treatments for the presence of granulation tissue and cleanliness by a experienced clinical observer (observer blind).

The intensity of pain was assessed on a 10 cm visual analogic scale (VAS) before and after ulcer therapy. The VAS was presented as a horizontal line on which the patient's pain intensity was represented by a point between the extremes of: 0 = no pain and 10 = the worst pain [17].

Statistical analysis

For statistical analysis Graph Pad software was used. Differences in percentage of reduction of the ulceration area was compared between Groups 1 and 2 (arterials) and 3 and 4 (venous) using Wilcoxon signed rank test. $P< 0.01$ values were considered as statistically significant.

Results

Table 2 presents the results obtained in the experimental and control groups. Practically all patients treated with the topical ozonated autohaemotherapy showed a marked improvement characterized by a significant reduction of the ulcer area. Moreover the ulcers became cleaner and appeared well oxygenated. The skin graft took well in about 60% of these patients. Pain markedly decreased from the second application of the ozonated blood.

By contrast the ulcers of similar size of control patients had only a minor improvement and no more than 30% of the skin grafts were accepted.

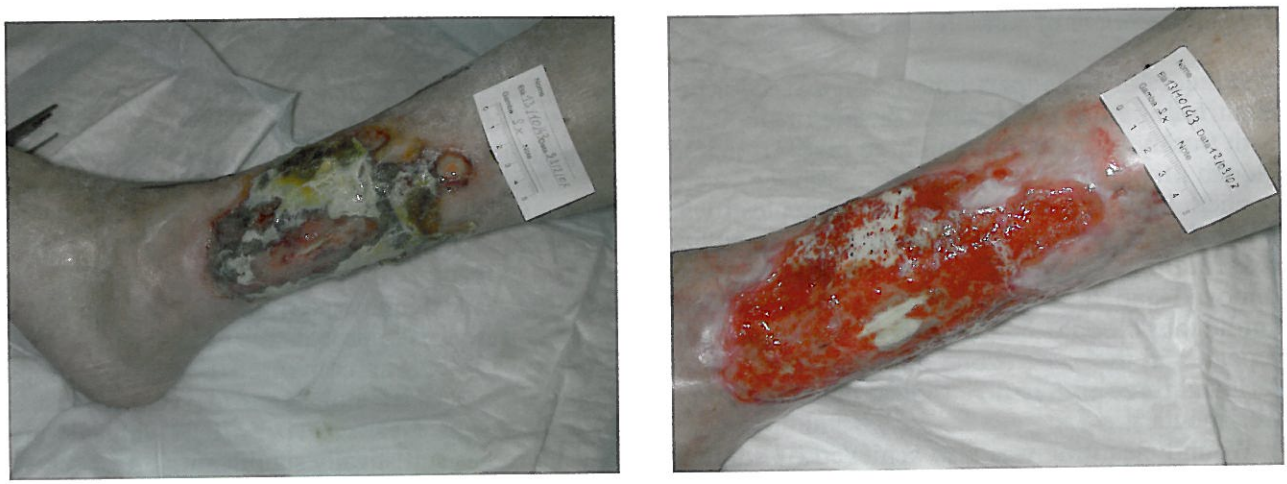
Figure 1 (A,B) shows the striking improvement of the arterial ulcer in terms of enhanced oxygenation and size reduction after three topical ozone autohaemotherapy treatments.

Figure 2 (A,B) shows the marked improvement of the venous ulcer regarding the decreased size and the improved aspect.

Table 2 Data on the reduction of the ulcer area and the reduction of pain

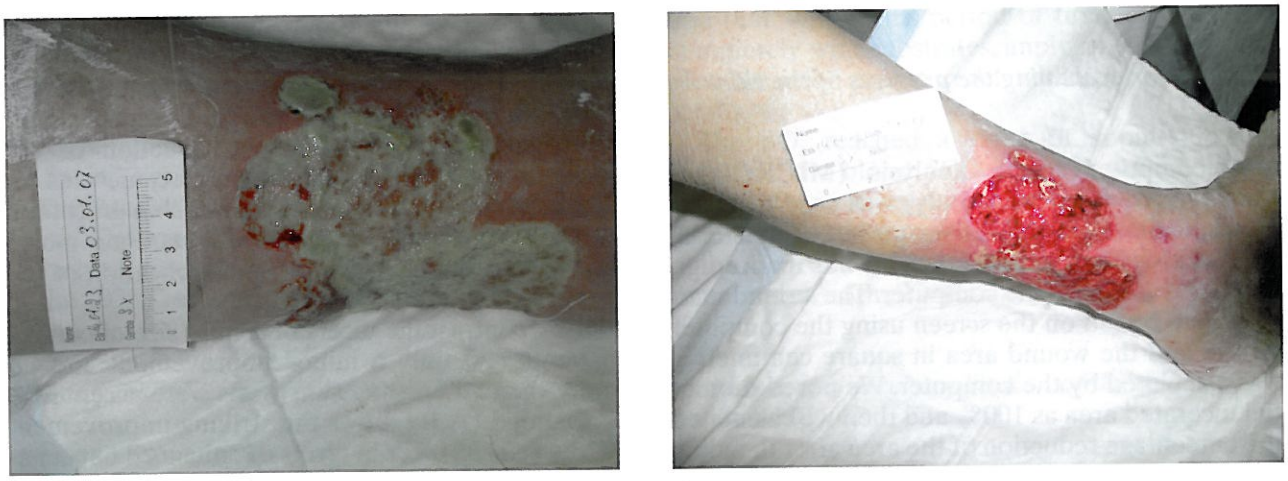
Pathology and treatment	*GROUP 1 Arterial ischaemia + topical OAHT*	*GROUP 2 Controls Arterial*	*GROUP 3 Venous stasis + topical OAHT*	*GROUP 4 Controls Venous*
Number of patients	8	8	8	8
Initial ulcer area (%)	100	100	100	100
Ulcer area after 3 treatments (%)	80(±5)	96(± 4)	84.2(± 6.2)	97(±5)
Difference as a %	20*	4	15.8*	3
Initial VAS	8(±2)	8(±2)	07(±3)	7(±3)
VAS after 3 treatments	1(± 2)*	6(± 2)	1(± 2)*	7(±3)

* p<0.01 Group 1 vs Group 2 and Group 3 vs Group 4.



A B

Figure 1 Chronic ischemic arterial ulcer before (A) and after (B) three topical autohaemotherapy treatments.



A B

Figure 2 Chronic venous stasis ulcer before (A) and after (B) three topical autohaemotherapy treatments.

Discussion

The Barbantini Clinic in Lucca, where the present work was performed, is a tertiary referral centre for patients with the worst skin ulcers in Tuscany and surrounding regions. Invariably the ulcers have become chronic, very painful, infected and with different degrees of necrosis.

During the last decade considerable attention has been dedicated to the treatment of venous and arterial leg ulcers by several approaches such as antibiotic therapy, hyperbaric oxygen, larval therapy [17] and negative pressure therapy [2-7]. Since mid 2003, we have used the most modern dressings like those suggested by Harding et Al [3] and Jones et Al [18]. However, owing to the infection and the extended area of the ulcers, improvement has been very slow in our patients. We soon realized that it was indispensable to eliminate infection and to reactivate tissue metabolism and cell proliferation. On the basis of previous studies [19-21], we hypothesized that the combination of ozone with critical growth factors released by the activated platelets present in the patient's blood may change the outcome. The application of platelet concentrate is well known [22-23], but our method of activation is far less time-consuming, inexpensive and absolutely safe as we use the patient's own blood. Moreover we have the further advantage of ozone's potent disinfectant activity and the stimulation of the metabolism due to blood oxygenation. Interestingly, one of us (M.L.I.) had the bold idea of testing the direct application of the blood immediately after one minute of ozonation. Since the first applications,

we realized that this approach was very effective in both reducing infection and promoting healing so that a skin graft could be successfully applied after repeating the treatment for three to four days.

The results shown in table 2 and figures 1 and 2 have clarified the striking difference between the use of conventional dressings and the new approach that we have named "topical ozonated autohaemotherapy".

The rapid disappearance of pain (table 2) and the enthusiastic cooperation and satisfaction of the patients encouraged us to present these data. Valacchi and Bocci [20] had already clarified the relevance of heparin in blood because, by using citrate as an anticoagulant, the amount of released TGF β1 and PDGF-AB from the ozonated blood was very much lower than heparin. At that time we also clearly showed the importance of the ozone concentrations that caused a drastic increase in growth factors when the ozone concentration was raised to 80 μg/ml per ml of blood. In this study we further increased the ozone concentration because we have regularly used 180 μg/ml per ml of blood. We are currently evaluating a new scheme for both optimal ozone concentration and the release of several other growth factors. These results will be presented in a subsequent paper.

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