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ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 5, pp.4132-4136, May, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

OXYGEN OZONE THERAPY IN THE INTEGRATED TREATMENT OF CHRONIC ULCER: A CASE SERIES REPORT

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Received 5th, April, 2015 Received in revised form 12th, April, 2015 Accepted 6th, May, 2015 Published online 28th, May, 2015

INTRODUCTION

Chronic ulcers (CU) present a major challenge to health care systems worldwide. In the United States alone, these wounds affect an estimated 2.5–4.5 million people (1). Predominantly a condition of the elderly, chronic leg and foot ulcers will continue to become more prevalent as the world population ages. The vascular etiology of CU include the venous stasis, arterial occlusive disease, thromboangioitis obliterans and lymphedema. Vasculitic causes of leg ulcers are leukocytoclastic vasculitis, autoimmune disease-related vasculitis, polyarteritis nodosa and Wegener's granulomatosis. Other causes of non healing ulcerations may be neoplastic diseases (for example leukemia cutis, lymphoma or primary skin neoplasms), traumatically-induced wounds, infectious wounds (with primary or secondary bacterial, fungal, viral, mycobacterial or parasitic etiology),hemathologic diseases (anti-phospholipid antibody syndrome or cryoglobulinemia) and metabolic diseases such as diabetes and gout(2). The most frequent site of ulceration is in the gaiter area, followed by the calf and the foot (3).

Chronic ulcers are associated with high treatment costs, decreased quality of life, and are a significant cause of morbidity. A frequent and distressing problem is the overwhelming rate of recurrence and the duration of the ulcer diathesis. Fittingly, the problem of chronic wounds has been defined as "The Silent Epidemic" (Smith & Nephew Foundation).

Clinical Hystory of CU

The duration of an ulcer varies from about nine months to a few years. This depends upon the age of the patient, the pathology, the side, the site of ulceration and the efficacy of the therapy. Nelson (4) examined 101 systematic reviews and observational studies and evaluated the quality of evidence for interventions. Topical antimicrobial agents, surgical and enzymatic debriding agents (5), collagen or alginate dressings, intermittent pneumatic compression, topically applied mesoglycan, keratinocyte growth factor 2 and topical negative pressure were included as approaches of variable effectiveness. Oral pentoxifylline(6), flavonoids, systemic mesoglycan and iontophoretic administration of calcitonin peptide(7) were likely to be beneficial. Unknown effectiveness was attributed to oral aspirin(8), sulodexide(9) , cultured allogenic dermal replacement, low level laser treatment, IV prostaglandin E1, oral rutosides, skin grafting, therapeutic ultrasound, oral thromboxane alpha 2 antagonists, zinc, silver treatment(10) and larval therapy. Other approaches such as light and magnetic therapies, topical warming-cooling and hypoclorous acid were only mentioned. It appears that so far a really effective method able to achieve healing and prevent recurrence is not available.

A new approach to the management of CU

In Germany and Italy, from 2006, many leg ulcers have been successfully treated with the ozonated autohaemotherapy (O_3-AHT) (11-13). It has taken several years to clarify the biochemical, molecular and pharmacologic events. It is useful to say that ozone is ten-fold more soluble in the water of plasma than oxygen: this fact implies that mixing human blood with an equivalent volume of a gas mixture composed of about 96% O2 and 4% O3 leads to a very rapid solubilization of ozone, which, owing to its high reactivity (E°= +2,076 V), reacts with hydrosoluble plasma antioxidants (ascorbic acid, uric acid, trace of reduced glutathione) and with the unsaturated lipids carried out by albumin as follows:

$$R\text{-}CH = HC\text{-}R + O_3 + H_2O \rightarrow H_2O_2 + 2\ R\text{-}CHO$$

This means that in a few minutes all the ozone dose is exhausted because it completely reacts and generates its messengers such as hydrogen peroxide and aldehydes derived from the unsaturated fatty acids peroxidation. The formation of aldehydes leads to the final formation of alkenals such as trans-4-hydroxy-2-nonenal (4-HNE from n-6) and a small quantity of trans-4-hydroxy-2-hexenal (from n-3)(14) Consequently ozone acts simply as a pro-drug and generates these two messengers. The unionized hydrogen peroxide immediately enters into the mass of erythrocytes and activates glycolysis with ATP increase and, most important, enhances the production of 2,3 di-phospho-glygerate (2,3-DPG), which is able to shift to the right the oxyhemoglobin dissociation curve, thus increasing the release of oxygen in the ischemic areas such as the ulcer's area (15). Moreover the infusion of ozonated blood implies an activation of nitric oxide release by the endothelium (16).

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Although the alkenals are intrinsically toxic, owing to the minimal ozone concentration, they are produced in the range of a few micromoles and undergo to either dilution, degradation by specific enzymes, elimination via the bile and urine but, most important, submicromolar quantities form adducts with either the Cys34 of albumin or the-SH group of glutathione (13,17). This step represents for the patient, who has been infused with his or her own ozonated blood, a calculated and well tolerated oxidative stress because the albumin adducts transport and deliver the alkenals to cells of many organs including those of the ulcer. Alkenals, inside the cell, binds to two-SH-groups (Cys272 and Cys288) of a large protein denominated Keap-1(Kelch-like ECH-associated protein1).

Normally Keap-1 is bound to Nrf2 and, as many other transcription factors, is floating freely in the cell cytoplasm. This complex has a half-life of about 20 min because is continuously digested by the proteasome. However, when the two SH groups of the Keap1 have bound two molecules of 4-HNE, the protein Nrf2 (nuclear factorE2-related factor 2) is released and translocates into the cell nucleus where, after making an heterodimer with a small Maf protein, binds to the antioxidant response elements (ARE) to regulate the downstream phase 2 gene expression and increase cell resistance to oxidative stress(18-22). This is the crucial event able to stimulate the upregulation of about 230 genes responsible for the transcription of a great number of antioxidant proteins (SOD, catalase, GSH-Px, GSH-Tr, etc), phase II enzymes and heme-oxygenase-1, which is a very protective enzyme. With the progress of ozonetherapy, these enzymes will be able to reverse the chronic oxidative stress induced by the chronic inflammation. At the same time, the enhanced oxygen release helps the healing of the ulcer. So far, millions of ozonated autohemotherapy, performed with small and precise ozone dosages, have never caused any side effects. In fact, most patients report a feeling of wellness which is likely due to a transitory increase of adrenocorticotrophic hormone-cortisol and endorphins (17).

Lastly, ozonetherapy has also a minimal cost. In terms of the ozonetherapy procedure, depending upon the patient's body weight, between 150-225 ml of blood are drawn by vacuum from an antecubital vein via a 19-21 G needle into a sterile glass, 500 ml bottle (Ozonosan, Iffezheim, Germany) containing 1 ml of 3.8% Na citrate solution as an anticoagulant for every 9 ml of withdrawn blood (9:1 ratio). A corresponding volume of a gas mixture (O2: 96%- O3: 4%), precisely measured regarding the ozone concentration via a spectrophotometrically reading on line at 253.7 nm (Hartley band), is immediately added with an initial ozone concentration of 20 mcg/ml gas per ml of blood.

Thus for a total volume of 200 ml blood the total ozone dose is equivalent to 4.0 mg. Ozone is produced by a last generation ozone generator fed with only medical oxygen. The gas and blood are immediately and gently mixed, avoiding foam formation, for no longer than ten minutes and then the oxygenated-ozonated blood is infused into the donor patient in about 15-20 minutes. It is emphasized that the transfusion must be only autologous and is absolutely safe. Depending upon the ulcer, the treatment is carried out twice weekly. for the initial 10 weeks. Normally the patient starts to note an improvement after 4-6 treatments. Afterwards it must be performed at least every week for maintaining the therapeutic effects. It is important to note that the initial low ozone concentration is slowly upgraded up to no more than 50 mcg per ml of blood.

The axiom: start low, go slow is proficiently used with this procedure. Indeed the oxidation properties of ozone represents a controlled oxidative stress for the patient and the low ozone concentrations are well tolerated because they yield a hormetic dose-response relationship(23-25) The above maintenance therapy is absolutely necessary and, if necessary, it can last several years. The explanation is due to the fact that ozone therapy is based on precise biochemical basis and, if it is suspended, the therapeutic effects tend to fade and possibly disappear within 6-9 months. The patient must be informed that the biochemical reactions have a rather short memory but this is not a drawback because patients are very compliant once they have noted the improvement. Indeed the positive patient's response is the best criterion to show that the therapy is effective.

Case Reports Presentation

We report a series of 40 patients (27 males and 13 females),with a median age of 70 years (range 33-83) suffering chronic single or multiple ulcerations defined as the presence of lesion longer than 8 weeks treated with O_3-AHT. Patients were considered to be affected by multiple lesions if each single ulceration surface was superior to 6 cm^2. All patients were classified as not responding to orthodox medical or surgical treatment, accordingly with a failed improvement or a worsening of lesion severity.Informed consent was obtained from all patients before the study. The protocol of study was approved by Ethical Committee of the Udine University Hospital.

The CLU etiology was multivariate including peripheral vascular disease, surgical complication, post trauma damages, diabetes (see Table 1). The O_3-AHT was performed collecting no more than 225 ml of autologous patient's blood in a dedicated 500 ml glass vacuum bottle (B.Braun Melsungen) containing Na citrate solution. An equivalent volume of O_2-O_3 mixture (O_2 96%-O_3 4%) was added to the collected blood and gently mixed for 10 min before re-infusing the solution back to the patient.

The treatment was carried out on ambulatory bases twice a week for at least the first 10 session, then the frequency was eventually reduced at once a week accordingly with clinical results and patient compliance. The treatment was free of charge.

Standard medical and surgical (curettages) therapy was maintained as usual during O_3-AHT treatment. At the beginning of O_3-AHT therapy, a picture of the lesion was taken and it was taken again regularly at five sessions interval to

Table 1 General data of patients. Legend: PVD= Peripheral Vascular Disease; AHT= Anti Hypertensive Therapy; O_2 HT= Oxygen Home Treatment; OHA= Oral Hypoglycemic Agents; PfA= Proposed for Amputation. Patients 2 and 10 had large ulcerations on both legs. Patient 21 had a CLU on both hands. Patient 27 had a post surgery peri anal ulceration.

Pts	Sex	Age	CU duration months	CU surface cm^2	N° of O_2/O_3 treatments	Residual CU surface cm^2	Co-morbidities	Ongoing Theraphy	Surgical proposal
1	F	65	36	36 110	35 120		Diabetes	Insulin, AHT	PfA
2	M	60	15	84 26	40		Vasculitis	Cortison, immunosuppressants	PfA
3	M	78	6	12	25		COPD, PVD, Hypertension	AHT, O_2 HT	
4	M	43	6	6	30		Dialysis, twice kidney graft failure		PfA
5	F	68	8	14	15	3.7	Hypertension	AHT	
6	F	65	36	4.6	25		Osteomyelitis post big toe amputation	OHA	
7	M	75	5	12	13			AHT	Plastic Surg
8	M	75	4	105	40	15	Diabetes, Obesity	OHA	PfA
9	M	75	8		20		PVD, bypass failure, Hypertension	AHT	PfA
10	M	63	4	200 118	50	43 24	Post traumatic infection		PfA
11	M	74	60	8	20		Surgical lesion		
12	F	70	48	19	10				
13	M	42	7	53	10		Post Merkel cancer excision	Repeated surgery	
14	F	80	3	56	10		Hypertension	AHT	
15	M	48	9	2	30		Osteomyelitis post fracture repair	Long lasting Antibiotic therapy	
16	M	72	25	10,3	22				
17	M	70	9	3	15		Diabetes	Insulin, AHT	
18	M	75	25	2	20		Post surgical, Diabetes	OHA	
19	F	70	14	4	12		Hypertension	AHT	
20	M	33	14	16	15		Post trauma loss of susbtances		
21	F*	83	6	17,6	20		Raynaud Syndrom Post		PfA
22	F	80	12	2	20		Hypertension	AHT	
23	M	80	20	24	30		Hypertension	AHT	
24	M	68	10	5	10	3			
25	F	70	200	60	40	51			
26	F	80	3	29	15		Perianal ulcera post surgery		
27	M	76	8	1	10		Hypertension		
28	M	73	20	15	15	2	Hypertension	AHT	
29	M	78	45	26	40		Diabetes	AHT	
30	F	80	12	25	27	4		AHT	
31	F	71	6	12	10			AHT	
32	M	76	32	15	18		Hypertension,Diabetes	AHT	
33	M	70	20	25	30	3	Diabetes	AHT	
34	M	74	16	14	12		Hypertension	AHT	
35	M	74	27	28	30	2	Hypertension,Diabetes	AHT	
36	M	70	32	46	36	3	Diabetes	AHT	
37	F	75	23	31	20			AHT	
38	M	70	14	27	12			AHT	
39	M	76	22	18	10		Diabetes	AHT	
40	M	77	29	16	18			AHT	

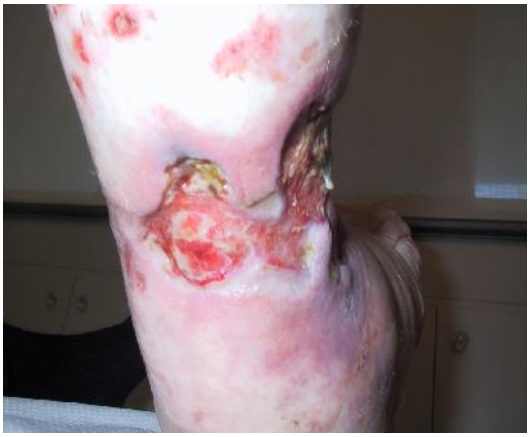
evaluate the clinical response. The picture was elaborated with a graphic software (CAD viewer 8, Guthrie CAD/GIS software Pty Ltd) to measure the surface of the lesion and monitor its evolution throughout the treatment.

The O_3-AHT was continued until healing of the wound, or in front of evidence that no further improvements were probable, or due to the patients refusal to continue the treatment, which is a rare event.

RESULTS

Thirty-seven patients were suffering legs ulceration, one patient presented a bilateral hand necrotic lesion due to Raynaud Syndrome following radiotherapy, a woman was affected by perianal ulceration after anal fistula surgery and a young man was suffering over the last eight months from chronic ulceration after excision of a gluteus Merkel tumor. (Table 1). Seven patients have been previously considered for leg amputation due to the uncontrollable pain in spite of epidural analgesia and systemic major opioid administration.

Ulcers have been open for a median time of 20 months, ranging from 3 to 200 months. Seven among all patients showed a multiple ulcer localisation. The median surface area of ulcerations was 19 cm^2 (range 0.5-200 cm^2). The patients underwent a number of O_3-AHT ranging from 10 to 120 with a median value of 20 sessions.



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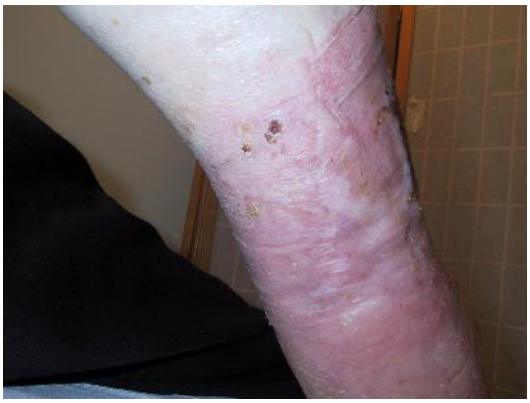

B

Fig.1 ischemic leg ulceration in a 78 years old man (patient n.29 in the table 1) before (a) and after (b) 40 treatments of O_3 AHT

In thirty- two cases a complete healing of the lesion was obtained while the remaining patients had a reduction of CU area of a median value of 79%. In figure 1 we reported the ulcer's picture of the patient n.29 before and after 40 treatments with O_3 -AHT. Among the patients that didn't reached a full recovery, one of them underwent a simple skin graft insertion after regeneration of muscle and subcutaneous tissue. Another patient, who at the beginning of treatment evidenced the exposure of peroneus longus and brevis tendon with a large loss of muscular tissue, stopped the treatment when the lesion area was reduced of 86% of its surface.

A third patient was suffering a wide leg lesion for 200 months, the treatment was suspended after 40 major O_3-AHT due to the unsatisfactory results (CU area reduction of only 15%)

In one case a 1 cm^2 residual area remained as a surface opening of a residual ostomielitic fistula. In two cases the ulcer appeared again after 10 months and they were successfully treated with the O_3-AHT, while 5 patients followed a maintenance therapy (once a month).

DISCUSSION

During the treatment of chronic ulcer a stepwise approach to wound management should address contributing factors such as peripheral arterial disease, infection and comorbid conditions. The local factors like edema, tissue viability, moisture balance and offloading are also important in the healing process.Poor wound healing after trauma, surgery, acute illness, or chronic disease conditions affects millions of people worldwide each year and is the consequence of poorly regulated elements of the healthy tissue repair response, including inflammation, angiogenesis, matrix deposition, and cell recruitment(26).The search for clinical strategies that might improve the body's natural repair mechanisms will need to be based on a thorough understanding of the basic biology of repair and regeneration.

A large number of experimental evidences suggest that the healing process in the chronic wounds is obstructed by local ischaemia due to hypoxia, high lactic acid concentration, an enhanced presence of reactive oxygen species and of pro-inflammatory cytokines. Deleterious vasoactive compounds, a low pH and lack of antioxidants are important factors enhancing a permanent tissue damage. Some experimental studies reported that the redox status of the cell is important if wounds are to modulate the oxidant signals that induce wound healing. The autologous infusion of ozonated blood is able to restore a physiological pH and the production of critical growth factors: Moreover the Nrf2 activation will promote the production of phase II proteins, antioxidant proteins and an enhanced release of GSH, thioredoxin and NADPH. Consequently the normalization of the antioxidant-redox cycling and the detoxification system slowly favours the tissue regeneration and the healing of the ulcer.As recently established(23) ozonetherapy is able to specifically treat oxidative stress related diseases. The improvement depends also upon the age of the patients and the presence of co-morbidities that may delay the healing. Indeed the ozonetherapeutic treatment is far more effective when started at the early time of ulceration. The patient is able to realize the progress and this is the reason of an excellent compliance.

Conflicts of interest

The authors have not received any funds for this work and report no conflicts of interest in this work.

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How to cite this article:
Emma Borrell *et al*., Oxygen Ozone Therapy in the Integrated Treatment of Chronic ulcer: a case Series Report. *International Journal of Recent Scientific Research Vol. 6, Issue, 5, pp.4132-4136, May, 2015*

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