Ozone Auto-Haemotherapy in Lower Limb Ulcerations

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Abstract

We report in this paper on the beneficial effects of Ozone Auto-Haemotherapy (OHT) in two patients that were afflicted with painful, intractable leg ulcers. One had diabetes mellitus type II, the other probably had a vasculitis, but this could not be established with certainty. Both patients had seen many specialists; a dermatologist, an internist and vascular surgeon, but to know avail. Their clinical course went from bad to worse. The increasing pain became intolerable and finally they came to our pain clinic. Chemical lumbar sympathectomy and epidural blockade with bupivacaine and morphine were somewhat successful in managing their pain, but ineffective in treating the ulcers. Only after instituting extended Ozone Auto-Haemotherapy did the ulcers heal.

Introduction

Ozone therapy is recent in medicine and early reports on the efficacy of ozone therapy in certain pathological condition have gone practically unnoticed. The reason for this is that ozone was branded as a pollutant and health hazard, particularly to the upper airways. Absence of sound data on physiological and biochemical activity presented a hindrance to further clinical experimentation. In recent years the picture has changed rapidly in this respect. Moreover therapeutic ranges in the dosage of ozone has been identified (1); however exceeding this level, ozone may be highly toxic.

This semi-standardization in the clinical application of ozone has helped to further the status of ozone as a very effective therapeutic agent in many pathological conditions, and in some cases the agent of choice. It has become evident that even low (therapeutic) concentrations of ozone pose a strong oxidant stress in the body by the formation of reactive oxygen species (ROS)(2). Ozone is rapidly converted to oxygen, limiting the duration of oxidant stress. The oxidant stress elicits a pronounced and lasting upregulation of the enzymatic anti-oxidant system. The oxidant stress of ozone involves a plethora of cells and systems in the body, which will be briefly reviewed.

In addition there are other physiological effects of ozone, not necessarily related to oxidant-antioxidant activity. We will refer to the latter as metabolic and rheological aspects of ozone therapy. We will briefly review and summarise in table format the spectrum of known bio-activity of ozone.

Early in the history of human ozone therapy, studies appeared of patients with peripheral occlusive artherosclorotic disease, whose symptoms of intermittent claudication improved significantly (3,4). The development of chronic ulcers is often on the basis of impaired microcirculation and micro-angiopathy, irrespective of their aetiology. The combination of biochemical, rheological and metabolic effects of OHT forms the rationale for its use in

patients suffering from chronic leg ulceration. We will describe briefly the pathogenesis and pathology of leg ulceration, the effects of ozone, and indicate how and where OHT may have beneficial effects and bring healing.
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Clinical Subjects

The first patient was a 62-year-old female, weight 55-kg, a non-insulin dependent diabetic and treated with oral anti-diabetic medication, who developed a non-healing ulcer on the lateral aspect of right leg proximal to lateral malleolus. She was otherwise in good health and a non-smoker. She was referred to the dermatologist, who treated her with topical medication and intermittent surgical curettage of the necrotic material accumulating in the wound.
The ulcer got steadily worse and the pain became unmanageable with anti-inflammatory and pain medication. When she came to our clinic the pain was so severe that she was not able to sleep at all. Focussing on the pain management a chemical lumbar sympathectomy was performed to improve her pain as well as local circulation through reduction of cathechole-amine levels. No improvement of the ulcer was observed although the pain was less severe.
A lumbar continuous epidural catheter was inserted and attached to a Baxter elastomeric infusion set for the purpose of controlled and extended delivery of 0.5 ml of bupivacaine of 0.15% with weekly refilling. Initially good analgesic effect was seen but after two months it was necessary to add morphine (2 mg a day) to the infusion to control the increasing pain. In the meantime the patient continued to see her dermatologist and internist, who decided to put her on regular insulin (15 units twice a day). Six months after the initial visit there was not only no improvement of the ulceration, instead it got worse. The vascular surgeon had already suggested to her to amputate her leg.
At that time it was decided to resort to ozone auto-heamotherapy (OHT). Initially the patient received OHT twice a week and after considerable improvement was achieved, this regimen was continued once a week. In total, the patient received 40 treatments before the ulcer was completely healed.

The second patient was a 57-year male, weight 80 kg, and of profession a carpenter. He had enjoyed very good health, was a non-drinker and a non-smoker and not on any medication. His symptoms started prior to visiting our clinic with extensive bilateral ulceration two years ago when he noticed on both legs multiple fluid-filled blisters, that upon discharging failed to heal or seal and then progressed to deep ulceration. The ulcers became worse over time and so did the general swelling and oedema.
The patient consulted many specialists, among them a dermatologist, internist, allergist and a vascular surgeon. Doppler flow studies were performed but no vascular abnormalities were found. Also an auto-immunological basis for his disease was excluded as well as contact dermatitis. Cultures were initially negative and local biopsies were non-specific for any type of disease. Despite the negative work up it was the consensus that the patient had a “vasculitis”, but of unknown aetiology. Nevertheless, he was put on cortisone medication which did not stop the continuous worsening of the ulceration and oedema. Six months after the onset of his symptoms he presented himself to our clinic as his pain became unbearable, despite oral morphine. By now he could only get a little sleep in a sitting position.
He received a lumbar epidural catheter with a continuous infusion of 25 mg of bupivacaine daily through a Baxter elastomeric infusion device, to which later morphine in a dosage of 3 mg daily had to be added to control his pain. This pain treatment was effective, but the ulceration and swelling of the legs worsened, the infection became uncontrollable to the point

that the surgeon suggested to the patient to consider amputation of his lower legs. As an alternative treatment we proposed to the patient to receive OHT; that was 4 months after his first visit to our clinic. Several ozone treatments already reduced the swelling and the pain. After 8 months of ozone treatments the ulcers on one leg have completely healed.

Pathology of leg ulcers

In the normal wound formation and healing processes many systems and cells are involved, through activation by ROS. Erythrocytes, plasma proteins, lipoproteins, mastcells, neutrophils, monocytes, lymphocytes, eosinophils etc. are all involved. Blood coagulation is also present.
The inflammatory phase is characterised by an early and late phase. This is followed by a granulation phase and finally by a matrix formation and remodelling phase. A brief review of the cells and systems involved in wound formation and healing will follow.

Traumatic injury and vessel disruption results in platelet aggregation and blood coagulation. These phenomena are limited, due to the intrinsic activities of the intact surrounding endothelium. Activated platelets and clotting products, forming a haemostatic plug, release a host of chemotactic and vaso-active substances on a local level. These substances are too numerous to mention, but here is a small selection(5,6,7): Histamine, bradykinin, serotonin, platelet activating factor (PAF), platelet derived growth factor (PDGF), tumor necrosis factors (TNF-α), transforming growth factors (TGF-α-β), fibroblast growth factors (FGF), eicosanoid products of arachidonic acid metabolism (prostacyclin, tromboxane B2 and leukotrienes (B4 and D4), adenosine phosphate. Fibrin, fibrinolysis products, fibronectin, proteases, with complement activation and generation of complement derived products C3a, C5a. Interleukines are generated (IL-1, IL-1β, IL-2, IL-6, IL-8, IFN-β). Neutrophils infiltrate the wound attracted by the various chemotactic factors, among them activated complement derived products (C3a, C5a). Many of these products mediate increased vascular constriction and permeability leading to oedema formation. Activated neutrophils release reactive oxygen species (ROS) and lipid peroxidation. By diapedesis they reach the peri-vascular space. In this stage histology often shows leukocyte and platelet plugs within the vascular lumen.

In the next phase the neutrophils, which are important in the inflammatory phase, are being more and more replaced by monocytes. The monocytes go on to become macrophages. The macrophages rid the wound of pathogenic organisms, tissue debris and effete neutrophils. Furthermore they release growth factors and chemotactic substances, including (PDGF), (TNF), and interferon-α-β (IFN). Thus the macrophages play an important role in transition between inflammation and repair. In this stage the fibrinolysis is activated and digestion of collagen and fibrin clot is facilitated by the release of proteases.

In the following phase of repair granulation tissue appears, which consist of newly formed vessels. They are embedded in a loose matrix of fibronectin, collage and hyaluronic acid, forming the granulation tissue that will support the new epidermis. Fibronectin and hyaluronic acid is mainly deposited by fibroblasts. Fibroblast proliferation and migration is signalled by chemotactic factors and growth factors such as FGF (fibroblast growth factor), TGF-β, PDGF (platelets and macrophages). Matrix proteins and thrombin, which also stimulate fibronectin and fibroplasia are formed. Also angiogenesis now appears, almost simultaneously with fibroplasia.

The final phase in the healing is re-epitheliazation, in which the epidermal growth factor (EGF) and keratinocyte growth factor (KGF) are involved.

In chronic ulceration however, the healing has failed. A simple definition of a chronic ulcer is that it is a defect in healing. The permanence of the defected healing process may further contribute to the exacerbation of the ulceration and superimposed infection and thus a vicious circle has been entered. At this stage the microcirculation is likely to be affected as severe angiopathy has developed. As a consequence, nutrient local blood flow is blocked, impeding the phagocytic cleansing of necrotic debris. Healing through granulation, fibroplasia, neo-vascularization and re-epithelization, all of which require an adequate blood flow, is arrested.

Patients with venous insufficiency or blockage (thrombosis) and/or peripheral occlusive artherosclerosic disease (POAD) are more prone to angiopathy and failure of the microcirculation. Vasculitis falls into the same category.

Subsequently the inflammatory stages may persist, endothelial damage may become more pronounced with swelling and denudation, presenting a thrombotic surface by exposing the sub-endothelial matrix. This chronically activates the intrinsic clotting cascade where plugs of leukocytes and platelets persist or are newly formed. Increases in vascular permeability and peri-vascular oedema formation further impede local blood flow. In this stage there is extravasation of fibrin as demonstrated with antifibrin-fibrinogen immuno-fluorescence (8), indicating a lack of normal fibrinolytic activity.

Thus, in chronic ulceration local ischaemic events seem to prevail and pre-existent vascular disease may sustain this condition. The ischaemic condition may not be altogether static, and from time to time a temporary (partial) re-perfusion of the affected area may occur. Intermittent ischaemia and re-perfusion may add insult to injury as this can result in a very deleterious activation of the xanthine/ xanthine oxidase pathway (9,10,11), generating more ROS for the tissues. At any rate the end stage of the above sketched scenario is ulceration and necrosis of the skin. Once these inflammatory conditions have become entrenched they may lead to a chronic persistence of the ulceration and superimposed infections. In this stage medical and topical therapy has often become ineffective. Of course surgical intervention is required if the condition of venous insufficiency or arterial occlusive disease is the underlying pathology.

ROS; oxidant and antioxidant system.

Oxidant stress, by reactive oxygen species (ROS) and resultant lipid peroxidation, appears to be an important cause of cell and tissue damage in many disorders. Within the body, extracellular oxidants include phagocytic cell products such as superoxide anion ($O_2^{\bullet}$) hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and hypochlorous acid (HOCl). Metals (iron and others) may catalyse reactions, which convert $O_2^{\bullet}$ and H_2O_2 to more toxic species such as $^{\bullet}OH$. Myeloperoxidas (MPO) may react with H_2O_2 to form HOCl. Ischemia-reperfusion injury is a prime model of ROS injury where xanthine dehydrogenase may be converted to xanthine oxidase leading to the generation of damaging O_2 metabolites.

The endogenous antioxidant defence systems include cellular anti-oxidant enzymes and circulating anti-oxidants. Common cellular anti-oxidants are (1) superoxide dismutase

(SOD), which converts super-oxide anion to hydrogen peroxide, (2) catalase, a scavenger of H_2O_2 and (3) glutathione peroxidase (GPX), which catalyses the reduction of organic hydroperoxide and H_2O_2 to non-toxic compounds. Reduced glutathione appears to inhibit lipid peroxidation.
In addition there are many hydro-soluble anti-oxidants (e.g. uric acid, L-ascorbic acid, plasma protein etc.), lipid-soluble antioxidants (vitamine E, A, etc.) and chelating proteins.

For the subject at hand we should bear in mind that erythrocytes (RBC) contain vast amounts of antioxidant enzymes. This notion will become relevant in the discussion of the therapeutic effect of ozonization of blood.

The effect of ozonized blood

Biochemical effects.
Ozone, by itself not an oxygen radical, has a strong oxidizing effect and generates oxidants (ROS). The oxidant stress by ozone involves many of the blood components: lipo-proteins, plasma proteins, lymphocytes, monocytes, granulocytes, platelets and erythrocytes. Ozone acts on any organ and surface with which it gets in contact (e.g.endothelial cells).
In a defence reaction to the generation of ROS, the various anti-oxidant systems are activated and go on to produce anti-oxidant enzymes and scavengers (12). Since the oxidizing effect of ozone is almost linearly related to its concentration in the blood, above a certain threshold it becomes very cytotoxic and produces haemolysis. The therapeutic range is rather narrow but has already been well described by now. The half-life of ozone is short. Ozone rapidly converts into oxygen via endothermic reaction, and its resident time is in the order of 10 minutes. The ozone oxidant stress is therefore of brief duration, but nevertheless, the antioxidant response is very pronounced where the latter is believed to outlast the former

For example, ozone pre-treatment followed by ischaemia-reperfusion injury showed a significant increase in organ SOD activity, preservation of glutathione, while H_2O_2 (13,14) levels were marginally increased. This may explain some of the observed therapeutic effects of ozone.
A summary of the aforementioned biological effects of ozone is given in table I, adapted from Bocci (15).

Table I Biochemical Effects of Ozone in Blood.

Erythrocytes	Platelets	Leukocytes	Endothelium	Haemostasis
ATP, EC, 2,3 DPG ↑	TGF ↑	PGE2 ↑	NO ↑	vWF ↑
O2 unloading ↑	PDGF ↑	TNF-α ↑	VEGF ?	(t-PA) ↑
SR ↓ , MF ↑	TXB2 ↑	INF-γ ↑		APTT ↑
PO2 arterial ↑→		IL2, IL6, IL8↑	Oedema ↓	TT ↑
PO2 venous ↑		BK, Hist. ?		

Table I. Ozone has a strong oxidising effect and elicits through generation of ROS many cellular responses, These in turn produce a host of chemotatic and vaso-active substances and growth factors as well as producing other stimulatory effects including those on the erythrocytes. In response the anti-oxidant system is in strongly stimulated. Abbreviations appear if full throughout the text

Rheological effects.
Ozone therapy has already established itself as a very effective treatment in some disorders. That ozone treatment can positively affect the microcirculation has been shown by earlier investigation in patients with severe claudicatio intermittens, all of which report a significant improvement. Ozone studies on filtrability of blood showed an improvement, perhaps on account of an increase in membrane fluidity (MF), while a reduction in sedimentation rate (SR) was found. We believe that the above rheological effects of ozone play a major role in the improvement of the microcirculation. These findings are presented in table II that has been adapted from Coppola et.al. (16). The data presented has been corroborated by similar findings from other authors (17,18).

Table II Rheological Effects of Ozone in Blood

Time post ozone	Haematocrit	Filterability whole blood	Viscosity blood	Viscosity plasma	Fibrinogen
O (control)					
15 min		↑	↓	↑	↓
60 min		↑	↓		

Table II. This table shows the rheological effects of ozone.
It is likely that each and all the parameters listed contributed to the healing of chronic ulcers in the patients, based on improved local micro-circulation, better oxygenation, facilitated O2 unloading and influx of anti-oxidants, importantly carried also by the erythrocytes.

Metabolic effects.
In several experimental studies of ischaemia-reperfusion injury in different organs: liver, kidney and brain, pre-treatment with ozonized blood was shown to have a striking protective effect. Biochemical markers of injury particularly occurring in the re-perfusion period were significantly less with prior ozone treatment. Injury assessment on the basis of morphology showed also a remarkable degree of protection. During ischaemia, as a consequence of ATP degradation, a significant increase in adenosine and xanthine production is seen. In the re-perfusion period, adenosine has a protective effect, but the ROS generation by xanthine/xanthine oxidase pathway has a deleterious effect. Ozone treatment prior to the ischaemic injury showed a significant reduction in the xanthine accumulation, while the adenosine levels were not affected. In a similar study it was found that the transaminases (AST, ALT) and lactate levels were attenuated, while a preservation of glutathione and an increase in SOD was observed. Also H_2O_2 levels did not increase. In another study on brain hypoxia, prior ozone treatment indicated that energy charge (EC) and ATP were well maintained and that lactate production was inhibited, producing a significant increase in survival time (19).
Finally a brief summary of these and other metabolic effects (20) are given in table III We also have listed some of the interstitial matrix factors and molecules that are essential in the healing process of wounds and that will benefit from a metabolic preservation and/or stimulation.

Table III Metabolic Effects of Ozone in Blood

ATP	↑	Hyaluronic acid	?
Energy Charge	↑	Fibronectin	?
Cholesterol	↓	FGF-β, EGF, KGF	?
Triglycerides	↓	Collagen I/III	?
Fatty acids	↓		
Low density lipids	↓		
xanthine	↓		

Table III. OHT exhibits a metabolic stimulation, perhaps more importantly preservation of ATP, energy charge, adenosine and reduction of lactate levels during ischaemia. Also significant is also the reduction in xanthine. All these factors minimise further injury in the re-perfusion period. Metabolic stimulation helps the formation of interstitial matrix factors where as lipids that can damage the endothelium are reduced.

In summarising we can say that ozone in blood, is a strong oxidising agent and causes:
1) stimulation the generation of antioxidants,
2) vasodilatation and hyperaemia (NO),
3) reduction in blood and plasma viscosity,
4) increase in erythrocyte membrane fluidity,
5) hyper-oxygenation and facilitated oxygen unloading to the tissue,
6) metabolic stimulation

Ozone auto-haemotherapy (OHT) protocol.

After cannulation of a large peripheral vein, 225 ml of blood was collected in a 500 ml bottle containing 950 mg of sodium citrate. Subsequently, 225 ml of O_2/O_3 mixture containing 50 mcg of O_3 per ml, was added to the collected blood and mixed for 15 min. by gently shaking the bottle. The ozonized blood was then re-infused to the patients over a 20-30 minute period. Initially the patient received OHT twice a week. After notable progress was made, treatment was reduced to once a week until healing of the ulceration was achieved.

Results

In our patients there was no underlying pathology that was amenable to surgical therapy. Regimens to improve the metabolic condition with medications remained unsuccessful. The patient with diabetes was put on insulin, the patient with vasculitis was started on cortisone therapy.

Extended OHT, however, resulted in complete healing of the ulceration. In the figures 1 and 3, photographs of the ulcers before or shortly after OHT are shown. The end results of OHT: the healing of the ulcers are shown in figures 2 and 4.

Fig1 1 and 2.Vasculitis patients before and after 70 OHT treatments, resulting in a complete healing

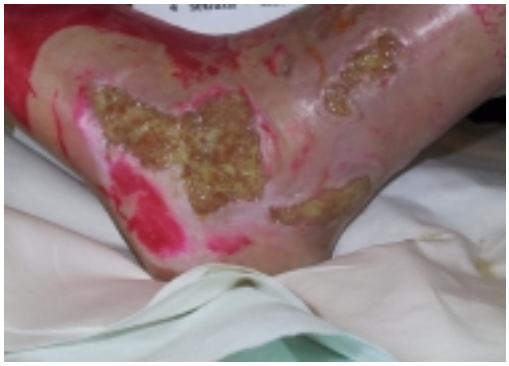

Fig 1

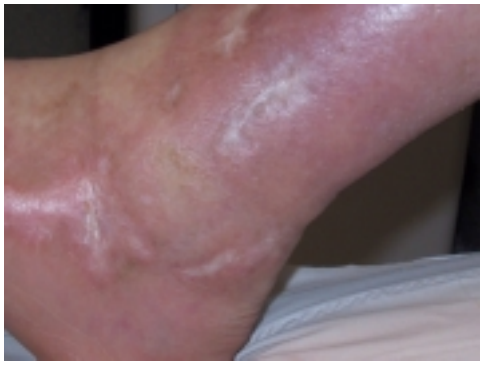

Fig 2

Fig. 3 and 4. Diabetic patient before and after 40 OHT treatments, resulting in a complete healing.

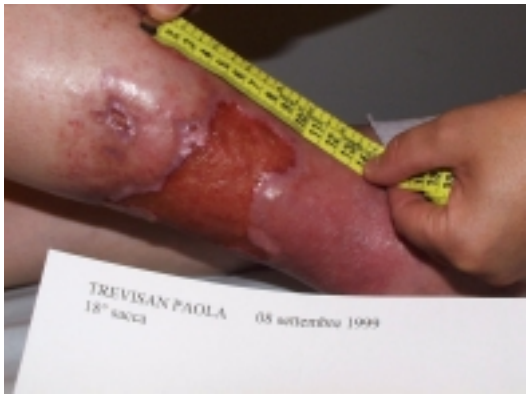

Fig 3

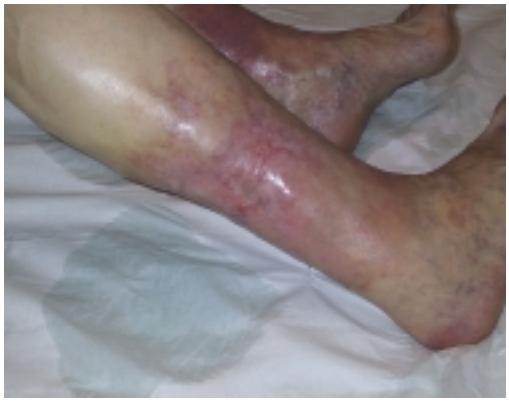

Fig 4

Discussion

We presented two patients with longstanding (6 months) chronic leg ulcers with different aetiology, in whom conventional topical and systemic therapy failed completely. OHT resulted in a complete healing of these chronic ulcers.
In reviewing some of the varied effects of ozone as described in the respective section above, we believe that the healing effect of OHT in these patients with chronic leg ulceration is the result of a multifactorial: biological, rheological and metabolic activity.

With respect to the biological effect as far as the oxidant-antioxidant system is concerned, we hypothesise that the balance over time is tipped in favour of the antioxidant system, helping

to control the ongoing ulceration and necrosis. In addition we believe the rheological changes as a result of OHT are important in the repair of chronic wounds.

In chronic ulceration there is often severe angiopathy (formation of plugs of leukocytes, platelets, clotting products etc) to the degree that the microcirculation may be critically impaired and made worse by endothelial swelling and peri-vascular oedema. One effect of OHT is vasodilatation on account of an ozone increased generation of nitric oxide (NO), a potent vasodilator. Ozone increases blood filterability (membrane fluidity is increased, sedimentation rate is decreased) and causes a decrease in blood and plasma viscosity (plasma macromolecules are decreased). Moreover ozone may induce a hypo-coagulatory state (21), thus decreasing the tendency to clot. The thrombine time (TT) is increased, von Willibrand factor (vWF) is stimulated, fibrinogen is decreased and the tissue plasminogen activator (t-PA) is increased. Activated partial thromboplastine time (APTT) is also prolonged. Moreover fibrinolysis, which is impaired in chronic wounds, may be enhanced.

Undoubtedly, all these factors resulting from OHT will improve the microcirculatory flow. The result is a more vigorous or renewed influx of erythrocytes. Erythrocytes carry vast amounts of anti-oxidants (SOD, catalase, GSH) that are capable of mopping up locally present reactive oxygen metabolites that are perpetuating chronic ulceration. An additional benefit of OHT is an increased concentration of 2,3 DPG in the erythrocytes, which results in a facilitated unloading of oxygen to the oxygen-starved tissues.

Benefits of OHT in the treatment of chronic ulcers may also be related to ozone's ability to preserve ATP and adenosine, while reducing lactate and xanthine levels during ischaemia. The obliterative micro-angiopathy seen in chronic ulcers is cause for ischaemia. This ischaemia may be intermittent in and not of static nature. Intermittent re-perfusion may occur from time to time. Re-perfusion has a deleterious effect on the tissue, but the reduction seen in xanthine levels after ozone exposure may limit or inhibit the re-perfusion injury. Finally, the positive metabolic aspects of ozone may enhance the formation of interstitial matrix factors and proteins that will benefit granulation, fibroplasia, neo-vascularization and the re-epithelaization process.

Conclusion

We have presented two non-surgical patients that were suffering from chronic leg ulceration for as long as two years and with whom conventional therapies failed altogether. OHT was instituted as a last resort to stave off a by the surgeon proposed amputation. OHT, initially given twice a week, had an almost immediate effect on swelling and oedema as well as on the intensity of the pain. The progress in healing was slow but steady. After many months of OHT a complete healing of the ulcers was the result.

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