Ozone medical applications in patients with diabetic foot

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Abstract

In Diabetes mellitus, long-term complications, that cause morbidity and premature mortality, is characterized by microvascular and macrovascular diseases. It has been demonstrated, in diabetic patients, the important role of oxidative stress for the development of complications. Ozone can exert its protective effects by means of an oxidative preconditioning, stimulating and/or preserving the endogenous antioxidant systems, by normalizing the oxygen metabolism and also due to its great germicide properties. The aim of this study is to evaluate ozone efficacy in the treatment of type 2 diabetes patients suffering of diabetic foot complications, and its effects in the oxidative stress, the hyperglycemia and the markers of endothelial damage, making a comparison with antibiotic therapy. A randomized controlled clinical trial, where all patients provided a signed informed consent before being enrolled, was performed with 100 patients divided into two groups: ozone, using local and rectal insufflation (with an ozone dose of 8 mg, ozone concentration: 40 mg/L) of the gas and control (using topical and systemic antibiotics), with 51 and 49 patients, respectively. The efficacy of the treatments was evaluated comparing the glycaemic figures, the area and perimeter of the lesion and the biochemical parameters involved in oxidative stress and endothelial damage, between both groups, after 20 days of treatment. The efficacy of the treatments was evaluated comparing the glycaemic figures, the area and perimeter of the lesion and the biochemical parameters involved in oxidative stress and endothelial damage, between both groups, after 20 days of treatment, as well as the clinical evaluation of the lesions and length of hospitalization. Ozone treatment improved glycemic control and prevented oxidative stress, standardized organic peroxides figures and activated superoxide dismutase. At the end of the treatments, a decrease of the area and perimeter, for both groups, was obtained, but the expected total recovery showed that patients treated with ozone needed half of the time to achieve a total recovery with regard to the control group. Ozone therapy not only reduced the number of patients submitted to amputation, but also decreased the area to be amputated. The length of hospitalization decreased in patients treated with ozone with regard to the control group. The pharmacodynamic effect of ozone, in the treatment of patients with neuroinfectious diabetic foot, can be ascribed to the possibility of being a superoxide scavenger. Nowadays, superoxide is considered a link element of the four metabolic routes associated to diabetes pathology and its complications. No side effects were presented. These results demonstrated that medical ozone treatment could be an alternative therapy of diabetes and its complications.

Introduction

Diabetes mellitus is characterized by metabolic abnormalities, a disorder of carbohydrate metabolism, with the presence of hyperglycemia and glycosuria, resulting from inadequate production or utilization of insulin. Long-term complications, that cause morbidity and premature mortality, is characterized by microvascular disease with capillary basement membrane thickening, macrovascular disease with accelerated atherosclerosis, neuropathy, neuromuscular dysfunction, embryopathy and decrease resistance to infections. Such chronic complications involve the eyes, kidneys, heart, nerves and blood vessels. Accelerated atherosclerosis produces 80 % of all diabetic mortality, three fourths of it owing to coronary disease. A more frequent concomitant of distal anesthesia is the development of neurotropic ulceration, particularly on the plantar aspect of the foot. Anesthesia leads to a worsening of any minor injury because of the absence of protective painful stimuli. All these events, in addition to pre-existing microvascular and macrovascular circulatory impairments, characterizes the underlying mechanisms that may lead to rapid gangrene after foot injury [1,2].

It has been demonstrated, in diabetic patients, the role of the reactive oxygen species (ROS) with an increase oxidative damage at the level of lipid peroxidation, DNA injury and protein damage [3-7]. Four main molecular mechanisms have been implicated in glucose-mediated vascular damage: increased polyol pathway flux; increased advanced glycation end-product (AGE) formation; activation of protein kinase C (PKC) isoforms and increased hexosamine pathway flux [8]. An hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain seems to be present. Also, a decrease in the antioxidant defense system, involving the erythrocyte superoxide dismutase and catalase [9,10], with a simultaneous decrease in vitamin C concentration in leukocytes have been mentioned [11].

Ozone can exert its protective effects by means of an oxidative preconditioning, stimulating and/or preserving the endogenous antioxidant systems and by blocking the xanthine/xanthine oxidase pathway for ROS generation, as it has been demonstrated in the damage induced by carbon tetrachloride and in the hepatic and renal ischemia-reperfusion [12-16]. In addition, it has been demonstrated that ozone therapy, by autohemotherapy, in patients with myocardial infarction, has a beneficial effect on blood lipid metabolism, decreasing blood cholesterol and provoking the activation of antioxidant protection system [17]. Ozone has been used with good results in the treatment of patients with diabetic foot, taking into account its germicide properties and its influence in the processes of oxygen metabolization, besides other effects [18].

Epidemiological studies suggest a prevalence of 300 millions of diabetic patients, mainly in type 2 diabetes, for the year 2010 [19,20]. Any treatment that is capable to normalize oxygen metabolism, to modulate the oxidative stress and to have germicide properties can improve the quality of life of these patients, as well as diminish patient consumption of medicines. Taking into account the ozone therapeutical properties, the aim of this study is to evaluate ozone efficacy in the treatment of type 2 diabetes patients suffering of diabetic foot complications, and its effects in the oxidative stress, the hyperglycemia and the markers of endothelial damage, making a comparison with antibiotic therapy.

Subjects and Methods

This is a randomized controlled clinical trial approved by an institutional review board (Scientific and Ethics Committees of the Institution). All patients provided a signed informed consent before being enrolled. Patients were given appropriate information of the study (benefits and possible side effects). Adult patients of both sex of any ethnic, with diagnosis of neuroinfectious diabetes foot,

according to the classification made by McCook *et al*. [21], suffering of ulcers of the feet and lower extremities, hospitalized in the Institute of Angiology and Vascular Surgery, were eligible to participate in the study. These patients must not meet any of the following criteria: severe septic conditions, hypersensibility to the medication that will be used, hepatic dysfunction, renal failure (serum creatinine level > 1.32 μmol/L), pregnancy, cancer or other serious disease, inability to cooperate with the requirements of the study, recent history of alcohol or drug abuse, current therapy with any immunosuppresive agent or anticonvulsant, concurrent participation in another clinical study or current treatment with an investigational drug. Patients were randomized to two different groups of treatment: 1-, control, 49 patients treated with antibiotic therapy (according to the germ present) systemic, using the conventional method of treatment and topically in the lesion (during 20 days), and 2-, ozone, 51 patients treated daily with ozone (generated by an OZOMED equipment, Cuba), 20 sessions, by rectal insufflation (with an ozone dose of 8 mg, ozone concentration: 40 mg/L) and locally (using a plastic bag, filled with ozone, during 1 hour and then the lesion was cured with ozonized sunflower oil-OLEOZON®). Debridement was indicated in essentially every wound and gauze dressings were used in them.

Blood samples for biochemical profile analysis were obtained after 12-hour overnight fast, at the beginning and 24 h after the last ozone and antibiotic treatments. Glucose, fructolysine, advanced oxidation protein products (AOPP), nitric oxide (NO), reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), total hydroperoxides, peroxidation potential (PP) and malondialdehyde (MDA) were measured in this protocol.

The main variables considered were:

- Clinical evaluation of the lesions.

1. Measurement of the area and perimeter of the lesions by means of a trace done in an acetate plate (planimetric analysis), in aseptic conditions, at the beginning and at the end of the study, as well as the variation of both parameters with respect to time. The resultant area and perimeter were quantified using a computer program (DIGIPAT).
2. Qualitative clinical evaluation of the lesions.
3. Length of hospitalization. The time necessary to obtain an aseptic lesion, with good granulation tissue and in a healing process or ready to receive a graft.

- Glucose figures, measured at the beginning and at the end of the study, taking into account that hyperglycemia is the primary factor associated to diabetes and its complications.

Secondary variables considered were:

- Serum determinations of fructolysine, AOPP, NO, GSH, GPx, CAT, SOD, total peroxides, PP and MDA.
- Side effects.

A good result was considered when a decrease in: area and perimeter of the lesion, length of hospitalization, glucose, fructolysine, AOPP, MDA, PP and total peroxides figures were achieved. Also, an increase in NO, GSH, GPX figures and an approach to physiological values of the equilibrium CAT/SOD were obtained. In the case of biochemical variables, laboratory data of healthy persons (n=50) were taken as normal reference values (negative control group).

Biochemical determinations

All biochemical parameters were determined by spectrophotometric methods using an Ultrospect Plus Spectrophotometer from Pharmacia LKB. Glucose concentration was determined by a colorimetric method, according to the procedure described by Schmidt *et al.* [22]. CAT activity was measured by following the decomposition of hydrogen peroxide at 240 nm at 10 sec intervals for one minute [23]. SOD and GPx were measured using kits supplied by Randox Laboratories Ltd., Ireland (Cat. No. SD125 and No. RS505). MDA were analyzed using the LPO-586 kit obtained

from Calbiochem (La Jolla, CA) [24]. Quantification of total hydroperoxides was measured by Bioxytech H2O2-560 kit (Oxis International Inc., Portland, OR, USA) using xylenol orange to form a stable colored complex, which can be measured at 560 nm. Total protein concentration was determined by the method of Bradford with bovine serum albumin as standard [25]. For the measurement of peroxidation potential (PP) the difference between the MDA figures, measured at 0 and 24 h after the induction, for each sample, were taken into account [26]. GSH was measured according to the method of Sedlak and Lindsay [27]. Nitrite/nitrate levels were determined by the Griess reaction by first converting nitrates to nitrites using nitrate reductase (Boehringer Mannheim Italy SpA, Milan, Italy) [28]. The AOPP were measured through the oxidation of iodide anion to diatomic iodine [29]. Relative fructolysine content (Amadori's product of glycated serum protein) was measured by reduction, through the redox indicator nitrobluetetrazolium (NBT) at 530 nm [30].

Statistical analysis

The OUTLIERS preliminary test for detection of error values was initially applied. Afterward, data were analyzed by one-way analysis of variance (ANOVA) followed by homogeneity variance test (Bartlett-Box). In addition, a multiple comparison test was used (Duncan test). Results are presented as means ± standard deviation, with a statistical significance of $p<0.05$.

Results and Discussion

In relation to the baseline characteristics (Table 1), both groups were similar at randomization ($p > 0.05$). There was a prevalence (44 %) of patients older than 60 years in both groupst. The previous history was characterized, mainly, by hypertension. The group of healthy persons taken as normal reference values (negative control group), according to biochemical variables, was in correspondence with age, sex and ethnic of both groups of patients enrolled in the study.

Table 1. Baseline patient characteristics

Characteristics		Control (n=49) n	%	Ozone (n=51) n	%
Age (years)	20 – 40	7	14	5	9
	40 – 60	20	40	17	32
	≥ 60	22	44	30	57
Ethnic	White	30	61	39	75
	Black	8	16	7	13
	Mixed race	11	22	6	11
Sex	Female	19	38	26	50
	Male	30	61	26	50
Previous History	Hypertension	23	46	20	38
	Renal dysfunction	2	4	2	3
	Cardiovascular disease	7	14	10	19
ETD (years)	X ± S.D.	18 ± 8		17 ± 11	
	Min.	1		1	
	Max.	42		50	

ETD. Evolution time of the disease, X mean value, S.D. standard deviation. Note. No significant statistical differences between both groups ($p > 0,05$), for these studied variables, were achieved.

The main blood glucose concentration, for all the patients enrolled in the study, at the beginning of the treatments, was 10.11 ± 4.20 mM, with no statistical significant differences between the groups. At the end of the treatments, in the ozone group, a significant decrease of blood glucose concentration ($p < 0.05$) was achieved, with regard to control group (Figure 1). At the beginning of

the study, the number of patients within the glucose reference values (3.33 – 8.88 mM), were 15 (30 %) and 14 (27 %) for the control and ozone groups, respectively. However, at the end of the treatments were 13 patients (26 %) and 29 (56 %) for the control and ozone groups, respectively, with significant differences between them. On the other hand, considering it as a healing criterion, the decrease of the glucose figures at the end of the treatments with respect to the initial one, 84 and 40 % of the patients treated with ozone and antibiotics, respectively, improved their glucose values, with significant differences between both groups.

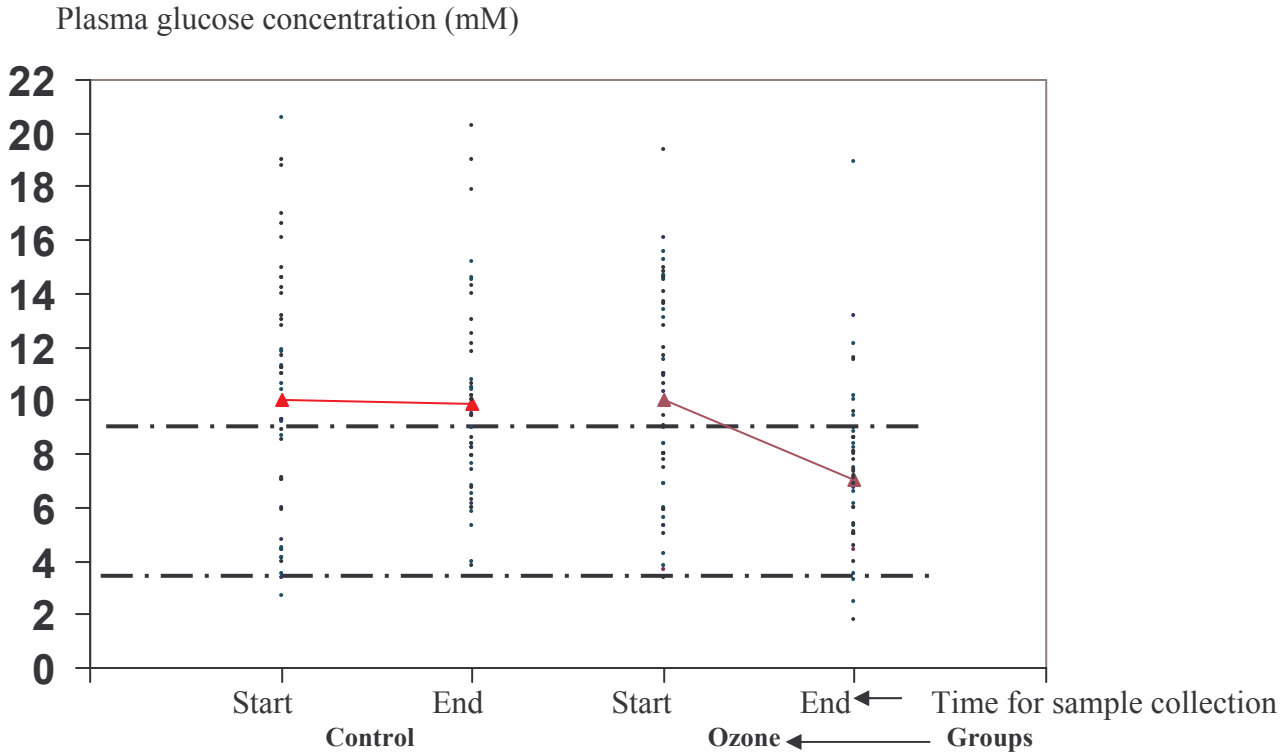

Fig. 1. Plasma glucose concentration (mM), at the beginning and at the end of the study (20 d), for control (n=49) and ozone (n=51) groups. The continuous lines correspond to the main glucose figures for each treatment. The discontinuous lines (•—) represent the normal reference interval for the Cuban people (3,33-8,88 mM)

The variation of the area and perimeter with respect to time had a significant increase in the ozone group in comparison with the control group (Table 2). The expected total recovery, as an improvement criterion, represents the days necessary to obtain a total healing (a trend to zero of the area and perimeter of the lesion), showed that patients treated with ozone needed half of the time to achieve a total recovery with regard to the control group.

No significant differences between both treatments were obtained for the variable clinical evaluation (qualitative clinical evaluation). The length of hospitalization decreased in patients treated with ozone with regard to the control group (Table 3). No side effects were observed in patients enrolled in the study.

Table 2. Measurement of the area and perimeter of the lesions, at the beginning and at the end of the study, the variation of both parameters respect to time, as well as the expected total recovery for both groups

Parameter		**Initial** (X ± SEM)	**Final** (X ± SEM)
Área (cm^2)	Antibiotic n=49	54.84 ± 0.39	40.72 ± 0.35
	Ozone n=51	57.97 ± 0.52	23.31 ± 0.36
	p^a	0.687	0.017
Perimeter (cm)	Antibiotic n=49	21.49 ± 0.11	17.34 ± 0.14
	Ozone n=51	18.49 ± 0.14	12.62 ± 0.13
	p^a	0.063	0.004
		Control n=49	**Ozone n=51**
Area Reduction (%)	%	50.30 ± 0,17	74.58 ± 0.35
	p^b	0.017	
Perimeter Reduction (%)	%	26.63 ± 0.17	41.52 ± 0.25
	p^b	0.000	
Healing rate respect to area ($cm^2\ d^{-1}$)	X ± SEM	1.21 ± 0.01	2.66 ± 0.05
	P^b	0.005	
Healing rate respect to perimeter ($cm\ d^{-1}$)	X ± SEM	0.24 ± 0.00	0.34 ± 0.00
	p^b	0.040	
Expected Total Recovery (d)[1]	X ± SEM	45 ± 11	21 ± 10
	p^b	0.002	

Initial and Final. Beginning and end of the treatment (after 20 days) with ozone or antibiotics (control).Data are mean ± SEM.
[1] Expected Total Recovery, is a healing criterion, according to the planimetric evaluation. Represents the expected days needed to achieve a total healing (trend to zero of the area and perimeter of the lesions).
p^a probability between groups, at the same time of treatment.
p^b probability between different treatments.

Table 3. Clinical evaluation and length of hospitalization for both groups of treatment

Parameters		**Control n=49**	**Ozone n=51**
Clinic Evaluation[1] (n / %)	Cured	34 / 69	39 / 78
	Not Cured	15 / 30	12 / 24
		N.S.	
Length of Hospitalization[2] (days)	X	34	26
	Min.-Max.	7 – 83	6 – 58
	S.D.	18	13
		[3]p=0,010	

(1) Qualitative evaluation made by the physician. (2) Time of hospitalization needed to achieve an aseptic lesion, with a good granulation tissue, ready to receive a graft.(3) Mc Nemar test, comparison between groups of treatment. N.S: no significant; S.D. Standard Deviation.

The biomarkers of the oxidative damage to proteins, antioxidant-prooxidant balance and nitric oxide is shown in Table 4

Table 4. Biomarkers of the oxidative damage to proteins, antioxidant-prooxidant balance and nitric oxide

Biomarkers \ Groups		Negative Control (n=60)	Antibiotic (n=49)	Ozone (n=51)
Fructolysine (relative fructolysine content)	Time 0	50 ± 5^{a}	1393 ± 125^{b}	1354 ± 110^{b}
	20 days	-	789 ± 60^{c}	250 ± 27^{d}
AOPP (μM of chloramine)	Time 0	12.13 ± 0.93^{a}	19.08 ± 0.84^{b}	21.90 ± 0.84^{b}
	20 days	-	19.66 ± 0.70^{b}	16.86 ± 0.36^{c}
GSH ($mg \cdot L^{-1}$)	Time 0	1020.3 ± 191.1^{a}	874.1 ± 97.6^{b}	867.8 ± 90.4^{b}
	20 days	-	515.1 ± 48.2^{c}	861.1 ± 57.3^{b}
MDA (μM)	Time 0	1.80 ± 0.07^{a}	8.78 ± 0.85^{b}	9.04 ± 0.44^{b}
	20 days	-	10.12 ± 0.68^{b}	9.92 ± 0.94^{b}
PP (μM)	Time 0	7.63 ± 1.29^{a}	13.09 ± 1.51^{b}	14.62 ± 1.18^{b}
	20 days	-	14.36 ± 1.96^{b}	10.40 ± 1.73^{c}
GPx ($U \cdot mL^{-1} \cdot min^{-1}$)	Time 0	30.28 ± 4.12^{a}	63.66 ± 7.25^{b}	66.35 ± 8.91^{b}
	20 days	-	59.22 ± 6.65^{b}	68.35 ± 8.38^{b}
SOD ($U \cdot mL^{-1} \cdot min^{-1}$)	Time 0	1.46 ± 0.14^{a}	0.97 ± 0.16^{b}	1.09 ± 0.13^{b}
	20 days	-	1.58 ± 0.16^{a}	6.87 ± 0.32^{c}
CAT ($U \cdot mL^{-1} \cdot min^{-1}$)	Time 0	161.57 ± 23.12^{a}	2880 ± 250^{b}	2112.86 ± 210^{c}
	20 days		2370 ± 247^{b}	3101.33 ± 290^{b}
NO_3/NO_2 (μM)	Time 0	67.82 ± 22.44^{a}	52.51 ± 9.78^{b}	50.06 ± 7.39^{b}
	20 days	-	75.31 ± 6.26^{a}	79.88 ± 6.26^{a}

AOPP, advanced oxidation protein products; GSH, reduced glutathione; MDA, Malondialdehyde; PP, peroxidation potential; GPx, glutathione preoxidase; SOD, superoxide dismutase; CAT, catalase; NO2/NO3, nitrites/nitrates. Data are mean ± SEM. Means having different superscript letters indicate significant difference ($p < 0.05$) between groups and within each group, comparing initial and final values.

Total hydroperoxides showed high figures in both groups (antibiotic 139.1 ± 29.7 μM and ozone 145.4 ± 31.6 μM), common in diabetic patients, with significant differences ($p < 0.01$) when comparing with the negative control group (103.7 ± 17.7 μM). At the end of the treatment, in the ozone group (106.3 ± 30.9 μM) no significant differences were obtained with respect to the negative control group. No change (between the initial and final figure) in the group treated with antibiotics was observed (Fig. 2).

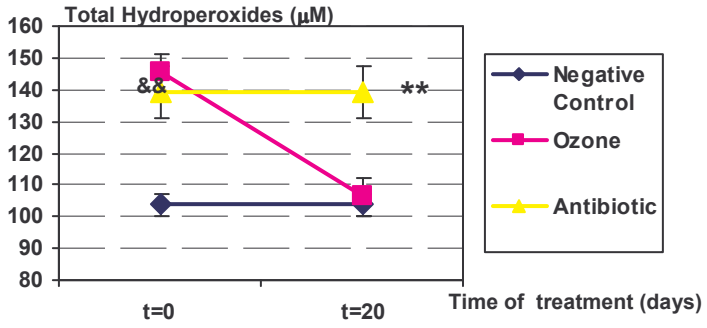

*Figure 2. Total hydroperoxides content before and after 20 days of treatment in the ozone (n=51) and antibiotic (n=49) groups. Data are mean ± SEM. ** significant differences ($p < 0.001$) between antibiotic and the remaining groups (ozone and negative control). && ($p < 0.001$) significant differences between antibiotic and ozone groups with regard to negative control group.*

Oxidative stress is one of the metabolic events associated to diabetes and its complications [19]. There are experimental and clinical evidences which prove that the generation of reactive oxygen species (ROI) is increased in both types of diabetes. The precise mechanisms by which oxidative stress may accelerate the development of complications in diabetes are only partly known [19]. In other studies [12-16, 31] we have demonstrated that using prophylactic ozone, by means of an oxidative preconditioning mechanism, it was possible to upregulate the expression of antioxidant enzymes. On the other hand, although ozone therapy has been used as an alternative medical approach for four decades, it has encountered skepticism, because of the lack of rational basis, appropriate controls and good clinical trials. In the present study, using a controlled, randomized clinical trial, we have tried to integrate some of the metabolic events associated with diabetes complications and its control by ozone therapy.

There is much evidence from experimental studies that the formation of ROI is a direct consequence of hyperglycemia and it is associated with the vascular complications in diabetic patients [32-34]. For that reason, it is necessary that any medication for the treatment of vascular diabetic complications must have the capacity to control hyperglycemia, with independence of other therapeutical effects that could be present. Our experimental results have shown that at the end of the study in the group treated with antibiotics, no modification in the glucose figures were obtained; however, the ozone group decreased the hyperglycemia and maintained its concentrations within the normal reference values. This “antidiabetic” effect produced by the ozone treatment seems to be associated to its antioxidant properties that might increase insulin sensitivity. Additional clinical observations have shown a close association between oxidative stress and insulin sensitivity [35-36]. If all these results and hypothesis are true, then we can postulate that an increase in the antioxidant capacity should improve insulin resistance. This has been demonstrated using α-lipoic acid in several preclinical and clinical experiments, where an improvement in glucose utilization and insulin resistance were achieved [37-39]. The preconditioning actions of ozone [12-16], as well as the improvement of the antioxidant defense systems and the ROS reduction favors an appropriate redox balance, suggesting that the hyperglycemia decrease, observed in type 2 diabetes patients, could be related to an increase in the insulin sensitivity and its signalling pathway. Nevertheless, other mechanisms could be present.

The severe vascular damage, in the neuroinfectious diabetes foot, associated to hyperglycemia, promotes ulcers and infections, inclusive amputations. For that reason, it is very important the control of this cascade of events and its cicatrization. Both treatments (antibiotic and ozone) favored the cicatrization. However, the ozone treatment exerted a more powerful effect. The superiority of the ozone treatment is a direct consequence of its “antidiabetc effects” (not present in the antibiotic therapy), its capacity to maintain the cellular redox balance (absent in the antibiotic

therapy as a direct effect) and its antimicrobial property, common for both treatments and the main reason for the use of antibiotic therapy. Ozone therapy not only reduced the number of patients submitted to amputation, but also decreased the area to be amputated. Amputation has a deep social significance, in relation to the patient quality of life; thus, any therapy that reduces the need to use it, even as the area to be amputated, represents great advantages.

Fructolysine is a precursor of the AOPP, which are induced by oxidative stress and also in turn, induces oxidative stress [40]. Glycolated proteins inactive enzymes, also affect the functions of bonding, transportation and protein structure [41]. Both treatments decreased fructolysine content at the end of the treatment, without achieving the negative control group, but the ozone group had significant differences with regard to the antibiotic group, approaching it to the negative control group. The aim of the antibiotic treatment is to reduce and eliminate the infectious process present in the diabetic foot. It is well known that during the infection there are ROS, which are also the result of inflammation, associated to activated neutrophils and macrophages [42-43]. The decrease observed in the fructolysine content, in the patients treated with antibiotics, suggests that this result is a consequence of the reduction or elimination of the infection, decreasing the ROS.

The H_2O_2 is a ROS produced during the glucose autoxidation and in the AOPP [44]. The ozone capacity to restore the concentrations of organic peroxides to normal levels, differing from the group treated with antibiotics, has an important significance in its “antidiabetic” effects. The H_2O_2 is capable to activate the transcription factor (NF-κB), which promotes the generation of cell adhesion molecules, cytokines and pro-coagulant tissue factor, mediators of the vascular complications present in the diabetic patients. It was demonstrated that this factor was inhibited by antioxidants as vitamin E and α-lipoic acid [19,45].

Both treatments can’t restore the glutathione concentrations up to the negative control group levels, neither to avoid the protein oxidation. With ozone treatment, GSH maintained its initial concentration at the end of the study, but the AOPP had a significant decrease. However, in the group treated with antibiotics, an additional depletion of GSH was observed, with no modification in the AOPP. The GSH decrease in this group is in relation with the GPx behavior, with the same analysis for the ozone group, though GPx concentrations, at the beginning of the study, were superior in comparison with the negative control group. The PP, as an expression of the antioxidant-oxidant balance, also presented positive results in the group treated with ozone with respect to the antibiotic group (it maintained its same level at the end of the treatment). A significant decrease with respect to its initial value was found in the ozone group, but it still maintained significant differences with regard to the negative control group.

It is well known, that SOD and CAT are scavenger enzymes of ROS, that can be inhibited during the oxidative stress. On the other hand, it has been demonstrated that serum total antioxidant activity, as an expression of the balance between the generation and inactivation of those oxidized metabolites, is reduced in diabetic patients [36]. The ratio CAT/SOD is considered as a biomarker of the glycemic control and as a risk factor in the development of diabetes complications [46]. While in the therapy with antibiotics, no modification was presented in the ratio CAT/SOD, maintaining higher levels at the end of the study, the ozone treatment was capable to decrease it, by 24 %, with respect to its initial value. It is also demonstrated that this ratio is proportional to the increase in glycoside hemoglobin, a biomarker of long-term glycemic control. This result obtained in the ozone group indicated an improvement in the glycemic control and it is in correspondance with fructolysine contents. The SOD is a scavenger of superoxide anion ($O_2^{\bullet-}$), producing H_2O_2, that is very well regulated with the ozone treatment. The overproduction of superoxide has been recently proposed as an important factor that unifies the four hypothesis about the physiopathologic mechanisms responsible of the diabetic complications [47]. A significant increase in SOD, even higher than the negative control group, was achieved in the ozone group, at the end of the treatment. These results indicate that overproduction of superoxide by the mitochondrial

electron-transport chain, must be reduced with the ozone treatment, decreasing the advanced glycation end-product, the activation of protein kinase C, the increase of hexosamine pathway flux and the activation of the polyol pathway. The increase in SOD activity, obtained with the ozone treatment, could be the result of a stimulation in the mechanism of gene expression that codifies for this enzyme [48-50].

The NO_2/NO_3 concentration, as a measure of NO, recovered its normal values, at the end of the study, for both treatments. This result suggests the importance of the control of the infectious process, regarding the similar behavior for both treatments and the main actions of the therapy with antibiotics, that does not modifies substantially the cellular redox state and the hyperglycemia, with differences with respect to the ozone group.

Clinically, patients treated with ozone therapy had a better and faster recovery of their lesions in comparison with the patients treated with antibiotic therapy (26 days vs 34 days). Any side effect was found. A preliminary economic evaluation showed that the use of ozone therapy, in the treatment of neuroinfectious diabetic foot, produced a decrease in the treatment cost of about 25 % in comparison with the use of antibiotics.

Conclusions

In summary, the ozone treatment, in patients with diabetes type 2 suffering of neuroinfectious diabetic foot, improved glycemic control by decreasing hyperglycemia, increased insulin sensitivity and prevented oxidative stress associated to diabetes mellitus and its complications, in agreement with the excellent results obtained clinically in these patients. Ozone therapy could be a future alternative in the therapy of diabetes and its complications.

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