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Effect of Ozone Therapy on Muscle Oxygenation

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ABSTRACT

Background and objective: Ozone therapy is being used to treat ischemic disorders. However, the underlying mechanisms for the success are unknown and the therapy has not been accepted fully within conventional medicine. This study sought to assess the effect of ozone therapy on resting muscle oxygenation.

Patients and design: Twenty-three (23) patients and 3 volunteers were recruited for this prospective study. Systemic ozone therapy was administered by autohemotransfusion on three alternate days over 1 week. Tissue oxygenation (mmHg) was directly measured in the tibialis anterior muscle using polarographic needle electrodes before and after the first and the third ozone therapy session.

Results: Globally, the differences in oxygenation were not statistically significant but there was a significant decrease in the percentage of low-oxygenated values ($pO_2 < 5$ mmHg) following ozone sessions ($p < 0.02$). The change in muscle oxygenation following ozone therapy was inversely correlated with age ($r = -0.398$; $p = 0.044$) and with the initial (baseline pretherapy) muscle oxygenation values ($r = -0.644$; $p < 0.001$), indicating that the more poorly oxygenated muscles benefited most from the therapy. A significant ($p = 0.031$) higher oxygenation in these tissues was observed 48 hours after the second session.

Conclusions: Ozone therapy can modify oxygenation in resting muscles, particularly of those that are most hypoxic. Our results suggest that ozone therapy could be used effectively as a complementary treatment of hypoxic and ischemic syndromes and that the therapy warrants further investigation for possible application in other clinical conditions.

INTRODUCTION

Ischemic syndromes can be produced by several disorders including respiratory and vascular diseases, diseases of connective tissues, anemia, and others. The principal characteristics are a deficiency in supply and/or utilization of oxygen in tissues. Ozone therapy (OT) is being used to treat ischemic disorders, particularly of the lower limbs (Romero et al., 1993;

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Rovira and Galindo 1991). However, few studies evaluating the mechanism of action in human tissues have been conducted, and the technique has yet to be accepted as conventional therapy.

The present pre–post therapy prospective study was undertaken to investigate the effect of OT on muscle oxygenation. Values of muscle oxygenation pre- and post-treatment were measured in the resting anterior tibialis muscle using the polarographic probe technique. This technique was designated as "gold standard" for tumor pO_2 measurement in a special workshop sponsored by the National Cancer Institute (Stone et al., 1993) and is a well-established method to assess tissue oxygen partial pressure (pO_2).

MATERIALS AND METHODS

Patients

Since June 1997, 36 patients have undergone systemic OT as part of their scheduled treatment for their various clinical conditions. All the patients were approached to participate in the polarographic probe aspect of the study. Twenty-three (23) patients agreed and the work we did with them forms the basis of this report. Three (3) healthy members of the staff of the hospital departments involved in the study volunteered to participate. Of the patients, 16 were receiving OT as an adjuvant for cancer and 7 received OT for other clinical conditions. No patient with lower-limb ischemia was included in this study. The study sample contained 20 males and 6 females, age range: 27 to 91 years (mean = 53 years). Patients and volunteers were from our university hospital (Dr. Negrín University Hospital, Canary Islands, Spain). The study was in accordance with the Helsinki agreement with respect to investigations in human subjects. The study was approved by the local ethics committee and informed consent was obtained from all participants prior to inclusion in the study.

Ozone therapy

Systemic OT was by autologous autohemotransfusion on 3 alternate days over 1 week. The procedure involved the extraction of 200 mL of venous blood into heparin (25 IU/mL) and $CaCl_2$ (5 mM). Using clinical-grade O_2, the O_3/O_2 gas mixture was prepared with an OZON 2000 device (Zotzmann + Stahl GmbH, Plüderhausen, Germany) and sterilized by passage through a sterile 0.20-μm filter. In a sterile, single-use 300-mL container, the blood was mixed with 200 mL of the O_3/O_2 gas mixture at a concentration of 60 μg/mL and, then, reintroduced into the patient slowly. The blood was extracorporeal 15–30 min and there were no adverse reactions.

Muscle pO_2 measurement

Muscle oxygenation was measured by a polarographic probe system the "pO_2 Histograph 6650." (Helzel Medical Systems, Kalterkirchen, Germany) The details of the technique have been described previously (Vaupel et al., 1991). Briefly, the polarographic probes quantify the oxygen availability in the interstitial space. The sampling area resembles a hemisphere proximal to the probe tip and with a radius of approximately 50 μm. The probe is 0.5 mm in diameter at the shaft and 0.3 mm in diameter at the tip. It is a glass-insulated gold microcathode of 12 μm in diameter, which is recessed and covered with a teflon membrane. The polarization potential is of the order of 700 mV with respect to the silver chloride anode placed on the skin of the patient's chest. For measurements, the probe is introduced at an angle of 20–30° into the muscle via a subcutaneous 20-gauge catheter previously put in place under local anesthesia and without vasoconstriction. The advance through the tissue is computer controlled and, typically, consists of a 1-mm forward motion and 0.3-mm reverse motion so as to decompress the tissue at the measurement site. The probe automatically performs 200 measurements with each value having a high spatial resolution and with a sampled volume of approximately 100 cells. The values obtained are expressed in mmHg.

Muscle pO_2 values have a non-Gaussian distribution and results are presented as the median of the 200 recorded pO_2 measurements together with the percentage of values that are below 5 mmHg. This latter parameter is calculated automatically by the device and indicates

the percentage of those values that are the most hypoxic. The procedure lasts approximately 15–20 min. No adverse effects relating to the pO_2 measurements were encountered.

Muscle oxygenation values were obtained on four occasions: (1) before session #1; (2) after session #1; (3) 48 hours after session #2 and before session #3; and (4) after session #3. For each participant the change in oxygenation (ΔpO_2) was calculated as the pO_2 value at each time-point relative to the presession #1 ("baseline") pO_2 value.

Statistical analysis

The SPSS 7.0 for Windows software package (SPSS-Ibérica, Madrid, Spain) was used throughout. The distribution of data was assessed by the Kolgomorov–Smirnov test. Two-tailed tests were applied for significance. The paired *t*-test was used to compare means of all median muscle values and of all the percentages of the <5 mmHg measurements. These data are expressed as means ± standard deviation (SD). The unpaired t-test was used to compare the ΔpO_2 between muscles above or below the median value of pO_2 prior to the commencement of the OT. These data are expressed as means and 95% confidence interval (CI) (95% CI). A general linear model for repeated measures was used to compare the different measurements of oxygenation and percentages of <5 mmHg values. The linear correlation was assessed by Pearson's *r*-test. Associations among more than two variables were assessed by linear regression. Differences were considered to be significant at the $p < 0.05$ level.

RESULTS

Muscle oxygenation

Initial muscle oxygenation was 23.9 ± 11.9 mmHg (median = 22.9 mmHg) and was not related to gender, age, hemoglobin levels, nor clinical status.

In the overall sample, the percentage of values <5 mmHg at baseline (11.4% ± 10.7%) was significantly decreased immediately after the OT sessions to 5% ± 5.4% ($p = 0.018$) after session #1 and to 5.2 % ± 5.5% ($p = 0.011$) after session #3. A 25%-decrease 48 hours after session #2 relative to baseline was not statistically significant. The analyses of repeated measurements showed a probability value on the borderline of significance in the percentage <5 mmHg values ($p = 0.084$).

Muscle oxygenation was 26.5 ± 10.8 mmHg after session #1, 27.8 ± 14.1 mmHg 48 hours after session #2 and 23.5 ± 9.4 mmHg after session #3. These differences were not statistically significant. The analyses of repeated measurements did not show significant changes in oxygenation. Relative to initial values, the percentages of subjects with muscle pO_2 increase were: 75% after session #1, 62% 48 hours after session #2 and 46% after session #3. Because the session #3 values represent the "worst-case scenario," these values were used for the subsequent regression analyses.

ΔpO_2

There was an inverse and significant correlation between individual ΔpO_2 and initial pO_2 value at each measurement time-point (i.e., a higher ΔpO_2 in those muscles that had had worse initial pO_2 values). This was corroborated by the comparison, at each measurement time-point, of the ΔpO_2 between muscles above and below the median pO_2 prior to OT. While the initially well-oxygenated muscles (those above the median) showed oxygenation decrease, the initially worse-oxygenated muscles (those below the median) increased in oxygenation after the OT, for example, by 50% (95% CI 20% to 85%; $p = 0.004$) after session #1; by 70% (95% CI 4% to 146%; $p = 0.033$) 48 hours after session #2; and by 66% (95% CI 20% to 112%; $p = 0.001$) after session #3 (Fig. 1).

The individual ΔpO_2 values after session #3 showed a significant inverse correlation with the initial pO_2 value: ($r = -0.644$, $p < 0.001$) (Fig. 2) and with age ($r = -0.398$, $p = 0.044$).

Linear regression showed that the individual effect of OT (ΔpO_2 after session #3) was inversely related to initial muscle oxygenation ($p < 0.001$) and to age ($p = 0.026$).

DISCUSSION

Although biomedical uses of ozone began at

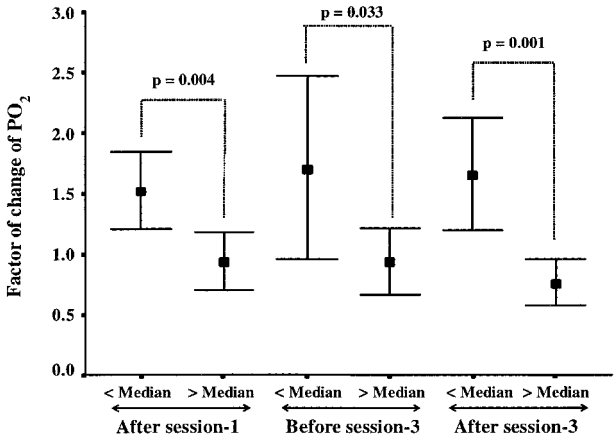

FIG. 1. Factor of change of pO_2 (ΔpO_2) and median pO_2. For each participant, the ΔpO_2 was calculated as the pO_2 value at each time-point relative to the "baseline" pO_2 value obtained prior to the start of the ozone therapy. This figure shows the ΔpO_2 at each measurement time-point after ozone therapy in muscles with baseline pO_2 value above or below the median of pO_2 value (22.9 mmHg) of the whole study group. The points represent the mean ΔpO_2 for both groups of muscles at each measurement time-point and the bars are the 95% confidence intervals. Postozone therapy, the mean of well-oxygenated muscles (baseline pO_2 above the median) decreased from 6% to 23% while the mean of "worst-oxygenated" muscles (baseline pO_2 below the median) increased more than 50%. These differences were significant at all the three measurement time-points. < Median = muscles with baseline pO_2 values below the median value; and > Median = muscles with baseline pO_2 values above the median value.

the end of the 19th century and were used during the first World War for healing infected wounds, recent interest in ozone, however, has been centered on its decrease in the tropospheric layer, environmental pollution, and lung toxicity.

OT by autohemotransfusion precludes airways involvement and, as such, avoids lung toxicity resulting from oxidative stress. More importantly OT, in appropriate concentrations, produces a transient oxidative stress that can stimulate blood antioxidants by upregulation (Bocci, 1996; León et al., 1998). This effect has been investigated in relation to its use to protect against damage by free radicals in heart (Hernández et al., 1995), renal (Barber et al., 1999), and hepatic (Peralta et al., 1999) disorders. We have used a concentration of 60 μg/mL following experimental data that suggested that the optimal concentration of ozone in the O_2/O_3 mixture should be 60–70 μg/mL, which has an acceptable lipid peroxide formation and hemolysis < 2.5% (Bocci, 1996).

Several studies have indicated that when only ozone-free oxygen is used, the clinical or biochemical response had not been observed. The clinical or the "pro-oxidant–antioxidant" biochemical response necessary to mediate the different effects of ozone therapy was produced only by the addition of ozone to the same oxygen volume (Giunta et al., 2001; León et al., 1998; Peralta et al., 1999). The objective of the present study was to assess if changes in tissue oxygenation occur during ozone therapy. Each patient was his or her own control and elective nonozonated autohemotransfusion was not performed.

The anterior tibialis muscle was chosen for the tissue measurements because: (1) it has been the most commonly used in other studies of pO_2 measurement and, hence, direct comparisons may be made with our current findings; (2) it is easy to carry out determinations at this site with minimum trauma to the patient; and (3) it is an anatomical area with frequent ischemic disorders and, as such, data

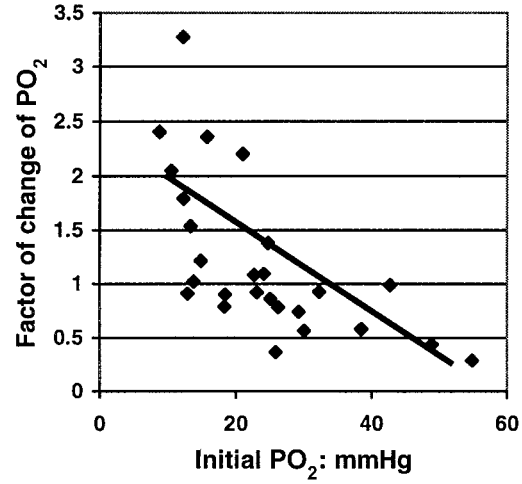

FIG. 2. Factor of change of pO_2 (ΔpO_2) and initial pO_2. Linear correlation between muscle "initial pO_2" and "ΔpO_2" after session #3 of ozone therapy. A ΔpO_2 value < 1 signifies decrease of oxygenation, and $\Delta pO_2 > 1$ signifies increase of muscle oxygenation after session #3. The data show a significant and inverse correlation ($r = -0.644$) indicating that the greatest therapy-associated changes in muscle pO_2 were in those muscles that had had the poorest baseline oxygenation.

obtained from this site could have direct applicability within the clinical assessments of these disorders.

Ozone, *per se*, does not enter the organism and its effects are mediated by rapid oxidation of blood substances in the transfusion recipient. Among the reactive oxygen species generated are the peroxidated lipoproteins and hydrogen peroxide that can activate the hexose monophosphate shunt (Bocci et al., 1998). Several mechanisms have been proposed to explain the vascular effect (Bocci, 1994; Giunta et al., 2001; Verrazzo et al., 1995). The increase of malonyl dialdehyde and lipid peroxidation can lead to charge modification and improved flexibility of erythrocyte membrane with an improvement of blood rheology. Substance liberation such as adenosine, nitric oxide and prostaglandins could collaborate in the effect on the microcirculation leading to a decrease in vascular resistance. Our hypothesis is that this effect could lead to blood-flow redistribution (i.e., a drop in pO_2 in well-oxygenated muscles in favor of the less-well-oxygenated). This possibility is supported by our data showing an inverse correlation between initial oxygenation and ΔpO_2 post-OT and agrees with our preliminary data indicating blood-flow increase of several day's duration following three successive OT sessions (Clavo et al., 1999). Another mechanism may be invoked to explain our results. Activation of the hexose monophosphate shunt can increase production of 2,3-diphosphoglycerate in erythrocytes (Bocci, 1997; Bocci et al., 1998) and lipid peroxidation of red cell membranes can alter intracellular pH (Giunta et al., 2001). Both factors would cause a shift to the right in the oxyhemoglobin dissociation curve and, as such, increase the release of oxygen to the tissues.

The observed decrease of pO_2 in well-oxygenated tissues is not of primary importance because these tissues have considerable functional reserve and, in addition, the effect may be secondary to blood-flow redistribution, as commented above.

However, a pO_2 increase in poorly oxygenated tissue (i.e., in "low mmHg" values) can be of considerable importance in hypoxic tissues. Our results show that, in the "worst-oxygenated" muscles, there was a mean increase of at least 50% in muscle pO_2 following OT and that this increase was maintained for at least 48 h after the session. The observed inverse correlation between the initial muscle pO_2 and ΔpO_2 for each measurement time-point indicates that a more beneficial effect could be expected in those tissues with a lower initial pO_2 value (i.e., those that would be in most need of a therapy to increase oxygenation). Despite the number of ozone sessions in the study being much less than that which would be considered necessary for an effective therapy, these results appear to confirm clinical experience in vascular disorders in which OT was administered several times per week over protracted periods of weeks or even months (Romero et al., 1993; Rovira and Galindo, 1991).

The baseline (pretherapy) mean pO_2 value of the muscles in our study was 23.9 mmHg, which is within the range of 16.2 and 27.2 mmHg described by other authors (Heinrich et al., 1990; Jung et al., 1990) for healthy anterior tibialis muscle. We did not observe a correlation between resting muscle pO_2 and age; a finding already described (Jung et al., 1990). However, we did find a significant and inverse correlation of the effect of OT with age. This could be explained as a decrease (or a slowing-down with age) of vascular adaptive responses and is in agreement with the greater vasoresponse capacity in young people that has been observed in studies of hyperoxia using nuclear magnetic resonance phase-contrast angiography (Watson et al., 2000). The implication is that a lower effect of systemic OT on tissue oxygenation could be expected in older patients.

Tissue pO_2 measurement using the polarographic probe technique is reliable in evaluating baseline status as well as the effect of treatment. In our study, basal muscle pO_2 assessed by this technique could have a predictive value with respect to the benefit from OT that could accrue to the individual patient being treated.

CONCLUSIONS

In conclusion, many aspects of ozone therapy in human subjects are, as yet, unexplored. The present study demonstrates, by direct muscle pO_2 measurement, that OT can modify the

level of oxygenation in resting muscles, particularly of those that are most hypoxic. The therapy appears to be safe and our results suggest that it could be used effectively as a complementary treatment of hypoxic and ischemic syndromes and that the therapy will gain greater clinical appreciation through further investigation.

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REFERENCES

Barber E, Menendez S, Leon OS, Barber MO, Merino N, Calunga JL, Cruz E, Bocci V. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. Mediators Inflamm 1999;8:37–41.

Bocci V. Autohaemotherapy after treatment of blood with ozone: A reappraisal. J Int Med Res 1994;22:131–143.

Bocci V. Does ozone therapy normalize the cellular redox balance? Implications for the therapy of human immunodeficiency virus infection and several other diseases. Med Hypotheses 1996;46:150–154.

Bocci V. Ozone as a bioregulator. Pharmacology and toxicology of ozone therapy today. J Biol Regul Homeost Agents 1997;10:31–53.

Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, Gerli R. Studies on the biological effects of ozone: 7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone. J Biol Regul Homeost Agents 1998;12:67–75.

Clavo B, Pérez JL, Lloret M, López L, Suárez G, Catalá L, Macías D, Santana M, Rodríguez V, Morera J, Pulido J, Rovira G, Günderoth M. Modification of tumor oxygenation by ozone therapy [in Spanish; abstr.]. Revista de Oncología 1999;1:17.

Giunta R, Coppola A, Luongo C, Sammartino A, Guastafierro S, Grassia A, Giunta L, Mascolo L, Tirelli A, Coppola L. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. Ann Hematol 2001;80:745–748.

Heinrich R, Günderoth-Palmowski M, Machac N. Effects of hemodilution with middle molecular HES solution on muscle tissue pO_2 in healthy volunteers. In: Ehrly AM, Fleckenstein W, Hauss J, Huch R, eds. Clinical Oxygen Pressure Measurement II. Berlin: Blackwell Ueberreuter Wissenschaft, 1990:30–37.

Hernández F, Menéndez S, Wong R. Decrease of blood cholesterol and stimulation of antioxidative response in cardiopathy patients treated with endovenous ozone therapy. Free Radical Biol Med 1995;19:115–119.

Jung F, Bock M, Heinrich R, Kolepke W, Krawzak HW, Kiesewtter H, Wenzel E. Intramuscular oxygen partial pressure in the tibialis anterior muscle of apparently healthy subjects. In: Ehrly AM, Fleckenstein W, Hauss J, Huch R, eds. Clinical Oxygen Pressure Measurement II. Berlin: Blackwell Ueberreuter Wissenschaft, 1990:15–24.

León OS, Menéndez S, Merino N, Castillo R, Sam S, Pérez L, Cruz E, Bocci V. Ozone oxidative preconditioning: A protection against cellular damage by free radicals. Mediators Inflamm 1998;7:289–294.

Peralta C, León OS, Xaus C, Prats N, Jalil EC, Sala-Planell E, Puig-Parellada P, Gelpi E, Roselló-Catafau J. Protective effect of ozone treatment on the injury associated with hepatic ischemia–reperfusion: antioxidant-prooxidant balance. Free Rad Res 1999;31:191–196.

Romero A, Menéndez S, Gómez M, Ley J. Ozone therapy in advanced stages of *atherosclerosis obliterans* [in Spanish]. Angiología. 1993;45:146–148.

Rovira G, Galindo N. Ozone therapy in the treatment of chronic wounds in the lower limbs [in Spanish]. Angiología 1991;2:47–50.

Stone HB, Brown JM, Phillips TL, Sutherland RM. Oxygen in human tumors: Correlations between methods of measurement and response to therapy. Radiat Res 1993;136:422–434.

Vaupel P, Schlenger K, Knoop C, Höckel M. Oxygenation of human tumors: Evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurements. Cancer Res 1991;51:3316–3322.

Verrazzo G, Coppola L, Luongo C, Sammartino A, Giunta R, Grassia A, Ragone R, Tirelli A. Hyperbaric oxygen, oxygen–ozone therapy, and rheologic parameters of blood in patients with peripheral occlusive arterial disease. Undersea Hyperb Med 1995;22:17–22.

Watson NA, Beards SC, Altaf N, Kassner A, Jackson A. The effect of hyperoxia on cerebral blood flow: A study in healthy volunteers using magnetic resonance phase-contrast angiography. Eur J Anaesthesiol 2000;17:152–159.

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