Original Article

Effect of ozone therapy on cerebral blood flow: A preliminary report.

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ABSTRACT

Background: Currently, ozone therapy is being used to treat ischemic disorders but the underlying mechanisms for the success are not well known. This study assesses the effect of ozone therapy on middle cerebral artery and common carotid artery blood flow.

Patients and methods*:* Seven study subjects were recruited for therapy performed with ozone-enriched autologous blood transfused on three alternate days over one week. Common carotid artery blood flow quantification (n = 14) was by color Doppler. Systolic and diastolic velocities in middle cerebral artery (n = 14) were by transcranial Doppler. Ultrasound assessments were conducted at three time-points: 1) basal (before ozone therapy) 2) after the 3rd session of ozone therapy 3) one week after the 3rd session of ozone therapy.

Results: Relative to baseline, common carotid blood-flow was increased by 75% after the 3rd session of ozone therapy ($p < 0.001$) and by 29% one week later ($p = 0.039$). In middle cerebral artery the systolic velocity was increased by 22% after the 3rd session ($p = 0.001$) and by 15% one week later ($p = 0.035$), while diastolic velocity was increased by 33% after the 3rd session ($p < 0.001$) and by 18% one week later ($p = 0.023$).

Conclusions*:* This preliminary Doppler study supports clinical experiences of improvement with ozone therapy in peripheral ischemic syndromes. Its potential usefulness as a complementary treatment in cerebral low perfusion syndromes merits further clinical evaluation.

Key Words: color Doppler, ischemia, low perfusion, transcranial Doppler

INTRODUCTION

Cerebral low perfusion syndromes have significant clinical and social repercussion. To seek more effective drugs and other methods to ameliorate this problem is an important field in neurological research. For many years now, ozone therapy has been a non-conventional therapy that has been used to treat ischemic disorders, particularly of the lower limbs (1-3). However, to-date, there has been a paucity of studies that have systematically evaluated blood flow changes resulting from ozone therapy.

The current study was undertaken redress this by investigating the effect of ozone therapy on middle cerebral artery (MCA) and common carotid artery (CCA) blood flow.

MATERIALS AND METHODS

Patients

Twenty-eight arteries (14 MCA and 14 CCA) were evaluated in this study in 7 subjects: 5 patients and 2 healthy volunteers. Patients and volunteers were from our university hospital. The patients were from the Radiation Oncology department who underwent elective ozone therapy and which was unrelated for treatment of cerebral vascular disease. The volunteers were members of the clinical staff of the departments involved in the investigation. There were 5 male and 2 females in the study. The age range was 58 years (range 34-78). Fully informed consent was obtained from all participants prior to inclusion into the study. The study was approved by the Institutional Ethical Committee.

Ozone therapy

Ozone therapy was by autologous blood transfusion on three alternate days over one week. The procedure involved the extraction of 200 ml venous blood into heparin (25 IU/ml) and $CaCl_2$ (5mM). Using clinical-grade O_2, the O_3/O_2 gas mixture was prepared with an OZON 2000 medical device (Zotzmann + Stahl GmbH, Plüderhausen, Germany). In a sterile single-use 300 ml container, the blood was mixed with 200 ml of O_3/O_2 gas mixture at a concentration of 60 µg/ml and then, re-introduced into the patient slowly via the antecubital vein, after being passed through a sterile 0.20 µm filter. Blood was extra-corporeal for about 15-30 minutes and there were no adverse reactions.

Doppler studies were conducted on three occasions; 1) before 1st session; 2) after 3rd session; and 3) one week after 3rd session.

Transcranial Doppler velocimetry

Systolic and diastolic velocities (cm/s) were measured in MCA by transcranial Doppler (TCD) in the trans-temporal approach, with a 2 MHz probe from an Angiodine-2 Fluo-Link 300® device. The absence of stenoses was confirmed with the patient alert, relaxed and seated. Insonation angle was <60º.

Common carotid blood-flow quantification

Blood flow quantification of CCA was performed using a Colour Doppler Philips Ultrasound P-800 unit®, with time-domain processing. This technique simultaneously evaluates velocity and vessel diameter and the data are presented as ml/min. The usefulness and validity of this technique has been described previously (4, 5). The absence of significant stenoses in extra-cranial carotid arteries was confirmed with the patient alert, relaxed and supine. A 7.5 MHz linear high-definition probe with a Doppler angle <60º was used. We obtained information (ml/min) on the volume blood flow of both CCA ≥2 cm before the carotid bifurcation.

All ultrasound studies were performed bilaterally by the same radiologist, to minimize inter-observer reproducibility (6). When an optimal stable flow image was obtained, recordings over at least three cardiac cycles were made. Each assessment was recorded at least three times so as to precluded operator or technical inaccuracy and median values were used in the statistical analyses.

They were not evaluated blood pressure or hemoglobin levels.

Statistical analysis

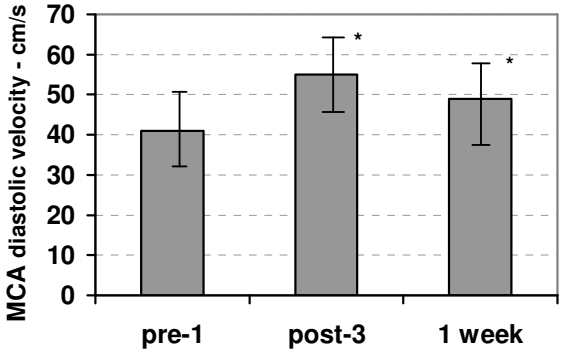
The SPSS 7.0 for Windows software package (SPSS-Ibérica, Madrid, Spain) was used throughout. Normality of distribution of data was assessed by the Kolgomorov-Smirnov test. Two-sided tests were applied. Data are expressed as means ± SD. The paired t-test was used to compare differences between baseline and the two time-point measurements following ozone therapy. Linear correlation was assessed by Pearson's r test. Differences were considered significant when $p < 0.05$.

RESULTS

Transcranial Doppler velocimetry

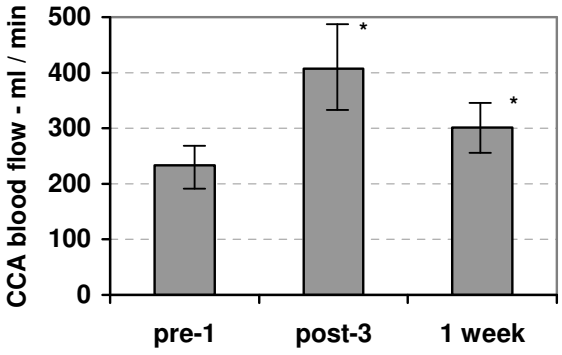
Baseline systolic velocity in MCA was 90.9 ± 6.1 cm/s. After the 3rd session this was increased to 111 ± 7.3cm/s (increase 22%, p = 0.001) and one week later was 104.3 ± 8 cm/s (increase 15%, p = 0.035).

Baseline diastolic velocity in MCA was 41.1 ± 4.4 cm/s. After the 3rd session this was increased to 54.6 ± 4.6 cm/s (increase 33%, p < 0.001) and one week later was 48.6 ± 5 cm/s (increase 18%, p = 0.023) (Fig. 1).

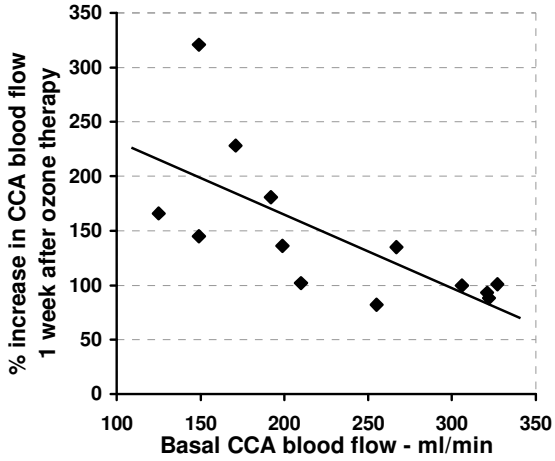


Common carotid blood-flow quantification

Baseline CCA blood flow was 233 ± 19 ml/min. After the 3rd session this increased to 407 ± 38 ml/min (increase 75%, p < 0.001) and one week later the flow was 301 ± 22 ml/min (increase 29%, p = 0.039) (Fig. 2).



Baseline CCA blood flow was directly correlated with MCA diastolic velocity ($r = 0.557$; $p = 0.039$) and inversely correlated with age ($r = -0.825$; $p < 0.001$). The percentage increase in CCA blood flow one week after the 3rd session was directly correlated with age ($r = 0.735$; $p = 0.004$) and inversely correlated with the initial values of CCA blood flow ($r = -0.691$; $p = 0.009$): a higher increase in CCA blood flow in older patients and lower basal perfusion (Fig. 3).



DISCUSSION

Although biomedical uses of ozone therapy can be traced back to the end of the 19th Century, many aspects of the effects of the therapy remain unexplored.

The therapy using ozone-enriched autologous blood transfusion precludes airways involvement and, as such, avoids lung toxicity resulting from oxidative stress. Ozone, *per se*, does not enter the organism and the effects that are observed are mediated by rapid oxidation of blood substances in the transfusion recipient. In appropriate concentrations, this can up-regulate blood antioxidant synthesis (7) and it is this property that has been most actively investigated with respect to protection against free radical damage involved in heart (8), renal (9), and liver (10) disorders. The mechanisms proposed to explain the vascular effect include vasoactive substance liberation as well as the improvement in erythrocyte flexibility and blood rheology (1, 11, 12).

Several studies that have included control groups of subjects have indicated that when ozone-free oxygen is used, the beneficial biochemical (7, 10) and rheological (1) responses are not observed.

Our study assessed changes in MCA and/or CCA blood flow occur during ozone therapy in which each patient was his/her own control and did not include non-ozonized blood transfusion.

Diastolic velocity increase in MCA is compatible with a decrease in vascular resistance, in rheological improvement (1, 12) and an overall blood flow increase, as indicated by the CCA measurements. The inverse correlation between percentage increase in CCA blood flow and initial values is compatible with a micro-vascular redistribution towards an oxygenation benefit in the poorer-irrigated tissues. We were tentatively able to demonstrate this in a previous study in our group (13) by direct measurement of muscle oxygenation using polarographic electrodes.

The number of ozone-therapy sessions in the present study is less than that which would be considered necessary for a full-fledged therapy; usually of the order of weeks or even months. However, our findings of a residual effect that is still significantly elevated over baseline one week after the brief therapeutic sessions support the clinical observations in vascular disorders of widely-separated sessions over extended periods (2, 3).

These rheological and vascular effects suggest that co-adjuvant ozone therapy could decrease vasoconstriction that is secondary to hyperoxia. Techniques such as carbogen breathing or hyperbaric chambers are used to increase the amount of O_2 dissolved in arterial blood. However when prolonged for >15-30 minutes, these therapies can lead to an increase in peripheral vascular resistance together with general vasoconstriction of most organs (14). Indeed, decreases in cerebral blood flow secondary to hyperoxia have been documented in humans by transcranial Doppler (15) and magnetic resonance (16) studies.

The above-mentioned effects of ozone therapy and data from the present study, specially a potential higher effect in older patients or with lower initial blood flow augur well for its use in cerebral low perfusion syndromes and stroke.

Further studies that include new technologies such as interstitial multi-channel laser Doppler to quantify fluctuations in micro-vascular perfusion during ozone therapy are in progress in order to clarify some of the remaining doubts regarding the efficacy of ozone therapy.

In conclusion, this preliminary Doppler study demonstrates, albeit in a small number of study subjects, that ozone therapy increases blood flow in CCA and MCA with a prolonged effect that it can

be assessed quite easily by TCD and carotid ultrasound. These data support clinical experiences of improvement with ozone therapy in peripheral ischemic syndromes. Its potential usefulness as a complementary treatment in cerebral low perfusion syndromes warrants further clinical investigation.

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FIGURE LEGENDS

Figure 1: Transcranial Doppler during ozone therapy

Diastolic velocity (in cm/s) in middle cerebral artery (MCA) was increased by 33% at the end of the 3rd session ($p < 0.001$) and an 18% increase persisted for 1 week after the 3rd session ($p = 0.023$). The error bars are the 95% Confidence Intervals. Significant differences ($p < 0.05$) were indicated with ***.**

Figure 2: Carotid blood flow during ozone therapy.

Blood flow quantification (in ml/min) in common carotid artery (CCA) was increased by 75% at the end of the 3rd session ($p < 0.001$) and a 29% increase persisted for 1 week after the 3rd session ($p = 0.039$). The error bars are the 95% Confidence Intervals. Significant differences ($p < 0.05$) were indicated with ***.**

Figure 3: Relationship between baseline blood flow and increase post ozone therapy.

The correlation in CCA blood flow between baseline values and the percentage increase one week after the 3rd session was highly significant ($r = -0.691$; $p = 0.009$) i.e. there is a higher percentage increase in CCA corresponding to a lower initial blood flow. Note: the percentages under 100% indicate a decrease in blood flow at this time-point.