Pergamon

Free Radical Biology & Medicine, Vol. 19, No. 1, pp. 115–119, 1995

0891-5849/95 $9.50 + .00

0891-5849(94)00201-0

Brief Communication

DECREASE OF BLOOD CHOLESTEROL AND STIMULATION OF ANTIOXIDATIVE RESPONSE IN CARDIOPATHY PATIENTS TREATED WITH ENDOVENOUS OZONE THERAPY

FRANK HERNÁNDEZ,* SILVIA MENÉNDEZ,* and ROLANDO WONG†

*Ozone Research Center and †Medical and Surgical Research Center, National Center for Scientific Research, Havana, Cuba

(*Received* 10 *December* 1993; *Revised* 16 *May* 1994; *Re-revised* 12 *September* 1994; *Accepted* 22 *September* 1994)

Abstract—Patients with cardiac infarction show a decrease in glutathione peroxidase and superoxide dismutase activities, which are beginners in the scavenger processes of lipid peroxide and superoxide radicals, respectively. In this study, we investigate the effects of endovenous ozone therapy on serum lipid pattern and on antioxidant defense system, such as the glutathione redox one, in the blood of patients with myocardial infarct. Twenty-two patients who had an infarction, between 3 months and 1 year before the study, were treated with ozone by autohemotherapy during 15 sessions. A statistically significant decrease in plasma total cholesterol and low density lipoprotein was observed. High biologically significant increases on erythrocyte glutathione peroxidase and glucose 6-phosphate dehydrogenase activities were found. There was no change in plasma lipid peroxidation level. It was concluded that endovenous ozone therapy in patients with myocardial infarction has a beneficial effect on blood lipid metabolism, provoking the activation of antioxidant protection system.

Keywords—Ozone, Oxidation, Cholesterol, Oxysterols, Cardiovascular disease, Atherosclerosis, Free radicals

INTRODUCTION

Over the last years there has been a growing interest in the concept that oxygen radicals play a role in the pathogenesis of myocardial ischemia.[1,2] It has been shown that peroxidation of arachidonic acid increases platelet aggregation, a feature in patients with myocardial infarction.[3] On the other hand, activation of mouse peritoneal macrophages in vivo results in a decrease of intracellular glutathione peroxidase activity, in association with an increasing capacity of the cells to generate reactive oxygen species, including hydroperoxides.[4] This macrophage activation occurs in both inflammatory processes and atherosclerotic diseases. A decrease in glutathione peroxidase and superoxide dismutase activities in patients with myocardial infarction[5] has been regarded. This fact has led to the use of scavenger agents in the infarction therapy.[6–8] We reported previously that ozone autohemotherapy produces stimulation of glutathione peroxidase activity,[9] and others have reported that it can also reduce the blood cholesterol level.[10]

Address correspondence to: Frank Hernández, Ozone Research Center, National Center for Scientific Research, P.O. Box 6990, Havana, Cuba.

The aim of this article is to know the effect of endovenous ozone therapy on serum lipid pattern and the activity of antioxidant defense system of patients that have suffered cardiac episode.

MATERIALS AND METHODS

Treatment

The study was carried out in a total of 22 patients who had given informed consent, and it was also approved by a Human Subjects Committee from the Cardiology Department of Medical and Surgical Research Center. All patients were of male sex, of the ages 46 to 76 years, that had suffered the last cardiac infarction between 3 months and 1 year before the study.

Autochemotherapy technique was applied to supply an appropriate ozone/oxygen mixture.[11] Briefly, 200 ml of blood were drawn in a transfusion flask and ozonized by bubbling ozone/oxygen mixture (50 mg/l). The ozonized blood was retransfused to the patient with a rapid flow. The treatment was applied 5 days a week up to 15 sessions.

In vitro experiment

To know the direct effect of ozone on blood components, whole blood, in patients beginning ozone

Table 1. Plasma Lipid Pattern Before and After Blood Ozonation In Vitro

Parameters	Before	After	Significance
CHO	5.50 ± 0.37	5.29 ± 0.39	0.2127
LDL	3.65 ± 0.44	3.54 ± 0.42	0.5076
HDL	0.98 ± 0.09	1.01 ± 0.11	1.0000
TG	1.84 ± 0.15	1.82 ± 0.15	0.3105

Values are expressed by the mean ± standard error of mean in mmol/l of plasma.

therapy by the first time, was ozonized in a transfusion flask, and the biochemical parameters were measured immediately. In this case, values obtained after ozonation were compared with those values from the same blood before ozonation.

In vivo experiment

Measurement of biochemical parameters were performed from blood samples obtained from patients after they had received 0, 5, and 15 sessions of ozone treatment. The effect of ozone in the organism was analyzed by comparison of the different values of each individual at 5 and 15 sessions with respect to its own values at 0 session.

Determinations

Blood was taken from individuals into anticoagulant tubes containing ACD (a mixture of citric acid, sodium citrate, and dextrose) and measurements made by duplicate. Reduced glutathione (GSH) and glucose 6 phosphate dehydrogenase (G6PDH) were measured in erythrocytes using the method of Beutler.[12] Glutathione peroxidase (GPx) activity was measured in erythrocytes by the modification of Faraj et al.[13] to the method of Thomson.[14]

One part of the blood was centrifuged at 1500 × g for 20 min and the plasma taken for analysis. Plasma lipid peroxidation (LPO) was measured according to the method of Satoh,[15] and plasma total cholesterol (CHO), high density lipoprotein (HDL), and tryglycerides (TG) were assayed using diagnostic kits from Boehringer (Nos. 290319, 400371, 726290, 701912, respectively).

Statistical analysis

Statistical analyses were carried out using Wilcoxon Rang Sum test[16] for mean values ± SEM (standard error of mean); a p value < 0.05 was taken as significant.

RESULTS AND DISCUSSION

In vitro experiment

The direct effect of ozone on plasma lipid pattern is evaluated in Table 1. There were no immediately significant changes on CHO, LDL, HDL, and TG levels from ozonized blood. However, it is interesting to point out that CHO and LDL tended to a slight decrease. In the same experiment, Table 2 shows the direct effect of ozone on blood biochemical parameters from glutathione redox system. LPO level was significantly increased, erythrocyte GPx activity had no significant change, but the erythrocyte G6PDH activity was significantly increased, and erythrocyte GSH level was significantly decreased after the ozonation of blood. This result suggests that in red cells GPx would be a non-first line antioxidant defence, and that this role is perhaps performed by Glutation S-transferase because a quick decrease in GSH level and an increment of G6PDH producing an enhancement on NADPH level were found. These facts are additional evidence of the biochemical selectivity of ozone when it reacts with biomolecules.[17]

In vivo experiment

The behavior of plasma lipid pattern during ozone treatment is given in Figure 1. It shows that after the 5 and 15 sessions of treatment, CHO level was significantly diminished (5.5% and 9.7%, respectively) from initial values. At the same sessions, LDL level also was significantly lower (15.4% and 19.8%, respectively), but in a higher extension, suggesting that decrease in total cholesterol was mainly due to cholesterol load in LDL particles. The CHO and LDL reductions were higher with the increasing number of treatment sessions. On the other hand, there were no significant changes in HDL and TG contents.

In a previous study we observed a significant increase of some biochemical parameters related to oxi-

Table 2. Profile of Blood Biochemical Parameters From Glutathione Redox System Before and After Blood Ozonation In Vitro

Parameters	Before	After	Significance
GPx	14.12 ± 1.32	14.20 ± 1.61	0.6009
G6PDH	1363 ± 100	1589 ± 97	0.0176
GSH	833 ± 44	713 ± 44	0.0074
LPO	1.96 ± 0.28	4.71 ± 0.72	0.0003

All parameters are expressed by the mean ± standard error of mean. GPx and G6PDH in International units per gram of hemoglobin, GSH in micromol/l of erythrocytes, and LPO in micromol/l of plasma.

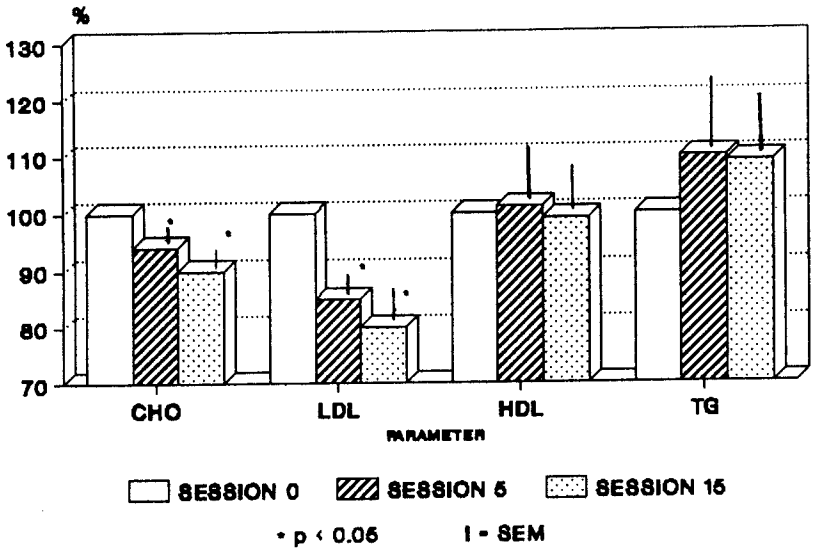

Fig. 1. Percentage values of lipid pattern after 5 and 15 endovenous ozone therapy.

dant defense system.[9] In this article we observed that erythrocyte G6PDH activity was significantly (22%) and nonsignificantly (13.4%) higher at the 5th and 15th treatment sessions, respectively (Fig. 2). Erythrocyte GPx activity rose, but not statistically significantly, during ozone therapy, with large extents of 41.8% and 78.9% after the 5th and 15th sessions, making these increases biologically significant. A high variability in the response of this enzyme from each patient was observed.

Rats exposed to low levels of ozone showed a 28% significant increase in lung GPx activity in measuring the activity, using an organic substrate.[18] GPx using cumene hydroperoxide or *tert*-butyl hydroperoxide (organic hydroperoxides) as substrate measure total activity, whereas hydrogen peroxide measures only selenium (Se)-dependent GPx activity.[19] In this study we could only measure Se-dependent GPx activity, maybe a higher and statistical change will be observed if total GPx activity had been measured.

Erythrocyte GSH and plasma LPO level (Fig. 2) had no noticeable changes during the ozone treatment. These results, analyzed together with those obtained in the in vitro experiment, evidence that the effect of ozone on blood cholesterol is associated with ozone scavenger enzymes stimulation. On the other hand, the

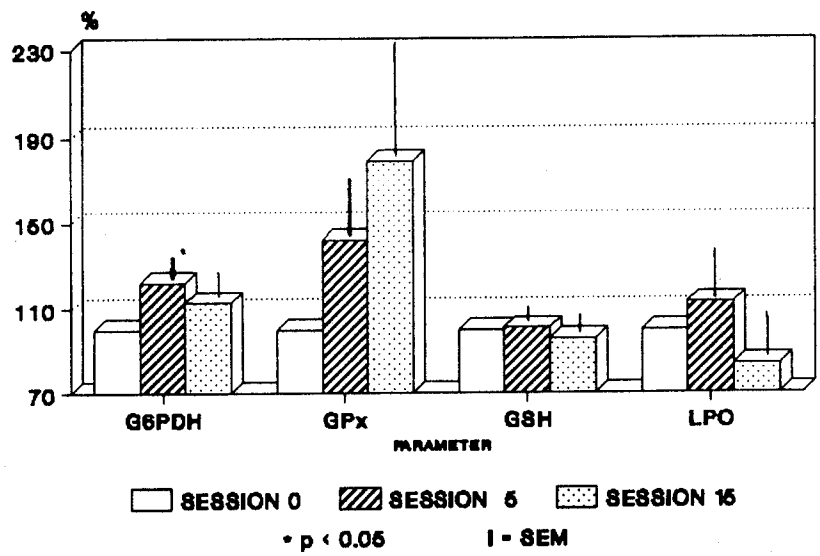

Fig. 2. Percentage values of biochemical parameters from glutathione redox system.

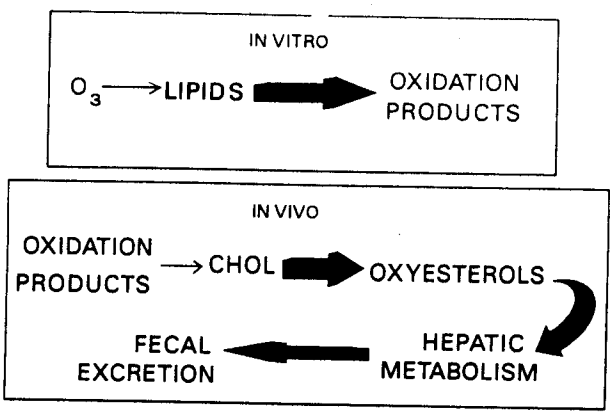

Fig. 3. Proposed mechanism for ozone effect on cholesterol metabolism.

actual mechanism by which ozone treatment decreases blood cholesterol is not clear. Nevertheless, there is an increasing knowledge about ozone reaction with organic molecules and its products that allows us to propose a possible mechanism of action taking into account our results.

The oxidation or lipid peroxidation of cholesterol leads to the production of B-ring oxidized oxysterols,[20–22] which are selectively transported in very low density lipoprotein (VLDL) and LDL particles.[23] These oxysterols are efficiently metabolized by liver, secreted in the bile, and subjected to intestinal microflora metabolism, with ultimate fecal excretion in the same manner as cholesterol.[24]

The question is how oxysterols are produced from ozone treatment in vivo. It can be supposed (Fig. 3) that ozone, when bubbled in the blood flask (in vitro step), reacts with unsaturated fatty acids from plasma tryglycerides, phospholipids, and esterified cholesterol, producing organic radicals and hydrogen peroxides, which are responsible for the initiation of cholesterol lipid peroxidation[25,26] when this blood is transfused (in vivo step) to the patient. Because ozone reacts at very fast rates at room temperature,[25,27,28] only the oxidized products derived from the reaction of ozone with blood in the transfusion flask enter the organism, and they are the ones that stimulate the antioxidant enzyme system and may be oxysterol-catabolizing enzymes as well. Therefore, decreasing scavenger enzyme activity in patients with cardiovascular diseases,[5] which seems to be a critical fact into pathogenesis of this disease, can be restored with ozone therapy.

The main objective is to give an appropriate ozone dose and treatment sequence to obtain a suitable oxidant defense system stimulation and ozone-decreasing blood cholesterol activity, as has been shown in this article. Perhaps other mechanisms can occur, but, taking into account our results and literature data, we think that the proposed mechanism is the more probable.

Because there is much other evidence that enhanced activity of the peroxide catabolism has beneficial effects on atherosclerotic diseases, our results suggest that stimulation of antioxidant enzymes by ozone therapy may be an important link between peroxide and cholesterol catabolism. We are at present investigating this theory.

REFERENCES

1. O'Neil, W.; Timmis, G.; Bourdillon, P.; Lai, P.; Ganghadarhan, V.; Walton, J.; Ramos, R.; Laufer, N.; Gordon, S.; Schork, M. A.; Pitt, B. A prospective randomized clinical trial of intracoronary streptokinase versus coronary angioplastic therapy of acute myocardial infarction. *N. Engl. J. Med.* **314**:812–828; 1986.
2. Ellis, S. G.; Wynne, J.; Braunwald, E.; Henschke, C. I.; Sandor, T.; Kloner, R. A. Response of reperfusion salvaged stunned myocardium to inotropic stimulation. *Am. Heart J.* **107**:13–19; 1984.
3. Smith, J. B.; Ingerman, C. M.; Silver, M. J. Effects of arachidonic acid and some of its metabolites on platelets. In: Silver, M. J.; Smith, J. B.; Kocsis, J. J., eds. *Prostaglandins in hematology.* New York: Spectrum Publications Inc.; 1977:277–291.
4. Parnham, M. J.; Leyck, S.; Dereu, N.; Winkelmann, J.; Graf, E. Ebselen (PZ 51): A GSH-peroxidase-like organoselenium compound with anti-inflammatory activity. *Adv. Inflam. Res.* **10**:397–400; 1985.
5. Loeper, J.; Goy, J.; Rozensztajn, L.; Bedu, O.; Moisson, P. Lipid peroxidation and protective enzymes during myocardial infarction. *Clin. Chim. Acta* **196**:119–126; 1991.
6. Parnham, M. J.; Graf, E.; Hoff, E.; Niemann, R. Selenium hydroperoxides and atherosclerosis. *Agents Actions* **22**:353–354; 1987.
7. Jolly, S. R.; Kane, W. J.; Baile, M. B.; Abrams, G. D.; Lucchesi, B. R. Canine myo-cardial reperfusion injury: Its reduction by the combined administration of superoxide dismutase and catalase. *Circ. Res.* **54**:277–285; 1984.
8. Ambrosio, G.; Becker, L. C.; Hutchins, G. M.; Weisman, H. F.; Weisfeklt, M. L. Reduction in experimental infarct size by recombinant human superoxide dismutase: Insights into the pathophysiology of reperfusion injury. *Circulation* **74**:1424–1433; 1986.
9. Hernández, F.; Menéndez, S.; Gómez, M.; Eng, L. Efecto de la ozonoterapia intravascular sobre el sistema de la glutation peroxidasa. *Rev. CENIC Ciencias Biol.* **20**:37–40; 1989.
10. Rilling, S. The basic clinical applications of ozone therapy. *Sci. Eng.* **7**:259–274; 1985.
11. Rilling, S.; Viebahn, R. *The application of ozone in medicine: A survey* (Vol. 3). Zurich; 1987:MI–MIX.
12. Beutler, E., ed. *Red cell metabolism: A manual of biochemical methods.* New York: Grune & Stratton. Inc; 1971:62–64.
13. Foraji, B.; Kang, H. K.; Valentine, J. L. Methods compared for determining gluthathione peroxidase activity in blood. *Cli. Chem.* **33**:539–543; 1987.
14. Thomson, C. D.; Rea, H. M.; Doesburg, V. M.; Robinson, M. F. Selenium concentrations and gluthathione peroxidase activities in whole blood of New Zealand residents. *Br. J. Nutr.* **37**:457–460; 1977.
15. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Cli. Chim. Acta* **90**:37–43; 1978.
16. Shayne, C. G.; Carrol, S. W. Statistics for toxicologists. In: Hayes, W.; ed. *Principles and methods of toxicology.* New York: Raven Press; 1982:273–320.

17. Mattassi, R. *Ozonoterapia.* Milano: Organizzazione Editoriale Medico Farmaceutica; 1985:33–57.
18. Lee, J. S.; Mastafa, M. G. Effects of short-term, single and combined exposure to low-level NO_2 and O_3 on lung tissue enzyme activities in rats. *J. Toxicol. Environ. Health.* **29:**293–305; 1990.
19. López-Torres, M.; Gil, P.; Barja de Quiroga, G. Effect of hyperoxia acclimation on catalase and glutathione peroxidase activities and in vivo peroxidation products in various tissues of the frog Rana ridibunda perezi. *J. Exp. Zool.* **248:**7–18; 1988.
20. Smith, L. L.; Johnson, B. H. Biological activities of oxysterols. *Free Radic. Biol Med.* **7:**285–332; 1989.
21. Morin, R. J.; Peng, S. K. The role of cholesterol oxidation products in the pathogenesis of atherosclerosis. *Ann. Clin. Lab. Sci.* **19:**225–237; 1989.
22. Smith, L. L.; Teng, J. I.; Lin, Y. Y.; Seitz, P. K.; McGhee, M. F. Lipid peroxidation of cholesterol. In: Yagi, K., ed. *Lipid peroxides in biology and medicine.* New York: Academic Press; 1982:89–105.
23. Peng, S. K.; Phillips, G. A.; Xia, G. Z.; Morin, R. J. Transport of cholesterol autoxidation products in rabbit lipoproteins. *Atherosclerosis* **64:**1–6; 1987.
24. Smith, L. L. Another cholesterol hypothesis: Cholesterol as antioxidant. *Free Radic. Biol. Med.* **11:**47–61; 1991.
25. Pryor, W. A.; Lightsey, J. W.; Prier, D. G. The production of free radicals in vivo from the action of xenobiotics: The initiation of antioxidation of polyunsaturated fatty acids by nitrogen dioxide and ozone. In: Yagi, K., ed. *Lipid peroxides in biology and medicine.* New York: Academic Press; 1982:1–22.
26. Pryor, W. A.; Church, D. F. Aldehydes, hydrogen peroxide and organic radicals as mediators of ozone toxicity. *Free Radic. Biol. Med.* **11:**41–46; 1991.
27. Pryor, W. A.; Giamalva, D. H.; Church, D. F. Kinetics of ozonation: 2. Amino acids and model compounds water and comparisons to reates in nonpolar solvents. *J. Ame. Chem. Soc.* **106:**7090–7100; 1984.
28. Pryor, W. A. How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? *Free Radic. Biol. Med.* **12:**83–88; 1992.