

Ozonotherapy protects from in-stent coronary neointimal proliferation. Role of redoxins



Barone A.^a, Otero-Losada M.^{a,*}, Grangeat A.M.^a, Cao G.^a, Azzato F.^a, Rodríguez A.^b, Milei J.^a

^a Institute of Cardiological Research, University of Buenos Aires, National Research Council Argentina, ININCA-UBA-CONICET, Argentina.

^b Otamendi Hospital, Post Graduate School of Medicine, Cardiac Unit, Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 8 June 2016

Accepted 27 July 2016

Available online 29 July 2016

Keywords:

Coronary angioplasty

Ozonotherapy

Thioredoxin-1

Neointimal hyperplasia

Restenosis

Endothelialization

ABSTRACT

Background: In-stent restenosis and poor re-endothelialization usually follow percutaneous transluminal coronary angioplasty, even using drug-eluting stents, due to inflammation and oxidative stress. Medical ozone has antioxidant and anti-inflammatory properties and has not been evaluated in this context.

Objectives: To evaluate whether ozonotherapy might reduce restenosis following bare metal stents implantation in relation to the redoxin system in pigs.

Methods: Twelve male Landrace pigs (51 ± 9 kg) underwent percutaneous transluminal circumflex coronary arteries bare metal stent implantation under heparine infusion and fluoroscopical guidance, using standard techniques. Pigs were randomized to ozonotherapy ($n = 6$) or placebo ($n = 6$) treatment. Before stenting (24 h) and twice a week for 30 days post-stenting, venous blood was collected, ozonized and reinfused. Same procedure was performed in placebo group except for ozonation. Both groups received antiplatelet treatment. Histopathology and immunohistochemistry studies were performed.

Results: Severe inflammatory reaction and restenosis with increase in the immunohistochemical expression of thioredoxin-1 were observed in placebo group 30 days after surgery. Oppositely, ozonotherapy drastically reduced inflammatory reaction and restenosis, and showed no increase in the Trx-1 immunohistochemical expression 30 days after surgery. Immunolabeling for Prx-2 was negative in both groups. Ozonated autohemotherapy strikingly reduced restenosis 30 days following PTCA with BMS implantation in pigs.

Conclusions: Stimulation of the redoxin system by ozone pretreatment might neutralize oxidative damage from the start and increase antioxidative buffering capacity post-injury, reducing further damage and so the demand for antioxidant enzymes. Our interpretation agrees with the ozone oxidative preconditioning mechanism, extensively investigated.

© 2016 Published by Elsevier Ireland Ltd.

1. Introduction

Percutaneous transluminal coronary angioplasty (PTCA) with stenting reduced restenosis to 10–20% overcoming limitations of balloon angioplasty. Restenosis results from elastic recoil and intimal hyperplasia as a response to vascular injury and may be as high as 40–50% by 6 months following dilatation of complex lesions [1]. While PTCA with stenting is currently the most popular treatment for coronary artery disease, in-stent restenosis remains its main limitation. Unfortunately, inhibition of neointimal hyperplasia with sirolimus-coated stents led to poor endothelialization [2].

Inflammation and oxidative stress are the basic mechanisms causing neointimal hyperplasia and are both involved in the pathogenesis of arterial diseases. Medical ozone, a 95% O₂–5% O₃ mixture, reverses oxidative stress following chronic inflammation [3], scavenges superoxides

due to activation of the redoxins superfamily and enhances peroxide detoxification in coronary artery disease [4]. Ozone up-regulates intracellular anti-oxidant enzymes, ameliorates ischemia-reperfusion injury and reduces antioxidant status and inflammation in kidneys [5] and ovaries [6], and exerts critical systemic anti-inflammatory effects [7].

Extracorporeal blood oxygenation and ozonation has been used with the aim of amplifying the results observed with ozone autohemotherapy since 1990 [8]. Ozone autohemotherapy has showed therapeutic efficacy in patients with atherosclerosis without side-effects, being recognized as worthy of testing in various diseases [8].

However, ozonotherapy potential to reduce neointimal hyperplasia after PCTA-stenting has not been evaluated.

The coronary arteries of domestic crossbred pigs respond similar to human coronary arteries after injury [9–11].

The purpose of this prospective, randomized, single-blinded study was to evaluate whether ozonotherapy might reduce restenosis following bare metal stents (BMS) implantation in relation to the redoxin system in pigs.

* Corresponding author at: ININCA-UBA-CONICET, Marcelo T. de Alvear 2270, Buenos Aires CP 1122, Argentina.

E-mail address: mol@fimed.uba.ar (M. Otero-Losada).

2. Methods

2.1. Animals

Twelve male Landrace pigs (51 ± 9 kg) underwent percutaneous transluminal circumflex coronary arteries (CCA) BMS implantation (BMS Coroflex Delta, B. Braun, Melsungen, Germany), under heparine infusion (100 UI/kg) and fluoroscopic guidance, using standard techniques. One stent (30 ± 3 mm \times 3.7 ± 0.2 mm) was impacted at high pressure in proximal CCA. Cinecoronariography was performed to evaluate coronary arterial tree during and post BMS implantation. Coronary flow was categorized in accordance with TIMI trial grades.

Pigs were randomized to ozonotherapy ($n = 6$) or placebo ($n = 6$) treatment. Before stenting (24 h) and twice a week for 30 days post-stenting, venous blood (100 mL) was collected, bubbled with an ozone-oxygen mixture to 20 μ g/mL ozone final concentration, and reinfused through the jugular vein (100 mL of ozonized blood containing 2 mg ozone). Same procedure was performed in placebo group except for the ozonation step. Both groups received antiplatelet treatment: aspirin 300 mg/day and clopidogrel 300 mg (24 h pre-stenting and 75 mg/day during follow-up). Animals were housed in an indoor facility and, 30 days after implantation, were deeply anesthetized with ketamine and euthanized by exsanguination. Immediately after, hearts were excised. Segments containing the stented coronary arteries were cut using a diamond disk saw. Stents were carefully removed and after 40-h fixation in 10% phosphate-buffered formaldehyde solution pH = 7.0, and segments were processed for histology. Serial sections (3 μ m thick) were stained with H&E and Masson trichrome. Histomorphometric analysis and planimetry were performed using image analysis software (Image-Pro Plus 4.5 for Windows). For immunohistochemistry, conventional streptavidin-biotin technique and primary polyclonal antibodies against CD68 (monocytes/macrophages), CD34 (endothelial cells), α -actin (smooth muscle cells, SMCs), thioredoxin (Trx)-1 and peroxiredoxin (Prx)-2 were used. Negative control sections were incubated with non-immune normal rabbit serum antibodies. Procedures followed the guidelines of the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Statistical analysis

Two-tailed unpaired t test (parametric variables) and Mann–Whitney test (non-parametric variables) were used for between-treatment data comparison and a p value <0.05 was considered statistically significant (SPSS™ 18.0, SPSS Inc., Chicago, Illinois).

3. Results

Coronary flow did not change after either stent implantation or intracoronary administration of ozonized solutions. TIMI 3 flow was observed in both groups. Compared with placebo, ozonotherapy reduced neointimal thickness (μ m) 127 ± 87 vs 648 ± 270 ($p < 0.03$), neointimal area (mm^2) 1.08 ± 0.68 vs 5.03 ± 0.17 ($p < 0.01$) and stenosis (%) 16.63 ± 11.17 vs 61.55 ± 19.90 ($p < 0.03$) and showed a trend to increase lumen area (mm^2) 5.52 ± 0.11 vs 3.10 ± 0.15 ($p < 0.06$, N.S.). Neointimal SMCs proliferation (α -actin immunopositivity), collagen deposition and extracellular matrix increase were the most striking lesions associated with mild neovascularization (Fig. 1).

In placebo group, two neointimal area layers were observed (Fig. 1). One deep layer showed globular SMCs, abundant extracellular matrix and no fibrosis, mild histiocytic infiltration and evident neovascularization encircling struts (hypocellular phase of inflammation). The other, superficial, layer showed abundant spindle or fusiform SMCs, fibromuscular stroma with scarce inflammatory cells (end stage of inflammation). Parietal wall showed variable mild inflammatory infiltration surrounding struts, comprising pale histiocytic cells near the stents and scarce foreign body-

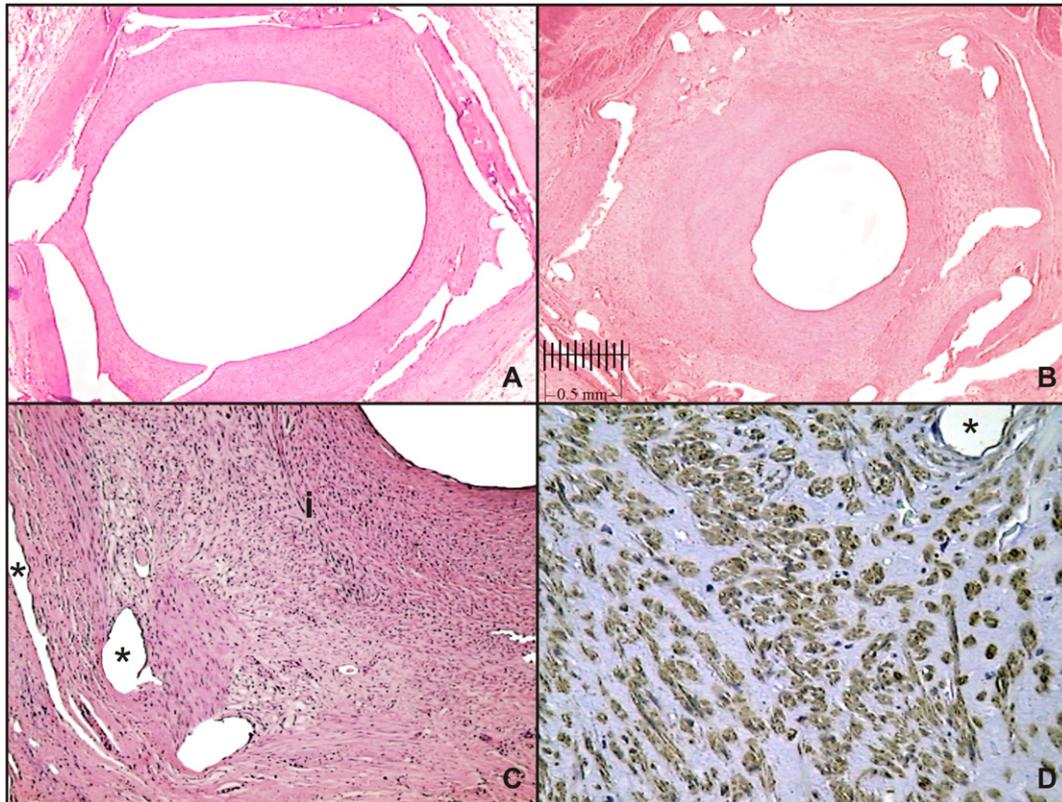


Fig. 1. Histology and morphology 30 days after stent implantation. A. Low-power image of mild–moderate neointimal thickening of a circumflex coronary artery 30 days after bare metal stent (BMS) implantation in an ozone-treated group. Lumen is fairly preserved. Optical negative spaces were formerly occupied by stent struts. H&E 4 \times . B. Low-power image of severe neointimal thickening of a circumflex coronary artery 30 days after BMS placement in placebo group. A marked concentric luminal stenosis is observed. H&E 4 \times . C. High-power image of a stented artery in placebo group showing severe neointimal healing response and marked concentric stenosis. Two layers can be clearly depicted: one internal (i) with marked collagen deposition, smooth muscle cells, scarce inflammatory infiltration and no vascularization, with preserved endoluminal surface, and the other, external, with striking smooth muscle cell proliferation, extracellular matrix increase and neovascularization. * indicates former occupation by stent strut. H&E 200 \times . D. High-power image of a stented artery in placebo group depicting two well-defined neointimal layers: one deep (external, left) with globular smooth muscle cells, absent/little fibrosis and abundant extracellular matrix limiting with the struts (*), and the other superficial (internal, right), with abundant spindle or fusiform smooth muscle cells, a fibromuscular stroma with scarce inflammatory cells and no neovascularization. Anti- α actin. 400 \times .

Download English Version:

<https://daneshyari.com/en/article/5963108>

Download Persian Version:

<https://daneshyari.com/article/5963108>

[Daneshyari.com](https://daneshyari.com)