

*Evaluation Study***Cleaning of the blood vessels**D. Kaliterna¹, Q. Zhu¹ and I. Bizic²¹*Poliklinika Poliderma, Zagreb, Croatia;* ²*Department of Vascular Surgery, University Hospital Rebro, Zagreb, Croatia*

Corresponding author:

Dinko Kaliterna, MD,
Ulica grada Vukovara 284d 4. kat
Zagreb (Almeria centar), Croatia
Tel: +385 1 4828 961
e-mail: poliderma@poliderma.hr

Keywords: *early atherosclerosis, ischemia, cardiovascular diseases, intravenous laser blood irradiation, glutathione, carnosine, phosphatidylcholine*

Received: 30 October 2023

Accepted: 31 January 2024

Copyright:

Journal of Applied Cosmetology ©2024

www.journalofappliedcosmetology.com

Copyright © by Journal of Applied Cosmetology

ISSN 2974-6140 (online) ISSN 0392-8543 (print).

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorised reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

ABSTRACT

The integrity of blood vessels is critical to vascular homeostasis, whose disruption is involved in atherosclerosis (AS), causing stroke and myocardial infarction. Therefore, detecting early AS is essential to avoid severe complications. An arteriograph is the best device for detecting early AS by measuring the rigidity of the blood vessels. To date, there are no safe and effective treatments to clean the blood vessels from lipid accumulation and calcium deposition, which are involved in the onset of AS. For this purpose, we have developed a 4-weeks non-pharmacological protocol, consisting of intravenous laser blood irradiation, injections of glutathione and carnosine, and oral supplementation with phosphatidylcholine, aimed at patients with risk factors for early AS. We observed a significant amelioration of lipids parameters and PWVao, an indicator of the existence of asymptomatic AS, confirming the efficacy of our protocol. Besides, all other health issues must be improved.

INTRODUCTION

The integrity of blood vessels is critical to vascular homeostasis, contributing to maintaining a non-thrombogenic environment. Endothelial dysfunction and a consequent loss of vascular integrity can contribute to atherogenesis by initiating the atherosclerotic process, whose complications are the leading cause of disability and mortality in Western countries (36). Atherosclerosis (AS) is a chronic inflammatory disease of the vascular wall of large and medium-sized arteries (27), leading to the formation of plaques characterized by a lipid-rich core surrounded by a fibrous cap, in which extracellular matrix (ECM) proteins are secreted (15). It is increasingly recognized that lipids, especially low-density lipoproteins (LDL) and cholesterol, and inflammation are two predominant mechanisms of AS (28), contributing to endothelial dysfunction. This activated endothelium leads to the over-production of reactive oxygen species (ROS) and to the decrease of nitric oxide (NO), which is essential for vascular homeostasis maintenance (14, 17). In addition, calcium deposition in neointimal lesions can occur and is associated with adverse cardiovascular events (33).

There are several risk factors involved in the development and progression of AS, including age, genetic, and environmental factors, i.e., hypercholesterolemia, high levels of LDL, low levels of high-density lipoproteins (HDL), diet, alcohol, smoking, diabetes, hypertension and an inactive lifestyle (3, 34, 36). Notably, it has been demonstrated that early lesions may regress if the LDL-cholesterol and other pro-atherogenic factors decrease or rapidly grow in hypercholesterolemia (21). In this context, early detection of atherosclerotic plaques is crucial to prevent complications.

The best tool for detecting arterial stiffness, which is directly associated with the increased risk for a cardiovascular event, is the Arteriograph (manufactured by TensioMed) (4). This medical device measures arterial function parameters, such as the aortic pulse wave velocity (PWVao), the central systolic blood pressure (SBP), and the augmentation index (AIx), allowing clinicians to identify those subjects with vascular damage before a stroke or myocardial infarction occurs.

Thus, early treatment in asymptomatic patients with high-risk coronary artery lesions can prevent the possible occurrence of lethal complications. Among non-pharmacological treatment strategies, intravascular laser irradiation of blood (ILIB), as an adjuvant therapy in the treatment of cardiovascular and peripheral ischemic vascular diseases, has been shown to have anti-inflammatory effects in addition to stimulating the NO production, which acts as a therapeutic agent to promote endothelial repair in the plaque site (31, 39). In addition, given that ROS are among the factors contributing to AS, antioxidant systems appear to be another promising therapeutic strategy; among them, glutathione (GSH) is one of the most significant cellular antioxidant defensive system members. It is essential for eliminating excess ROS and protecting the

myocardium in the presence of pathological cardiovascular factors (22, 30). Another natural antioxidant and free-radical scavenger is carnosine (10), which has been shown to have not only anti-inflammatory but also neuroprotective properties (5, 41) and heavy metal chelating activity (40). Interestingly, along with GSH, carnosine reduces lipid peroxidation (16, 24). Besides antioxidant systems, several animal trials have shown the role of dietary phosphatidylcholine (PC) supplementation in reducing plasma cholesterol levels and enhancing immune development (13, 26, 35). The atheroprotective effect of PC administration is due to decreased serum levels of LDL, triglyceride, cholesterol, and APOB (1, 37).

Of note, it is very important to detect early AS, which affects even young men (25). While color-Doppler ultrasound of the carotid arteries can't measure the rigidity of the blood vessels, an Arteriograph can measure the rigidity of the blood vessels, detecting early AS and thus determining the risk of stroke and heart attack.

Given the above considerations, we have developed a special protocol (see materials and methods) for patients with risk factors for early AS. The main goal was to evaluate the efficacy of our 4-week treatment by assessing lipid blood parameters and using an arcograph.

MATERIALS AND METHODS

Study population

We evaluated 19 men aged 40 to 50 years old with the following cardiovascular risk factors: 1) LDL cholesterol > 130 mg/dl, 2) current smokers (since at least 1 year), and 3) overweight, defined for a body mass index [BMI] > 25 kg/m² according to the WHO classification, derived from weight and height.

Measurements

All patients underwent a lipid profile blood assessment (total cholesterol, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, and triglycerides) at baseline and after 4 weeks from the application of the protocol (see below). After an overnight fast, blood samples were collected in the morning to determine lipids by automated enzymatic colorimetric method).

In addition, all patients underwent the evaluation of PWVao with Arteriograph at baseline and after 4 weeks from the application of the protocol (see below). An arteriograph is the gold standard for measuring PWVao, a parameter that can indicate the existence of asymptomatic atherosclerosis (<https://tensiomed.com/>). An arteriograph is equipped with an inflatable cuff placed on the patient's upper arm and inflated 45 mmHg above the individual's SBP. A pressure sensor detects pressure variations, transferring the signal to a computer. The PWVao acquirement is based on generating two systolic peaks: the first peak results from the systolic volume ejection in the aorta, whereas the second and lower peak is given by wave pressure reflection from peripheral arteries. Considering that return time (RT) is the difference between the first peak and the second reflected systolic peak, PWVao is calculated as the "distance measured from jugular to symphysis" / (RT/2) (32). The characteristics of the aortic wall determine the PWVao. The stiffer the aortic wall, the faster the PWVao is. PWVao is an independent predictor of coronary heart disease and stroke in asymptomatic, apparently healthy subjects, and it is a strong indicator of cardiovascular risk and is used increasingly in clinical practice. Moreover, it is also related to the sub-clinical coronary atherosclerosis independently from conventional risk factors, including blood pressure indices. PWVao is considered normal under 9 m/s (32).

Protocol set up

Below are step-by-step treatments that characterize our 4-week designed protocol.

- Intravenous laser blood irradiation (ILIB): The ILIB procedure was performed in the treatment room. A puncture of the cubital vein with the help of a disposable needle with a light pipe inside was made. After this,

with the help of a special adapter, a light was attached to the laser light source – a device for intravenous blood irradiation. After the procedure, a needle with a light pipe was removed from the vein and is being recycled. This procedure lasted about 20-30 minutes. After the procedure, a patient rested comfortably for less than 30 minutes. Two treatment sessions were conducted twice a week for 8 sessions.

- Glutathione injections: directly injected into the muscles, efficiently bypassing the digestive system and ensuring that patients get benefits much faster and in higher concentrations. Multiple doses at 1400 mg each were split thrice weekly for 12 injections.

- Carnosine injections consist of intravenous administration of L-carnosine at a dosage of 1.2 g/day for 4 weeks.

- Oral PC supplementation: A daily PC supplementation (2400 mg) was provided to all patients for 4 weeks.

Statistics

To summarize patients' clinical characteristics, we used descriptive statistics. Continuous data were expressed as mean $\pm$ standard deviation (SD) when normally distributed or as medians (quartiles) for non-normal distribution. The paired two-sided Student's t-test was used to compare lipids levels and PWVao before and after 4 weeks of protocol application. Statistical analysis was performed with SPSS (SPSS, Inc., Chicago, IL, USA) for Windows 27. A p-value < 0.05 was considered significant.

RESULTS

The 19 patients studied showed a mean age of 45.4 ± 3.6 years. The mean BMI of the patients studied was 28.2 ± 3.6 kg/m². The mean duration of the smoking habit was 14.2 ± 4.6 years, and the mean number of cigarettes per day was 10 ± 5 .

At baseline, the mean levels of blood lipids were: total cholesterol 220 ± 12 mg/dl, HDL cholesterol 38 ± 8 mg/dl, LDL cholesterol 150 ± 6 mg/dl, and triglycerides $160 [140 - 200]$ mg/dl. The mean PWVao revealed by the Arteriograph was 11 ± 3 m/s.

After 4 weeks from the application of the protocol, a significant amelioration of the lipids parameters and PWVao was observed. In particular, total cholesterol was 190 ± 14 mg/dl, HDL cholesterol 42 ± 7 mg/dl, LDL cholesterol 118 ± 10 mg/dl, and triglycerides $142 [130 - 180]$ mg/dl, while the mean PWVao revealed by the Arteriograph was 8 ± 3 m/s. A significant difference ($p < 0.05$) was observed comparing the values of all the abovementioned parameters evaluated after 4 weeks of protocol with those assessed at baseline.

DISCUSSION

The endothelium plays a crucial role in the regulation of vascular homeostasis. Structural alterations of the vascular wall of medium and large arteries lead to the formation of atherosclerotic plaques (9), the pathophysiological drivers of clinical atherosclerotic cardiovascular disease, a persistently leading cause of death worldwide (36). AS is a slow but progressive process triggered by the accumulation of LDL-cholesterol in the intima of arterial vessels, acting as a chronic stimulator of the innate and adaptive immune response (6). However, a "multiple-hit" hypothesis has been recently proposed for the development of AS, in which multiple mechanisms, including an aberrant lipid profile, inflammation, vascular injury, oxidative stress, hemodynamic forces, epigenetics, and others, interact with each other leading to pro-atherogenic signaling (42).

Over the past three decades, a substantial reduction in cardiovascular mortality has been achieved mainly through LDL-cholesterol-lowering regimes and therapies targeting other traditional risk factors for

cardiovascular disease, such as hypertension, smoking, diabetes mellitus, and obesity (15). However, a substantial global burden of cardiovascular disease still remains, and early, non-invasive, and effective treatments aimed at high-risk patients are needed.

In this study, we have developed a 4-week protocol for selected patients with risk factors for early AS (n=19) consisting of multiple non-pharmacological interventions, including ILIB, glutathione and carnosine injections, and oral PC supplementation. Among the several anti-atherosclerotic properties of ILIB, improving endothelial function via increasing NO bioavailability is the most interesting (11). NO is a well-known mediator acting as a vasodilator and antithrombotic factor, and its deficiency is a hallmark of endothelial dysfunction (2, 19). Thus, enhancing the NO release from the endothelium by ILIB stimulation can reverse the atherosclerotic process. Glutathione and carnosine are two critical antioxidant systems. Glutathione, on the one hand, improves vascular integrity by lowering oxidative stress and defending cells from free radicals, which are involved in the atherogenic process (30). On the other side, the positive biological effects of carnosine are also explained by its ability to form complexes with bivalent metals (8), whose ions take an active part in many metabolic processes and can activate free-radical reactions. Hence, they have an essential role as significant antioxidants. Finally, several studies have suggested that PC supplementation can help in improving blood lipid profile (12, 18), and a high dose of PC supplementation (2400 mg) can significantly decrease triglyceride levels and may help reduce LDL-cholesterol levels (23). Notably, none of these treatments showed side effects (16, 24, 23, 30, 39), leading us to consider their use in a standardized clinical protocol.

Four weeks after the application of the protocol, a significant amelioration of the lipids parameters, including total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides, was observed, indicating an improvement in the management of dyslipidemia, which is the leading risk factor for AS. In addition, a significant decrease in PWVao was also observed, confirming the benefits of the applied non-pharmacological treatments on aortic walls. The vasoprotective effects of this treatment include the recovery of vascular homeostasis. This is probably due to the generation of NO, the maintenance of normal vascular tone, the reduction of oxidative stress, and the inhibition of pro-inflammatory responses (20).

Thus, our approach of a multiple non-pharmacological protocol aiming at recuperating vascular homeostasis holds promise for anti-AS therapies. However, besides this therapeutic strategy, all patients must implement lifestyle modifications, including exercise, healthy habitual eating, and smoking cessation, which are known to maintain vascular homeostasis and prevent atherosclerotic cardiovascular diseases (7, 29).

Further studies with a larger sample size are needed to confirm the efficacy of our therapeutic strategy in patients with risk factors for early AS.

REFERENCES

1. Aldana-Hernández P, Azarcoya-Barrera J, van der Veen JN, Leonard KA, Zhao YY, Nelson R, Goruk S, Field CJ, Curtis JM, Richard C, Jacobs RL. Dietary phosphatidylcholine supplementation reduces atherosclerosis in Ldlr-/- male mice. *J Nutr Biochem* 2021; 92:108617.
2. Atochin DN, Huang PL. Role of endothelial nitric oxide in cerebrovascular regulation, *Curr Pharm Biotechnol* 12 (2011) 1334–42.
3. Bays HE, Taub PR, Epstein E, Michos ED, Ferraro RA, Bailey AL, Kelli HM, Ferdinand KC, Echols MR, Weintraub H, et al. Ten things to know about ten cardiovascular disease risk factors. *Am. J. Prev. Cardiol* 2021; 5:100149.
4. Baulmann J, Schillings U, Rickert S, Uen S, Düsing R, Illyes M, Cziraki A, Nickering G, Mengden T. A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. *J Hypertens*. 2008; 26(3):523-8.

5. Bellia F, Vecchio G, Cuzzocrea S, Calabrese V, Rizzarelli E. Neuroprotective features of carnosine in oxidative driven diseases. *Mol Aspects Med* 2011; 32(4-6):258-66.
6. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. *Circ Res* 2014; 114:1852–1866.
7. Bernhard D, Pfister G, Huck CW, Kind M, Salvenmoser W, Bonn GK, et al. Disruption of vascular endothelial homeostasis by tobacco smoke: impact on atherosclerosis. *FASEB J* (2003) 17(15):2302–4.
8. Brown CE, Antholine WE. Chelation chemistry of carnosine. Evidence that mixed complexes may occur in vivo. *Journal of Physical Chemistry* 1979; 83(26):3314–3319.
9. Camaré C, Pucelle M, Nègre-Salvayre A, Salvayre R. Angiogenesis in the atherosclerotic plaque. *Redox Biol* 2017; 12:18-34.
10. Chasovnikova LV, Formazyuk VE, Sergienko VI, Boldyrev AA, Severin SE. The antioxidative properties of carnosine and other drugs. *Biochem Int* 1990; 20(6):1097-103.
11. Chen CH, Hung HS, Hsu SH. Low-energy laser irradiation increases endothelial cell proliferation, migration, and eNOS gene expression, possibly via the PI3K signal pathway. *Lasers Surg Med*. 2008 Jan;40(1):46-54.
12. Childs MT, Bowlin JA, Ogilvie JT, Hazzard WR, Albers JJ. The contrasting effects of a dietary soya lecithin product and corn oil on lipoprotein lipids in normolipidemic and familial hypercholesterolemic subjects. *Atherosclerosis*. 1981; 38(1-2):217-28.
13. Dellschaft NS, Richard C, Lewis ED, Goruk S, Jacobs RL, Curtis JM, et al. The dietary form of choline during lactation affects maternal immune function in rats. *Eur J Nutr* 2018; 57:2189–99.
14. Endemann DH, Schiffrin EL. Endothelial dysfunction. *J Am Soc Nephrol* 2004; 15:1983–92.
15. Engelen SE, Robinson AJB, Zurke YX, Monaco C. Therapeutic strategies targeting inflammation and immunity in atherosclerosis: how to proceed? *Nat Rev Cardiol*. 2022 Aug;19(8):522-542.
16. Gaucher C, Boudier A, Bonetti J, Clarot I, Leroy P, Parent M. Glutathione: antioxidant properties dedicated to nanotechnologies. *Antioxidants*. 2018; 7:62.
17. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001; 104:2673–8.
18. Høie LH, Morgenstern EC, Gruenwald J, Graubaum HJ, Busch R, Lüder W, Zunft HJ. A double-blind placebo-controlled clinical trial compares the cholesterol-lowering effects of two different soy protein preparations in hypercholesterolemic subjects. *Eur J Nutr*. 2005 Mar;44(2):65-71.
19. Huang PL. Endothelial nitric oxide synthase and endothelial dysfunction, *Curr Hypertens Rep* 5 (2003) 473–80.
20. Huwiler A, Pfeilschifter J. Recuperation of vascular homeostasis. *Circ Res* (2021) 129(2):237–9.
21. Insull W. Jr. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *Am. J. Med*. 2009; 122(1 Suppl):S3–S14.
22. Jaganjac M, Milkovic L, Sunjic SB, Zarkovic N. The NRF2, thioredoxin, and glutathione system in tumorigenesis and anticancer therapies. *Antioxidants* 2020; 9:1151.
23. Jan M, Thondre PS, El-Chab A, Lightowler HJ. The effect of dietary phosphatidylcholine supplementation on lipid profile in mild hyperlipidaemic individuals. *Proceedings of the Nutrition Society*. 2017;76(OCE4):E219.
24. Kim MY, Kim EJ, Kim YN, Choi C, Lee BH. Effects of α -lipoic acid and L-carnosine supplementation on antioxidant activities and lipid profiles in rats. *Nutr Res Pract*. 2011 Oct;5(5):421-8.
25. Kotsis V, Antza C, Doundoulakis I, Stabouli S. Markers of Early Vascular Ageing. *Curr Pharm Des*. 2017;23(22):3200-3204.
26. Lewis E, Richard C, Goruk S, Wadge E, Curtis J, Jacobs R, et al. feeding a mixture of choline forms during lactation improves offspring growth and maternal lymphocyte response to ex vivo immune challenges. *Nutrients* 2017; 9:713.
27. Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420(6917):868–874.
28. Libby P. The changing landscape of atherosclerosis. *Nature* (2021) 592(7855):524–33.
29. Man AWC, Li H, Xia N. Impact of lifestyles (Diet and exercise) on vascular health: Oxidative stress and endothelial function. *Oxid Med Cell Longev* 2020 (2020) p:1496462.
30. Matuz-Mares D, Riveros-Rosas H, Vilchis-Landeros MM, Vazquez-Meza H. Glutathione participation in the prevention of cardiovascular diseases. *Antioxidants* 2021; 10:1220.
31. Mikhaylov VA. The use of Intravenous Laser Blood Irradiation (ILBI) at 630-640 nm to prevent vascular diseases and to increase life expectancy. *Laser Ther*. 2015 Mar 31;24(1):15-26.
32. Milan A, Zocaro G, Leone D, Tosello F, Buraioli I, Schiavone D, Veglio F. Current assessment of pulse wave velocity: comprehensive review of validation studies. *J Hypertens*. 2019 Aug;37(8):1547-1557.

33. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, Greenland P. Coronary artery calcium score and risk classification for coronary heart disease prediction. *JAMA* 2010; 303:1610–1616.
34. Rafieian-Kopaei M, Setorki M, Douadi M, Baradaran A, Nasri H. Atherosclerosis: Process, indicators, risk factors and new hopes. *Int. J. Prev. Med.* 2014; 5:927–946.
35. Richard C, Lewis E, Goruk S, Wadge E, Curtis J, Jacobs R, et al. Feeding a mixture of choline forms to lactating dams improves the development of the immune system in sprague-dawley rat offspring. *Nutrients* 2017; 9:567.
36. Roth GA, Mensah GA, Johnson CO et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. *J Am Coll Cardiol* 2020; 76:2982–3021.
37. Samochowiec L, Kadłubowska D, Rózwicka ´ L. Investigations in experimental atherosclerosis Part 1. The effects of phosphatidylcholine (EPL) on experimental atherosclerosis in white rats. *Atherosclerosis* 1976; 23:305–17.
38. Samochowiec L, Kadłubowska D, Rózewicka ´ L, Kuzna ´ W, Szyszka K. Investigations in experimental atherosclerosis Part 2. The effect of phosphatidylcholine (EPL) on experimental atherosclerotic changes in miniature pigs. *Atherosclerosis* 1976; 23:319–31.
39. Tomé RFF, Silva DFB, Dos Santos CAO, de Vasconcelos Neves G, Rolim AKA, de Castro Gomes DQ. ILIB (intravascular laser irradiation of blood) as an adjuvant therapy in the treatment of patients with chronic systemic diseases-an integrative literature review. *Lasers Med Sci* 2020; 35(9):1899-1907.
40. Torreggiani A, Tamba M, Fini G. Binding of copper (II) to carnosine: Raman and IR spectroscopic study. *Biopolymers* 2000; 57:149–159.
41. Xing L, Chee ME, Zhang H, Zhang W, Mine Y. Carnosine—a natural bioactive dipeptide: Bioaccessibility, bioavailability and health benefits. *J. Food Bioact.* 2019, 5, 8–17.
42. Xu S, Lyu QR, Ilyas I, Tian XY, Weng J. Vascular homeostasis in atherosclerosis: A holistic overview. *Front Immunol.* 2022; 13:976722.