Effect of endovascular coronary low-level laser therapy during angioplasty on the release of endothelin-1 and nitric oxide

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Abstract

Background. Nitric oxide (NO) and endothelin-1 are potentially significant factors contributing to the pathogenesis of post-angioplasty restenosis. It may be postulated that low-level laser therapy (LLLT) can favorably influence the process of restenosis by affecting those factors.

Objectives. The aim of the study was to evaluate the effect of LLLT applied during percutaneous coronary intervention (PCI) on the factors participating in the homeostasis of vascular tone – NO and endothelin-1.

Material and methods. In a randomized, prospective study of 52 subjects undergoing PCI, an additional 808 nm intravascular LLLT was applied at a dose of 9 J/cm^2 in the lesion part. The control group was 49 subjects with PCI only. We assessed the concentration of nitrites/nitrates reflecting NO metabolism as well as endothelin-1 in both groups before PCI, and at 6 h, 12 h and 1 month after the procedure. In addition, half a year after PCI, a follow-up angiography was performed.

Results. Statistically higher nitrite/nitrate concentrations were observed in the laser group as compared to the control group in all tests except the pre-PCI assays. Endothelin-1 levels were significantly higher in the laser group 6 h after PCI with a significant decrease in subsequent tests, which was not observed in the control group. The restenosis rate was 15.0% in the laser group and 32.4% in the control group (however the difference was not statistically significant).

Conclusions. LLLT applied during the PCI procedure can influence the process of restenosis by modifying NO and endothelin-1 concentrations.

Key words: low-level laser therapy, coronary angioplasty, restenosis, nitric oxide, endothelin-1

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One of the main factors affecting the long-term efficacy of percutaneous coronary intervention (PCI) is restenosis in the treated region. The mechanism of this phenomenon is multifactorial and has not yet been fully explored. Nitric oxide (NO) and endothelin-1, factors involved in vascular homeostasis, play an important role in the pathogenesis of restenosis. NO dilates vessels, shows anti-aggregating properties and exerts a beneficial effect on the regeneration and proliferation of vascular endothelium, thus inhibiting restenosis.[1,2] Endothelin-1 contracts vessels, shows pro-aggregating, mitogenic and atherogenic effects, and accelerates the processes leading to restenosis.[3,4] There are clinical trials, including self-reported ones, whose results indicate that low-level laser therapy (LLLT) during PCI inhibits restenosis.[5–7] In this paper, we present the effects of LLLT on NO and endothelin-1 concentrations in patients undergoing PCI.

Material and methods

The study group

The study was conducted on a group of 101 subjects (26 women and 75 men; mean age 57 ± 11 years) with stable coronary artery disease who had undergone successful coronary angioplasty. When balloon angioplasty alone was effective (i.e. residual stenosis did not exceed 30%), a stent was not implanted. When residual stenosis was greater than 30%, or if there was a significant coronary artery dissection, a stent implantation was additionally performed. In this study, only bare-metal stents (BMS) were applied. In the study group, 29 subjects underwent balloon angioplasty alone and 72 subjects had stents implanted.

The exclusion criteria for this study included unstable coronary artery disease, diabetes or other conditions which may affect patient survival. In addition, the study excluded cases of angioplasty for stenosis in the left main coronary artery or an ostial lesion, at the bifurcation, with significant calcification and chronic total occlusion, and when the reference artery diameter was less than 2.5 mm.

Prior to the procedure and during the follow-up, all patients received dual anti-platelet therapy (acetylsalicylic acid and P2Y12 inhibitor), statin, and, if possible, angiotensin converting enzyme inhibitors and β-blockers.

A coin flip method was used to randomize the qualified subjects into 2 groups. In effect, 52 subjects received PCI with LLLT. The remaining 49 patients constituted a control group. In both groups, there were no statistically significant differences in potential risk factors for restenosis such as sex, age, presence of hypertension, previous myocardial infarction, history of smoking or lipid profile disorders (Table 1).

After 6 months, a control coronary angiography was performed to assess the degree of stenosis in the treated region. Quantitative coronary angiography (QCA), supplied with the angiography equipment (General Electric, Fairfield, Connecticut, USA) was used for the measurement of reference values of coronary artery diameter stenosis grade and length. These measurements were performed before and after angioplasty and follow-up angiography after 6 months.

The measured values included the percentage of the stenosis, MLD (minimum luminal diameter) and RD (reference diameter). MLD values were used in calculating the increase in luminal diameter immediately after the procedure (acute gain) or loss of luminal diameter at 6 months (late lumen loss) and the late lumen loss index, which is the quotient of the late lumen loss value by acute gain value.[8] Restenosis was diagnosed when a follow-up examination revealed a narrowing of at least 50%.

Table 1. Demographic, clinical and procedural characteristics of analyzed groups

Parameter	Laser group	Control group	p-values
Total number of patients women men	52 12 40	49 14 35	0.687
Age for the total group (years)	57.3 ± 11.3	60.7 ± 9.5	0.106
Coronary artery disease CCS I CCS II CCS III CCS IV	 12 21 12 7	 6 22 14 7	0.757
Arterial hypertension	24	30	0.505
Tobacco smoking	34	31	0.956
Total cholesterol [mg/dL] LDL cholesterol [mg/dL] HDL cholesterol [mg/dL] Triglycerides [mg/dL]	199.9 ± 52.0 123.4 ± 43.5 46.4 ± 9.7 151.5 ± 87.2	198.3 ± 45.4 123.7 ± 38.6 42.7 ± 8.1 156.4 ± 83.6	0.870 0.871 0.160 0.773
Dilated artery LAD RCA Cx	 29 12 11	 23 13 13	0.667
Lesion supplied with balloons LAD RCA Cx	 7 4 3	 3 6 6	0.227
Lesion supplied with stents LAD RCA Cx	 22 9 7	 20 7 7	0.940

CCS – Canadian Cardiovascular Society class; LAD – left anterior descending artery; RCA – right coronary artery; Cx – circumflex coronary artery.

Ethics statement

All experiments were conducted and approved in accordance with the guidelines of the Bioethics Committee at Wroclaw Medical University and adhered to the principles of the Declaration of Helsinki and Title 45 U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects (revised November 13, 2001, effective December 13, 2001). All participants provided their written consent to participate in the study. The written consent had been approved by the Ethics Committee.

Radiation therapy

The radiation procedure was performed with the use of our own method. After the successful expansion of the vessel, a low-pressure balloon catheter was introduced into the region of the lesion. The catheter had an inbuilt optical fiber and an integrated diffuser at the end, corresponding to the length of the balloon. The diffuser emitted laser radiation perpendicularly to the long axis of the catheter and, as the balloon filled, it centered the diffuser in the vessel lumen. The area of the lesion was irradiated with a 5 mm margin proximally and distally to the stenosis. Optical fiber and a semiconductor laser (Optel, Poland) emitting 808 nm radiation were optically connected (pig-tailed). Laser radiation power was 100 mW/cm^2 in the diffuser, and the total energy of the irradiated coronary artery surface was 9 J/cm^2. This radiation technique has also been presented in other publications.[4,7,9–11]

Biochemical tests

Plasma concentration of endothelin-1 was measured and assessed with an enzyme-linked immunosorbent assay (ELISA) by Biomedica (Vienna, Austria).

Since nitric oxide is oxidized to nitrites (NO_2^-) and nitrates (NO_3^-), its concentration was obtained by use of an indirect method for determination of the NO_3^- and NO_2^- ions in a colorimetric assay based on tests by R&D Systems (Minneapolis, USA). Blood was sampled before the angioplasty and then at 6 and 12 h after the PCI and 1 month after the procedure.

Statistical analysis

Statistical analysis was performed by STATISTICA PL v. 6.0 software package (StatSoft, Poland). The data was expressed as mean ± SD. The distribution of variables was verified with the Shapiro-Wilk test. In the case of independent quantitative variables with normal distribution, Student's t-test or ANOVA analysis were used, as appropriate. For non-parametric analyses, the U Mann-Whitney test or non-parametric Kruskal-Wallis ANOVA test were used whereas in the case of dependent quantitative variables, the Wilcoxon matched pairs test or non-parametric Friedman ANOVA variance analysis were used. Statistically significant differences were marked with Newman-Keuls post-hoc test. The level of $p < 0.05$ was considered statistically significant.

Results

In the period between angioplasty and a follow-up angiography at 6 months, 1 subject died. The patient was in the control group and the cause of death was a myocardial infarction in a region other than the coronary artery which had been expanded. After 6 months, angiography was performed in 40 patients from the laser group and restenosis was found in 6 cases (15.0%). In the control

Table 2. Comparison of the angiographic data obtained by quantitative coronary angiography (QCA)

Parameter	Laser Group	Control group	p-values
Reference diameter of blood vessel [mm]	3.20 ± 0.44	3.11 ± 0.43	0.756
MLD before procedure [mm]	0.83 ± 0.45	0.85 ± 0.42	0.685
MLD following the procedure [mm]	2.71 ± 0.38	2.52 ± 0.37	0.314
MLD in control angiography [mm]	2.18 ± 0.70	1.76 ± 0.74	< 0.05
Acute gain after PCI [mm]	1.88 ± 0.68	1.67 ± 0.73	0.462
Late Lumen Loss in control examination [mm]	0.53 ± 0.68	0.76 ± 0.76	< 0.01
Late Lumen Los Index in control examination	0.28 ± 0.39	0.46 ± 0.43	< 0.005

MLD – minimum luminal diameter; PCI – percutaneous coronary intervention.

Table 3. Results of biochemical analyses

Parameter	Examination	Laser group	Control group	p-values
Endothelin-1 [pg/mL]	0	1.01 ± 0.48	0.80 ± 0.51	0.082
	6 h	1.20 ± 0.26	0.66 ± 0.25	p < 0.001
	12 h	0.89 ± 0.40	0.96 ± 0.57	0.149
	1 m	0.79 ± 0.31	0.60 ± 0.13	0.090
Nitrites/nitrates [µmol/L]	0	39.7 ± 1.80	41.9 ± 2.1	0.064
	6 h	29.9 ± 1.1	27.0 ± 0.6	p < 0.001
	12 h	32.0 ± 1.4	25.0 ± 1.0	p < 0.001
	1 m	63.7 ± 4.5	38.4 ± 2.2	p < 0.001

group, angiography was performed in 37 cases and restenosis was found in 12 patients (32.4%). These differences were not statistically significant.

In the irradiated group, smaller average narrowing of the lumen (32.0% vs 43.5% in the control, $p < 0.05$) resulting from a smaller average stenosis in patients diagnosed with restenosis (59.1% vs 78.8%, respectively, $p < 0.01$) was observed. The differences in the 2 groups of patients without restenosis were not statistically significant (27.5% vs 32.2%, $p > 0.05$).

Vessel QCA values including the average measurements of lumen size, its growth and loss before and after the treatment and in the follow-up for both groups, are shown in Table 2 and the biochemical data is presented in Table 3.

Endothelin-1 levels between the two groups showed statistically significant differences at the 6th hour. The analysis of the concentration of endothelin-1 in each of the groups revealed statistically significant differences between the results obtained after 6 h, after 12 h, and 1 month after the procedure in the laser group. In the control group, no significant differences were observed between the tests. The observed differences between the 2 groups, within the groups, and between successive tests are presented in Fig. 1.

The comparison of the nitrite/nitrate levels in both groups revealed statistically significantly higher values in the laser-treated group relative to the control group between each of the tests except the one performed prior to the procedure. The nitrite/nitrate levels revealed statistically significant differences between each test in each group and in all cases. The results are presented in Fig. 2.

Discussion

This is the first study to demonstrate that the protective effect of LLLT on restenosis following PCI may be mediated by preventing the decrease in nitric oxide bioavailability and attenuating the increase in endothelin-1 levels.

The negative effect of endothelin-1 on blood vessels may be associated not only with its vasoconstrictory properties, but also with its mitogenic effects. There have been a few reports of an increase in the concentration of endothelin-1 after angioplasty, which results from mechanical damage to the vascular wall, but it does not appear significant in all papers.[12–14] In this study, there was no statistically significant increase in the levels of endothelin-1 after PCI in either group, although the difference between groups at 6 h after surgery was significant – a higher mean concentration was observed in the laser-treated group. This effect can probably be associated with a slightly higher vascular injury in the irradiated patients, because in addition to angioplasty, a special balloon was used for exposure. In subsequent tests, there was a significant reduction in mean plasma endothelin-1 but still no differences were observed between the two groups.

Nitric oxide shows beneficial effects during angioplasty.[1] It may also positively influence the regeneration and proliferation of endothelial cells and inhibit the pathological remodeling of the artery.[2] In this study, we observed a decrease in the average nitrite/nitrate concentrations in both groups within several hours (6 and 12 h) after the procedure, which may be associated with endothelial damage. However, the decrease was significantly smaller in the irradiated group, which can be explained with the stimulating effects of laser radiation. Furthermore, the follow-up after 1 month revealed that mean nitrite/nitrate levels in the laser-treated group significantly exceeded the initial values, while in the second group they were lower than at baseline.

Laser radiation may exert biochemical effects as it has an impact on cytochromes, resulting in increased NO production, as shown in several reports.[15–17] It was further observed in animal studies, where a relationship between the increase in NO concentration as a result of irradiation, and the reduction of restenosis were observed.[18,19] Our results of this human study confirm those reports.

So far, there have been only a few publications regarding the use of LLLT during angioplasty. Previous experimental studies involved radiation at 632 nm wavelength (helium-neon laser), then radiation at 650 nm (semiconductor laser) in animals and clinical trials.[5,6,20–23] In our model, we used radiation at 808 nm wavelength, since the available literature has no reports on the most effective radiation wavelength and energy dose in this type of procedure.[7,24] The need for a single radiation therapy forced the use of a relatively large dose of radiation. It should, however, be noted that a certain amount of the radiation

Fig. 1. Comparison of the endothelin-1 levels between analyzed groups at particular steps of study protocol

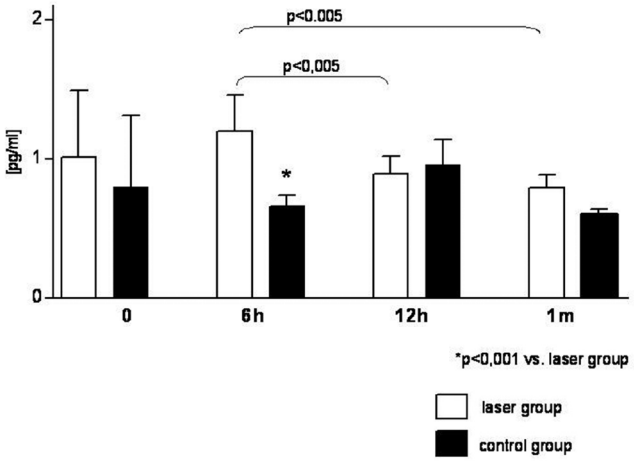
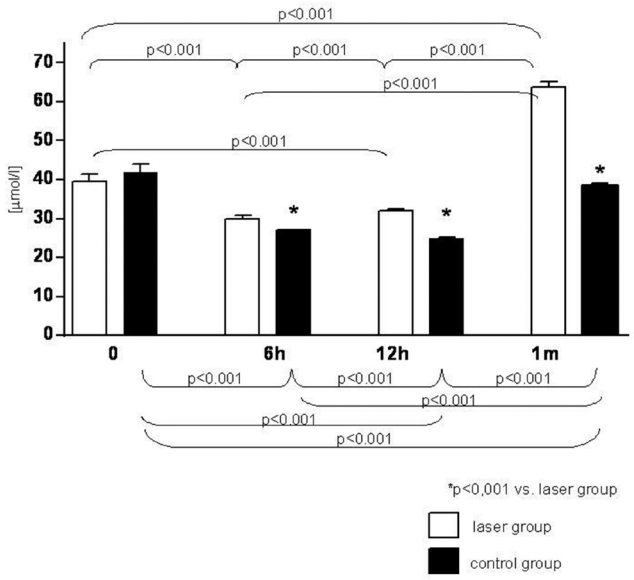


Fig. 2. Comparison of the nitrite/nitrate levels between analyzed groups at particular steps of study protocol



is absorbed or reflected (isolated cells, calcification of the stent structure if applicable), which reduces the effective dose. Unfortunately, this amount is not possible to estimate. It seems that it is important to use an energy capacity that does not exceed 100 mW/cm^2. At a higher power density values may cause an adverse thermal effect, which intensifies the process of restenosis.

To conclude, the results of our study show that LLLT applied during the PCI procedure can influence the process of restenosis by modifying NO and endothelin-1 release.

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