Modulatory effect of laser irradiation on mastoparan-7-induced contraction

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Abstract. Mastoparan-7 activates guanine nucleotide-binding proteins (G-proteins) and stimulates both apoptosis and increases in cytoplasmic calcium concentration and may induce smooth muscle contraction. The primary aim of the present study was to evaluate the modulatory effect of laser stimulation on vascular smooth muscle contraction induced by direct stimulation of G-protein with mastoparan-7. Experiments were performed on isolated and perfused tail arteries of Wistar rats. Contraction force in the model was measured by increased levels of perfusion pressure with a constant flow. Irradiation treatment was applied directly to the blood vessels. The laser was applied in increasing doses of 10 mW (E=1.8 J), 30 mW (E=5.5 J) and 110 mW (E=19.8 J). Time of exposure was 3 min for each irradiation. In the laser-stimulated arteries, a significant and dose-dependent decrease was observed. The half maximal effective concentration values were $4.43\pm2.2\times10^{-8}$, $2.4\pm0.56\times10^{-7}$, $3.2\pm0.72\times10^{-7}$ and $7.7\pm0.3\times10^{-7}$ M/l in the control and 10, 30 and 110 mW laser irradiation groups, respectively. Significant (P<0.001) changes were identified for all laser treatment groups in comparison with the control. When analyzing the function of calcium ion (Ca^{2+}) stores was analyzed, a significant inhibition of influx from both intra- and extracellular Ca^{2+} stores was observed. The results from the present study suggested that contraction induced by direct activation of G-protein with mastoparan-7 may by effectively inhibited by laser radiation, and that the effect was associated with an inhibition of Ca^{2+} influx from both intracellular and extracellular Ca^{2+} stores.

Introduction

Laser biostimulation with low and mid-level laser therapy has been used for a number of years as a well-known method of healing soft and hard tissues (1-8). In addition, the anti-inflammatory effects of this therapy have been described extensively (2,6,9). Low-level laser irradiation (LLLI) is able to modify different biological pathways, including increases in mitochondrial respiration, and ATP synthesis proliferation of mesenchymal stem cells and cardiac stem cells (10,11).

The potential mechanism of the effect of light on biological structures, in particular vascular smooth muscle, have been examined previously; experimental studies have suggested that lasers of power <100 mW cause relaxation of smooth muscle of blood vessels (12-15). Two studies (11,13) have demonstrated that LLLI may cause photorelaxation of blood vessels, including coronary arteries, and may prevent their restenosis following percutaneous transluminal coronary angioplasty (11,16). A number of experimental studies analyzed the effect of LLLI on vascular smooth muscle cells following pretreatment with α-adrenoceptors agonists (adrenalin and phenylephrine) and peptides, including vasopressin or endothelin-1 (17,18). To the best of our knowledge, the effect of non-receptor stimulation has not been analyzed previously.

Mastoparan-7 is a basic tetradecapeptide isolated from wasp venom. The mechanism of its action is associated with the activation of guanine nucleotide-binding proteins (G-proteins). The peptide has been demonstrated to stimulate phospholipase C (PLC) in several cellular compartments such as rat mast cells, rat hepatocytes and human HL-60 leukemia cells. By contrast, inhibition of PLC by mastoparan has been demonstrated in SH-SY5Y human neuroblastoma cells and in human astrocytoma cells (19). In vascular smooth cells, it is able to increase contractility (20-22).

Calcium ions (Ca^{2+}) serve a central role in general cell processes. According to pathological factors, Ca^{2+} concentration changes occur in various cell compartments, which may induce apoptosis (23-25). An increase in Ca^{2+} describes one of final elements of programmed cell death and physiological concentrations may differ significantly in cell compartments.

Our previous studies confirmed that mastoparan-7 is able to increase the calcium load in smooth muscle cytoplasm by activation of calcium influx from intra and extracellular calcium stores in an isolated resistant artery model (20,21). In addition, during ischemia and reperfusion, the pathway associated with direct stimulation of G-proteins remains active (22).

The primary aim of the present study was to evaluate the modulatory effect of low and moderate laser irradiation on vascular smooth muscle contraction induced by direct stimulation of G-proteins with mastoparan-7.

Materials and methods

Animals. Experiments were performed on isolated and perfused tail arteries of Wistar rats (10 males; age, 8 weeks; body weight, 250-270 g; Hodowla Zwierząt Laboratoryjnych). Animals were housed at 20-21˚C with a humidity of 50-60% and 12-h light/dark cycles and had *ad libitum* access to food and water. Prior to tail artery isolation, rats were anesthetized by intraperitoneal injection of 1,200 mg/kg urethane and euthanized by cervical dislocation. The study protocol was approved by the Local Ethics Committee for Experiments on Animals, (Bydgoszcz, Poland). All studies were performed in accordance with the United States of America National Institutes of Health guidelines (26).

Drugs and solutions. Mastoparan-7 (G-protein activator), mastoparan-17 (negative control), and Krebs solution containing NaCl (71.8 mM/l), KCl (4.7 mM/l), $CaCl_2$ (1.7 mM/l) $NaHCO_3$ (28.4 mM/l), $MgSO_4$ (2.4 mM/l), KH_2PO_4 (1.2 mM/l) and glucose (11.1 mM/l) were purchased from Sigma Aldrich; Merck KGaA.

Study design and conduction. Following dissection from the surrounding tissues, 2-3-cm long segments of rat tail arteries were cannulated and connected to a perfusion device. The distal part was held in place with 500 mg weight and the tail was placed in a 20 ml container filled with oxygenated Krebs solution at 37˚C (pH 7.4). The perfusion pressure was continuously measured. Perfusion solution flow was gradually increased using a peristaltic pump to 1 ml/min, until the optimum perfusion pressure of 2-4 kPa was reached (25). The study utilized a semiconductor laser (400 mW, wavelength =810 nm), operating in continuous-wave mode. Subsequent to achieving maximal vasospasm, the arteries were rinsed and stabilized for a period of 30 min prior to exposure to laser irradiation. The arteries were placed on a plate and the laser header was positioned on a tripod ~1 cm from the irradiated tissue. The irradiation was applied directly on the blood vessels without utilization of a glass chamber. The laser was applied in increasing doses of 10 mW ('L1'; E=1.8 J), 30 mW ('L2'; E=5.5 J), 110 mW ('L3'; E=19.8 J). Time of exposition was 3 min for each irradiation (17). In experiments performed on endothelium-denudated arteries, the endothelium was

removed with the compressed air (temperature=37˚C, pressure = 1.0-1.1 atm). The absence of endothelium was confirmed with an acetylcholine test (single dose of acetylcholine 10^{-5} M/l). To evaluate constriction associated with the intracellular calcium, experiments were performed in calcium-free Krebs solution (phase 1) and after switching to the Krebs solution analysis of constriction related to calcium influx from extracellular space was performed (phase 2).

Data analysis and statistical procedures. Investigations were performed using a TSZ-04 system (Experimetria Kft.). Perfusion pressure was measured on BPR-01 and BPR-02 (Experimetria Kft.) devices, and vascular smooth muscle tension was measured using an FSG-01 transducer connected with a Graphtec GL820 midi LOGGER digital recorder (Graphtec Corporation). All transducers used in the experiments were produced by Experimetria Kft. Concentration-response curves (CRCs) were calculated according to the van Rossum method (27). Maximum response of tissue (E_{max}) was calculated as a proportion of the maximal response for phenylephrine. The half maximal effective concentration (EC_{50}) was estimated using classical pharmacologic methods with the pD_2 value, the negative logarithm of the EC_{50}. The CRC and E_{max} values were used in all calculations estimating the statistical significance. Mastoparan-17 was included in all experiments as the negative control.

Results are presented as mean ± standard deviation. Tukey's Honest Significant Difference test was used following two-way analysis of variance for multiple comparisons of means. P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using STATISTICA 12.5 (StatSoft, Inc.).

Results

Concentration-response curve obtained for mastoparan-7 presents a sigmoidal association. In the laser-irradiated arteries, a significant rightward shift with significant decrease in maximal responses was observed. This effect was dependent on the power of laser irradiation (Fig. 1). For all samples in which the effect was measured as ≥20%, the difference was deemed statistically significant. The EC_{50} values were $4.43 \pm 2.2 \times 10^{-8}$, $2.4 \pm 0.56 \times 10^{-7}$, $3.2 \pm 0.72 \times 10^{-7}$ and $7.7 \pm 0.3 \times 10^{-7}$ M/l in the control and 10, 30 and 110 mW laser irradiation groups, respectively. Significant (P<0.001) changes were observed for all laser power groups in comparison with the control.

In experiments performed on the endothelium-denudated arteries, the EC_{50} values were $2.25 \pm 2.5 \times 10^{-9}$, $2.73 \pm 1.9 \times 10^{-9}$, $2.63 \pm 2.1 \times 10^{-9}$ and $2.15 \pm 1.8 \times 10^{-9}$ M/l in the control and 10, 30 and 110 mW laser irradiation groups, respectively. Values did not differ significantly. All E_{max}, EC_{50} and pD_2 values are presented in Table I. Changes in trends in pD_2 values are presented in Fig. 2.

When analyzing the changes in perfusion pressure as a result of intracellular Ca^{2+} influx during mastoparan-7-induced contraction in the control group, a significant increase was observed in comparison with the negative control, mastoparan-17 (P<0.001). Following laser irradiation at 10, 30 and 110 mW, a significant decrease in vascular reaction
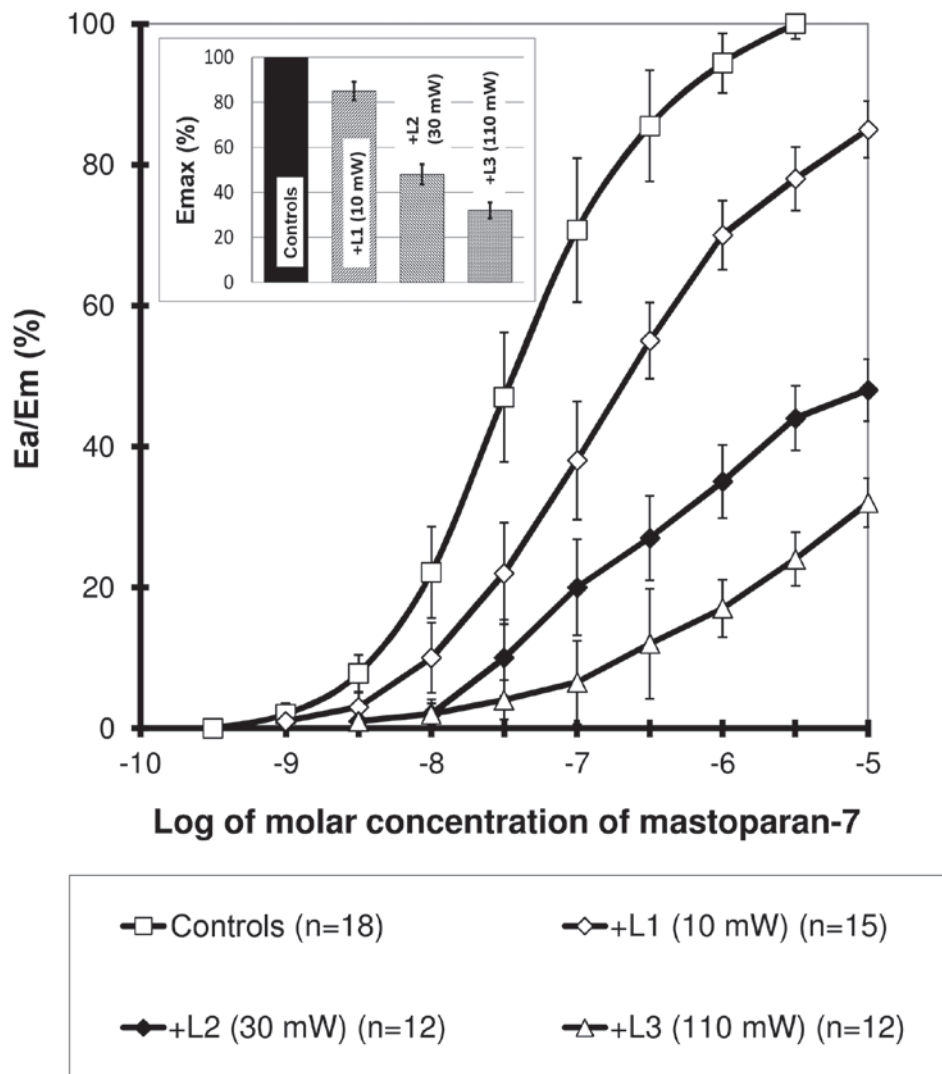
Figure 1. Concentration response curves and E_{max} values for mastoparan-7 in the control and in laser-irradiated arteries (10, 30 and 110 mW). E_{max}, maximum response of tissue.

was observed following influx of extracellular Ca^{2+} into the cytoplasm, whereas in intracellular Ca^{2+}, a significant decrease was only identified at 30 and 110 mW (Table II; Fig. 3). No additional benefit in increasing the laser power from 30 to 110 mW was observed.

Discussion

Arterial smooth muscle function has been investigated in a number of different studies, involving typical pharmacological stimulation with the use of receptor agonists including α_1-adrenoceptors, vasopressin type 1 receptors or the L-type calcium channel (20-25,28,29). Direct stimulation of G-proteins with mastoparan-7 was described in certain studies (20-22,29,30). The present study investigated the possibility of the induction of photorelaxation in laser-pretreated arteries. Results from our previous studies confirmed the occurrence of photorelaxation in arteries stimulated with receptor agonists (17,18,29,30). Constriction of vascular smooth muscle cells in the presence of mastoparan-7 was demonstrated by Kanagy and Webb (29), and Birnbaumer (30) described experiments on spiral cutting fragments of the common carotid artery.

In the present study, laser irradiation significantly inhibited mastoparan-7-induced constriction of vascular smooth muscle cells. In addition, proportional to the laser power, a decrease in perfusion pressure caused by intra- and extracellular Ca^{2+} influx inhibition was observed. Mastoparan-7 leads to degranulation of mast cells by activation of phospholipase A_2 at a concentration of $5x10^{-5}$ M/l (31).

Previous studies performed on vessels suggested that laser photorelaxation in vessels stimulated pharmacologically is partially endothelium-dependent process (17,18), therefore degradation of the vascular endothelium may able to eliminate the inhibitory effect of laser irradiation on vessels reactivity. In the experiments performed in the present study, removal of the vascular endothelium prevented the inhibitory effect of laser irradiation on vessel walls stimulated by G-protein with mastoparan-7; consequently, we concluded that laser vasodilation in mastoparan-7-induced arteries may be an endothelium-dependent process. Similar effects were observed for endothelin-1 and phenylephrine stimulation of entothelin-1 and α_1-adrenoceptors, respectively (17).

Similar effects of LLLI-induced vasorelaxation were described by Gal *et al* (12) and Steg *et al* (13). Results of their

Table I. EC50, maximal response and relative potency for mastoparan-7 in controls and in the 10, 30 and 110 mW laser irradiation groups for normal and endothelium-denudated arteries.

A, Normal vessels

Groups	N^a	$\%E_{max}{}^b$	EC_{50} (M/l)	pD_2	RP^c	P-value
Control	18	100	$4.43\pm2.2\times10^{-8}$	7.35 ± 0.21	1.000	-
Laser treatment, mW						
+L1 (10)	15	85 ± 8	$2.4\pm0.56\times10^{-7}$	6.62 ± 0.13	0.185	<0.0001
+L2 (30)	12	48 ± 8	$3.2\pm0.72\times10^{-7}$	6.49 ± 0.09	0.138	<0.0001
+L3 (110)	12	32 ± 7	$7.7\pm0.3\times10^{-7}$	6.11 ± 0.20	0.058	<0.0001

B, Endothelium-denudated artery

Groups	N^a	$\%E_{max}{}^b$	EC_{50} (M/l)	pD_2	RP^c	P-value
Control	18	100	$2.25\pm2.5\times10^{-9}$	8.65 ± 0.24	1.000	-
Laser treatment, mW						
+L1 (10)	15	85 ± 8	$2.73\pm1.9\times10^{-9}$	8.56 ± 0.21	1.213	ns
+L2 (30)	12	48 ± 8	$2.63\pm2.1\times10^{-9}$	8.58 ± 0.09	1.169	ns
+L3 (110)	12	32 ± 7	$2.15\pm1.8\times10^{-9}$	8.67 ± 0.20	0.956	ns

[a]Number of concentration-response curves used for calculations. [b]E_{max}, calculated as a percentage of maximal response for controls. [c]RP, calculated as EC_{50} for controls/EC_{50}. E_{max}, maximum response of tissue; EC_{50}, half maximal effective concentration; pD_2, negative logarithm of the EC_{50}; RP, relative potency; ns, not significant.
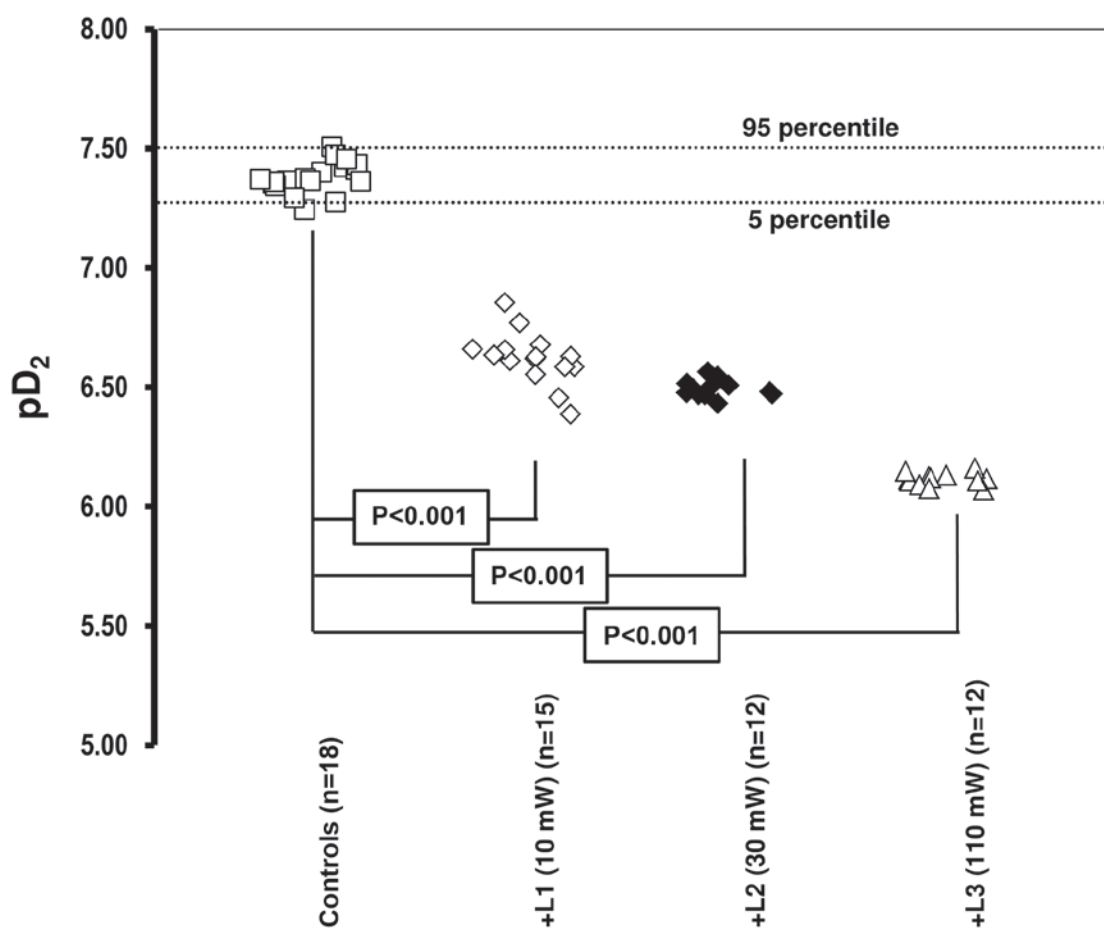

Figure 2. pD_2 values for mastoparan-7 in the control and laser irradiated (10, 30 and 110 mW) groups.

Table II. Maximal perfusion pressure for mastoparan-7 induced contraction activated by Ca^{2+} influx from intracellular (phase 1) and extracellular Ca^{2+} stores (phase 2) in the control and laser irradiated (10, 30 and 110 mW) groups.

Groups	Phase 1		Phase 2	
	n	Perfusion pressure, mmHg	n	Perfusion pressure, mmHg
Control	16	19.1±3.2	16	31.2±5.9
Laser treatment, mW				
+L1 (10)	12	15.2±1.7[f]	12	21.9±3.1[a]
+L2 (30)	12	12.4±3.5[a,c]	12	11.2±3.8[b,d]
+L3 (110)	12	11.8±5.6[a,e]	12	12.3±5.7[b,d]
Mastoparan-17	10	12.2±5.4[a]	10	10.3±4.7[b]

[a]$P<0.05$ and [b]$P<0.001$ vs. control; [c]$P<0.05$ vs. L1; [d]$P<0.0001$ vs. L1; [e]$P=0.06$ vs. L1; [f]$P=0.07$ vs. control. Data are presented as the mean ± standard deviation.
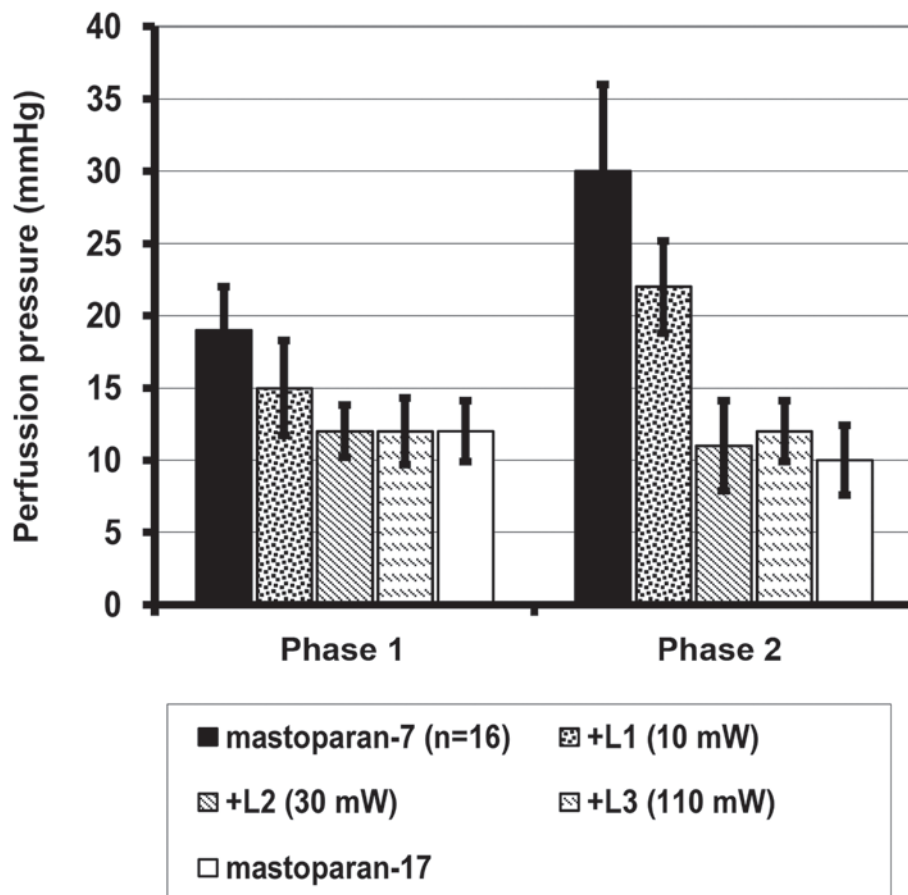

Figure 3. Effect of guanine nucleotide-binding protein activation by mastoparan 7 on perfusion pressure, accompanied by changes in intra- and extracellular Ca^{2+} levels in comparison with the negative control (mastoparan-17) and in laser irradiated arteries (10, 30 and 110 mW).

studies indicated that laser irradiation at a power <100 mW was responsible for constant smooth muscle relaxation, whereas continuous wave laser at a power <1 W induced vasoconstriction (14). By contrast, the opposite effect of endothelium removal was described by Gal *et al* (12) and Steg *et al* (13), who postulated that the vasodilatory effect of laser treatment was not endothelium-dependent. These studies were able to induce smooth muscle dilation in endothelium-denudated arteries using *in vitro* and *in vivo* experiments. In addition, Steg *et al* (13)

observed relaxation of smooth muscles induced by low level pulse lasers, even if the muscles had not been previously contracted (13). Maegawa *et al* (32) also described vasodilation of arterioles in a study investigating the effect of LLLI smooth muscle reactivity. This study suggested that the observed effects were partially due to NO release from the vascular endothelium, particularly in the initial phase, whereas in the late phase LLLI induced a decrease of Ca^{2+} in microvascular smooth muscles. Generally, the process of LLLI-induced vasodilation appears to

be a result of processes occurring in the large and small vessels, pre-capillary sphincteromas and microcirculation.

In conclusion, the results of the present study suggest that contraction induced by direct activation of G-protein with mastoparan-7 may by effectively inhibited by laser irradiation, and may be an endothelium-dependent process. In addition, this effect was laser power-dependent, and associated with the inhibition of Ca^{2+}influx from both intra and extracellular calcium stores.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article excluding raw data, which are available from the corresponding author on reasonable request.

Authors' contributions

EG, MMM, HMN, WH and GG conceived and designed the study. EG, MW and GG performed the experiments. EG, MMM, HMN, MW and GG collected the data. EG, MMM, HMN, IB, MK, AK, WH and GG analyzed the data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Local Ethics Committee for Experiments on Animals, Bydgoszcz, Poland. All studies were performed in accordance with the United States of America National Institutes of Health guidelines.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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