MORPHOLOGY AND PATHOMORPHOLOGY

Role of Nitric Oxide in the Reaction of Arterial Vessels to Laser Irradiation

V. M. Chertok, A. E. Kotsyuba, and E. V. Bespalova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 6, pp. 699-703, June, 2008
Original article submitted October 4, 2007

Reactivity of arterial vessels in the small intestine mesentery to irradiation with a helium-neon laser before and after NO synthase blockade was studied by means of biomicroscopy. Blood flow velocity and vascular diameter increased under conditions of laser irradiation. During irradiation, arterial vasodilation was inversely related to the initial diameter. After treatment with NO synthase inhibitor, the dilatory response of vessels to laser irradiation was completely abolished (arteries, diameter >80 μ) or decreased by 2 times (arterioles, diameter <50 μ).

Key Words: *microcirculation; biomicroscopy; laser radiation; NO synthase blockade; L-arginine*

Low-intensity laser radiation (LILR) increases the blood flow rate, causes microvascular dilation, and improves microcirculation, which determinesstrong therapeutic effect of laser irradiation in clinical practice [4,5]. Recent studies showed that NO plays a role in this process. NO is involved in the realization of vasomotor reactions [2,4,7]. As differentiated from great vessels, NO has little role in the reactivity of arterioles [9]. LILR-induced microcirculatory changes are associated with the influence or interaction of various signal substances [3,8].

Here we studied the role of NO in arterial reactivity to LILR.

MATERIALS AND METHODS

Experiments were performed on 52 outbred albino rats weighing 250-270 g. The animals were anesthetized with intramuscular injection of nembutal (5 mg/100 g) and placed on a microscopic stage under thermostatic conditions. Mesenteric arteries of the small intestine were studied under normal conditions and during exposure to various factors. The arteries in group 1 animals were examined after continuous irradiation with a LGN-108 helium-neon laser for 10 min (λ=0.63 μ, output power 2 mW). Microvessels in group 2 rats were examined after injection of a NO synthase inhibitor L-NAME (2 mg/kg into the femoral vein). Group 3 rats received intravenous injection of 100 mg/kg L-arginine 5 min after treatment with L-NAME. Group 4 animals were irradiated 5 min after injection of L-NAME into the femoral vein.

The vascular diameter and blood flow velocity in animals of each group were measured on an Allegro-MC automatic image analysis system. The methods of sampling and quantitative biomicroscopy were described previously [1,7]. Each experimental group consisted of at least 5 animals. The diameter and blood flow velocity in various arteries of each rat were measured over 3 min after preparation (control values).

Department of Human Anatomy, Vladivostok State Medical University, Russia. ***Address for correspondence:*** chertokv@mail.ru. V. M. Chertok

0007-4888/08/1456-0751

RESULTS

Laser irradiation markedly affected reactivity of arterial vessels in group 1 rats. Significant increase in blood flow velocity over the first minutes after irradiation was accompanied by typical morphological signs. Optical density of blood flow increased. The peripheral plasma layer near the vascular wall was narrowed and then disappeared. We revealed the appearance of additional anastomoses and increase in the vascular diameter (Fig. 1, *a-d*).

The observed changes were most pronounced 3-10 min after irradiation. The vascular diameter increased more slowly and less significantly than blood flow velocity (Fig. 2, *a*, *b*). Arterial vasodilation was inversely related to the initial diameter of microvessels. The arterioles with a diameter of 30-60 μ were most sensitive to LILR. After laser irradiation, the diameter of arterioles increased more significantly than that of large vessels. Changes in the diameter of arteries with an initial diameter of more that 100 μ were observed 10 min after irradiation and did not exceed 12% ($p<0.05$). However, the diameter of vessels with an initial diameter of less than 60 μ exceeded the basal level by more than 20% ($p<0.05$). The exceptions were thin precapillary arterioles. The majority of these vessels exhibited poor response to laser irradiation. Only 6-8 of 18-20 arterioles demonstrated pronounced responses to LILR. Deviations in blood flow velocity and diameter of these arterioles were 10-16%.

High sensitivity of arterioles to LILR was reported previously. Arterial smooth muscle cells serve as the major acceptor of laser energy in microcirculatory vessels [5]. Differences in the reactivity of various arterioles to laser irradiation are associated with structural and functional characteristics of the contractile apparatus in these vessels. Recent studies showed that blood cells play a role of the acceptor. For example, LILR energy absorption by leukocytes is accompanied by increased production of bioactive substances (*e.g.*, NO) improving microcirculation [3,4]. However, there are no data that vessels of various diameters have different sensitivity to leukocyte priming products.

Intravenous injection of L-NAME to group 2 animals was followed by a short-term increase in blood flow velocity and vascular diameter. The vascular resistance tended to increase, while blood flow decreased under these conditions (Fig. 2, *a*, *b*). Blood volume in several microvessels decreased 5-7 min after treatment. Lightening and narrowing of the axial flow were accompanied by an increase in the peripheral plasma layer near the vascular wall (Fig. 3, *a*, *b*). Erythrocyte flow in small arteries (10-30 μ in diameter) became granular and was replaced with the plasma, which resulted in "lightening" or "disappearance" of vessels.

Large arteries in these rats were less sensitive to NO synthase blockade. Blood flow velocity in these vessels remained practically unchanged ($p>0.05$). After administration of the test substance, the diameter of large vessels decreased less significantly compared to that of small arterioles (by 6-8 and 12-17%, respectively, $p<0.05$). The observed changes in vascular reactivity are probably related to vasoconstriction and increase in blood pressure, which results from intravenous injection of NO synthase inhibitor [6]. It cannot be excluded that this process also involves an endothelium-derived vasoconstricting factor (*e.g.*, endothelin). The effect of this substance is balanced by NO under normal conditions, but results in significant vasoconstriction at low concentration of NO [10]. Vascular tone and blood pressure are determined by an equilibrium between the vasoconstrictor and vasodilator effect of vasoactive substances on smooth muscle cells of the vascular wall.

Further treatment with L-arginine (group 3) abolished changes induced by NO synthase inhibitor (Fig. 2, *a*, *b*). However, the diameter of large and small vessels remained below the control value (by 3-4 and 6-7%, respectively).

Vascular reactivity of the microcirculatory bed in group 4 rats was studied under laser irradiation, which occurred 5 min after intravenous injection of L-NAME (Fig. 2, *a*, *b*). The diagrams illustrate hemodynamic parameters of arterioles with different diameter, which are exposed to laser irradiation before and after administration of NO synthase inhibitor. L-NAME modulated the response of various microvessels to laser exposure. Variations in blood flow velocity in group 4 rats were similar to those in group 1 animals (laser irradiation without NO synthase blockade). However, the increase in blood flow velocity was less significant and did not result in the vasodilator response. After treatment with NO synthase inhibitor, the dilatory response of vessels to laser irradiation was completely abolished (arteries, diameter >80 μ) or decreased by 2 times (arterioles, diameter <50 μ).

These findings indicate that NO mediates the response of microcirculatory vessels to LILR. Our results are consistent with published data that the laser radiation-induced increase in blood flow velocity is followed by an increase in endothelial shear stress and NO synthesis. NO causes relaxation of vascular smooth muscles [7]. We believe that vascular reactivity under LILR is determined not only by NO, but also by other substances. NO primarily

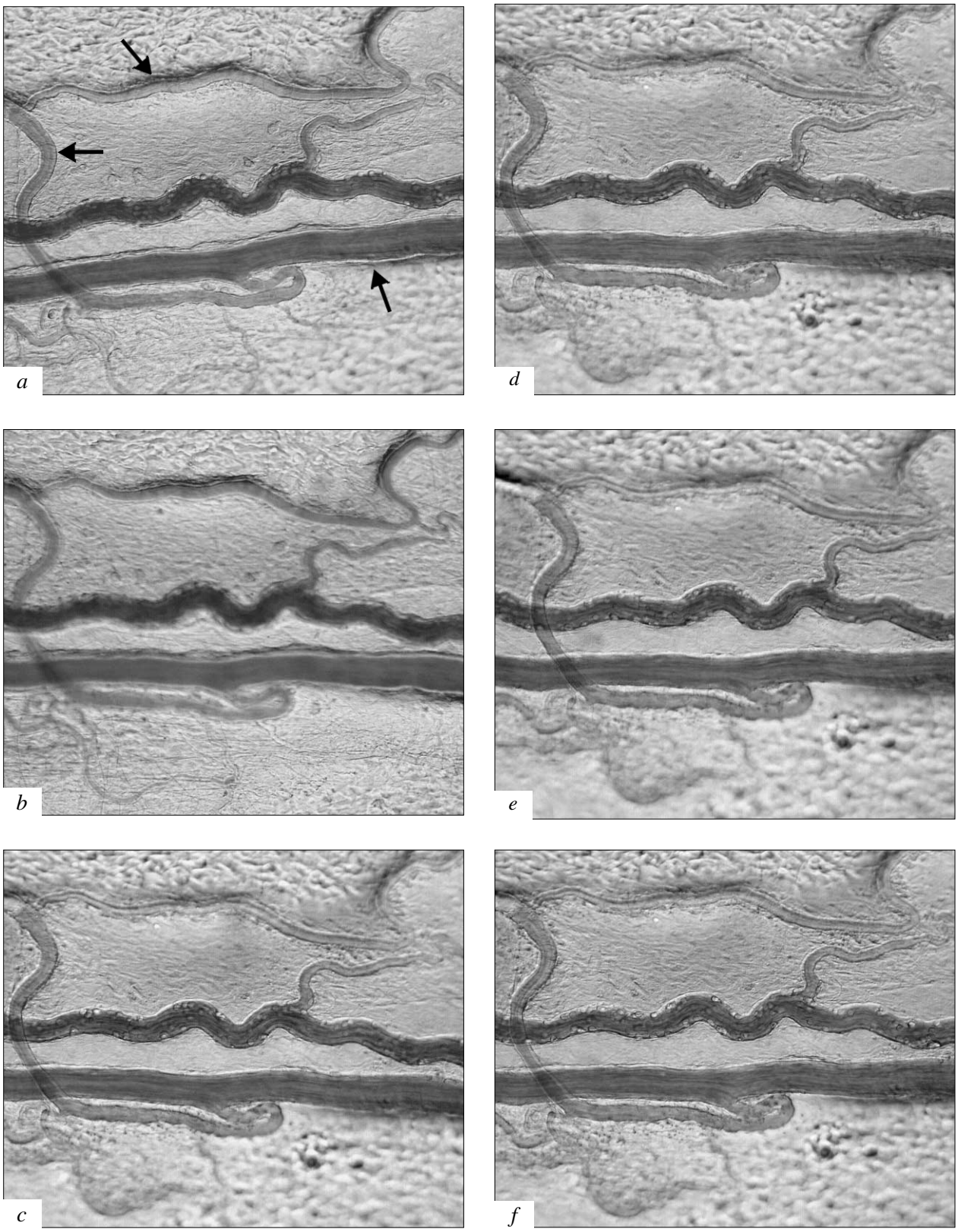

Fig. 1. Arterial bed of the small intestine mesentery under control conditions (*a*) and 30 sec (*b*), 1 min (*c*), 2 min (*d*), 3 min (*e*), and 5 min (*f*) after laser exposure. Biomicroscopy, ×100.

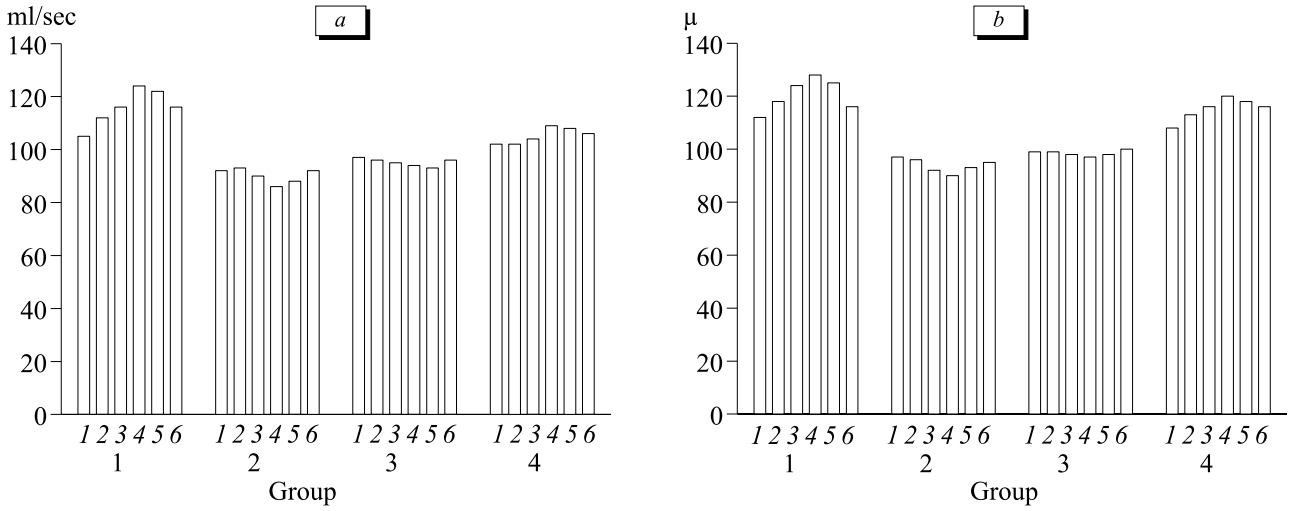

Fig. 2. Blood flow velocity (*a*) and diameter of arterial vessels (*b*) under various conditions. Diameters: 130-140 (*1*), 100-120 (*2*), 60-80 (*3*), 40-50 (*4*), 20-30 (*5*), and 10-15 μ (*6*).

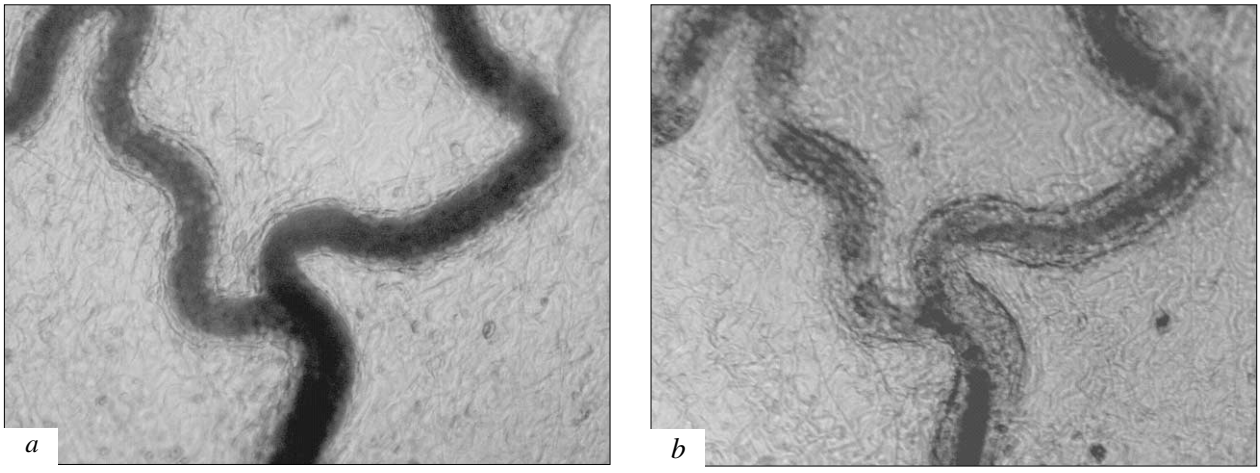

Fig. 3. Arteriolar response 5 (*a*) and 7 min (*b*) after intravenous injection of L-NAME. Biomicroscopy, ×100.

affects the arterioles with a diameter < 50 μ, where we observed more pronounced changes in reactivity in response to combined exposure to NO synthase blockade and LILR.

REFERENCES

1. A. A. Afanas'ev, A. E. Kotsyuba, and V. M. Chertok, *Tikhookeansk. Med. Zh.*, No. 3, 65-68 (2002).
2. G. E. Brill' and A. G. Brill', *Lazer. Med.*, No. 1, 39-42 (1997).
3. N. D. Devyatkov, S. M. Zubkova, I. B. Laprun, and N. S. Makeeva, *Uspekhi Sovrem. Biol.*, **103**, No. 6, 31-43 (1998).
4. G. I. Klebanov and Yu. A. Vladimorov, *Ibid.*, **119**, No. 5, 462-475 (1999).
5. V. I. Kozlov, T. M. Soboleva, G. A. Azizov, *et al.*, *Fiziol. Zh.*, **77**, 55-67 (1991).
6. L. I. Osadchii, T. V. Balueva, and I. V. Sergeev, *Ibid.*, **89**, 810-816 (2003).
7. V. M. Chertok, A. E. Kotsyuba, and E. V. Bespalova, *Tikhookeansk. Med. Zh.*, No. 3, 48-52 (2007).
8. A. F. Haas, J. W. Wong, C. K. Iwanashi, *et al.*, *Free Rad. Biol. Med.*, **25**, No. 9, 998-1005 (1998).
9. A. Koller, D. Sun, A. Huang, and G. Kaley, *Am. J. Physiol.*, **267**, H326-H332 (1994).
10. E. H. Ohlstein and B. L. Stroer, *J. Neurosurg.*, **77**, No. 2, 274-278 (1992).