SIMULATION OF MICROCIRCULATORY DISORDERS BY THE USE OF AN ULTRAVIOLET LASER

A. M. Chernukh,* V. S. Shinkarenko, L. S. Ivanov, O. V. Alekseev, and V. S. Sinyakov

UDC 616.16-005-092.9-02:615.849.19

Responses of venules, capillaries, arterioles, and mast cells of the rat mesentery to irradiation by the LGI-21 pulsed ultraviolet laser (λ = 337 nm) was studied by intravital microscopy. The diameter of the laser beam varied from 2 to 100 μ. The processes studied were recorded and analyzed by a photographic and videorecording method. Depending on the intensity and duration, irradiation caused either an increase in permeability of the vessel walls, or thrombus formation, or hemorrhage. Besides injury to the vessel wall, an important role in thrombus formation was played by factors appearing on destruction of erythrocytes and other blood cells. The change in diameter and permeability of the vessels during laser irradiation of the mast cells could be associated with liberation of the histamine and serotonin contained in them.

KEY WORDS: microcirculation, vascular permeability; hemmorrhage; thrombus formation; lasers.

Laser techniques are playing an increasingly important role in the study of mechanisms of hemoaggregation and thrombus formation [6, 8]. The use of lasers to irradiate mast cells [2] in order to shed further light on their role in the regulation of the state of the microcirculation is a promising development.

The object of this investigation was to assess the possibility of using lasers for simulating and analyzing typical forms of microcirculatory disturbances.

EXPERIMENTAL METHOD

Experiments were carried out on nonibred albino rats weighing 250-270 g. The anesthetized rat was placed on the constant-temperature stage of the microscope. Through an incision in the abdominal wall a loop of small intestine was brought out and its mesentery was placed on the light guide of an intravital microscope. To prevent the mesentery from drying it was covered with a layer of PMS-500 (polymethylsiloxane). Observations on the mesenteric microvessels were made under different powers of magnification (10, 58, UV, and 90× objectives). The apparatus described previously [3], consisting of an intravital microscope and coupled laser, was used. The LGI-21 molecular nitrogen ultraviolet laser with a wavelength of 337 nm and a mean output power of 2 mW was used (output pulsed power 1600 W for a pulse duration of 10 nsec). The optical coupling system ensured that the laser beam passed through the center of the objective. The diameter of the injuring beam could be smoothly controlled within the range 2-100 μ by means of a special diaphragm. The structure chosen to be irradiated was placed opposite the laser sight, built into the ocular of the microscope. Vessel walls in different parts of the microcirculation were injured: venules, arterioles, and capillaries; individual erythrocytes in the lumen of the vessels and mast cells were destroyed.

*Academician of the Academy of Medical Sciences of the USSR.

Laboratory of General Pathology and Experimental Therapy, and Bioengineering Laboratory, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 1, pp. 16-19, January, 1976. Original article submitted April 11, 1975.

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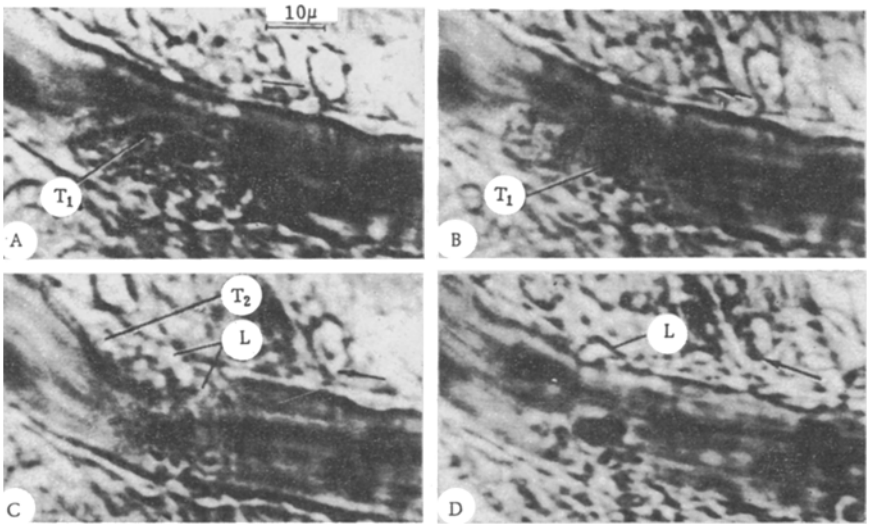

Fig. 1. Venule of rat mesentery after laser irradiation. Television biomicroscopy. Photographs from monitor screen during reproduction of videorecording. T1 and T2) Thrombi; L) leukocytes. Arrow points in direction of blood flow. Explanation in text.

The degree of sensitivity of the irradiated object was estimated from the number of pulses of radiation required to obtain a given effect in different parts. Increased permeability in the area of irradiation was judged from the settling of particles of ink injected into the blood stream (2 ml/kg body weight) immediately before irradiation in the vessel wall. The intravital microscopic picture observed was recorded by a videorecording method [4] and photographed. The source of light for photomicrography was the ISSh-500 flash tube, giving flashes with a duration of 10^{-6}-10^{-7} sec. Because of the short duration of the flash, an effect of "stopping the blood flow" could be obtained, so that individual moving blood cells could be distinguished on the photograph.

EXPERIMENTAL RESULTS

Injuries to components of the microcirculation of different intensity and character could be obtained by the method described. With high intensities of irradiation local disturbance of integrity of the vessel wall (arterioles, venules, capillaries) was observed and it led either to profuse bleeding or (irradiation at lower intensity) to extravasation of single blood cells. During the development of these disturbances intra- and extravascular coagulation of blood was observed in the region of injury.

Laser irradiation of the wall of a venule caused disturbances the character of which depended on the caliber of the vessel and the time of irradiation. An increase in permeability could be observed at the site of injury with the formation of a mural thrombus or hemorrhage followed by thrombus formation. Irradiation with the laser at low intensities, aimed at an area of local constriction of the venule, led to marked dilatation of the vessel. Irradiation not disturbing the integrity of the vessel wall led most frequently to thrombus formation. Rapdily developing processes of thrombus formation and destruction were analyzed by repeated reproduction of the videorecording. In Fig. 1A a thrombus formed after injury to the wall of a venule by the laser beam (5 sec, 10 pulses/sec), and consisting mainly of erythrocytes, can be seen in the lumen of a venule. Part of the thrombus is detached after 3 sec (Fig. IB) and is carried away by the blood flow. The thrombus is quickly reformed by the addition of new blood cells, and 2 sec later it is the same size as before, and it remained so for 40 sec. After a further detachment, the thrombus was not restored. In this experiment, 5 min after the first irradiation, a second injury was inflicted on the opposite side of the vessel, leading to the appearance of a red thrombus at that site (Fig. 1C), including also leukocytes. When this thrombus was detached 13 sec later, the leukocytes remained in situ, some of them were washed away by the blood stream, whereas others were removed from the lumen of the vessel by diapedesis (Fig. 1D). In some experiments, after a single injury to the wall of a venule, a repeated succession of formation and and detachment of thrombi could be observed. Most frequently the process ended with the formation of a mural thrombus, but in some cases by total thrombosis of the vessle lumen.

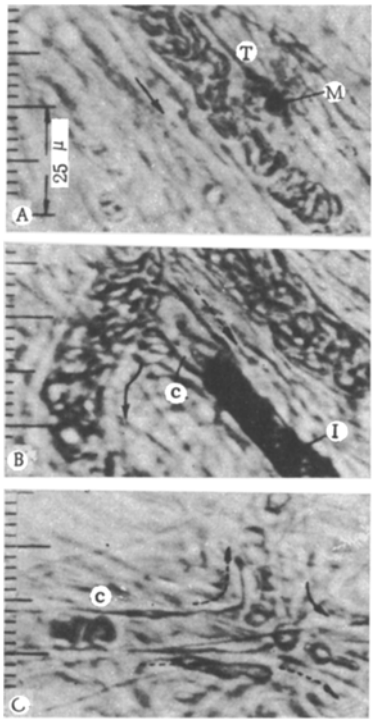

Fig. 2. Microvessels of rat mesentery after laser irradiation. Biomicroscopy. A) Arteriole 1 min after irradiation (10 sec, 10 pulses/sec): formation of thrombus (T) from erythrocytes and platelets, deposition of ink (M) in the injured area; B) capillary (c) 10 min after laser injury (7 equal pulses) to the wall and erythrocytes in its lumen: deposition of ink at site of injury (I) and around it, with stasis; C) aggregation of erythrocytes and platelets in capillary (c) and stasis after destruction of one erythrocyte by the laser beam (one pulse). Broken arrow shows direction of blood flow before irradiation; ordinary arrow shows direction of blood flow after irradiation, 580×.

At the site of laser irradiation of the wall of an arteriole, as a rule blood cells escaped from the lumen through its wall and an intravascular thrombus was formed, with consequent slowing of the blood flow in the large (60-100 μ) arterioles and stasis in the smaller arterioles. The departure of the blood cells soon stopped but the increased filtration of plasma continued, as shown by the accumulation of ink in the injured part of the wall (Fig. 2A). The vessel wall became thickened at the site of injury, the zone of deposition of ink in it gradually widened, and lysis of the erythrocytes which had left the vessel were observed.

A few single pulses of laser irradiation were sufficient to destroy the capillary wall and subsequently to cause hemorrhage. If the intensity of the radiation was low, disturbance of the permeability of the capillary walls was observed. If the erythrocytes were not destroyed in this case, the blood flow in the capillary continued, although accumulation of ink in its wall was observed. If the capillary wall and erythrocytes in its lumen were injured simultaneously, erythrocytes and platelets were concentrated around the injured cells. The resulting conglomerate closed the lumen of the capillary and led to the development of irreversible stasis (Fig. 2B). In some experiments one or two single pulses were sufficient to destroy separate erythrocytes in the capillary lumen without disturbing the permeability of its walls. Destruction of an erythrocyte led to aggregation of surrounding erythrocytes and platelets, and then to stoppage of the blood flow (Fig. 2C). Gradually this conglomerate could be shifted along the capillary by the impact of the blood, and in this case the blood flow was restored.

In a special series of experiments an attempt was made to obtain standard changes in the microcirculation by directing the laser beam on to mast cells. If the mast cell was in the immediate vicinity of a microvessel, after its irradiation increased permeability of the vessel walls was observed, sometimes accompanied by thrombus formation (Fig. 3). No visible changes in the velocity of the blood flow could be detected. In the case of consecutive injuries to the mast cell and the wall of an adjacent microvessel, instantaneous aggregation of erythrocytes and platelets took place at the site of injury to the wall. The cells adhered to each other to form a band attached at one end to the wall at the site of injury and with the other end stretching in the direction of the blood flow. As a rule erythrocytes adhered to the wall at the site of injury.

The experiments showed that by means of the laser different forms of disturbances of the microcirculation can be obtained, so that the opportunities for investigation into the experimental treatment of these disturbances are widened. The use of ultraviolet radiation enables not only the intensity of the beam but also its character to be controlled smoothly. Evidently the harmful action of the ultraviolet laser is based not only on the thermal- and photoeffect, but also on the specific wave characteristics. The fact that the wavelength of the radiation lies in the region of the absorption spectra of the most important biologically active substances points to the specificity of its action.

Because of the nature of the biological object used and of the narrow localization and low intensity of the irradiation given in this investigation, the energy characteristics could not be determined.

Injury to the vessel wall is evidently not the only cause of thrombus formation in the microvessels.

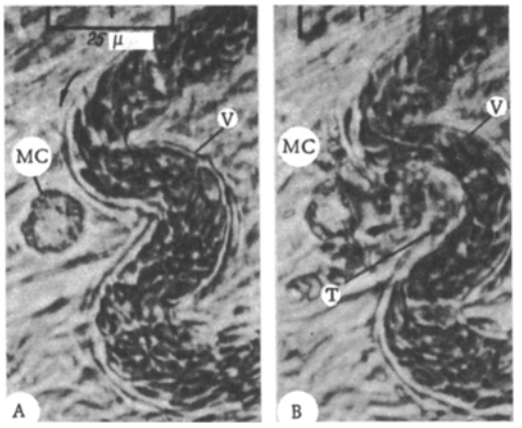

Fig. 3. Formation of mural thrombus (T) in a venule (V) after laser injury to a mast cell (MC) by single pulses: A) before irradiation, B) after irradiation. Arrow indicates direction of blood flow. 580×.

Destruction of erythrocytes, which is accompanied by the liberation of ADP, stimulating adhesion of the platelets and their subsequent aggregation [8], plays an important role in this process. The character of the blood flow [7] and, possibly, the presence of leukocytes in the thrombus [5], also play a role in thrombus formation.

The observed disturbance of permeability of the microvessels after destruction of mast cells is evidently connected with liberation of the histamine and serotonin contained in them [3]. However, there is evidence of the dual role of the mast cells in changes in vascular permeability [1].

The use of lasers in microcirculatory research provides a fine and precisely controllable tool with which to act upon different parts of the microcirculatory system.

LITERATURE CITED

1. M. P. Gorizontova, O. V. Alekseev, and A. M. Chernukh, Byull. Éksperim. Biol. Med., No. 3, 22 (1975).
2. N. V. Tsyganova, Byull. Éksperim. Biol. Med., No. 12, 29 (1973).
3. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1975).
4. A. M. Chernukh, V. S. Shinkarenko, and L. S. Ivanov, Pat. Fiziol., No. 2, 78 (1975).
5. M. M. Guest, T. P. Bond, and M. A. Crawford, Bibl. Anat. (Basel), No. 12, 131 (1973).
6. F. N. McKenzie, K. E. Arfors, T. Hovig, et al., Bibl. Anat. (Basel), No. 12, 186 (1973).
7. P. D. Stein and H. N. Sabbah, Circulat. Res., 35, 608 (1974).
8. M. P. Wiedeman, Microvasc. Res., 8, 132 (1974).