Effect of Low-Intensity Laser Radiation on Cells

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Low-intensity (0.89 μ) laser irradiation of mast cells in rat mesentery in various combinations of power and exposure modifies their functional activity. Enhanced degranulation was observed at 18-25 mW beam power and 15-30 sec exposure. The modes of radiation that did not promote degranulation, changed the form of the cells, which indicates a membranotropic effect of low-intensity laser radiation.

Key Words: *mast cells; membrane; low-intensity laser radiation*

Low-intensity laser radiation (LIL) is widely applied in clinical practice. The use of LIL is based on a number of positive effects: it stimulates the immune system, promotes wound healing, and alleviates various symptoms. Clinical use of LIL is far ahead of theoretical concepts on the mechanisms of its effects in biological objects.

Our aim was to investigate the effect of various modes of radiation on specific functions of mast cells.

MATERIALS AND METHODS

The study was carried out on the mesentery of Wistar rats (body weight 130-180 g) subjected to various doses of LIL. The specimens of mast cells for microscopic study were prepared according to following procedure: 1) fixation of irradiated mesenteric fragment with 40% neutral formalin during 5 min; 2) isolation of the examined mesenteric fragment; and 3) preparation of film specimens on slides. Mast cells were stained with toluidine blue (pH 2.0) for 5 min. An immersion objective of a light microscope was used to evaluate morphological alterations. The numbers of nondegranulated and degranulated cells per 100 cells were counted. To study the effect of intracellular Ca^{2+} concentration on LIL-induced mast cell degranulation, the examined mesenteric fragment was treated with verapamil (1.5×10^{-5} mM, 2 min). Degranulation was categorized as follows: 1) weak degranulation (below 10 released granules), 2) pronounced degranulation (more than 10 released granules), and 3) total degranulation.

Usually the first type corresponds to apocrine degranulation (the granules are released from one cell pole), while the second and third types correspond to holocrine degranulation (massive granule release from entire cell surface) [1].

The experiments were carried out using Ulei-2 laser with a radiator area of 0.5 cm^2 and wavelength 0.89 μ. Laser was operated in 6 impulse modes, in which the mean impulse power was 8, 12, 15, 18, 21, and 24 mW, respectively. The time scale was 8, 15, 30, 60, 120, 240, and 480 sec.

RESULTS

Two experimental series were carried out to find the optimal conditions for LIL effect on mast cell degranulation. Degranulation activity was studied as a function of irradiation power (series I) or duration (series II).

We determined the dependence of mast cell degranulation on the mean impulse irradiation power ranged from 7.5 to 25 mW and applied for 15 sec (Fig. 1). Activation of mast cell degranulation was observed after irradiation with 18, 21, and 25 mW power. Under these conditions, 66-73% mast cells in the irradiated fragment of the mesentery were degranulated in comparison with 40% degranulation in intact mesentery. The high degree of degranulation was primarily due

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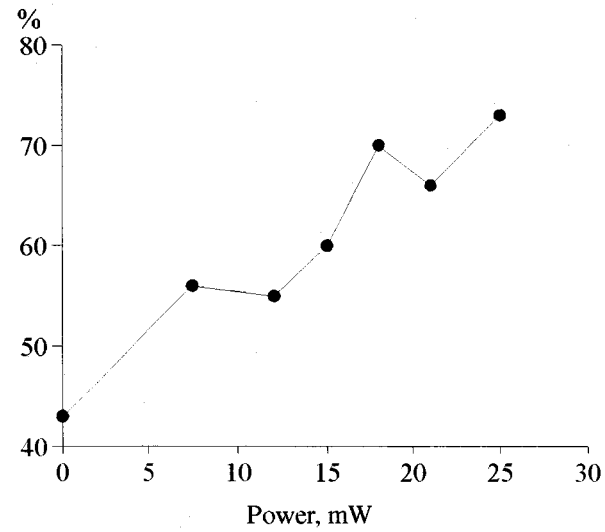

Fig. 1. Mast cell degranulation as a function of radiation power at 15-sec exposure of the blood.

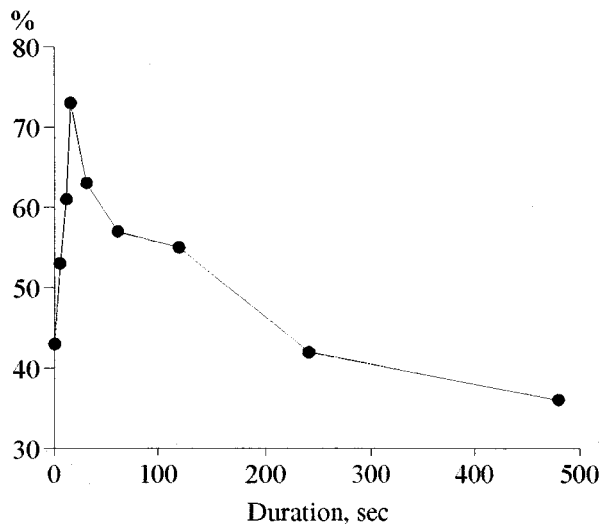

Fig. 2. Mast cell degranulation as a function of exposure of the blood at 25-mW laser power.

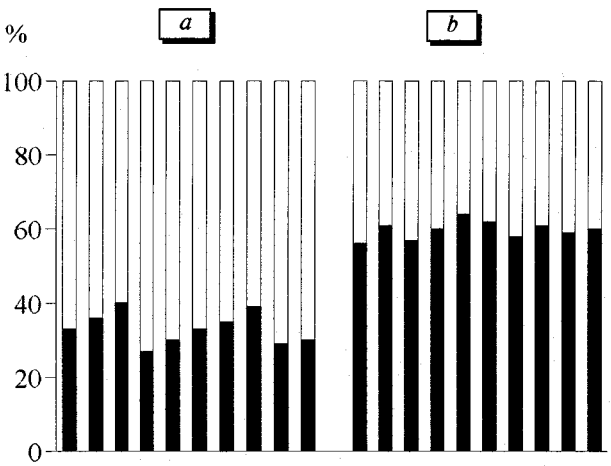

Fig. 3. Number of nondegranulated (solid bars) and degranulated (light bars) mast cells before (*a*) and after (*b*) blockade of Ca channels with verapamil under the effect of 15-sec laser irradiation at 25 mW.

to abundant cells with the second or third type of degranulation. Irradiation with low power (7.5-15 mW) slightly stimulated degranulation of mast cell, the first and second type of granule exocytosis prevailed. This power affected the shape rather than degranulation of mast cell.

In the second experimental series we studied the dependence of degranulation on the duration of LIL (mean impulse power 25 mW) (Fig. 2). The most efficient duration of irradiation was 15-30 sec.

The revealed dependencies advance the question about the mechanism of mast cell degranulation under the action of laser irradiation.

The first step in this direction was elucidation of the role of Ca^{2+} ions in this process. To this end, verapamil (1.5×10^{-5} mM) was applied onto a fragment of small intestine mesentery for 2-3 min prior to laser irradiation (25 mW, 15 sec). Blockade of Ca channels prevented degranulation of mast cells by laser irradiation at this power (Fig. 3).

Degranulation of mast cells caused by various degranulators is an active Ca-dependent process [4] involving actin polymerization, which in the inactive state encloses the granules as a reticular formation. There are two types of ionic channels responsible for calcium entry into the cell: non-selective cation channels and specific Ca^{2+} channels [5]. Specific Ca^{2+} channels react to various stimuli and determine mast cell sensitivity to Ca^{2+} ions. Actin polymerization begins during the plateau phase of intracellular calcium concentration, when Ca^{2+} ions are released from the intracellular stores. This process is initiated by Ca^{2+} entry into the cells due to activation of specific Ca^{2+} channels [5]. This implies interrelation between these processes during drastic enhancement of degranulation under the effect of LIL. Verapamil, a potent Ca^{2+} channel blocker preventing Ca^{2+} entry into cells, abolished degranulation induced by irradiation (18-25 mW, 15-30 sec). Therefore, it may be proposed that the effect of laser irradiation on mast cells with subsequent pronounced degranulation is mediated via membrane structures, namely, via Ca^{2+} channels. LIL with 780 nm modulates Ca^{2+} transport in mitochondria [3].

The effect of LIL on cell membranes was also revealed in experiments with variation of power and duration of laser radiation. The radiation at 18-25 mW applied during 15-30 sec was most efficient. Low power (less than 18 mW) and prolonged exposure did not induce degranulation, but possessed a membranotropic effect (changes cell shape). We found elliptic and stellar mast cells, while normal mast cells had a round shape. These alterations seem to result from the changes in the phospholipid bilayer, its fluidity, and redistribution of actin in these cells. Probably, LIL affects some enzyme systems on the cell surface. For instance, He-Ne laser irradiation reduces platelet ag-

gregation due to changes in the cell receptor apparatus (15-min irradiation of the blood *in vitro* at the light guide tip power of 1 mW) [2].

Our findings suggest that:

— LIL-induced degranulation of mast cells results from the membrane-targeted action of radiation;

— degranulation depends on the power and duration of laser radiation, 18-25 mW power and 15-30-sec exposure were most effective;

LIL-induced degranulation depends on Ca channel function in mast cell membrane.

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