Long-term effect of low energy laser irradiation on infarction and reperfusion injury in the rat heart

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Abstract

Yaakobi, Tali, Yariv Shoshany, Sara Levkovitz, Ofer Rubin, Shlomo A. Ben Haim, and Uri Oron. Long-term effect of low-energy laser irradiation on infarction and reperfusion injury in the rat heart. J Appl Physiol 90: 2411–2419, 2001.—Low-energy laser irradiation (LELI) has been found to modulate biological processes. The present study investigated the effect of LELI on infarct size after chronic myocardial infarction (MI) and ischemia-reperfusion injury in rats. The left anterior descending (LAD) coronary artery was ligated in 83 rats to create MI or ischemia-reperfusion injury. The hearts of the laser-irradiated (LI) rats received irradiation after LAD coronary artery occlusion and 3 days post-MI. At 14, 21, and 45 days post-LAD coronary artery permanent occlusion, infarct sizes (percentage of left ventricular volume) in the non-laser-irradiated (NLI) rats were 52 ± 12 (SD), 47 ± 11, and 34 ± 7%, respectively, whereas in the LI rats they were significantly lower, being 20 ± 8, 15 ± 6, and 10 ± 4%, respectively. Left ventricular dilatation (LVD) in the chronic infarcted rats was significantly reduced (50–60%) in LI compared with NLI rats. LVD in the ischemia-reperfusion-injured LI rats was significantly reduced to a value that did not differ from intact normal noninfarcted rats. Laser irradiation caused a significant 2.2-fold elevation in the content of inducible heat shock proteins (specifically HSP70i) and 3.1-fold elevation in newly formed blood vessels in the heart compared with NLI rats. It is concluded that LELI caused a profound reduction in infarct size and LVD in the rat heart after chronic MI and caused complete reduction of LVD in ischemic-reperfused heart. This phenomenon may be partially explained by the cardioprotective effect of the HSP70i and enhanced angiogenesis in the myocardium after laser irradiation.

ischemia; myocardial infarction; angiogenesis; heat shock proteins; laser

The sequential histological and physiological changes that take place in the rat myocardium after occlusion of the left anterior descending (LAD) coronary artery in experimental animals, including rats, have been well documented (10, 11, 25, 26). It has been shown that, after chronic myocardial infarction (MI) in the rat, an impairment of the left ventricular function (reduced power output and elevated filling pressure) occurs that is related to the loss of viable myocardium (11, 26).

The possible involvement of myocardial stress proteins in myocardial protection after ischemic injury has been recently reviewed (4). The anti-ischemic (22) and antiapoptotic (34) properties of the most important of these proteins, the heat shock proteins (HSP) [specifically, the inducible 70-kDa HSP (HSP70i)], have been demonstrated by using the transgenic mouse model that overexpresses this protein (22).

Novel approaches for enhancing angiogenesis in the ischemic myocardium by introducing growth factors (mainly of the family of vascular endothelial growth factors) were adapted and found to have beneficial effect on patients with severe angina (20). Furthermore, the significance of angiogenesis with regard to ischemic heart disease has been addressed in a recent review (32). Low-energy lasers have been found to modulate various biological processes in tissue cultures and animal models (12, 16, 17). For example, laser irradiation has been found to increase mitochondrial respiration and ATP synthesis (24, 38), accelerate wound healing (12), and promote the process of skeletal muscle regeneration after injury (5, 7, 37). Inflammatory response was markedly decreased by laser irradiation (6), and neof ormation of blood vessels in the injured zone of skeletal muscles was elevated (5). Recently, our laboratory has shown that low-energy laser irradiation (LELI) induces cell cycle regulatory protein synthesis in satellite cells from skeletal muscles because of activation of early cell cycle regulatory genes (2).

The effect of LELI on the heart muscle and cardiomyocytes has been studied to a limited extent. Zhu et al. (39) recently showed that argon dye laser (660-nm wavelength) improves the functional recovery of cold-stored, isolated rat cardiomyocytes. Those authors also found that the end-storage ATP and end-reperfusion catalase activities in isolated cardiomyocytes that were irradiated with the argon dye laser were significantly higher than those in the untreated cells.

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We therefore hypothesized that 1) an increase in the content of any cytoprotective molecule, such as HSPs in the myocardium, as a consequence of LELI immediately post-LAD coronary artery ligation, might have a beneficial effect on cardiomyocytes under ischemic conditions; and 2) exposing the heart to a second LELI (on day 3 after LAD ligation) might cause enhancement of new blood vessel formation and thereby contribute to a better recovery of cardiomyocytes under ischemic conditions.

METHODS

Experimental Procedures

Chronic MI. Studies were performed on 65 eight- to ten-week-old (250–350 g) male Sprague-Dawley rats. The rats were anesthetized with Avertin (1 ml of 1.25% tribromoethanol in saline per 100 g body wt), and the operative technique for LAD coronary artery occlusion was carried out essentially as described previously (30). Anesthesia with Avertin was found to be superior to other common drugs for anesthesia and led to better survival of rats. In brief, thoracotomy was performed after longitudinal incision of the left side of the thorax and insertion of a silk thread (4-0) through the intercostal muscles around the area (~15 mm in diameter) of the thoracotomy in the chest. A thoracotomy was made between the fifth and sixth ribs, and the heart was exteriorized by exerting pressure on the chest (rats were not ventilated during surgery). The LAD was occluded 3 mm distally from where it branches off the aorta by using 5-0 polypropylene thread (Ethicon, Cincinnati, OH). The exact location of ligation of the LAD coronary artery (distance from where it branched off from the aorta and its anatomy) was determined after preliminary studies. The chest was then squeezed to inhibit pneumothorax and closed by using the prepared guided suture as described above. The above procedure from thoracotomy up to its closure lasted 30–60 s. Postoperatively, food and water were supplied ad libitum. Mortality rate after the above procedure was 42%.

Ischemia-reperfusion injury. LAD coronary artery occlusion in the ischemia-reperfusion injury to the rat heart was performed on 18 rats similarly to that described in the chronic MI experimental model. The LAD coronary artery was ligated with 4-0 silk thread (Ethicon) by using a flat and tight (as for the chronic LAD coronary artery occlusion) knot suture. One tip of the thread was left protruding from the chest muscles during closure after LAD coronary artery occlusion to enable release of the occlusion. At 45 min post-MI, the rats were lightly anesthetized with halothane and the suture around the LAD coronary artery was released and removed by gently pulling the protruding tip of the thread. In ~20% of the rats the “pull force” was very low, indicating release of the flat suture after occlusion. These rats were discarded from the study. In preliminary experiments, the above process was performed, and blood flow in the occluded artery after suture removal was evident by injection of yellow dye into the aorta. Halothane was found to be the most effective short-term anesthetic in terms of reduced mortality compared with ether or chloroform. Rats were killed 2 wk post-MI. In a review article (8) discussing the point of perfusion and reperfusion time periods in experimental animal models of 90 min of LAD coronary artery occlusion and 3 and 6 h of reperfusion were discussed. Thus a 45-min period in a smaller animal (rat) is sufficient to cause similar damage to cardiomyocytes.

Laser Irradiation

A diode (Ga-As) laser (wavelength of 804 nm with a power output of 38 mW and a beam diameter of 1.5 × 3.5 mm after collimation) was employed (Lasotronic, Zug, Switzerland). The laser device was equipped with a blunt tip (1.5-mm diameter) and placed directly on the intercostal muscles (after removal of the skin) at a perpendicular angle to the medial and lateral side of the left chest wall at the point above the beating heart. Rats were randomly assigned to either control non-laser-irradiated (NLI) or laser-irradiated (LI) rat groups. The above laser irradiation did not cause elevation of the temperature in the irradiated tissue as measured previously in skeletal muscles (5, 6). Furthermore, no elevation of temperature was noticed in the myocardium of rats that were irradiated with the above laser for 3 min as measured by a sensitive (± 0.1°C) thermocouple with a probe inserted in the irradiated area of the myocardium.

Five anesthetized rats whose chests had been opened at the midline were used to determine the dispersion and power of the laser beam after penetration through the intercostal muscles. An infrared viewer (Lasotronic, Zug, Switzerland) and infrared-sensitive detecting card (Newport, Irvine, CA) were used to trace the infrared irradiation area. A NOVA power-energy laser monitor equipped with a special detecting probe (Ophir Optronics, Jerusalem, Israel) was used to measure the power output of the laser and the power of the laser irradiation after penetration through the chest muscles. The laser monitor probe and the infrared sensor card were placed in the rats’ chests at the same distance and angle of the heart. This was performed to detect, as closely as possible, the area of irradiation and its power on the heart during laser irradiation of a closed chest, as described above. The area of irradiation (1.1 cm²) was elliptical in shape (1.8-cm long axis × 1.1-cm short axis) and was determined by planimetry. The measured power of irradiation in the above area was 5.0 ± 0.7 mW; thus the power density of the irradiation on the myocardium was 4.5 ± 0.6 mW/cm². It could be assumed, therefore, that the dispersed laser beam would cover the infarcted area, including most of the lateral wall of the left ventricle (LV) of the rats. Laser irradiation was performed as described above 10–15 min after LAD coronary artery occlusion for a duration of 1 min after the rats were breathing regularly. Total energy given to the tissue thus was 0.27 J/cm². On day 3 post-LAD coronary artery occlusion, the rats were lightly anesthetized with halothane (ICI, Cheshire, UK), the skin sutures over the chest were removed, and the intercostal muscles below were exposed. Laser irradiation was performed as above, but time of exposure was lengthened to 3 min. On the basis of the above data, the total energy given on the third day was 0.81 J/cm². Control infarcted sham-operated rats underwent the same process as the treated rats (anesthetized and chest exposed), and the laser was applied to the chest but was not connected to a power source. Irradiation immediately post-LAD coronary artery ligation was aimed at enhancing ATP content (24, 38) or stimulating other factors that induce cytoprotective effects in the cardiomyocytes and, at 3 days postligation, at promoting angiogenesis, which commences in the rat at this time interval (13). The ischemic-reperfused rats were laser irradiated at the same power density and timing as the chronic MI rats. The above regime of laser irradiation (energy and length of irradiation, timing and number of irradiations, and so forth) was determined after several preliminary experiments to yield optimal beneficial effects of irradiation on infarct size (Table 1). However, not all possible combinations of the above parameters of variation of laser irradiation (i.e., exposure...
Table 1. Effect of various power densities of laser irradiation on reduction of infarct size in the rat heart

<table>
<thead>
<tr>
<th>Power Density, mW/cm²</th>
<th>Reduction of Infarct Size, %Control Nonirradiated Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>14*</td>
</tr>
<tr>
<td>5.0</td>
<td>62*</td>
</tr>
<tr>
<td>20.0</td>
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The left anterior descending coronary artery was ligated in 3 groups (5–7 rats each) that received laser irradiation at 3 different power densities. Infarct size was calculated and expressed as percent reduction of infarct size from control group of rats that were not laser irradiated. *Statistically significant, P < 0.05.

time, energy, wavelength, and so forth) on the reduction of infarct size were investigated. Experiments to explore the effect of single laser irradiation on the infarct size were performed using 12 rats. In these experiments, laser irradiation was performed as described above 30 min post-LAD coronary artery occlusion at power densities of 5 and 12 mW/cm². Infarct size was determined as described below.

Histology and Immunohistochemistry

At 7, 14, 21, or 45 days post-LAD coronary artery occlusion, the rats were killed with an overdose of chloroform and the hearts were excised while still beating. The excised hearts (NLI and LI rats) were immediately soaked in cold saline for 10 s to remove excess blood from the ventricles and then fixed in neutral buffered formalin (4% vol/vol) for 48 h. Transverse slices (2.0–2.5 mm thick) were prepared from each heart and the endocardium calculated from all the LI rats. This indicates that, even though the laser irradiation is applied epicardially, the endocardial region of the myocardium is affected to the same extent by the laser as the epicardial region.

The area at risk was determined on nine rats essentially as described by Black and Rodger (8). Rats were anesthetized with Avertin, and the LAD coronary artery was occluded as described in Chronic MI. Four rats (randomly chosen) served as control NLI rats, whereas the rest (5 rats) served as LI rats. Laser irradiation was performed after LAD coronary artery occlusion as described in Laser Irradiation. A 1.5% (wt/vol) solution of Evans blue in saline was injected into the aorta. The hearts were then excised and fixed in 4% neutral buffered formalin. Transverse slices (width of 2 mm) were prepared from each heart. The percentage of the area at risk out of the total area of the left ventricle (LV) was calculated for the section in the middle of the area at risk by using computerized histomorphometric methods as described in Laser Irradiation for the infarct size (see Fig. 5). It was found that the area at risk was 60.3 ± 6.4 (SD) and 62.4 ± 9.3% in the NLI and LI rats, respectively.

Histology and Immunohistochemistry

Histological sections (8 μm) were prepared in a plane parallel to the atrioventricular groove and in the middle of the infarct. Location and tightness (>1-mm loop diameter) of the suture were checked in all sections in each slide to be taken from each muscle according to the magnitude of variability of the parameters measured within the sections on each slide, slides of the same animal, and between NLI and LI hearts. According to this test, six histological cross sections of the infarcted zone of each rat (from 3 microscopic slides and 2 sections per slide) were randomly chosen for morphometric analysis. Infarct size was defined as the percent of the infarcted (necrotic) area out of the total area of the LV. Left ventricular dilatation (LVD) was defined as the volume occupied by the left ventricular chamber (in percent) relative to the total volume of the LV. Results obtained from the morphometric analysis were finally statistically analyzed by using ANOVA (33) test with statistical significance level of P < 0.05.

Histomorphometric Measurements and Statistics

The size of the infarcted area and other parameters were measured using quantitative histomorphometric methods (36). Most of the parameters measured associated with infarct size have been basically previously described by Roberts et al. (29). The measurements were performed on Masson’s trichrome-stained histological sections using image analysis software Sigma Scan Pro (Sigma Chemical, St. Louis, MO). The examiner of the tissue sections was blinded to the treatment (NLI or LI rats). Three-level nested ANOVA (33) was carried out on the morphometric results of a preliminary experiment to determine the number of slides and number of sections in each slide to be taken from each muscle according to the magnitude of variability of the parameters measured within the sections on each slide, slides of the same animal, and between NLI and LI hearts. According to this test, six histological cross sections of the infarcted zone of each rat (from 3 microscopic slides and 2 sections per slide) were randomly chosen for morphometric analysis. Infarct size was defined as the percent of the infarcted (necrotic) area out of the total area of the LV. Left ventricular dilatation (LVD) was defined as the volume occupied by the left ventricular chamber (in percent) relative to the total volume of the LV. The results obtained from the morphometric analysis were finally statistically analyzed by using ANOVA (33) test with statistical significance level of P < 0.05.

Analysis of Newly Formed Blood Vessels

For determination of angiogenesis, 11 (5 NLI and 6 LI) LAD coronary artery-ligated rats were used. The rats were laser irradiated as described in Laser Irradiation after LAD coronary artery occlusion and again 3 days later. The rats were lightly anesthetized with halothane 5 days post-LAD coronary artery occlusion, and 40 mg/kg body wt BrdU (Sigma Chemical) in saline were injected three times every 4 h intraperitoneally to label proliferating cells. The rats were killed on day 6 post-LAD occlusion. The hearts were excised, and 3-mm transverse sections from the middle of the infarcted area were processed for histology as described in Histology and Immunohistochemistry. The above regime and time points were chosen to trace the effect of the second laser irradiation and to follow neoformation of blood vessels that commenced in the infarcted area at 3 days post-LAD coronary artery occlusion (13). Two histological sections from
each heart were randomly selected for analysis of blood vessel formation in the infarcted area. The sections were viewed at a direct \( \times 400 \) magnification by using a Zeiss microscope equipped with a video screen. Two observers (blind to the treatment: NLI or LI rats) analyzed the blood vessels in the entire area of the LV of the infarcted rats. The total number of capillaries (\(<10 \mu m \) in diameter) and arterioles (20–100 \( \mu m \) in diameter) were counted in the LV area. On the same section, the newly formed blood vessels were also counted. These vessels were defined as vessels that contained more than one endothelial cell positively immunolabeled (see above) for BrdU (see Fig. 8). The total number of BrdU-labeled (dividing cells) endothelial cells (confined to blood vessels) in the LV area were counted as well. The results were expressed per LV area of each microscopic section in the heart because of low variability in the area at risk (as described in Histology and Immunocytochemistry) and similar heart size of the rats. Furthermore, they express the newly formed blood vessels both in the perinfarcted and infarcted regions of the LV in this particular time point (6 days) post-LAD coronary artery ligation. The results were statistically analyzed using ANOVA.

Western blotting analysis of HSP70i. For HSP determination, chronic MI was induced in a group of 10 rats as described in Chronic MI. Six rats were laser irradiated immediately after LAD coronary artery occlusion, and the rest (4 rats) served as control NLI rats. Five hours post-LAD coronary artery occlusion (this time interval was found to be optimal on the basis of preliminary studies at 1 and 24 h post-LAD coronary artery occlusion), the rats were killed immediately after LELI was 19.8 ± 6%, whereas in the LI rats it was 20 ± 8, 15 ± 6, and 10 ± 4%, respectively. Thus the reduction of infarct size after LELI was 64–70% at 14–45 days, respectively. LVD was also significantly lower at 14, 21, and 45 days post-LAD coronary artery occlusion in LI rats compared with NLI rats (Fig. 2). At 14, 21, and 45 days post-LAD coronary artery occlusion the LVD of the NLI rats was 36 ± 7, 30 ± 9, and 25 ± 7%, respectively, whereas in the LI rats the values were 18 ± 4, 10 ± 3, and 14 ± 3% for the respective time intervals. At 14, 21, and 45 days post-LAD coronary artery occlusion, LVD of the ischemic reperfused rat hearts is significantly different from the noninfarcted intact rat hearts in which the LVD was 11 ± 4% (Fig. 2). The infarct size and LVD of the ischemic reperfused rat hearts are presented in Figs. 3 and 4. It can be seen that, at 2 wk after ischemia-reperfusion injury to the heart, infarct size in the NLI rats was 19.8 ± 7% (SD), whereas in the LI rats it was 11.7 ± 3.4% (not statistically different). However, LVD in the LI rats was only 12 ± 2.5%, which was significantly lower than the 18 ± 3% found in the NLI rats (Fig. 4). When laser irradiation at 3

![Fig. 1. Histograms of the infarct size of myocardial infarction (MI)-induced non-laser-irradiated (NLI; hatched bars) and laser-irradiated (LI; solid bars) rats at different time intervals post-MI. Values are means ± SD of 10–12 rats at each time interval. LV, left ventricle.](Image master1D prime ver. 3.01, Amersham Pharmacia, Buckinghamshire, UK) was used to determine the optical density of the blots.

**RESULTS**

**Effect of laser irradiation on infarct size and LVD.** The effect of laser irradiation on infarct size at various time intervals post-LAD coronary artery ligation is presented in Fig. 1. Infarct size in the LI rats was significantly smaller than in NLI rats. At 14, 21, and 45 days post-LAD coronary artery ligation, infarct sizes in the NLI rats were 52 ± 12 (SD), 47 ± 11, and 34 ± 7%, respectively, whereas infarct sizes in the LI rats were 20 ± 8, 15 ± 6, and 10 ± 4%, respectively. The results were statistically analyzed using ANOVA.

**Fig. 2. Histograms of LV dilatation of intact (open bar), MI-induced NLI (hatched bars), and LI (solid bars) rats at different time intervals post-MI. Values are means ± SD of 10–12 rats at each time interval. Significantly different from NLI rats: *P < 0.05; **P < 0.01.**
days was omitted and the irradiation was performed only immediately post-LAD coronary artery occlusion (at a power density of 12 mW/cm²), the reduction in infarct size (2 wk post-MI) was only 46% (significant, \( P < 0.05 \)) compared with 68% when two irradiations were applied. Single laser irradiation (power density of 5 mW/cm²) 30 min post-LAD coronary artery ligation resulted in 21% reduction (not statistically significant) of infarct size.

**Effect of Laser Irradiation on Histology, Desmin Expression, and HSP Synthesis in the Infarcted Area**

Figure 5 represents typical histological characteristics of MI-induced NLI rats compared with LI ones. Large infarct and thinning of LV anterior and lateral walls characterized the NLI hearts (Fig. 5b) compared with small infarcts (Fig. 5c) in the LI hearts. A marked LVD was noticed in the NLI hearts compared with minimal LVD (similar to noninfarcted heart) in the LI rats (Fig. 5, a–c). A similar phenomenon was observed in the LI hearts after ischemia-reperfusion injury, but the infarcts were more diffuse than in the acute MI model (Fig. 5, d and e). The histology of the infarcted area in the NLI rats revealed a typical collagenous scarring and thinning of the ventricular wall (Fig. 6e), compared with aligned and fused cells and less collagen and thinning in the LI hearts (Fig. 6f). The cells within the infarcted area in NLI, MI-induced rats did not demonstrate positive immunoreaction to desmin (Fig. 6e), compared with ~20% of the cells in the infarcted area of the LI rat hearts that did immunoreact to desmin (Fig. 6f).

The effect of LELI on the amount of HSP70i 5 h postirradiation is presented in Fig. 7. It can be seen that, in the noninfarcted myocardium, there is a small amount of HSP70i, which becomes elevated by 5 h postinduction of MI. However, in LI, rats the content of HSP70i is much higher. Densitometric analysis of the Western blots confirmed that the amount of HSP70i in LI rats was 2.2-fold (significant, \( P < 0.05 \)) higher than in the NLI rats.

**Effect of Laser Irradiation on Angiogenesis**

The number of total blood vessels counted in the histological sections of the LV of the LI rats was 62 ± 10 (SE) compared with 27 ± 4 in the NLI rats (Figs. 8 and 9). Neovascularization as manifested by BrdU-labeled vessels was 3.1-fold (significant, \( P < 0.01 \)) higher in the LI rats than in the NLI ones. The number of dividing (BrdU-labeled) endothelial cells in histological sections of the LV of LI rats was 28 ± 6, which was significantly higher than the value (11 ± 5) in the NLI rats (Fig. 8).

**DISCUSSION**

The infarct size in control NLI rats decreased with time after infarction from 57 to ~34%. This phenomenon corroborates with previous studies that found gradual shrinkage of the relative volume of scar tissue with time after LAD coronary artery occlusion (13). The results of the present study indicate that the beneficial effects of LELI on infarct size and LVD in the rat after chronic MI or ischemia-reperfusion injury progressed with time post-LAD coronary artery occlu-
tion in the rats and that they were most prominent at the longest time interval tested. Thus, at 45 days post-LAD coronary artery occlusion, reduction of infarct size in the LI rats was 70% compared with NLI rats. The fact that the LVD in the LI rats completely returned to the normal value of an intact noninfarcted rat (from 2 wk on post-MI) further supports the profound enhancement in recovery of the myocardium of the LI rats post-MI compared with NLI rats. It was previously demonstrated that reduction in stroke volume in rats occurs only when the infarct size is larger than ~46% of the myocardium (27). Thus it may be postulated that, if laser irradiation in the present study caused reduction of infarct size from a range of 34–52% (2–6 wk post MI) to 10–20%, the irradiated rats may show a better functional performance of the heart over the NLI rats. LELI given within a short time interval after occlusion of the coronary arteries may attenuate the very rapid decrease in ATP and the consequent irreversible adverse effects that take place in the cardiomyocyte mitochondria in the ischemic zone. Indeed, LELI has been found to increase mitochondrial respiration and ATP synthesis (24, 38). Thus the injured laser-irradiated cells may have a much slower rate of degeneration because of an increase in ATP production in the LI rats. It was previously demonstrated that, after coronary occlusion in rats, the basic coronary flow was completely normalized within 7 days (23). Thus it may be postulated that there is a rapid gradual growth of new blood vessels in untreated ischemic heart. In the LI rats, in which formation of new blood vessels is enhanced as shown in the present study, angiogenesis is even faster enabling some portion of the cardiomyocytes to synthesize ATP. The

Fig. 6. Light micrographs and immunohistochemic desmin localization of infarcted zones of NLI (a, c, and e) and LI hearts (b, d, and f) 3 wk post-MI. Note that the infarcted area in the NLI is filled with scar connective tissue (a and c) as opposed to aligned fused cells (arrowhead) in the infarcted area of the LI rats (b and d). Cells in the infarcted zone of the LI hearts are positively stained for desmin (arrowhead) in f as opposed to no staining in the infarcted zone (IF) of the NLI ones in e. ED, endocardium; EP, epicardium. Masson’s trichrome stain was used. Bars in a and b = 2 mm; bars in c–f = 100 μm.

Fig. 7. Western blot of samples prepared from ischemic portion of the heart harvested from LI (B and D) and NLI (A and C) rats that were killed 5 h after infarction. The samples were probed with a polyclonal antibody recognizing inducible heat shock protein 70.

Fig. 8. Histograms of the number of blood vessels (top), 5-bromo-2’-deoxyuridine (BrdU)-labeled blood vessels (middle), and BrdU-labeled endothelial cells (bottom) in the area of the LV in a histological section of the LV of NLI (hatched bars) and LI (solid bars) rats 6 days after left anterior descending coronary artery occlusion. Values are means ± SE of 12 and 10 histological sections in NLI and LI rats, respectively. Significantly different from NLI rats: *P < 0.05; **P < 0.01
decrease in the number of injured cardiomyocytes may also markedly reduce the inflammatory response after MI in the myocardium, as was previously shown by our laboratory for toad skeletal muscles after injury (6). This phenomenon may, in turn, reduce the adverse effects that have been attributed to leukocyte infiltration as part of the complex sequential processes that occur post-MI (31). Furthermore, we show in the present study that LELI given post-LAD coronary artery ligation caused a rapid elevation in the content of HSP70i in the ischemic area over the nonirradiated myocardium. The HSP family has been shown to play a major role in cytoprotection of cells and enhanced protection against ischemic injury in the heart (4, 22). Thus laser irradiation, via elevation of HSP70i content in the cardiomyocytes, could lead to salvage of a higher percentage of cells in the ischemic zone of LI rats compared with NLI rats. Indeed, it was previously shown that infarct size was negatively correlated with myocardial HSP70i content (15). Furthermore, it should be emphasized that the elevation of HSP70i synthesis in the ischemic myocardium by LELI is a novel phenomenon that is demonstrated in the present study for the first time. So far it has been shown that induction of HSP in vivo could only be achieved by heat stress.

The present study also indicates that LELI has beneficial effects when ischemia in the myocardium is transient and followed by reperfusion injury. Indeed, in the LI rats, there was a marked reduction of the diffused infarct in the myocardium and a return to normal values (as in noninfarcted heart) of LVD as soon as 2 wk post-MI. These results indicate that LELI may have a mitigating influence on the deleterious effects of the free radicals that are formed in the ischemic zone after reperfusion or reduce their content. Indeed, it was recently shown that argon dye laser caused elevation of catalase activity in isolated cardiomyocytes (39). Thus it can be postulated that the direct or indirect elevation of catalase activity, and/or of other enzymes that act as antioxidants, by LELI may contribute to a decrease in the content of superoxides in the reperfused injured or ischemic heart. Indeed, brief periods of ischemia (as in ischemic preconditioning) have been recently suggested to trigger production of low levels of reactive oxygen species, which induce antioxidant defenses that can reduce subsequent ischemic damage (21).

Another mechanism that might be associated with reduction of formation of scar tissue after MI in the rats is the possible transformation of more presumptive fibroblasts in the infarcted area into myofibroblasts after LELI. Pourreau-Schneider et al. (28) have previously shown, using electron microscopic methods and immunohistochemistry, that a direct and massive transformation of cultured fibroblasts into myofibroblasts was observed in cultures 24 h after He-Ne laser irradiation, whereas control cultures contained only resting and active fibroblasts. Indeed, in the present study, cells that were positively stained for desmin, which is attributed to myogenic cells that synthesize de novo proteins (1), were much more numerous in the infarcted area of the LI rats than in the NLI ones. This phenomenon may suggest increased survival of cardiomyocytes in LI relative to NLI hearts and possible regenerative capability of partially injured cardiomyocytes to synthesize new myogenic proteins and express desmin-positive staining. It may also be postulated that a much higher percentage of presumptive myoblasts or myofibroblasts is transformed into myoblasts due to LELI (28) and positively react to desmin. The possibility that scar tissue formation is inhibited by LELI rather than a mainly cardioprotective effect of the LELI cannot be ruled out. Indeed, it has been previously shown that LELI enhanced the proliferation of fibroblasts in vitro (35) and enhanced wound healing (12). Thus it seems more likely that the complex process of fibrosis post-MI may be attenuated by the LELI rather than inhibited.

Irradiation of the heart on day 3 post-MI was aimed at enhancing the process of angiogenesis that commences on the third day, after occlusion of the left coronary artery in the rat (26). Quantitative methods
have shown previously that formation of blood vessels in the regenerating zone after skeletal muscle injury is enhanced twofold by applying laser irradiation (7). The results of the present study also indicate a significant (3.1-fold) increase in the number of newly formed blood vessels and proliferating endothelial cells in the infarcted LV and also in the noninfarcted LV. Such a profound increase may be due to stimulation of endothelial cell proliferation by LELI (Y. Krispel, U. Oron, and N. Mirsky, unpublished data) as was found also for example for satellite cells of skeletal muscle origin (2). Thus cardiomyocytes that were salvaged from the ischemic injury by the first laser irradiation that caused elevation of HSP70i may have been fitted from the better oxygen and nutrient supply through an enhanced neof ormation of blood vessels in the noninfarcted area. The significant contribution of the laser irradiation at the third day post-LAD coronary artery occlusion can be explained by the reduction of only 21% (when power density was 5 mW/cm²) in infarct size when this irradiation was omitted. Yet, application of single irradiation at 12 mW/cm² probably has better cardioprotective properties because reduction of infarct size was 46% compared with control.

The results of the present study may also have clinical relevance. On the basis of our laboratory’s previous results showing that direct LELI on myoblasts in culture does not affect their differentiation in vitro (2) and that the use of LELI in humans has no known deleterious effects (12, 16), it can be postulated that the use of LELI post-MI would appear to be safe. LELI can be delivered to the myocardium in humans via fiber optics in the catheter of the nonfluoroscopic in vivo navigation and mapping technology currently in use in experimental animals and in humans (3, 14, 18). The LELI energy can also be applied during or after the procedure of balloon angiography, using a catheter with a central canal bearing a fiber optic, through which the laser energy can be transversely delivered (360°) to the infarcted area. As an adjunct procedure to other, increasingly popular approaches of repairing ischemic myocardium (angioplasty, stenting, grafting, transmyocardial revascularization, and so forth), the cardioprotective effects of LELI may similarly be beneficial at minimal or no additional risk.

In conclusion, the results of the present study demonstrate that LELI causes a profound reduction in the size of necrotic and scar tissue and complete recovery of LVD after induction of acute MI in rats. These phenomena can most probably be explained as a result of the cardioprotective effects of the LELI at early stages post-LAD coronary artery occlusion, (most probably as a consequence of elevation of HSP70i content in the irradiated myocardium) and enhancement of neof ormation of blood vessels later on. However, the precise molecular mechanisms associated with the above phenomena remain to be further elucidated.

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