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Low-Level Laser Therapy Accelerates Collateral Circulation and Enhances Microcirculation

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ABSTRACT

Objective: **To evaluate the efficacy of low-level laser therapy (LLLT) on collateral circulation and microcirculation if a blood vessel is occluded.** ***Background data:*** **Investigators have attempted prostaglandin and ultrasound therapy to promote improvements in the vascular bed of deprived tissue after an injury, which may lead to occlusion of the blood vessels.** ***Materials and Methods:*** **Thirty-four adult rabbits were used in this study, two of them considered 0-h reading group, while the rest were divided into two equal groups, with 16 rabbits each: control and those treated with LLLT. Each rabbit underwent two surgical operations; the medial aspect of each thigh was slit, the skin incised and the femoral artery exposed and ligated. The site of the operation in the treated group was irradiated directly following the operation and for 3 d after, one session daily for 10 min/session. The laser system used was a gallium-aluminum-arsenide (Ga-Al-As) diode laser with a wavelength of 904 nm and power of 10 mW. Blood samples collected from the femoral artery above the site of the ligation were sent for examination with high-performance liquid chromatography (HPLC) to determine the levels of adenosine, growth hormone (GH) and fibroblast growth factor (FGF). Tissue specimens collected from the site of the operation, consisting of the artery and its surrounding muscle fibers, were sent for histopathological examination to determine the fiber/capillary (F/C) ratio and capillary diameter. Blood samples and tissue specimens were collected at 4, 8, 12, 16, 20, 24, 48 and 72 h postoperatively from the animals of both groups, control and treated.** ***Results:*** **Rapid increases in the level of adenosine, GH, and FGF occurred. The F/C ratio and capillary diameter peaked at 12–16 h; their levels declined gradually, reaching normal values 72 h after irradiation in the treated group. Numerous collateral blood vessels proliferated the area, with marked increases in the diameters of the original blood vessels.** ***Conclusions:*** **The results indicated that LLLT accelerated collateral circulation and enhanced microcirculation and seemed to be unique in the normalization of the functional features of the injured area, which could lead to occlusion of the regional blood vessels.**

INTRODUCTION

NEW CAPILLARIES are always formed from preexisting ones by a process of budding. This occurs by growth during embryonic development (angiogenesis)[1] and after tissue injury through collateral circulation.[2] Endothelial budding is induced by a growth factor formed by parenchymal cells in hypoxia, possibly as a result of anaerobic glycolysis.[1,2]

Control of blood flow to a tissue can be divided into two phases: (1) acute, which is achieved by rapid changes in the diameter of arterioles, metarterioles, or precapillary sphincters; and (2) long-term, which results from an increase or decrease in the number of actual blood vessels supplying the tissue.[3]

During the healing process of an injured muscle, fibers begin to increase in length and diameter many times the normal values, which is associated with the formation of new capillaries. If new capillaries are not formed during the regenerative process, the capillary density will be reduced and the healing process altered.[4] New capillaries are formed only if the muscle maintains a normal blood supply, which comes from collateral circulation. Regeneration of any muscle depends upon the presence of factors such as adenosine, growth hormone (GH), and fibroblast growth factor (FGF), also called the *angiogenic factors.*[5]

Low-level laser therapy enhances vasodilatation and proliferation of the microvasculature.[6] Laser irradiation is also thought to increase the level of oxygen content of tissue.[7]

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MATERIALS AND METHODS

Thirty-four adult New Zealand rabbits with average body weights of 1.5–2 kg were used in this study. They were divided into two equal groups: a control group and a group treated with LLLT, each group consisting of 16 rabbits, with the remaining two rabbits considered 0 h reading group.

Each rabbit underwent two surgical operations: one in the medial aspect of each thigh. The operations were carried out under general anesthesia using a combination of acepromazine malet (10 mg/kg body weight) Calmivet, 0.5 mg, Many-Venice-70200, Lura, France), ketamine hydrochloride (10 mg/kg body weight) (Ketallar, 50 mg/mL, Park Davis, Gwent, U.K.), and Xylazin (5 mg/kg body weight) (Rompon, 20 mg/mL, DeH 28, Pantex, Holland), injected intramuscularly.[8] The femoral artery is the main arterial supply of the lower limb.[9] An incision was made halfway down the medial aspect of the thigh to involve the skin, superficial fascia, and the thin fascia lata. Then the two muscles—the sartorius, anteriorly, and the adductor lounges, posteriorly—were separated bluntly to expose the femoral artery, which was ligated at the level of the midshaft of the femur using 4-0 catgut (Chordasowa Resor, bills aseptica, PH-Eue, Berlin, Germany).

Blood samples were collected from the femoral artery above the ligation and examined with high-performance liquid chromatography (HPLC, Stamps. Analytical Laboratory Instrument, Japan) to determine the levels of adenosine, according to the United States Pharmacopoeia,[10] GH, according to Smith and Lee,[11] and FGF levels, according to the British Pharmacopoeia.[12]

Tissue specimens were taken from the site of ligation (transverse sections), which included the artery and its surrounding muscle fibers. The specimens were fixed and stained with hematoxyline and eosin (H&E) stain. The specimens were examined histopathologically to determine the fiber/capillary (F/C) ratio and the capillary's diameter, according to Tomanek et al.[13,14] The capillary diameter (7–10 capillaries) was measured during each period, depending on the degree of healing, using a micrometer (Micro, Bausch & Lomb, United States).

Blood samples and tissue specimens at 0-h were collected from the two rabbits, which were considered the 0-hour reading group. Two rabbits from each group, the control and laser-treated, were identified for collection of blood samples, and tissue specimens were measured at 4, 8, 12, 16, 20, 24, 48, and 72 h postoperatively. Those in the treated group were irradiated immediately postoperatively and for 3 d thereafter. They received one session daily for 10 min/session using a gallium-aluminum-arsenide (Ga-Al-As) diode laser system (Russian-Palisb, Moskovesky Polisib, Moscow, Russia) with a 904-nm wavelength at 10 mW. The laser beam was applied directly on the site of the operation.

The values obtained from blood samples and tissue specimen examinations were estimated statistically using ANOVA and LSD to compare the responses of both the control and laser-treated groups throughout the entire period of the study.[15]

RESULTS

In the treated group, HPLC showed a rapid increase in the levels of adenosine, GH, and FGF, reaching peak at 12–16 h and gradually dropping to normal values by 72 h (Tables 1–3).

The histopathological examination indicated an increase in the F/C ratio and the capillary diameter in the treated group, which corrolated with the HPLC readings (Tables 4 and 5).

Statistical analysis revealed a very highly significant ($p < 0.001$) response in the animals of the treated group from the occlusion of the femoral artery, beginning at the fourth hour after irradiation and peaking at the twelfth hour. The normalization of the injured area and whole body function commenced early in a very highly significant manner ($p < 0.001$), beginning at the twentieth hour and continuing until the end of the study. The results obtained from the treated group were very highly significant ($p < 0.001$), when compared with those of the control group for the same periods.

The level of adenosine and GH and F/C ratio reached peak 12 h after irradiation with the laser ($p < 0.001$), while in the

Table 1. Mean and Standard Deviations of Adenosine Concentration for Control and Laser-Treated Groups

Time (h)	*Control (nM/mL)*	*Laser (nM/mL)*	*Level of significance*
0	2.475 ± 0.04	2.475 ± 0.43	NS
4	3.76 ± 0.22	5.520 ± 0.03	**
8	5.435 ± 0.04	8.910 ± 0.04	**
12	5.518 ± 0.15	9.760 ± 0.02	**
16	5.435 ± 0.05	9.705 ± 0.01	**
20	9.166 ± 0.27	7.010 ± 0.02	**
24	9.652 ± 0.39	4.310 ± 0.06	***
48	7.547 ± 0.34	3.180 ± 0.03	**
72	7.496 ± 0.24	2.480 ± 0.034	**

NS, not significant ($p > 0.05$); *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.01$).

Table 2. Mean and Standard Deviations of Growth Hormone Concentrations for Control and Laser-Treated Groups

Time (h)	*Control (ng/mL)*	*Laser (ng/mL)*	*Level of significance*
0	0.96 ± 0.72	0.96 ± 0.8	NS
4	1.68 ± 0.16	2.80 ± 0.04	*
8	2.74 ± 0.73	3.96 ± 0.81	**
12	3.51 ± 0.10	4.99 ± 0.84	**
16	3.76 ± 0.09	4.81 ± 0.07	**
20	4.65 ± 0.07	3.10 ± 0.73	**
24	4.91 ± 0.08	2.56 ± 0.74	**
48	4.82 ± 0.07	1.73 ± 0.07	**
72	4.44 ± 0.08	0.98 ± 0.83	**

NS, not significant ($p > 0.05$); *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.01$).

Table 3. Mean and Standard Deviations of Fibroblast Growth Factor Concentrations for Control and Laser-Treated Groups

Time (h)	*Control (ng/mL)*	*Laser (ng/mL)*	*Level of significance*
0	0.0031 ± 0.0017	0.0031 ± 0.001	NS
4	0.0051 ± 0.001	0.0140 ± 0.002	NS
8	0.0077 ± 0.001	0.0320 ± 0.002	**
12	0.0096 ± 0.0014	0.0486 ± 0.006	**
16	0.0101 ± 0.0014	0.0398 ± 0.003	**
20	0.0234 ± 0.0017	0.0291 ± 0.0014	NS
24	0.0288 ± 0.003	0.0120 ± 0.001	NS
48	0.0310 ± 0.002	0.0093 ± 0.011	*
72	0.0449 ± 0.001	0.0042 ± 0.001	*

NS, not significant ($p > 0.05$); *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.01$).

Table 4. Mean and Standard Deviations of Fiber/Capillary Ratio for Control and Laser-Treated Groups

Time (h)	*Control (μm)*	*Laser (μm)*	*Level of significance*
0	1:0.38 ± 0.01	1:0.38 ± 0.01	NS
4	1:0.41 ± 0.05	1:0.9 ± 0.19	**
8	1:1.025 ± 0.32	1:1.5 ± 0.11	**
12	1:1.66 ± 0.08	1:2.2 ± 0.37	**
16	1:1.65 ± 0.07	1:1.2 ± 0.67	**
20	1:1.1 ± 0.08	1:0.9 ± 0.2	*
24	1:0.78 ± 0.07	1:0.5 ± 0.18	*
48	1:0.75 ± 0.07	1:0.4 ± 0.19	*
72	1:0.58 ± 0.101	1:0.35 ± 0.06	*

NS, not significant ($p > 0.05$); *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.01$).

TABLE 5. MEAN AND STANDARD DEVIATIONS OF CAPILLARY DIAMETER FOR CONTROL AND LASER-TREATED GROUPS

Time (h)	*Control (μm)*	*Laser (μm)*	*Level of significance*
0	0.44 ± 0.045	0.44 ± 0.037	NS
4	0.46 ± 0.063	0.68 ± 0.056	NS
8	0.5 ± 0.86	0.78 ± 0.056	NS
12	0.53 ± 0.063	0.95 ± 0.031	*
16	0.59 ± 0.576	0.94 ± 0.054	*
20	0.65 ± 0.089	0.83 ± 0.067	NS
24	0.68 ± 0.067	0.72 ± 0.077	NS
48	0.64 ± 0.091	0.63 ± 0.161	NS
72	0.62 ± 0.092	0.49 ± 0.06	NS

NS, not significant ($p > 0.05$); *, significant ($p < 0.05$); **, highly significant ($p < 0.01$); ***, very highly significant ($p < 0.01$).

control group they peaked 24 h postoperatively. The level of the FGF was highly significant ($p < 0.01$), reaching a peak at 4–12 h in the treated group. Normalization began 16 h after irradiation in the treated group and continued until the end of the experiment ($p < 0.01$). They also revealed highly significant variations ($p < 0.01$) when compared with those obtained from the control group for the same periods. There was a rapid increase in the capillary diameter 4–12 h after irradiation, and normalization began at 16 h and continued until the end of the experiment in the treated group. When these results were compared with those obtained from the animals of the control group for the same periods, they showed significant variations ($p < 0.5$) in the diameter of the capillaries to the end of the experiment.

DISCUSSION

The factors that cause a large artery to develop into a more or less constant shape are not completely understood. Probably in the earliest embryonic stages, the formation of the vessels takes place through heredity. Later, the shape and growth of the blood vessels are determined by local chemical and hemodynamic factors.[16] Many authors have proved increased vascularization of the sites with LLLT. This result has been shown to be a laser-specific reaction.[6]

Growth hormone stimulates G-protein, which facilitates cyclic adenosine monophosphate (cAMP). The latter, in turn, stimulates cyclic deoxyribonucleic acid (cDNA); this product will stimulate the messenger ribonucleic acid (mRNA), which enhances and increases protein synthesis. Prostaglandins exaggerate the stimulatory effect of GH, cAMP, and proteinkinase.[5]

FGF stimulates phosphodiesterase that converts pentos phosphate shunt to yield nicotinamide diphosphate (NADPH).[5] This compound converts to nicotinamide dihydrogen (NAD) and adenosine triphosphate (ATP). The latter, in turn, breaks down to adenosine diphosphate (ADP) and a phosphorous molecule, while the oxygen molecules convert to free radicals. Prostaglandins enhance the stimulatory effect of FGF.[17]

Low-level lasers also activate ATP, ATPase, and the conversion of adenosine triphosphate to adenosine. Adenosine stimulates the conversion of cAMP to nitric oxide (NO) or the vascular endothelial growth factor (VEGF).[18]

Adenosine, GH, FGE, and VEGF are angiogenic factors and promote new vessel growth in the same manner.[3] Endothelial budding is induced by the angiogenic factors, which are secreted by the parenchymal cells in hypoxic states, possibly as a product of anaerobic glycolysis formed at the site of these cells and diffused in all directions.[1,2]

The success of collateral circulation and rapid appearance of the microcirculation as a part of the compensatory mechanism of the body against the sudden occlusion of the femoral artery may be due to the early anastamosis between the perforating branches of the profunda femoris artery with the articular and muscular branches of the same artery with those of the popliteal artery when the femoral artery is ligated above the adductor canal.[9] Thus, we can explain the rapid increase in the number and diameter of the capillaries within the first hours until the peak at the twelfth hour after irradiation with low-level lasers and the subsequent decrease to near-normal values.

The results of the present study agreed with those obtained by Maegawa et al.,[19] who investigated the effect of low-level lasers on the rat mesenteric microcirculation *in vivo*. They provide a potent dilation of the arterioles irradiated with laser, followed by a marked increase in the arteriolar blood flow.

CONCLUSIONS

Low-level laser emission increased tissue oxygenation, morphofunctional activity, and substantial expansion of the microcirculatory bed. They, in turn, accelerated the restoration of functions, stimulation of adaptational ability, and stabilization of the hormonal status.

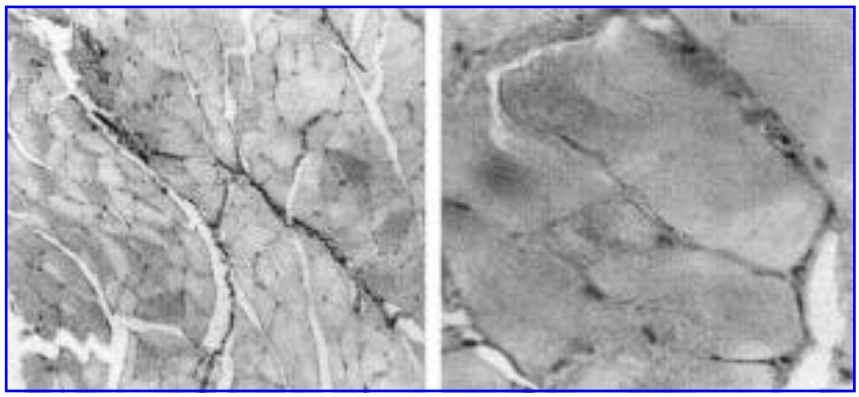

FIG. 1. Gutters of the blood vessels and the surrounding muscle fibers at the time of operation (0 time). (Left, H&E × 4.5; right, H&E × 10.)

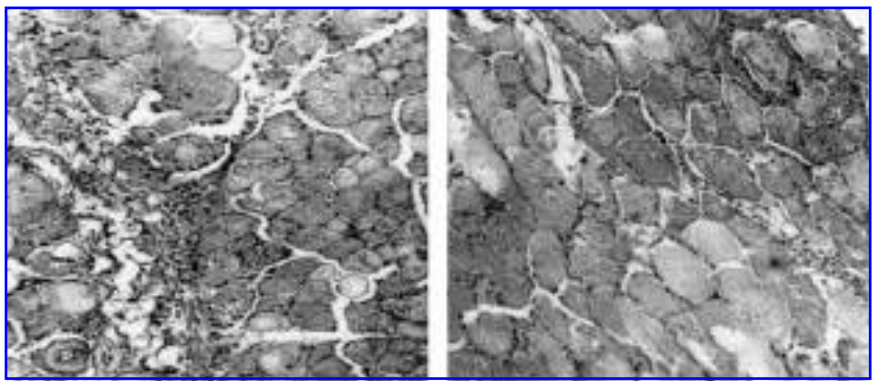

FIG. 2. Highly vascularized area in the treated group 12 h after irradiation with laser (left, H&E × 10), with significant increase in the diameter of the blood vessels (right, H&E × 20).

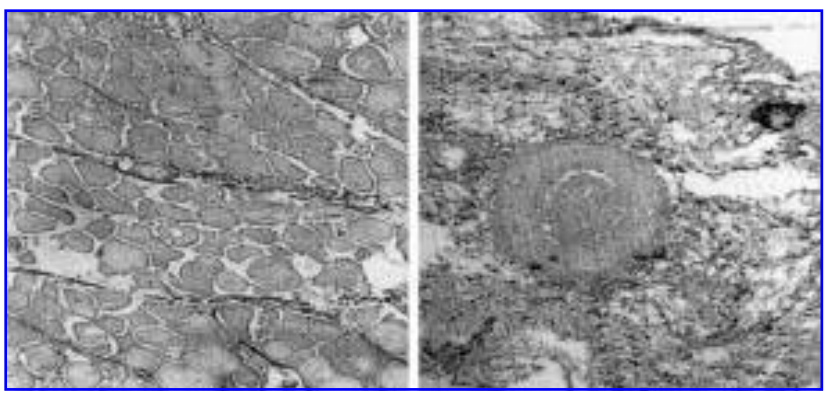

FIG. 3. Beginning of the normalization of the blood vessels and surrounding muscle fibers in the animals of the treated group 16 h after irradiation (left, H&E × 20). Appearance of well-developed arteries in the animals of the treated group 72 h after irradiation (right, H&E × 40).

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