THE EFFECTS OF DIODE LASER LLLT ON FLAP SURVIVAL: MEASUREMENT OF FLAP MICROCIRCULATION WITH LASER SPECKLE FLOWMETRY

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A laser speckle flowmetry technique has been developed to enable visualization of the distribution of skin blood flow and has been used to measure the microcirculation in various angiopathies as well as to monitor blood flow changes and other haemodynamics in skin flaps. The principal author’s institute has recently acquired such a laser speckle flowmetry system. The present preliminary study was designed to test the efficacy of the gallium aluminium arsenide (GaAlAs) diode laser on flap survival in the rat model using laser speckle flowmetry. Caudal-based random pattern flaps were raised on the back of two groups of Wistar rats, 10 rats in each group. The first group served as the control and underwent sham irradiation, otherwise they were handled in exactly the same way as the second group. The blood flow in all flaps was measured with laser blood flowmetry using the laser speckle method. Blood flow was measured in the flaps of the experimental animals and the unirradiated controls preirradiation, and the flaps were sutured back in place. The experimental group received laser irradiation from a GaAlAs diode laser (60 mW, 830 nm, continuous wave) for one minute on a point at the centre of the flap base (energy density $36 \, \text{J/cm}^2$). Laser speckle flowmetry was then performed on all animals immediately postirradiation and 30 minutes postirradiation. The following points were noted. There was no significant change in the flow rate of the flaps in the unirradiated animals. Immediately following the one minute irradiation in the experimental animals a decrease in the blood flow rate was seen compared with the unirradiated controls, but at 30 minutes postirradiation the blood flow rate in the flaps increased in the irradiated animals compared with controls. Five days postirradiation the survival area of the diode laser irradiated flaps was greater than the control flaps ($1.12:1$). It was concluded that GaAlAs diode LLLT increased early perfusion of the experimental flaps, thereby possibly accelerating early stage wound repair while at the same time controlling the inflammatory response, thus giving the irradiated flaps a better and earlier ‘take’.

Key words: random caudal flap, laser speckle flowmetry, LLLT, flap take

Introduction

Following the late Endré Mester’s first and subsequent publications on applications of low incident doses of light energy to enhance wound healing, revascularization and neovascularization, a number of publications have appeared on the effects of laser energy at low incident doses on blood flow rate and volume. A variety of flaps and grafts have been developed to repair tissue defects, but one of the most problematic conditions for the plastic and reconstructive surgeon is the appearance of flap necrosis in vascularly compromised grafts and flaps. Conventional conservative methodology is often insufficient and in extreme cases the flap is totally lost. The authors have reported before on the efficacy of 830 nm diode laser therapy in promoting healing in caudal based flaps in the rat model, comparing a group of rats treated using diode LLLT with a group treated with noncoherent but monochromatic light source and an unirradiated control group. In these studies an increased rate of perfusion was seen in the LLLT-treated flap compared with the non-laser diode and control flaps as assessed by fluorescein angiography one hour after irradiation, increased vascularization was demonstrated by transillumination of excised skin specimens at seven days and statistically significantly better flap survival area was seen with less flap necrosis in the LLLT group compared with the others, while there was no statistical difference seen between the non-laser diode group and the unirradiated controls. There have been no studies on blood flow rate before and immediately after LLLT irradiation however. Recently the principal author’s institute acquired a new system for the accurate measurement of blood flow rate using laser speckle flowmetry, which provides a much clearer visual record of...
of overall blood flow rate than conventional laser Doppler flowmetry. The present preliminary study was therefore designed to examine the differences in blood flow rate before, immediately after and 30 minutes after LLLT in flaps on the rat model, compared with identically timed measurements in an unirradiated control group of animals.

Materials and Methods

Laser System

The laser used was a gallium aluminium arsenide (GaAlAs) diode laser system (OhLase-3D1, Proli Japan, Figure 1) developed by the Japan Medical Laser Laboratory, with an output power in continuous wave of 60 mW at 830 nm with an incident power density at the probe tip of approximately 3 W/cm². The system consists of a control console, from which time parameters are set and recorded, connected to the hand-held probe by a flexible cable. The probe is usually held in contact with the target tissue, where appropriate, thus eliminating any intermediate loss of incident energy and minimizing reflection losses at the target tissue surface. However in the present experiment to remove any tactile stimulation from probe contact, the laser was mounted in a clamp 1 cm from the target point.

Rat Model

Figure 2 shows the design of the caudally-based random flap, with the target point indicated for laser irradiation in the experimental animals at a medial point 2 cm distal from the base of the flap. Two groups of ten male Wistar rats (approximately 3 weeks old, 250 - 300 g) were anaesthetized by intraperitoneal injection of sodium pentobarbital 0.1 mg/100 g sufficient to maintain anaesthesia throughout the entire experimental stage (1 ~ 2 hrs). All 20 animals then had 9 cm by 3 cm flaps raised on their dorsum according to the pattern seen in Figure 2 and demonstrated in Figure 3a, 3b and 3c. The first laser speckle flowmetry measurement (also known as colour Doppler flowmetry) was performed at this time on all animals. The flowmeter head was positioned above the animal and a mask was used over the back of the animal oriented as shown with an aperture the same size as the flap to eliminate any artifacts from the skin surrounding the flap (Figure 4a) while the reading was monitored on the colour CRT and recorded on magnetic media for later analysis (Figure 4b). The laser speckle flowmeter was model LMAP-10 from M and M Co., Ltd, Japan, utilizing a 20 mW beam generated by a diode laser at a wavelength of 780 nm. The flaps were sutured back in place (Figure 3d) after the initial assessment of the flap microcirculation in both control and experimental animals.

The GaAlAs diode laser was positioned in a clamp 1 cm from the target tissue in all 20 animals. The 10 experimental animals then received 1 min laser irradiation (incident power density ≥ 600 mW/cm², incident energy density ≥ 36 J/cm²) and the second laser speckle flowmetry assessment was immediately performed at the same point in time in both control and unirradiated animals. Thirty minutes later, the third and final flow-
metry assessment was performed on both groups of animals.

The animals were allowed to recover, placed in separate cages and were fed and watered normally ad libitum. Five days after irradiation, the degree of survival and necrosis of the flaps was assessed by an independent pathologist who was unaware to which group each animal belonged.

Results

Figure 5 shows representative laser speckle flowmetry of a control animal (upper) and an experimental animal (lower) preirradiation. The base of the flap is to the top of the illustration in both cases. Figure 6 shows the same experimental animal as in Figure 5 before irradiation (upper) and immediately after 1 minute irradiation (lower). A noticeable decrease in blood flow in the flap is clearly visible postirradiation from the laser speckle pattern. Figure 7 shows the condition of the flap in the same control animal as in Figure 5 (upper) and the same LLLT-treated animal (lower) as shown in Figures 5 and 6, 30 minutes postirradiation. The blood flow rate in the irradiated flap is now greater than in the unirradiated control flap, with areas of elevated flow seen in the distal zone of the flap. In particular, there is a significant in-
crease in the flow rate and volume in the experimental animal comparing the right-hand side of Figures 5 and 7. There was no significant difference in the flowmetry data for the unirradiated controls at any point in the experiment.

Figure 8 is the condition of the flaps on the same animals as before (control, left; LLLT-treated, right), five days postoperatively. The LLLT-treated flap has better survival with less necrosis than the control flap, which is showing signs of compromise even at the proximal part of the flap (base of the flap now to the bottom of the illustration). The average survival length of the LLLT-treated flaps was 61.3 mm compared with 54.8 mm in the unirradiated controls.

Discussion

Previous studies by the author and his colleagues concentrated on effects on the blood flow at least a minute after LLLT, as assessed by fluorescein angiography.\(^{(2,3)}\) Many other reports have used thermography as a way of assessing changes in the microvasculature.\(^{(4,5)}\) In these reports the assessment is also made some time after LLLT has been given because it takes time to set up the thermography equipment, and there is also usually an acclimatization period to allow the patient to become
acustomed to the temperature of the thermography suite. Thermography in addition only gives a general idea of increased blood flow as it measures the temperature rise caused by the increased flow rate in the superficial microvasculature, and a large number of environmental variables can cause artifacts in thermographic assessment. On the other hand thermographic equipment is relatively inexpensive compared with laser-based flowmetry systems and thermography itself is also improving in accuracy and speed of measurement. Laser Doppler flowmetry (LDF) was reviewed by Nilsson in an earlier issue of this journal,(6) but, as he stated, conventional LDF is useful for evaluation of a comparatively small area of tissue, and was at the time of writing displayed as digital data and as a plotted line graph. Also it was not feasible to move the probe around due to the variations in readings in areas which appear equally perfused due to alterations in the rhythmical patterns in blood flow seen in the skin (vasomotion) and also irregular variations in skin temperature and their effect on blood flow through vasodilation or vasoconstriction.

Laser speckle flowmetry on the other hand is capable of covering a larger area than conventional LDF, and because of advances in digitizing techniques, can be displayed in real time in colour on a CRT in addition to being stored as both a visual form and in pure data on magnetic or other media for subsequent detailed evaluation. Speckle flowmetry depends on the same phenomenon as LDF for its action. The laser speckle pattern is recognized only in laser beams where there is an intense photon density at the target surface resulting in a constantly shifting interference pattern. Laser speckles have been shown to be capable of penetrating through some centimetres of minced beef packed into glass dishes, and visible on the other side.(7) The flap and flap bed microvasculature in the present study is some millimetres under the skin surface, and therefore well within the range of laser speckles. Depending on the rate and volume of the blood flow into which it is incident, some of the laser energy is backscattered but is shifted spectrally by the fast-moving blood cells, referred to as Doppler shifting or broadening. This Doppler broadening is known to be linearly associated with both blood cell velocity and concentration.(6) By comparing the spectral shift with a portion of the original incident beam as a reference beam, the flow rate and volume can be calculated, digitized, colour simulated and displayed in real time on a CRT.

The apparent drop in blood flow rate as seen in the colour Doppler flowmetric distribution in the animal seen Figure 6 compared with the preirradiation flow in the same animal in Figure 5 immediately after irradiation is an interesting phenomenon. It is not seen on normal thermographic evaluation as that tends to be carried out a few minutes after irradiation, and is a phenomenon appearing at first contrary to conventional wisdom, which is based on the very many reports connecting LLLT sessions with elevation of local and systemic skin temperatures associated with increased photomodulated flow in the microvasculature. This immediate drop in the blood flow rate postirradiation with a HeNe laser has been reported before, however, as a function of temperature change in the visible red light laser-irradiated human fingertip measured by highly sensitive radiant infrared temperature recorders.(8) It is possibly associated with the possible short-term photomodulated depolarization of the parasympathetic nervous system coupled with polarization of the sympathetic system, which is associated with vasoconstriction.

However, the later stages of increased blood flow rate and volume are much more familiar, and coupled with the better flap survival for the LLLT irradiated animals, these data are in complete accordance with the authors' and other previously reported findings and conclusions,(9,10) that the early stage increased perfusion of the LLLT-irradiated flap accelerated wound healing, controlled the local inflammatory response and helped flap survival.

This report is presented only as a preliminary report as the principal author’s institute has only recently acquired the laser speckle flowmetry system. Further detailed studies are planned in both animal models and in man to evaluate the usefulness of this noninvasive real time monitoring system in LLLT for a variety of vascular disorders and conditions.
References