A STUDY ON THE POSSIBLE HAEMORRHAGIC EFFECTS OF EXTENDED INFRARED DIODE LASER IRRADIATION ON ENCAPSULATED AND EXPOSED SYNOVIAL MEMBRANE ARTICULAR TISSUE IN THE RAT

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Low reactive-level laser therapy (LLLT) has been reported as having a beneficial effect in the therapy of rheumatoid arthritis. Some concerns have been expressed about the possible photothermal damage to articular tissue, for example the synovial membrane, following extended doses of LLLT such as are usually applied. The present study was designed to assess qualitatively and quantitatively the possible photothermal damage to articular membranous tissue following an extended exposure (30 min) of infrared laser radiation on the rat synovial membrane, both through the capsule and with the capsule dissected away to allow direct exposure. A third group of unirradiated animals acted as the control. The laser was a gallium aluminium arsenide (GaAlAs) diode laser system, delivering 60 mW, to a total spot size from three diodes of approximately 0.3 cm², power density ≈ 0.2 W/cm², incident energy 108 J, energy density 360 J/cm². Evaluation of any photoinduced thermal damage was made both macroscopically and with blinded microscopic histological assessment. No damage was macroscopically visible, and no difference was found in the histology of the control, indirectly and directly-irradiated synovial membrane specimens. The authors conclude that, at the wavelength and delivery parameters given, even with extended dosage, laser therapy has no adverse photothermal effects on encapsulated or exposed synovial membrane *in vivo*.

KEY WORDS LLLT Photobioactivation Laser safety Laser therapy dosimetry

Introduction

The extended studies of Goldman in the U.S.A. and Oyamada and Asada in Japan on the effectiveness of LLLT in the therapy of rheumatoid arthritis, although spanning some 11 years,¹⁻³ all demonstrate a real effect of LLLT in both relieving pain, reducing swelling and other inflammatory-mediated responses, and enabling greater range of motion (ROM) in the irradiated joint. However, concerns about the possible photothermal damage to long-term LLLT-irradiated tissue have been recently expressed in the literature. With output powers of 1-3 mW, the helium neon laser requires long exposure times to achieve any effect, and photoinduced side-effects following over-irradiation have been a concern.⁴

Accordingly, the present study was designed to assess macroscopically and microscopically the

possible photothermal effects of prolonged diode laser irradiation (30 min) of exposed encapsulated and unencapsulated rat synovial membrane *in vivo*, at comparatively high output powers (60 mW) and incident power and energy densities compared with the ranges reported in the literature, usually measured in mW/cm² and mJ/cm².^{5,6}

Materials and Methods

The laser used was the OhLase-3D1 (Proli, Japan, Ltd), a semiconductor (diode) infrared laser system (gallium aluminium arsenide—GaAlAs), delivering 60 mW from three diode chips, giving a spot size of 0.3 cm².

Experimental Method

The study called for 15 male Wistar strain rats, of approximately the same age and weight (average, 300 g). The animals were anaesthetized by peritoneal injection of 3 mg/100 g Nembutal® (50 mg/ml

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sodium pentobarbital, Abbot Labs, U.S.A.). Following anaesthesia, the animals were divided into three groups (groups A, B and C) of five rats per group. Groups A and B were the experimental laser-irradiated groups, and group C was the identically-handled but unirradiated control group.

Group A (Figure 1a). The skin over both hind leg knee joints was dissected away in all animals, giving 10 models. The laser was clamped in a holder to ensure a constant distance between the target joint and the laser probe head, and therefore a constant incident dose. The synovial capsule was left intact and the synovial membrane in both joints irradiated with the GaAlAs laser at the parameters given before, for an irradiation time of 30 min. The total irradiated area was 0.3 cm², giving a power density of 0.2 W/cm², and an energy density at 30 min exposure time of 360 J/cm².

Group B (Figure 1b). Following similar dissecting away of the skin of both hind knee joints as in group A, the joint capsule was also incised and retracted to expose the membrane. With the laser clamped in place as described above, both joints on each of the five animals were irradiated in noncontact mode at the same parameters as for group A above.

Group C. In group C, one hind leg on each animal was treated similarly to group A, and the other similar to group B, with without irradiation. The tissue specimens from this unirradiated group were used as control.

One hour after the end of irradiation of groups A and B, each irradiated area was carefully checked macroscopically for any sign of heat damage, followed by microscopic histological assessment.

Macroscopic Assessment. (a) The irradiated areas of the articular tissues were carefully examined by

the naked eye. Any sign of thermal damage was marked as positive, as was any indication of laser-induced haemorrhage: no such damage was scored as negative. Figures 2 and 3 show the typical condition of groups A and B encapsulated and exposed synovial membrane, with no macroscopically-visible thermally-induced lesion or degradative change.

(b) The irradiated capsule and synovial membrane tissue was then excised from all three groups, and examined macroscopically for thermal damage. Positive or negative findings were recorded.

(c) The excised tissue was coded, and placed in 10% formalin for 24 h, after which the specimens were routinely prepared and stained using haematoxylin-eosin: microscopic evaluation of the histology was performed and any tissue abnormalities noted and recorded. The histological assessment was performed by an independent laboratory on randomized samples.

Results

Tables 1 and 2 summarize the macroscopic and histological findings. No discolouration or bleeding could be macroscopically observed in any of the irradiated tissues from both the capsule-irradiated group (group A) and the membrane-irradiated group (group B) compared with the unirradiated group C.

Figures 4 and 5 show typical histological specimens for groups A and B, respectively. No alteration in architecture was found in any of the specimens, and there was no difference in the histological findings between the groups A, B and C.

Discussion

From previous *in vivo* experimental work on the effect of LLLT on nerve cell sodium-potassium-

Figure 1. Schematic of design for experimental and control joints. **A:** Incision of overlying tissue to allow laser irradiation of synovial membrane through intact joint capsule. **B:** Same incision of overlying tissue as before, but capsule incised and retracted to allow direct laser irradiation of synovial membrane. Control joints received no irradiation but were otherwise treated identically to the experimental groups

Table 1. Macroscopic and microscopic assessment of photoinduced synovial membrane damage following 30 min of IR laser irradiation through the intact membrane capsule

Group	Animal	Leg	Assessment of photoinduced damage (<i>In vivo</i> macroscopic assessment)						Histological change (microscopic)
			Joint capsule		Synovial membrane		Excised synovial membrane		
			Thermal	Bleeding	Thermal	Bleeding	Thermal	Bleeding	
			damage		damage		damage		
Group A: 30 min	No. 1	L hind leg	-	-	-	-	-	-	-
		R hind leg	-	-	-	-	-	-	-
	No. 2	L hind leg	-	-	-	-	-	-	-
		R hind leg	-	-	-	-	-	-	-
	No. 3	L hind leg	-	-	-	-	-	-	-
		R hind leg	-	-	-	-	-	-	-
	No. 4	L hind leg	-	-	-	-	-	-	-
		R hind leg	-	-	-	-	-	-	-
	No. 5	L hind leg	-	-	-	-	-	-	-
		R hind leg	-	-	-	-	-	-	-

*Sample histology seen in Figure 4.

Table 2. Macroscopic and microscopic assessment of photoinduced damage to the synovial membrane following 30 min of direct IR laser irradiation

Group	Animal	Leg	Assessment of photoinduced damage (<i>In vivo</i> macroscopic assessment)				Histological change (microscopic)
			Synovial membrane		Excised synovial membrane		
			Thermal damage	Bleeding	Thermal damage	Bleeding	
Group B: 30 min	No. 1	L hind leg	—	—	—	—	—
		R hind leg	—	—	—	—	—
	No. 2	L hind leg	—	—	—	—	—
		R hind leg	—	—	—	—	—
	No. 3	L hind leg	—	—	—	—	—
		R hind leg	—	—	—	—	—
	No. 4	L hind leg	—	—	—	—	—
		R hind leg	—	—	—	—	—
	No. 5	L hind leg	—	—	—	—	—
		R hind leg	—	—	—	—	—*

*Sample histology seen in Figure 5.

Figure 2. Representative view of exposed group A joint with joint capsule intact, following laser irradiation of synovial membrane through capsule. No visible photothermal changes and no irradiation-caused haemorrhage. (GaAlAs laser, 830 nm, 60 mW c/w, spot size 0.3 cm², irradiation time 30 min)

Figure 3. Representative view of exposed group B joint with joint capsule incised and retracted, following direct laser irradiation of synovial membrane. No visible photothermal changes and no irradiation-caused haemorrhage. (Laser parameters same as for Figure 2)

Figure 4. Section of excised IR indirect laser-irradiated synovial membrane from a group A animal (animal 4—irradiation through joint capsule). Laser parameters as for Figure 2 above. No abnormality of tissue architecture seen. (HE stain, original magnification $\times 100$)

Figure 5. Section of directly IR laser-irradiated synovial membrane from a group B animal (animal 5). Laser parameters as for Figure 2 above. No abnormality of tissue architecture seen. (HE stain, original magnification $\times 100$)

adenosine triphosphatase (Na-K-ATPase) activity,⁷ the maximal activity in the irradiated rat sciatic nerve was found after a 15-s exposure time with an incident power density (PD) of $\approx 3 \text{ W/cm}^2$. As the incident PD in the present trial was 0.2 W/cm^2 , the most effective irradiation time should therefore be in the region of $(15 \times 15) \text{ s}$, or 3.75 min. The irradiation time of 30 min was thus chosen as a fair example of an extended dose, just under 10 times 'normal'. A 3 mW HeNe laser with a spot size of 2 cm^2 , a representative dose as seen in the literature, will give an incident power density of 1.5 mW/cm^2 , approximately similar to the irradiance delivered by solar radiation at sea level, measured at $630 \pm 5 \text{ nm}$. As the incident irradiance from the system used in the trial is 0.2 W/cm^2 , a difference of over two orders of magnitude, the authors feel that the parameters as described in the study represent a well-extended dose, compared with the average

normal LLLT dosage reported in the literature, especially from HeNe systems.

Despite this extended dose, no damage or tissue degradative changes were seen in either the encapsulated or exposed irradiated synovial membrane, compared with the unirradiated control tissue. Blinded histological assessment also failed to show any alteration of the normal membrane tissue in both experimental and the control group.

Conclusions

From the evaluation of the data, it can be concluded that, for the parameters in this study, near IR laser irradiation at a wavelength of 830 nm, continuous wave, total irradiated area from the triple diode beams of 0.3 cm^2 , output power 60 mW, power density 0.2 W/cm^2 , irradiation time, 30 min, and an energy density 360 J/cm^2 of the rat synovial capsule and the synovial membrane *in vivo* did not cause any adverse photothermal effects, such as discolouration, haemorrhage, or degradative changes of the collagen matrix. The photothermal safety of the IR laser at these parameters has therefore been further demonstrated in *in vivo* synovial membrane tissue in the rat model.

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