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Short Communication

In vivo clinical and histological thermal effect of a 445 nm diode laser on oral soft tissues during a biopsy

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Abstract: Blue diode laser emits a 445 nm wavelength light, which is extremely effective with hemoglobin, and has an optimal hemostatic effect. This work analyzes its thermal effects and clinical efficacy, when used to perform excisional biopsies. Forty-two excisional biopsies were performed with the laser; then, the extent of perincisional thermal effects was evaluated and patients were followed up. Average alteration on epithelial tissue was 507.07 µm, and on connective tissue was 320.39 µm. In all the cases, it was possible to obtain a histological diagnosis. Diode lasers can be safely used to perform biopsies; various intra- and postoperative advantages were confirmed.

Keywords; blue laser, diode laser, excisional biopsy, histological evaluation, oral biopsy, oral lesion

Introduction

Laser devices are still the object of experimental research, but are used in several clinical activities, and for this reason are being continuously optimized. In addition to oral surgery, both low and high intensity laser devices are used in the dental field [1].

Laser light, emitted by stimulated emission, has a different wavelength based on the active medium, thus the basic consideration when using these devices for clinical purposes is interaction between beam and target tissues through selective absorption. Human tissues contain chromophores, which are able to absorb radiation in the visible or ultraviolet spectrum; the laser wavelength interacts with specific tissue chromophores, which are melanin, water, hemoglobin, and hydroxyapatite. Currently, selection of laser devices is based on interaction between chromophores and target tissue via selective photothermolysis, to maximize effects on target tissues, and reduce surrounding tissue alteration [2].

Diode lasers are very practical devices due to their small size, low cost, and optimal cutting properties. Blue lasers, recently adopted in medicine, can emit high-energy light through a beam with a wavelength within the visible blue light range. This light is selectively absorbed by oxygenated hemoglobin, thus giving it optimal properties of interaction with highly vascularized tissues.

Diode lasers have numerous applications and can be used to perform oral soft tissue biopsies. Lasers are used in surgery for the many advantages that they offer such as: accurate cutting; intraoperative bleeding control, improved operator view; optimal ergonomics; and potential analgesic effect. Additional benefits include healing without sutures; better postoperative course, with a reduction in complications; antibacterial potential; and tissue biostimulation [3].

Compared to a traditional cold blade, however, laser devices may have some critical issues, such as cost of the device, and the learning curve required for new devices. Moreover, laser beams can alter the targeted tissues due to photothermal effects.

Concerning the thermal effect during laser biopsies, various wavelengths have been tested, to ascertain the safety of their use during oral biopsy procedures, which require optimal readability of the specimen, but blue diode lasers have not been tested yet with an adequate sample size [4].

The aim of this "*in vivo*" study was to evaluate thermal effects on perincisional tissue, when using a 445 nm diode laser to conduct a biopsy, and to define the safety and clinical efficacy of the device.

Materials and Methods

This uncontrolled clinical trial obtained a certificate of good clinical practice (EGCP19/3015). The study was conducted according to the guidelines of the World Medical Association Helsinki Declaration, and a local ethics committee (No. 4011).

Forty patients were selected and an excisional biopsy was performed on all patients, with two patients undergoing double surgery for two lesions.

The inclusion criteria specified that patients must: have clinically benign oral soft tissue lesions; have lesions not extended to the bone plane; not be under 12 years of age; and have no severe systemic disorders. Patients with potentially malignant epithelial lesions, enucleable lesions, or severe systemic disorders, were excluded from the study.

All biopsies were performed by the same skilled operator, with a 445 ± 5 nm blue diode laser (Eltech K-Laser srl, Treviso, Italy) with the same parameters: a power of 2.5 watts in Continuous Wave (CW), a fluence of 3,100 J/cm^2, and an optical fiber with a diameter of 320 µm.

Infiltrative perilesional anesthesia was performed with 1.8 mL of mepivacaine solution (Mepivacaina Pierrel, 30 mg/mL, Pierrel Spa, Milan, Italy). All lesions were tractioned with a suture; the excision was performed with an incision of all sides of the lesion.

No sutures were applied, hemostasis was monitored for all patients, and they were followed-up at one week, one month and three months, to check healing and to detect any recurrence. During postoperative course, patients did not take any medications, they only applied 0.2% chlorhexidine spray (Corsodyl, GlaxoSmithKline Consumer Healthcare Spa, Branzate, Milan, Italy) to the postoperative site, tid, for one week.

A blinded anatomopathologist then retrospectively performed the histological analysis of the specimens with an optical microscope (Leica Leitz Camera, Leica Camera AG, Wetzlar, Germany) with a computerized digital camera (Olympus Camedia 5050, Olympus Inc., Tokyo, Japan), capturing 5 Mp images (24-bit color depth, stored as JPG files) of perincisional margin sections at ×100 magnification.

The maximum extent of thermal alterations was quantified to the epithelium and connective tissues separately, such as structural and cellular alterations of the tissues, with ImageScope software (ImageScope, Leica Biosystem, Milan, Italy).

The mean and population standard deviation were calculated using Excel software (Microsoft, Redmond, WA, USA) for both epithelial and connective tissue thermal alterations, and then compared with a student *t*-test.

Results

Fifty-three per cent of patients were male and 47% female. The most frequent site of lesions was the buccal mucosa (Fig. 1). Optimal intraoperative and postoperative hemostasis was achieved in all cases, without sutures.

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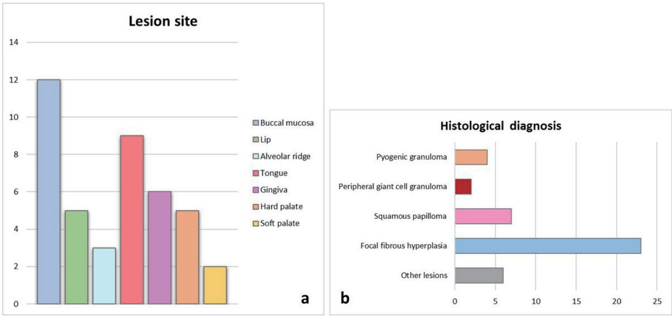

Fig. 1 (a) excised lesions site; (b) specimen histological diagnosis

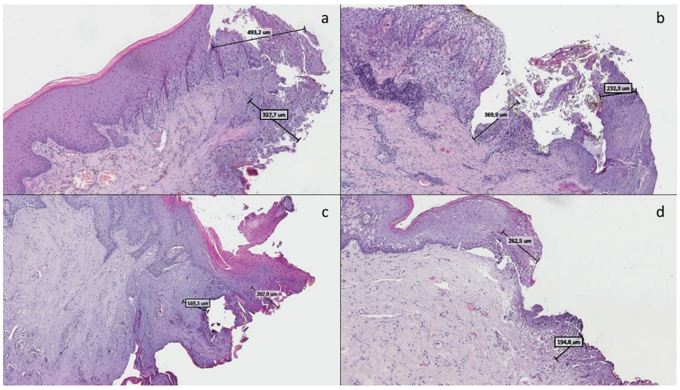

Fig. 2 Specimen (a) 24, (b) 35, (c) 29, (d) 30, histological evaluation, stain H&E, magnification 10×

Table 1 Histological diagnosis and thermal effect of extent of the samples analyzed, with mean and standard deviation

Case	Histological diagnosis	Epithelial thermal effect (μm)	Connective tissue thermal effect (μm)
1.	Peripheral giant cell granuloma	898.7	217.5
2.	Squamous papilloma	136.1	181.7
3.	Pyogenic granuloma	831.5	926.9
4.	Focal fibrous hyperplasia	690.8	555.3
5.	Focal fibrous hyperplasia	1,188.0	585.5
6.1	Pyogenic granuloma	666.8	828.4
6.2	Focal fibrous hyperplasia	331.7	266.1
7.	Squamous papilloma	477.1	248.4
8.	Squamous papilloma	427.1	311.7
9.	Focal fibrous hyperplasia	861.5	599.2
10.	Squamous papilloma	262.4	261.5
11.	Focal fibrous hyperplasia	314.9	167.5
12.	Focal fibrous hyperplasia	518.9	355.3
13.	Focal fibrous hyperplasia	250.0	256.4
14.	Lymphoepithelial cyst	126.0	158.0
15.	Focal fibrous hyperplasia	not evaluable	not evaluable
16.	Keratosis with no dysplasia	244.0	170.3
17.	Keratosis with no dysplasia	373.2	164.8
18.	Pyogenic granuloma	752.6	307.7
19.	Inflammatory focal fibrous hyperplasia	778.5	176.3
20.	Focal fibrous hyperplasia	623.5	126.6
21.	Focal fibrous hyperplasia	1,138.0	246.9
22.1	Focal fibrous hyperplasia	536.4	246.8
22.2	Mucocele	not evaluable	not evaluable
23.	Focal fibrous hyperplasia	176.4	265.3
24.	Peripheral giant cell granuloma	493.2	327.7
25.	Ectopic lymphoid tissue	not evaluable	not evaluable
26.	Squamous papilloma	338.5	185.0
27.	Focal fibrous hyperplasia	563.0	136.3
28.	Pyogenic granuloma	424.2	191.9
29.	Squamous papilloma	207.9	169.3
30.	Focal fibrous hyperplasia	262.5	194.8
31.	Squamous papilloma	not evaluable	not evaluable
32.	Focal fibrous hyperplasia	372.5	111.6
33.	Focal fibrous hyperplasia	1,106.0	476.1
34.	Focal fibrous hyperplasia	345.2	290.5
35.	Inflammatory focal fibrous hyperplasia	369.9	232.3
36.	Focal fibrous hyperplasia	778.3	611.3
37.	Granulation tissue	399.5	854.1
38.	Focal fibrous hyperplasia	344.7	280.0
39.	Focal fibrous hyperplasia	504.6	190.9
40.	Focal fibrous hyperplasia	154.9	298.9
	Mean (μm)	507.08	320.39
	Standard deviation (μm)	±283.76	±209.04

All cases displayed regular healing at the 7-day follow-up, with no postoperative complications.

At the one-month follow-up, healing was complete in all cases and there was no relapse at the 3-month follow-up.

In all 42 samples, it was possible to formulate a definitive histological diagnosis (Fig. 1); the histological evaluations of the thermal effects of laser biopsy were achieved for 38 specimens (Fig. 2), 4 specimens were not oriented correctly after sample processing.

The mean extent of thermal effects on epithelial tissue was 507.07 μm, with a standard deviation of ±280 μm. Connective tissue showed a lower mean thermal effect extent, at 320.39 μm, with a standard deviation of ±206.28 μm. At the epithelial level, an alteration extent of greater than 1 mm was found in only 3 cases (Table 1).

T-student test, showed the difference between means to be statistically significant, with a P value ≤ 0.001 ($P = 0.00085$).

Discussion

Tissue sample obtained through a biopsy is unique and non-replicable, therefore it is essential that it is not altered by the process. Laser devices, when used in biopsies, may cause an alteration of the excised tissue. Indeed, some authors have previously defined this procedure as ineffective, due to potential compromise of the specimen, and the obscuring of any dysplastic changes in the tissue [5]. Several "*ex vivo*" and "*in vivo*" studies have been performed to define soft tissue alterations.

The thermal effect varies according to laser device parameters, at the level of the incised tissue, thus it is very important to establish the type of biopsy being performed. Incisional biopsies that are performed with potentially malignant epithelial lesions, require the perincisional margins to be fully readable, in order to microscopically identify any infiltration in dysplastic lesions [6].

The study shows that 445 nm blue diode laser has optimal intraoperative and postoperative hemostatic effects. In fact, in all cases, visibility during surgery was maintained and no local hemostatic medications or sutures were applied. This outcome is related to selective photothermolysis, which guarantees optimal hemostatic properties at this wavelength, as it is absorbed by hemoglobin, and there is no need to administer a vasoconstrictor. The device tested here demonstrates optimal cutting properties, especially with vascularized tissues, because its power of 2.5 watts can cut soft oral tissues with speed and precision, compared to a CO_2 laser.

Clinically, the device was safe, as all cases showed a postoperative course without complications and at one month, healing was complete in all cases, with no cases of relapse at three months.

The results suggest that the 445 nm diode laser can be considered as safe for performing excisional biopsies; in all 42 samples it was possible to formulate a histological diagnosis of certainty, and achieve complete excision of pathological tissue, both superficial and deep.

Alterations due to thermal effects at the perincisional margin, were found in all cases, and did not prevent histological evaluations (Fig. 3). The mean extent of alterations was greater in the epithelium than in the connective tissue, and this was related to the interaction between the beam and vascularized tissues, which by absorbing energy, reduced alteration of the surrounding tissue. However, with extensively vascularized lesions, such as pyogenic granuloma, the histologically observed effect was greater, due to the intraoperative photocoagulation necessary to ensure hemostasis during surgery, and the poorly vascularized lesions, such as squamous papilloma, showed very limited alterations.

Comparing this study to the same laser device in an "*ex vivo*" context, some differences can be noted. For the specimen groups at both 2 W and 3 W in continuous wave (CW), the mean epithelial thermal effect was more extensive when studied "*in vivo*" than "*ex vivo*", while the connective

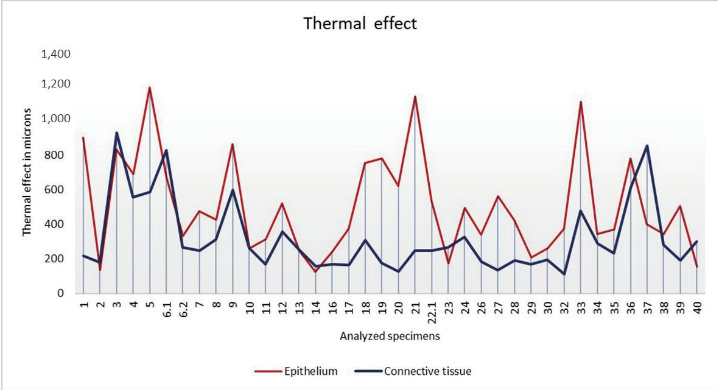

Fig. 3 Extent of thermal effect in micrometers on epithelium (red line), and connective tissue (blue line)

tissue thermal effect was reduced in this study [7]. This difference may be assigned to an absence of a vascular flow, as oxygenated hemoglobin in the connective tissue of pig cadaver tongues does not show selective photothermolysis.

The device tested here also appears to be safer than a 10,600 nm super-pulsed CO_2 laser, when evaluated "*in vivo*", at 4.2 W in pulsed wave (PW) [8]. The higher tissue alteration caused by the CO_2 laser is a result of the greater power needed to achieve beam cutting action, especially for vascularized tissues, as the wavelength of the CO_2 laser has water as a target.

Romeo et al. showed a lower extent of thermal effect, with both an 808 nm diode laser and a KTP 532 nm laser "*in vivo*" when compared to the study device, but the sample size of their study was smaller [9].

Compared to the mean thermal effect of the first 10 specimens in this study, the mean of all 38 specimens is lower, and this trend may be attributable to the learning curve required for new devices, even when used by a skilled operator [10]. This aspect confirms the need to use laser devices correctly, with the right intraoperative parameters, and with correct fiber movements, to limit tissue carbonization.

Therefore, the 445 nm blue diode laser has proven to be an effective and efficient device, safe for surgical use on oral soft tissues, with several clinical advantages. The optimal hemostatic effect could be exploited with high-risk patients, such as those with coagulation disorders, but further selective studies are needed for this purpose.

In relation to the histological thermal effects, this laser device was found to be both safe and advantageous for excisional biopsies of clinically benign lesions in oral soft tissues. Therefore, for excisional biopsies of clinically unsuspected oral soft tissue lesions, laser devices may be viewed as the gold standard, when compared to a traditional cold blade. For clinically suspicious lesions, known as potentially malignant epithelial lesions, this device could be used to perform an excisional biopsy, widening the excision margins by at least 0.5 mm. Based on the study parameters, this would avoid thermal alterations of the lesion margins; however future studies will be needed to confirm this hypothesis.

Conflict of interest

None.

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