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Original Research

Penetration Profiles of a Class IV Therapeutic Laser and a Photobiomodulation Therapy Device in Equine Skin

Stelio Pacca Loureiro Luna [a,*], Allen Schoen [b], Pedro Henrique Esteves Trindade [a], Paula Barreto da Rocha [a]

[a] School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (Unesp), Botucatu, São Paulo, Brazil
[b] Center for Integrative Animal Health, Salt Spring Island, British Columbia, Canada

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ABSTRACT

Photobiomodulation therapy (PBMT) effects depend on the energy settings and laser penetration. We investigated the penetration time profiles of two different light therapy devices, at the dark and light skin regions in horses. Six light skin and six dark skin adult clinically healthy Arab and Quarter horses were used. A cutometer was used to measure the width of the skin fold from both sides of the cervical area, followed by three measurements of the thickness of the same skin fold by transversal and longitudinal ultrasonography (US). The depth of light penetration was compared based on the percentage of penetration versus power, between a portable PBMT device versus a class IV laser device. The laser mean power output was measured with an optical power meter system for 120 seconds after penetrating the skin. Skin width and laser penetration were compared among equipment by paired “t” test. There was no difference in the width of the skin fold between measurements acquired by the cutometer against either longitudinal or transversal US or between the US measurements at cervical versus metacarpus area. Light penetration was greater in both kinds of skins in the PBMT (0.01303 ± 0.00778) versus class IV laser (0.00122 ± SD 0.00070) ($P < .001$). The PBMT device provided a greater energy penetration than the class IV laser in unclipped light and dark skin, suggesting that the former may produce a better therapeutic effect. The color of the skin changes penetration profiles of PBMT.

1. Introduction

Photobiomodulation therapy (PBMT) is currently used for tissue injury repair [1,2]. Several beneficial effects have been attributed to PBMT such as an acceleration of nerve regeneration and function [1] and modulation of the synthesis and organization of collagen in skeletal muscles and tendons [2].

Most studies investigating penetration of PBMT (using laser and/or LED light) are performed in vitro on skin flaps, by measuring immediate penetration depth and energy loss [3,4]. Data in vitro are not necessarily reproducible in vivo as absorption and reduced scattering coefficients are apparently smaller in vivo than in vitro [5].

Tendinitis and desmitis are some of the main musculoskeletal disorders [6] leading to decreased performance and lameness in horses [7]. PBMT is indicated to treat tendinitis and desmitis. The effects are dependent on the energy settings. PBMT may also induce tendinopathy [8,9]; therefore, it is essential to determine the optimal settings to achieve a beneficial therapeutic effect.

Considering the plethora of light therapy devices available in the market, an important matter is to select the most appropriate equipment to guarantee sufficient penetration into the target tissue in situ. Although both a class I PBMT equipment and a class IV therapeutic laser may be used for tissue repair, there are no studies comparing light penetration between these devices and also

Animal welfare/ethical statement: The authors certify that legal and ethical requirements have been met with regard to the humane treatment of animals according to the legislation of the Brazilian Council of Control in Animal Experimentation (http://www.mctic.gov.br/mctic/opencms/institucional/concea/index.html). The study was approved by the Animal Research Ethical Committee from the School of Veterinary Medicine and Animal Science, University of São Paulo State, under the protocol number 0103/2018.

Conflict of interest statement: A. Schoen is an occasional consultant for Multi Radiance Medical (Solon, OH), a laser device manufacturer, but did not participate in data acquisition, management, and interpretation of data or statistical analysis. Other authors do not have conflict of interest. Data storage and documentation: data are available on request.

* Corresponding author at: Stelio Pacca Loureiro Luna, School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (Unesp), Botucatu, São Paulo, Brazil.

E-mail address: stelio.pacca@unesp.br (S.P.L. Luna).

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according to skin color. This information is relevant for the clinician when selecting the equipment for laser therapy.

The aim of this study was to investigate the penetration time profiles of two different light therapy devices, at the skin of the cervical region in dark and light skin horses. The hypothesis of the study is that the ACTIVet PRO PBMT device produces a greater light penetration than the LightForce class IV laser device and the penetration in light skin is greater than in dark skin in both cases.

2. Material and Methods

The study was approved by the Institutional Animal Research Ethical Committee under the protocol number 0103/2018.

Six adult Arabian mares and six Quarter horses (five mares and one stallion) ranging from 330 and 570 kg were included in this prospective study. Their skin color was subjectively analyzed by photographs and classified as light to medium dark or dark skin [10] (Fig. 1). Six horses had light to medium dark skin (two Arabians and four Quarter horses) and the other six had dark skin (four Arabians and two Quarter horses). Horses were kept on pasture grass, supplemented with hay and concentrated food, and water ad libitum. Horses were considered healthy after clinical examination and were restrained in a covered stock for measurement procedures, where concentrated food was supplied between measurements. At the period of measurements, another horse, which shared the paddock with the one that was restrained in the stocks, was maintained at the same room to provide company and reduce stress. The order of all horses was randomized by using the website https://www.random.org/. Once the horse was adapted and comfortable in the stock, a cutaneous fold was produced by grasping the skin at the proximal lateral area of the cervical region with two intestinal clamps. A cutometer was used to measure the width of the skin fold from both sides of the cervical area, followed by three sequential measurements of the thickness of the same skin fold by transversal and longitudinal ultrasonography (US). The width of the skin covering the palmar surface of the metacarpus was also measured by US in six horses to compare with the width of the skin at the cervical area.

Each horse was submitted to light therapy using one equipment at the previously measured cervical skin fold at one side and the other equipment in the other side. The cervical side where the equipment were used was randomized. Therefore in six horses, one equipment was used in the left side and the other one in the right side and in the other six horses the other way round. The depth of penetration was compared based on the percentage of penetration versus power, between ACTIVet PRO portable PBMT device (ACTIVet PRO portable PBMT device: Multi Radiance Medical, Solon, OH) (450 mW mean power output [MPO]) versus a LightForce class IV laser device (LightForce class IV laser device: LiteCure, Newark, DE) (9 W mean output power). The parameters of both light therapy devices are described in Table 1.

The laser MPO was measured with an optical power meter system (Optical Power Meter System: Thorlabs Instruments, Newton, NJ) consisting of a PM200 display unit with a sample rate of 6 Hz and accuracy of 1%, and two different sensors. The S322C sensor was used to measure the total power of devices (without any barrier), and also to test the power through plastic film. The S322C sensor had an aperture area of 4 cm^2 with an optical power range from 100 mW to 200 W and an accuracy of 5%, according to the manufacturer's specification. The S121C sensor was used to measure the percentage of power from devices after penetrating through plastic film and skin. The S121C sensor had an aperture area of 1 cm^2 with an optical power range from 500 nW to 500 mW and an accuracy of 5%, according to the manufacturer's specification.

Fig. 1. Classification of skin color subjectively analyzed by photographs and classified as light to medium dark (left) or dark skin (right).

In step 1, the energy output was measured directly. The two light therapy devices were tested for MPO during 120 seconds of exposure with no obstacles between the laser source and the optical power meter. In step 2, the energy output was measured after penetrating a plastic film. The two lasers were tested for MPO during 120 seconds of exposure with a transparent plastic film between the laser source and the optical power meter. In step 3, the energy output was measured after penetrating the skin. With the

Table 1
Detailed parameters for ACTIVet PRO and LightForce devices.

Parameters	ACTIVet PRO	LightForce
Class	1M	4
Number of lasers	1 Super-pulsed infrared	1
Wavelength (nm)	905 (±1)	980 (±10)
Frequency (Hz)	250	Continuous output
Peak power (W)	50	
Average mean optical output (mW)	1.25	9,000
Power density (mW/cm^2)	2.84	566
Energy density (J/cm^2)	0.34	67.92
Dose (J)	0.15	—
Spot size of laser (cm^2)	0.44	—
Number of red LEDs	3 Red	—
Wavelength of red LEDs (nm)	640 (±10)	—
Frequency (Hz)	2	—
Average optical output (mW)—each	66.67	—
Power density (mW/cm^2)-each	74.08	—
Energy density (J/cm^2)-each	8.89	—
Dose (J)-each	8.00	—
Spot size of red LED (cm^2)—each	0.9	—
Number of infrared LEDs	3 Infrared	—
Wavelength of infrared LEDs (nm)	875 (±10)	—
Frequency (Hz)	16	—
Average optical output (mW)—each	83.33	—
Power density (mW/cm^2)-each	92.59	—
Energy density (J/cm^2)-each	11.11	—
Dose (J)-each	10.00	—
Spot Size of LED (cm^2)-each	0.9	—
Magnetic Field (mT)	35	—
Irradiation time (sec)	120	120
Total energy irradiated to the skin surface (J)	54.15	1080 J
Aperture of the device (cm^2)	4	15.90
Application mode	Cluster probe held stationary in skin contact with a 90-degree angle and slight pressure	Scanning probe in skin contact with a 90-degree angle and slight pressure

probe in stationary skin contact for the ACTIVet PRO (following manufacturer's instruction manual) and in scanning skin contact using a crystal roller ball at the tip of the probe for the LightForce (also following manufacturer's instructions manual), the power of the light therapy devices was measured during 120 seconds of exposure. The percentage of power penetrating the system (skin-plastic film) from each device was measured.

2.1. Statistical Analysis

For each variable, normality was assessed by Shapiro—Wilk test and graphical analysis. Skin width and laser penetration were compared among equipment by paired "t" test (GraphPad software [GraphPad; San Diego, CA]). The results are presented as mean ± SD and *P* value was set at .05.

3. Results

There was no statistical difference for the width of the skin fold between measurements performed by the cutometer against either longitudinal or transversal US. No differences were observed between the width measured by longitudinal and transversal US either at the cervical or metacarpus region. There were no differences between the skin width measured by US of the cervical area and the skin width measured by US at the metacarpus area.

Light penetration was greater in dark skin, light skin, and in both kinds of skin grouped in the PBMT device versus class IV laser (Fig. 2).

The sample size was estimated according to the difference between the mean percentage of light penetration of the two devices, in both kinds of skin grouped. A test power of 80% and an alpha level of 5% were considered.

4. Discussion

Adequate light penetration is fundamental to provide a beneficial treatment and is directly related to the therapeutic outcome [11]. The PBMT device produced a more than tenfold penetration than class IV laser device. When both light and dark skin types were considered, the penetration was 17 and 8 times greater into light and dark skin, respectively, suggesting that a better effectiveness might be achieved when the former device (class I) is used therapeutically. Light absorption is directly linked to the depth of penetration. It depends on the distance between the source of photobiostimulation and the target tissue, the integrity or degree of damaged tissue, and most importantly the wavelength [12,13].

The PBMT device provided light penetration even without clipping the area, showing that it may be used in the intact skin where clipping is not possible, like in show horses. As this device is portable, it may be used in different venues, like competitions.

A previous study [14] tested the percentage of light penetration on skin samples of equine cadavers. However, the skin thickness ranged from 1.5 to 3.5 mm, different from this study where the thickness ranged from 3.2 to 6.8 cm. Therefore, due to this large difference between skin thickness and the outcomes of this study, they cannot be directly compared. In addition, there is the question of the impact of bioelectrical conductivity in live tissue versus dead tissue.

It is well recognized that PBMT can improve tissue repair, by contributing to tendon healing [15] and reducing tendon inflammation in man. This might be achieved by accelerating the extracellular matrix reorganization of the inflamed tendon [16], reducing the release of proinflammatory cytokines, such as TNF-α and, therefore, regenerating the tendon after rupture [17]. According to these beneficial effects, PBMT is indicated to treat tendinopathy in man [18] and in laboratory animals, such as rats [18], and improve the biomechanical properties in tendinopathies [19]. However, improvement of histological parameters is not necessarily achieved either in experimentally [20] or even clinically induced [21] tendon lesions in horses, and this failure may be related to the device settings.

Conversion of in vitro studies to in vivo scenario may be biased by different reflection, absorption, and scattering coefficients [5]. It has been reported that clipping and cleaning the skin with alcohol increases energy penetration through the tissues, but only alcohol applied to the unclipped skin does not [12]. In this study, the skin was unclipped, suggesting that even under this condition, good penetration was achieved. In agreement to a previous study [14], coat color modified penetration, that is, dark skin, reduced penetration, however, the differences in light energy penetration between dark and light skin in our study was not so accentuated for both devices as reported previously by using a class IV laser [14]. Our data and previous data [14] strongly suggest that adjustments of laser energy output settings should be performed, according to the skin color, to guarantee sufficient energy supplied to tendons.

A limitation of previous studies was that either in vitro tissues [14] or the whole leg [12] was used for the calculation of energy penetration. Considering that hemoglobin present in the blood vessels is chromophore, it is expected that in vivo equine skin would reduce penetration of light and therefore the energy

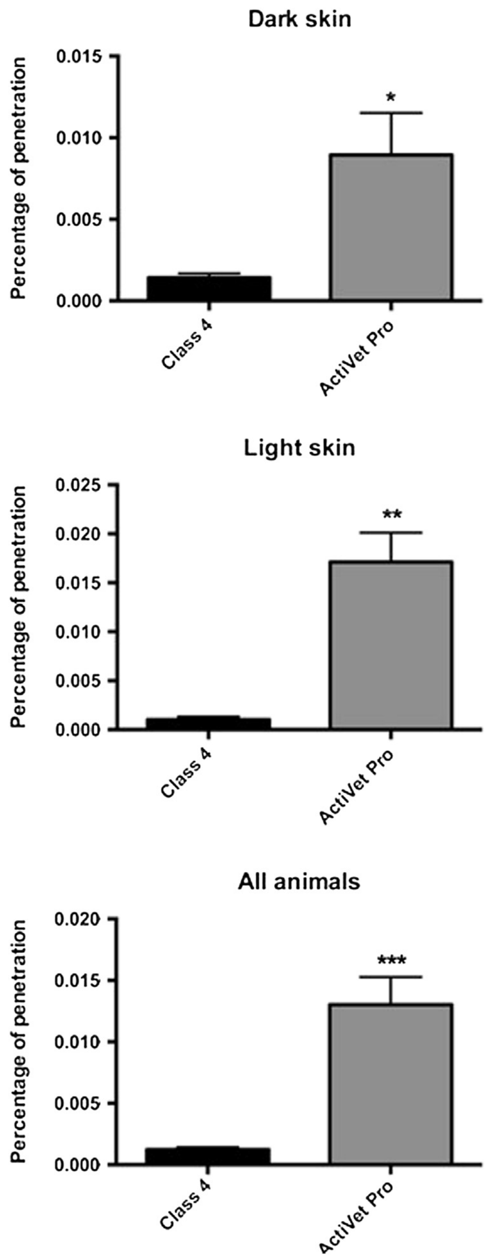

Fig. 2. Mean (SD) of percentage of light penetration of the class IV versus ACTIVet PRO devices in dark skin, light skin, and both kinds of skins grouped. $^{*}P < .05$; $^{**}P < .01$; and $^{***}P < .001$.

absorbed by the target tissue [14]. This limitation was overcome in our study. However, our limitation was that no comparisons were performed between clipped and unclipped areas; therefore, the differences in the percentage of energy between the two devices might not be so prominent when the areas had been clipped. Yet, ideally in show horses, there is a great advantage to not have clipped areas.

The model used in this study seemed to be appropriate to perform an in vivo evaluation of the depth of light penetration, and results may be clinically applicable. The cervical skin area was loose enough to provide sufficient space to position the light devices and the equipment used for measurements. The width of the skin was measured by two different methods (cutometer and US) that showed very similar results and guaranteed the reliability of the data. Another substantial result was that the differences of skin width from different regions (cervical or metacarpal area) were negligible. Therefore, it appears that these results are applicable to the target tissue area where tendon lesions are observed. This study provides evidence that the PBMT class I laser offers greater skin depth penetration around lower leg tendon issues than class IV laser.

We conclude that the PBMT device provided a greater penetration than a class IV laser in unclipped light and dark skin, suggesting that the former may produce a better therapeutic clinical effect. The color of the skin changes penetration profiles of PBMT. Further investigation is warranted to compare the outcome after treatment of injuries by using these devices clinically.

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