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Effects of Laser Photobiomodulation on TGF-ß and VEGF Expression in Burn Wound: Systematic Review and Meta-Analysis in the Animal Model

Influencia de la Fotobiomodulación por Láser en la Expresión de TGF-ß y VEGF en Heridas por Quemadura: Revisión Sistemática y Meta-Análisis en Modelo Animal

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SUMMARY: Laser photobiomodulation (laser PBM) is known to be able to accelerate burn wound healing in the animal model; however little evidence exists on the action of laser PBM on the expression of important proteins in wound healing in the animal model, such as VEGF and TGF-ß1. The aim of this study was to carry out a systematic review in order to analyse the effect of laser PBM on VEGF and TGF-ß expression during burn wound repair in the animal model. A systematic review was carried out of the EMBASE, PubMed/MEDLINE and LILACS databases. The studies included were preclinical studies that analysed the action of laser PBM on the expression of VEGF and TGF-ß (1, 2, 3) during burn wound repair in the animal model. The SYRCLE risk of bias tool was used. Random effect models were used to estimate the combined effect. Increased VEGF expression was observed with the use of laser PBM at 4.93 J/cm^2 per point in the first two weeks after induction of the burn wound, with greater size of effect in the second week (SDM = 5.72; 95% CI: 3.14 to 8.31, I^2 = 0 %; very low certainty of evidence). We also observed that the effect of laser PBM on TGF-ß1 expression was greater than in the control in the first week (SDM = -0.45; 95% CI: -1.91 to 1.02, I^2 = 51 %; very low certainty of evidence), but diminished in the third week after induction of the lesion (SDM = -2.50; 95% CI: 3.98 to -1.01, I^2 = 0 %; very low certainty of evidence). Laser PBM has an effect on TGF-ß1 and VEGF expression, promoting burn wound repair in the animal model.

KEY WORDS: Laser photobiomodulation; Burn wound; Animal model; VEGF; TGF-ß.

INTRODUCTION

Burn wounds constitute an important public health problem, determining physical and psychological sequelae and causing a negative impact on patients' quality of life (Novelli *et al*., 2009); it is therefore important to find effective therapies to reduce cicatrization time. Laser photobiomodulation (PBM) is a non-invasive treatment widely used in medical practice, including wound healing (Chung *et al*., 2012). Phototherapy is able to induce photobiological processes in the cells (Karu, 1987). Laser PBM photoactivates cell mechanisms to promote the normalisation of injured areas by oedema reduction, analgesia and acceleration of tissue repair. The action mechanism of laser PBM occurs through the mitochondria, which promote biomodulation of the tissues, increase the respiratory chain and adenosine triphosphate synthesis (Chung *et al*.), modulate reactive oxygen species (ROS) and induce transcription factors (Chen *et al*., 2011). These transcription factors cause the synthesis of proteins which in turn trigger other effects, such as: greater cell proliferation and migration; modulation of the levels of cytokines, growth factors and inflammatory mediators; and greater tissue oxygenation (Karu & Kolyakov, 2005).

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Wound cicatrization consists of three phases, inflammatory, proliferative and tissue remodelling, which overlap in time (Singer & Clark, 1999). Photostimulation can be used during the inflammatory and proliferative phases, since laser is effective in reducing the inflammatory infiltrate (Mokoena *et al*., 2018), as well as promoting neovascularization (Deana *et al*., 2021). The formation of new blood vessels during wound healing is also fundamental, as they sustain the newly-formed granulation tissue (Singer & Clark). The induction of angiogenesis is attributed to the acidic and basic fibroblast growth factors, endothelial growth factor, transforming growth factor ß (TGF-ß), angiogenin, angiotropin, etc (Iruela-Arispe & Dvorak, 1997). Furthermore, it has been shown that cells irradiated with laser present a greater distribution of filaments in the cytoskeleton, endoplasmic reticulum, and a more conspicuous nucleus, indicating an increase in protein synthesis (Szezerbaty *et al*., 2018).

Laser PBM has been shown to be effective during burn wound healing in the animal model, with observations of increased neovascularization, accelerated wound retraction and increased deposition of collagen fibres (Deana *et al*.). Nevertheless, there is still little evidence regarding the action of laser PBM in the expression of important proteins in the wound healing process in the animal model, such as VEGF and TGF-ß. The aim of this study was to carry out a systematic review in order to analyse the effect of laser PBM on the expression of VEGF and TGF-ß (1, 2, 3) during burn wound repair in the animal model. The research question for this study was: What is the effect of laser PBM on VEGF and TGF-ß expression in burn wound repair in the animal model?

MATERIAL AND METHOD

Protocol. The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guide was used to report this systematic review (Liberati *et al*., 2009). The present report focuses on the results obtained for the action of laser PBM on VEGF and TGF-ß expression. This study was registered in PROSPERO, ID: CRD42019147098.

Eligibility criteria. The studies included were original studies which evaluated the effect of laser PBM on expression of the proteins VEGF and TGF-ß (1, 2, 3). Studies written in English, Spanish and Portuguese were included, with no date limit. In vitro studies, studies using high level laser, LED, studies in humans, therapies with other kinds of light, studies in animals with systemic diseases and works which evaluated laser PBM associated with another therapy were excluded.

Information sources and search. A systematic search was carried out in EMBASE, PubMed/MEDLINE and LILACS. The references of the studies included were also reviewed. The following search strategy was used: ((("Burns") OR burn*)) AND (((photobiomodulation*)) OR ((((((LLLT) OR low light laser therapy) OR laser*[tiab]) OR "Laser Therapy") OR "Low-Level Light Therapy"))) AND ((((((healing) OR complication) OR infect*)) AND (("Wound Healing") OR wound))).

Study selection. All the references identified were extracted to an EndNote X9 database to facilitate handling and eliminate duplicate articles. Two independent reviewers examined the titles and abstracts of the studies recovered by the search strategy to identify studies that might comply with the inclusion criteria. Titles and abstracts were selected using the Rayyan software (http://rayyan.qcri.org). Two independent reviewers reviewed the full texts of all the relevant and potentially relevant studies that met the inclusion criteria, and those for which there was insufficient information in the title and abstract to reach a clear decision.

The following data were collected: animal model used and type of burn, treatment parameters, follow-up time, outcome measurements analysed, sample size calculation or analysis of the power of the study, and use of a report template.

Risk of bias. The risks of bias were assessed using the SYRCLE risk of bias tool for animal studies (SYRCLE RoB) (Hooijmans *et al*., 2014). The following domains were acquired: 1. Random sequence generation (selection bias); 2. Baseline characteristics (selection bias); 3. Allocation concealment (selection bias); 4. Random housing (performance bias); 5. Blinding (performance bias); 6. Random outcome assessment (detection bias); 7. Blinding (detection bias); 8. Incomplete outcome data (attrition bias); 9. Selective outcome reporting (reporting bias); 10. Other sources of bias (other bias). The potential risk of bias for each study was classified as "No" (high risk), "Uncertain" (unclear risk) or "Yes" (low risk).

Data synthesis strategy. The synthesis of the findings was presented in narrative form. Information on the study population, the laser parameters used, the follow-up period and the results measured in the wound were presented in Tables. The meta-analysis was carried out by calculation of the standardised mean differences (SMD) for continuous outcomes. The WebPlotDigitizer 4.4 for Mac software was used for extraction of continuous data presented in figures (box plots). We pooled studies that compared the effect of laser PBM vs. control in the VEGF and TGF-ß1 expression. In studies that presented more than one energy density (ED),

the values closest to other grouped studies was used. Subgroup analysis was carried out by follow-up period, classified as follows: 1 week (analysis from day 1 to day 7), 2 weeks (analysis from day 8 to day 14), 3 weeks (analysis from day 15 to day 21). Forest plots were constructed showing the summary and 95 % confidence interval (CI) estimated in the meta-analysis, together with the results of the individual studies. We used a random effects model (DerSimonian-Laird method), as we expected variation in the effects due to differences in preclinical studies. The heterogeneity between the studies was examined by I^2 statistical categorization as follows: <30 % unimportant; 30 %-50 % moderate; 50 %-75 % substantial; 75 %-100 % considerable (Liberati *et al*.; Higgins & Green, 2011). To explore the possible publication bias, a funnel plot was planned when the number of grouped studies was greater than or equal to 10. The software used was Review Manager 5.4 (Cochrane IMS, Copenhagen, Denmark).

The principle of the GRADE system was used to evaluate the general quality of the body of evidence associated with the principal result, and we constructed a "Summary of Findings" (SoF) table using the GRADEpro GDT software (http://gdt.guidelinedevelopment.org) (Langendam *et al*., 2013; Hooijmans *et al*., 2018). The GRADE approach evaluates the quality of a body of evidence based on the degree of certainty that the element assessed has an effect or association. The quality of the set of proofs was assessed with reference to: the general risk of bias of the studies included; indirect evidence; inconsistency; imprecision; publication bias; and size of the effect. The quality of the evidence was downgraded by one or two levels for each of these factors, reducing the certainty of the evidence. The quality of the set of proofs was classified for each of the primary results as high, moderate, low or very low.

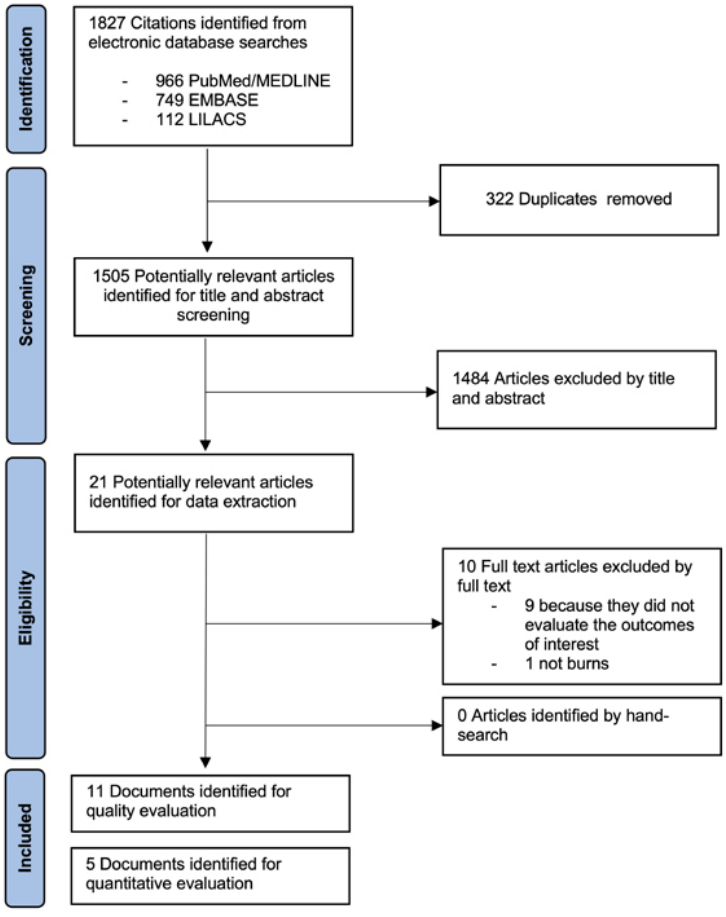

Fig. 1. Flow diagram showing the study selection process.

RESULTS

Search results. The search identified 1,827 references (Fig. 1). After 322 duplicates had been excluded and the titles and abstracts had been reviewed, 21 articles were read in full text. Subsequently, 10 studies were excluded in the full text stage because they did not analyse the outcomes evaluated in the present study; thus a total of 11 studies were included in the qualitative synthesis and 5 in the quantitative analysis.

Characteristics of the studies included. Almost all the studies included were carried out in Brazil (9/11) using Wistar rats and red laser; the two exceptions were published in India and used Sprague-Dawley rats and infra-red laser (Gupta *et al*., 2015; Yadav *et al*., 2017). The dosimetry per point used in the studies included varied between 0.2 J/cm^2 and 25 J/cm^2. All the studies used metal plates to induce the burn wound. The majority of the studies (9/11) induced a second degree burn and only two studies a third-degree burn (Table I). To process the samples and analyse VEGF and TGF-ß1 expression, the majority of the studies (8/11) used the Western Blot and densitometry methods (Renno *et al*., 2011; Belli *et al*., 2014; Chiarotto *et al*., 2014; Catarino *et al*., 2015; Gupta *et al*.; Jácomo *et al*., 2015; Trajano *et al*., 2015; Maligieri *et al*., 2017). Two other studies used immunohistochemistry (2/11) (Brassolatti *et al*., 2016, 2018) and one study (1/11) used ELISA (Yadav *et al*.). None of the studies reported the sample calculation or presented a power analysis. No study used statements or checklists to report results. The principal results reported by the studies on the action of laser PBM on VEGF and TGF-ß1 expression are presented in Table II.

Table I. Characteristics of the studies included.

Author	Country	Animal model, sex	N Animals	Age of animals	Method of wound induction	Degree of wound	Laser equipment model	ED (J/cm^2)	Application technique
Belli *et al.*, 2014	Brazil	Winstar rats, male	72	150 days	Metal plate heated to 120 ºC	2	670 nm InGaP Laser	4.93	No direct contact, point application
Brassolati *et al.*, 2016	Brazil	Winstar rats, male	30	12 weeks	Metal plate heated to 150 ºC	3	670 nm InGaP Laser	12.5 25	Direct contact, point application
Brassolati *et al.*, 2018	Brazil	Winstar rats, male	40	12 weeks	Metal plate heated to 150 ºC	3	670 nm InGaP Laser	25	Direct contact, point application
Catarino *et al.*, 2015	Brazil	Winstar rats, male	72	120 days	Metal plate heated to 120 ºC	2	670 nm InGaP Laser	4.93	No direct contact, point application
Chiarotto *et al.*, 2014	Brazil	Winstar rats, male	63	Adults	Metal plate heated to 120 ºC	2	670 nm InGaP Laser; 830 nm GaAl As	4.93 4.48	No direct contact, point application
Gupta *et al.*, 2015	India	Sprague-Dawley	36	Adults	Metal plate heated to 85 ºC	2	904 nm, Ga-As	0.2	Focused point of light
Jácomo *et al.*, 2015	Brazil	Winstar rats, male	72	120 days	Metal plate heated to 120 ºC	2	670 nm InGaP Laser	4.93	No direct contact, point application
Maligieri *et al.*, 2017	Brazil	Winstar rats, male	54	120 days	Metal plate heated to 120 ºC	2	670 nm InGaP Laser	4.93 9.86	No direct contact, point application
Renno *et al.*, 2011	Brazil	Winstar rats, male	32	NR	Heated instrument, 2 x 2	2	660 nm AlGaInP	20	Point application
Trajano et al., 2015	Brazil	Winstar rats, male	38	NR	Metal plate heated to 80 ºC	2	660 nm AlGaInP	20	Grade technique
Yadav *et al.*, 2018	India	Sprague-Dawley	36	Adults	Metal plate heated to 85 ºC	2	904 nm, Ga-As	0.2	Focused point of light

NR: not reported; ED: energy density

Table II. Principal findings reported by the primary studies.

Author	Principal results
Belli *et al.*, 2014	Laser PBM increased TGF-ß1 expression in the initial stage of the repair process, and diminished during the follow-up period. The groups that received treatment presented a significant increase in VEGF expression, with a reduction in the final part of follow-up (21 days).
Brassolati *et al.*, 2016	Laser PBM treatment at 25 J/cm^2 presented an increase in VEGF expression, while laser PBM treatment at 12.5 J/cm^2 was ineffective.
Brassolati *et al.*, 2018	Laser PBM treatment at 25 J/cm^2 determined an increase in VEGF expression.
Catarino *et al.*, 2015	Laser PBM increased TGF-ß1 expression in the initial stage of the repair process, and diminished during the 21-days' follow-up period. VEGF expression was significantly greater in the laser PBM group than the control, and was most marked at days 14 and 21 after the burn wound.
Chiarotto *et al.*, 2014	Laser PBM increased TGF-ß1 expression in the initial period of healing, and diminished during the follow-up period. Laser PBM also effectively increased VEGF expression on day 14, assisting tissue repair. Expression of the protein diminished at the end of the follow-up period (21 days).
Gupta *et al.*, 2015	Laser PBM increased VEGF expression compared to the control in an early stage of tissue repair (4 and 7 days).
Jácomo *et al.*, 2015	Laser PBM increased TGF-ß1 expression in the initial period of repair, and diminished during the follow-up period. Laser PBM also effectively increased VEGF expression on day 14, assisting tissue repair. Expression of the protein diminished at the end of the follow-up period (21 days).
Maligieri *et al.*, 2017	The groups treated with laser PBM presented a gradual reduction in TGF-ß1 expression compared with the untreated group. Laser PBM treatment at 9.86 J/cm^2 presented greater TGF-ß1 expression compared with the lower dosimetry (4.43 J/cm^2). A similar result was observed for VEGF expression, with an increase in this protein reported only with the higher dosimetry (9.86 J/cm^2).
Renno *et al.*, 2011	In the initial stage no differences were reported between the laser PBM and control groups, however after 14 days VEGF expression increased in the experimental group.
Trajano et al., 2015	Early application of laser PBM determined a greater increase in TGF-ß expression than later application, however in both cases TGF-ß expression was higher than in the control.
Yadav *et al.*, 2018	TGF-ß1 expression was higher in the groups treated with laser PBM than in the control.

Risks of bias. All the studies presented a high risk of bias in the general evaluation (Fig. 2). This was due mainly to the difficulty of blinding the operator, since the laser equipment emits light, sound or both; all the studies therefore presented a high risk of bias for this domain. High risk of bias was also observed in 7/11 studies for other sources of bias because the authors did not carry out laser treatment simulation on the animals in the control groups (Belli *et al*.; Brassolatti *et al*., 2016, 2018; Catarino *et al*.; Chiarotto *et al*.; Maligieri *et al*.; Renno *et al*.), which might have affected wound healing. In the domains "allocation concealment", "random housing" and "random outcome assessment", all the studies were classified as having unclear risk of bias, since they did not provide sufficient information to evaluate these domains. Some studies claimed that they carried out random sequence generation, however none of these explained how the sequence was generated, so all the studies were classified as having unclear risk of bias. Only 2/11 studies reported blinding the results assessor (Brassolatti *et al*., 2016, 2018) and were classified with low risk of bias; all the other studies presented insufficient information to judge this domain. All the studies presented low risk of bias for baseline characteristics and selective reporting of results.

Analysis of the effects of Laser PBM on VEGF expression. Five studies were grouped in the meta-analysis for the first two weeks, and 4 studies for the third week. All these studies used male Wistar rats, infra-red laser with similar ED, and the Western Blot method to process the samples. In the first two weeks of treatment, it was observed that laser PBM increased VEGF expression in burn wounds in the animal model: first week (SDM = 2.34; 95 % CI: 0.27 to 4.40; I^2 = 49 %; certainty of evidence very low); second week (SDM = 5.72; 95% CI: 3.14 to 8.31, I^2 = 0 %; very low certainty of evidence). In the third week after induction of the burn wound, it was unclear whether laser PBM increased or diminished VEGF expression (SDM = -0.16; 95 % CI: -1.69 to 1.37; I^2 = 51 %; very low certainty of evidence) (Fig. 3).

Analysis of the effects of laser PBM on TGF-ß1 expression. Five studies were grouped in the meta-analysis for the first two weeks, and 4 studies for the third week. All these studies used male Wistar rats, infra-red laser with similar ED, and the Western Blot method to process the samples. In the first week the meta-analysis showed that laser PBM favoured an increase in TGF-ß1 expression (SDM = 2.15; 95 % CI: 0.11 to 4.20, I^2 = 55 %; very low certainty of evidence). In the second week it was uncertain whether Laser PBM favoured an increase or a reduction in TGF-ß1 expression (SDM = -0.45; 95 % CI: -1.91 to 1.02, I^2 = 51 %; certainty of evidence very low). In the third week the meta-analysis showed that animals treated with laser PBM presented lower TGF-ß1 expression than the control (SDM = -2.50; 95 % CI: -3.98 to -1.01; I^2 = 0 %; very low certainty of evidence) (Fig. 4).

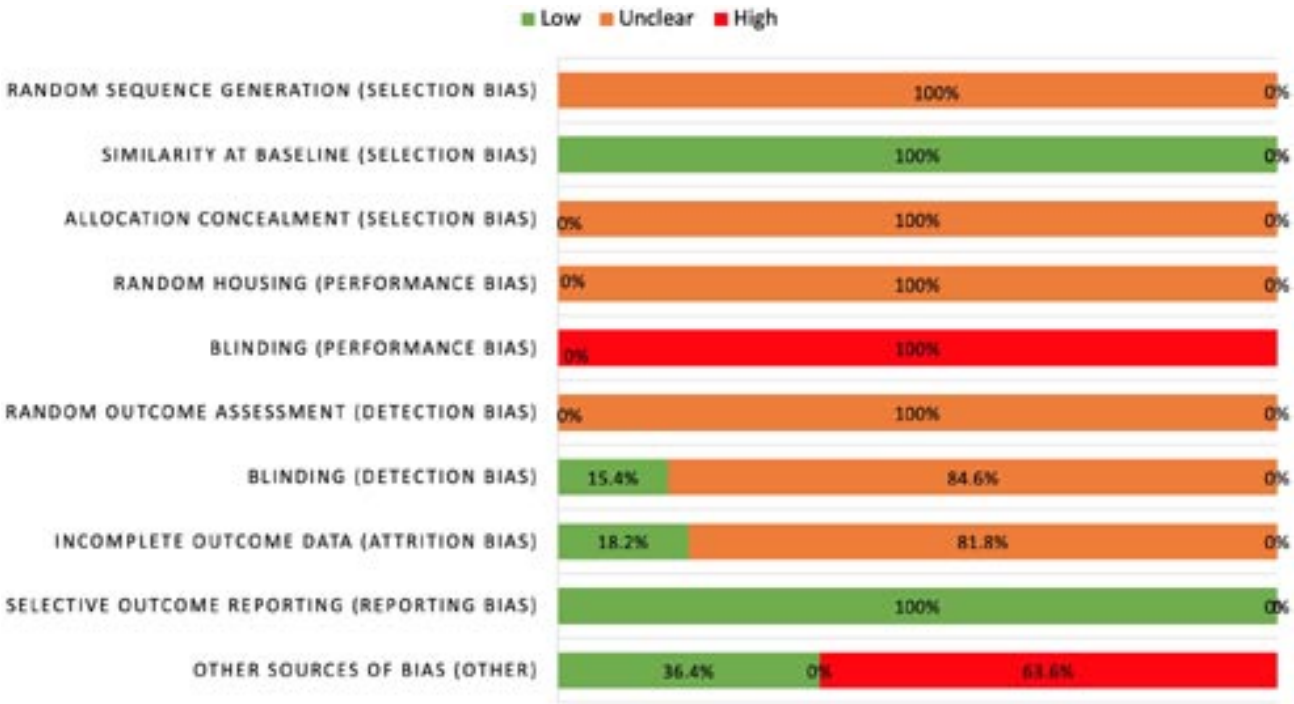

Fig. 2. Risk of bias of the studies included, according to the SYRCLE tool.

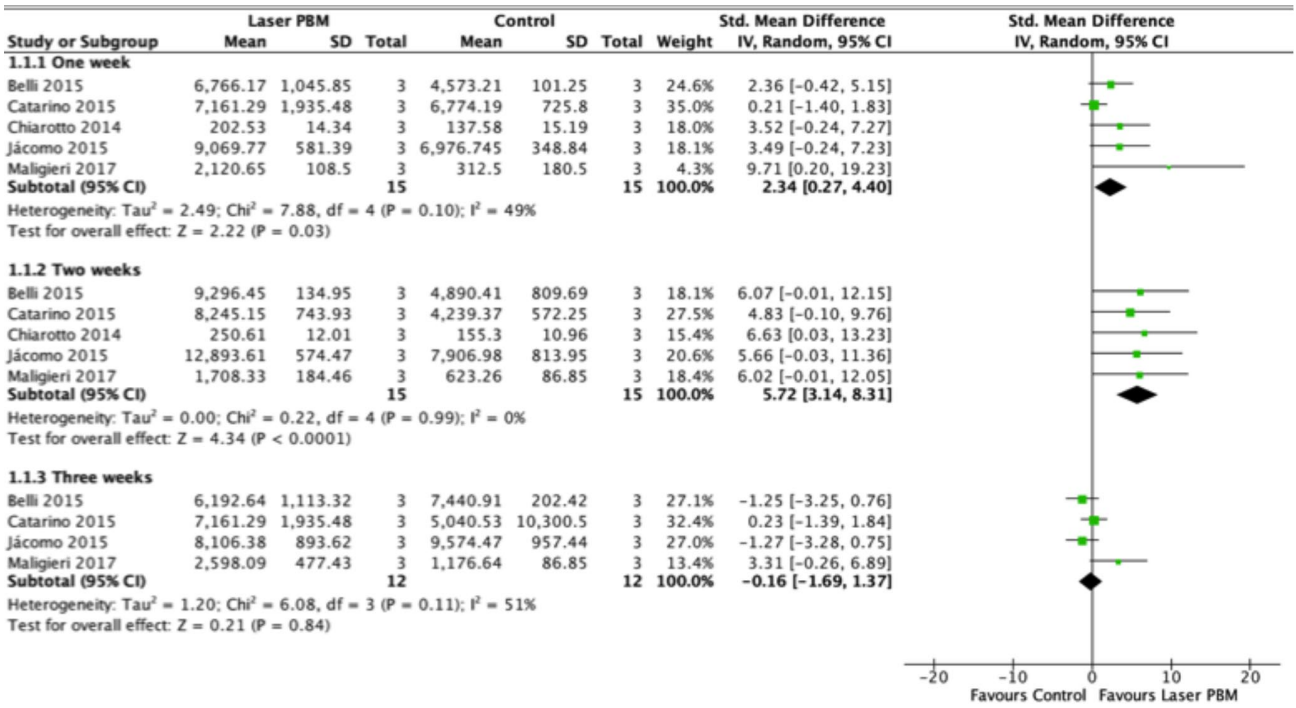

Fig. 3. Forest plot showing the comparison of laser PBM vs. control for VEGF expression.

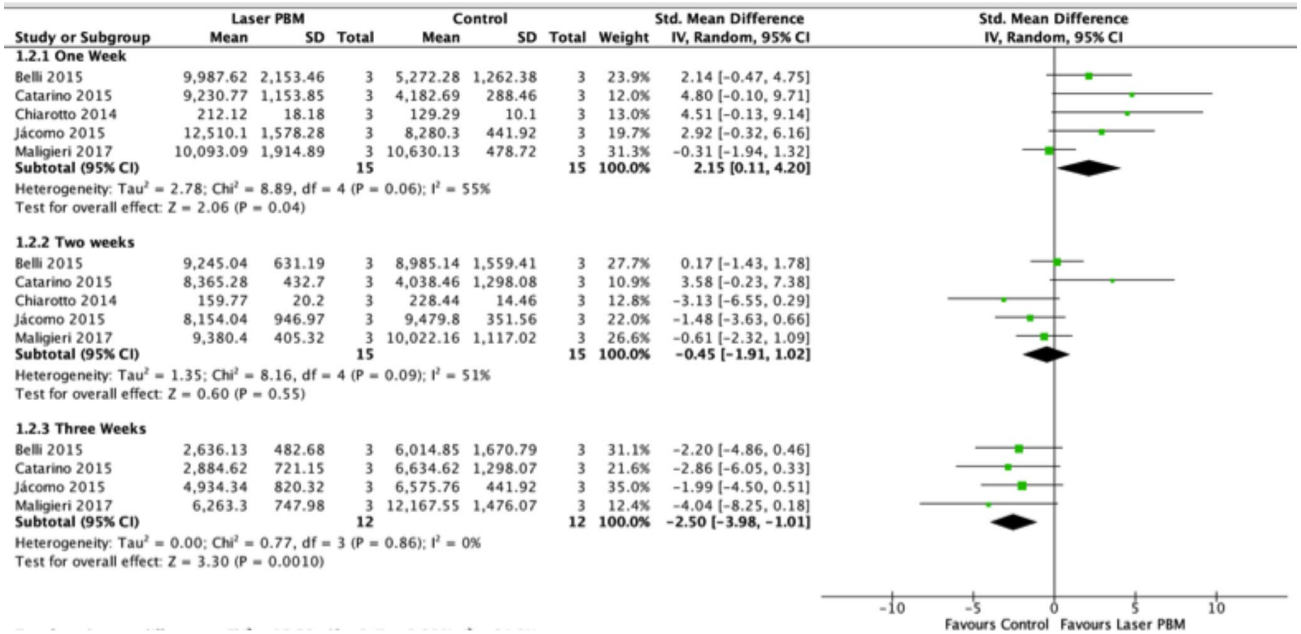

Fig. 4. Forest plot showing the comparison of laser PBM vs. control for TGF-ß1 expression.

Publication bias. Publication bias was not analysed as no comparison presented data from more than 10 studies.

Summary of the quality of the evidence. We analysed the quality of the evidence for all the outcomes (Table III). All the studies in the meta-analysis were experimental and in parallel; however, some methodological problems were identified which diminished the certainty of the evidence. We downgraded the quality by two levels for indirect evidence because the study population differed from the population of interest, and because wound healing in rats is dissimilar to cicatrization in humans. One meta-analysis

Table III. Summary of quality of evidence (GRADE SoF table).

Outcome	Participants, interventions, comparators	Follow-up	Participants (studies)	Quality of evidence	Comparator	Intervention (Laser PBM) *vs.* comparator (control) mean
VEGF	Animal model: Wistar rats Laser PBM *vs.* Control	1 week	30 participants (5 studies)	⊕OOO **VERY LOW** because of risk of bias,[a] inconsistency[b] indirectness[c] and imprecision[d]	The mean VEGF expression across the control group ranged from **137.58 to 6,9767.45**	SMD **2.34 higher** (CI 95 % 0.27 higher to 4.40 higher)
VEGF	Animal model: Wistar rats Laser PBM *vs.* Control	2 weeks	30 participants (5 studies)	⊕OOO **VERY LOW** because of risk of bias,[a] indirectness[c] and imprecision[d]	The mean VEGF expression across the control group ranged from **155.3 to 7,906.98**	MD **5.72 higher** (CI 95% 3.14 higher to 8.31 higher)
VEGF	Animal model: Wistar rats Laser PBM *vs.* Control	3 weeks	24 participants (4 studies)	⊕OOO **VERY LOW** because of risk of bias,[a] inconsistency[b] indirectness[c] and imprecision[d]	The mean VEGF expression across the control group ranged from **1,176.64** to **9,574.47**	SMD **0.16 lower** (CI 95% 1.69 lower to 1.37 higher)
TGF-ß1	Animal model: Wistar rats Laser PBM *vs.* Control	1 week	30 participants (5 studies)	⊕OOO **VERY LOW** because of risk of bias,[a] inconsistency[b] indirectness[c] and imprecision[d]	The mean TGF- ß1 expression across the control group ranged from **129.29 to 10,630.13**	SMD **2.15 higher** (CI 95% 0.11 higher to 4.20 higher)
TGF-ß1	Animal model: Wistar rats Laser PBM *vs.* Control	2 weeks	30 participants (5 studies)	⊕OOO **VERY LOW** because of risk of bias,[a] inconsistency[b] indirectness[c] and imprecision[d]	The mean TGF-ß1 expression across the control group ranged from **228.44 to 10,022.16**	SMD **0.45 lower** (CI 95% 1.91 lower to 1.02 higher)
TGF-ß1	Animal model: Wistar rats Laser PBM *vs.* Control	3 weeks	24 participants (4 studies)	⊕OOO **VERY LOW** because of risk of bias,[a] indirectness[c] and imprecision[d]	The mean TGF-ß1 expression across the control group ranged from **6,014.85 to 12,167.55**	SMD **2.50 lower** (CI 95% 3.98 lower to 1.01 lower)

Abbreviations: TGF-ß1, Transforming growth factor beta-1; VEGF, Vascular Endothelial Growth Factor; CI, Confidence interval; Laser PBM, Laser photobiomodulation; SMD, Standardized mean difference.

Explanations

a. The Grade of the evidence was reduced by one level due to the high risk of bias in operator blinding and other sources of bias.

b. The Grade of the evidence was reduced by one level due to the heterogeneity of the studies.

c. The Grade of the evidence was reduced by two levels due to very serious concerns about the indirectness and the transferability of the evidence.

d. The Grade of the evidence was reduced by one level due to serious concerns about imprecision, because of the small number of participants.

presented moderate heterogeneity and three presented substantial heterogeneity, so the certainty of the evidence was reduced by one level for inconsistency. We also downgraded the evidence for imprecision in all the outcomes because it did not comply with optimal information size (OIS).

DISCUSSION

Various growth factors play important roles in wound healing, such as TGF-ß, VEGF, fibroblast growth factors (FGF) and platelet-derived growth factor (PDGF) (Singer & Clark). TGF-ß is a prototype multifunctional cytokine responsible for inhibiting or stimulating cell growth and proliferation (Morikawa *et al*., 2016), immunosuppression, angiogenesis and wound cicatrization (Massagué, 2012). Its activity affects principally the epithelial cells, and the absence of TGF-ß activity may weaken the wound repair process (Murphy-Ullrich & Poczatek, 2000). Increased activity on the other hand can result in hypertrophic scars, fibrotic diseases and suppression of the immune system (Murphy-Ullrich & Poczatek).

TGF-ß1 plays an important role in mediating wound cicatrization, principally through the SMAD signalling pathway by increasing ATP and TGF-ß expression to improve wound cicatrization and stimulate normal cell processes. TGF-ß1 is a potent regulator of the inflammatory process, since it can attract neutrophils and macrophages to the site of the lesion, which play an important part in tissue repair (Atkins *et al*., 2006). It has been shown that laser PBM can increase TGF-ß1 expression in an initial phase of burn lesions; it then diminishes gradually during the burn wound repair process (Belli *et al*.; Catarino *et al*.; Jácomo *et al*.).

These findings coincide with the results obtained in our meta-analysis, which show an increase in TGF-ß1 expression in an initial phase of tissue repair and lower TGF-ß1 expression in a more advanced stage. The diminution of TGF-ß1 expression may reduce the time of tissue repair by shortening the inflammatory period (Jácomo *et al*.).

Laser PBM can accelerate tissue repair processes through its biomodulation effect (Bagnato, 2008). Laser PBM activates cicatrization by stimulating cells like fibroblasts and keratinocytes to carry out their normal function, differentiating and increasing collagen synthesis, angiogenesis and growth factors (Keskiner *et al*., 2016). However, because the effect of laser therapy is dose-dependent, an appropriate irradiation protocol is required for it to be effective (Hawkins & Abrahamse, 2006). Because many different parameters are used for burn wound treatment with laser PBM, it may be difficult to find an effective protocol or determine which is most effective. Previous studies compared different dosimetries, confirming that the effect of laser PBM is dose-dependent. Maligieri *et al*. indicated that treatment with laser PBM at 9.86 J/cm^2 had a greater impact on TGF-ß1 and VEGF expression than a lower dosimetry (4.43 J/cm^2). Brassolati *et al*. (2016) also reported that laser PBM treatment at 25 J/cm^2 increased VEGF expression, whereas a dose of 12.5 J/cm^2 had no effect. In our meta-analysis we grouped studies that used similar wavelengths; we showed that irradiation with red laser at 4.93 J/cm^2 (per point) influenced VEGF and TGF-ß1 expression, promoting burn wound repair in the animal model. Previous studies in fibroblasts irradiated with GaInAlAs at 660 nm have shown that laser diode at 5 J/cm^2 promoted cell viability and VEGF expression at 24, 48 and 72 hours after irradiation, with a 1.98-fold increase in the number of transcriptions after 72 hours (Szezerbaty *et al*.). In a previous study, our team showed that burn wound irradiation with dosimetry of 11 to 20 J/cm^2 (per session) was effective in stimulating angiogenesis, with large size of effect and moderate certainty of evidence (Deana *et al*.). The results of our meta-analysis corroborated these findings, since we observed an increase in VEGF expression in the first two weeks after burn wound infliction, favouring angiogenesis in the animals treated with laser PBM. VEGF is an important proangiogenic cytokine which stimulates multiple components of the angiogenic cascade (Bao *et al*., 2009). Angiogenesis is fundamental in the tissue repair process, as it is responsible for supplying oxygen and nutrients to the injured tissues (Gupta *et al*.).

We carried out a critical evaluation of the studies included through a risk of bias analysis using the SYRCLE RoB tool. We found that all the studies presented high risk of bias in the general evaluation, as well as deficient reporting. This suggests that there are important aspects of the execution of studies in animals that are being ignored, and should be implemented in future investigations in order to deliver better quality evidence. We noted that not one of the studies included stated that a reporting template had been used; this deficiency made evaluation of the quality of the studies included very difficult. There are many templates available for use in reporting studies in animals. They help the researcher to write a more transparent publication, and can facilitate evaluation of the quality of studies in SR. They can also improve the reproducibility of studies in animals and the certainty of their evidence.

All the outcomes analysed presented very low quality of evidence. Quality refers to our confidence in the estimated effects (Guyatt *et al*., 2011). In other words, the real effect found may differ substantially from the estimated effect (Guyatt *et al*.). Studies carried out in animals provide evidence of the effectiveness of new therapies, and their possible adverse effects, to allow these therapies to be used in humans subsequently. It is therefore essential to adopt measurements in preclinical studies that will offer the best possible certainty of evidence. The use of report templates to produce clearer publications, and the selection of an appropriate sample size, are simple strategies which can be adopted to improve the quality of reporting in animal studies, thus improving the certainty of evidence in future studies.
Study limitations; implications for clinical practice and for research. We identified some limitations in our review process on which we should comment. First, limitations derived from the systematic nature of the review: although we searched in the most important databases in the field of health sciences, we may not have identified every relevant article. However, we believe that we minimised this limitation by the sensitive search strategy used, the additional search by hand of the references, and the double independent review process followed. Furthermore, we only selected studies published in English, Spanish or Portuguese, the languages in which the reviewers are competent; nonetheless, no study found was excluded on the basis of language. Secondly, all the studies included presented a high risk of bias. Finally, very low certainty of evidence was found for all the outcomes evaluated. Studies with low or very low certainty of evidence generate uncertainty as to the effects of the intervention. For this reason, carrying out trials in humans supported by preclinical studies with low certainty of evidence may be a waste of both time and human and financial resources. They may also cause frustration due to the false expectations generated with respect to a treatment that in principle appears promising. There is therefore a need for preclinical studies performed with greater transparency, scientific rigor and certainty of evidence to support investigations in human beings.

CONCLUSION

Laser PBM at a dose of 4.93 J/cm^2 per point has an effect on TGF-ß1 and VEGF expression, promoting burn wound repair in the animal model. TGF-ß1 expression increases in the initial stage and then diminishes gradually during the repair process. Laser PBM also increases VEGF expression during the first two weeks of treatment. Because the studies presented very low certainty of evidence, our results must be treated with caution.

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ALVES, N.; ZAROR, C.; DEL SOL, M.; BAGNATO, V. S. & DEANA, N. F. Influencia de la fotobiomodulación por láser en la expresión de TGF-ß y VEGF en heridas por quemadura: Revisión sistemática y meta-análisis en modelo animal. *Int. J. Morphol., 40(1)*:194-203, 2022.

RESUMEN: Es sabido que la fotobiomodulación por láser (FBM láser) puede acelerar el proceso de curación de heridas por quemadura en modelo animal, sin embargo aún se carece de mayor evidencia sobre la acción de la FBM láser en la expresión de proteínas importantes en el proceso de curación de heridas en modelo animal, como VEGF y TGF-ß1. Así, el objetivo de este estudio fue realizar una revisión sistemática a fin de analizar el efecto de la FBM láser sobre la expresión de VEGF, TGF-ß durante el proceso de reparación de heridas por quemadura en modelo animal. Se realizó una búsqueda sistemática en las bases de datos EMBASE, PubMed/MEDLINE y LILACS. Se incluyeron estudios preclínicos que analizaron la acción de la FBM láser en la expresión de VEGF, TGF-ß (1, 2, 3) durante el proceso de reparación de heridas por quemadura en modelo animal. Se utilizó la herramienta de riesgo de sesgo SYRCLE. Se utilizaron modelos de efectos aleatorios para estimar el efecto combinado. Observamos aumento de la expresión de VEGF con el uso de FBM láser 4.93 J/cm^2 por punto, en las dos primeras semanas tras inducción de la herida por quemadura, con mayor tamaño de efecto en la segunda semana (SDM = 5,72; IC del 95%: 3,14 a 8,31, I^2 = 0 %; certeza de la evidencia muy baja). También se observó el efecto de la FBM láser en la expresión del TGF-ß1 que fue mayor que el control en la primera semana (SDM = -0,45; IC del 95%: -1,91 a 1,02, I^2 = 51 %; certeza de la evidencia muy baja), disminuyendo en la tercera semana tras inducción de la lesión (SDM = -2,50; IC del 95%: -3,98 a -1,01; I^2 = 0 %; certeza de la evidencia baja). La TFB por láser ejerce influencia en la expresión de TGF-ß1 y VEGF favoreciendo el proceso de reparación de heridas por quemadura en modelo animal.

PALABRAS CLAVE: Fotobiomodulación; Laser diodo; Heridas por quemadura; Modelo animal; VEGF, TGF- ß.

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