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ORIGINAL ARTICLE

Effect of photobiomodulation on endothelial cell exposed to *Bothrops jararaca* venom

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Abstract Bleeding is a common feature in envenoming caused by *Bothrops* snake venom due to extensive damage to capillaries and venules, producing alterations in capillary endothelial cell morphology. It has been demonstrated, in vivo, that photobiomodulation (PBM) decreases hemorrhage after venom inoculation; however, the mechanism is unknown. Thus, the objective was to investigate the effects of PBM on a murine endothelial cell line (tEnd) exposed to *Bothrops jararaca* venom (BjV). Cells were exposed to BjV and irradiated once with either 660- or 780-nm wavelength laser light at energy densities of 4 and 5 J/cm^2, respectively, and irradiation time of 10 s. Cell integrity was analyzed by crystal violet and cell viability/mitochondrial metabolism by MTT assay. The release of lactic dehydrogenase (LDH) was quantified as a measure of cell damage. In addition, cytokine IL1-β levels were measured in the supernatant. PBM at 660 and 780 nm wavelength was able to increase cellular viability and decrease the release of LDH and the loss of cellular integrity. In addition, the concentration of pro-inflammatory cytokine IL1-β was reduced after PBM by both wavelengths. The data reported herein indicates that irradiation with red or near-infrared laser resulted in protection on endothelial cells after exposure to *Bothrops* venom and could be, at least in part, a reasonable explanation by the beneficial effects of PBM inhibiting the local effects induced by *Bothrops* venoms, in vivo.

Keywords *Bothrops jararaca* · Cellular viability · Cellular cytotoxicity · Photobiomodulation · Cell integrity · IL1-β

Introduction

Accidents caused by snakebites are a public health problem in tropical regions around the world [1]. *Bothrops* genus is responsible for the majority of the accidents in South America, according to the epidemiological evidences [1, 2]. Local and systemic bleeding is a common feature of *Bothrops* snake envenomation and has been constantly associated with prominent local tissue damage and severe hemodynamic alterations [3]. The bleeding in *Bothrops* envenomation is due to extensive damage to capillaries and venules, with prominent alterations in capillary endothelial cell structure [4–6]. Moreover, the degradation of vascular basement membrane components has been proposed as a key event for the onset of capillary vessel disruption [6]. Such alterations are attributed to the action of hemorrhage toxins present in the venom, named metalloproteinase, which present a key role in the hemorrhage process [5, 7].

Bothrops jararaca (BjV) is an abundant venomous snake responsible for the snakebites in Brazil [8]. BjV is a complex mixture of toxins, such as phospholipase A2, bradykinin potentiate peptides, serine proteases, and desintegrins, among others, from which the most abundant are metalloproteinases [9]. Clinically, the venom from this snake interferes with the hemostatic system, interacting with blood coagulation factors and platelet aggregation, leading to persistent bleeding [10]. Furthermore, BjV causes visible signs at the bite site, such as

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hemorrhage, dermonecrosis, and an intense inflammatory reaction, which is associated with the release of inflammatory mediators, such as the cytokines interleukin-1β (IL-1β), IL-6, TNF-α, and interferon-γ [8–12].

Presently, the most effective treatment for *Bothrops* snakebite accidents remains antivenom (AV) administration, which efficiently neutralizes the systemic toxic effects, preventing death [13]. However, local reaction and systemic hemorrhage cannot be prevented by serotherapy, even when large AV amounts are rapidly administered after the bite [6, 14–16]. Moreover, delay in administering the serum may further reduce its effectiveness, leading to irreversible and disabling damage [15]. The lack of protection of AV therapy and the severity of the local effects caused by these venoms encouraged the research for alternative treatment to inhibit the intense local reaction and complement the action of currently utilized AV therapy. Among the new alternative treatments that are being studied, photobiomodulation (PBM) therapy shows to be promising.

A number of publications from our and other groups have reported the benefits of PBM therapy on local effect induced by *Bothrops* venom. These studies carried out with PBM therapy showed a decrease of myonecrosis [17, 18], inflammation, and pain [19, 20] after the injection of different species of *Bothrops* venom, in vivo. Furthermore, Nadur-Andrade et al. [21] showed that PBM treatment decreased hemorrhage induced by *B. moojeni* venom in mice. Although different studies have shown the efficacy of PBM treatment against local reaction induced by *Bothrops* venoms, the effects of PBM on isolated endothelial cells exposed to these venoms are unknown.

The purpose of this study was to evaluate the action of PBM irradiation using either visible red or near-infrared wavelength on endothelial cells exposed to BjV in terms of integrity, viability, and cytotoxicity and on the release of the pro-inflammatory cytokine (IL-1-β).

Material and methods

Bothrops jararaca venom

The lyophilized crude BjV was obtained from the Arthropod Laboratory of Butantan Institute. The venom was stored at 4 °C until diluted in culture medium immediately before use.

Cell culture

The murine endothelial (tEnd) cell line was kindly provided by Dr. Bruno Lomonte, from Clodomiro Picado Institute, University of Costa Rica. tEnd cells were maintained at subconfluent densities in growth medium consisting of Dulbecco's modified Eagle medium (DMEM, Cultilab, Campinas, SP, Brazil) supplemented with 10 % fetal bovine serum (FBS, Cultilab) and 1 % antibiotic-antimycotic (Cultilab) (at 37 °C with 5 % CO_2). The cells were passaged every 2 days. tEnd cells were plated in 96-well plates (1×10^4 cells/well) and incubated for 48 h for cell adherence. After this period, the cells were incubated with BjV at concentrations of 5, 10, 25, and 50 μg/mL in culture medium for 30, 60, and 120 min.

Laser irradiation

Cells were irradiated with gallium aluminum arsenide (GaAIAs, 780-nm, near-infrared) and aluminium gallium indium phosphide (InGaAIP, 660-nm, red) lasers (MM Optics Ltd., São Carlos, SP-Brazil). The parameter settings are listed in Table 1 and exposure time was 10 s. The power loss due to measure through the bottom of the plastic dish was corrected by calculating the loss of 22 %. The optical power output of the lasers was measured with a Newport multifunction optical meter (model 1835C, Newport Corp., Irvine, CA, USA). Cells were irradiated immediately after addition of the venom in the culture and were applied directly into the well from the bottom plate. Irradiation was performed in an environment with partial obscurity to avoid interference of other light sources.

Irradiated BjV

To verify if the laser irradiation could change the venom toxicity, lyophilized BjV was diluted in medium and irradiated before the incubation with tEnd cells, using the same laser parameters as used to irradiate the cell culture. The purpose of this protocol was to clarify if the laser irradiation could modify the venom components and therefore alter the biological activity of the venom.

Cell attachment assay

After the cell incubation with venom (30, 60, and 120 min), the culture supernatants were removed and the monolayer washed with 100 μL of PBS, and then 40 μL of crystal violet (0.5 %) was added in acetic acid (30 %) per well. After 15 min, the plates were washed and laid to dry. Next, 100 μL of absolute methanol (Merck) was added to each well and the optical density (OD) was read in an ELISA reader at 620 nm. The cell detachment caused was defined as the percentage decrease in the OD observed in the monolayer subjected to the action of venom in relation to unstimulated endothelial cells.

Table 1 Laser parameter setting

Parameters	Red laser		Near-infrared laser	
	Parameters used	Effective value	Parameters used	Effective value
Wavelength (nm)	660	660	780	780
Output Power (mW)	16	14.08	20	17.6
Beam spot area (cm^2)	0.04	0.04	0.04	0.04
Power density (mW/cm^2)	400	352	500	440
Energy density (J/cm^2)	4	3.52	5	4.4
CW	Punctual	Punctual	Punctual	Punctual

The parameters used refer to the parameter output from the laser, and the effective value refers to the parameters that the cells were exposed

Cell viability assay

Mitochondrial activity was measured to assess endothelial cell viability. This analysis was based on cell mitochondrial measured by the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT)-based cytotoxicity assay (Sigma Aldrich, St. Louis, MO, USA). After incubation with venom for 30, 60, and 120 min, the cells were washed with 100 μL of PBS, MTT was added to the cell culture to a final concentration of 0.5 μg/mL, and the cells were incubated for 3 h, at 37 °C. After the incubation time, 100 μL of isopropanol was added to each well to dissolve the formazan crystals. The absorbance of the supernatant was then measured spectrophotometrically in an ELISA reader at 620 nm.

Cell integrity assay

To confirm the cytotoxicity caused by the venom, the enzymatic activity of lactate dehydrogenase (LDH) present in the supernatant of cultures was determined as the parameter of cell integrity. LDH activity was determined by using a commercial kit (Labtest, MG, Brazil) in 20 μL of cell supernatant by the addition of 200 μL of substrate containing 200 mM NaCl, 0.2 mM pyruvate, and 1.6 mM NADH. The absorbance of the supernatant was then measured spectrophotometrically in an ELISA reader at 340 nm. The results were expressed by the decrease of the OD values, resulting from the oxidation of NADH in the presence of pyruvate. Each sample was assayed in triplicate wells, in at least three independent experiments.

Evaluation of the inflammatory mediator IL-1β

Supernatants of tEnd cells were used for determination of IL-1β levels quantified using the enzyme-linked immunosorbent assay, as per the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA.) Briefly, 96-well plates were coated with 50 mL of the first captured monoclonal antibody anti-IL-1 β (2 mg/mL) and incubated for 18 h, at 37 °C. Following this period, 200 mL of blocking buffer, containing 5 % bovine serum albumin in PBS/Tween 20 (Sigma, St. Louis, MO, USA), was added to the wells and the plates were incubated overnight at 4 °C. After washing, 50 μL of either samples or standards were dispensed into each well and the plates incubated for 2 h at 37 °C. Wells were washed, and bounded IL-1β was detected by the addition of the biotinylated monoclonal antibodies anti-IL-1β (5 μg/mL, 50 μL/well). After washing the plates, the volume of 100 μL streptavidin-peroxidase was added and left for 1 h at room temperature (22 °C) followed by further washes. The reading was performed in a SpectraMax Plus 384 spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA, USA) at a wavelength of 450 nm with correction at 570 nm. The sample concentrations were calculated from standard curves obtained with recombinant cytokines.

Statistical analysis

The statistical significance among the control and venom-treated groups was determined by one-way analysis of variance (ANOVA) followed by the Tukey test. A value of $p < 0.05$ indicated statistical significance.

Results

Effect of BjV on integrity of tEnd cells

Endothelial cell line (tEnd) was exposed to different concentrations (5, 10, 25, and 50 μg/mL) of BjV for 30, 60, and 120 min to determine its ability to induce cell detachment (Fig. 1). At a concentration of 5 μg/mL, BjV caused a detachment of 32 % at 120 min; in the other periods, there were no alteration of the monolayer integrity at a concentration of 5 μg/mL (Fig. 1a–c). At higher concentrations (10, 25, and 50 μg/mL), BjV was able to cause detachment of the monolayer in all periods analyzed, compared to control cell (Fig. 1a–c). At 120 min, the concentrations of 25 and 50 μg/mL caused a detachment of the cells, which was statistically different from the other concentrations studied (Fig. 1c).

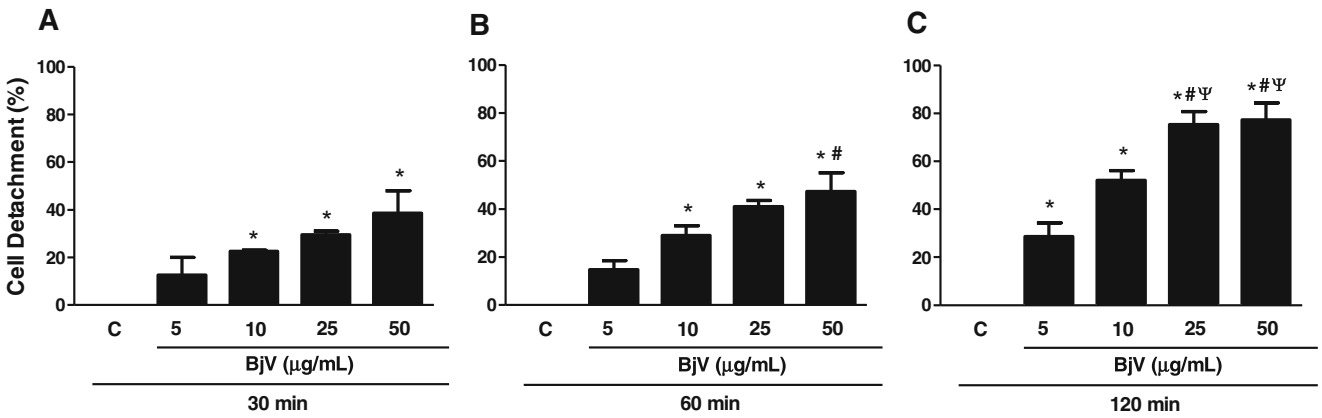

Fig. 1 Effect of BjV on integrity of tEnd cells. tEnd cells were seeded and allowed to adhere in 96-well plates for 48 h. After this period, the venom was added and incubated with venom in different concentrations (5, 10, 25, and 50 μg/mL) or medium alone (control) for **a** 30, **b** 60, and **c** 120 min. Cell detachment was determined by crystal violet assay. Each value represents the mean ± SEM of three independent experiments, $^{*}p < 0.05$ vs. control; $^{\#}p < 0.05$ vs. 5 μg/mL; $^{\Psi}p < 0.05$ vs. 10 μg/mL

Effect of BjV on viability of tEnd cells

When the tEnd cell line was incubated with BjV for 30 min, no cytotoxicity was observed (Fig. 1a). Exposure of tEnd cells to BjV for 60 and 120 min significantly reduced cell viability at all concentrations studied (5, 10, 25, and 50 μg/mL) when compared to control group (Fig. 2b, c). There was no statistical difference in cell viability among the studied concentrations at a 60-min period of incubation (Fig. 2b). However, at 120 min the concentrations of 10, 25, and 50 μg/mL caused a statistically significant reduction in cell viability compared to the concentration of 5 μg/mL (Fig. 2c).

Based on the results above, all subsequent experiments were performed with a concentration of 10 μg/mL of BjV. This was the concentration which caused an effect on integrity and viability of cells with a possible effect of PBM which could be verified.

Effect of irradiated BjV on integrity and viability of tEnd cells

To assess whether PBM would be able to modify components of the venom, the lyophilized venom solution of crude BjV (10 μg/mL) was irradiated before exposed to tEnd cell. Results showed that there were no differences in the effects caused by BjV and irradiated BjV (iBjV) on cellular integrity or viability of tEnd cells (Table 2).

Effect of PBM on integrity of tEnd cells exposed to BjV

There were no significant differences in cellular detachment between non-irradiated versus irradiated control cells (Fig. 3a–c). Likewise, PBM for both wavelengths (660 and 780 nm) at 30 min (Fig. 3a) did not induce any significant change in cellular integrity of endothelial cells. However, PBM on tEnd cells caused a reduction in cell detachment by 20 and 18 %, with 660 nm wavelength, and 27 and 19 %, with 780 nm wavelength at 60 and 120 min, respectively, when compared to the venom group (Fig. 3b, c).

Effect of PBM on viability of tEnd cells exposed to BjV

There were no significant differences in cell viability that was irradiated with PBM at 30 min of BjV incubation (Fig. 4a). However, PBM increases cell viability by 40 and 31 % of tEnd cell at wavelengths 660 and 780 nm, respectively, within 60 min of BjV incubation (Fig. 4b). Moreover, at 120 min after the venom incubation, PBM caused an increase of cellular viability that was 22 and 24 % higher than BjV by 660 and 780 nm wavelengths, respectively (Fig. 4b, c).

Effect of PBM on cytotoxicity of tEnd cells exposed to BjV

The loss of membrane integrity after BjV incubation and laser irradiation was monitored by analyzing the cell culture media on the release of the cytoplasmic enzyme LDH. As illustrated in Fig. 5, cells that were incubated with venom showed increased levels of LDH in the supernatant at 30 and 60 min, compared to control cells. The PBM irradiation at 660 nm was able to promote a reduction in LDH release by 33 and 49 % at 60 and 120 min, respectively (Fig. 5b, c). Moreover, PBM irradiation at 780 nm was able to promote a reduction in LDH release by 28 and 54 %, at 60 and 120 min, respectively. The same statistically significant difference was not observed at 30 min by either 660 or 780 nm laser irradiation (Fig. 5a).

Effect of PBM on IL-1β release of tEnd cells exposed to BjV

To investigate the effect of PBM on the release of IL-1β by endothelial cells, cultures of tEnd cell cultures were stimulated with BjV and the proinflammatory cytokine IL-1β was

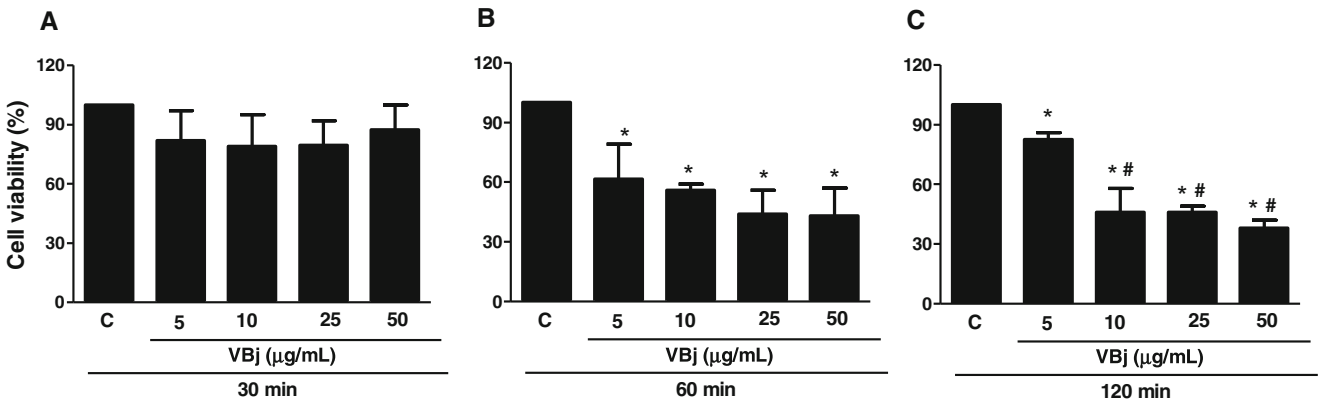

Fig. 2 Effect of BjV on cellular viability of tEnd cells. tEnd cells were seeded and allowed to adhere in 96-well plates for 48 h. After this period, the venom was added and incubated with venom in concentrations of 5, 10, 25, and 50 μg/mL or medium alone (control) for **a** 30, **b** 60, and **c** 120 min. Cellular viability was determined by MTT assay. Each value represents the mean ± SEM of three independent experiments, $*p < 0.05$ vs. control, $\#p < 0.05$ vs. 5 μg/mL

determined in the cell culture supernatants. As illustrated in Fig. 6, cells that were incubated with BjV showed increased release levels of IL-1β in the supernatant from 30 min up to 120 min of venom incubation. PBM at 660 nm, significantly reduced BjV-induced release of IL-1β at 120 min, whereas PBM irradiation at 780 nm, markedly decreased IL-1β from 60 min up to 120 min, in comparison to BjV (Fig. 6).

Discussion

In this work, the endothelial tEnd cell line was used to examine the direct effect of BjV on endothelial cells. Our results demonstrated that BjV induced a loss of cell integrity and decreased cell viability of tEnd cell from 30 min up to 120 min. These results are in agreement with cytotoxic activities from botropic crude venom and venom components, such as myotoxins and metalloproteinases, studied in endothelial cell line [22–24]. The endothelium has a protective role in the cardiovascular system, releasing substances that modulate smooth muscle contraction, its proliferation, inflammation, platelet adhesion, and aggregation and control homeostasis [25]. Therefore, in terms of lesion or dysfunction of this tissue, there are repercussions on the vascular structure, on the adjacent tissue, and finally, on the cardiovascular system [26].

Experimental studies have demonstrated the effectiveness of PBM treatment in reducing local reactions induced by several species of *Bothrops* venom in vivo [17–21, 27]. Moreover, a previous study from our laboratory showed that PBM treatment decreased the hemorrhagic effect caused by *B. moojeni* venom in vivo [21], but the mechanisms underlining this effect are unknown. Here, the effect of two types of PBM, a red laser at wavelength of 660 nm and an near-infrared laser at wavelength of 780 nm, using two different energy densities of 4 and 5 J/cm^2 on the cytotoxicity caused by BjV on endothelial cell, was evaluated. The laser dose was chosen based on the literature, which shows a beneficial effect for either red or near-infrared laser as low as 5 J/cm^2 [28]. In an attempt to investigate if the PBM could modify the venom components and therefore decrease its toxicity, we irradiated the lyophilized venom solution before the incubation with tEnd cells. Results demonstrated that irradiated venom provoked the same level of effects as the non-irradiated venom. This result indicates that most probably the laser irradiation does not modify the venom components or structure, resulting in similar results as reported in an experimental model in vivo [19].

Table 2 Effect of irradiated BjV on endothelial cellular viability and integrity

Time (min)	Cellular viability (%)			Cell detachment (%)		
	Control	BjV	iBjV	Control	BjV	iBjV
30	71.3 ± 1.5	70.3 ± 3.8	68.6 ± 2.5	22.7 ± 0.9	26.6 ± 1.5	24.6 ± 2.3
60	60.3 ± 3.7	54.3 ± 2.3	53.3 ± 1.8	54.4 ± 3.5	56.7 ± 1.8	57.3 ± 1.2
120	32 ± 2.2	27.3 ± 2.2	27.6 ± 2.6	61 ± 4	65.7 ± 2.2	64.7 ± 2.7

tEnd cells were plated into 96-well plates and incubated for 24 h for cellular adhesion. After this period, the irradiated venom (iBjV) was added (10 μg/mL) and was incubated for 30, 60, and 120 min. Cells incubated with medium were used as control. Cellular viability was determined by MTT assay and cell integrity by crystal violet assay

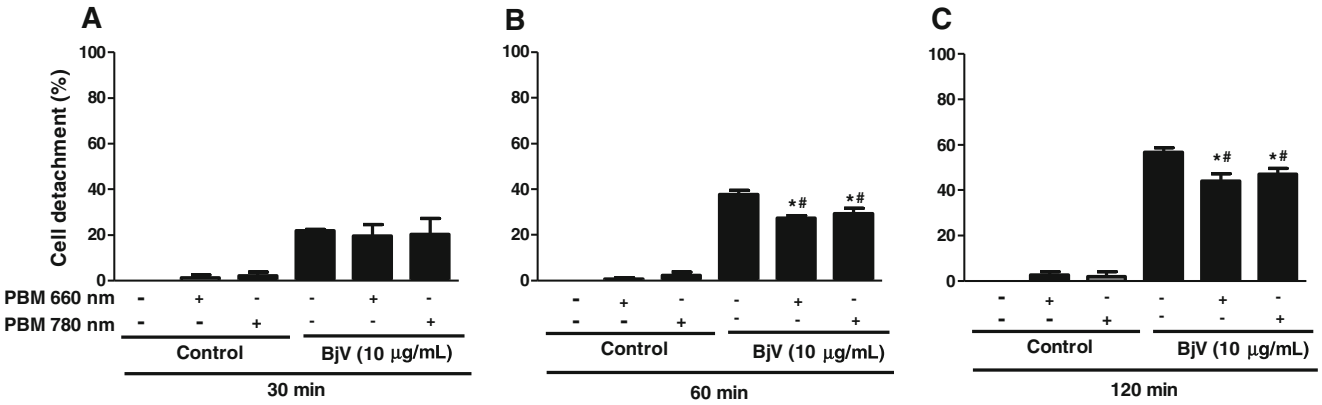

Fig. 3 Effect of PBM on integrity of endothelial cells exposed to *B. jararaca* venom (BjV). tEnd cells were plated in 96-well plates and incubated for 48 h for cell attachment. After this time, the venom (10 μg/mL) was added and cells were immediately irradiated in a wavelength of 660 or 780 nm and were incubated for 30, 60, and 120 min. Cell integrity was determined by crystal violet assay. *BjV B. jararaca* venom, *PBM* photobiomodulation. Each value represents the mean ± SEM of three independent experiments, ANOVA. *$p < 0.05$ vs. control, #$p < 0.05$ vs. venom

In the present study, PBM reduced the detachment caused by BjV on endothelial cells at 660 nm. Detachment of endothelial cells is one of the early effects caused by botropic venom and is associated with the loss of cell integrity and the presence of bleeding [29]. Local and systemic bleeding caused by botropic venom is related to an indirect action of venom metalloproteinases on the basement membrane of the capillary and related extracellular matrix components that provide stability to the microvessel structure in the vasculature [30]. It has been demonstrated that the mechanisms involved in the pathogenesis of venom-induced hemorrhage that causes the escape of erythrocytes to the interstitial space occurs mainly due to a per rhexis mechanism with the development of gaps or lesions within the endothelial cells [30]. In addition, the earliest ultrastructural changes observed in capillary blood vessels, caused by metalloproteinase BaH1 isolated from *B. asper* venom, were a drop in the number of pinocytotic vesicles and a detachment of some endothelial cells from the basal lamina [31]. In our experimental model, it is possible that the laser reduced the detachment of endothelial cells by preventing gap formation and subsequent lesions within endothelial cells, a hypothesis that should be addressed in future studies.

With respect to the decrease in cellular cytotoxicity of tEnd cells observed following exposure to BjV, our data suggest a direct cytotoxic activity of the venom in vitro. This hypothesis is supported by the fact that LDH is one of the main cytoplasm-containing enzymes that in normal circumstances cannot permeate cell membrane. So, the increase of LDH is often viewed as one of the hallmarks of cell membrane disintegration and necrosis [32]. In the current study, irradiation with 660 and 780 nm wavelengths caused an increase in cellular viability and reduction in LDH release after exposure to the venom, indicating that the PBM protected the cell against the ability of BjV to disrupt the integrity of the plasma membrane. A similar result was found by Dourado et al. [27] and

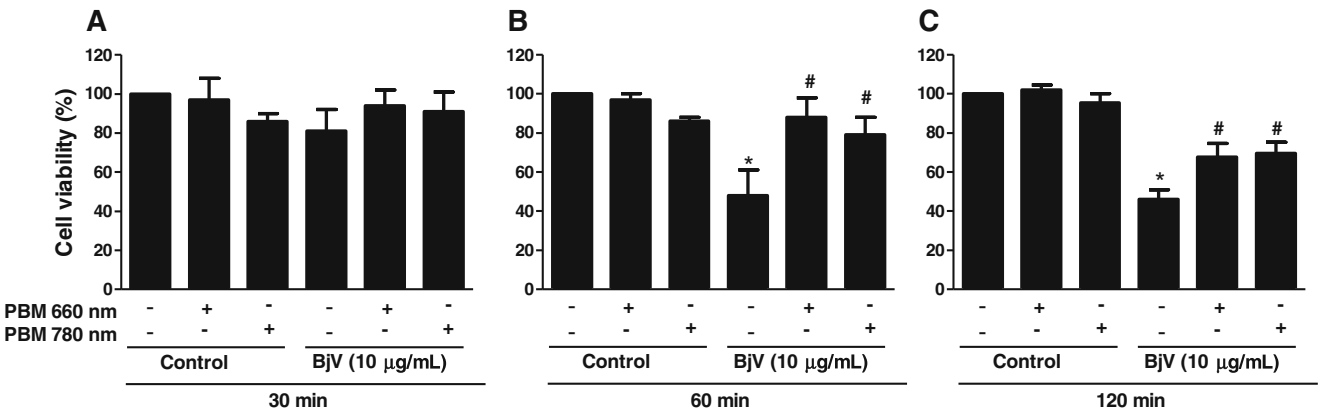

Fig. 4 Effect of PBM on viability of endothelial cells exposed to *B. jararaca* venom (BjV). tEnd cells were plated into 96-well plates and incubated for 48 h for cellular adhesion. After this period, the venom was added (10 μg/mL) and cells were immediately irradiated with LLL in wavelengths of 660 and 780 nm or cells received only medium (control) and were incubated for 30, 60, and 120 min. Cellular viability was determined by MTT assay. *BjV B. jararaca* venom, *PBM* photobiomodulation. Each value represents the mean ± SEM of three independent experiments. *$p < 0.05$ vs. control, #$p < 0.05$ vs. BjV group

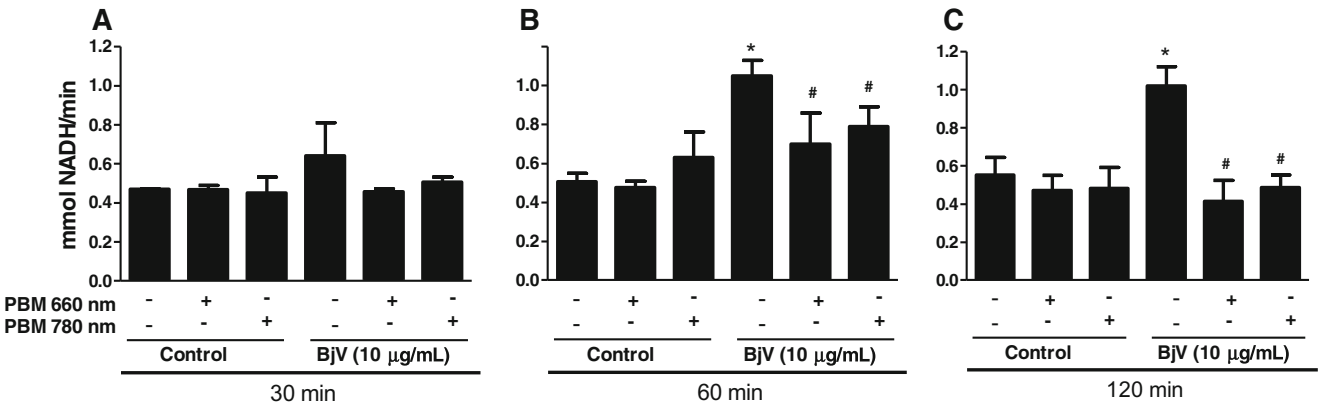

Fig. 5 Effect of PBM on cytotoxicity of endothelial cells exposed to *B. jararaca* venom (BjV). tEnd cells were plated in 96-well plates and incubated for 48 h for cell attachment. After this period, BjV (10 mg/mL) was added to the cell culture and was immediately irradiated with LLL at 660 or 780 nm and incubated for 30, 60, and 120 min. The supernatant was collected, and the release of lactate dehydrogenase (LDH) was determined by the release of NADH. *BjV B. jararaca* venom, *PBM* photobiomodulation. Each value represents the mean ± SEM of three independent experiments. *$p < 0.05$ vs. control, #$p < 0.05$ vs. BjV group

Barbosa et al. [18] in an in vivo study investigating the effects of PBM therapy on gastrocnemius muscle after *B. moojeni* and *B. jararacussu* venom injection, respectively.

In addition to hemorrhage, inflammatory response is also an important feature in the local injury caused by *Bothrops* venoms, which leads to necrosis of difficult regeneration [30]. A number of reports have established the involvement of pro-inflammatory cytokines in the local inflammation induced by botropic venoms, such as IL-1β, IL-6, and TNF-α, [10, 33, 34]. Further investigations have also shown that messenger RNAs (mRNAs) coding for IL-1β, IL-6, and TNF-α were up-regulated after injection of jararhagin, a snake venom metalloproteinase isolated from BjV, in mouse gastrocnemius muscle [35]. In addition, jararhagin can directly stimulate the expression of mRNA encoding for proinflammatory cytokines on endothelial cells [36]. In this aspect, endothelial cells are key regulators of the inflammatory response since in the case of injury they line blood vessel, control adhesion, and migration of leukocytes and exchange fluids from the bloodstream to the injured tissue [37]. In order to determine the contribution of the PBM in reducing the inflammatory reaction caused by BjV on endothelial cells, we evaluated the effect of PBM on IL-1β release. Results demonstrated that elevated concentrations of IL-1β were detected in tEnd cell supernatants exposed to BjV and that PBM was able to reduce the release of IL-1β induced by BjV by both red and near-infrared lasers. The result is consistent with several studies that show a reduction of this cytokine by PBM therapy in vivo [38–40]. Taking into account that BjV is able to stimulate endothelial cells to produce inflammatory cytokines and PBM is able to reduce expression, we hypothesize that light irradiation is important in the reduction of local inflammation.

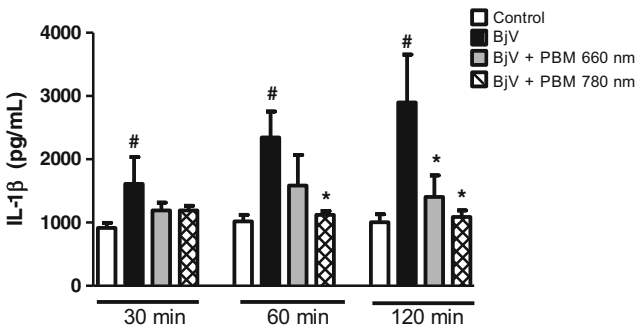

Fig. 6 Effect of PBM on the release of IL-1β by endothelial cells exposed to *B. jararaca* venom (BjV). tEnd cells were plated in 96-well plates and incubated for 48 h for cell attachment. After this period, BjV (10 mg mL) was added to the cell culture and was immediately irradiated with the red laser at 660 nm or near-infrared laser at 780 nm and incubated for 30, 60, and 120 min. The concentrations of IL-1β were measured by ELISA in the supernatant of cells. *BjV B. jararaca* venom, *PBM* photobiomodulation. #$p < 0.05$ vs. control group *$p < 0.05$ vs. BjV group (ANOVA)

In spite of the fact that many studies of PBM therapy on the local reaction caused by botropic venom have demonstrated its beneficial effect in vivo, little is known about how PBM is able to affect the cellular system involved in botropic venom-induced local effects as well as the molecular mechanisms involved in this process. The present study demonstrated positive changes in cytotoxicity and integrity of tEnd cells as well as decrease in IL-1β concentration after the PBM (660 and 780 nm). There are relatively few data available on the effects of PBM on endothelial cells. However, it has been reported that PBM enhances mitochondrial respiration and also activates the redox-sensitive NFkB signaling via generation of intracellular reactive oxygen species (ROS) and promotes proliferation of many cell types [41, 42]. Nuclear factor kappa B (NF-kB) is a transcription factor regulating expression of multiple genes and has been shown to govern various cellular functions, including inflammatory and stress-induced responses [43, 44]. Therefore, we hypothesize that the biomodulatory effect caused by PBM could be related to an

activation of ROS that initiates a signaling cascade that promotes cellular changes observed in our study.

In conclusion, this study clearly indicates that irradiation with either red or near-infrared laser resulted in cytoprotection on endothelial cells after venom exposure, involving preservation of plasma membrane and inhibition of IL-1β release. Further studies should be performed to provide a better understanding about the mechanisms involving such effects in order to underline the development of complementary strategies for treatment of local snake envenoming.

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