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Evaluation of DNA Damage Induced by Therapeutic Low-Level Red Laser

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Abstract

Biological effects of monochromatic lights on cells have aroused interest regarding an active and non-innocuous effect on human skin. The aim of this work was to evaluate DNA damage induced by low-level red laser at doses and frequencies used in therapeutic protocols. For this purpose, *E. coli* cultures and bacterial plasmids were used to assess bacterial survival, filamentation, DNA lesions and *in vitro* DNA repair induced by low-level red laser exposure at low doses in continuous wave and pulsed emission mode. Data indicate that low-level red laser does not affect the survival of *E. coli* cultures, topological forms of DNA, and does not induce DNA lesions targeted by endonuclease IV, formamidopyrimidine DNA glycosylase and endonuclease III, but rather that it induces bacterial filamentation in wild type and DNA repair-deficient *E. coli* cultures and DNA lesions targeted by exonuclease III. Monochromatic red light could activate survival and/or adaptive mechanisms against harmful radiations.

Keywords: DNA; Enzyme; *Escherichia coli*; Laser; Monochromatic light

Introduction

Photobiological effects of low-level lasers, in continuous wave and pulsed emission mode, are considered to occur following absorption of light energy and production of a photosignal, which is subsequently transduced into the cell [1]. For red lasers, the laser light chromophores are related to mitochondrial respiratory chain (as cytichrome c, for example), which could generate singlet oxygen and, in turn, stimulate processes such as synthesis of RNA and DNA [2]. This biostimulative effect sustains some clinical protocols, as those suggested for treatment of inflammations [3], pain [4] and wound healing [5]. In fact, low-level lasers have been reported to increase both the speed and quality of the healing process of wounds in humans [6-8]. Although the exact mechanisms by which low-level lasers exert their effects on skin have not been clarified, some studies have demonstrated increasing of skin cell mitotic activity [9,10], as well as collagen deposition, angiogenesis and alteration on cytokine expression [11,12].

However, there is still some skepticism whether the biological effects of low-level lasers are measurable or even if they are of relevant importance to disease treatments. Therefore, performing experimental studies to verify the presence and absence of effects in biological systems exposed to low-level lasers at different conditions (dose, emission mode, power, and wavelength) is justified. On the other hand, there are examples whereby these lasers are capable of altering some biochemical process, changing cellular metabolism [1] and photobiological side-effects by the production of reactive oxygen and nitrogen species with subsequent free radical reactions with biomolecules and cellular function modifications [13,14]. Adverse effects on cells and data about DNA damage after exposure to these lights have been reported in eukaryotic [15,16] and prokaryotic cells [17-21] but experimental data about effects on DNA are scarce considering commercially-available low-level laser devices (with different powers, wavelengths and emission modes). Moreover, the biological effects of monochromatic red and near-infrared light on human skin have interest and this solar spectrum range could not be non-active or innocuous but rather to induce protective/adaptive mechanisms, with important participation on the effects of polychromatic solar radiation on human skin [22].

E. coli cultures both proficient and deficient in DNA repair mechanisms is used to evaluate the effects of physical and chemical [18,23] agents on DNA [19,20,24]. There is no eukaryotic cell line with genetic characteristics similar to these *E. coli* strains, and studies based on these cells permit evaluating the participation of each gene-coding enzymes and proteins related to DNA repair on a biological effect of interest. For a long time, *E. coli* survival both proficient and deficient in mechanisms of DNA repair has been used as a valuable and practical experimental model for studying biological effects of ionizing and non ionizing radiations [25]. Bacterial filamentation is part of SOS response, which is a global response induced in cells upon DNA damage [25]. Bacterial cells undergoing filamentation present an anomalous growth and they continue to elongate but there is not septa formation [26]. This bacterial morphological change is used to evaluate actions of environmental agents, both natural and man-made, which induce DNA lesions [19,20,27]. Topological forms of plasmid DNA are evaluated by electrophoretic profile into alkaline agarose gels [28] and this technique is used to evaluate the ability of genotoxic agents to induce alkali-labile DNA lesions by direct and indirect mechanisms [19]. In addition, the action of specific DNA repair enzymes on DNA is evaluated *in vitro* by an electrophoretic profile of plasmid DNA into agarose gels, and it is used to study DNA lesions induced by genotoxic agents [21].

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As the effectiveness and potential side-effects on biological systems are still unclear and of interest, as well as safety and clinical applications of non-ionizing monochromatic lights are increasing, effects of low-level red laser on *E. coli* cultures, plasmids and DNA repair were studied at doses and frequencies used in therapeutic protocols.

Material and Methods

Low-level red laser

A therapeutic low-level red laser (Laser HTM Compact model, AlGaInP, 10 mW), with emission in 658 nm, was purchased from HTM Eletrônica (São Paulo, Brazil).

E. coli survival assay

Survival of *E. coli* AB1157 (wild type), BW527 (endonuclease IV deficient), BW9091 (exonuclease III deficient), BH20 (formamidopyrimidine DNA glycosylase deficient) and BW375 (endonuclease III deficient) cultures exposed to low-level red laser was evaluated in exponential and stationary growth phase. Aliquots (50 µL, five aliquots for each dose and frequency) of *E. coli* suspensions (1-2 × 10^8 cells/mL, in 0.9% NaCl solution) were exposed to low-level red laser (658 nm, spot size of 12.566 mm^2) at different doses (0.13, 0.52 and 1.04 J), in continuous wave (power output of 10mW, power density of 79.6 mW/cm^2) and in pulsed emission mode (2.5, 250 and 2500 Hz, 50% duty cycle). *E. coli* suspensions not exposed to laser were used as controls. Aliquots of *E. coli* suspensions were diluted in sterile saline (0.9% NaCl), spread onto Petri dishes containing solidified rich medium (1.5% agar). Colonies formed after overnight incubation at 37°C were counted and the survival fractions were calculated as described elsewhere [18].

E. coli filamentation assay

E. coli AB1157, BW527, BW9091, BH20 and BW375 cultures in exponential and stationary growth phase were exposed to low-level red laser as described in the *E. coli* survival assay, aliquots (20 µL) were spread onto microscope slides and stained by Gram method [29]. *E. coli* suspensions not exposed to laser were used as controls. *E. coli* cells were visualized by light microscopy (40x magnification), photographed and *E. coli* filamentation were quantified by Image ProPlus software to determine the percentage of bacterial filamentation as described elsewhere [20].

Plasmid exposure to laser and alkaline electrophoretic profile assay

pBSK plasmids were obtained from DH5αF'Iq (*recA*$^-$) strain hosting this plasmid by a standard procedure [30]. Plasmids were exposed to low-level red laser as described to *E. coli* suspensions and plasmids not exposed to laser were used as controls. After that, each sample was mixed with loading buffer and applied in 0.8% alkaline agarose gels into a horizontal electrophoresis chamber containing alkaline electrophoresis buffer [30]. After electrophoresis, gels were neutralized, stained with ethidium bromide (0.5 µg/mL) and the plasmids forms were viewed under fluorescence using an ultraviolet trans-illumination system. Gels were digitalized and the plasmid forms were semiquantified using the Image J for Windows computer program.

DNA repair enzyme assay

Endonuclease IV, exonuclease III, formamidopyrimidine DNA glycosylase and endonuclease III were used to evaluate DNA repair of lesions induced by low-level red laser exposure in DNA molecules [21]. Plasmids were exposed to low-level red laser as described in the bacterial assays. Immediately afterwards, plasmids (200 ng, approximately) were mixed with appropriated enzyme buffer, enzyme (2 units for each enzyme) and incubated (37°C, 30 minutes). After that, plasmid samples were mixed with loading buffer, applied in agarose horizontal gel electrophoresis, stained with ethidium bromide, visualized under ultraviolet trans-illumination system and plasmid forms were semiquantified as in the electrophoretic profile assay.

Statistical analysis

Data are reported as mean and standard deviation (mean ± SD) of the survival fraction, percentage of bacterial filaments and a percentage of plasmid forms. The one-way variance test analysis was performed to verify possible statistical differences followed by Bonferroni post-test with $p<0.05$ as a less significant level. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows XP, GraphPad Software, San Diego, California, USA).

Results

Survival of *E. coli* cultures exposed to laser

Survival fractions of *E. Coli* AB1157, BW527, BW9091, BH20 and BW375, cultures in exponential growth phase exposed to low-level red laser at different doses in different emission modes are shown in Table 1. There is no alteration of survival fractions of these *E. coli* cultures when exposed to laser. To verify whether the growth phase interferes on laser-induced biological effects, survival of *E. coli* cultures in the stationary growth phase were evaluated. Table 2 shows the survival fractions of *E. coli* AB1157, BW527, BW9091, BH20 and BW375 cultures in the stationary growth phase exposed to low-level red laser at different doses in different emission modes. Similar to data shown on Table 1, no survival fraction alteration was observed in these *E. coli* cultures exposed to laser.

	Survival Fractions			
Dose (J)	**Continuous**	**2.5 Hz**	**250 Hz**	**2500 Hz**
AB1157				
0.13	1.1 ± 0.12	1.2 ± 0.20	1.2 ± 0.18	0.9 ± 0.23
0.52	0.9 ± 0.14	0.9 ± 0.08	0.9 ± 0.09	0.8 ± 0.03
1.04	0.9 ± 0.22	1.3 ± 0.08	1.1 ± 0.30	0.8 ± 0.24
BW527				
0.13	0.8 ± 0.11	0.8 ± 0.24	0.8 ± 0.10	0.9 ± 0.12
0.52	0.8 ± 0.10	0.8 ± 0.19	0.7 ± 0.12	0.7 ± 0.13
1.04	0.7 ± 0.13	0.9 ± 0.15	1.0 ± 0.02	0.8 ± 0.08
BW9091				
0.13	1.0 ± 0.22	1.2 ± 0.28	1.3 ± 0.08	1.0 ± 0.08
0.52	1.0 ± 0.02	1.1 ± 0.14	1.1 ± 0.20	1.1 ± 0.03
1.04	1.1 ± 0.23	1.0 ± 0.12	1.2 ± 0.24	0.9 ± 0.06
BH20				
0.13	0.9 ± 0.10	1.1 ± 0.24	1.0 ± 0.22	1.4 ± 0.40
0.52	1.0 ± 0.22	1.6 ± 0.18	1.0 ± 0.13	1.2 ± 0.29
1.04	1.1 ± 0.27	1.5 ± 0.34	1.4 ± 0.35	1.2 ± 0.33
BW375				
0.13	0.8 ± 0.14	0.8 ± 0.15	0.8 ± 0.13	0.8 ± 0.16
0.52	0.9 ± 0.11	0.7 ± 0.16	0.8 ± 0.12	0.6 ± 0.14
1.04	0.9 ± 0.14	0.6 ± 0.20	0.8 ± 0.09	0.7 ± 0.10

Table 1: Survival fractions of *E. coli* cultures exposed to low-level red laser in exponential growth phase.

Survival Fractions				
Dose (J)	**Continuous**	**2.5 Hz**	**250 Hz**	**2500 Hz**
AB1157				
0.13	1.1 ± 0.05	1.3 ± 0.17	1.0 ± 0.26	1.2 ± 0.18
0.52	1.0 ± 0.26	0.9 ± 0.23	1.1 ± 0.19	1.1 ± 0.16
1.04	1.2 ± 0.05	1.1 ± 0.22	0.9 ± 0.20	1.1 ± 0.17
BW527				
0.13	0.9 ± 0.21	1.0 ± 0.10	0.8 ± 0.17	1.1 ± 0.18
0.52	0.9 ± 0.25	1.1 ± 0.21	0.9 ± 0.15	1.1 ± 0.10
1.04	0.9 ± 0.14	1.1 ± 0.10	1.3 ± 0.09	1.1±0.21
BW9091				
0.13	1.0 ± 0.23	1.2 ± 0.23	1.3 ± 0.24	1.0 ± 0.22
0.52	1.1 ± 0.20	1.1 ± 0.30	1.2 ± 0.32	1.1 ± 0.21
1.04	1.0 ± 0.31	0.8 ± 0.31	1.2 ± 0.26	1.0 ± 0.17
BH20				
0.13	1.0 ± 0.15	1.0 ± 0.14	1.1 ± 0.20	0.9 ±0.19
0.52	1.1 ± 0.10	1.1 ± 0.11	1.3 ± 0.11	1.1 ± 0.25
1.04	1.3 ± 0.17	1.1 ± 0.14	1.0 ± 0.21	0.9 ± 0.14
BW375				
0.13	0.8 ± 0.20	0.8 ± 0.18	0.8 ± 0.06	1.0 ± 0.18
0.52	0.8 ± 0.04	0.7 ± 0.21	0.8 ± 0.10	0.8 ± 0.08
1.04	0.8 ± 0.08	0.8 ± 0.06	0.8 ± 0.07	0.8 ± 0.19

Table 2: Survival fractions of *E. coli* cultures exposed to low-level red laser in stationary growth phase.

Filamentation in E. coli cultures exposed to laser

Table 3 shows the percentage of filamentation in *E. coli* AB1157, BW527, BW9091, BH20 and BW375 cultures in exponential growth phase exposed to low-level red laser at different doses in different emission modes. The data in this table indicate that laser exposure induces filamentation in exponential *E. coli* AB1157, BW527, BH20, BW375 and BW9091 cultures at all emission modes evaluated (continuous wave and pulsed). Also, *E. coli* cultures in stationary growth phase were exposed to low-level red laser to verify whether laser-induced *E. coli* filamentation depends on *E. coli* growth phase. Similar to that obtained with *E. coli* cultures in exponential growth phase, data in the Table 4 shows that red laser induces filamentation in *E. coli* AB1157, BW527, BW9091, BH20 and BW375 cultures in stationary growth phase in emission mode.

Electrophoretic profile of plasmids in alkaline gels

Alkaline agarose gel electrophoresis of bacterial plasmids exposed to low-level red laser at different doses in continuous and pulsed (2.5 Hz) emission modes are shown on Figures 1b and 2b. These photographs indicate that laser exposure induces no alterations on the electrophoretic profile of plasmids (lanes 2, 3 and 4) when compared with control (lane 1). These findings were confirmed by quantifications of plasmid forms (Figures 1a and 2a) indicating no alteration on electrophoretic profile. Similar electrophoretic profiles were obtained when bacterial plasmids were exposed to laser in pulsed mode emission at 250 and 2500 Hz (data not shown).

DNA repair enzyme assay

Figures 3b and 4b shows photographs of agarose gel electrophoresis of plasmids after low-level laser exposure in continuous and pulsed (2.5 Hz) emission mode, respectively, and incubation with endonuclease IV. Analysis of these figures suggests no action of endonuclease IV on plasmid DNA exposed or not exposed (control) to low-level red laser. Quantification of plasmid forms (supercoiled and open circle form) confirms this qualitative analysis (Figure 3a and 4a). Similar results were obtained with formamidopyrimidine DNA glycosylase (Figures

Percentage of filamentation				
Dose (J)	**Continuous**	**2.5 Hz**	**250 Hz**	**2500 Hz**
AB1157				
0.13	1.0 ± 0.50	2.3 ± 0.58*	1.0 ± 0.40	1.7 ± 0.48
0.52	2.0 ± 0.82*	3.0 ± 1.00*	1.7 ± 0.59	2.7 ± 0.55*
1.04	5.0 ± 1.00*	7.3 ± 1.58*	7.0 ± 1.00*	2.0 ± 0.37*
BW527				
0.13	8.7 ± 1.15*	8.0 ± 1.00*	6.7 ± 1.10*	6.0 ± 1.01
0.52	4.0 ± 1.00	4.4 ± 1.15	7.3 ± 0.59*	4.7 ± 0.54
1.04	4.3 ± 0.53	5.0 ± 1.02	3.3 ± 0.56	4.6 ± 1.52
BW9091				
0.13	18.3 ± 1.25*	14.0 ± 2.60	22.3 ± 2.64*	11.3 ± 1.50
0.52	28.0 ± 2.65*	18.3 ± 2.08*	18.7 ± 1.53*	19.0 ± 1.73*
1.04	39.7 ± 2.52*	30.7 ± 2.10*	39.3 ± 2.11*	15.3 ± 1.52*
BH20				
0.13	6.0 ± 1.00*	7.3 ± 1.15*	7.0 ± 0.04*	3.3 ± 0.59
0.52	6.3 ± 0.58*	4.7 ± 0.60*	6.3 ± 1.11*	4.3 ± 1.20*
1.04	9.7 ± 1.53*	5.0 ± 1.10*	8.3 ± 2.08*	4.3 ± 1.50*
BW375				
0.13	7.7 ± 2.08	11.7 ± 1.50*	11.0 ± 1.73*	19.0 ± 2.65*
0.52	19.3 ± 2.10*	19.7 ± 0.53*	12.3 ± 3.06*	19.0 ± 1.00*
1.04	23.3 ± 1.43*	22.7 ± 2.52*	27.3 ± 2.50*	23.0 ± 2.55*

Controls: 0.2 ± 0.03 for AB1157, 4.7 ± 0.60 for BW527, 10.7 ± 2.08 for BW9091, 1.7 ± 0.58 for BH20, 6.7 ± 1.54 for BW375. (*) $p<0.05$ when compared with control group. Error bars indicate the standard deviation of the mean for n=2 independent experiments

Table 3: Percentage of filamentation in *E. coli* cultures exposed to low-level red laser in exponential growth phase.

Percentage of filamentation				
Dose (J)	**Continuous**	**2.5 Hz**	**250 Hz**	**2500 Hz**
AB1157				
0.13	1.7 ± 0.58*	1.0 ± 0.08*	4.7 ± 0.58*	1.7 ± 0.48*
0.52	2.0 ± 1.05*	1.0 ± 0.52*	4.0 ± 1.00*	2.0 ± 0.50*
1.04	3.7 ± 1.53*	11.0 ± 1.00*	12.7 ± 2.08*	6.0 ± 1.00*
BW527				
0.13	5.7 ± 0.58*	4.7 ± 1.15*	5.7 ±1.43*	6.0 ± 1.00*
0.52	3.3 ± 0.48	3.7 ± 0.57	3.7 ± 0.59	5.3 ± 0.54*
1.04	5.3 ± 1.53*	6.3 ± 1.50*	7.3 ± 0.60*	5.3 ± 0.55*
BW9091				
0.13	63.3 ± 4.16*	54.3 ± 4.51*	64.3 ± 5.12*	24.3 ± 4.93*
0.52	86.7 ± 4.93*	90.3 ± 4.41*	94.7 ± 2.52*	32.7 ± 3.79*
1.04	78.3 ± 3.06*	85.7 ± 5.13*	54.7 ± 4.73*	81.7 ± 7.09*
BH20				
0.13	5.3 ± 0.60	1.7 ± 0.57	3.3 ± 0.54	3.0 ± 0.99
0.52	4.3 ± 0.58	4.0 ± 1.04	2.0 ± 1.00	3.3 ± 0.59
1.04	4.0 ± 1.00	2.3 ± 1.58	2.7 ± 0.68	4.0 ± 0.02
BW375				
0.13	8.0 ± 2.00*	5.0 ± 1.00	6.7 ± 1.14	6.0 ±1.00
0.52	7.3 ± 0.60*	7.0 ± 1.01*	6.0 ± 1.98*	8.3 ± 0.48*
1.04	12.0 ± 2.00*	8.7 ± 1.15*	8.0 ± 1.02*	8.0 ± 2.05*

Controls: 0.1 ± 0.04 for AB1157, 2.7 ± 0.58 for BW527, 14.0 ± 3.61 for BW9091, 3.7 ± 0.58 for BH20, 4.7 ± 0.49 for BW375. (*) $p<0.05$ when compared with control group. Error bars indicate the standard deviation of the mean for n=2 independent experiments

Table 4: Percentage of filamentation in *E. coli* cultures exposed to low-level red laser in stationary growth phase.

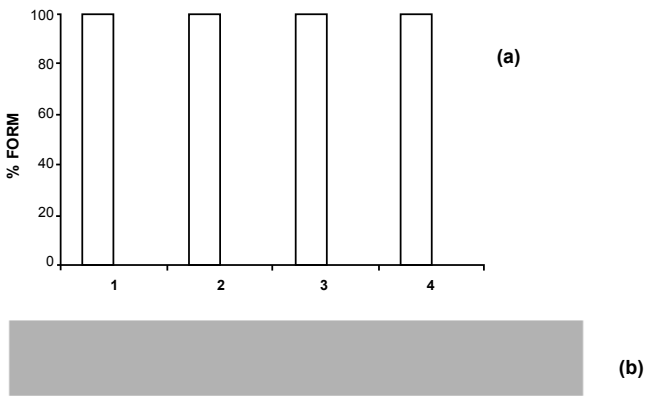

Figure 1: Percentage of bacterial plasmid forms (a) and photograph (b) of alkaline agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in continuous wave mode. Lanes: (1) pBSK (control); (2) pBSK+continuous wave laser 0.13 J; (3) pBSK+continuous wave laser 0.52 J; (4) pBSK+continuous wave laser 1.04 J. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

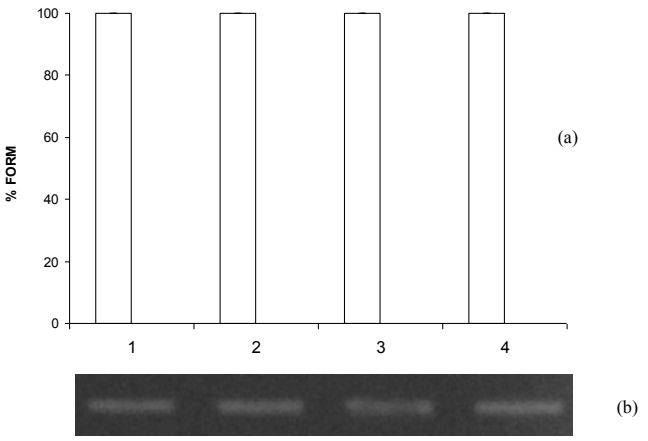

Figure 2 : Percentage of bacterial plasmid forms (a) and photograph (b) of alkaline agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in 2.5 Hz pulsed emission mode. Lanes: (1) pBSK (control); (2) pBSK+continuous wave laser 0.13 J; (3) pBSK+continuous wave laser 0.52 J; (4) pBSK+continuous wave laser 1.04 J. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

5b and 6b) and endonuclease III (Figures 7b and 8b). No action of these enzymes on plasmids exposed to laser was confirmed by quantification of plasmid forms (Figures 5a,6a,7a and 8a). However, exonuclease III presented action on plasmid DNA exposed to low-level red laser in continuous and pulsed (2.5 Hz) emission mode (Figures 9b and 10b). The action of exonuclease III on plasmids exposed to the laser was confirmed by quantitative analysis of plasmid forms (Figures 9a and 10a).

Discussion

Our research shows that low-level red laser in continuous wave and pulsed emission mode was not lethal to *E. coli* cultures in exponential (Table 1) and stationary growth phase (Table 2) when therapeutic doses were used on wild type (AB1157), endonuclease IV deficient (BW527), exonuclease III deficient (BW9091), formamidopyrimidine DNA glycosylase deficient (BH20) or endonuclease III deficient (BW375) cells. Our results are important to justify the safety of low-level lasers in clinical protocols as those are carried out to treat wounds, inflammation processes and pain. Low-level infrared (810 nm) laser was also incapable of modifying B14 cell viability [31]. However, low-level red lasers have been suggested to induce free radical production [31,32] and antioxidants eliminate the light effect of laser-induced increase of

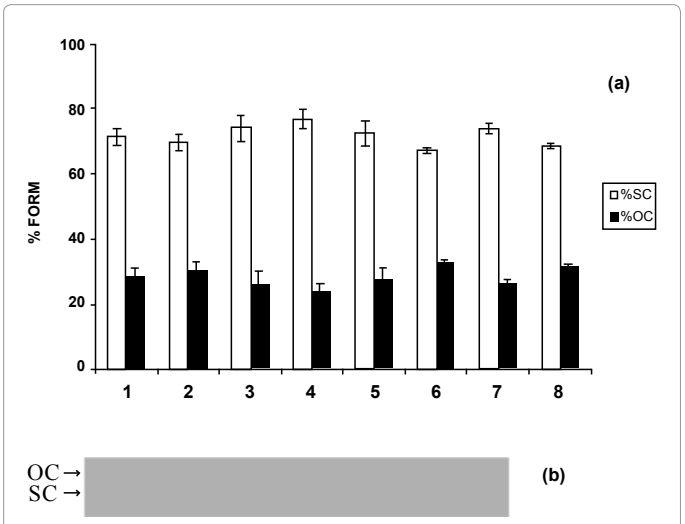

Figure 3: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in continuous wave mode and incubated with endonuclease IV. Lanes: (1) pBSK; (2) pBSK+endonuclease IV; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+endonuclease IV; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+endonuclease IV; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+endonuclease IV. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

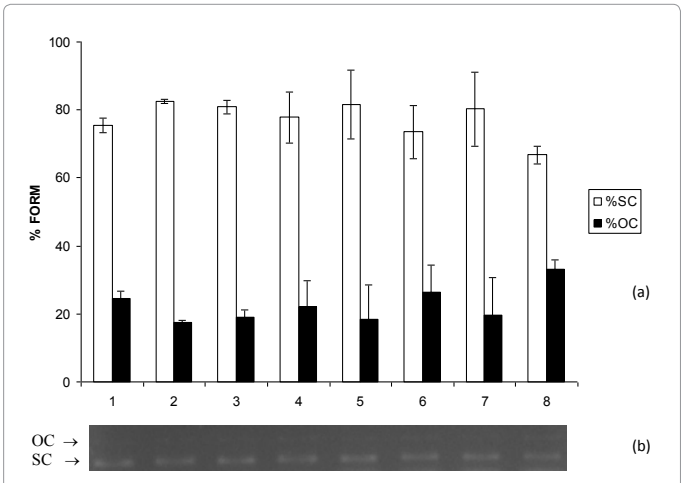

Figure 4: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in 2.5 Hz pulsed emission mode and incubated with endonuclease IV. Lanes: (1) pBSK; (2) pBSK+endonuclease IV; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+endonuclease IV; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+endonuclease IV; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+endonuclease IV. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

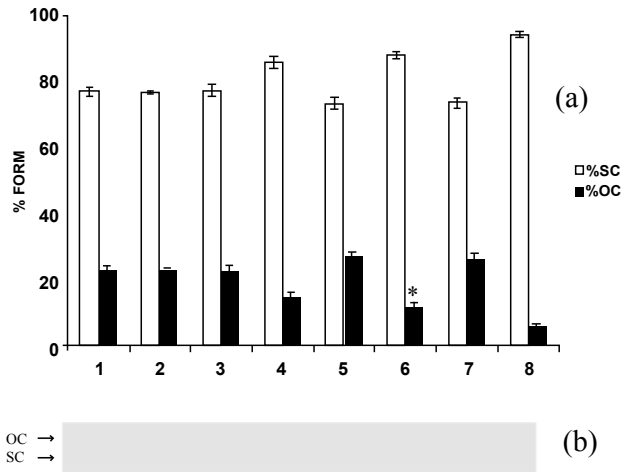

Figure 5: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in continuous wave mode and incubated with formamidopyrimidine DNA glycosylase/MutM protein (fpg). Lanes: (1) pBSK; (2) pBSK+fpg; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+fpg; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+fpg; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+fpg. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

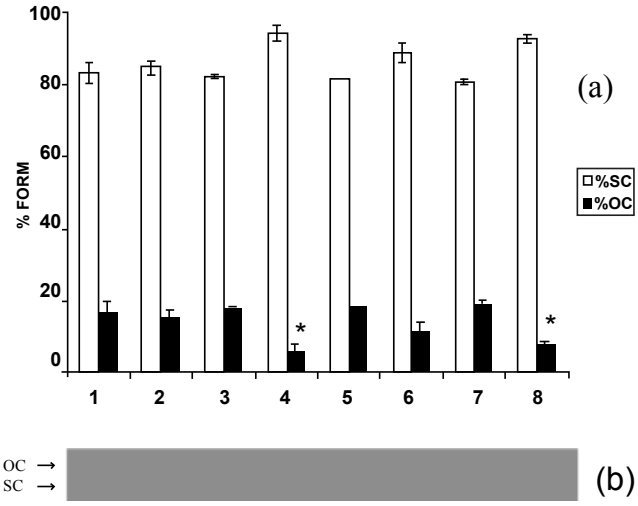

Figure 6: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in 2.5 Hz pulsed emission mode and incubated with formamidopyrimidine DNA glycosylase/MutM protein (fpg). Lanes: (1) pBSK; (2) pBSK+fpg; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+fpg; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+fpg; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+fpg. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

cell attachment [33]. Different exposure conditions, cells (*E. coli*), as well as presence of other DNA repair mechanisms in these cells, such as error prone mechanisms (SOS responses), could explain the absence of toxicity of low-level red laser on *E. coli* cultures evaluated.

E. coli filamentation assay was performed to verify whether low-level red laser effects on DNA could induce SOS responses. In fact, low-level red laser exposure at therapeutic doses induces filamentous phenotype in *E. coli* AB1157, BW527, BW9091, BH20 and BW375 cultures in exponential (Table 3) growth phase. These data are in accordance with previous results obtained by low-level infrared laser

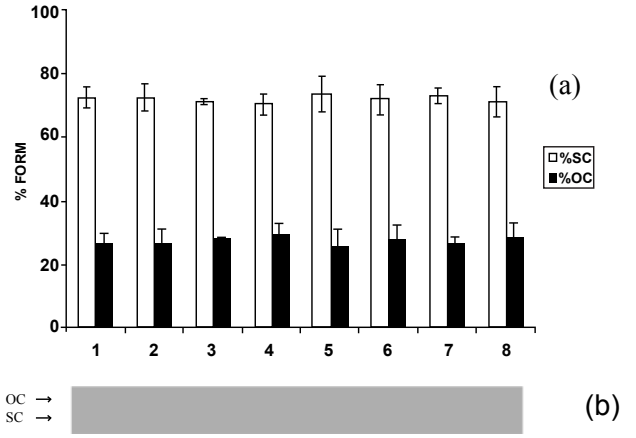

Figure 7: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in continuous wave mode and incubated with endonuclease III. Lanes: (1) pBSK; (2) pBSK+endonuclease III; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+endonuclease III; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+endonuclease III; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+endonuclease III. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent.

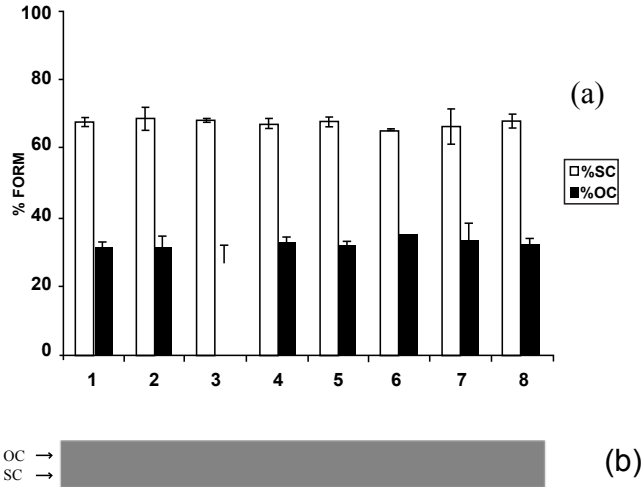

Figure 8: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in 2.5 Hz pulsed emission mode and incubated with endonuclease III. Lanes: (1) pBSK; (2) pBSK+endonuclease III; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+endonuclease III; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+endonuclease III; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+endonuclease III. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

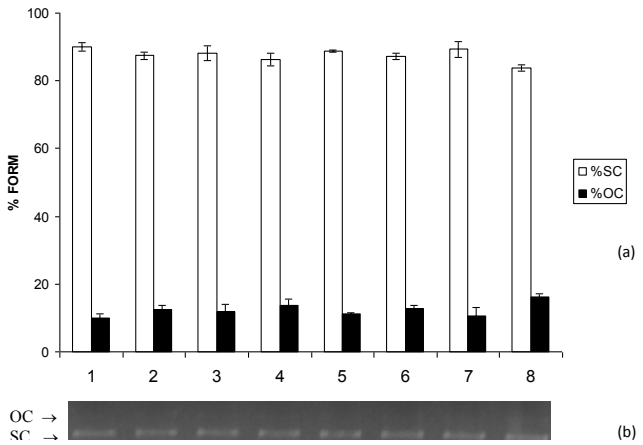

Figure 9: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in continuous wave mode and incubated with exonuclease III. Lanes: (1) pBSK; (2) pBSK+exonuclease III; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+exonuclease III; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+exonuclease III; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+exonuclease III. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

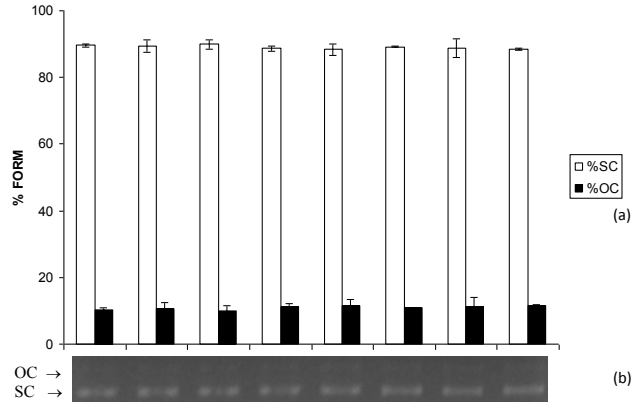

Figure 10: Percentage of bacterial plasmid forms (a) and photograph (b) of neutral agarose gel after electrophoresis of pBSK plasmids exposed to low-level red laser in 2.5 Hz pulsed emission mode and incubated with exonuclease III. Lanes: (1) pBSK; (2) pBSK+exonuclease III; (3) pBSK+pulsed laser 0.13 J; (4) pBSK+pulsed laser 0.13 J+exonuclease III; (5) pBSK+pulsed laser 0.52 J; (6) pBSK+pulsed laser 0.52 J+exonuclease III; (7) pBSK+pulsed laser 1.04 J; (8) pBSK+pulsed laser 1.04 J+exonuclease III. (□) SC (supercoiled); (■) OC (open circle). Numbers (1) through (8) for the histogram refer to gel lanes. Error bars indicate the standard deviation of the mean for n=3 independent experiments.

[20]. Filamentous phenotype occurs in over-stressed, sick and dying members of a bacterial population, as a vital survival strategy for bacterial survival [34] and following ultraviolet radiation exposure [35]. Laser-induced stimulation of cell replication in *E. coli* cultures depends on the culture conditions, determining the particular metabolic state necessary for the division [36]. Then, filamentation in *E. Coli* cultures in the stationary growth phase were also evaluated. Table 4 shows that the percentage of filamentation induced by low-level red laser in *E. Coli* cultures in the stationary growth phase are significant to all *E. coli* strains evaluated. Comparison between Tables 3 and 4 shows that *E. coli* filamentation is dependent on the culture growth phase for *E. coli* BW9091 and BW375 suggesting that low-level red effects depend on the physiological conditions. These *E. coli* mutants present highest filamentation percentages, mainly in continuous wave and in pulsed emission mode at the higher frequency (2500 Hz). It was demonstrated that low-level lasers present different effects on *Escherichia coli* cultures in continuous wave and pulsed emission mode [37]. At least in part, these differences could explain highest filamentation inductions in *E. coli* mutants exposed to laser in continuous wave and pulsed emission mode at the higher frequency evaluated. On the other hand, data from the filamentation assay reinforce that non-functional genes related to DNA repair could be important to laser-induced effects at doses used in therapy [19]. Also, these data suggest that functional gene products, in special these related to DNA repair of oxidative lesions, could be important to laser effects on cells because *E. coli* BW9091 cultures in the stationary growth phase presented highest filamentation percentages. *E. coli* BW375 presented highest filamentation percentages in exponential growth phase, while other *E. coli* strains (AB1157, BW527 and BH20) presented similar filamentation percentages in both growth phases. *nth*, *nfo* and *xthA fpg* gene products play role key in repair pathway involved in repair of DNA lesions induced by oxidizing agents [38]. Hydrogen peroxide [39], oxidative stress conditions [40] and low-level infrared laser induce filamentation in *E. coli* cultures [20]. Laser exposure at therapeutic doses has been demonstrated to induce DNA damage repair genes, in particular, those involved in the repair of lesions induced by free radicals [41,42]. In fact, redox status in mitochondria is regulated after low-level laser exposure and some components of respiratory chain components (flavine dehydrogenases, cytochromes and cytochrome oxidase) could be associated with this process [32]. In bacterial cells, citochrome bd is considered the chromophore to red near infrared light [37]. Thus, our data demonstrate highest filamentation inductions in *E. coli* cells deficient on repair of oxidative DNA lesions reinforce that low-level red laser could induce DNA lesions by free radical generation.

Low-level red laser in continuous and pulsed mode emission at the lower frequency evaluated (2.5 Hz) is not capable to induce single- and double-strand breaks, alkali labile sites and abasic sites in DNA (lanes 2, 3 and 4 compared with lane 1 in Figures 1 and 2. These results are in accordance with previous data from a study with a laser at 658 nm [18] and with data reported in another study where infrared (810 nm) laser was incapable of inducing DNA lesions at fluences similar to those used in our study [31]. However, alterations in electrophoretic profile of plasmids exposed to infrared [19] were described. This discrepancy could be related to different wavelengths used. Analysis of Figures 3-8 suggests that red laser exposure could induce DNA lesions not targeted by endonuclease IV, formamidopyrimidine DNA glycosylase or endonuclease III. However, Figures 9 and 10 are indicative that low-level intensity red laser could induce DNA lesions targeted by exonuclease III. This enzyme is involved in dark DNA repair [25], being an important mechanism in repairing DNA lesions, such as apurinic/apyrimidic sites and 3'-oxidative damages induced by oxidant agents. Figures 9 and 10 suggest that low-level red laser induces DNA lesions by oxidative mechanisms. Although exonuclease III deficient cells are more sensitive to oxidative damage, these cells present endonuclease III, another enzyme involved in repair of oxidative lesions in DNA [25] and all other DNA mechanisms, including those related to SOS response. This could explain why low-level red laser is not capable of altering the survival of BW9091 (Tables 1 and 2) but inducing bacterial filamentation (Tables 3 and 4). Thus, these findings and data from other authors [13,31,32] are in accordance with the hypothesis

that some laser-induced biological effects could occur by free radical generation and reinforce the importance to consider low-level lasers capable of inducing, by direct or indirect pathways, DNA repair and changes in gene expression. On the other hand, pre-exposure to visible-to-near infrared light protects human dermal fibroblast against ultraviolet cytotoxity [43,44], sunburn [45] and gamma radiation. Thus, it is possible that pre-exposure to red and near-infrared light activate a protective/adaptive mechanism against non-ionizing and ionizing radiations.

Conclusion

Low-level red laser at therapeutic doses and in different emission modes has not effect on survival of *E. coli* wild type, endonuclease IV, exonuclease III, formamidopyrimidine DNA glycosylase and endonuclease III deficient cells but it induces filamentation in the cultures of these *E. coli* strains and DNA lesions targeted by exonuclease III. Thus, exposure to monochromatic red light could be important to active/induce survival mechanisms against harmful radiations.

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