Low-power laser irradiation did not stimulate breast cancer cells following ionizing radiation

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ABSTRACT

Cancer has become a public health problem worldwide. Radiotherapy may be a treatment to a number of types of cancer, frequently using gamma-radiation with sources such as ^{137}Cs and ^{60}Co, with varying doses, dose rates, and exposure times to obtain a better as a stimulant for cell proliferation and tissue healing process. However, its effects on cancer cells are not yet well elucidated. The purpose of this work was to evaluate the effects of the LPL on breast cancer cultures after ionizing radiation. The breast cancer-MDA-MB-231 cells were gamma irradiated by a ^{60}Co source, with dose of 2.5 Gy. After 24h, cells were submitted to LPL irradiation using a red laser emitting at λ= 660 nm, with output power of 40 mW and exposure time of 30 s and 60 s. The plates were uniformly irradiated, with energy of 1.2 J and 2.4 J, respectively. Cell viability was analyzed using the exclusion method with trypan blue. Our results show that breast cancer cells submitted to LPL after ionizing radiation remained 95 % viable. No statistically significant differences were observed between laser and control untreated cells, ($P > 0.05$). These findings suggest that LPL did not influenced cancer cells viability.

Keywords: cell viability, Low Power Laser, Ionizing Radiation, Cancer Cells.

1- INTRODUCTION

Cancer is a group of diseases described as an uncontrolled growth of body cells that divide without stopping and spread around tissues[1]. Although all types of cancer have become a public health question in the world, it is undeniable that the breast cancer is the most common type of cancer that affects women in many countries, and it is a frequent cause of death among patients worldwide[2].

The treatment options usually involve surgery, chemotherapy, radiotherapy, and hormone therapy. However, radiotherapy is the treatment frequently used after lumpectomy or mastectomy surgery to eliminate any residual cells, by exposing the tumor to ionizing radiation (IR). Usually gamma rays sources are used, such as ^{60}Co and ^{137}Cs, which is an electromagnetic radiation of high-energy photons that affect DNA structure[3].

Application of IR in cancer treatment triggers deleterious effects in biological tissue by generation of reactive oxygen species (ROS) through water radiolysis and oxidative stress. ROS, such as superoxide, hydroxyl radicals and hydrogen peroxide induce DNA and RNA alterations, resulting in senescence, apoptosis or necrosis. In addition, IR cause important side effects like dermatitis, fibrosis and mucositis[4].

Low Power Laser (LPL) has been applied in the last years as an interesting approach for bio modulation of inflammatory processes, wound healing, [5, 6] and analgesia [7, 8]. Also, depending on light parameters LPL can prevent cytotoxic effects when applied before IR [9, 10]. Regarding cancer cells, effects of LPL have not been well elucidated yet [11, 12], mainly following IR. Thus, the aim of the present study was to evaluate the effects of LPL in MDA-MB-231 cells after exposure to ionizing radiation by a ^{60}Co source. The cells were submitted to LPL irradiation after 24 h, using a red laser emitting at λ=660 nm.

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2- MATERIALS AND METHODS

Cell Cultures

Experiments were conducted with MDA-MB-231 cells (human breast cancer; ATCC® HTB-26). The cells were cultured in DMEN (Gibco, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), 100 U/mL penicillin (Sigma, USA) and 100 µg/mL Streptomycin (Sigma, USA) at 37º C with 5 % CO_2 in humidified air. The cells were passaged using 0.25 % trypsin and 0.03% ethylenediaminetetraacetic acid (EDTA) (Sigma, USA) in phosphate buffered saline (PBS) solution. After the cells reached confluence, they were plated with $1x10^5$ cells concentration. The experiments were realized in triplicate in three different days.

Ionizing Radiation (IR)

For ionizing radiation, it was used ^{60}Co source (Gamma Cell- Atomomic Energy of Canada, LTD) with the dose of 2.5 Gy. The dose- rate was 1.76 Gy/min.

Low–Power Laser (LPL)

After twenty- four-H of the IR, the cells were exposed to LPL with a He- Ne laser (λ= 660 nm) according Table 1. The plates were uniformly irradiated, with energy of 1.2 J and 2.4 J, corresponding to exposure times of 30 s and 60 s, respectively. This is day is considered in our results as 0 h.

Table1. Parameters used for irradiation with LPL.

Energy density (J/cm^2)	Spot area (cm^2)	Time (s)	Output power (mW)	Energy (J)
Control (zero)	-	-	-	-
30	0.04	30	40	1.2
60	0.04	60	40	2.4

Viability assay

Twenty four-h after LPL, the cells were removed with 200 µL trypsin solution, centrifuged with 1500 rpm for five minutes and resuspended in 80 µL PBS solution with 10 µL of trypan blue and 10 µL of cells. The viability of cells was checked by trypan blue exclusion test and counted with a haemocytometer. The formula utilized was

$$N= (total\ number\ of\ viable\ cells\ x\ diluation\ x\ 10^4\ )/4$$

We checked the viability of cells with trypan blue exclusion for 24 h and 48h after LPL.

Statiscal Analysis

Statiscal analysis was performed in Graph Pad Prism 5.0 software. Data are presented as means ± standard error of mean (SEM). For continuous variables, means were compared by one way analysis of variance (ANOVA) and Dunn`s Multiple Comparison post- hoc testing. The level of statistical significance was $p < 0.05$.

3- RESULTS

The figure 1 shows the mean values of control group and ionizing radiation group with dose 2.5 Gy in all experimental times.

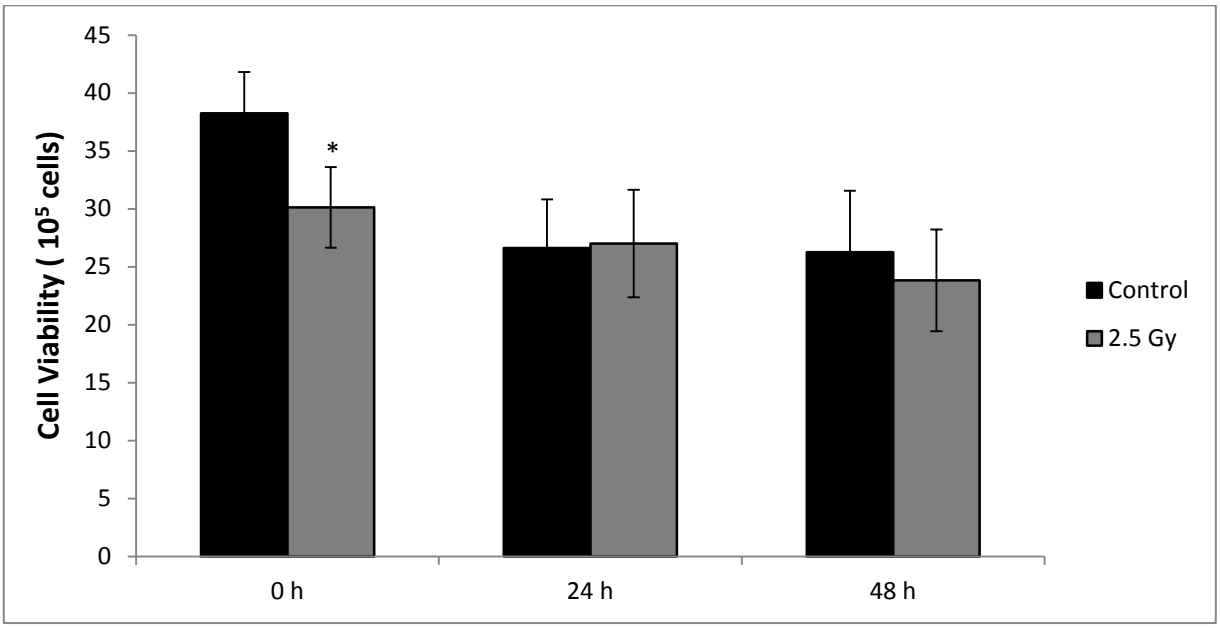

Figure 1 – Mean values ± SEM of cell viability of control group and 2.5 Gy group. The signal * represents statistically significant differences from control group in each experimental period. ($p < 0.05$).

The results in Fig.1 indicate that IR effects in cancer cells with dose 2.5 Gy influenced only their viability in 0h. In the others experimental periods the cell viability was similar between groups. These results indicate that cancer cells MDA-MB-231 were resistant to IR effects probably because its elevate proliferation rate [13].

The figure 2 shows the mean values of control group cell viability and the cells received only the LPL with the energies of 1.2 and 2.4 J.

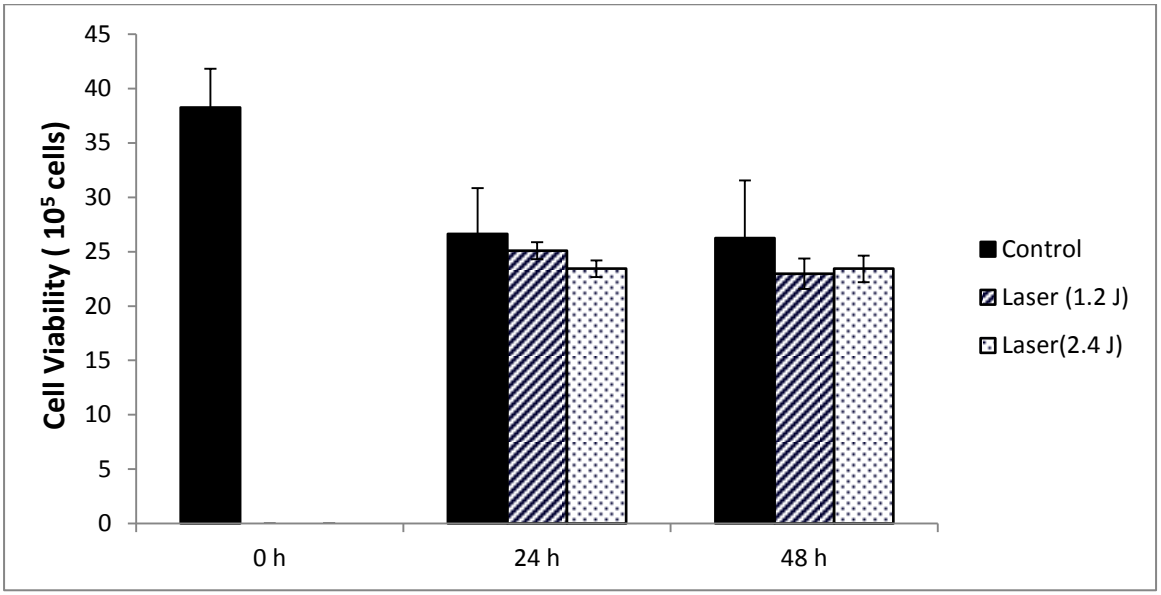

Figure 2 – Mean values of cell viability of control group and laser groups with energies of 1.2 and 2.4 J in all experimental periods. The bars represent the SEM.

The results in Fig.2 indicate that mean values of laser groups were similar to control group. The LPL did not influence cell viability in all experimental periods independent energy utilized 1.2 J or 2.4 J.
The figure 3 shows the mean values of group of received only I.R with dose 2.5 Gy and the groups that received I.R and LPL with energies of 1.2 and 2.4 J.

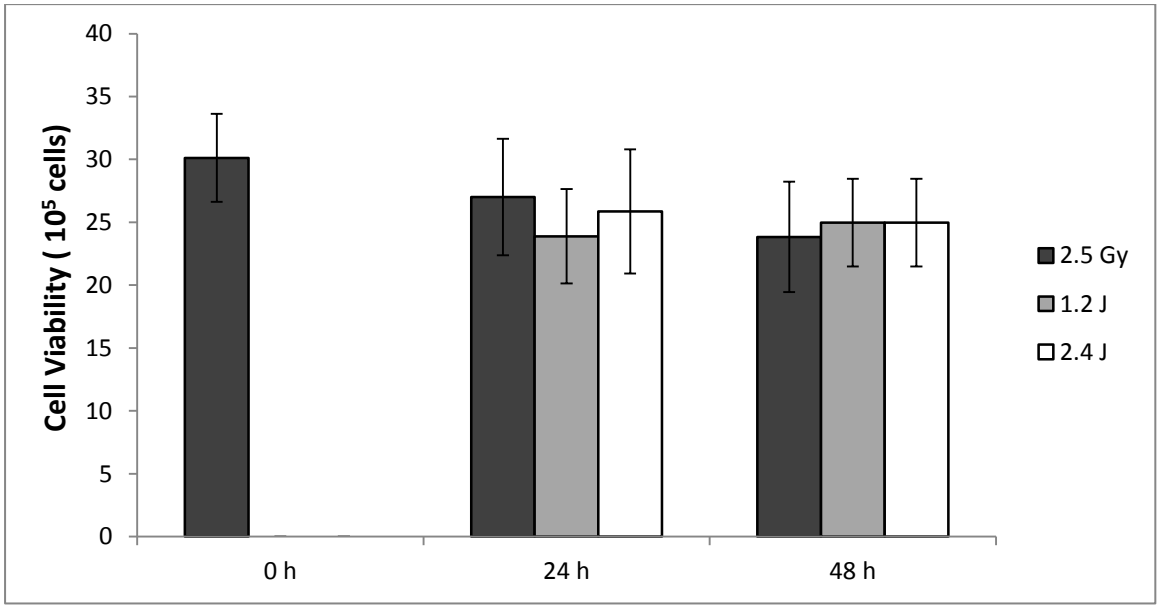

Figure 3 – Mean values ± SEM of cell viability of 2.5 Gy group and groups that received IR and LPL with energies of 1.2 and 2.4 J in all experimental times.

The association IR with dose 2.5 Gy and LPL with energies 1.2 and 2.4 J did not influence in cell viability according the Fig.3. In experimental period of 24 h, the results of 1.2 J (23. 88889 ± 3. 752982) and 2.4 J (25.86111 ± 4.933039) group were similar to control group (26.625 ± 4.204825). In experimental period of 48 h, the results of 1.2 J and 2.4 J groups showed similar to control group and it don't exhibited statistical difference.

4- DISCUSSION

IR exposures in biological structures release some free radicals that can change cells components and behavior. These modifications can stimulate oxidative damage that results in apoptosis and cellular necrosis[14]. The usual IR dose in radiotherapy varies between 1.5 and 2.5 Gy. The severity of the effects of IR depends on accumulated radiation dose, exposition time, dose rate, irradiated area, and the radiation intensity [15]. The IR is frequently used for cancer treatment, the radiosensitivity of cell proliferation varies according to the phase of its cycle and different strains [16] .

The literature has reported that IR commonly damage DNA molecules directly and induces loss or base changing, double-strand breaking and hydrogen bonds. These damages can undergo repair mechanism, however if these alterations are severe it will be difficult to change them physiologically [17].

The Fig 2 represents LPL effects in groups submitted to energies 1.2 and 2.4 J. The outcomes demonstrate that LPL does not influence cell viability in all experimental times. However, the literature reports that LPL can stimulate its proliferation. In fact, Schaffer et.al (1997)[18], Kreisler et.al (2003)[19] and Powell et.al (2010)[20], verified cell proliferation according to different parameters. Nevertheless, other authors, such as Frigo et.al (2009)[21] and Murayama et.al (2012)[22] did not observe higher proliferation compared with control group when cancer cells were LPL irradiated.

The divergence of results may be attributed to different energy densities used. In this work, we applied middle doses (1.2 and 2.4 J) compared to the literature. Frigo et.al (2009) [21], using 150 J/cm² in B16F10 cells can`t found increase cell viability, however, when they irradiated with 1050 J/cm² the same strain cell, the results were opposite.

Some authors proposed that modulating effects of LPL also depends on cell culture physiological state before LPL irradiation. Probably, LPL can be more efficient on cells under stressing conditions, which increases cell viability [23, 24]. According to figure 3, there was no statistical difference between 1.2 J and 2.4 J groups (IR+LPL) in all experimental times compared with 2.5 Gy group. These results suggest that even when the cells were under stress after IR exposure, the LPL did not stimulate cell viability.

There are few literature reviews about LPL in association with IR. Some authors, such as Karu et.al (1994) [9], observed increase survival fraction when utilized LPL with density energy of 100 J/m² in HeLa cells before IR exposed with doses between 0.2 to 10 Gy. On the other hand, Djavid et.al (2015)[25], demonstrated the level of survival curve decreased when utilized LPL with wavelengths of 685 and 830 nm in NIH 3T3 cells before IR with doses 2, 4 and 6 Gy.

Alternative treatments are required to minimize adverse effects on healthy tissue around cancer cells irradiated by IR. Many studies have shown that LPL can induce adaptive reactions to IR in different strains cells, and it stimulates non-cancerous cells proliferation. It is well known that laser therapy can reduce the incidence of mucositis[26] and dermatitis[27] radiotherapy-induced. These results are promising since the purpose of study was to accelerate tissue regeneration and repair process of healthy tissue without influence the cancer cells.

5- CONCLUSIONS

Under the conditions used in this study, our data suggest that LPL using red laser and energies of 1.2 J and 2.4 J did not stimulate cancer cell proliferation. Thus, LPL could be used in oncologic patients to minimize adverse effects of ionizing radiation. Given the effect of LPL to not increase cancer cell viability, further study to investigate the real underlying mechanisms is necessary.

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