Effect of irradiation with different laser wavelengths on oxidative stress of non-hepatectomized rats[1]

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ABSTRACT

PURPOSE: To assess the effect of two laser wavelengths, either separate or combined, on intact rat livers.

METHOD: Nineteen male Wistar rats (200-300 g) were submitted to laser irradiation at 5 different sites on the liver surface.Wavelengths 660 and 780 nm were used, with a dose of irradiation of 60 J/cm^2/site.The animals were divided into the groups:control (C) and animals irradiated with 660 nm laser (L1), with 780 nm laser (L2) or withboth wavelengths (L3).Mitochondrial function, mitochondrial swelling, and hepatocellular malondialdehyde (MDA) levels were determined.Data were analyzed by the Mann-Whitney test, with the level of significance set at 5%.

RESULTS: There was a reduction of ADP-activated respiration (state 3) in group L1 compared to group C (p=0.0016), whereas the values of group L2 were similar to control.Group L3 also showed a reduction of state 3 (p=0.0159).There was a reduction of RCR in group L1 compared to control (p=0.0001) and to group L2 (p=0.0040).Mitochondrial swelling only differed between group L3 and control (p=0.0286).There was a increase in MDA levels in group L3 compared to control (p=0.0476) and to group L2 (p=0.0286) and in group L1 compared to group L2 (p=0.0132).

CONCLUSION: Although laser irradiation reduced mitochondrial function,it did not interfere with the hepatocellular energy status.

Key words: Laser. Mitochondria. Liver. Rats.

Introduction

The literature has reported the stimulatory effect of laser on hepatic regeneration in livers submitted to partial hepatectomy. Several studies have shown an effective interaction between light and biological tissues, the liver in particular[1-5].Conventional light, as well as light-emitting diodes (LED) and laser, with their different optical properties concerning light production and delivery have similar effects on cell organelles, leading to increased efficiency of wound healing, of hepatocellular mitochondrial energy capacity, and of hepatic regeneration[2,3-6]. The partial loss of hepatic tissue rapidly triggers the regenerative process until the original weight is recovered[7,8]. The detection of the response of the liver to various challenges such as hepatitis, intoxication, hepatic resections and liver transplantation is of a mythological nature[9,10] and raises extremely interesting aspects about the hepatic coherencein meeting challenges and overcoming obstacles. Laser phototherapy has proved to be effective for neovascularization, fibroblast proliferation, bone repair and nerve regeneration, also with broad applications to oncology in the form of photodynamic therapy[2-4]. The mechanisms of interaction between laser and matterare characterized as photochemical, photothermal and photomechanic processes. Photobiostimulation is based on the most subtle level of light interaction with tissues, with the photon transmitting its energy to a photosensitive compound. Laser light in the visible range of the spectrum is assumed to lead to conformational changes in the cytochrome structure of hepatocytes, thus inducing increased ATP production that expresses an increase in the energy metabolism of the liver of hepatectomized rats[11-13]. It has been demonstrated that both high and low power laser light has the ability to increase the mitotic index and mitochondrial function of the liver remnant after partial resection in non-cirrhotic rats[5]. However, there is no clear knowledge about the effect of laser light on non-hepatectomized livers free from regenerative mechanisms that might interact with this light.

Thus, the objective of the present study was to assess the effect of laser light at two different wavelengths applied separately or in combination on intact livers with no level of hepatic resection.

Method

Based on the sample size calculation of Kelley and Maxwell[14], 19 male Wistar rats weighing 200-300 g were submitted to irradiation by placing the distal end of the application device in light contact with the liver surface at 5 different sites. The apparatus used for liver irradiation was a CW laser, model BDP 660, class III b instrumentwith continuous emission of visible light of low power and equipped with a semiconductor active medium and a fused fiber optic transducer 80 mm in length and 7 mm in diameter from M.M. Optics LTDA (São Carlos, São Paulo). The wavelengths used were 660 and 780nm, of high penetrability into biological tissue and regulated at a power of 40mW, with an irradiation dose of 60J/cm^2 per/ irradiation site. The animals were divided into the following groups: control (C), group irradiated with 660nm laser (L1), group irradiated with 780nm laser (L2), and group irradiated with both wavelengths (L3).

The animals were submitted to general anesthesia with intramuscular xylazine (Dopaser, Gepec, Belo Horizonte, Brazil)/ketamine (Ketalar, Pfizer, São Paulo, Brazil) at the dose of 80/16 mg/kg, placed in dorsal decubitus on a wood support with their paws fixed in extension, followed by shaving of the thoracoabdominal area. The surgical procedure was perfomed in a closed environment under controlled temperature (23°C). The liver was exposed by median laparotomy and irradiated with laser as described above and the animals were sacrificed 15 min after the last application.

Mitochondrial function was analyzed by the polarographic method[15], by the spectrophotometric determination of mitochondrial swelling[16] and by the determination of hepatocellular levels of malondialdehyde (MDA)[17].

The results of mitochondrial function are reported as a fraction compared to group C and MDA levels are reported as μM(10^{-2})/mg protein. The results were analyzed statistically by the nonparametric Mann-Whitney test, with the level of significance set at 5% ($p<0.05$).

Results

Regarding mitochondrial function, there was a reduction of ADP-activated respiration (state 3) in group L1 compared to group C ($p=0.0016$) while the values for group L2 were similar to control. Group L3 also showed a reduction of state 3 ($p=0.0159$), while state 4 did not differ significantly between the groups studied (panel B). As shown in Figure 1, panel C, there was a significant reduction of RCR in group L1 compared to control ($p=0.0001$) and to group L2 ($p=0.0040$). Mitochondrial swelling showed a significant difference only between group L3 and group C (p=o. o286).

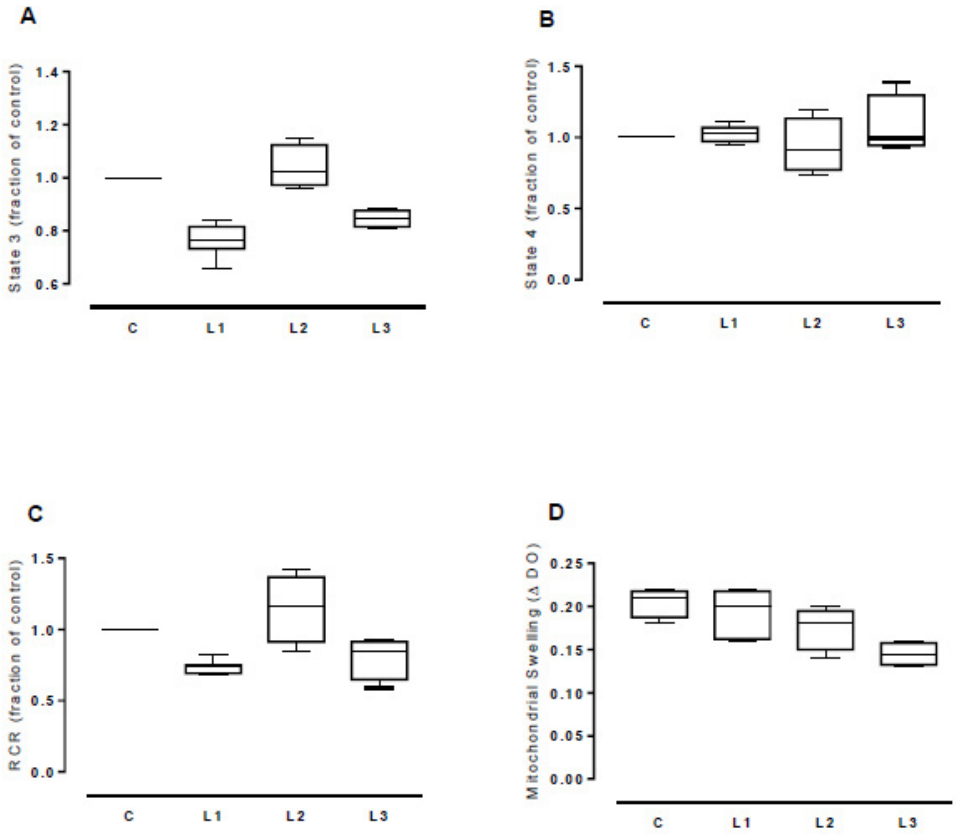

FIGURE 1 - Panel A: State 3 of the mitochondrial respiration chain of the various groups studied: L1 vs C (p= 0.0016), L3 vs C (p=0.0159). Panel B: State 4 of the mitochondrial respiration chain of the various groups studied. There was no significant difference between the various groups. Panel C: A significant reduction of RCR in group L1 compared to Control (p=0.0001) and togroupL2(p=0.0040). Panel D: Mitochondrial swelling differed significantly only between group L3 and control (p=0.0289).

There was a significant increase in MDA levels in L3 compared to control (p=0.0476) and to group L2 (p=0.0286) and in L1 compared to L2 (p=0.0132), as shown in Figure 2.

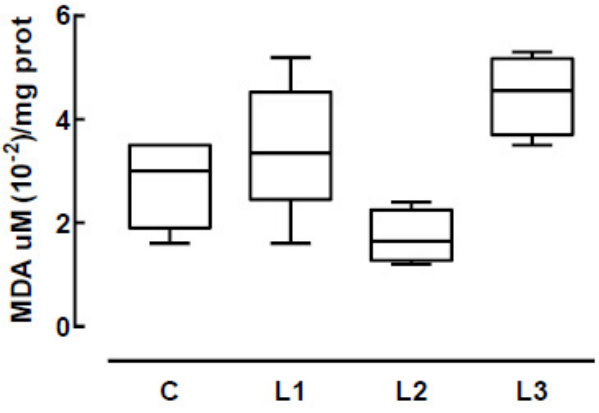

FIGURE 2 - Hepatocellular malondialdehyde (MDA) levels of the four groups studied showing a significant increase in L3 compared to control (p=0.0476) and togroup L2 (p=0.0286) and in L1 compared to L2(p=0.0132).

Discussion

In the present study we assessed hepatic mitochondrial function and MDA levels in intact livers in response to the use of laser as a source of light in a simulation of laser therapy at two different wavelengths applied separately or in combination, based on the basic understanding of some fundamental elements of the interaction between light and matter[1,6]. The main objective was to determine the effect of laser light on hepatic energy capacity based on previous studies from our laboratory about hepatic function and mitochondrial function[5,18,19].

The liver, as matter in its nature, consists of atoms that form molecules, structures, tissues, organs and organisms. Light, in turn, is of a dual nature and can be explained as a wave or particle since it behaves like both. The energy carried by light can be understood as an oscillation of electric and magnetic fields with no mass. When interacting with the charges of matter, those fields cause them to move by oscillating, especially electrons, which are lighter than atoms nuclei and, in turn, generate other electric and magnetic fields, producing different effects[2,4,20-22].

Some of these effects are more relevant to the understanding of the processes occurring in matter when illuminated, i.e., the energy transfer from light to molecules (absorption) and vice-versa (emission), and the propagation changes in light direction (scattering). These three phenomena are essential in order to understand how light interacts with biological structures. A fundamental concept of phototherapy, called biological optical window or therapeutic optical window, represents the range of wavelengths that are best suitable for those applications[1,2,4,20-22].

The biological optical window comprises the end of the ultraviolet spectrum, the visible light range and the beginning of the near infrared range, which are considered to be the acceptable region for the use of phototherapy and biophotonics' applications in general.

This window is limited by the effects that the remaining wavelengths produce on biological environment. From the ultraviolet light to the higher wavelengths (starting approximately at 380 nm), the energy of the photons becomes so high that it may ionize molecules, possibly impairing processes such as cell reproduction and damaging DNA. On the other hand, starting approximately at 1200 nm in the direction of lower wavelengths, the absorption of electromagnetic radiation by water is so relevant that the resulting thermal effects can no longer be ignored. This leaves approximately the 400-1200 nm range to be used for phototherapy. However, the association of high scattering and high absorption occurring for most biomolecules between 300 and 500 nm, although not prohibitive, causes most wavelengths in this range not to exceed the barrier of a few hundred microns of penetration into biological tissue. This leaves a useful range of approximately 550-1200 nm for the so-called "biological window". Understanding this interaction and these concepts is of fundamental importance for the understanding of how any biological effects are promoted by light.[2,4,20-22].

Thus, in the present study we applied two laser light wavelengths within this biological window, 660 and 780 nm, and their combination to the intact liver not submitted to any chemical or surgical procedure. We observed a reduction of energy capacity in the groups submitted to the lower wavelength alone or in combination with the other at the same dose. Mitochondrial edema and MDA levels increased significantly after the combined application. However, an overall analysis revealed that the state of the mitochondrial membrane and the mitochondrial RCR edema remained biologically stable. Although the ADP-activated rate was reduced in groups L1 and L3, the energy status of the liver was preserved under laser application, a fact that supports the possibility of using this application as therapy under the conditions of the present experiment.

Conclusion

Although laser light reduced mitochondrial function at 660 nm wavelength and with the combination of 660+780 nm wavelength, it did not interfere in a negative manner with hepatocellular energy state. Additionally, the increase in MDA in L3 showed that laser induced an increase in oxidative stress in the liver with a consequent increase in oxygen free radicals.

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