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Evaluation of Laser Phototherapy in the Inflammatory Process of the Rat's TMJ Induced by Carrageenan

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Abstract

Aim: The aim of this study was to evaluate, by light microscopy, the effects of laser phototherapy (LPT) at 780 nm or a combination of 660 and 790 nm, on the inflammatory process of the rat temporomandibular joint (TMJ) induced by carrageen. ***Background:*** Temporomandibular disorders (TMDs) are frequent in the population and generally present an inflammatory component. Previous studies have evidenced positive effects of laser phototherapy on TMDs. However, its mechanism of action on the inflammation of the TMJ is not known yet. ***Materials and Methods:*** Eighty-five Wistar rats were divided into 9 groups: G1, Saline; G2, Saline + LPT IR; G3, Saline + LPT IR + R; G4, Carrageenan; G5, Carrageenan + LPT IR; G6, Carrageenan + LPT IR + R; G7, previous LPT + Carrageenan; G8, previous LPT + carrageenan + LPT IR; and G9, previous LPT + carrageenan + LPT IR + R, and then subdivided in subgroups of 3 and 7 days. After animal death, specimens were taken, routinely cut and stained with HE, Sirius Red, and Toluidine Blue. Descriptive analysis of components of the TMJ was done. The synovial cell layers were counted. ***Results:*** Injection of saline did not produced inflammatory reaction and the irradiated groups did not present differences compared to non-irradiated ones. After carrageenan injection, intense inflammatory infiltration and synovial cell layers proliferation were observed. The infrared irradiated group presented less inflammation and less synovial cell layers number compared to other groups. Previous laser irradiation did not improve the results. ***Conclusion:*** It was concluded that the LPT presented positive effects on inflammatory infiltration reduction and accelerated the inflammation process, mainly with IR laser irradiation. The number of synovial cell layers was reduced on irradiated group.

Introduction

Temporomandibular joint disorders (TMDs) are multifactorial conditions in which the most common symptoms are pain (joint, muscles); limited movement or locking of the jaw; and grating sounds on the temporomandibular joint (TMJ) when opening or closing the mouth.[1] TMDs are the most common nontooth-related chronic orofacial pain conditions that often present an inflammatory component. However, description of the pathogenesis of the inflammation of the TMJs has been based on data from studies of other synovial joints. Few studies have assessed only the immediate events of the inflammatory process on TMJs.[2]

Anatomic and functional aspects of the TMJ may influence its response as a synovial joint. Its surface, for example, presents a dense fibrous tissue covering, whereas most other synovial joints are covered only by hyaline cartilage. Moreover, the concentration of some inflammatory substances in the TMJ differs from those in other joints. For example, substance P levels, a peptide related to calcitonin and

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neuropeptide Y, are higher in TMJ arthritis than on the knee joint, probably due to a denser innervation.[3]

Treatment of the TMDs includes both the maintenance of the function and pain control. The nonsteroidal anti-inflammatory drugs (NSAIDs) are generally the first pharmacological option of treatment.[4]

Carrageenan is a polysaccharide widely used for the induction of experimental inflammation on animal models, and it is the most common method for drug testing or assessment of new anti-inflammatory therapies.[5] Some studies have reported the successful utilization of this model to induce both rat paw and joint inflammation.[6,7]

Alterations of the synovial membrane have been reported in cases of osteoarthritis of the TMJ. The thickness of synovial cell layers is one histological change commonly assessed on inflamed TMJs. Some studies have graded the number of cell layers and reported its proliferation on TMJs showing disc displacement, osteoarthritis, and following induced trauma.[8–10]

Laser phototherapy (LPT) is an option for the treatment of musculoskeletal disorders due to its analgesic, anti-inflammatory and regenerative effects.[11] This therapy has been used on TMDs and showed good patient acceptance and reduction of the use of drugs.[12]

Previous studies have shown positive effects of LPT on both TMDs[13,14] and rat paw edema.[15] NSAIDs have been used to treat both acute and chronic inflammatory articular disorders. However, this drug may reduce the inflammation but it may also increase the risk of complications, such as gastric ulcer and nephrotoxicity.[16] In this way, the use of LPT is an alternative anti-inflammatory treatment without those undesirable reactions.

Although previous studies reported the use of LPT in TMDs, the anti-inflammatory mechanism of this therapy is poorly understood. LPT may be a noninvasive treatment for TMDs patients that reduce the pain without side effects. It is necessary to understand the effects of LPT on inflamed TMJs in order to provide more precise information to contribute to a better clinical practice.

The aim of this study was to evaluate, by light microscopy, the effects of laser phototherapy (LPT) on the inflammatory reaction induced by carrageenan on TMJs of rodents.

Materials and Methods

Following approval by the Animal Experimentation Ethics Committee of the School of Dentistry of the Federal University of Bahia (Protocol 014/2006), 85 healthy young adult male Wistar rats, average 3 months old (~200 g), were obtained from the Central Animal House of the School of Veterinary Medicine of the Federal University of Bahia and kept at the Health Sciences Institute of Federal University of Bahia. The animals were kept in groups of five in acrylic cages lined with wood chips and maintained at 22°C in a day/night light cycle. The animals were fed a standard laboratory diet (Labina®, Purina, São Paulo, Brazil) and had water available *ad libidum*. After a regular quarantine period, the animals were randomly distributed into nine major groups. Each group was then divided into two subgroups according to the timing of the animal death (3 and 7 days, Table 1).

Table 1. Division and Description of the Experimental Groups

Group	*Irradiation*	*Agent*	*LPT*	*Time (days)*	*Number of animals*
G1	No previous irradiation	Saline solution	No LPT	3	5
			LPT IR	3	5
G2	No previous irradiation	Saline solution	LPT IR	7	5
G3	No previous irradiation	Saline solution	LPT IR + R	3	5
				7	5
G4	No previous irradiation	Carrageenan	No LPT	3	5
				7	5
G5	No previous irradiation	Carrageenan	LPT IR	3	5
				7	5
G6	No previous irradiation	Carrageenan	LPT IR + R	3	5
				7	5
G7	Previous irradiation	Carrageenan	No LPT	3	5
				7	5
G8	Previous irradiation	Carrageenan	LPT IR	3	5
				7	5
G9	Previous irradiation	Carrageenan	LPT IR + R	3	5
				7	5

Induction of TMJ inflammation

Under intraperitoneal general anesthesia (Ketamine; Vetaset®, 0.06 ml/100 g, Fort Dodge, Campinas, SP, Brazil) and Xylazine (Copazine®, 0.03 ml/100 g, Coopers, São Paulo, SP, Brazil), each animal had its left preauricular area shaven and cleaned with 2% chlorohexidine solution and were injected with 20 μl of carrageenan (C1867, Sigma-Aldrich®, St. Louis, MO) in 1% saline solution. Animals of the control group were injected with 20 μl of 0.9% saline solution. A 30-gauge needle was then connected to a Hamilton syringe (50 μl) by a cannula (polyethylene tube, PE50). The needle was then introduced posterior to the zygomatic process of temporal bone and moved anteriorly towards the superior joint space until contact with the posterolateral side of the condyle.

Laser phototherapy (LPT)

A diode laser [Twin Laser®; MMoptics, São Carlos, SP, Brazil, CW, beam area = 0.04 cm^2, λ 780 nm, 50 mW (0.05 W), round shaped beam, 1.25 W/cm^2; total irradiated area 0.04 cm^2, or λ 660 nm, 40 mW (0.04 W), round shaped beam, 1 W/cm^2], total irradiated area 0.04 cm^2, was used. LPT started 24 h after induction of the inflammation and was repeated at 48 h intervals during experimental periods of 3 and 7 days. Groups G2, G5, and G8 were irradiated with 225 J/cm^2; 9J, λ 780 nm, 50 mW, 3 min. Groups G3, G6, and G9 received 112 J/cm^2, 4.5 J, λ 780 nm, 50 mW, 1 min and 30 sec, and 120 J/cm^2, 4.8 J, λ 660 nm, 40 mW, 2 min. The irradiation was performed transcutaneously in a single perpendicular contact point at the same local of the inflammation induction identified by palpation.

Histological processing

At the end of the experimental period, the animals were killed by an overdose of general anesthetic. The skin of the head was removed and an intermaxillar fixation was made with acrylic resin on the right molar teeth. The head then was

fixed in a buffered 10% formalin solution for 48 h. The specimens were decalcified during 48 h in 5% formic acid and coronal sections were then obtained. The local of the sectioning was set between the orbitary cavity and auditory condute. This distance was divided into three parts and the cut realized near the auditory conduct. The specimens were routinely processed to wax, cut, stained with hematoxylin eosin (HE) and toluidine blue, and underwent histological analysis at the Laboratory of Surgical Pathology of the School of Dentistry of Federal University of Bahia and at the Department of Maxillofacial Surgery and Traumatology of the School of Dentistry of the University of São Paulo. The description of morphological alteration, inflammatory reaction, and vascularity was carried out on the area of the condyle and articular capsule. The histological criteria used on this study may be seen on Table 2.

Histomorphometry

The number of synovial layers was determined on two selected portions named internal and external from each TMJ of all rats (Fig. 1).The cell layers were counted in each portion and then an average per subgroup of five rats was calculated. Histopathologic grading was performed according to a previous report by Muto et al.[10] The synovial hyperplasia was graduated from 0 to 2: Grade 0: staining of 1–3 cell layers; Grade 1: staining of 4–6 cell layers; Grade 2: staining of 7 or more cell layers.

Results

Group 1 (Saline solution)

At the third day, the articular components of the TMJ (condyle, articular disc, and articular fossa) presented no changes (Fig. 2). The injection of saline solution did not cause any change. The condyle presented well-defined layers: A fibrous articular surface had a proliferative zone; fibrocartilage, and subchondral bone (Fig. 3).

Group 2 (Saline solution + LPT IR)

At both days 3 and 7, the articular tissues presented characteristics of normality similar to that seen on Group 1 with discrete vascularization.

Group 3 (Saline solution + LPT IR + R)

At day 3, the articular tissues presented characteristics of normality as Group 1, and a moderated vascularization was noticed. Normality aspect persisted up until day 7 (Fig. 4), when a discrete vascularization was seen.

Group 4 (Carrageenan)

At day 3, an intense inflammatory infiltration was observed near to both the articular capsule and condyle. Inflammation was predominantly of mixed characteristic and extended adjacent to the muscular fibers (Fig. 5). Fibrinous exudate was observed at the condylar region. Areas of bone reabsorption were observed at the lateral side of the condyle (Fig. 6). Discrete vascularization was present on the articular region. Proliferation of the synovial lining and focal areas of hyperplasia were noticed. Granulation tissue was observed. At day 7, an intense inflammatory reaction, predominantly lymphocytic, was observed at the articular capsule. A few congested blood vessels were also seen (Fig. 7). The cartilage layers of the condyle and the muscular tissue did not present alterations. Degenerative changes with signs of bone reabsorption were observed in the lateral condyle. Discrete vascularization was detectable as well as focal hyperplasia and villi.

Group 5 (Carrageenan + LPT IR)

At the third day, a discrete inflammatory infiltration, predominantly lymphocytic, was observed at both the lateral side of the condyle and on the articular capsule (Fig. 8). Congested blood vessels were observed in the area near to the condyle. Areas of reabsorption were present in two cases at the lateral side of the condyle. Discrete vascularization was observed. Hyperplasic focal areas were observed at the synovial membrane. At day 7, a discrete inflammatory reaction, mainly lymphocytic, was observed at the articular capsule (Fig. 9). In three cases, areas of reabsorption at the lateral of the condyle were observed. There was a discrete vascularization on the area. Some areas of proliferation in the synovial membrane were observed.

Group 6 (Carrageenan + LPT IR + R)

At day 3, an intense and chronic inflammatory infiltrate was present on both the articular capsule and condylar

Table 2. Criteria of Histologycal Evaluation

Criterion	*Area*	*Discrete*	*Moderate*	*Intense*
Acute Inflammation	Articular capsule and condyle	Presence of <25% of neutrophyles in relation to the total of cells in the observed area	Presence of <25–50% of neutrophyles in relation to the total of cells in the observed area	Presence of >50% of neutrophyles in relation to the total of cells in the observed area
Chronic Inflammation	Articular capsule and condyle	Presence of <25% of limphocytes in relation to the total of cells in the observed area	Presence of <25–50% of limphocytes in relation to the total of cells in the observed area	Presence of >50% of limphocytes in relation to the total of cells in the observed area
Vascularity	Articular capsule and condyle	Presence of <25% of blood vessels in the observed area	Presence of < 25–50% of blood vessels in the observed area	Presence of >50% of blood vessels in the observed area

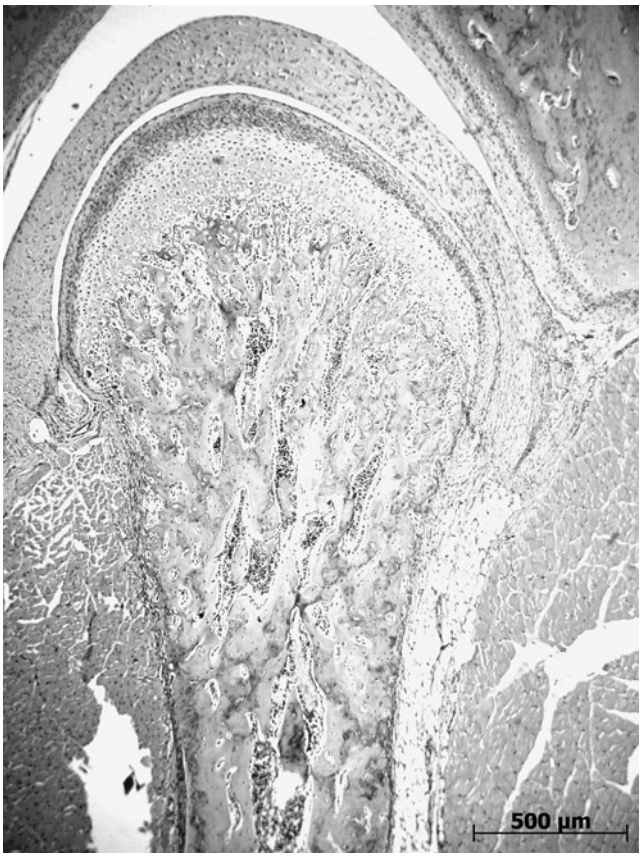

FIG. 1. Photomicrography showing the selected portions from the temporomandibular joint where cell layers of the synovial membrane were counted. I, Internal portion; E, external portion. Stained with HE.

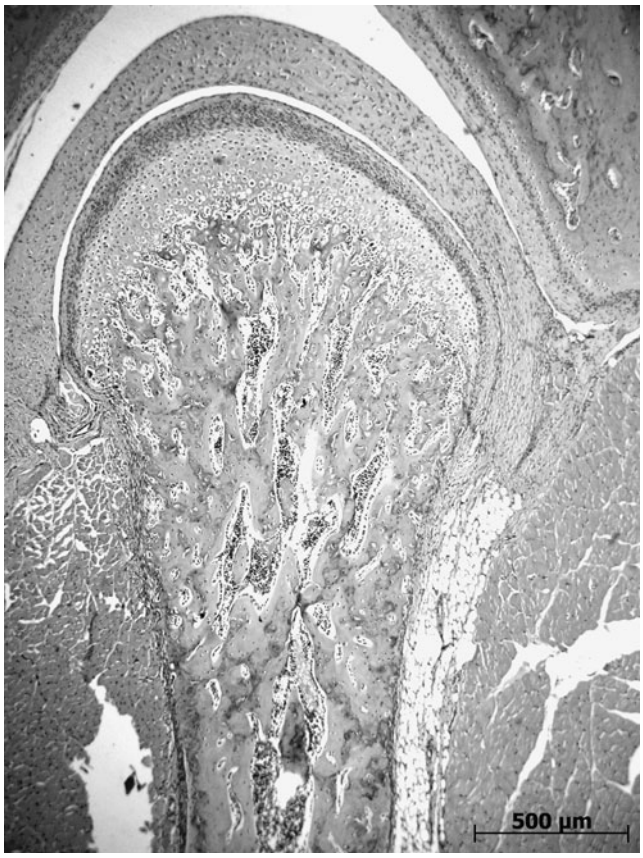

FIG. 2. Photomicrography of specimen of Group 1 (saline solution): normal articular components of the temporomandibular joint: AC, articular capsule; D, articular disc; C, condyle; M, adjacent muscular tissue. Stained with HE.

region extending along the muscular tissue (Fig. 10). A fibrinous-hemorrhagic exudate was observed at both the condylar region and among muscular fibers. Reabsorption was seen at the lateral portion of the condyle and, at the center area, giant cells were observed. Discrete vascularization was present. Extensive areas of hyperplasia area and villous were observed on the synovial membrane. At the seventh day, a moderate mononuclear cellular infiltrate and congested blood vessels were observed at the condylar neck and on the articular capsule (Fig. 11). Bone reabsorption was observed on the lateral side of the condyle in three cases. Presence of some areas of proliferation of the synovial lining was also observed.

Group 7 (LPT + Carrageenan)

At day 3, the presence of discrete inflammation in the articular capsule, mixed and intense inflammatory infiltration in the condylar necks, as well as congested blood vessels were observed on the condylar neck (Fig. 12). At the lateral condyle, giant cells and osteoclasts were observed, as well as a discrete vascularization. Discrete hyperplasia was present in the synovial membrane. At day 7, an intense chronic inflammatory infiltrate was observed on both the condylar neck and in the articular capsule (Fig. 13). Bone reabsorption in the condyle was observed. Villous hyperplasia was present.

Group 8 (LPT + Carrageenan + LPT IR)

At the third day, a moderate inflammation was present on the condylar neck near the articular capsule and along the

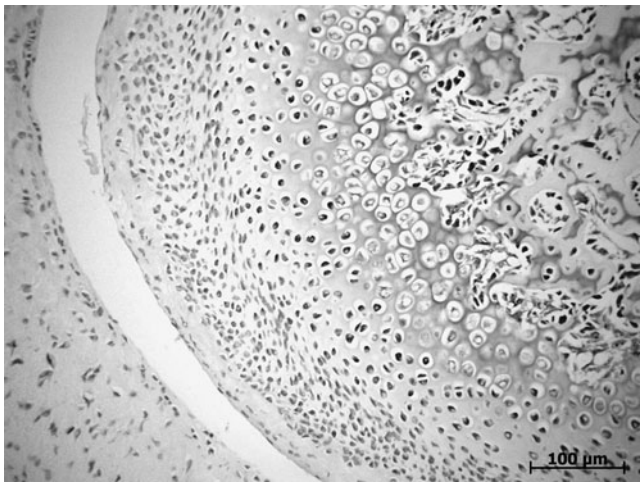

FIG. 3. Photomicrography of specimen of Group 1 (saline solution): condyle layers well defined: FT, fibrinous tissue; F, fibrocartilage; P, proliferative zone; and B, subchondral bone. Stained with TB.

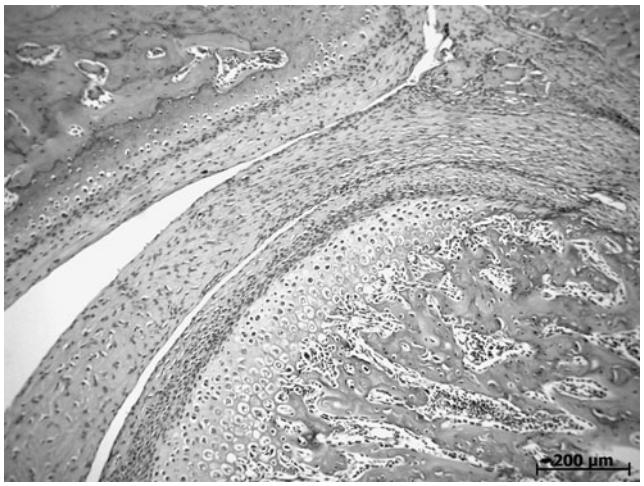

FIG. 4. Photomicrography of specimen of Group 3 (saline solution + LPT IR + R) at day 7. Normal aspect of the condyle (C) with well defined layers; D, articular disc; F, articular fossa; S, supradiscal space. Stained with HE.

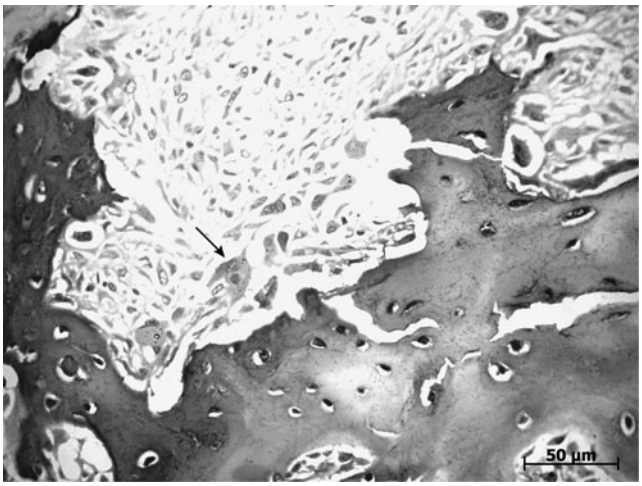

FIG. 6. Photomicrography of specimen of Group 4 (Carrageenan) at the third day, showing bone reabsorption of the condyle and the presence of osteoclast (*arrow*). Stained with HE.

muscular tissue (Fig. 14).The presence of congested blood vessels was also observed, and giant cells were seen on the condylar area. Vascularization was discrete and the synovial membrane presented discrete hyperplasia. Villous change was seen in one case. At the seventh day, the presence of a moderate inflammatory infiltrate in the condylar neck was observed. Discrete inflammation was seen on the capsule (Fig. 15). Congested blood vessels and areas of bone reabsorption were observed (two cases). Moderate vascularization was present and discrete proliferation of the synovial lining and villous areas were noticed.

Group 9 (LPT + Carrageenan + LPT IR + R)

At day 3, a moderate lymphocytic inflammatory reaction was observed on the condylar neck (Fig. 16).Discrete vascularization and villous hyperplasia were also observed. At day 7, a moderate inflammatory process was observed on the articular capsule and near the condylar neck (Fig. 17). Bone reabsorption was observed at the lateral side of the condyle in three cases. Presence of congested blood vessels and discrete vascularization were observed on the articular region. Some proliferative areas were also observed.

Histomorphometry

Alterations in thickness of the synovial membrane were observed on different groups and periods of observation. The result of the grading may be seen on Table 3.

Discussion

TMDs are painful and highly prevalent conditions on worldwide populations and often present an inflammatory component.[17] TMJs present special features that differ from

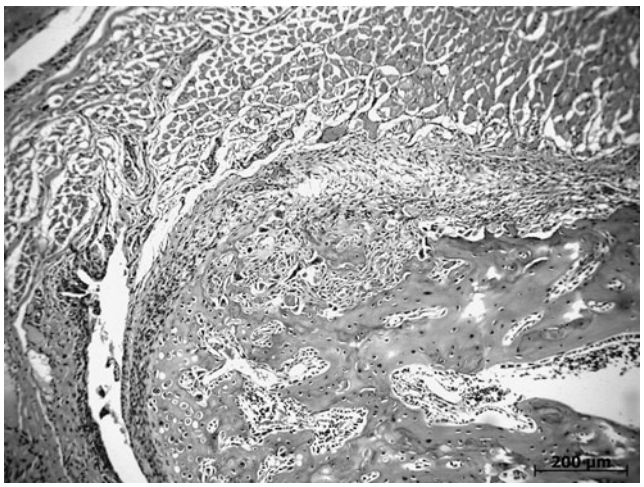

FIG. 5. Photomicrography of specimen of Group 4 (Carrageenan) at day 3, showing mixed inflammatory inflammation at the articular capsule extending to the muscular fibers. Stained with HE.

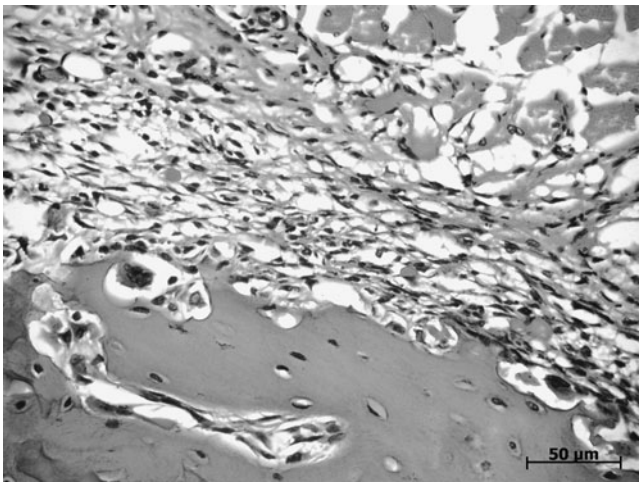

FIG. 7. Photomicrography of specimen of Group 4 (Carrageenan) at the seventh day, showing the presence of intense and chronic inflammatory infiltrate at the articular capsule and congested blood vessels. Stained with HE.

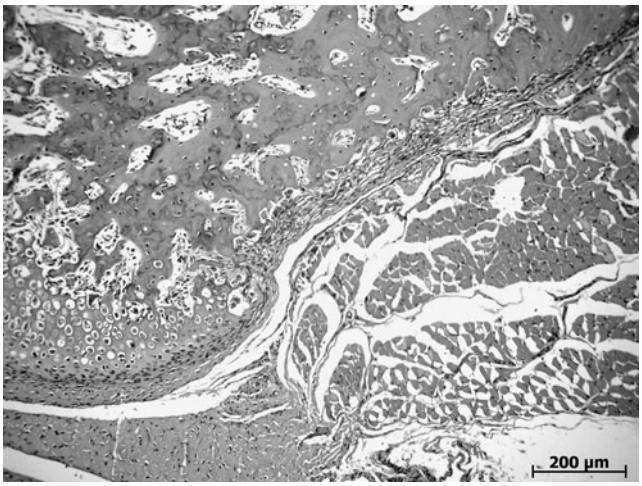

FIG. 8. Photomicrography of specimen of Group 5 (Carrageenan + LPT IR) at day 3, showing a discrete and chronic inflammatory infiltrate at the lateral side of the condyle. Stained with HE.

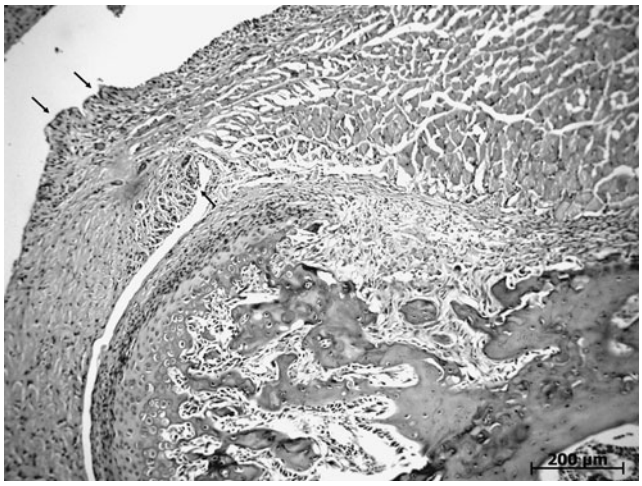

FIG. 10. Photomicrography of specimen of Group 6 (Carrageenan + LPT IR + R) at day 3, showing a chronic inflammatory infiltrate at the articular capsule extending along muscular fibers and presence of villous and hyperplasia on the synovial membrana (*arrows*). Stained with HE.

other synovial joints, including fibrous tissue covering of hyaline cartilage, teeth occlusion interference, and the condyle position.[3] Therefore, the study of an inflammation experimental model in rats TMJ is an important tool to understand the mechanisms and the effects of different treatments on this specific joint.

In the present study, we used carrageenan as a phlogistic agent. Carrageenan has been widely used to evaluate the anti-inflammatory effects of drugs as NSAIDs and corticosteroids. Carrageenan may induce a highly reproducible local antigenic response and its effects were reported in many studies.[5,18,19,20]

A single local injection of 1% carrageenan results in an inflammatory reaction in the TMJ and periarticular tissues, with hyperplasia of the synovial membrane.[7] The dose and dilution of carrageenan used in this study were reported in previous studies that proved its inflammatory effect.[7,21]

LPT has been used in clinical practice for the treatment of the pain related to TMDs. However, the action mechanism and the anti-inflammatory effect on TMJs remain unclear. This is the first study that evaluated LPT histological effect in the carrageenan-induced inflammation in TMJs of rats.

The irradiation of other joints, such as rat's knee, with infrared laser, and the irradiation of superficial tissues, such as the rat's paw, with red laser, caused a positive effect on the induced inflammation.[22,23]

An adequate dosage of LPT produces anti-inflammatory effects and pain relief over that seen with placebo group. The effect on laboratory studies during the first hours after injury equals that of NSAIDs when optimal doses are administered.[24] In a previous systematic review, Bjordal et al.[25]

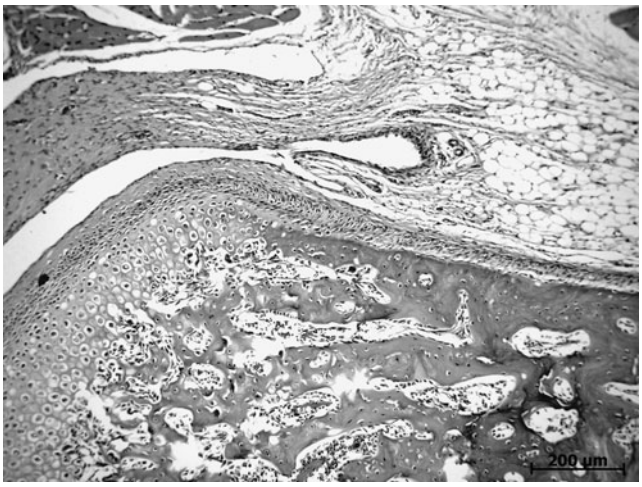

FIG. 9. Photomicrography of specimen of Group 5 (Carrageenan + LPT IR) at day 7, showing the presence of discrete chronic inflammatory infiltrate at the articular capsule. Stained with HE.

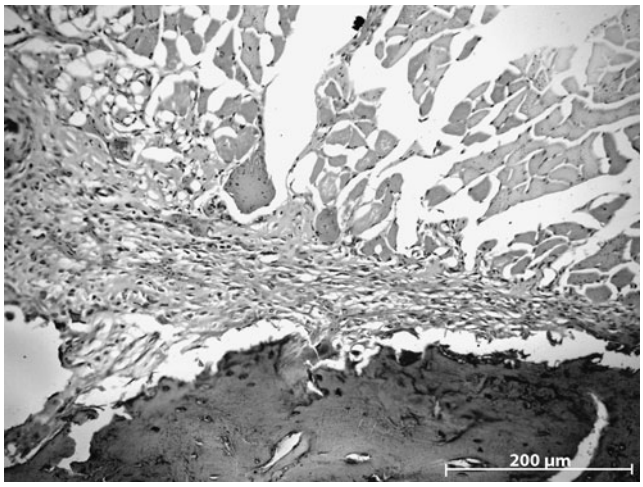

FIG. 11. Photomicrography of specimen of Group 6 (Carrageenan + LPT IR + R) at day 7, showing congested blood vessels and chronic inflammation at the articular capsule. Stained with HE.

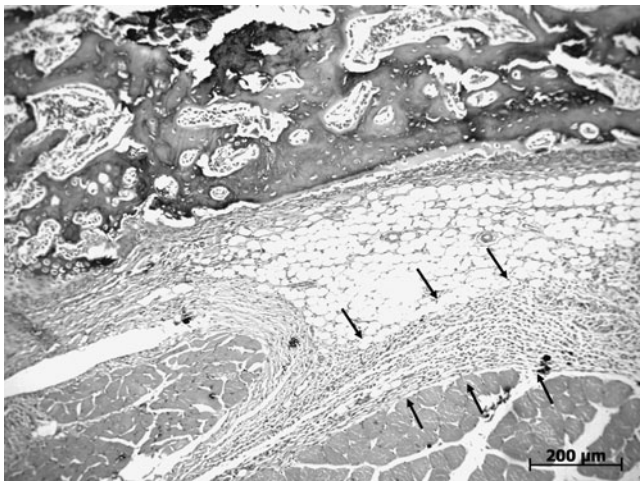

FIG. 12. Photomicrography of specimen of Group 7 (LPT + Carrageenan) at day 3, showing the presence of mixed and intense inflammatory reaction on the condylar neck, extending along the muscular tissue. Stained with HE.

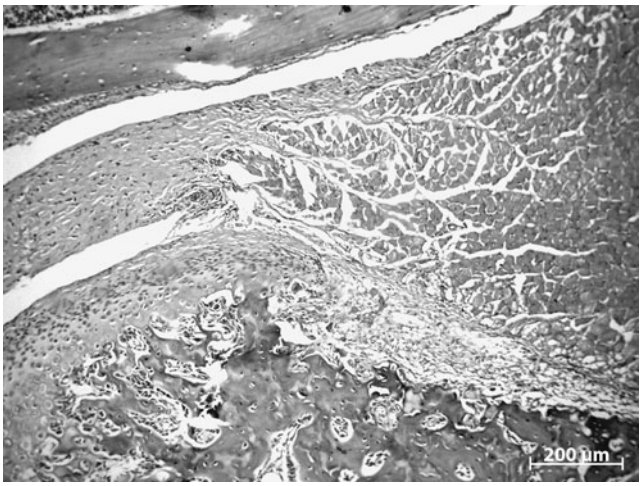

FIG. 14. Photomicrography of specimen of Group 8 (LPT + Carrageenan + LPT IR) at day 3, showing an inflammatory, predominantly lymphocytic, infiltrate at the articular capsule and muscular tissue. Stained with HE.

demonstrated that the optimal parameters for infrared lasers (λ820–830 nm) are intensity of 30–210 mW/cm^2 and doses ranging from 6 to 24 J per session on the treatment of chronic joint disorders. The World Association of Laser Therapy (WALT) recommends for treating TMD the use of infrared laser, λ780–820 nm, dose 6 J/cm^2 in one or two points, at 48 h intervals during 3 or 4 weeks. In this study, a diode laser (λ780 nm, 9 J, in one point, at 48 h intervals) was used, in agreement with the literature reports.

The wavelength is one variable that alters the light penetration and absorption on biological tissue. Infrared laser light has been demonstrated to have a typical penetration depth of nearly 3 mm, while red laser light has a penetration depth of 1 mm.[25] The contact of the needle with the rat condyle during the inflammation induction occurred in a depth of 3 mm.[26] Thus, it may be suggested that the infrared laser presented a depth of penetration similar to the localization of the rat TMJ. In this study, the infrared laser isolated showed better results on the reduction of the inflammation and the number of layers of the synovial membrane when compared with the LPT associating the two wavelengths. The dose of the infrared laser when associated to the red laser was that of 4.5 J, half of the dose used with the infrared isolated. Studies that had evaluated the cutaneous tissue healing reported better results with the association of red and infrared laser.[27] However, in this study, probably due the depth, the infrared laser isolated showed better results. The clinical experience of our team indicates that the use of infrared laser on TMJ and the use of red laser, on muscles, result in better response on the reduction of the pain in patients with TMDs.

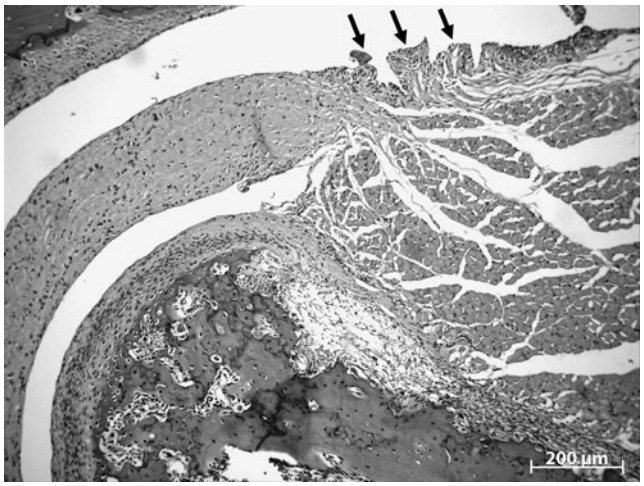

FIG. 13. Photomicrography of specimen of Group 7 (LPT + Carrageenan) at day 7, showing the presence of intense chronic inflammatory infiltrate at the articular capsule, as well as villous hyperplasia at the synovial membrane (*arrows*). Stained with HE.

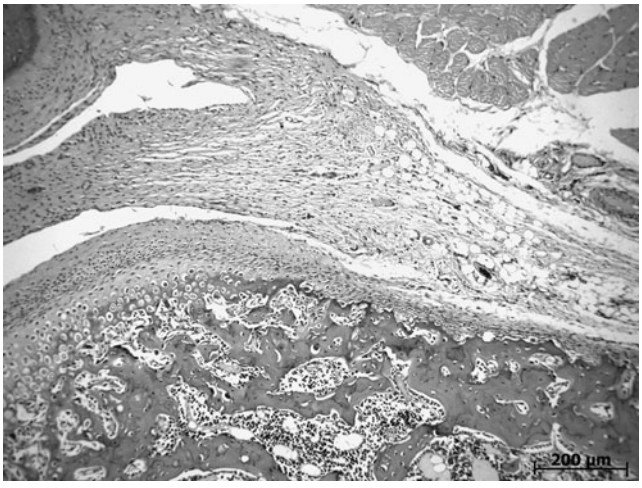

FIG. 15. Photomicrography of specimen of Group 8 (LPT + Carrageenan + LPT IR) at day 7, showing discrete chronic inflammation at the capsule and discrete hyperplasia of synovial membrane. Stained with HE.

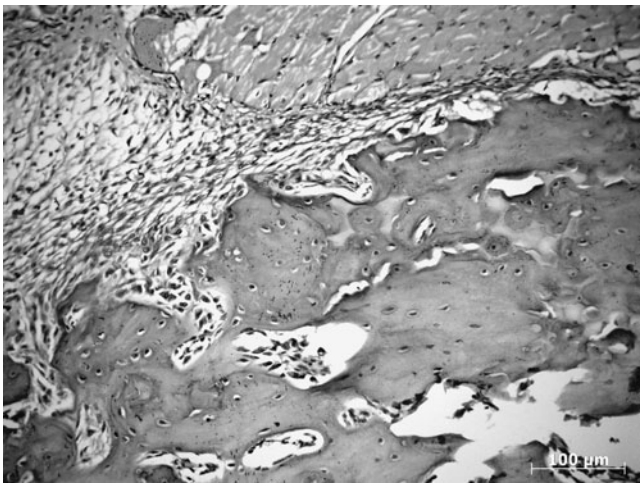

FIG. 16. Photomicrography of specimen of Group 9 (LPT + Carrageenan + LPT IR + R) at day 3, showing moderate lymphocytic inflammatory infiltrate at the capsule and the muscular tissue. Stained with HE.

The experimental model of this study, the Wistar rat, is a small animal and the structure of the articular layers is similar of the human one. The structures of the TMJs on the groups where saline solution was injected did not present alterations and the characteristics of normality of the TMJ components described in literature were observed.[28] The irradiation with infrared laser isolated or associated to red laser in the groups with saline solution did not alter the structure of the TMJ. Thus, the LPT used in healthy TMJ did not produce histological difference compared to the nonirradiated group.

A single local injection of 20 μl of 1% carrageenan, in this study, resulted in an inflammatory response on the TMJ. After 3 days, we observed an intense and mixed inflammatory infiltration and, after 7 days, an intense inflammation, predominantly lymphocytic, was seen on the region of the articular capsule and at the condylar neck. Areas of cellular proliferation of the synovial membrane and villous were observed after the induction of the inflammation. This pattern was used as control of the inflammatory response.

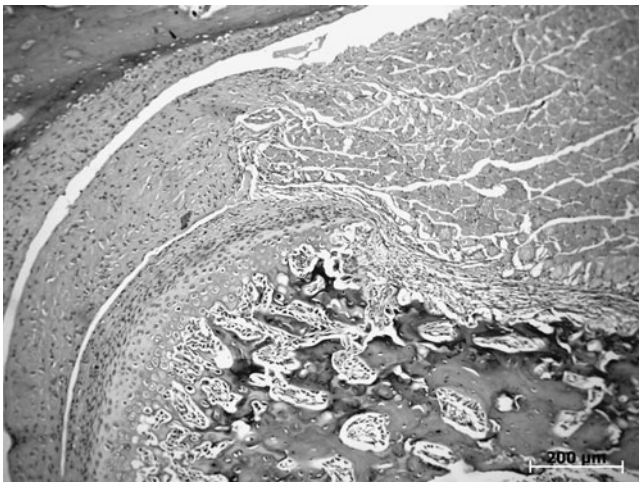

FIG. 17. Photomicrography of specimen of Group 9 (LPT + Carrageenan + LPT IR + R) at day 7, showing a moderate chronic inflammation of the capsule and muscular tissue. Stained with HE.

Table 3. Results of the Scoring of the Synovial Layers Determined on Two Selected Parts From Each TMJ (Internal and External)

Group	*Period (day)*	*Area*	*Grade 0*	*Grade 1*
G1 saline	3	External	X	
		Internal	X	
G2 saline + LPT IR	3	External	X	
		Internal	X	
	7	External	X	
		Internal	X	
G3 saline + LPT IR + R	3	External	X	
		Internal	X	
	7	External	X	
		Internal	X	
G4 Carrageenan	3	External		X
		Internal		X
	7	External		X
		Internal		X
G5 Carrageenan + LPT IR	3	External	X	
		Internal	X	
	7	External	X	
		Internal	X	
G6 Carrageenan + LPT IR + R	3	External		X
		Internal		X
	7	External	X	
		Internal	X	
G7 pre laser + Carrageenan	3	External		X
		Internal		X
	7	External		X
		Internal		X
G8 pre laser + Carrageenan + LPT IR	3	External	X	
		Internal		X
	7	External	X	
		Internal		X
G9 pre laser + Carrageenan + LPT IR + R	3	External		X
		Internal		X
	7	External	X	
		Internal	X	

In relation to the groups treated with LPT, the group irradiated with infrared laser isolated (G5) showed the best result. LPT with λ780 nm resulted in the reduction of the number of inflammatory cells and on the quickening of the inflammatory process. Some studies reported that the LPT (λ632.8 nm) posses anti-inflammatory effect on induced arthritis in joints of the back limbs of rats,[29,30] and others demonstrated the anti-inflammatory effect of LPT on carrageenan-induced inflammation in rat paw[31] or in pleurisy.[32] The effect of LPT in the inflammation induced by carrageenan on TMJ was not described previously.

The use of a previous laser irradiation, without LPT after the induction (G7) presented similar results to the nontreated group (G4), demonstrating that the irradiation 24 h before the induction in healthy tissue did not alter the inflammatory process. Moreover, the association between previous irradiation and LPT after inflammation induction did not result in better effects. In general, the previous irradiation did not improve the final result of the groups, so its practice seems to be

not justified. The preventive use of LPT on healthy TMJ did not result on any protective effect. It is possible that some type of deficiency is required for the benefits of LPT to be observed.

The synovial membrane thickness on both normal and pathologic TMJs was analyzed in previous studies.[9,33] It is known that normal synovial membrane of rat TMJ presents one to three cell layers. After induced trauma by forced hypermobility, a proliferation in cell layers is observed resulting in areas of hyperplasia.[10] The present study used the gradation proposed by Muto et al.[10] and others studies that followed this method found membrane hyperplasia, Grade 1, after induction of inflammation by masseter resection or hypermobility.[8,34]

In this study, all the saline solution subgroups (G1, G2, and G3) did not present alteration in the number of layers (grade 0), while in the carrageen group without treatment (G4), the number of layers was bigger (grade 1), demonstrating that the carrageenan-induced inflammation caused proliferation of the membrane cells. After the irradiation with the IR laser (G5), the number of layers was similar to the observed on the saline solution group, on both external and internal observation areas and periods of observation. The IR laser irradiation showed the best result on relation to the number of membrane cell layers.

The clinical importance of the histological alterations on synovial membrane of the TMJ still remains to be determined. However, it is possible that it may be related to the pain resulting of inflammatory reaction.[9]

The use of the LPT on this study resulted in a positive effect on induced inflammatory process in the rat TMJ, especially with the use of λ780 nm and 225 J/cm^2 To reach clinical success of the use of LPT on TMDs, it is necessary to have a full understanding of the mechanisms of action of the LPT, and the establishment of adequate clinical protocols as well as the limitations of the use of this therapy on the TMJ.

Our results are indicative that LPT caused positive effects on the reduction of the inflammatory reaction and accelerated the inflammation process, mainly with the use of IR laser.

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Author Disclosure Statement

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