

## 11 - PAIN AND EDEMA EVALUATION IN SUBMITTED RATS TO EXPERIMENTAL OSTEOARTHRITIS AND TREATED WITH LOW LEVEL LASER THERAPY

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### INTRODUCTION

Rheumatic diseases are the major cause of morbidity and disability in the population, being pointed an increase in the prevalence of musculoskeletal disorders with the age. What takes the implications for the health cares, especially due to the population aging (BADLEY; TENNANT, 1992).

The osteoarthritis is a chronic disease, multifactorial that drives to the progressive functional inability (COIMBRA et al., 2002), with pain presence influencing the functional activities level (VASCONCELOS; DIAS; DIAS, 2006). According to Kafka (2002), the osteoarthritis is produced initially by fissures in the cartilage comes of applied loads in a very fast way.

Patients with osteoarthritis shows deficit of force comparing with healthy people of similar age, and even comparing to the non affected side; being possible that such fact happens due to the reflex inhibition, or to the exercise lack (BAYRAMOGLU; TOPRAK; SOZAY, 2007; SUETTA et al., 2007). Besides, individuals with severe knee osteoarthritis present proprioceptive deficit, when compared with individuals with less severe osteoarthritis (BAYRAMOGLU; TOPRAK; SOZAY, 2007; EMRANI et al., 2006), increase in the sensibility to the heat, pain to the pressure and the cold (KOSEK; ORDEBERG, 2000).

According to Coimbra et al. (2002), the osteoarthritis treatment should look for the functional improvement, mechanics and clinical, tends as base: educational programs, therapeutic exercises, orthosis, physical agents (as the physical therapy resources), drugs and surgical treatments.

A good number of animal models representing human arthritis have been developed in the last years, and the model of chronic arthritis created by into the joint injection of complete Freund adjuvante in rats, it is characterized by chronic inflammation and joint pain (HSIEH, 2006). According to Gomis et al. (2007), in knee arthritis model, the nociceptive answers increases when joint moving due to the inflammation induced locally. For Hsu et al. (2006), among the inflammatory mediators, the IL-20 play important part in the inflammation and angiogenesis generation, besides others like TNF- $\alpha$ , IL-1 $\alpha$ , IL-6.

During the inflammatory process, it happens a sequence of events, it which some types of cells prevail and they exercise deep effects in the initial phase (platelets, endothelial cells and neutrophils), and in late phase (macrophages, lymphocytes, fibroblasts), these are involved with the pain production, chemotact, regulation of vascular tone, the repair and neovascularisation (SCOTT et al. 2004).

According to Scott et al. (2004) non-steroidal anti-inflammatory drugs (NSAIDS) have been used, in several forms, for more than 3500 years. To every year about 120 billion aspirin tablets are consumed in the world, and in older people, it contributes to 100.000 hospital admissions and 16.500 deaths each year, due to complications related to the gastrointestinal tract. Several physical modalities are used with the "anti-inflammatory" purpose, even so they are not still totally established.

Among the modalities, does it make an appointment to the low level laser therapy, which presents anti-inflammatory effects, how decrease in the liberation of TNF $\alpha$  (AIMBIRE et al., 2006), COX-2 (ALBERTINI et al. 2007) and PGE<sub>2</sub> (MIZUTANI et al., 2004), besides reduction of inflammatory edema for probable liberation of adrenal hormones (ALBERTINI et al., 2004). Bjordal et al. (2003) tell that the laser is a tool that significantly reduces the pain and it improves the joint chronic disorders; Enwemeka et al. (2004) confirms that the laser is effective in the pain reduction, even so different wavelengths can influence the therapeutic results.

For the reasons exposed above, the need of studies is observed that found the physical modalities use in joint inflammatory frames. Then, the aim of this study went evaluate the effect of the low level laser therapy, 660 nm, in the pain and edema evolution in rats Wistar submitted to the experimental osteoarthritis.

### MATERIALS AND METHODS

#### *Animals and Experimental Groups*

Female rats Wistar (n=10), with 20  $\pm$  2 weeks of age, obtained in Central vivarium of the State University of the Paraná West - UNIOESTE, Cascavel/PR. The rats were housed in a 12 h light-dark cycle, four rats per cage, with free access to standard rat food and water. The experiment was carried according to the ethical precepts defined by the Brazilian School of Animal Experimentation (COBEA) (ANDERSEN et al., 2004). The animals were randomly divided in two groups:

- GS (group sham; n=5) - submitted to the right knee arthritis induction and to the sham treatment;
- GL (group laser; n=5) - submitted to the right knee arthritis induction and irradiated with laser 10 J/cm<sup>2</sup>.

#### *Induction of Arthritic Inflammation*

The animals were sedate with inhalation of ethyl ether, later on the trichotomy of the right knee was accomplished. For the arthritis production, 0,1 ml of complete Freund adjuvant (CFA) was used, intra-articular tibio-femoral injections in the right knee.

#### *Test of Functional Inability (Paw Elevation Time)*

The test was characterized basically by a metallic cylinder in movement, and a computer software with connection to an adapted metallic boot to the rat paw, described originally by Tonussi and Ferreira (1992).

In this test, rats are placed on a revolving cylinder (30 cm diameter; 3 rpm) for 1-min periods and a computer-assisted device measures the total time that a specific hind paw was not in contact with the cylinder surface, i.e. the paw elevation time (PET). According to Bressan, Cunha and Tonussi (2006), animals that didn't suffer any intervention invasiva, and they don't present march alterations, the PET it is about of 10 s, considering than this value only increases in the affected limb.

The experiment began with the training of the animals on the cylinder, and the following day they were logged the values of PET. Soon after, there was the procedure of induction of the arthritis, happening reevaluations after 10 days of the injection, and finally after the 22<sup>nd</sup> day of lesion, what corresponded to the 10<sup>th</sup> day of treatment (interval of two days among to 5<sup>th</sup> and 6<sup>th</sup> treatment session).

#### *Edema evaluation*

To quantify the edema in the experimental lesion area, previous the lesion, the diameter of the right knees was evaluated, with caliper positioned accomplishing the measures in lateral-medial sides (GOULD, et al. 2007; HSIEH, 2006), in the

moments similar to the PET.

#### Laser Equipment

In the 10<sup>th</sup> day after the arthritis induction, the treatment began using the laser of the DMC® mark, with wavelength of 660 nm, previously calibrated. The treatment happened in a punctual and continuous form, on the medial knee joint, being the animals maintained in thermoplastic-PVC container, originally described by Lirani (2004). The sham group suffered similar procedure, even so with the turned off equipment. The procedure was daily, for 10 days, with pause for two days among to 5<sup>th</sup> and 6<sup>th</sup> sessions.

#### Statistical Analysis

The data were showed by the descriptive statistics (average, standard deviation) and analyzed within the groups with paired, two-tailed Student *t* tests. Being accepted the significance level  $p < 0,05$ .

## RESULTS

The results obtained in the Paw Elevation Time (PET) evaluation and edema is presented in the table I and II, respectively.

Table I - Presentation of PET data, in the different moments of evaluation.

	Before Lesion	Before Treatment	After 5 <sup>th</sup> treatment	Before 6 <sup>th</sup> treatment	After 10 <sup>th</sup> treatment
GS	8,87 ± 2,08 s	15,93 ± 1,70 s	14,37 ± 5,41 s	14,35 ± 5,51 s	13,56 ± 2,93 s
GL	9,35 ± 2,32 s	14,87 ± 1,94 s	10,26 ± 1,09 s	10,59 ± 0,62 s	10,22 ± 0,65 s

For GS, when comparing with the before lesion value, the moment before treatment presented significant increase ( $p=0,0053$ ) of PET in 79,51%, but for the following moments (after 5<sup>th</sup> treatment, before 6<sup>th</sup> treatment and after 10<sup>th</sup> treatment), there was just increase tendency, respectively of 61,93% ( $p=0,0658$ ), 61,71% ( $p=0,0679$ ), and 51,81% ( $p=0,0716$ ). When comparing the values obtained after the lesion accomplishment, that is to say, in the moment before treatment, with the moment after to 5<sup>th</sup> therapy, non significant decrease of 9,79% was observed ( $p=0,4786$ ), comparing with the moment previous to 6<sup>th</sup> therapy the decrease was not also significant of 9,92% ( $p=0,4945$ ), and finally when comparing with the end of the treatment period, the decrease was not also significant of 14,88% ( $p=0,0895$ ).

For GL, when comparing with the value before lesion, the moment before treatment presented significant increase ( $p=0,0023$ ) of PET in 59,04%, for the following moments there was just light increase, not significant, when comparing with the moment after to 5<sup>th</sup> therapy (9,73%,  $p=0,4842$ ), before to 6<sup>th</sup> therapy (13,26%,  $p=0,3089$ ), and after the end of the therapies (9,30%,  $p=0,4245$ ). However, when comparing the values obtained in the moment before treatment with the remaining moments, there was significant decrease in every moment, respectively, after 5<sup>th</sup> therapy -31,00% ( $p=0,0093$ ), before 6<sup>th</sup> therapy -28,78% ( $p=0,0042$ ), and at the end of the treatment -31,27% ( $p=0,0038$ ).

Table II - Presentation of the edema relative data, in the different moments of evaluation.

	Before Lesion	Before Treatment	After 5 <sup>th</sup> treatment	Before 6 <sup>th</sup> treatment	After 10 <sup>th</sup> treatment
GS	0,98 ± 0,09 s	1,31 ± 0,12 s	1,24 ± 0,21 s	1,22 ± 0,11 s	1,16 ± 0,10 s
GL	1,12 ± 0,10 s	1,30 ± 0,08 s	1,14 ± 0,04 s	1,12 ± 0,05 s	1,09 ± 0,05 s

With regard to the edema, for the sham group when comparing with the value before lesion, the moment before treatment presented significant increase ( $p=0,0201$ ) in 34,36%, but for the following moments (after 5<sup>th</sup> treatment, before 6<sup>th</sup> treatment and after 10<sup>th</sup> treatment), there was not significant increase, respectively in 26,38% ( $p=0,1243$ ), presenting again non significant for the comparison with before 6<sup>th</sup> treatment in 25,15% ( $p=0,0307$ ), and at the end of the 10<sup>th</sup> therapy with 18,81% ( $p=0,0486$ ). When comparing the values obtained after the lesion accomplishment, that is to say, in the moment before treatment, with the moment after to 5<sup>th</sup> therapy, non significant decrease of 5,94% was observed ( $p=0,1962$ ), comparing with the moment previous to 6<sup>th</sup> therapy the decrease was not also significant of 6,85% ( $p=0,2450$ ), and finally when comparing with the end of the treatment period, the decrease was not also significant of 11,57% ( $p=0,0807$ ).

For GL, when comparing with the value before lesion, the moment before treatment presented significant edema increase ( $p=0,0077$ ) in 16,31%, for the following moments there just was not significant increase, when comparing with the moment after to 5<sup>th</sup> therapy (2,33%,  $p=0,5632$ ), before to 6<sup>th</sup> therapy (0,18%,  $p=0,9431$ ), and after the end of the therapies (-1,97%,  $p=0,7308$ ). However, when comparing the values obtained in the moment before treatment with the remaining moments, there was significant decrease in every moment, respectively, after 5<sup>th</sup> therapy -12,02% ( $p=0,0081$ ), before 6<sup>th</sup> therapy -13,87% ( $p=0,0018$ ), and at the end of the treatment -15,72% ( $p=0,0081$ ).

## DISCUSSION

The model of arthritis induction used in the present study, through the adjuvant injection has been used thoroughly to evaluate experimentally the pathophysiology of human rheumatoid arthritis, because of similarities among the two syndromes, being an useful instrument to investigate new analgesic and anti-inflammatory drugs. In this model, the articular hyperalgesia precedes the limb edema development (FRANCISCHI; PEREIRA; CASTRO, 1997).

Brosseau et al. (2005) they evaluated low level laser therapy in the pain symptoms of the hands osteoarthritis. They used the laser with 860 nm, 3 J/cm<sup>2</sup>, in fingers with osteoarthritis in chronic stage, three times a week, for 6 weeks. They tell that the treatment was not significantly better than the placebo to reduce the intensity of the pain, morning rigidity, or to improve the range of movement, strength and functional state. However, Gur et al. (2003) evaluating the laser 904 nm use, in patients with knee osteoarthritis, for 14 weeks, they observed that the treated patients, with 2 or 3 J, presented improvement in the pain, functional state and life quality, compared with the sham group.

In the present study, for both groups the injection of complete Freund adjuvant of produced pain and edema 10 days after the injection, even so, when evaluating with PET, the following moments of evaluation there was not significant difference comparing with the values before treatment, there was just tendency of larger times. However, the evaluations showed that for the sham group, there was not significant pain decrease, because, the values of PET for the moments after to 5<sup>th</sup> therapy, previous to 6<sup>th</sup> therapy and after to 10<sup>th</sup> therapy, they went similar to those of the moment before 1<sup>st</sup> therapy. Already for the treated group with laser there was significant decrease in every following moment when comparing with the before treatment.

Such fact agree with Laakso and Cabot (2005), that they evaluated the pain due to inflammatory process in rats paw, submitted the treatment with 780 nm laser, with doses of 1 and 2,5 J/cm<sup>2</sup>. They observed dependent dose effect, and 1 J/cm<sup>2</sup> didn't produce any effect, but, 2,5 J/cm<sup>2</sup> decreased the pressure pain threshold. For Castano et al. (2007), that they evaluated the

use of 810 nm laser different doses and irradiances, on rats with knee arthritis induced, they tell that the irradiation time is an important factor for the anti-inflammatory effects presence of the laser therapy, as edema reduction and levels of PGE<sub>2</sub> decrease.

The low level laser therapy it is shown useful in the mucopolysaccharide induction, in osteoarthritis experimental model, which are responsible for to maintain the fibers of collagen united and to assure the cartilage integrity. This form the laser increasing the arthritic cartilage biosynthesis, it results in improvement of the histopathologic alterations (LIN; HUANG; CHAI, 2006), the laser also increases the shock proteins amount and this form it protects the arthritic cartilage (LIN et al., 2004).

Campana et al. (2004) they evaluated the effects of the laser on plasma fibrinogen in the rats with arthritis induced by urate crystals. They treated with HeNe (6 mW), 8 J/cm<sup>2</sup>, for 3 consecutive days. They tell that the laser presented effect anti-inflammatory in the arthritis induced, determined by the fibrinogen levels and for the histological evaluation.

Albertini et al. (2004) they evaluated the alteration in the acute inflammatory edema, after carragenina subplantar injection in rats paws, and they observed that irradiating the 650 nm laser, in the doses of 1 and 2,5 J/cm<sup>2</sup> reduced the edema, 27% (p<0,05) and 45,4% (p<0,01), respectively, and the dose of 2,5 J/cm<sup>2</sup> produced similar anti-inflammatory effects those produced by diclofenaco in the dose of 1 mg/kg. However, in adrenalectomized rats, the irradiation didn't inhibit the edema, suggesting that the laser exert its anti-inflammatory effects for stimulating the release of adrenal corticosteroid hormones.

In the present study the edema evolution, it was altered by the laser therapy presence, because for the sham group the edema was produced, and it presented significant variation with relationship at the levels evaluated before lesion, for the moments before 6th therapy and after 10<sup>th</sup> therapy, and when comparing with the moment after lesion, in none of the following evaluations it happened significant decrease. In the treated group, the edema evolution showed that after the lesion accomplishment (before therapy) in any following moment there was significant increase when comparing with the values before lesion, and when it was compared the moment after lesion, the following evaluations showed significant decrease of the found values.

### CONCLUSION

This form, it is pointed out that the low level laser therapy presented pain and edema effects, with significant decrease of the same ones in arthritic rats.

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#### **PAIN AND EDEMA EVALUATION IN SUBMITTED RATS TO EXPERIMENTAL OSTEOARTHRITIS AND TREATED WITH LOW LEVEL LASER THERAPY**

##### **ABSTRACT:**

The osteoarthritis is a chronic disease, which leads to multiple functional incapacity progressive, with pain presence influencing the functional activity level. The aim of this study was to evaluate the effect of laser, 660 nm, in the development of pain and swelling in rats subjected to experimental osteoarthritis. Ten female rats were used, divided into two groups: GS-induction of arthritis in the right knee and sham treatment; GL-induction of arthritis in the right knee and laser irradiated with 10 J / cm<sup>2</sup>. For the induction of arthritis was injected 0,1 ml of Freund Complete Adjuvant of the femoral-tibial articular space. The pain evaluation occurred through Paw Elevation Time (PET), when the animal walking for one minute in a metal cylinder in motion, at times before injury, after 10 days of the injection, after the 5<sup>th</sup> therapy, prior to 6<sup>th</sup> therapy and the end of the 10<sup>th</sup> therapy. The edema evaluation was performed with metallic caliper medium-laterally to the knee joint, at times similar to PET. Treatment with laser began in the 10<sup>th</sup> day after the arthritis induction on the medial interline joint. The procedure was daily, for 10 days, intervals of two days between the 5<sup>th</sup> and 6<sup>th</sup> sessions. The results showed that for PET to GS there was significant increase after injury, with a tendency to increase in subsequent time, without significant diminishing compare post-injury with subsequent moments, to GL there was increased in the post-injury, which decreased significantly in subsequent periods. For the edema, GS presented significant appearance of it, which has not diminished; for GL the edema was also present, but there was a significant reduction of the same. We concluded that the laser produce effects on pain and edema, with diminishing of them.

KEYWORDS: low level laser therapy, edema, pain.

#### **ÉVALUATION DE LA DOULEUR ET OEDÈME DANS SOURIS SOUMIS À ARTHRITE EXPÉRIMENTALE QU'IL EST TRAITÉ AVEC LASER DE BASSE PUISSANCE**

##### **RÉSUMÉ:**

L'arthrite prend à l'incapacité progressive et douleur. Il a été eu l'intention à d'évaluer l'effet du laser de basse puissance, 660 nm, dans la douleur et évolution de l'oedème dans les souris Wistar a soumis à l'osteoarthrits expérimental. Il est été séparé 10 souris virils, avec 12±2 semaines d'âge, dans deux groupes, : GP - arthrite provoquée et traitement du placebo; GL - arthrite provoquée et irradié avec laser 10 J/cm<sup>2</sup>. L'arthrite a été induite en injectant 0,1 ml d'Adjuvante Complet de Freund dans l'espace articulaire tibio - fémoral du genou droit. La douleur a été évaluée par le Temps d'Élévation de la Patte (TEP) dans les moments: la pré lésion; après 10 jours de l'injection; après la 5<sup>ème</sup> thérapie, antérieure à 6<sup>ème</sup> thérapie; et à la fin de la 10<sup>ème</sup> thérapie. L'oedème a été évalué par une attelle-étrier métallique placé moyen-latéralement au genou, dans les mêmes moments de l'évaluation de TEP. Les lasertherapy ont commencé dans le 10<sup>ème</sup> jour après l'énumération de l'arthrite, d'une façon ponctuel et continu, sur l'interligne articulé intermédiaire. La procédure était journalière, pour 10 jours, a espacé pour deux jours parmi à 5<sup>ème</sup> et 6<sup>ème</sup> sessions. Dans GP le TEP a augmenté après la lésion considérablement et il a soigné à l'augmentation dans les moments subséquents, sans baisse considérable par rapport aux valeurs après - lésion. Dans GL il y avait l'augmentation dans la période après - lésion et baisse considérable dans les périodes subséquentes. Dans GP la formation considérable de l'oedème a été observée sans réduction. Dans GL il y avait aussi formation considérable de l'oedème, mais cela a réduit considérablement. Il est terminé que le laser de basse puissance a présenté des effets sur la douleur et oedème, avec baisse considérable des mêmes dans les souris arthritiques.

MOTS-CLEF: laser de basse puissance, oedème, douleur.

**EVALUACIÓN DEL DOLOR Y EDEMA EM RATONES SUBMETIDOS A LA OSTEOARTRITES EXPERIMENTAL Y TRATADOS COM LASER BAJA POTENCIA****RESUMEN:**

La osteoartritis es una enfermedad crónica, multifactorial que conduce a la incapacidad funcional progresiva, con presencia del dolor influyendo el nivel de las actividades funcionales. El objetivo de este estudio fue evaluar el efecto del laser, 660 nm, en el dolor y evolución del edema en ratones, sujetos al osteoartritis experimental. Fueron usados 10 ratones hembras, divididas en dos grupos: GS - sujeto a la inducción del artritis en la rodilla derecha y al tratamiento apacible. GL - sujeto a la inducción del artritis en la rodilla derecha y irradiado con laser 10 J/cm<sup>2</sup>. Para la inducción del artritis fue inyectado 0,1ml del Adjuvante Completo del Freund en el espacio articular tibia-femoral derecha. La evaluación del dolor ocurrió por medio del Tiempo de la Elevación de la Pata (TEP), cuando el animal caminaba por 1 minuto en un cilindro metálico en movimiento, en los momentos prelesión, después de 10 días de la inyección, después la 5ª terapia, previo la 6ª terapia y al final de la 10ª terapia. La evaluación del edema fue realizada con paquímetro metálico posicionado lateralmente a la articulación de la rodilla, en los momentos semejantes al TEP. El tratamiento con laser empezó en el 10º día después la inducción del artritis, por la interlinea articular medial. El procedimiento fue diaria, por 10 días, alternado por dos días entre la 5ª y 6ª sesiones. Los resultados para TEP mostraron que para GS hubo aumento significativo del tiempo después de la lesión, con tendencia del aumento en los tiempos subsiguientes, sin disminución significativa al comparar los valores pós-lesión con los momentos subsiguientes, para GL hubo el aumento en el período pos-lesión el cual disminuyó significativamente en los períodos subsiguientes. Para el edema, GS presentó significativo del mismo, el cual no disminuyó; en GL el edema también fue presente, pero hubo reducción significativa del mismo. Se concluye que el laser presentó efectos sobre el dolor y edema, con disminución significativa de ambos.

**PALABRAS CLAVE:** laser de baja potencia, edema, dolor.

**AVALIAÇÃO DA DOR E EDEMA EM RATOS SUBMETIDOS À OSTEOARTRITE EXPERIMENTAL E TRATADOS COM LASER DE BAIXA POTÊNCIA****RESUMO:**

A osteoartrite é uma doença crônica, multifatorial que conduz à incapacidade funcional progressiva, com presença de dor influenciando o nível das atividades funcionais. O objetivo deste estudo foi avaliar o efeito do laser, 660 nm, na dor e evolução do edema em ratos submetidos à osteoartrite experimental. Foram utilizados 10 ratas fêmeas, divididas em dois grupos: GS - indução de artrite em joelho direito e tratamento placebo; GL - indução de artrite em joelho direito e irradiado com laser 10 J/cm<sup>2</sup>. Para a indução da artrite foi injetado 0,1 ml de Adjuvante Completo de Freund no espaço articular tibia-femoral direito. A avaliação da dor ocorreu através do Tempo de Elevação da Pata (TEP), quando o animal caminhava durante 1 minuto em um cilindro metálico em movimento, nos momentos pré-lesão, após 10 dias da injeção, após a 5ª terapia, previo a 6ª terapia e ao final da 10ª terapia. A avaliação do edema foi realizada com paquímetro metálico médio-lateralmente à articulação do joelho, nos momentos semelhantes ao TEP. O tratamento com laser iniciou no 10º dia após a indução de artrite sobre a interlinha articular medial. O procedimento foi diário, por 10 dias, intervalado por dois dias entre a 5ª e 6ª sessões. Os resultados para TEP mostraram que para GS houve aumento significativo do tempo após a lesão, com tendência de aumento nos tempos subsequentes, sem diminuição significativa ao comparar os valores pós-lesão com os momentos subsequentes, para GL houve aumento no período pós-lesão, o qual diminuiu significativamente nos períodos subsequentes. Para o edema, GS apresentou aumento significativo do mesmo, o qual não diminuiu; em GL o edema também foi presente, mas houve redução significativa do mesmo. Conclui-se que o laser apresentou efeitos sobre a dor e edema, com diminuição significativa dos mesmos.

**PALAVRAS-CHAVE:** laser de baixa potência, edema, dor.