Efeito da Aplicação da energia vermelha monocromática (MIRE®) sobre a cicatrização de feridas cutâneas em ratos diabéticos

Monochromatic infrared energy application effect (MIRE®) of skin healing wounds in diabetic rats

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ABSTRACT
Introduction: The monochromatic infrared energy use (MIRE®) as a resource to promote healing shows excellent prospects. Objective: Determine the MIRE (890nm) application effect in skin wounds healing in diabetic rats. Methods: Experimental type study, with qualitative and quantitative sample data evaluation. Skin wounds 1 cm2 were made in the dorsal region Rattus norvegicus diabetics, who were randomly distributed in 14 animals groups and treated for seven to 14 days with 0.9% saline (control group - C) phototherapy (MIRE - wavelength = 890 nm; energy density = 24,96J / cm2; and irradiation time = 600 seconds) or clostebol acetate / neomycin sulfate (Trofodermin® - Default). Comparisons between groups were performed by ANOVA followed by post- Newman-Keuls test. Results: No statistically significant differences were found between the groups in the wounds contraction on the 4th day after wounds production. There was significantly higher wound contraction ($p < 0.05$) in standard group (0.66 ± 0.02) compared to MIRE® (0.27 ± 0.03) and control (0.43 ± 0, 07) on the 7th monitoring day.On 14th treatment day, the wound contraction was higher ($p < 0.05$) in standard group (0.87 ± 0.04) and MIRE® (0.81 ± 0.04) compared with control (0 68 ± 0.06). A semi- quantitative histological analysis revealed increased vascular proliferation in MIRE®. Conclusion: The results indicate that treatment with MIRE® favored the open wounds healing in diabetic rats.

Keywords: Phototherapy, Wound Healing, Diabetes Mellitus

RESUMO
Introdução: O uso da energia infravermelha monocromática (MIRE®) como recurso para promover a cura apresenta excelentes perspectivas. Objetivo: Determinar o efeito da aplicação de MIRE (890nm) na cicatrização de feridas cutâneas em ratos diabéticos. Métodos: Estudo do tipo experimental, com avaliação qualitativa e quantitativa dos dados amostrais. Feridas cutâneas de 1 cm^2 foram feitas na região dorsal de *Rattus norvegicus* diabéticos, os quais foram distribuídos aleatoriamente em grupos de 14 animais e tratados por sete a 14 dias com solução salina 0,9% (grupo controle - C) fototerapia (MIRE - comprimento de onda = 890 nm; densidade de energia = 24,96J / cm^2; e tempo de irradiação = 600 segundos) ou acetato de clostebol / sulfato de neomicina (Trofodermin® - Padrão). As comparações entre os grupos foram realizadas por ANOVA seguida do teste pós-Newman-Keuls, nível de significância de $p<0,05$. Resultados: Não foram encontradas diferenças estatisticamente significantes entre os grupos na contração das feridas no 4º dia após a produção das feridas. Houve contração da ferida significativamente maior ($p < 0,05$) no grupo padrão (0,66 ± 0,02) em comparação ao MIRE® (0,27 ± 0,03) e controle (0,43 ± 0,07) no 7º dia de monitoramento. No 14º dia de tratamento, a contração da ferida foi maior ($p < 0,05$) no grupo padrão (0,87 ± 0,04) e MIRE® (0,81 ± 0,04) em comparação com o controle (0 68 ± 0,06). Uma análise histológica semiquantitativa revelou aumento da proliferação vascular no MIRE®. Conclusão: Os resultados indicam que o tratamento com MIRE® favoreceu a cicatrização de feridas abertas em ratos diabéticos.

Palavras-chave: fototerapia, cicatrização, Diabetes Mellitus

1 INTRODUCTION

According to the International Diabetes Federation (2013) there are about 382 million people with diabetes worldwide and this number will increase by 55% by 2035.

The extremity ulcers risk development in patients with diabetes can be as high as 25% (BOULTON et al, 2005), and the healing delay that occurs in diabetic foot ulcers case is a serious worldwide problem, both financial and social. The diseases treatment costs related to scar deficiency justify the medicine search and bandages capable of interacting with the damaged tissue in order to speed up the tissue repair process. Coherent light (laser) and not coherent (LED-Light Emitting Diodes) use has become popular as a therapeutic modality in a clinical applications variety, among which is the healing promotion (Lowe et al, 1998;. SMITH, 2005). In addition to this application, lightsources are used by various health professionals with different purposes. In this sense, they are used by physiotherapists to treat acute and chronic pain variety and promote various ulcerations healing; by dentists to treat inflamed oral tissue; by dermatologists totreat edema, indolent ulcers, burns and dermatitis, and by rheumatologists and other professionals from different specialties (VO-DINH, 2003). It is interesting to note that the clinical evidence to support the LEDs efficacy in treating wounds in humans is emerging (SUSSMAN; Bates-Jensen, 2006). The low power therapy field is characterized by a methodologies variety and several light sources uses (lasers and LEDs), with different adjustment parameters, among which we highlight differences in wavelength, the output intensity,in continuous or pulsed operation mode, and pulse parameters (VO-DINH, 2003).

Parameters such as application and intensity angle of phototherapy, and tissue factors (such as color and skin density) affect energy absorption in the tissue, producing different interaction forms with matter (SUSSMAN; Bates-Jensen, 2006). In this context, it has been demonstrated that infrared energy monochromatic mode (MIRE® - monochromatic infrared energy), commercially available and approved by the Food and Drug Administration (FDA), increases the nitric oxide release in the whole blood and normal adults plasma (HORWITZ et al, 1999). And since nitric oxide appears to play role in regulatory forces modulation in various cellular activities on the inflammatory stage, and in the proliferative healing phase (CHILDRESS; STECHMILLER, 2002), the MIRE® use has proved to be a promising technology that refers to accelerating the healing process (Horwitz 1999). Furthermore, diabetes mellitus is a disease which

admittedly is associated with chronic scarring problems (Fahey et al, 1991; Greenhalgh, 2003), related to decreased nitric oxide bioavailability (HALFOUN et al., 2003).

Considering the above, the MIRE® use can constitute resource with excellent prospects to promote healing. Thus, this study aimed to evaluate the monochromatic infrared energy - MIRE (890nm) application effect on skin wounds healing of diabetic rats.

2 METHODS

Experimental type study, with sample data qualitative and quantitative evaluation performed in University Center UNINOVAFAPI Experimental Surgery Laboratory.

42 rats were studied (Rattus norvegicus albinos, Rodentia mammalia) Wistar rats (70-80 days), coming from the University Center UNINOVAFAPI animal facilities. The animals were kept in controlled temperature conditions (25 ± 1 ° C) and lighting (12 light and 12 dark hours), with free access to water and standard commercial feed for rats (Labina - Purina). All experiments were performed in accordance with the Law 11,794 provisions of October 8, 2008, and other rules applicable to the animals in research use, especially the National Animal Experimentation Council Control Normative Resolutions - CONCEA. The research project was submitted for consideration and approval by the University Center Research Ethics Committee UNINOVAFAPI.

Animals with streptozotocin-induced diabetes after skin wounds production, were randomized into three experimental 14 animals groups: control (C), consisting of animals subjected to daily dressing with saline; Standard group (P) consisting of animals treated with Trofodermin® (clostebol acetate / neomycin sulfate); phototherapy group (F) consisting of animals treated with phototherapy Anodyne (MIRE®). Each group was divided into two subgroups, subject to procedures described for 7 or 14 days, periods already used in experimental healing by Amorim study et al. (2006). The diabetes induction was performed in all animals after fasting for 12 hours with free access to water, by intravenous administration streptozotocin 40mg / kg (Sigma Chemical, USA), dissolved in 10 mM citrate buffer pH 4.5 and (Machado et al., 2000; Lerco et al., 2003). Ater 30 minutes from treatment the animals were fed normally. Fasting glucose assessments were made on the fifth day after diabetes induction and every seven days during follow-up using test strips accue chek. They were included in the experiment only animals showing blood glucose above 250 mg / dL (Machado et al., 2000). 1 cm^2 wound skin in the lateral dorsal midline region the was produced based on the surgical technique

proposed by Oliveira et al. (2000) under anesthesia with ketamine 40 mg / kg and xylazine 5 mg / kg body weight i.p. The wounds were made with iris scissors and scalpel blade cast using the mold, removing skin, subcutaneous tissue and fat while maintaining the muscle tissue integrity. Wounds treatment was started immediately after surgery once a day, always at the same time, remaining wounds without dressing throughout the experiment. The treatment for each group was administered topically, in the lesion area (Santos et al., 2002), with the products designated for each group. The wounds were cleaned with saline 0.9% immediately before each group foreseen treatment (Santos et al, 2002;. Rahal et al., 2001).

Therapy with Anodyne (MIRE®, 890 nm) was performed by applying on alternate days 4 LEDs, for 10 minutes at each treatment session, with 8 bars (80% of total capacity), taking care that the therapy pads were placed slightly on the skin, without exerting excessive pressure to avoid burns. Since each diode emits 10.4 mW infrared energy and fourth diodes were used, each irradiating corresponded to 41.6 mW for 10 minutes (24.96 J / cm2) (Table 1).

Table 1: irradiation parameters used in the experiment with MIRE®

Parameters	MIRE®
Wave-length	890 nm
Power	10,4 mW X 4 =41,6mW
Energy density	24,96 J/cm^2
Irradiation area	1cm^2
Irradiation Time	600s

Lesions were assessed daily at the treatment application time considering the following features: secretion presence or absence , re-epithelialization, granulation tissue formation, fibrin presence, bleeding occurrence, edema presence and injuries measures from photographic images. After the certain period described for the subgroups treatment (7 and 14 days), euthanasia was performed by sodium thiopental administration overdose (100 mg / kg) intraperitoneally (MASSONE, 2003. Then, each animal was placed on a surgical board and injury was imaged using digital camera Nikon brand maintained on a tripod at a fixed distance. subsequently, the image was imported into morphometry software ImageJ®, which was bounded on the wound periphery and calculated your area. For subgroups animals the treated for 14 days after euthanasia and injury photography , the surgical wound was dissected with a 1 cm of skin margin in around the lesion with depth to the animal dorsal musculature. The piece was placed in formaldehyde solution 10% buffered for further processing and histological analysis, which evaluated: vascular

proliferation, polymorphonuclear and mononuclear cells presence , fibroblast proliferation, the collagen fibers presence and re-epithelialization.

The wound contraction rate was calculated for each wound in accordance with the formula: ICF = 100 x (Wo - Wi) / Wo, where Wo = initial wound area; Wi = wound area on the collection day for histological analysis.

The data were presented as mean and standard mean error. Comparisons between groups were performed by variance analysis (ANOVA) followed by post-test Newman-Keuls. The data were processed using the GraphPad Prism 5.0 (Ayres et al., 2007). The significance level was set at $p < 0.05$.

3 RESULTS

3.1 MORPHOMETRIC ANALYSIS

Table 1 shows the wounds contraction average rates in the fourth, seventh and the fourteenth day after the skin lesions production. There were no statistically significant differences between groups in the wounds contraction on the 4th day of treatment. On the 7th monitoring day there was a wound area reduction significantly higher ($p < 0.05$) in the standard group (0.66 ± 0.02) compared to MIRE® groups (0.27 ± 0.03) and control (0.43 ± 0.07). And on the 14th treatment day the wound contraction was significantly higher in default groups animals (0.87 ± 0.04) and MIRE® (0.81 ± 0.04) compared withthe control group (0.68 ± 0.06). There were no statistically significant differences betweenthe standard and MIRE® groups (Table 1).

Table 1: Index cutaneous wound healing in diabetic rats treated with monochromatic infrared energy application (MIRE®), Trofodermin® (Standard) or Saline 0.9% (Control) on the 4th, 7th and 14th treatment days.

Analysis periods	Control Group (SF 0,9%)	MIRE® Group	Standard group
4º dia	0,19±0,02	0,22±0,06	0,16±0,06
7º dia	0,39±0,07	0,27±0,03	0,6±0,02*
14º dia	0,68±0,06	0,81±0,04**	0,87±0,04**

*p<0,05 compared to control groups and pattern.
**p<0,05 in the control group

3.2 CLINIC ANALYSIS

The results for the clinical wounds healing are shown in Table 2. Similar results were observed in MIRE® and standard groups. The different animal groups showed wound clean and without purulent secretion after four, seven or 14 treatment days.

Table 2: clinical wound healing aspects in skin wounds in Rattus norvegicus diabetics treated with monochromatic infrared energy application (MIRE®), Trofodermin (Padrão®) or Saline 0.9% (Control) on the 4th, 7th and 14th days after wound production.

Clinical parameter	Treatment					
	Control Group (SF 0,9%)		MIRE® Group		Standard Group	
secretion (%)	07 days	14 dias	07 days	14 dias	07 days	14 days
4º day	0,0	0,0	0,0	0,0	0,0	0,0
7º day	0,0	0,0	0,0	0,0	0,0	0,0
14º day	-	0,0	-	0,0	-	0,0
Re-epithelialization (%)						
4º day	0,0	0,0	0,0	0,0	0,0	0,0
7º day	60,0	60,0	0,0	66,7	0,0	60,0
14º day	-	80,0	-	100,0	-	100,0
Granulation Tissue (%)						
4º day	100,0	100,0	100,0	100	100,0	100,0
7º day	100 ,0	100,0	100,0	100	100,0	100,0
14º day	-	100,0		-	-	100,0
			100,0			
Fibrin (%)						
4º day	0,0	20,0		0,0	0,0	0,0
			16,7			
7º day	0,0	0,0		0,0	0,0	20,0
			66,7			
14º day	-	40,0		-	-	0,0
			0,0			
Edema (%)						
4º day	50,0	0,0	37,5	33,3	20,0	40,0
7º day	20,0	20,0	37,5	0,0	20,0	0,0
14º day	-	20,0	-	0,0	-	0,0

3.3 HISTOLOGICAL ANALYSIS

Histological day 14 analysis revealed the inflammatory infiltrate presence moderate intensity in all groups. However, the semi-quantitative analysis revealed increased vascular proliferation MIRE®. No wound in the different groups studied was healed in that period.

4 DISCUSSION

Several studies have shown that photobiomodulation by low intensity light facilitates healing of wounds (Conlan et al., 1996), promotes the collagen production (SILVA, 2013; He et al, 2012), fibroblast cell proliferation (TANIGUCHI et al, 2009) and endothelial cells (. Chen et al, 2008) and reduces oxidative stress (LIM et al., 2008).

Although there are more than 2,000 publications related to the phototherapy use (VO-DINH, 2003), there is still skepticism about its efficacy in wound healing, since several studies differ how the type and dosimetry used (Minatel et al., 2009a). In this study, the MIRE® influence on cutaneous wound healing was assessed using an

experimental model in diabetic mice was observed that the wound contraction was similar between MIRE® and control groups on 7th treatment day. As suggested by Kilik et al (2014), a possible explanation for this finding is the fact that the inflammatory diabetes phase is slowed down and the phototherapy effect is not capable of increasing metabolic demand appropriately in the early application days. The greatest reduction in wound area observed between the group animals treated with MIRE® 24.96 J / cm2 using irradiation with 41.6 mW for 10 minutes on alternate days (0.81 ± 0.04) compared to the control group (0.68 ± 0.06) at treatment day 14 indicates that irradiation with infrared light using appropriate treatment parameters may accelerate skin healing in diabetic rats. A similar wound contraction rate on the 14th treatment day observed in standard groups (0.87 ± 0.04) and MIRE® (0.81 ± 0.04) corroborates with this hypothesis. However, these findings are different from those found by He et al (2012) evaluating the monochromatic infrared energy effect application in three weekly applications with 85% of themaximum energy intensity offered by the device for 30 minutes, which found nodifference in skin healing diabetic rats rate by comparing the treatment group to the control group at 7 and 14 observation days. And Lowe (1998), when analyzing the irradiation effects of a matrix with multiple current sources (0.18, 0.54, and 1.45 J / cm2;Gallium-Aluminum-Arsenic, 890 nm) on the healing in murine skin, also found that using irradiation with 0.18 and 054 J / cm2 did not affect the rate and scarring, while useof 1.45 J / cm2 to elicit an inhibitory effect. It is noteworthy that both the above studies used different treatment protocols that the present research.

And, a possible explanation for the MIRE® effect absence, seen in He et al. (2012) and Lowe et al. (1998) studies , above reffered , it can be assigned to the treatmentprotocol used and not the effect lack of monochromatic infrared energy on skin healing. Corroborates this hypothesis the fact that clinical studies results using different clinical irradiation protocols with LEDs in wavelength of 890nm, pointing to beneficial phototherapy effects on the skin wounds healing (MINATEL et al., 2009a; CAETANO et al, 2009) and case reports (MINATEL et al., 2009b; HORWITZ; BURKE; CARNEGIE, 1999; NATHER et al, 2007). Additionally, Hussein (2011) demonstrated that daily pulsed laser application at a 890 nm wavelength, 10mW power and frequency KLTZ 20 for 5 minutes for 7 days resulted in an increased healing skin rate in rabbits. Accordingly, Dadpay et al (2012), in carry out biomechanical testing on skin wounds, observed increase maximum stress and elasticity modulus, as well as acceleration speed in wound healing rate in healthy and diabetic mice using the pulsed infrared laser 0.2 J

/ cm2 with a 890 nm (80 Hz) wavelength. The mechanism proposed to explain the healing rate increase is the light interaction with photoreceptor endogenous enzymes that initiate the intracellular signaling pathways activation and alter cellular and tissue metabolism and cellular proliferation (POYTON; Ball, 2011). It has also been proposed that photobiomodulation could increase the nitric oxide bioavailability by stock intracellular release , especially heme proteins (hemoglobin or myoglobin, for example) (LOHR et al, 2009;. Shiva; Gladwin 2009), and induces the mitochondrial cytochrome-c oxidase activating the reduction reaction of nitrite in nitric oxide (POYTON et al, 2009). In addition, the increasing speed secondary capillary blood flow in MIRE® irradiation previously demonstrated by Mak and Cheing (2012) in healthy humans, might also contribute to increased healing rate by increasing the oxygen and nutrients supply in skin wound. The biggest vascular proliferation MIRE® found in the histological analysis corroborates previous studies that showed increased angiogenesis by the phototherapy use (KILÍK et al., 2014; SCHINDL et al., 2003; MIRSKY et al., 2003; KIPSHIDZE et al., 2001). In this sense, it is emphasized that Schindl et al. (2003) observed that the laser use with 670nm wavelength and energy intensity of 8 J / cm2, 20 and 65 mW / cm2, caused endothelial cell proliferation. And Hipshidze (2001) showed an increase in the vascular endothelial growth factor release (VEGF) by muscle cells and fibroblasts in vitro using 632 nm laser wavelength with an intensity from 0.10 to 6.3 J / cm2. Similarly,Sousa et al. (2012) showed an increase in angiogenesis in vivo in skin wounds in rats using LEDs in 700 nm ± 20, 530 ± 20 nm and 460 ± 20 nm wavelength, and pointed to the increase in nitric oxide levels as allegedly responsible for this finding, effect which they said would not be determined by the the coherence light phenomenon.

5 CONCLUSION

The results indicate that treatment with MIRE® favor the cutaneous wounds healing in diabetic mice and increased vascular proliferation contributes as a mechanism for facilitating the skin wounds healing. Further studies are needed to compare different treatment protocols, use up to 21 day treatment period as already used in the study as the Amorim et al. (2006) in normal rats not have difficulty in healing as diabetic animals and also phototherapy using different modes in order to allow identifying the most effective treatment form and to fully clarify mechanisms involved in the MIRE healing effect.

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