Effect of Laser Therapy on Immune Cells Infiltrate After Excisional Wounds in Diabetic Rats

Cristiano de Loura Santana, Daniela de F atima Teixeira Silva, Amanda Pires de Souza, Marcos Vinicius Jacinto, Sandra Kalil Bussadori, Raquel Agnelli Mesquita-Ferrari, Kristianne Porta Santos Fernandes, and Cristiane Miranda Franc,a_ Postgraduate Program in Biophotonics Applied to Health Sciences, University Nove de Julho, S~ao Paulo, SP, Brazil Background and Objective: Diabetes alters innate and specific immunity, causing an imbalanced tissue repair process. Very active neutrophils and macrophages are found for a long time in chronic wounds in those individuals. The aim of this study was to evaluate the response of the main effector cells of immunity (neutrophils, macrophages, and T lymphocytes) and to compare the effects of two laser therapy regimens in the postoperative treatment of excision wounds. Method: Diabetes was induced in female Wistar rats and a punch was used to cause wounds in the dorsum of each individual. The animals were randomly allocated to a control group (CG), in which the wound was untreated, a single-dose laser group (SLG), in which the wound was submitted to single dose of laser therapy at wavelength of 660 nm, output power of 30mW, energy density of 4 J/cm2, and 26-second exposure time, and a fractionateddose laser group (FLG), submitted to 1 J/cm2 of laser therapy on Days 1, 3, 8, and 10. Euthanasia was performed on five animals from each group Days 1, 3, 8, 10, 15, and 22. The wound was removed and routinely processed for immunohistochemistry against elastase, CD3, CD68, and CD206 antibodies. The samples were photographed and labeled cells were counted by a blinded observer. The Kruskal-Wallis test was used for the statistical analysis. Results: Neutrophils were predominant in the SLG on Day 1, whereas these cells were mostly found in the CG on Day 3 (P<0.05). The T lymphocyte count was similar in all groups in the throughout the experiment. On Day 3, the SLG exhibited a greater number of total macrophages (CD68b) (P<0.05), whereas the macrophage count was similar among the different groups on the other evaluation days. The CD206bcell counts revealed that the SLG had more M2 macrophages than the CG on Day 8 (P<0.05), whereas the FLG exhibited more M2 macrophages than the CG on Day 10 (P<0.05). Conclusion: The present findings demonstrate that laser therapy can alter the composition of inflammatory infiltrate in diabetic wounds, leading to a more balanced response transiting from a rapid neutrophil infiltration through to M2 macrophage polarization, especially with a single application of 4 J/cm2 in the immediate postoperative period. Lasers Surg. Med. 48:45-51, 2016. 2015 Wiley Periodicals, Inc.