Irradiation at 830 nm Stimulates Nitric Oxide Production and Inhibits Pro-Inflammatory Cytokines in Diabetic Wounded Fibroblast Cells

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Background and Objective: Wound healing in diabetic patients remains a chief problem in the clinical setting and there is a strong need for the development of new, safe, reliable therapies. This study aimed to establish the effect of irradiating diabetic wounded fibroblast cells (WS1) in vitro on pro-inflammatory cytokines and the production of nitric oxide (NO).

Materials and Methods: Normal, wounded and diabetic wounded WS1 cells were exposed to an 830 nm laser with 5 J/cm^2 and incubated for a pre-determined amount of time. Changes in cellular viability, proliferation and apoptosis were evaluated by the Trypan blue assay, VisionBlue^{TM} fluorescence assay and caspase 3/7 activity respectively. Changes in cytokines (interleukin—IL-6, IL-1β and tumour necrosis factor-alpha, TNF-α) were determined by ELISA. NO was determined spectrophotometrically and reactive oxygen species (ROS) was evaluated by immunofluorescent staining.

Results: Diabetic wounded WS1 cells showed no significant change in viability, a significant increase in proliferation at 24 and 48 hours ($P<0.001$ and $P<0.01$ respectively) and a decrease in apoptosis 24 hours post-irradiation ($P<0.01$). TNF-α levels were significantly decreased at both 1 and 24 hours ($P<0.05$), while IL-1β was only decreased at 24 hours ($P<0.05$). There was no significant change in IL-6. There was an increase in ROS and NO ($P<0.01$) 15 minutes post-irradiation.

Conclusion: Results show that irradiation of diabetic wounded fibroblast cells at 830 nm with 5 J/cm^2 has a positive effect on wound healing in vitro. There was a decrease in pro-inflammatory cytokines (IL-1β and TNF-α) and irradiation stimulated the release of ROS and NO due to what appears to be direct photochemical processes. Lasers Surg. Med. 42:494–502, 2010.

Key words: IL-1β; IL-6; lasers; NO; ROS; TNF-α

INTRODUCTION

The process of wound healing is a highly co-ordinated process that involves a series of overlapping events controlled by a variety of cells, growth factors, cytokines and metabolic enzymes released at the wound site. Dysregulation of this co-ordinated event leads to impaired wound healing; an abnormality which is frequently seen in conditions such as diabetes. There are many causes of chronic wounds, with diabetes, pressure ulcers and venous stasis as the three most common causes [1]. Impaired wound healing is an incapacitating complication of diabetes often necessitating amputation and poses a serious challenge in clinical practice.

Growth factors and cytokines such as interleukin-1-beta (IL-1β), IL-6 and tumour necrosis factor-alpha (TNF-α) have diverse modes of action and are released during wound repair [2]. IL-1β and TNF-α are both well-known pro-inflammatory cytokines and have similar functions or effects; however, they do not share chemical or structural resemblance and their effects are interceded by specific receptors. Together with IL-1, TNF-α is the first cytokine known to be upregulated during the inflammatory phase of wound healing and contributes to the oxidative stress within the wound by generating reactive oxygen species (ROS) [3]. IL-6 is induced during acute phase reactions and usually expressed in response to or together with IL-1 and TNF-α [4]. However, contradictory effects have been reported [5]; it suppresses TNF-α, IL-1 and IL-12. Its vital role in wound healing is its ability to cause cell differentiation and proliferation. TNF-α is the most critical accelerator of diabetes [6].

ROS and reactive nitrogen species (RNS) act as molecular messengers during cell signalling; however, they have a biphasic effect, being both beneficial and detrimental depending on their concentration. ROS and RNS are generated during wound healing and are important mediators in this carefully controlled process, however in chronic wounds there is an uncontrolled production of these molecules. Nitric oxide (NO) is significantly reduced in chronic ulcers and impaired healing of diabetic wounds is

Contract grant sponsor: University of Johannesburg (UJ); Contract grant sponsor: National Research Foundation (NRF) of South Africa; Contract grant sponsor: Medical Research Council (MRC) of South Africa.
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Accepted 5 August 2009
Published online 15 July 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20812