Comparison of topical low dose photodynamic therapy using 5-aminolevulinic acid and methylene blue in wound healing by multiphoton microscopy in-vivo

Zuhayri H.1, Nikolaev V.V.1,2, Lepekhina T.B.1, Zakharova O.A.1, Knyazkova A.I.1,2, Borisov A.V.1, Kistenev Yu.V.1, N.A Krivova 1
1Tomsk State University, Tomsk, Russia,
2V.E. Zuev Institute of Atmospheric Optics SB RAS, Tomsk, Russia

Introduction
Low dose photodynamic therapy (LDPDT) is a promising and non-invasive therapy for the healing of skin wounds that can reduce treatment time, accelerating tissue repair and promote healing using an optimum dose of laser light.

Study design
Animal model: Experiments have been done with CD1 mice, wounds were obtained surgically 0.5 cm x 0.5 cm (Fig 1).

Low dose photodynamic therapy protocol: has done with aminolevulinic acid (5-ALA) and Methylene blue (MB) with AlGaNp Laser: λ = 630 nm, P=5mW and two laser doses 1J/cm² and 4J/cm².

Observation days: 1,3,7,14

Multi-photon microscope: The measurements were carried out on the MPTflex equipment by Jenlab (Germany) (Fig 2) with its techniques; Second harmonic generation (SHG) and Autofluorescence (AF). The image size was 70 μm x 70 μm and images were recorded on a 512 x 512 pixel matrix. The excitation wavelength was 760 nm. The analysis was done with specific software package “Becker&Hickl”.

Results

Visual observation

Fig 3. Digital photograph assessment of healing progression from day 0 to day 14. (a) control group, (b) LDPDT-5ALA 1J/cm², (c) LDPDT-5ALA 4J/cm², (d) LDPDT-MB 1J/cm², (e) LDPDT-MB 4J/cm²

In vivo MPM imaging

Fig 4. SHG and AF images of wound healing. (a) Control group, (b) LDPDT-5ALA 1J/cm², (c) LDPDT-5ALA 4J/cm², (d) LDPDT-MB 1J/cm², (e) LDPDT-MB 4J/cm²

SAAID index

As a specific parameter for wound healing and its course the second-harmonic generation-to-autofluorescence aging index of dermis (SAAID) was used, which defines as follows:

\[ \text{SAAID} = \frac{\text{SHG-AF}}{\text{SHG+AF}} \]

Fig 5. SAAID for control group.

Fig 6. SAAID for LDPDT-5ALA 4J/cm².

Fig 7. SAAID for LDPDT-5ALA 4J/cm² and LDPDT-MB 4J/cm² groups on day 14.

Conclusion
Transformation in collagen structure was observed during wound healing from 1st day to 14th day. Also, the LDPDT accelerates the process of wound healing using both of 5-ALA and MB, with better results for LDPDT-MB 4J/cm².

Different behavior of SAAID compared to different days of the wound healing process for different groups was be observed, so SAAID is a good factor to monitor wound healing process.