H_2O_2 production during wound healing in bovine corneal endothelial cells in culture does not affect migration

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Introduction

The production of oxidant species during wound healing has been studied extensively. In this regard, Lisse et al. has shown that, in scratch assays of human keratinocytes in culture, DPI treatment inhibit H2O2 production and wound clousure, suggesting a role for this molecule in the migration modulation. In addition, Abbafy et al. found that the nitric oxide is critical for Xenopus embryonic wound healing. To further understand the role of oxidants, we have studied the production and role of reactive oxigen species (ROS), specifically the H2O2 during wound healing in bovine corneal endothelial cells (BCEC) in culture.

Results

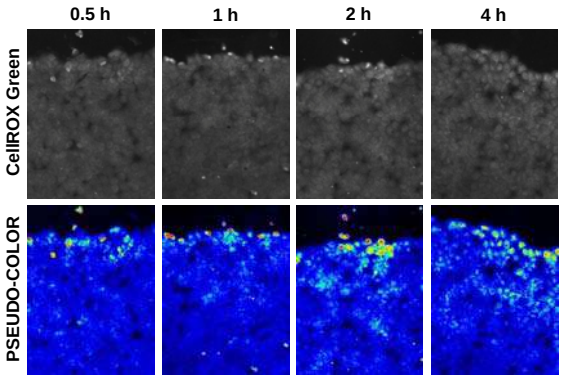

Figure 1. CellROX Green images at 0.5, 1, 2 and 4 hours after injury. Original images in grey and pseudo-color are shown.

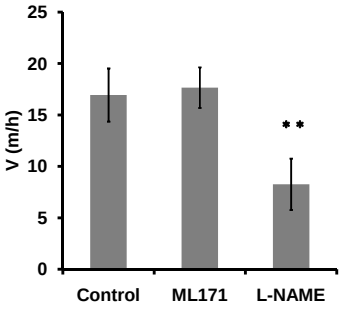

Figure 3. Healing rate in absence or presence of the NADPH Oxidase inhibitor (ML171) and NO Synthase inhibitor (L-NAME).
$(**)$ $p < 0.01$

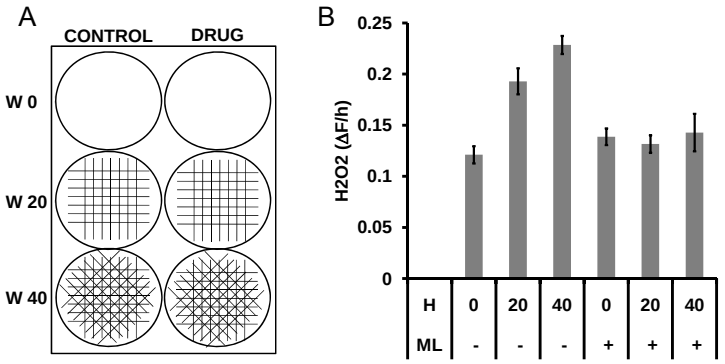

Figure 2. (A) 6-well plate experiment diagram showing wounds quantity and distribution. **(B)** Determination of H2O2 with Amplex Red, the graph shows relative fluorescence per hour in absence or presence of the NADPH Oxidase inhibitor (ML171).

Conclusions

Our results suggest that during wound healing in BCEC in culture, H2O2 is produced in wound border in a way dependent of NADPH oxidase. Nevertheless, contrary to Lisse findings, our results suggest that H2O2 do not altere wound healing rate in BCEC. On the other hand, the experiments using the NOS inhibitor L-NAME suggest a role of NO in migration during healing. Further investigation is required to comprehend the ROS function in wound healing.

Materials and Methods

Cell Culture and Wounding Procedure. Bovine eyes, obtained were processed as described in Justet et al. 2019. Linear wounds were made using a pipette tip. For 6-well plate experiments 0, 20 or 40 wounds were performed in absence or presence of the drug of interest as it is schematized in the Figure 2A. *ROS, H_2O_2 and peroxynitrite determination.* Unspecific ROS were monitored with the unspecific probe CellROX Green. H_2O_2 was determined using Amplex Red and Horseradish Peroxidase. Peroxynitrite was monitored using fluorescein-boronate (Fl-B) (Ríos et al. 2016). *Enzyme inhibition.* NADPH oxidases and NO synthase were inhibited by 2-Acetylphenothiazine (ML-171) and N omega-Nitro-L-arginine methyl ester hydrochloride (L-NAME) incubation, respectively. *Migration.* The migrated distance (ΔL) and mean velocity was calculated as described in Justet et al. 2016.

Bibliography

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