Cutaneous hypoxia: effect of oxygen-loaded nanodroplets in human keratinocytes and fibroblasts

1)Piazza S. 2)Fumagalli M. 3)Prato M. 4)Sangiovanni E. 5)Finesso N. 6)Troia A. 7)Garibaldi G. 8)Basilico N. 9)Dell'agli M.

Università degli studi di Milano

Skin is an important barrier against many environmental insults. The epidermis is composed by several cell types (such as keratinocytes, the most abundant epidermal cells), strictly involved in the protection and integrity of skin. Wound healing process can be divided into four phases: haemostasis, inflammation, proliferation and remodelling; during this repair process, keratinocytes and fibroblast contribute to the re-epithelisation and remodelling phases. If the process is blocked in the inflammatory state precluding the passage to proliferation, this can lead to chronic wound (Eming et al., 2007). Chronic wounds can be classified into three main categories: venous ulcers, pressure ulcers, and diabetic ulcers. Many chronic wounds occur in presence of local tissue hypoxia due to vasculopathies (such as atherosclerosis), this local hypoxia disrupt wound healing promoting inflammatory cascades (Toledo-Pereyra et al., 2004); moreover, also re-epithelisation, fibroblast proliferation and synthesis of collagen are impaired. Hypoxia is a common physiologic and pathophysiologic stimulus that activates the expression of genes through oxygen-sensitive transcription factors, including hypoxia-inducible factor (HIF) and NF-κB, a transcription factor strictly involved in inflammatory processes (Cumminns et al., 2005; Koong et al., 1994). Since the therapies currently used are characterized by side effects, it is necessary to search for new strategies to counteract hypoxia-based wound. Oxygen-loaded nanodroplets, constituted by 2H,3H-decafluoropentane as core fluorocarbon and dextran or chitosan as shell polysaccharides, have been developed and well characterized; these nanodroplets act as efficient, biocompatible and stable oxygen delivery systems (Prato et al., 2015). Recent studies have demonstrated that these nanocarriers are able to abrogate hypoxia-dependent dysregulation of MMP/TIMP balance in different cell types (such as keratinocytes) (Khadjavi et al., 2015). In addition, in literature, different studies have demonstrated the anti-inflammatory activity of chitosan (Tu et al., 2016).

The aim of this work was to study the effects of oxygen-loaded nanodroplets in human keratinocytes and fibroblasts during hypoxic and inflammatory conditions.

Oxygen nanodroplets were prepared as previously described (Prato et al., 2015) and used at three different concentrations (5%, 10% and 25% v/v) in human keratinocytes and fibroblasts; NF-κB driven transcription was assayed transfecting cells with a reporter plasmid.

Dextran and chitosan oxygen nanodroplets inhibited TNF□-induced NF-κB driven transcription in concentration-dependent manner after 6-hour treatment, both in keratinocytes and fibroblast, while without the pro-inflammatory mediator nanocarries were inactive. After 24-hours treatment only dextran oxygen-loaded nanodroplets decreased these parameters in concentration dependent-way in keratinocytes, while in the absence of TNF□, chitosan oxygen-loaded

nanodroplets showed a statistical significant inhibition at 5% and 10% v/v. Since the effect of oxygen-loaded nanodroplets and the effect of oxygen-free nanodroplets was comparable, the anti-inflammatory activity observed can be associated to dextran or chitosan shell whereas the effect of the oxygen release is negligible.

These results suggest that in normoxic condition dextran and chitosan shell exert an anti-inflammatory activity; the effect of oxygen nanodroplets in hypoxia conditions is currently under investigation.

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