Expression of Cyclooxygenase-2 (COX-2) in Human Fibroblasts after Induced Oxidative Stress

A. Alonso-Varona, 1, I. Azcoitia 2, B. Castro 2, M. Del Olmo 2, J. Bejar 3, T. Palomares 1

Corresponding Author: e-mail

1 Univ. Basque Country, Leioa, Vizcaya; Spain, 2 Histocell, Zamudio, Vizcaya, Spain; Cruces Hospital, Baracaldo, Vizcaya, Spain.

Introduction

Chronic ulcers are an example of abnormal wound healing showing chronic inflammation which can result in delayed healing. One of the reasons that can influence the chronic state of the ulcer edge is a continuous state of oxidative stress (1) partially due to a release of toxic metabolites, proteolytic enzymes and toxic free radicals accumulated in the damaged tissue (2). In order to evaluate this biological process, we used an experimental model based on human fibroblasts, obtained from dermis, exposed to H2O2. The aim of this work was to study the implication of COX-2 in this oxidative process.

Materials and Methods

Human fibroblasts were exposed in vitro to different concentrations of H2O2 (0.5-1.5 mM) for 1 hour. After the stress, we determined ROS levels (using 2,7-dichlorofluoresceine), changes in intracellular levels of GSH (labelled with monochlorobimane), changes in proliferative activity (MTT assay) and in COX-2 expression (PCR).

Results

Exposure of human fibroblasts to H2O2 induces a dose-dependent increase of ROS levels (4, 7 and 10 times higher with 0.5, 1 and 1.5 mM, respectively), reaching the maximum at 30 min. In concordance, a dose-dependent GSH reduction (up to 40%) was observed between 15 and 30 min., after which we found a rebound of GSH levels (55% increase at 4h). As expected, proliferation was reduced in a dose-dependent manner (46, 60 and 90% reduction with 0.5, 1 and 1.5 mM, respectively, at 72h). The induced oxidative stress produces a significant expression of COX-2, observed from 1 to 24 h after the stress.

Discussion and Conclusions

In summary, this in vitro model can be useful to evaluate the implication of COX-2 in inflammatory process and to analyze the efficacy of antioxidant agents used in the treatment of chronic ulcers.

References


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