

Tumor necrosis factor-alpha - mediated 2-hydroxyethyl methacrylate cytotoxic and inflammatory effect on human gingival fibroblasts

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2-Hydroxyethyl methacrylate (HEMA), deriving from polymerized dental resinous biomaterials, can diffuse throughout the dentin organic matrix, preventing collagen collapse, but also at gingival and tooth pulp level [1]. HEMA could induce toxic effects, such as tissue inflammation, also at relatively low concentrations.

Our study aimed to investigate the cytotoxic and inflammatory effect exerted on human gingival fibroblasts (HGFs) by a low HEMA concentration evaluating cell viability by Trypan blue dye exclusion test, early apoptosis and reactive oxygen species (ROS) production by flow cytometry and gene expression of specific proteins involved in the inflammatory process, such as tumor necrosis factor-alpha (TNF- α) and cyclooxygenase-2 (COX-2), by real-time reverse transcription polymerase chain reaction (real-time RT PCR).

Cultured HGFs, obtained from fragments of gingival tissue, were exposed to 3 mM HEMA in Dulbecco's modified Eagle's medium for 0, 24 or 96 hours.

In our experimental model, both 24- and 96-hour HEMA treatment decreased cell viability of about 20%. In parallel Annexin-V/PI assay, which detects apoptosis, indicated a 18% of Annexin-V positive cells after 24- and 96-hour HEMA incubation. After 24-hour HEMA treatment we observed an increase of ROS persisting up to 96 hours. Interestingly, 24-hour HEMA treatment increased TNF- α gene expression of about 80% and COX-2 mRNA levels of about 70% compared to control. After 96-hour HEMA incubation, TNF- α gene expression was about sixfold and COX-2 mRNA levels were about fivefold compared to control. Increase of TNF- α and COX-2 gene expression was hence HEMA exposure time-dependent.

Since TNF- α - induced inflammation has been shown to be mediated by the activation of COX-2 transcription in HGFs [2], we can hypothesize that, in our experimental model, 24- or 96-hour HEMA treatment in HGFs induces a ROS-mediated cytotoxicity and an inflammatory process modulated by increase of TNF- α gene expression, which could rapidly produce the observed up-regulation of COX-2 transcription.

Thus, the knowledge of molecular mechanisms underlying cellular response to dental resinous biomaterials, identifying threshold over which these compounds become toxic, could allow to set up protocols for a more effective clinical practice and for a better performance of tested materials.

[1] Schweickl H, Spagnuolo G, Schmalz G. J Dent Res 2006; 85: 870-7.

[2] Nakao S, Ogata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H. Mol Cell Biochem 2002; 238: 11-8.

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