Biomaterials 33 (2012) 5177–5186

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

The influence of glutathione on redox regulation by antioxidant proteins and apoptosis in macrophages exposed to 2-hydroxyethyl methacrylate (HEMA)

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ARTICLE INFO

Article history:
Received 23 March 2012
Accepted 2 April 2012
Available online 24 April 2012

Keywords:
Resin monomer
HEMA
Reactive oxygen species
Glutathione
Antioxidant protein
Apoptosis

ABSTRACT

Resin monomers like 2-hydroxyethyl methacrylate (HEMA) disturb cell functions including responses of the innate immune system, mineralization and differentiation, or induce cell death via apoptosis. These phenomena are associated with oxidative stress and a reduction in the concentration of the antioxidant glutathione (GSH), resulting in imbalanced redox homeostasis. Thus far, the precise mechanism of how resin monomers interfere with cellular redox regulation is unknown. The present study provides insight into the induction of apoptosis and the differential expression of antioxidant enzymes depending on the availability of GSH. Buthionine sulfoximine (BSO) was used to inhibit GSH synthesis, while 2-oxothiazolidine-4-carboxylate (OTC), and N-acetylcysteine (NAC) as prodrugs supported GSH synthesis in RAW264.7 mouse macrophages exposed to HEMA (0–8 mM) for 24 h. The level of GSH was significantly decreased after cells were preincubated with BSO, and the formation of reactive oxygen species (ROS) increased in cultures subsequently exposed to HEMA. Apoptosis was drastically increased by BSO in HEMA-exposed cell cultures as well, but OTC and NAC retracted HEMA-induced cell death. These results show that dental monomer-induced apoptosis is causally related to the availability of GSH. The hydrogen peroxide decomposing enzymes glutathione peroxidase (GPx1/2) and catalase were differentially regulated in HEMA-exposed cultures. Expression of GPx1/2 was inhibited by HEMA and further reduced in the presence of BSO. SOD1 (superoxide dismutase) expression was inhibited in the presence of HEMA, and was decreased to an even greater extent by BSO, possibly due to H_2O_2-feedback inhibition. The expression of catalase was considerably up-regulated in HEMA-exposed cultures, implying that H_2O_2 is the type of ROS that is significantly increased in monomer-exposed cells. OTC and NAC counteracted the effect of HEMA on GPx1/2, SOD1, and catalase expression. HO-1 (heme oxygenase) expression was strongly enhanced by HEMA, suggesting the need for further antioxidants like bilirubin to support enzyme activities that directly regulate H_2O_2 equilibrium. Expression of the oxidoreductase thioredoxin (TRX1), the second major thiol-dependent antioxidant system in eukaryotic cells, was slightly reduced, while the oxygen-sensing protein HIF-1α was downregulated in HEMA-exposed cell cultures. These results indicate that cells and tissues actively respond to monomer-induced oxidative stress by the differential expression of enzymatic antioxidants.

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1. Introduction

Resin monomers including 2-hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) are well-known components of composite resins and adhesives in dentistry, but bone cements and scaffolds for tissue regeneration may also incorporate methacrylates [1,2]. Due to insufficient monomer-polymer conversion, residual monomers of composite materials leach into their surrounding aqueous environment and accordingly act on adjacent tissues [3]. Contact allergies have been observed clinically, but unreacted monomers may also affect tissues of the oral cavity including the dental pulp, either immediately in the case of direct pulp capping procedures, or eventually possibly diffuse from restorative composite materials through dentinal tubules. Based on experiments from in vitro cell culture experiments of multiple target cells, resin monomers specifically interfere with various cellular functions, for instance, by inhibiting cytokine production, mineralization, cell differentiation, or inducing apoptosis related to the modification of signal transduction pathways and expression of

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0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2012.04.013