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Short Communication

Cytotoxic Effect of Eugenol on The Expression of Molecular and Osteogenic Differentiation of Human Dental Pulp Cells

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ABSTRACT

The cytotoxic effect of eugenol on the expression of molecular markers related to the osteogenic differentiation of human dental pulp cells such as collagen synthesis and the expression of two osteogenesis-related genes, alkaline phosphatase (ALP) and bone sialoprotein (BSP), was studied using human dental pulp cells (D824 cells). Cellular growth and survival were decreased by treatment of cells with eugenol in a concentration-dependent manner. The incorporation rate of [3H] proline into the acid-insoluble fraction and the synthesis of type I–V collagens were also reduced by treatment of cells with eugenol in a concentration-dependent fashion. The mRNA expression of ALP was scarcely affected in cells exposed to eugenol, whereas the mRNA and protein expression of BSP was down-regulated depending on the concentrations of eugenol. The results suggest that because collagen synthesis and BSP expression play a critical role in hard tissue formation, eugenol used for endodontic treatment may give rise to cytotoxic effects to the normal function of stem cells reported to exist in human dental pulp tissue and periodontal ligament. Eugenol (4-allyl-2-methoxyphenol) is the main component of oil of cloves (Eugenia caryophyllate). It is used as a fragrance and flavoring agent, an insect attractant, and as a topical antiseptic and anti-inflammatory analgesic in dentistry. Mixed with zinc oxide into a thick paste, eugenol is also used in dentistry as a component of periodontal dressings, impression materials, and endodontic medications. Some of the endodontic medications administered to the teeth can reach the pulp tissue or the periodontium after penetrating the enamel and dentin or passing through apical foramens, respectively [1]. If the endodontic medications were cytotoxic, they could disturb the normal function of stem cells reported to exist in human dental pulp tissue [2] and periodontal ligament [3]. It is, therefore, important to study the cytotoxicity of chemical agents used for endodontic treatment. Despite the extensive clinical use in dentistry, eugenol is cytotoxic to several types of human cells, including dental pulp cells [4], gingival fibroblasts [5], and periodontal ligament fibroblasts [6]. The cytotoxicity of eugenol shown in almost all the studies was determined by the growth or viability of cells treated with eugenol.

INTRODUCTION

In the present study, we investigated the cytotoxic effect of eugenol on the expression of molecular markers related to the osteogenic differentiation of human dental pulp cells such as collagen synthesis and the expression of two osteogenesis-related genes, alkaline phosphatase (ALP) and bone sialoprotein (BSP), in human dental pulp cells. Goldberg et al. [7] have demonstrated that the osteogenesis-related proteins including collagen, ALP, and BSP are synthesized during physiological and reparative dentinogenesis. Collagen, particularly type I, composes 90% of the dentin matrix. The collagen matrix provides not only the scaffold to promote and develop a mineralized tissue, but also an excellent natural support for non-collagenous proteins such as BSP and dentin sialoprotein [7]. ALP is an essential factor in dentin mineralization and in the formation of acellular cementum [8]. BSP is one of the non-collagenous proteins found in bone, dentin, and dental pulps [9], and considered as an early marker of differentiating osteoblasts [10] and odontoblast-like cells [11]. It binds to the specific residues of type I collagen [12] and serves as a potent nucleator of hydroxyapatite formation on the collagen fibrils [11]. Human dental pulp cells (D824 cells) derived from the dental pulp tissue obtained from a lower third molar extracted from a woman (22 years old) were grown as described previously [13]. D824 cells have the capability of forming mineralized nodules in vitro and recruiting odontoblast-like cells and dentin-like tissue in immunocompromised mice [13]. All experiments were carried out using D824 cells at 10–15 passages. Eugenol (>95% pure) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and dissolved at 200 mM in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Tokyo, Japan). The solution was diluted with culture medium to the desired concentrations and applied to D824 cells. Cell survival was determined by the colony-forming efficiency of cells treated with eugenol. Cells (500) were plated in triplicate onto 60-mm dishes and incubated overnight. The cells were treated with eugenol at varying concentrations for 24 h. Control cultures were incubated with DMSO medium. After two washings with 2 ml of fresh medium, cells were incubated for 13 days for colony formation. Cells were fixed with absolute methanol and stained with a 10% Giemsa solution.

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