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Immunomodulatory/anti-inflammatory effect of ZOE-based dental materials

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ABSTRACT

Objective. The study assessed the cytotoxicity and immunomodulatory/anti-inflammatory effect of extract from zinc oxide–eugenol (ZOE)-based dental materials during setting using immortalized human dental pulp stem cells (IHDPSCs) and mouse bone marrow monocytes (IMBMMs), and identified the responsible extract component.

Methods. In accord with the ISO 10993-12, we extracted a mixture of ZOE cement and sealer after a specified time. The extract was analyzed by two types of mass spectrometry (ICP-MS and GC–MS). Cell viability was evaluated with extract and serial concentrations of $ZnCl_2$, $ZnSO_4$, and eugenol liquid by WST assay. The immunomodulatory/anti-inflammatory effect of a ZOE component was determined by RT-PCR to detect the downregulatory effect of inflammatory mRNA expression after lipopolysaccharide (LPS)-induced inflammation.

Results. Zn^{2+} and eugenol (2–20 ppm) were detected in the ZOE cement and sealer extracts. During the early stage of setting, significant cytotoxicity was observed in IHDPSCs and IMBMMs ($p < 0.05$). The half maximal effective concentration of Zn^{2+} was 5–8 ppm, whereas that of eugenol could not be detected within 80 ppm. After extract treatment, the expression of inflammatory mRNA was significantly lower in inflamed IHDPSCs, but not inflamed IMBMMs, than in the LPS control ($p < 0.05$). However, eugenol, not Zn^{2+}, at 5–20 ppm downregulated inflammatory mRNA expression in the inflamed IMBMMs with and without the exchange of LPS-pretreated medium.

Significance. ZOE was highly cytotoxic, especially during setting, to both cells due to Zn^{2+} while the immunomodulatory/anti-inflammatory effect of ZOE was induced by eugenol.

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1. Introduction

Dental materials containing zinc oxide–eugenol (ZOE) have been widely used in dentistry for temporary restoration, cementation, and root canal filler because of its easy handability, low cost, excellent cavity-sealing ability, and therapeutic effects including immunomodulatory/anti-inflammatory and a sedative effect on target teeth [1]. However, the use of ZOE has certain disadvantages, such as cytotoxicity, harm to surrounding tissue including dental pulp stem cell and bone marrow derived monocytes, and inhibition of the resin polymerization [2]. Therefore, it has been suggested that the direct contact of ZOE with oral tissue should be prohibited due to irritation to tissue around pulp and oral mucosa [3,4].

Indirect contact between ZOE-based dental materials and the involved or surrounding dental tissues was also considered to have adverse potential [2,3]. Extracts from set ZOE-based materials are cytotoxic to dental pulp stem cell, gingival fibroblasts and keratinocytes in vitro [1,4,5]. Furthermore, freshly set ZOE-based materials are more cytotoxic than the final set state because the degree of cytotoxicity decreases substantially as setting takes place. In the clinical setting, freshly set ZOE can come in contact with the dentinal tubes or periapical lesion, so possible adverse effects to the dental pulp are a concern, as well as effects on periodontal ligament and related alveolar bone tissue. However, ZOE extract-induced cytotoxicity to dental pulp cells and bone marrow-derived immunomodulatory cells during or after setting has not been investigated.

Eugenol is a major ingredient of the extract from ZOE-based materials. Eugenol is believed to play a major role in inducing adverse effects [6–8]. An investigation of the relationship between cytotoxicity and eugenol released from these materials suggested a strong correlation [9]. However, the pattern of eugenol release and cytotoxicity was not always consistent, suggesting the involvement of other factors, such as zinc ion (Zn^{2+}) [4].

Immunomodulatory/anti-inflammatory properties are expected from ZOE-based materials owing to the presence of eugenol [10–13]. When the integrity of dental hard tissue is breached, elements of external origin, such as bacteria and noxious environmental stimuli, can invade the pulp or alveolar bone tissue. Acting as antigens, bacterial constituents potentially prompt a variety of immune reactions, and immunomodulatory cells including bone marrow derived monocytes (renamed macrophages) are recruited to the area of the damaged tissue. Therefore, the therapeutic use of ZOE-based dental materials requires regulation of immunomodulatory cells including monocytes (termed as macrophages in tissue) and dental pulp stem cells (DPSCs) which are responsible for pulp or periapical tissues' innate immunity [14,15]. When the innate immune response is triggered by pathogen-associated molecules like lipopolysaccharide (LPS), proinflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) are produced by immunomodulatory cells [16,17]. It has been believed that an immunomodulatory/anti-inflammatory effect depends on eugenol concentration [10,18]. However, whether the immunomodulatory/anti-inflammatory properties of eugenol are correlated with its concentration detected in quantitative analyses of extract has not been investigated with monocytes and DPSCs.

Therefore, the aim of this study was to assess the cytotoxicity and immunomodulatory/anti-inflammatory effect of ZOE extract on immortalized human DPSCs dental pulp stem cells (IHDPSCs) and immortalized mouse bone marrow monocytes (IMBMMs) during the setting of ZOE-based material and to identify the component of the extract that induced these effects. The null hypothesis is that cytotoxicity and immunomodulatory/anti-inflammatory effects are induced by both Zn^{2+} and eugenol.

2. Materials and methods

2.1. Extract of ZOE cement

Intermediate Restorative Material (IRM; Lot No. 131022; Dentsply, Tulsa, OK, USA) and Tubli-Seal (Lot No. 3-1340; Kerr Corp., Romulus, MI, USA) were chosen from among the various forms of commercially available ZOE-based products because they had been shown to be very cytotoxic in previous studies [1,19,20]. Each product was used after being checked for its expiration date and was stored under manufacturers' recommended conditions throughout the experiment. Extracts from IRM and Tubli-Seal were prepared according to the international standard [21,22]. Briefly, after powder and liquid were mixed for 2 min on a mixing pad according to the manufacturers' instructions (23 °C, relative humidity 20%), the mixed specimen was immediately incubated at 37 °C in a model VS-9160C humidified incubator (Vision Scientific, Gyeonggi-Do, Korea). When the desired time from the start of mixing (see Table 1) was reached, the hardened specimen was immersed in a sterilized glass bottle containing distilled water (DW). DW was chosen for extraction instead of serum-free cell culture medium to avoid medium-related analytic interference, which could lead to errors between analytic assays and biological tests.

An extraction ratio for the sample was set at 1 mL DW per 0.2 g of mixed cement according to international standards for irregularly shaped specimens [21]. Each sample was extracted at 37 °C for 24 h in a model SI-600R incubator shaker (Jeio Tech, Seoul, Korea) at 80 rpm. After filtration with a sterilized 0.20-μm nitrocellulose filter (HP045AN, Advantec, Tokyo, Japan), the extract was ready for analytical and biological analyses.

Concerning the extraction starting time for IRM, 3 min was set in order to mimic early exposure on dentin when cementation or filling is performed about 3 min after start of mixing. The middle stage and setting stage time was 6 min and 10 min, respectively, based on the manufacturers' instructions and the relevant ISO standard [22]. For Tubli-Seal, a time of 3 min (T3) was set to mimic early exposure on periapical lesion when filling was performed in root canal, typically about 3 min after start of mixing. A time of 20 min (T20) and 60 min (T60) was chosen to mimic the conditions of the middle stage and setting stage, respectively, based on the manufacturers' instructions and the ISO standard [23].