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Genotoxicity of dental resin polymerization initiators *in vitro*

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The polymerization initiators for resins cured using visible light usually consist of a photosensitizer, primarily camphorquinone (CQ), and a reducing agent, which is often a tertiary amine (DMPT, DMAEMA), while the initiator used for self-curing resins consists of benzoyl peroxide (BPO) and a tertiary amine (DMPT). The genotoxicities of camphorquinone (CQ), benzoyl peroxide (BPO), dimethyl-para-toluidine (DMPT), 2-dimethylamino-ethyl-methacrylate (DMAEMA), and 1-allyl-2-thiourea (ATU) were examined using the bioluminescent bacterial genotoxicity test. 4-Nitroquinoline-N-oxide (4NQO) was prepared for comparison with these chemicals. Acetone solutions of the five polymerization initiators and 4NQO were prepared.

Benzoyl peroxide (BPO), dimethyl-para-toluidine (DMPT), and 1-allyl-2-thiourea (ATU) showed significant genotoxic activity at 24 h in the bioluminescent bacterial genotoxicity test, at concentrations of approximately 5 μM, 4 mM, and 1 mM, respectively. 2-Dimethyloamino-ethyl-methacrylate (DMAEMA) did not have genotoxic activity and CQ had questionable genotoxic activity. In comparison, 4NQO had strong genotoxicity, at 4 μM, roughly the same as that of BPO. Therefore, BPO should be used carefully in clinical dentistry.

1. Introduction

Dental resins are widely used as restorative materials in dentistry due to their ease of handling and esthetic merits. However, drawbacks such as water sorption [1, 2] and dissolution of the residual monomer [3–6] have been reported. Moreover, leaching of the polymerization initiators, which are present in smaller amounts than the monomer, is problematic because of their toxicity [7–15].

For visible light (VL)-cured resins, the polymerization initiator usually consists of a photo-sensitizer, primarily camphorquinone (CQ), and a reducing agent, often tertiary amines (DMPT, DMAEMA), while that for self-curing resins consists of benzoyl peroxide (BPO) and a tertiary amine (DMPT).

Radicals are generated when CQ and the tertiary amines in the VL resin are irradiated, or when BPO is mixed with a tertiary amine. These radicals not only attack double bonds in the resin monomer, but also the double bonds of polyunsaturated fatty acids and phospholipids in living systems [9]. Consequently, the membranes in erythrocytes, lysosomes, mitochondria, and microsomes are damaged [10–15]. Moreover, these radicals can attack DNA. Consequently, these radicals have important genotoxic effects in living organisms.

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Genotoxicity can be examined using the Ames test [16–18]. In the 1980s, Ulitzur *et al*. [19–22] devised the bioluminescent bacterial genotoxicity test for genotoxic agents, and this was commercialized under the name Mutatox® (AZUR Environmental, US). The Mutatox test has been adopted as a rapid, simple alternative to the Ames test; however, samples subjected to the Mutatox test must be diluted, cultured, and measured individually using a special glass cuvette in a luminescence measurer [23–25]. Consequently, this test is laborious and time-consuming.

In 1999, Shiraishi *et al*. [26] simplified the method using 96-well plates to dilute, culture, and measure the samples, as well as a computer to process all the data, to allow the bioluminescent bacterial genotoxicity test to be used to monitor the genotoxicity of environmental materials.

The aim of this study was to determine the genotoxicity of polymerization initiators used in dental resins using the bioluminescent bacterial genotoxicity test as modified by Shiraishi *et al*. [26].

2. Materials and methods

2.1. Test chemicals

Five polymerization initiators, camphorquinone (CQ), benzoyl peroxide (BPO), dimethyl-para-toluidine (DMPT), 2-dimethylamino-ethyl-methacrylate (DMAEMA), and 1-allyl-2-thiourea (ATU) were purchased from Tokyo Kasei Co. (Tokyo, Japan) and used without further purification. 4-Nitroquinoline-N-oxide (4NQO, Kanto Chemical Co., Tokyo, Japan) was prepared for comparison. Acetone (Kanto Chemical Co., Tokyo, Japan) solutions of the five polymerization initiators and 4NQO were prepared for the bioluminescent bacterial genotoxicity test and Microtox® test.

2.2. Bioluminescent bacterial genotoxicity test

The bioluminescent bacteria used for this test were dark mutants of marine luminous bacteria (*Vibrio fischeri* M169, Microbics, Tokyo, Japan), which were incubated for 6 h at 27°C in Marine Broth 2216 (Difco, Tokyo, Japan). The bacterial concentration was kept at ca. 2×10^9 /ml by adding testing medium consisting of 0.4 g peptone, 3.5 g glycerol, 20.0 g NaCl, 29.0 g $MgSO_4{\cdot}7H_2O$, 0.9 g KCl, and 0.1 g K_2HPO_4 in 1000 ml of distilled water with 4.5 ml of 1 M MOPS buffer solution (Dojindo, Kumamoto, Japan).

The acetone solutions were diluted 50 fold with the testing medium and 40 μl was added to each well in the first file of a 96-well plate (Sumitomo Bakelite Co., Tokyo, Japan) with an 8-barrel pipette together with 160 μl of the test medium, while 100 μl of the test medium were added to the wells in rows 2 to 12. A 2-fold dilution series was created by removing 100 μl from each well in the first file and adding it to the adjacent well in the second file and repeating the process for each file. Consequently, the sample in the twelfth file was diluted 2^{-11} times compared with the first file. To each well were added 100 μl of the test bacteria solution to give a total volume of 200 μl. The plate was incubated at 27°C and the chemiluminescence intensity per second of each well was measured with a Luminescencer-2000 (ATTO Co., Tokyo, Japan) every 2 h, beginning 16 h after the beginning of incubation and ending 8 h later.

Since the chemiluminescence intensity is influenced by acetone, when the ratio (T/B) of the chemiluminescence of the chemicals (T) to that of acetone (B) exceeded 10, the chemical was considered genotoxic at that concentration.

2.3. Microtox test

The Microtox test is based on the principle that toxic substances inhibit the luminescence of luminous bacteria. This test is used to assess chemical toxicity and is viewed as a simple, rapid method for evaluating acute toxicity. The luminous bacteria *Photobacterium phosphoreum* (Microbics, Tokyo, Japan) was incubated in Marine Broth 2216 (Difco, Tokyo, Japan) for 6 h at 27°C.

Tests were conducted using a method similar to the bioluminescent bacterial genotoxicity test. A two-fold dilution series of the chemicals was established on 96-well plates with bacteria and testing medium. Chemical luminescence intensity was measured 5 min after mixing the materials by vortexing the 96-well plate, with a luminescence measurer. The toxicity of the chemicals was determined from the inhibition rate (%) of chemiluminescence intensity relative to the control (5% acetone). Then, a regression line was calculated from the concentration to show the quantity-response relationship and inhibition rate. Finally, the IC_{50} was calculated as the concentration resulting in 50% luminescence inhibition.

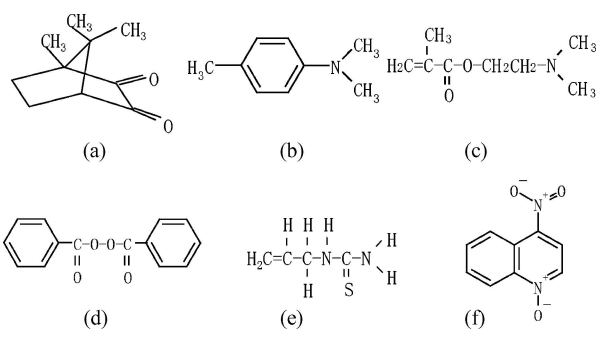

Figure 1 Molecule structure of five polymerization initiators: (a) camphorquinone; (b) dimethyl-para-toluidine; (c) 2-dimethylamino-ethyl-methacrylate; (d) benzoyl peroxide; (e) 1-allyl-2-thiourea, and (f) 4-Nitroquinoline-N-Oxide.

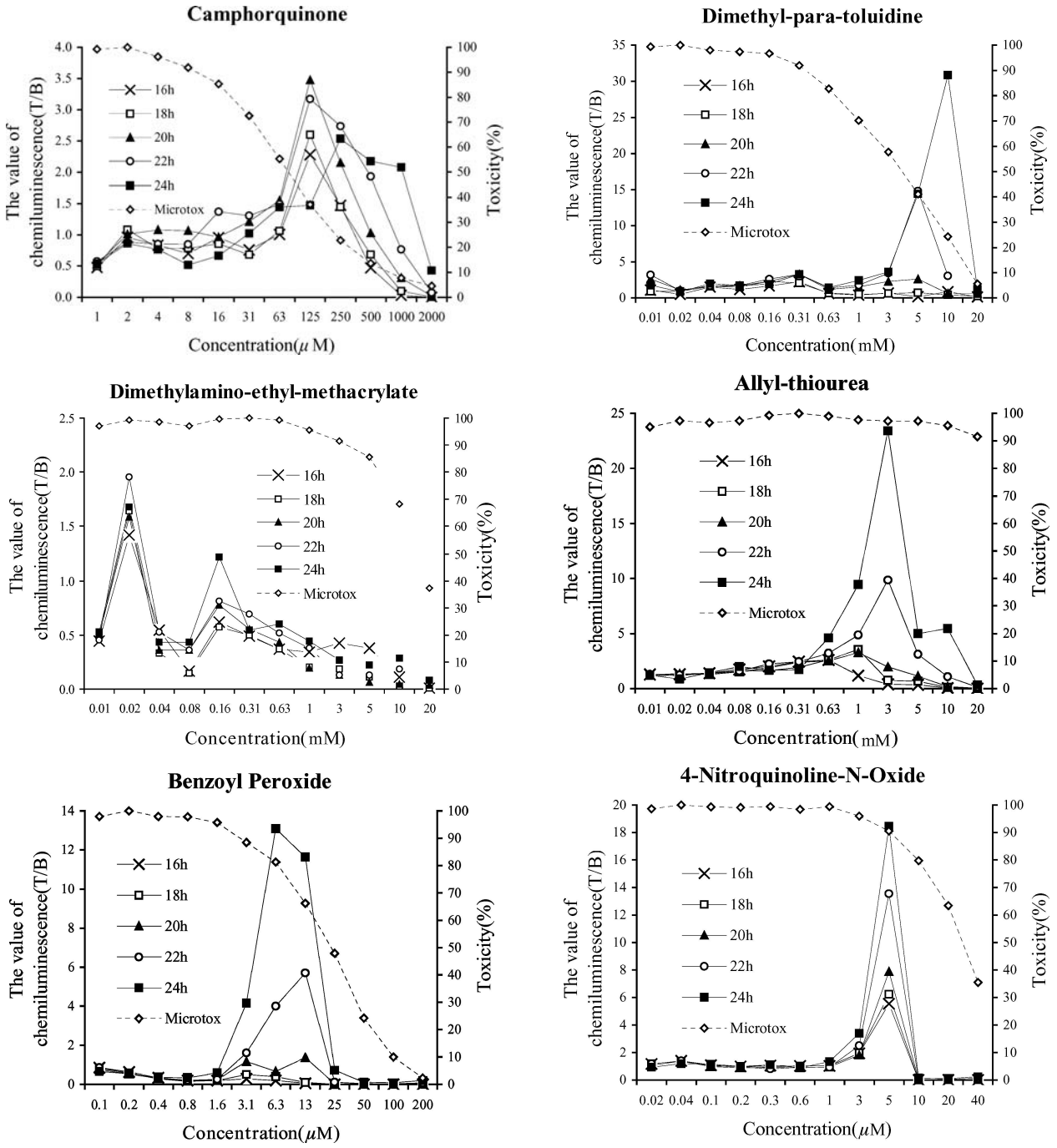

Figure 2 The genotoxicities and toxicities of the five polymerization initiators (camphorquinone, dimethyl-para-toluidine, dimethyloamino-ethyl-methacrylate, allyl-thiourea, and benzoyl peroxide) and 4-Nitroquinoline-N-Oxide.

3. Results

The genotoxicities and toxicities of the five polymerization initiators are summarized in Fig. 2. Benzoyl peroxide (BPO), dimethyl-para-toluidine (DMPT), and 1-allyl-2-thiourea (ATU) showed significant genotoxic activity at 24 h in bioluminescent bacterial genotoxicity test, at concentrations of ca. 5 μM, 4 mM, and 1 mM respectively. 2-Dimethylamino-ethyl-methacrylate (DMAEMA) did not show genotoxic activity. The chemiluminescence of camphorquinone (CQ) was about 3.5 (20 h) at concentrations $\geq$125 μM, implying that the toxicity to bacteria is marked at concentrations over 125 μM. Therefore, the genotoxic activity of CQ was doubtful in this experiment. By comparison, 4-nitroquinoline-N-oxide (4NQO) had strong genotoxicity at concentrations near 4 μM.

The toxicities (IC_{50}) of CQ, DMPT, DMAEMA, BPO, and 4NQO were approximately 80 μM, 4 mM,

15 mM, 20 μM, and 30 μM, respectively, while ATU showed no toxicity over the range studied.

4. Discussion

Various materials are used in dentistry, and their harmful effects on the living system are seen mainly as pulp damage, contact dermatitis, and allergic responses [27–29]. The work of Olea *et al*. [30] on detecting BPA in dental resin has led to recognition of the potential problems posed by endocrine disruptor chemicals in dentistry [31, 32]. An endocrine disruptor causes effects at very low concentrations and can have genotoxic effects at similar levels [19–22].

Dental resins usually contain about 0.2–1.5 wt% polymerization initiator relative to the monomer [33]. Unpolymerized polymerization initiators will diffuse into the mouth. Moin *et al*. [34] reported that the polymerization initiator (0.05–0.15 wt% of dental resin) diffused into an organic solvent (methanol) from the cured resin over a 24-h period. Therefore, it is important to evaluate the safety of polymerization initiators in comparison with other environmental chemicals.

The bioluminescence test can be used to evaluate three groups of genotoxic agents: (1) direct mutagens that are either base-substitution or frame-shift agents, (2) DNA-damaging agents and DNA-synthesis inhibitors, and (3) DNA-intercalating agents [19–22].

In this study, 4NQO was used as a positive control. 4NQO is very genotoxic; it is toxic at about 30 μM and genotoxic at about 4 μM.

BPO was toxic at about 20 μM and genotoxic at about 5 μM, making it similar to 4NQO. Therefore, careful attention should be paid to the use of BPO in dentistry. Compared with BPO, the toxicity of DMPT was about 4 mM and the genotoxicity about 4 mM, and the genotoxicity of ATU was about 1 mM. The genotoxicities of DMPT and ATU were thought to be weak. DMAEMA did not appear to be genotoxic, and the genotoxicity of CQ was doubtful.

Dental resins that are cured using polymerization initiators are very helpful and used widely in clinical dentistry. This study evaluated the genotoxic risks of polymerization initiators from dental resins *in vitro*; however, this may not be sufficient to determine whether these compounds are genotoxic. More data based on physiological and biochemical tests, and *in vivo* studies are needed.

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