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<th>Comparison of temporal changes in components of formalin guaiacol under several storage conditions</th>
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Abstract

This study examined the effects of storage conditions such as time course, temperature, fluorescent light, and darkness on the components and antibacterial activity of formalin guaiacol (FG) used in endodontic treatment. We measured the quantities of formaldehyde and guaiacol in FG and antibacterial activities against *Staphylococcus aureus*, *Porphyromonas gingivalis*, and *Porphyromonas endodontalis*. The components and antibacterial activity of FG in the brown or transparent tightly sealed containers were not affected by temperature or fluorescent light throughout the 4 week test. However, in the loosely sealed containers, formaldehyde and guaiacol in FG sample decreased remarkably within one week, not only in a temperature- and time-dependent manner, but also under fluorescent light at 20°C. Furthermore, the antibacterial activities in the FG sample were significantly attenuated in parallel with the decrease in formaldehyde levels. Fluorescent light caused color changes and crystallization of FG samples in the transparent containers. These results suggest that it is important to replace fresh FG every 5 to 7 days for endodontic treatment and that, in the dental office, it is advisable to store fresh FG in tightly sealed containers every 2 weeks to maintain its efficacy.

Key words: Formalin guaiacol—Antibacterial activity—Temporal changes

INTRODUCTION

Formalin guaiacol (FG), which is used as a root canal treatment, contains formaldehyde, guaiacol, and ethanol, which have an antibacterial activity, a sedative effect, and a solvent property, respectively. Some of these components are very volatile, so that it is critical to preserve the material appropriately for effective treatment. Several studies have shown that some anaerobic bacteria, especially *Porphyromonas gingivalis* and *Porphyromonas*
endodontalis, are closely related to the presence of acute symptoms in endodontic infections \(^{1,6,7,16}\). A change of the major components of FG may have an undesirable effect on antibacterial activity against endodontic pathogens and a sedative effect. It has been reported that the quantity of formaldehyde in formalin cresol, which is a root canal disinfectant, changed under storage conditions, \(e.g.,\) container, period, and temperature \(^{10,14}\).

Clearly, the storage of drugs, especially drugs containing volatile components, is very important in maintaining their efficiency. In this study, we investigated the effects of various storage conditions on the components and the antibacterial activity of FG, based on the results from questionnaires on the management of FG from approximately 200 dentists in Japan.

**MATERIALS AND METHODS**

1. **Chemicals**

   The following chemicals were used: FG (Neo Dental Chemical Products Co., Ltd., Tokyo, Japan), guaiacol (Wako Pure Chemical Industries Ltd., Osaka, Japan), formaldehyde (Wako Pure Chemical Industries Ltd.), 2,4-dinitrophenylhydrazine (DNPH; Nacalai Tesque Inc., Kyoto, Japan), hemin (Sigma Chemical Co., St. Louis, MO, USA), menadione (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), defibrillated horse blood (Kojinbio, Tokyo, Japan), and Trypticase soy agar (Becton Dickinson Systems Co., MD, USA). All other chemicals used were of analytical reagent grade.

2. **Glass containers**

   Two types of glass containers (5 ml) with screw caps were prepared for storing FG samples: one was brown, and the other was transparent (colorless). FG was divided into 0.5 ml aliquots and put into tightly or loosely sealed containers. A 0.5 ml aliquot of FG contains 75 mg formaldehyde, 200 mg guaiacol, 100 mg ethanol, 20 mg methanol, and 105 mg water. In the temperature test, the brown containers with/without tight seals were stored in a dark place at 4°, 20°, 30°, or 37°C. In the light test, the brown or transparent containers with/without tight seals were placed under fluorescent light at 15 W at 20°C. Throughout the 4 weeks of tests, the FG samples were sampled at one week intervals and used to measure the quantities of guaiacol and formaldehyde and their antibacterial activities.

3. **Analysis of guaiacol and formaldehyde in FG**

   FG solutions were diluted a hundred fold with methanol for measuring the quantities of guaiacol and formaldehyde. For guaiacol analysis, 10 µl of diluted sample was directly injected into high performance liquid chromatography (HPLC) \(^{15}\). For formaldehyde analysis, 100 µl of diluted sample was mixed with 100 µl of 2 mM DNPH dissolved in 0.5 M sulfuric acid \(^{13}\). After 1 min, 10 µl of this mixture was injected into the HPLC. The HPLC system consisted of a delivery pump (LC6A; Shimadzu Co., Kyoto, Japan), a reverse-phase column (Chemcosorb 7-ODS-H, 4.6×250 mm i.d.; Chemco Scientific Co., Osaka, Japan) maintained at 40°C, and an UV spectrophotometric detector (SPD-6A; Shimadzu Co.). The condition for measurements of guaiacol and formaldehyde is shown in Table 1. Containers were sequenced and weighed before and after filling FG (0.5 ml). Then, the altered weight of FG at each time point was calculated by the difference between the total weight and the container’s own. The net quantity of guaiacol or formaldehyde included in the altered FG was calculated using the equation shown in Fig. 1.

4. **Antibacterial activity test**

   Preparing culture media and measuring antibacterial activity were performed as described by Ishihara et al. \(^{8}\). Briefly, the medium consisted of Trypticase soy agar (30 g/liter) supplemented with 5 µg/ml hemin, 0.5 µg/ml menadione, and 10% defibrillated horse blood. The Trypticase soy agar was autoclaved and cooled to 50°C, and sterile hemin, menadione, and defibrillated horse blood were
Table 1 Analytical conditions for guaiacol and formaldehyde in HPLC system

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<tr>
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<th>Guaiacol</th>
<th>Formaldehyde</th>
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<tr>
<td>Mobile phase</td>
<td>methanol:water (60:40)</td>
<td>acetonitrile:water (55:45)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8 ml/min</td>
<td>1.0 ml/min</td>
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<tr>
<td>UV wavelength</td>
<td>270nm</td>
<td>350nm</td>
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\[
\text{Net quantity of FA or GA} = \frac{\text{StC(\(\mu\)g)} \times \text{AS(FA) or (GA)}}{\text{AS(FA) or (GA)}} \times \frac{\text{altered weight of FG}}{1,000}
\]

StC: standard concentration when injected into HPLC, AS: area of sample peak, ASt: area of standard peak, (FA): formaldehyde, (GA): guaiacol, altered weight of FG: FG quantity at each time point.

Fig. 1 A formula for calculating net weight of formaldehyde or guaiacol at each time point.

added. The paper disc method was used to measure the antibacterial activity of FG against Staphylococcus aureus, Porphyromonas gingivalis, and Porphyromonas endodontalis. The sterilized filter paper (6 mm, i.d.) was put into the center of Trypticase blood agar plate (90×15 mm) suspending bacterium at approximately 10⁷ cells per ml, and 8μl of FG sample was dropped onto it. Two Porphyromonas species were incubated for 96 hr at 37°C in an anaerobic chamber gassed with 10% CO₂-10% H₂-80% N₂, whereas S. aureus was incubated for 48 hr in an aerobic atmosphere at 37°C. The incubation was carried out on triplicate plates. The diameter in the blocking area was accurately measured using a slide caliper. Antibacterial activity was indicated by the percentage, with the zero week condition (control) taken as 100%.

RESULTS

1. Effects of storage conditions on the components of FG

In the brown or transparent tightly sealed containers, the quantities of formaldehyde and guaiacol in FG samples were not affected by temperature, fluorescent light, or darkness even after 4 weeks. When the loosely sealed containers were stored in the dark, the quantities of formaldehyde decreased in a temperature and time dependent manner (Fig. 2). The percentages remaining at 4°, 20°, 30°, or 37°C were respectively as follows: in the 1st week, 87, 92, 81, 68%; in the 2nd week, 83, 74, 70, 14%; in the 3rd week, 73, 49, 56, 5.6%; in the 4th week, 69, 30, 26, 0%. The quantities of guaiacol also decreased, but the decreasing ratios were smaller than those of formaldehyde (Fig. 3). When the brown or transparent loosely sealed containers were placed under fluorescent light at 20°C, formaldehyde and guaiacol also decreased to 30% and 72% of the control in the brown containers and to 22% and 77% in the transparent containers after 4 weeks. There were no differences in the decreases in two compounds between the dark and fluorescent light at 20°C.

2. Effects of fluorescent light on the visual changes in FG

In the brown tightly sealed containers, the appearance of the FG solution did not change under any conditions. Fluorescent light caused color changes and crystallization in FG samples in both loosely and tightly sealed transparent containers.
3. Temporal changes in antibacterial activities of FG

In the brown or transparent tightly sealed containers, there were no differences in the antibacterial activities against *S. aureus*, *P. gingivalis*, and *P. endodontalis* under any conditions of temperature and fluorescent light. Figure 4 shows the results of FG samples stored under fluorescent light. However, in the loosely sealed containers, the antibacterial activities were significantly attenuated in parallel with the decrease in formaldehyde levels (Fig. 5).

**DISCUSSION**

In this study, we clarified that it is very important to store FG in an airtight container shaded from light for preventing loss of components and antibacterial activity.

In the temperature test, storage in the loosely sealed containers at 37°C rapidly reduced the quantity of formaldehyde and attenuated the antibacterial activity against endodontic pathogens, *P. gingivalis* and *P. endodontalis* (Figs. 2 and 5). The quantities of guaiacol also decreased in a temperature and time dependent manner, but the decreasing ratios were smaller than those of formaldehyde (Fig. 3). This result indicates that guaiacol is relatively more stable to temperature than formaldehyde. In the tightly sealed containers, however, the quantities of formaldehyde and guaiacol did not decrease even at 37°C for 4 weeks. These results are consistent with the view reported previously for formaldehyde in formalin cresol. Our findings suggest that sealing the container tightly was the most significant factor in preventing loss of the volatile components in the FG and that temperature (4° to 37°C) was not a direct factor; however, it could influence loss of the volatile components.

Fluorescent light includes ultraviolet (253.7 nm), which can induce photochemical reactions. Our findings demonstrated that fluorescent light did not affect the quantity of volatile components in the tightly sealed containers (Fig. 4); it was involved only in changes in the nature of the components such as coloring and crystallization in the transparent containers.

In the experiment examining the antibacterial activity against *S. aureus*, *P. gingivalis*, and *P. endodontalis*, the loosely sealed containers lost their effectiveness in parallel with the...
decrease in the quantity of formaldehyde, which itself has a strong antibacterial activity\(^3,4\) (Fig. 5). It is generally recognized that formaldehyde also has a tissue stimulating action\(^17\) and a carcinogenic activity\(^2,9,12\). However, some research has demonstrated that the carcinogenesis of formaldehyde is closely related to dose and time dependency. Kerns et al.\(^12\) reported that, when rats were exposed by inhalation to 22.4\(\mu g\)/kg of formaldehyde gas.
6 hr/day, 5 days/week, for 24 months, only a 0.8% incidence of squamous cell carcinomas was induced in the nasal cavities, although a 44% incidence was observed at the higher exposure dose of 57.2 g/kg. No nasal tumors were observed in the lower exposure groups (<8 g/kg). A number of studies have also shown that formaldehyde inhalation was weakly associated with an increase in the frequency of polypoid adenomas at its lower concentration. On the other hand, the dosage and period of formaldehyde used for the dental treatment are extremely smaller and shorter than those in carcinogenic experiments. Practically, when FG containing 15% formaldehyde is used to treat a patient with body weight of 50 kg for the endodontic treatment at a dose of 5 µl/day, 1 day/week, for 1 month, the total amount of formaldehyde used is about 60 µg/kg. This value corresponds to a volume of 1:500 compared to that of in vivo experiments reported by Kerns et al. and is below the carcinogenic threshold.

Guaiacol scavenges superoxide radicals and activates the cell proliferation at very low concentrations (10^{-12} to 10^{-10} M). This result indicates that guaiacol greatly contributes to reconstruction of the injured tissues. Moreover, guaiacol may play a role of relieving the irritation and carcinogenesis of formaldehyde. Consequently, FG may be regarded as a safe therapeutic agent in the dental treatment.

From these results, we conclude that, when being applied for endodontic treatments, we strongly recommend that FG should be exchanged in the root canal for fresh FG every 5 to 7 days. Moreover, in the dental office, it is advisable to exchange FG in a tightly sealed container for fresh FG every 2 weeks to maintain the efficiency of FG, and it should be kept at a low ambient temperature.

REFERENCES


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