Dentin Is Dissolved by High Concentrations of L-Ascorbic Acid 2-[3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-yl-hydrogen Phosphate] Potassium Salt with or without Hydrogen Peroxide

Masahiro KOZUKA and Yasuhisa TSUJIMOTO*

Research Institute of Oral Science, Department of Endodontics, Nihon University School of Dentistry at Matsudo; 870–1 Sakaecho, Nishi-2, Matsudo, Chiba 271–8587, Japan. Received October 27, 2003; accepted February 17, 2004

L-Ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-yl-hydrogen phosphate] potassium salt (EPC-K_1) is a conjugate of vitamin C and vitamin E that is water-soluble and stable at room temperature. EPC-K_1 has been developed as a hydroxyl radical (·OH) scavenger and antioxidant. In a previous tooth whitening experiment, it was accidentally found that tooth (dentin) blocks were dissolved by EPC-K_1 with H_2O_2. In the current study, high concentrations of EPC-K_1 (2.5, 25 mM) with 3% H_2O_2 dissolved and caused the collapse of dentin blocks. Similar concentrations of EPC-K_1 without 3% H_2O_2, however, dissolved the dentin blocks without collapse over a 3-week period. In these cases, a ·OH-like signal was detected using an ESR spin-trapping method. The volume of calcium in solution (including the dentin block) increased on the addition of EPC-K_1 in a concentration-dependent manner. In addition, the calcium : phosphorus ratio changed from 2 : 1 in sound dentin to 1 : 2 in the collapsed dentin block. High concentrations of EPC-K_1 are therefore considered to have calcium chelating and dentin dissolving activity. The dentin dissolving activity was enhanced when EPC-K_1 was used with H_2O_2. EPC-K_1 had no protective effect when used in tooth whitening with H_2O_2.

Key words dentin; hydroxyl radical; ESR

In a previous study, it was reported[1,2] that hydroxyl radicals (·OH) generated *via* hydrogen peroxide (H_2O_2) were responsible for tooth whitening. However, the detailed mechanism(s) of tooth whitening have not yet been elucidated. It is well known that ·OH are scavenged by ·OH scavengers such as methanol, dimethyl sulfoxide (DMSO) and L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-1-benzopyran-6-yl-hydrogen phosphate] potassium salt (EPC-K_1).[3—11] EPC-K_1, a conjugate of vitamin C and vitamin E that is water-soluble and stable at room temperature, was developed as a ·OH scavenger and antioxidant agent by Senju Pharmaceuticals (Osaka, Japan).

In the tooth whitening pilot study, we used EPC-K_1 to investigate the relationship between whitening effect and ·OH. At that time we accidentally found that the tooth (dentin) blocks were dissolved by H_2O_2 with EPC-K_1. EPC-K_1 has been developed as an antioxidant for the living body. There is a huge problem if EPC-K_1 is able to dissolve dentin blocks, because tooth components are almost the same as bone. If damage to organic and/or inorganic components occurs with EPC-K_1, the components of the human body will become unbalanced.

The aim of the current study is to examine the effects of EPC-K_1 with and without H_2O_2 on dentin blocks over time.

MATERIALS AND METHODS

Chemicals EPC-K_1 was kindly provided by Senju Pharmaceuticals (Osaka, Japan). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), which is used as a trapping agent for free radicals, was obtained from Dojin Chemicals (Kumamoto, Japan). Ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA), used as an irrigant for the removal of a smear layer, was obtained from Dojin Chemicals (Kumamoto, Japan). H_2O_2 which is used as a tooth whitening agent, and ethanol and DMSO, which are used as ·OH scavengers, were obtained from Wako Pure Chemical Industries, Ltd., (Osaka, Japan).

Dentin Blocks The dentin blocks were prepared as described by Tsujimoto *et al.*[12] Four blocks were obtained from the root of an extracted human single cone tooth. The pulp and smear layers were removed by file, then 15% EDTA was applied on the dentin wall for 2 min. The sample was washed with pure water (PW) and blot dried with Kimwipe (Kimberly-Clark Co. Ltd., Japan) paper. The sample was then weighed. Three blocks were used for each examination.

Free Radical Measurement The ESR spin-trapping method[11,13,14] was used to measure free radical generation from each solution. The final concentration of DMPO in 200 μl of the reaction mixture was adjusted to 89.0 mM for this experiment. The ESR spectra were measured using a JES FA 300 (JEOL, Tokyo, Japan). The measurement conditions were as follows: microwave power, 8 mW; magnetic field, 335.0±5 mT; sweep time, 2 min; modulation frequency, 100 kHz; and time constant, 0.03 s.

Calcium Volume Measurement The calcium volumes of each solution around the dentin blocks were measured over time using an inductively coupled plasma atomic emission spectrometer (SPS 1700R; Seiko Instrument Inc., Tokyo, Japan). The measurement conditions were as follows: modulation frequency, 27.12 MHz; microwave power, 1.30 kW; plasma flow (argon gas), 16.0 l/min.

Changes in pH The pH of each solution was measured using an ISFET pH Meter KS723 (Shindengen Electric Manufacturing Co., Ltd., Tokyo, Japan).

Wet Tooth Block Weight The wet tooth block weight was measured using a R2000D electric scale (Sartorious, Germany).

* To whom correspondence should be addressed. e-mail: tsuji@mascat.nihon-u.ac.jp

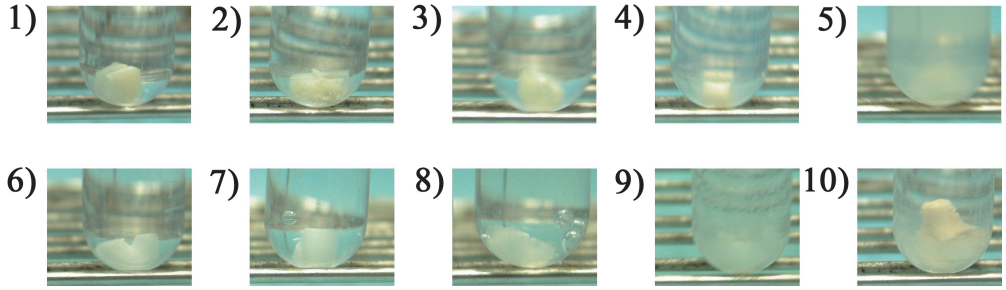

Fig. 1. The Conditions in Each Solution Including Dentin Block

(1) PW, (2) 0.025 mM EPC-K_1, (3) 0.25 mM EPC-K_1, (4) 2.5 mM EPC-K_1, (5) 25 mM EPC-K_1, (6) 3% H_2O_2, (7) 3% H_2O_2 with 0.025 mM EPC-K_1, (8) 3% H_2O_2 with 0.25 mM EPC-K_1, (9) 3% H_2O_2 with 2.5 mM EPC-K_1, (10) 3% H_2O_2 with 25 mM EPC-K_1.

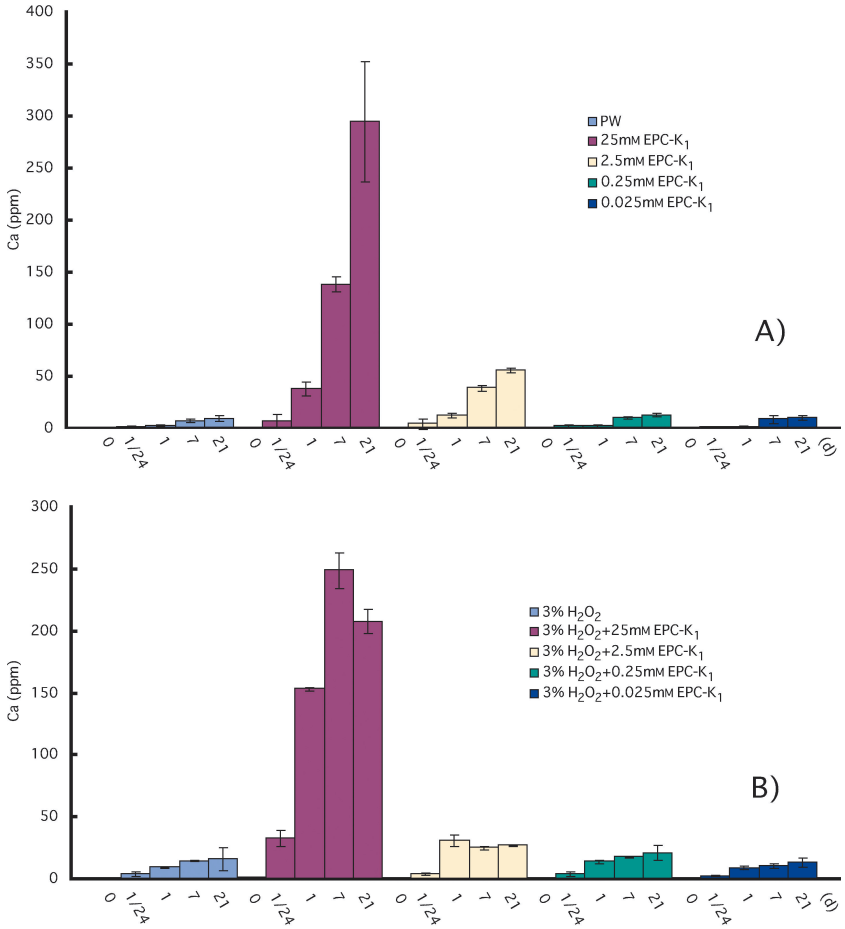

Fig. 2. Relationship between Each Solution and Calcium Volume

(A) Calcium volume in PW with/without each concentration of EPC-K_1. (B) Calcium volume in H_2O_2 with/without each concentration of EPC-K_1.

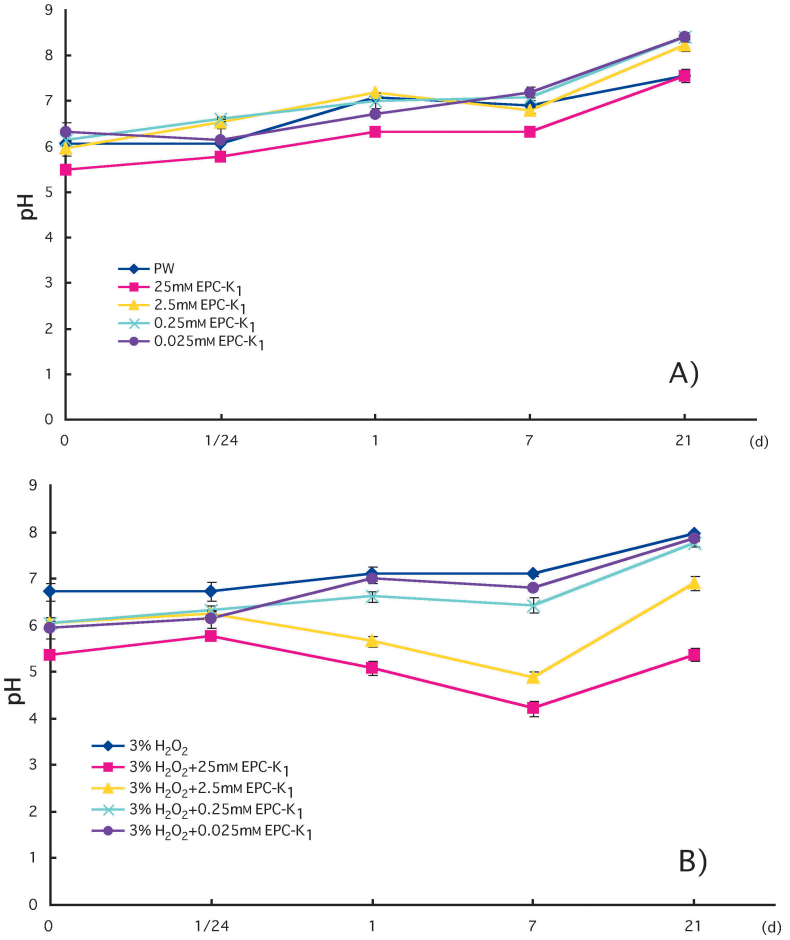

Fig. 3. Changes in pH

(A) PW with/without each concentration of EPC-K_1. (B) H_2O_2 with/without each concentration of EPC-K_1.

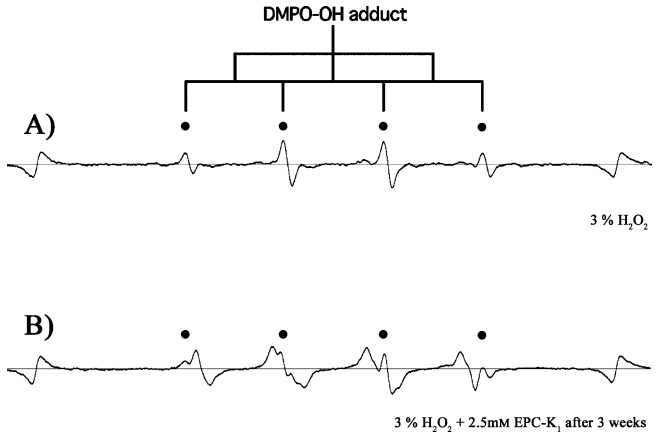

Fig. 4. Typical ESR Signal

(A) ·OH signal from 3% H_2O_2. (B) ·OH-like signal from 3% H_2O_2 with 2.5 mM EPC-K_1.

RESULTS

State of the Dentin Blocks The typical state of the dentin blocks in each solution after 3 weeks is shown in Fig. 1 (results at 1 and 2 weeks not shown). The dentin blocks and solutions in PW and EPC-K_1 showed little change after 3 weeks; however, the color of the solutions in 2.5 and 25 mM EPC-K_1 changed to milk-white. The color of the dentin blocks became white after 3 weeks in 3% H_2O_2 and 3% H_2O_2 with EPC-K_1. However, after 3 weeks in 3% H_2O_2 with 2.5 and 25 mM EPC-K_1 the dentin blocks had collapsed. An exceptional amount of collapse was observed in 3% H_2O_2 with 25 mM EPC-K_1 after 3 weeks.

Calcium Volume of the Solutions The calcium volume in the solutions is shown in Figs. 2A and B. Calcium volume increased after the addition of EPC-K_1 in a concentration-dependent manner. An exceptional amount of calcium was detected after the addition of 25 mM EPC-K_1.

Changes in pH The change in pH of each solution (including the dentin block) is shown in Fig. 3. In the case of EPC-K_1, the pH of the solution (approximately pH 6) increased gradually after mixing. After 3 weeks, the pH was approximately 8 (Fig. 3A). The pH of the solution was approximately 6 after mixing with 3% H_2O_2 and differing concentrations of EPC-K_1. The pH increased to pH 7—8 in 3% H_2O_2, 3% H_2O_2 with 0.025 mM and 0.25 mM EPC-K_1. However, in 3% H_2O_2 with 2.5 and 25 mM EPC-K_1 the pH decreased to pH 4—5 at 1 week and then increased to pH 6 after 3 weeks (Fig. 3B).

Wet Dentin Block Weight The wet dentin block weight showed little change except when 3% H_2O_2 was used with 25 mM EPC-K_1 for 3 weeks. This happened because the dentin block collapsed (data not shown).

Analysis of Collapsed Dentin Blocks The calcium: phosphorus ratio of the collapsed tooth block was measured using an inductively coupled plasma atomic emission spectrometer (SPS 1700R; Seiko Instrument Inc., Tokyo, Japan). The calcium : phosphorus ratio was found to be 1 : 2 in 3% H_2O_2 with 25 mM EPC-K_1.

Free Radical Measurement Generated free radicals were measured using an ESR spin-trapping method.[11,13,14] ·OH were detected from 3% H_2O_2 (Fig. 4), and a carbon center radical (·C) was detected when ethanol or DMSO was added to 3% H_2O_2 (data not shown). This means that free ·OH was produced followed by conversion to the DMPO-C-center.[15] ·OH was not detected with every concentration of EPC-K_1, however ·OH-like signals were observed at 3 weeks with high concentrations of EPC-K_1 (2.5, 25 mM) (Fig. 4). ·OH-like signals were also detected at 3 weeks in H_2O_2 solutions with 2.5 and 25 mM EPC-K_1. Higher ·OH-like signals were detected with 2.5 and 25 mM EPC-K_1 with H_2O_2 than without H_2O_2.

DISCUSSION

EPC-K_1 is composed of vitamin C and vitamin E joined by a phosphodiester linkage. EPC-K_1 has been reported to have potent ·OH scavenging activity, anti-lipid peroxidation activity,[10] and can chelate iron and copper.[11] In addition, Kuribayashi *et al.*[16] suggested that EPC-K_1 might be useful in the treatment of ischemia-reperfusion. It was thought that EPC-K_1 could be used to treat the inflammation of dental pulp and gingiva, and in tooth replantation and transplantation. It has not been reported what kind of effect EPC-K_1 has on the tooth component. As mentioned above, we found accidentally that EPC-K_1 dissolved dentin during the tooth whitening experiment. It is therefore very important to clarify the effects of EPC-K_1 before it is applied clinically.

The results of the current study show that high concentrations of EPC-K_1 (2.5, 25 mM) with 3% H_2O_2 dissolved and caused the collapse of dentin blocks. When the pH of the solution was measured, it was found to be pH 5—6 for 24 h, then decreased to pH 4—5 in the first week, and then rose after 3 weeks to pH 5.5—7.

High concentrations of EPC-K_1 (2.5, 25 mM) without 3% H_2O_2, on the other hand, dissolved the dentin blocks without causing a collapse. In this case, the initial pH was approximately 6 and then increased gradually to pH 8 after 3 weeks.

The ·OH was generated from H_2O_2 *via* Fenton reaction[17,18] because of H_2O_2 generally includes a small volume of metal in the manufacture process. In this experiment, the EPC-K_1 concentrations ranged from 0.025—25 mM. This was because the ·OH generated from 3% H_2O_2 was completely eliminated by the addition of 0.025—25 mM EPC-K_1 in a preliminary study (data not shown). The pH experiment is an important aspect because teeth are dissolved by acidic solutions such as phosphoric acid, which is used as a tooth etching agent. The pH was not dependent on dentin being dissolved by EPC-K_1; the pH decreased to pH 4—5 in the first week and then rose to pH 5.5—7 after 3 weeks when high concentrations of EPC-K_1 with H_2O_2 were used. This observation is difficult to explain, although it is suggested that the dissolving of dentin by EPC-K_1 is enhanced by H_2O_2. A further study to clarify this issue is currently underway.

In a previous tooth whitening study,[2] the ·OH generated from H_2O_2 degraded the amino acids that make up organic matter, although the structure of hydroxyl apatite, like the inorganic material in dentin, did not change after application of H_2O_2. The weight of the dentin block decreased after application of H_2O_2, but the calcium volume did not change. It has been suggested that the ·OH generated from H_2O_2 degraded the organic matter of dentin, and then destroyed its structure. In this study, a ·OH-like signal was detected with 2.5 and

25 mM EPC-K_1 with and without H_2O_2. In addition, dentin bleaching (whitening) by the ·OH generated from H_2O_2 was not prevented by the addition of EPC-K_1, a ·OH scavenger. There have been no reports on free radical generation from EPC-K_1 solutions, and it is unknown how ·OH-like radicals are produced. There is no evidence that the ·OH-like signal influences the dissolving of dentin, and this phenomenon therefore requires clarification in a further study.

In sound dentin, the calcium : phosphorus ratio is usually 2 : 1, but in this study the calcium : phosphorus ratio was 1 : 2 in the collapsed tooth blocks treated with 25 mM EPC-K_1 with 3% H_2O_2. It is suggested that EPC-K_1 not only chelates iron and copper, as reported previously,[10,11] but can also chelate calcium.

From the results of this study, it is suggested that H_2O_2 generates ·OH which attack an organic material, such as dental collagen, and that the calcium component of dentin was lost over time by the chelating activity of EPC-K_1. It is therefore evident that high concentrations of EPC-K_1 should not be used for long periods of time during tooth replantation, treatment of periapical lesions, tooth transplants and implants. This is because the alveolar bone might be damaged by EPC-K_1. High concentrations of EPC-K_1 for protection against bone resorption and/or the chelation of calcium from bone or teeth should be used with caution.

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