Blue Light from Dental Resin Curing Unit Causes Light-Induced Vasoconstriction in Isolated Rat Aorta

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Abstract

Aims: Currently, the blue light of resin curing unit is frequently used in dental treatment. However, the influence of the blue light irradiation on oral tissue is not clear. The aim of this study was to elucidate the effect of dental blue light irradiation on vascular smooth muscle.

Methods: Isolated rat descending aorta was suspended in a superfusion chamber and superfused continuously with Krebs-Ringer solution. The changes in isometric force of blue light irradiation were assessed using vessel strips superfused with the Krebs-Ringer solution alone or the Krebs-Ringer solutions containing 10 μM phentolamine, 100 mM dimethyl sulfoxide, 10 unit/mL superoxide dismutase, or 5 mM L-histidine. The reactive oxygen species (ROS) scavenging activity of antioxidants was examined using the electron spin resonance technique respectively.

Results: We first demonstrated that the vasoconstriction was induced by irradiated of blue light using dental resin curing unit. The vasoconstriction was inhibited by including in the Krebs-Ringer solution an α-receptor blocker that inhibits the neurotransmitter. This phenomenon was controlled with the addition of ROS scavengers.

Conclusions: Blue light irradiation of multiple times in dental treatment might have the potential to accelerate aging of pulpal blood vessel from ischemia through dental pulp vasoconstriction via ROS generation. In addition, prolonged and/or repeated blue light irradiation could cause ROS-induced oxidative stress such as ischemia-reperfusion injury on pulpal blood vessel. Therefore, pre-intake of antioxidants is suggested to avoid effects such as aging of dental pulp due to blue light irradiation-induced ROS in dental clinic treatments.

Key Words: Blue light, Reactive oxygen species, Dental curing lights, Vasoconstriction, Antioxidant

Introduction

It is common to use a dental resin curing blue light during treatments in dental clinics. These dental resin curing units were produced with the development in 1965 of a light-excited polymerization composite resin to improve the disadvantages that chemical-cured composite resins impose on operability [1,2]. In 1973, Ultraviolet (UV) rays were being used as curing lights. To settle problems with UV light, including biological toxicity and optical polymerization depth, the current dental resin curing unit using visible light was developed in 1980 [3,4].

Current dental resin curing units use Quartz Tungsten Halogen (QTH), xenon, or Light Emitting Diodes (LED) as the illuminator. The peak wavelength of any of these units stands between 400–510 nm [5]. With the rapid development of cosmetic dental restorative techniques, dental resin curing blue light dramatically increased the use of light units to photo-cure resin composites. The standard intensity of QTH units has been approximately 600 mW/cm^2, which can adequately cure most dental composites to a depth of 2 mm in approximately 40 s [6,7].

In ophthalmology, light with a wavelength of 380–530 nm is called high-energy visible light [8]. This wavelength range of blue light has been shown to cause photo-aging and age-related macular degeneration of the retina. This blue light effect is noted by a "Blue Light Hazard", and the use of blue light protective goggles has been proposed [9,10]. The wavelengths between 400–510 nm are commonly used in the dental resin curing unit. Hence, the eyes of the clinician and the patient are protected with goggles during treatment with blue light. However, the defenses of maxillofacial tissues except eyes have not been considered, and there are few reports that concern the influence of blue light on oral tissues.

In recent years, blue light irradiation has been applied for 10 min or more using resin curing light as an in-office tooth bleaching technique [6,11,12]. Therefore, it is important to consider not only the influence on dental materials, but, also, on dental pulp. It was reported that the temperature of teeth

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rose with increasing irradiation time for every photo-curing unit [5,6].

Dental pulp is rich in nerve fibers and blood vessels. It has been reported that vasodilation in the pulp of a closed system surrounded by hard tissue such as dentin can influence the pressure in the dental pulp cavity and induce intense acute tooth pain [6,13]. Furthermore, light of various wavelengths has been recognized to induce vasodilation or vasoconstriction in the regulation of vascular smooth muscle circulation [6,14]. A few studies have also shown that irradiation of mammalian cells with visible light induces cellular damage primarily by generating Reactive Oxygen Species (ROS) [6,14-16]. We have already reported that blue light irradiation toward gingival fibroblasts and vascular smooth muscle cells induce ROS generation. Although we have shown that the first target of the blue light irradiation is the mitochondria, causing apoptosis, the kinds of functional changes that are caused by blue light irradiation of these tissues remain unclear [6,17]. In this study, we first demonstrated that ROS-dependent Noradrenaline (NA) release induced by the blue light irradiation from the dental resin curing unit causes vasoconstriction.

Materials and Methods

Reagents

5-(2,2-dimethyl-1,3-propoxycyclophosphoryl)-5-methyl-1-pyrroline-*N*-oxide (CYPMPO) was purchased from Radical Research (Tokyo, Japan). Superoxide dismutase (SOD), 2,2,6,6-tetramethyl-4-piperidinol (4-OH-TEMP) and L-histidine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rose bengal, H_2O_2, titanium (IV) oxide, anatase form (TiO_2), and dimethyl sulfoxide (DMSO) were purchased from Wako Chemicals (Osaka, Japan). Phentolamine mesilate was obtained from Novartis Pharma K.K. (Tokyo, Japan). All reagents were of analytical grade.

Animal and aorta preparation

Seven-week-old male Wistar rats were purchased from Japan SLC (Shizuoka, Japan). Animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed access to food and water *ad libitum*. Our previous protocol for the preparation of the vessels was modified according to the following procedure [18]. Descending aortas were taken from rats under anesthesia with sodium pentobarbital (50 mg/kg, *i.p.*). The aortas were surgically removed and placed into cold Krebs-Ringer solution with the following composition in millimoles per liter: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$, and 11.0 glucose, aerated with 95% O_2-5% CO_2 (pH 7.4). The vessels were cleaned of adherent connective tissue and cut into helical strips (0.2 cm width, 1.5 cm length).

The procedures used in this study were in accordance with the guidelines of the US National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication NO. 85–23, revised 1996) and the protocols were approved by our Kanagawa Dental University Graduate School Institutional Animal Care Committee (Yokosuka, Japan).

Light unit and superfusion measurements

A QTH unit (Jetlite 1000, J. Morita USA Inc., Irvine, CA, USA) was used to irradiate the vessels from a distance of 1.0 cm and was filtered to provide blue light with wavelengths between 400–520 nm. The effect of blue light irradiation was determined using a superfusion technique described previously [18,19]. A helical strip of vessel was suspended in a jacketed (37°C) superfusion chamber and superfused continuously (1.5 mL/min) with aerated (95% O_2, 5% CO_2) Krebs-Ringer solution. The strips were connected to a force transducer MLT050/A (ADInstruments, Colorado, USA) and changes in isometric force signals were converted to digital signals by Power Lab 2/20 (ADInstruments, Colorado, USA). These were recorded onto a computer through the recording software Chart v5.01 (ADInstruments, Colorado, USA). Sampling commenced after a 100 min equilibration period, consisting of three 30 min periods where resting tension was set at 3.0 g followed by 10 min set at 0.0 g [18]. The changes in isometric force caused by the blue light irradiation were recorded for 10 min after a basal recording of 10 min (*Figure 1*). The control was recorded without the blue light irradiation for 10 min. The effects of blue light irradiation were assessed using vessel strips superfused with the Krebs-Ringer solution alone or the Krebs-Ringer solutions containing 10 μM phentolamine, 100 mM DMSO, 10 unit/mL SOD, or 5 mM L-histidine, respectively. The treatment of these reagents

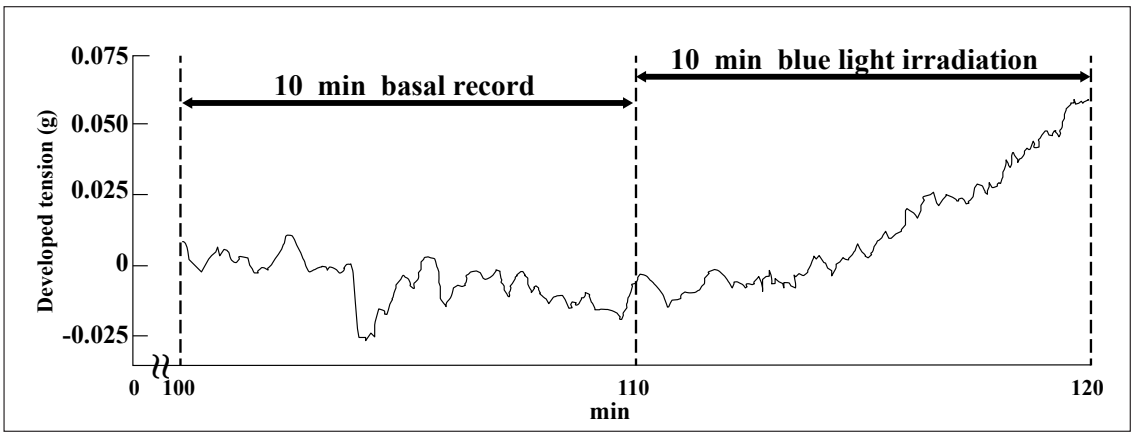

Figure 1. *Typical superfusion chart of vessels irradiated with blue light.*
A helical vessel strip was suspended in a jacketed (37°C) superfusion chamber and superfused continuously (1.5 ml/min) with aerated (95% O_2, 5% CO_2) Krebs-Ringer solution. The strips were connected to a force transducer and changes in isometric force signals were recorded on a computer. Sampling commenced after a 100 min equilibration period. The changes in isometric force were recorded with irradiation by the blue light for 10 min after a 10 min basal record.

were applied for 20 min from basal record (100-110 min) to the completion of blue light irradiation (110-120 min).

***In vitro* electron spin resonance (ESR) measurements**

Hydroxyl Radicals ($HO^{\bullet}$) were generated by H_2O_2 irradiated with UV light (emission: 310–400 nm, 20 s; 400 mW/cm^2; SUPERCURE-203S, RU-360, Radical Research, Tokyo, Japan) using CYPMPO as an $HO^{\bullet}$ spin trap, as previously described [20-22]. Superoxide radicals ($O_2^{\bullet-}$) were generated by TiO_2 photocatalysis (UV, emission: 310–400 nm, 60 s; 100 mW/cm^2; SUPERCURE-203S, RU-360, Radical Research, Tokyo, Japan) with H_2O_2 also described previously [23]. We verified the generation of singlet oxygen ($^1O_2$) by the photochemical reaction of rose bengal illuminated for 5 min (550 nm, 18,000 lux) [24,25]. ESR spin trapping was conducted with the ROS-generating system containing CYPMPO as a spin trap for $O_2^{\bullet-}$ or $HO^{\bullet}$, 4-OH-TEMP as a spin trap for $^1O_2$ [26]. ESR was performed with a JES-RE1X (JEOL, Tokyo, Japan) connected to a WIN-RAD ESR Data Analyzer (Radical Research, Tokyo, Japan) at the following instrument settings:

(1) $O_2^{\bullet-}$ and $HO^{\bullet}$: microwave power, 8.00 mW; magnetic field, 335.6 ± 7.5 mT; field modulation width, 0.079 mT; sweep time, 1 minute; and time constant, 0.03 s,

(2) $^1O_2$: microwave power, 8.00 mW; magnetic field, 335.6 ± 5.0 mT; field modulation width, 0.1 mT; sweep time, 1 minute; and time constant, 0.03 s. All experiments were repeated a minimum of three times.

Statistical analysis

Results are expressed as a mean ± standard deviation. Dunnett's multiple comparison test and Student's *t*-test were used for statistical analysis. A P-value of less than 0.05 was considered statistically significant.

Results

Tension developed by blue light irradiation in rat aorta and the effect of phentolamine

We examined the tension that developed in the rat aorta during irradiation with the blue light of the dental resin curing unit. Vasoconstriction was observed simultaneously in a time-dependent manner with blue light irradiation and significant increases in tension were observed in the blue light irradiation group compared with the control group (*Figure 1*). NA is released from sympathetic nerve endings in vascular smooth muscle and causes vasoconstriction to bind to its α-receptor. We therefore examined the effect of a blocker of α-receptors, phentolamine, against the developed tension by the blue light irradiation. The tension resulting from blue light irradiation was inhibited in the group that had 10 μM phentolamine added to the Krebs-Ringer solution (*Figure 2*).

***In vitro* ESR spectrum of ROS in the presence of various scavengers**

Using ESR, we confirmed the scavenging activity of DMSO, SOD, and L-histidine, which are the specific ROS scavengers of $HO^{\bullet}$, $O_2^{\bullet-}$, and $^1O_2$, respectively. Each observed ROS was significantly scavenged by the addition of 100 mM DMSO for $HO^{\bullet}$, 10 unit/mL SOD for $O_2^{\bullet-}$, and 5 mM L-histidine for $^1O_2$ (*Figure 3*).

The effect of ROS scavengers on vasoconstriction from the blue light irradiation of rat aorta

We assessed the inhibitory effect of ROS in rat vascular smooth muscle strips on vasoconstriction developed by blue light irradiation from the dental resin curing unit. For ROS scavengers, we used 100 mM DMSO for $HO^{\bullet}$, 10 unit/mL SOD for $O_2^{\bullet-}$, and 5 mM L-histidine for $^1O_2$. A significant increase in tension was observed in the groups receiving blue light irradiation compared with the control group that did not receive irradiation. There were inhibition in vasoconstriction observed in the irradiated groups that included 100 mM DMSO, 10 unit/mL SOD, or 5 mM L-histidine in the Krebs-Ringer solution (*Figure 4*).

Discussion

We previously reported that the blue light irradiation exert cytotoxicity to mediate ROS generation on human smooth muscle cells and gingival fibroblasts [6,17]. The purpose of this study was to examine what kind of biological response was observed in blood vessels by dental blue light irradiation. We first demonstrated that vasoconstriction in superfused isolated rat aorta was induced by 10 min of blue light irradiation using the dental resin curing unit (*Figure 1*). These results suggest the possibility that the blue light irradiation of teeth in bleaching and/or preservative restoration can constrict the numerous small blood vessels in dental pulp in the oral cavity. Consequently, vasoconstriction can occur using blue light irradiation from the dental resin curing unit during dental treatments such as placements of resin-based composites and tooth bleaching.

This blue light irradiation-induced vasoconstriction was inhibited by including in the Krebs-Ringer solution an α-receptor blocker that inhibits the neurotransmitter NA (*Figure 2*). Vasoconstriction was also inhibited with the addition of various ROS scavengers to the Krebs-Ringer solution (*Figures 3,4*). It has been already reported that NA release induced by ROS, such as $^1O_2$ and $HO^{\bullet}$, produced at

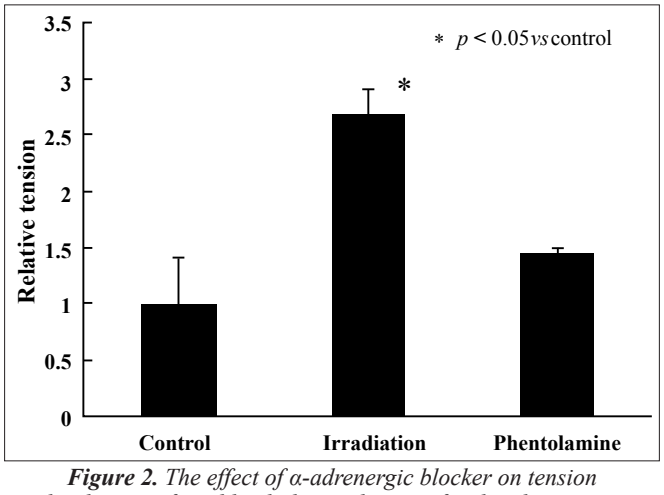

Figure 2. *The effect of α-adrenergic blocker on tension development from blue light irradiation of isolated rat aorta. (a) Control: relative tension change without blue light irradiation. (b) Irradiation: relative tension change on the control with blue light irradiation. (c) Phentolamine: relative tension change on the control with blue light irradiation in the presence of 10 μM phentolamine. Phentolamine was applied for 20 min from basal record (100-110 min) to the completion of blue light irradiation (110-120 min). Results are expressed as the difference from the maximum value of the experimental period and the basal period value and are represented as a mean ± standard deviation (n=4–5). An * indicates a significant difference (p<0.05) versus the corresponding control value. Experimental conditions are described in Materials and Methods.*

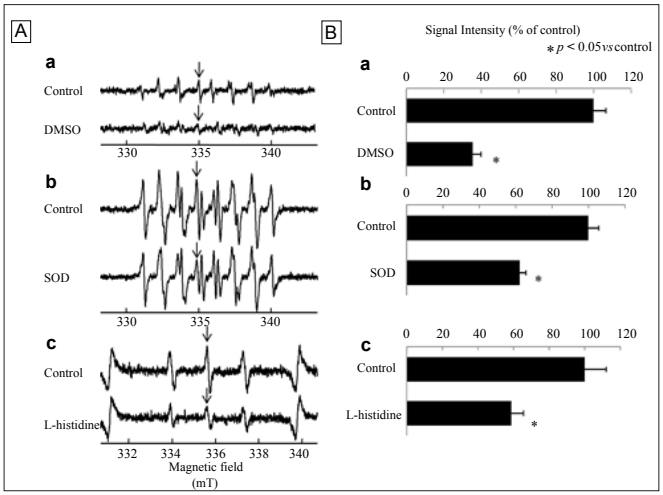

Figure 3. *In vitro ESR spectrum of ROS in the presence of scavengers. (A) Typical in vitro ESR spectrum of each ROS. (a) HO• generated by H_2O_2 with UV irradiation for 20 s with CYPMPO (5.0 mM) as the spin trap with or without 100 mM DMSO. (b) $O_2^{\bullet-}$ generated by photoexcited TiO_2 with UV irradiation for 60 s with CYPMPO (5.0 mM) with or without 10 unit/mL SOD. (c) $^1O_2$ generated by the photochemical reaction of rose bengal illuminated for 5 min (18,000 lux) with or without 5 mM L-histidine. The arrows pointing down indicate the compared signal intensities. (B) The effect of each ROS scavenger on an ROS. (a) Scavenging activity of 100 mM DMSO on HO• generation. (b) Scavenging activity of 10 unit/mL SOD on $O_2^{\bullet-}$ generation. (c) Scavenging activity of 5 mM L-histidine on $^1O_2$ generation. The signal intensity was normalized to 100% of the control. The data are expressed as a mean ± standard deviation (n=3). An * indicates a significant difference (p<0.05) versus the corresponding control value.*

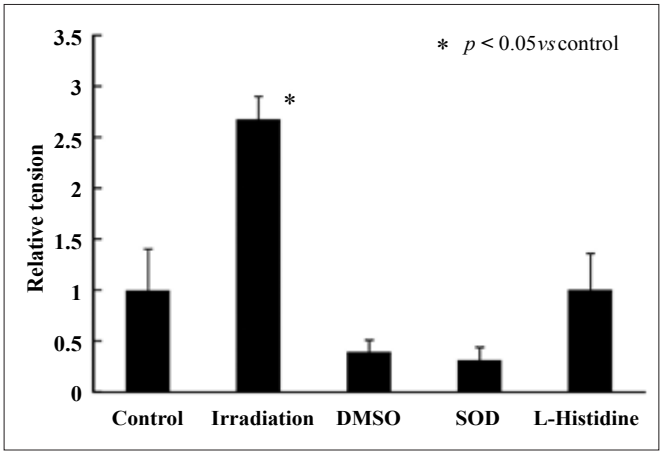

Figure 4. *The effect of ROS scavengers on vasoconstriction by blue light irradiation.*
*(a) Control: relative tension change without blue light irradiation. (b) Irradiation: relative tension change on the control with blue light irradiation. (c) DMSO: relative tension change on the control with blue light irradiation in the presence of 100 mM DMSO. (d) SOD: relative tension change on the control with blue light irradiation in the presence of 10 unit/mL SOD. (e) L-histidine: relative tension change on the control with blue light irradiation in the presence of 5 mM L-histidine. These reagents were applied for 20 min from basal record (100-110 min) to the completion of blue light irradiation (110-120 min).Results are expressed as the difference from the maximum value of the experimental period and the basal period value and are represented as a mean ± standard deviation (n=4–5). An * indicates a significant difference (p<0.05) versus the corresponding control value. Experimental conditions are described in Materials and Methods.*

the nerve endings promotes vasoconstriction, and it has been reported that ROS such as $O_2^{\bullet-}$ are involved in vasoconstriction through the release of calcium from the sarcoplasmic reticulum in vascular smooth muscle cells [18,19,27]. It has been reported that $^1O_2$ is produced by photoexcitation of pigment [18,28]. In this study, ROS scavengers inhibited the development of tension in the vascular smooth muscles. Hence, it is suggested that ROS generation are induced by photoexcitation caused by blue light irradiation. The α-receptor blocker also inhibited tension development, therefore, it can be implied that ROS-dependent NA release is induced by blue light irradiation. Moreover, we previously reported the possibility of flavin and flavin-containing oxidases within peroxisomes and mitochondria are targets of blue light irradiation [17]. This is because flavins have excitation maxima at around 450 nm. Therefore, ROS generation by photoexcited flavin in mitochondria might cause injury to sympathetic cells and induce dysfunction of NA release from nerve termini.

It has been reported that ROS are released by the hydrogen peroxide (H_2O_2)-containing tooth bleaching agents, the resin composite itself, or are generated by free monomers during resin curing during dental treatments [29-32]. $^1O_2$ is generated by the photoexcitation of the red pigment of rose bengal [18,28,33,34]. Blood, including erythrocytes, has red pigment and circulates through the intravascular lumen in vivo; however, this study was performed *in vitro* using isolated blood vessels without circulation. Hence, the production of ROS using tooth bleaching agents, resin composites, or blue light excitation of red pigment in the blood might potentially increase vasoconstriction through ROS-dependent NA release during blue light irradiation in dental treatments.

Conclusions

The blue light irradiation of the dental resin curing unit could causes vasoconstriction in the blood vessels via ROS generation. Prolonged and/or repetitive blue light irradiation might induce temporary ischemia through vasoconstriction in dental pulp that is not the treatment target. Ischemia is characterized by decreased adenosine triphosphate in the local tissue and this increases hypoxanthine. ROS such as $O_2^{\bullet-}$ and HO• arise from reactions with hypoxanthine and blood that is provided after reperfusion [35]. Therefore, dental blue light might not only induce vasoconstriction by ROS generation with blue light irradiation, but also the generation of ROS associated with the recovery from vasoconstriction. The ischemia-reperfusion injury accompany with oxidative stress induced by ROS generated in this way causes not only direct biological dysfunction but also has potential to accelerate the aging of the pulp. Therefore, it is possible that the pre-intake of antioxidants could hold potential in avoiding the effects of ROS induced by blue light irradiation in dental clinic treatments, such as the aging of dental pulp.

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Conflict of interest

The authors declare that they have no competing interests.

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