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

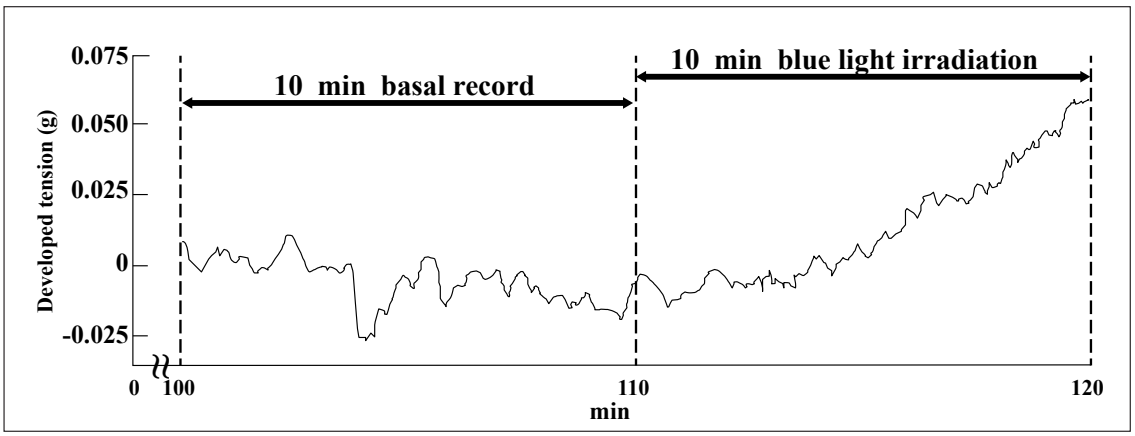

Figure 1. *Typical superfusion chart of vessels irradiated with blue light.*
A helical vessel strip was suspended in a jacketed (37°C) superfusion chamber and superfused continuously (1.5 ml/min) with aerated (95% O_2, 5% CO_2) Krebs-Ringer solution. The strips were connected to a force transducer and 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.

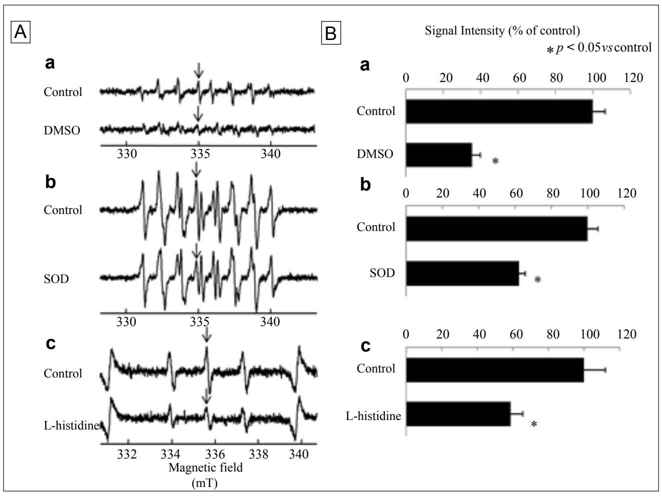

Figure 3. *In vitro ESR spectrum of ROS in the presence of scavengers. (A) Typical in vitro ESR spectrum of each ROS. (a) HO generated by H_2O_2 with UV irradiation for 20 s with CYPMPO (5.0 mM) as the spin trap with or without 100 mM DMSO. (b) O_2 generated by photoexcited TiO_2 with UV irradiation 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 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 ± VWDDUHYLDWLRQLLFDWHVDVLJLFDWLIIHUHFH SYHUVVWKHFRUUHVSRLJFRWURYDOH*

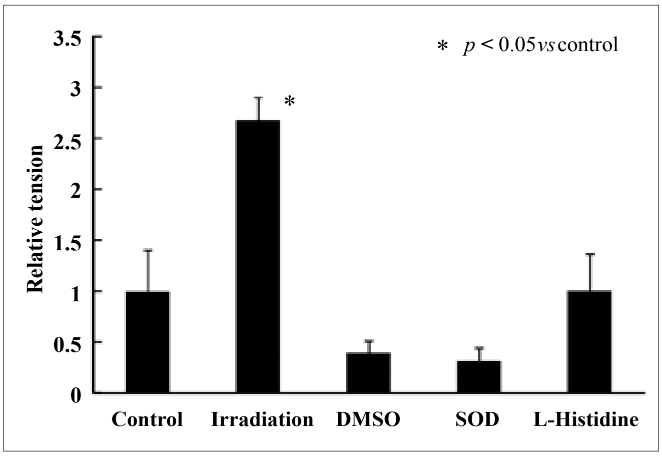

Figure 4. *The effect of ROS scavengers on vasoconstriction by blue light irradiation. (a) Control: relative tension change without blue light irradiation. (b) Irradiation: relative tension change on the control with blue light irradiation. (c) DMSO: relative tension change on the control with blue light 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 HYLDWLRLLFDWHVDVLJLFDWLIIHUHFHS versus the corresponding control value. Experimental conditions are described in Material and Methods.*

the nerve endings promotes vasoconstriction, and it has been reported that ROS such as O_2 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 EEOXHOLJKWLUUDGLDWLRQ7KH.UHFHSWRUEORFNHUD tension development, therefore, it can be implied that ROS-dependent NA release is induced by blue light irradiation. 0RUHRYHU SUHYLRXVO\UHSRUWHG WKH SRVVLELOL DQG DYLQFRQWDLQLQJ R[LGDVHV ZWKLQ SHUR[LVR mitochondria are targets of blue light irradiation [17]. This LV EHFDXVH DYLQV KDYH H[FLWDWLRQ PD[LPD DW DU QP 7KHUHIRUH 526 JHQHUDWLRQ E\SKRWRH[FLWHG DYL 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 LQ YLWUR 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 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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