DENTAL MATERIALS 24 (2008) 1070–1076

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Intracellular reactive oxygen species in monocytes generated by photosensitive chromophores activated with blue light

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ARTICLE INFO

Article history:
Received 15 October 2007
Accepted 18 December 2007

Keywords:
ROS
Photodynamic therapy
Endodontic infection
Oxidative stress
Cytotoxicity

ABSTRACT

Objectives. Disinfection of the tooth pulp-canal system is imperative to successful endodontic therapy. Yet, studies suggest that 30–50% of current endodontic treatments fail from residual bacterial infection. Photodynamic therapy using red-light chromophores (630 nm) to induce antimicrobial death mediated by generated reactive oxygen species (ROS) has been reported, but red-light also may thermally damage resident tissues. In the current study, we tested the hypothesis that several blue light chromophores (380–500 nm) generate intracellular reactive oxygen species but are not cytotoxic to mammalian cells.

Methods. THP1 monocytes were exposed to 10 μM of four chromophores (chlorin e6, pheophorbide-a, pheophorbide-a-PLL, and riboflavin) for 30 min before activation with blue light (27 J/cm^2, 60 s). After activation, intracellular ROS were measured using a dihydrofluorescein diacetate technique, and cytotoxicity was determined by measuring mitochondrial activity with the MTT method.

Results. All photosensitizers produced intracellular ROS levels that were dependent on both the presence of the photosensitizer and blue light exposure. Riboflavin and pheophorbide-a-PLL produced the highest levels of ROS. Photosensitizers except riboflavin exhibited cytotoxicity above 10 μM, and all except pheophorbide-a-PLL were more cytotoxic after blue light irradiation.

Significance. The current study demonstrated the possible utility of blue light chromophores as producers of ROS that would be useful for endodontic disinfection.

1. Introduction

Without treatment, endodontic infection leads to alveolar bone destruction, swelling, and severe pain [1]. Growing evidence suggests that chronic endodontic infections, accelerate coronary artery disease, increase the infection risk of orthopedic implants, and exacerbate other chronic inflammatory diseases [2–4]. Endodontic infections are treated by remov-

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doi:10.1016/j.dental.2007.12.004

ing infected tissues from the pulp canal system, disinfecting the canals, then filling the canal space to prevent re-infection. Several disinfectants are currently used to eliminate bacterial contamination including peroxides, hypochlorite, chlorhexidine, calcium hydroxide, or antibiotics [5]. However, epidemiological studies suggest that 30–50% of root canal treatments fail from residual infection [6–8]. This percentage of failures strongly suggests that current endodontic antimicrobial techniques are not adequate and that improved or supplemental disinfection strategies are needed.

Photodynamic therapy (PDT) has been recently proposed to treat endodontic infections [9–11]. Photodynamic therapy (PDT) uses a chemical chromophore, commonly called a photosensitizer that is taken up by target cells then activated to initiate bacterial disinfection. Generally, absorption of the light triggers excitation of the photosensitizer, which then either kills cells directly through formation of highly reactive free radicals (Type I mechanism), or reacts with molecular oxygen to create secondary reactive oxygen species (ROS) that disrupt cell function (Type II mechanism) [12]. Bacteria are generally more susceptible to light-activated photosensitizers than mammalian cells because bacteria have fewer rescue systems to help them survive an oxidative insult. Preferential uptake of the photosensitizer by bacteria may be enhanced by linking it with a targeting moiety via a poly-lysine polymer [13].

Photodynamic approaches using methylene blue, toluidine blue, or tolonium chloride activated with high-power red-light lasers (630 nm, 30 min, 200 J) have been shown to be effective antimicrobials of bacteria that are prominent in endodontic infections [9]. Other studies suggest that activated-photosensitizers are even effective against antibiotic-resistant strains and may act faster than current endodontic disinfectants [14,15]. However, red light significantly heats tissues and increases the risk of thermal trauma during PDT therapy [16]. Red-light photosensitizers also may require relatively long exposure times for activation that are not practical clinically and may exacerbate the problem of heat generation. Furthermore, red-light photosensitizers such as methylene blue or toluidine blue may permanently stain teeth [17,18]. Finally, high intensity red-light lasers are expensive and not commonly available in dental practices.

On the contrary, high-intensity (500–1000 mW/cm^2), blue light (380–500 nm) sources which are routinely used for curing resin-based materials, are readily available in all dental offices. Blue light sources are less expensive and more compact than red-light lasers that are currently used for PDT. Because of its shorter wavelength, blue light carries more energy that is available to promote formation of reactive oxygen species after shorter exposure times. Thus, blue light would be an attractive trigger for activation of photosensitizers to disinfect root canals. However, for blue-light PDT to be useful in endodontic treatments, photosensitizers that adsorb blue light need to be discovered and tested [19,20].

Because photodynamic effects are mediated by ROS, unwanted cytotoxicity from ROS in resident cells must be considered in the development of any new photosensitizer [21]. Monocytes and macrophages play prominent roles in the immune response during endodontic infections [22]. Therefore, in the current study, we assessed the ability of four candidate blue light photosensitizers to generate ROS in human monocytes and assessed the ability of the monocytes to survive application of these photosensitizers. Our hypothesis was that we could irradiate photosensitizers with blue light to trigger intracellular ROS that were not cytotoxic to monocytes.

2. Materials and methods

2.1. Photosensitizers

Four chemicals were used as photosensitizers: chlorin e6 (Frontier Scientific Inc., Logan, UT), pheophorbide-a (Frontier Scientific Inc., Logan, UT), riboflavin (Sigma–Aldrich, Buchs, Switzerland), and pheophorbide-a-poly-lysine synthesized as described previously [13]. Solutions of the photosensitizers in 5 μM dimethyl sulfoxide (DMSO, Sigma–Aldrich) were prepared and the absorption spectra were confirmed using a spectrophotomer (Cintra 40 UV/VIS, GBC, Dandenong, Australia). For each photosensitizer a stock solution (2 mmol/L) was prepared in phosphate-buffered saline (PBS) after initially dissolving the powder in 50 μL DMSO. The final concentration of DMSO in the stock solutions was 0.025%. These compounds absorbed light in the blue range (Table 1). For experiments in cells, stock solutions were diluted on the day of the experiments to the desired concentrations with PBS and were stored in the dark at −20 °C.

2.2. Cell-culture

Human THP1 monocytes (ATCC TIB 202, American Type Culture Collection, Rockville, MD) were maintained in suspension in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), β-mercaptoethanol (50 μmol/L), and 2 mmol/L glutamine (Sigma, St. Louis, MO). Seventy-two hours prior to experiments, the β-mercaptoethanol was withheld to avoid confounding effects of this reducing agent.

For experiments that measured ROS, monocytes were resuspended in Hallam's buffer (145 mM NaCl, 5 mM KCl, 1 mM

Table 1 – Absorption characteristics of photosensitizers

Photosensitizer	Absorption maxima[a] (nm)	Absorption range (nm)
Chlorin e6	400	350–450
	490	475–520
	665	650–680
Pheophorbide-a[b]	410	350–430
	510	490–520
	540	520–555
	615	600–630
	670	640–680
Riboflavin	450	400–500

[a] In 5 μM DMSO.
[b] Absorption spectrum of pheophorbide-a-PLL was equivalent to pheophorbide-a.