Effect of a Light-Curing Unit on the PGE2 Concentration from the Pulp Fiber in an Animal Model

Decky J. Indrani[1]*, Ratna S. Dewi[2], Rachmitasari Noviana[3], Permanawati Permanawati[3], Bernard Kurniawan[4]

1. Department of Dental Materials Science, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
2. Department of Prosthodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
3. Primate Research Center, Agricultural Institute, Bogor, Indonesia.
4. Undergraduate Program of Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

Abstract

Prostaglandin E2 (PGE2) has been suggested as an indicator of irreversible pulpitis in permanent teeth. The aim of the present study was to investigate the PGE2 concentration detected in pulp fiber harvested from teeth in a primate following light exposure to resin composite restorations.

The investigation involved three monkeys from which twelve PGE2 specimens were obtained. In each monkey, three teeth were involved for which each tooth was restored by resin composite and polymerized with eithe the pulse lighting in 10 sec, the pulse lighting in 20 sec or the continuous lighting in 20 sec as comparison and a tooth without treatment was used as control. An immunoassay was used to detect the Optical Density level then converted to PGE2 concentrations.

The application of the pulse lighting in either 10 or 20 sec, the continuous-lighting curing units in 20 sec and the control showed the mean (log) PGE2 concentration of ranging from 6.37±0.93 to 24.9±6.81 pg/mL, respectively.

PGE2 correlated positively with light exposure from the pulse lighting in 10 and 20 sec, as well as, the continuous lighting in 20 sec to the of restored resin composites.

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Introduction

The development of advanced technology in dentistry has improved resin polymerization process by light that further increases the use of bonded composite resins for dental restoration.[1,2] Current light-curing units have focused on using light-emitting diodes (LEDs) in the blue light range. LEDs use junctions of doped semiconductors (p-n junctions) based on gallium nitride to maintain the light and reduce heat. Previous studies have shown that LEDs are more efficient than Quartz Tungsten Halogen lamps in converting energy to light, and their light emission matches the absorption spectrum of camphorquinone more closely.[3,4]

In recent years, light-curing units use light-emitting diodes (LED-LCU) with higher irradiance to produce higher energy output compared to those in previous generations of LCUs to obtain brief curing time[5,6]. However, applying external heat with a magnitude that reaches a critical level could raise pulp chamber temperature that may cause irreversible pulp damage.[7-9] Furthermore, pulp damage could may possibly cause pulpal inflammation. The detrimental effect caused by high heat in pulp chamber during restorative treatment has been a concern to clinicians.[10]

Accurate postoperative diagnosis of an inflammatory pulp tissue in teeth restored with light-cured resin composites is difficult. This has led to the use of response to inflammation in most human tissues. During an inflammatory process, an array of endogenous chemical mediators of inflammation is released. Among these are prostaglandins E2 (PGE2), a group of arachidonic fatty acid derivatives that are enzymatically removed from the cell membrane.[11] Increases of PGE2 due to inflammation were observed in human[12,13]. Morphologically, non-human primates

*Corresponding author:
Decky J. Indrani
Department of Dental Materials Science
Faculty of Dentistry, Universitas Indonesia
E-mail: deckyji@gmail.com

are close to humans and the widely used non-human primates in studies are rhesus monkey. The primates *Macacaque* has a body weight of approximately 3–7 kg, shows the hematologic and biochemical characteristics close to humans. Likewise, the cytokine production mechanism of tooth formation present a degree of similarity to humans. Changes in PGE2 levels have been dectected as responses of inflammation in dental pulp of non-human primates.[12,13]

For an inflammation to occur in a non-human primate pulp chamber, it is necessary to apply stimulation to the teeth. Our ealier study has constructed an experimental dental curing unit using a pulse-lighting mode with an irradiance of 900 mW/cm^2 in 20 sec and compared it to a commercially available curing with continuous lighting with light exposure in same duration.[14] We also reported that the restorations polymerized by the curing units are fully cured.[15] While we investigated the temperature change of the curing units and the pulp chamber temperature in extracted teeth, and ensure that restorations are fully cured, there have been scant studies on the PGE2 production related to applications of the curing units. Therefore, the purpose of this in-vitro study was to analyze the influence of irradiances of light curing units on the production of PGE2 in non-human primates.

Materials and methods

Preparation of the primates and the light curing units

We restored each tooth with resin composite material followed by polymerization. Teeth of the primates involved in the study were grouped together with the curing units as follows,

- Group 1: mandibular right first molar, restored with resin composite and polymerized using the pulse lighting of 900 mW/cm^2 in 10 sec
- Group 2: mandibular left first molar, restored with resin composite and polymerized using the pulse lighting of 900 mW/cm^2 in 20 sec
- Group 3: mandibular righ third molar, restored with resin composite and polymerized using the continuous lighting of 900 mW/cm^2 in 20 sec (as comparison)
- Group 4: mandibular left third molar with no restoration (as control)

After an hour, each tooth was extracted and immediately stored in saline solution until later use; a total of 12 teeth were obtained. After the extraction process, the *primates* were given topical antiseptics, topical analgesics, an antibiotic and ketoprofen injection followed by suturing with 4/0 vycril yarn. The primates were under observation prior to entering the recovery stage.

PGE2 quantification

We bisected each tooth using a dental burr to collect pulp fiber. The pulp fiber was removed with tweezers and weighed. Each pulp fiber was subsequently stored in a microcentrifuge tube containing 0.2 mL of phosphate-buffered saline solution. To quantify PGE2, the pulp fiber were removed from storage in batches.

First, a serial dilution of PGE2 stock solution of a calibrator diluent (RD5-56, R&D Systems, Canada) and an immunoassay using a plate-based Enzyme-Linked Immunosorbent Assay (ELISA kits, R&D Systems, Canada) were conducted in duplo at a wavelength of 450 nm to perform absorbancies for Optical Density (OD) standard. After the assay, the PGE2 and OD standard values were plot to obtain a calibration curve that produced a regression (y-axis) described by a logistic equation, for which the quality of fit (R^2 value) should be assessed. A calibration curve with a regression yielding R^2 values of greater than 0·95 is accepted. Finally, the OD standard values were substituted on the regression (y-axis) to determin mean (log) of the PGE2 expressed as PGE2 concentration in units of pg/mL.

Statistical analysis

All data were expressed as group mean ± SD. The normality of the data was analyzed using Kolmogorov-Smirnov test with a confidence level of 95%. Kruskal Wallis was performed, and Mann Whitney post-hoc was applied to determine significant differences. All statistical tests with P-value <0.05 were considered significant. Data was processed using SPSS statistical analysis software.

Results

The resin composites restored in the primate teeth and polymerized by the curit units with modes and duration-dependent manners that stimulated the pulp fiber have resulted the PGE2 concentration determined by the OD and PGE2 standard values on the regression (y-axis).

Immunoassay

The OD standard values based on the serial dilution of the PGE2 standard at 450 nm is summerized in Table 1.

PGE2 standard (pg/mL)	Mean OD standard
2000	0.055
1000	0.095
500	0.14
250	0.177
125	0.239
62.5	0.319
31.25	0.401
15.6	0.497
7.8	0.583
3.9	0.629

Table 1. The OD based on PGE2 standard values

Table 1 showed mean OD standard values ranging from 0,055 to 0,629. Plot of the OD on the PGE2 standard concentration demonstrated a calibration curve as is shown in Fig 1.

Fig. 1 demonstrated the PGE2 standard on the linear scale (*y*-axis) against the changes in OD standard values on the logarithmic scale (*x*-axis). The calibration curve determined a regression y = 2088,2e-10.09x, which shows R^2 value of greater than 0·95; the regression value means that the calibration curve was accepted.

PGE2 Concentration in pulp fiber from the primate teeth

Substitution of the mean OD values and the PGE2 standard concentrations into the regression equation resulted the mean (log) PGE2 concentration (PGE2 concentration) is as shown in Table 2.

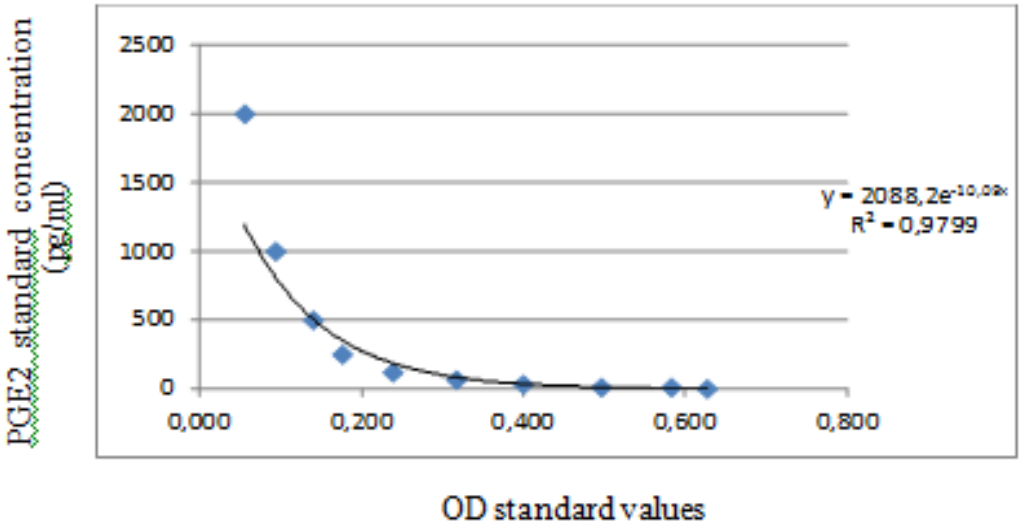

Figure 1. Plot of OD standard values on PGE2 standard concentration

Curing unit Mode	Time of lighting (sec)	PGE2 concentration (pg/mL)	P-value
Pulse-lighting	10	11.38±0.73	0.015
	20	14.93±0.95	
Continuous-lighting (Comparison)	20	24.9±6.81	
Without lighting (Control)	20	6.37±0.93	

Table 2. PGE2 concentration in relation to using the light-curing mode

Light curing unit mode	Pulse lighting 10 sec	Pulse lighting 20 sec	Continuous lighting 20 sec (Comparison)
Pulse lighting 10 sec	---	---	---
Pulse lighting 20 sec	0.046 *	---	---
Continuous lighting 20 sec (Comparison)	0.046 *	0.05	---
Without lighting (Control)	0.046 *	0.05	0.05

* = significant

Table 3. Significancy of the PGE2 concentration between groups

The data in Table 2 shows that PGE2 concentration resulted in relation to to using of the light-curing mode for polymerization of the resin composites varied from 6.37±0.93 to 24.9±6.81 pg/mL. the use of continuous lighting (comparison), respectively. Whereas, PGE2 concentration in relation to the use of pulse lighting in both 10 and 20 sec, i.e., 11.38±0.73 and 14.93±0.95 pg/mL, respectivey, were in the mid range. The statistical analysis detected significant different (P<0.015) among the PGE2 concentration groups. Further siginificancy of the PGE2 concentration between groups are summerized in Table 3.

As evident from Table 3, significant differences (*P*=0.046) in the PGE2 concentrations were detected between the using of the pulse lighting in 10 and each curing unit. On the other hand, there were insignificant differences (*P*=0.05) in the PGE2 concentrations between the using of the pulse lighting in 20 sec and the comparison and the the control, as well as, between the pulse lighting in 20 sec and the control.

Discussion

This study did not investigate the role played by PGE2 in the inflammatory process of the primate teeth stimulated by the using of the light curing units to polymerize restored resin composites in the primates, but to assess the presence of PGE2 in pulp fiber. Previous studies have quantified PGE2 from pulp and periodntal tissues, but never before has it been quantified in pulp fiber obtained from teeth of non-human primate related to the use of a light curing units. However, the results from this study were in concomitant with previous reports on PGE2 concentration in either human or non-human primates. Periapical tissue from unerupted third molars specimens from patients with clinical signs and symptoms of acute apical periodontitis showed significantly higher levels of PGE2 than that of chronic apical periodontitis, and both were higher than control, i.e., normal periapical tissues.[16] An increase was observed of PGE2 in human in reversible pulpitis compared with healthy pulps and with the irreversible pulpitis.[12]

By analyzing the increase in PGE2 concentration (see Table 2), there appears to be evidence to support the using of the light-curing units. It appears that the untreated teeth (control) have a capacity to produce PGE2 endogenously. After the resin composite restored in teeth and were polymerized by each light curing unit, higher PGE2 concentration were found compared to that of the control. The data were good illustration for production of the PGE2 after light curing the restored teeth in the primates.

The PGE2 concentration was not proportional to the mode, pulse and continuous light curing, although the irradiance was same, i.e. 900 mW/cm^2. Pulse lighting, as it applies to the curing unit, is a way of delivering energy. The microcontroller that was programmed by pulse, regulates energy flow. On one period of lighting, heat reduction is accomplished through pulses. The pulse-lighting curing unit emitted lower heat that likely suppressed inflammation in the pulp fiber, consequently, significantly lower PGE2 concentration was produced. Due to lower PGE2

production, this was to say that the pulse lighting was preferable when compared to the commercially available continuous-lighting curing unit.

With respect to the pulse-lighting mode, prolonged irradiance in 20 sec than 10 sec resulted higher PGE2 concentration. A proportional relationship was found between the time and PGE concentration. When using an irradiance of 900 mW/cm^2 in 10 sec for polymerizing the restored resin composite, lower PGE concentration was produced, which was likely due to lower energy density (irradiance x time) of 9,000 mJ/cm^2 than that when using in 20 sec resulting 18,000 mJ/cm^2 that cuase significantly higher PGE2 concentration. Therefore, using the 10 sec light curing seemed be adequate to polymerize the resin composite in the primate teeth.

Due to their close relation to humans the findings of the PGE2 concentration from the primates in this study were as expected. Nevertheless, analysis of whether the pulse lighting had an influence on the PGE2 concentration detected in the pulp fiber specimens showed a just-significant difference (*P* =0.05) values. One could, however, have expected the a just-significant difference (*P* =0.05) was significant or event more significant for the PGE2 concentrations between the using of the pulse lighting in 20 sec either with the comparison or the control. The discrepencies in the statistical results could probably because the number of specimens that was limited which cause the statistical analysis may 'dilute' the strength of significancy that show unsuccessful statistical results.

Regardless of the statistical analysis, it appeard that the results from this study showed a succession of pulses rather than the continuously lighting. The pulse lighting revealed with lower PGE concentration compared to those with using the continuously lighting. To confirm the association of the lighting curing unit and the production of PGE2 assumed to be presented within pulp fiber of human teeth, further study may conduct clinical trial for determining post-operative tooth sensitiviy in patients.

Conclusions

This study produce PGE2 concentration collected in pulp fiber derived from the primate teeth with the use of one lighting mode over the other, continuous and pulse, as well as, the lighting time, 10 and 20 sec. For polymerizing resin composite restored in the primates teeth, all PGE concentration increase compared to that without treatment (control). The continuous-lighting curing unit produced the PGE2 concentration higher than that when using the pulse-lighting curing unit. Regarding the pulse-lighting, production of the PGE2 concentration in 10 sec lighting was lower that that in 20 sec.

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Declaration of Interest

The authors declare that there is no conflicts of interest.

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