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Dental curing light: non-ionizing electromagnetic radiation and its possible genotoxic effects

Luz de tratamento dentário: radiação eletromagnética não ionizante e seus possíveis efeitos genotóxicos

Luz de tratamiento dental: radiación electromagnética no ionizante y sus posibles efectos genotóticos

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Abstract

Objective: In this scientific study, we aimed to evaluate genotoxic effects in rats (*Rattus norvegicus*), related to different periods of exposures to the LED curing light. Methodology: For the genotoxicity evaluation, the rats received lights from the LED photopolymerizer for 40 sec, 10 min and 7-and-a-half minutes, while the negative and positive control groups were treated with distilled water and cyclophosphamide by intraperitoneal, respectively. A sample of peripheral blood was collected from the animals for the comet assay. The bone marrow was collected from each rat for the micronucleus test. Results: It was observed that in the comet assay and micronucleus test, the animals exposed to LED for 10 min, showed genotoxic damage, and they have not presented toxicity degree in the periods of 40 sec and 7-and-a-half minutes. Conclusion: It is possible to conclude that, there was genotoxic effects on the animals' teeth when exposed to the LED curing light in 10 min. However, in the periods of 40 sec, and 7- and-a-half minutes, have been not observed genotoxic effects. This means these times are safe for professional dentists in clinical care.

Keywords: Photopolymerizer; Genotoxicity; Comet assay; Micronucleus; Non-ionizing radiation**.**

Resumo

Objetivo: Neste estudo científico, objetivou-se avaliar os efeitos genotóxicos em ratos (*Rattus norvegicus*), relacionados a diferentes períodos de exposição à luz de cura LED. Metodologia: Para avaliação da genotoxicidade, os ratos receberam luzes do fotopolimerizador LED por 40 seg, 10 min e 7 minutos e meio, enquanto os grupos controle negativo e positivo foram tratados com água destilada e ciclofosfamida por via intraperitoneal, respectivamente. Uma amostra de sangue periférico foi coletada dos animais para o ensaio do cometa. A medula óssea foi coletada de cada rato para o teste de micronúcleo. Resultados: Observou-se que no ensaio do cometa e teste do micronúcleo, os animais expostos ao LED por 10 min, apresentaram dano genotóxico, não tendo apresentado grau de toxicidade nos períodos de 40 seg e 7 minutos e meio. Conclusão: É possível concluir que houve efeito genotóxico nos dentes dos animais quando expostos à luz de polimerização LED em 10 min. Porém, nos períodos de 40 seg, e 7 e meio minutos, não foram observados efeitos genotóxicos. Isso significa que esses horários são seguros para dentistas profissionais em atendimento clínico.

Palavras-chave: Fotopolimerizador; Genotoxicidade; Ensaio do cometa; Micronúcleo; Radiação não ionizante.

Resumen

Objetivo: En este estudio científico, nuestro objetivo fue evaluar los efectos genotóxicos en ratas (Rattus norvegicus), relacionados con diferentes períodos de exposición a la luz de curado LED. Metodología: Para la evaluación de genotoxicidad, las ratas recibieron luces del fotopolimerizador LED durante 40 segundos, 10 minutos y 7 minutos y medio, mientras que los grupos de control negativo y positivo fueron tratados con agua destilada y ciclofosfamida por vía intraperitoneal, respectivamente. Se recogió una muestra de sangre periférica de los animales para el ensayo cometa. Se recogió la médula ósea de cada rata para la prueba de micronúcleos. Resultados: Se observó que en el ensayo cometa y prueba de micronúcleos, los animales expuestos a LED por 10 min, presentaron daño genotóxico, y no han presentado grado de toxicidad en los períodos de 40 seg y 7 minutos y medio. Conclusión: Es posible concluir que hubo efectos genotóxicos en los dientes de los animales cuando se expusieron a la luz de curado LED en 10 min. Sin embargo, en los periodos de 40 segundos y 7 minutos y medio, no se han observado efectos genotóxicos. Esto significa que estos tiempos son seguros para los dentistas profesionales en la atención clínica.

Palabras clave: Fotopolimerizador; Genotoxicidad; Ensayo de cometa; Micronúcleo; Radiación no ionizante.

1. Introduction

The Dental curing light is an essential instrument for the dental surgeon at dental restorations. Photopolymerization consists in activating the polymerization reaction of resinous material when exposed to a light source. It begins by the light absorption from a photo-initiator, usually camphorquinone (Oliveira, 2014).

Camphoroquinone is the photo-initiator present in most of composed resins available on the market and it is capable to absorb energy into the specific wavelength of light, where initiates the polymerization process. The polymerization of photoactivated composed resins by visible light, begins when camphorquinone is exposed to light with a wavelength varying from 450 to 500 nanometers(nm), absorbing the photons, so that enter in a reactive state (Franco, 2007).

Dental curing light devices are differentiated by the type of light source used, by its variation in the wavelength interval, and by the type of pulse and light intensity (Soares, Peçanha, Batitucci, Neto, Batitucci, & Batitucci, 2005). Light is measured in wavelengths and expressed in units of nanometers. The wavelengths are often designated according to their

associated color, such as: blue color (ranges from 400-700 nm); green color (ranges from 470-550 nm); red color (ranges from 630-700 nm) and the infrared (ranges from 700- 1200 nm). In general, the longer is the wavelength, deeper is the tissue penetration (de Paula, 2010).

In relation to photopolymerization, so many factors may influence in the success or failure of resinous materials in the oral cavity (Assis, 2014). Therefore, the photoactivation process depends of the irradiation (mW/cm2) emitted by the light source and the exposure time. If dental materials do not receive enough energy, the degree of monomer conversion will be low, resulting in a possible increase in cytotoxicity, as well as a reduction in hardness and elastic modulus (Guiraldo *et al*., 2014).

The identification and study of the action of a physical, chemical or biological agent that can cause any type of damage to the genetic material, refers to genotoxicity. Genotoxicity tests provide an indication of changes associated with deoxyribonucleic acid (DNA), DNA breaks or mitotic anomalies (do Sacramento, 2012).

There is a great difficulty to obtain in the literature, toxicological effects caused by the use of the LEDs curing light over the biological tissues of the people. However, dentists should be conscious that radiation absorbed by endogenous and exogenous substances that accumulate in the eyes and skin(hands) of the operators, as well as in oral mucosa of the patients might cause different phototoxic and photoallergic reactions (Petroucic, Fontana, Hetem & Fontana, 2004).

Health care workers are exposed to the same risks (chemical, physical and ergonomic) as other workers in general way. Changes have been happening recently in the risk behaviors concepts, in relation to the biosafety of health professionals, which required alterations in the Standard Operating Procedures (SOP), with the purpose to establish the basic guidelines for health and safety measures implementation to protect workers. (Brasil, 2011; Neri, Milazzo, Ugolini, Milic, Campolongo, Pasqualetti, & Bonassi, 2015).

Faced with this perspective, taking into consideration the interest in verifying the light interaction over the patient's dental organ, hands and eyes of the professional dentist in clinical care, the objective of this scientific study was evaluate the occurrence of genotoxic alterations through the light emitted by the LED photopolymerizer in different periods of time, as well as establish a minimum time necessary of light exposure for the no appearance of toxicity, using the comet and micronucleus assays. Therefore, the hypothesis of this research is that the LED curing light causes a genotoxic action in Rattus norvegicus.

2. Methodology

The study is configured as a trial, randomized and controlled trial with a quantitative approach due to the results evidenced in figures and analysis occur through statistics and percentages. The experiment protocol was based on the methodology of Hayashi, et al. (1994); Hayashi, Morita, Kodama, Sofuni, & Ishidate Jr (1990); Hamada et al. (2015) for the micronucleus test and Hartmann *el al*. (2003); Brendler-Schwaab, Hartmann, Pfuhler & Speit (2005); Collins *et al*. (2008); da Silva, dos Santos & Takahashi (2010); Azqueta *et al*. (2011); Azqueta & Collins (2013) for the comet assay.

2.1 Ethical Procedures

This present research followed the guidelines recommended by the Law 11.794 of the Ethics Committee on Animal Use, and was submitted for approval by the Committee on Animal Research and Ethics of the *Universidade Estadual do Piauí* (UESPI), under Protocol number 01060/2018.

2.2 Animals and Research Location

In this study, an *in vivo* test was carried out to evaluate possible genotoxic effects in Rattus norvegicus in exposure to the LED photopolymerizer at the Laboratório do Núcleo de Pesquisa em Biotecnologia e Biodiversidade of UESPI in Teresina - Piauí.

The sample was consisted by 120 rats (60 males / 60 females), weighing 200g to 250g from the "Biotério-UESPI". All the animals were kept at collective cages (03 in each box) separated by gender, with normal diet and water ad libitum in an environment at 25 ºC.

The rats were divided into three groups: positive and negative control groups and the experimental groups of non-ionizing exposure to the LED curing light, in periods of 40 sec, 10 min and 7-and-a-half minutes.

In the 24 animals for the control with positive parameter (cyclophosphamide 50 mg / kg), was administered by intraperitoneally and for the control with negative parameter (distilled water), was administered the same dosage as the positive control for more 24 animals, totaling 48 animals for the comet and micronucleus assay.

In the experimental groups, the animals were submitted to the LED curing light under the upper anterior teeth. The animals were exposed for 40 sec representing the patient, others

animals were exposed for 10 min representing the dental surgeon and in other period in which the animals were exposed for 7 and-a-half-min, the dental surgeon was represented again.

In the period of 40 sec, both for the comet and micronucleus assay, was used 24 animals. In the 10 min, for the tests mentioned above, was used 24 animals and in the time of 7 and-a-half-min was used more 24 animals. In this research was totaled 72 animals in the experimental groups.

2.3 Applications of Light Emitting Diode

Initially, all the animals were anesthetized with Xylazine (5 to 10 mg/Kg) combined with Ketamine (60 to 75 mg / Kg) for improve muscle relaxation, and analgesia for duration of the effect.

After anesthesia, the rats of the experimental groups (periods of 40 sec, 10 min and 7-and-a-half-min) were removed from the cages and placed in a plastic tray, in which was used a needle-holder to facilitate the opening of the mouth and also for the LED exposure. During the application of the light, the optical top of the LED curing light was kept leaned in the animals' upper central incisor teeth and the device remained stationary with a help of a holder, where the operator was only necessary to activated the time exposure for each animal.

At each period of one-minute exposure, one-minute of interval was realized, followed by a new exposure minute, and so on, until was completed the predetermined time for each group (Petroucic *et al.*, 2004).

2.4 Sample preparation

2.4.1 Comet Assay

The Comet assay was carried out with peripheral blood retired from the animal's tail, after 4 hours of exposure(damage) and after 24 hours of exposure(repair). At the end of each period, was collected 40uL of this sample and transferred to microtubes containing 120uL of agarose (1.5%) at 37 °C. That mixture was homogenized and transferred to pre-coated microscope slides with 5% agarose. After that, the slides were covered with coverslips and placed on the refrigerator for 20 min at 30 °C. The coverslips were removed and the slides were immersed in a lysis solution (1ml of Triton X-100 and 10ml of DMSO) during 1 hour in the dark at 3 °C.

The slides were placed in an electrophoresis trough and neutralized. They were dried and fixed with 100% ethanol for 10 min and stored for subsequent analysis. Finally, the slides were stained with Gel Red and covered with a coverslip.

The slides were prepared in duplicates by using an immunofluorescence microscope(40x magnification) in a blind assay and was used a 420- 490 nm filter and a 520 nm barrier filter, then, 200 cells per animal have been counted (100 for acute exposure /100 for chronic exposure). This test analyzes the DNA through the stature of tail, which is a proportional parameter to the size of the damage caused, where the mere visualization of the comet, already identifies DNA damages.

The DNA damage detection can be done in different ways, e.g,: measure the length of a fragmented migrant DNA with a measuring eyepiece, or rank visually in different levels the cells damage analyzed, obtaining arbitrary value which expresses the general damage suffered by a cell population (Neri *et al*., 2015).

For interpretation of results, the DNA damage was evaluated by the percentage of DNA measure in the tail (% of DNA - measure of the proportion of total DNA present in the tail) and the tail length (tail length times the percentage of DNA in the tail). These parameters were calculated in 100 nucleiodes / sample. For this, it was used the Open Comet software, that has been validated on both alkaline and neutral comet assays across different levels of damage. Due to automation is more accurate, less prone to human bias, and faster than manual analysis, it achieves high accuracy with significantly reduced analysis time. (Kumaravel, Vilhar, Faux & Jha, 2009; Gyori, Venkatachalam, Hsu & Clement, 2014).

2.4.2 Micronucleus Test

Bone marrow cells were collected immediately after the animals' euthanasia. For this, a syringe(01 mL) was filled with fetal bovine serum(FBS) and the needle was inserted into the aperture of one end of the femur ,thus, the fetal serum was spilled through the canal, pushing towards other side the medullary component, where was the Falcon tube, previously marked with animal's code. Then, the marrow material in fetal bovine serum was resuspended until reaches homogeneity. The suspension was centrifuged by 5 min at 1,000 revolutions per minute (RPM), excluding the supernatant with a Pasteur pipette at the end of the procedure. The sample was completed with fetal bovine serum (0.5 mL), and suspended until its homogenization. The smears were prepared by adding 02 drops of the suspension in a slide (previously marked with the animal's code) with help of another slide (2 slides per animal) at

a 45 degree angle, being dried at open air. Staining was performed 24 h after the slides confection in Giemsa for 3 min, and the analysis was accomplished in a blind field by using an optical microscope at 100x magnification (immersion objective) in a short period of time by the same observer. Micronuclei frequency was determined in 2,000 cells of polychromatic erythrocytes per animal. (Hayashi, *et al.*, 1994; Hayashi, Morita, Kodama, Sofuni, & Ishidate Jr, 1990; Hamada *et al.*, 2015).

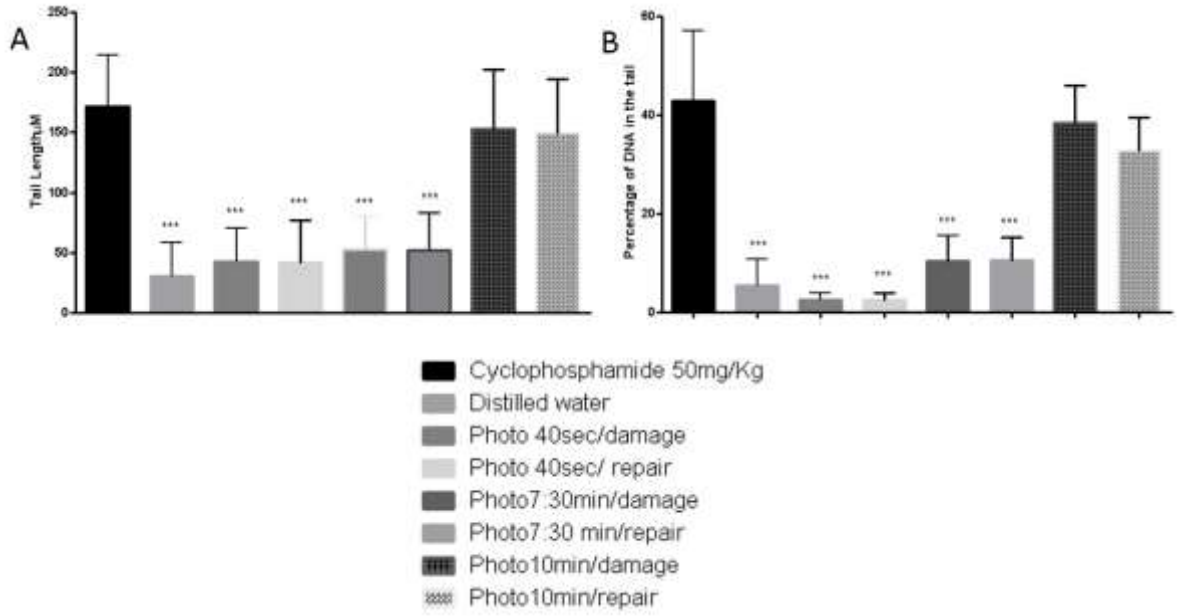
2.5 Data Organization and Analysis

The collected data were analyzed by the Graph Pad Prism software version 5.0 for the submission of the statistical analysis using the One - Way ANOVA and the Student t-test. The results were expressed as averages, that is, standard deviation of the three independent experiments. For all tests, a significance level of 5% was considered. The result was considered positive when there was a statically significant increase ($p < 0.05$).

3. Results

According to Figure 1, it is possible to observe the records of the DNA tail lengths and the percentages of DNA in the tail after analysis of the animals in the Open Comet software, respectively, in exposures of 40 sec, 10 min and 7-and-a-half-minutes.

Figure 1. Records of tail lengths(A) and percentages of DNA in the tail(B) after analysis of the treated animals in the Open Comet software, respectively, with cyclophosphamide (50mg / Kg), distilled water and exposures to the photopolymerizer for 40 sec, 10 min and 7-and-a-half-minutes.

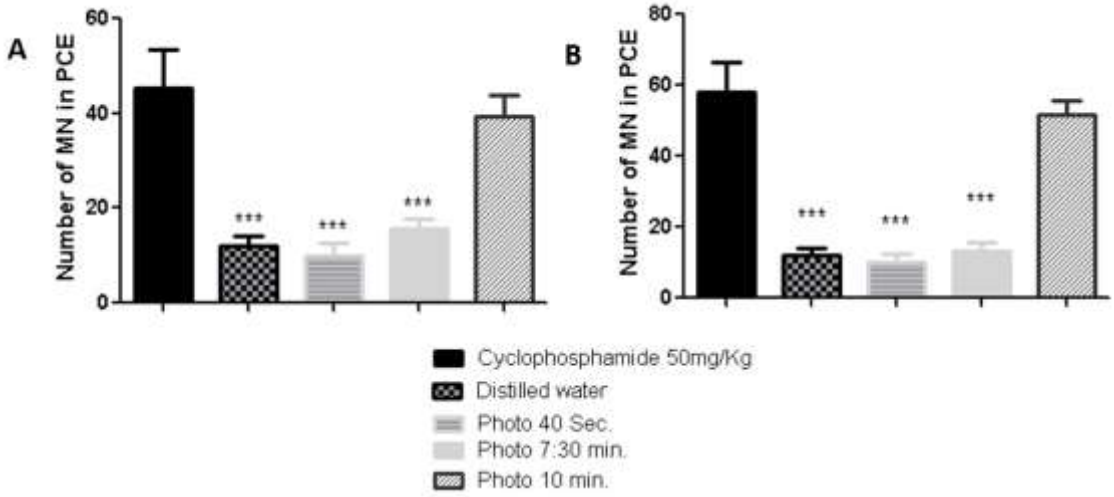


Subtitle: ******* = p <0.001 when compared to Cyclophosphamide group (50mg / Kg)
Source: Authors.

The statistical test of the comet demonstrated that there was a significant difference, showing the exposure time of 10 minutes to the photopolymerizer (both in damage and repair) had genotoxic effects in rats, but there were no significant differences in the negative control group and in groups treated with LED curing light in 40 sec and 7-and-a-half-minutes.

Figure 2 presents micronuclei averages found in 2,000 polychromatic erythrocytes, after exposures of 40 sec, 10 min and 7-and-a-half-minutes.

Figure 2. Micronuclei average found in 2,000 polychromatic erythrocytes after acute exposure(A) and chronic exposure(B) of the treated animals, respectively, with cyclophosphamide (50mg / Kg), distilled water, and exposures to the photopolymerizer for 40 sec, 10 min and 7-and-a-half-minutes.



Subtitle: ******* = p <0.001 when compared to Cyclophosphamide group (50mg / Kg)
Source: Authors.

The statistical test of the micronucleus demonstrated that the exposure time of 10 min to the LED curing light (acute and chronic exposure) presented toxicological effects in the rats in comparison to cyclophosphamide, which is a toxicological parameter. However, there were no significant differences in the negative control groups and in the treated groups for 40 sec and 7-and-a-half-minutes in micronucleus exposure (acute and chronic).

4. Discussion

In this study, was evaluated genotoxic effects in the rats' teeth on blue LED curing light exposure, confirming with the research in which the epithelial cells of human retinal pigment were exposed to different LEDs radiations of different colors that varied from blue color with 468 nm wavelength, green color with 525 nm, and red color with 616 nm. The wavelength in the white color was not mentioned in this research. It was observed that LED blue light exposure decreases cell viability from 75 to 99%; increased cell death from 66% to 89%; increased the production of reactive oxygen species and DNA damage (Gea, Schilirò, Iacomussi, Degan, Bonetta & Gilli, 2018; Yoshida, Yoshino, Makita, Maehata, Higashi, Miyamoto, Wada-Takahashi, Takahashi, Takahashi & Lee, 2013).

Meanwhile, in another study is reported that light emitted by the LED above 400 nm is not harmful to the hand's skin of professionals and dental assistants or to the patient's oral tissues. This is explained or by the presence of optical filters or by the light transmission system or by the way they are used as well (Petroucic *et al*., 2004).

The LED-based devices were launched with the main objective of emitting cool light, but was confirmed in this study that when the device has a high light intensity, it also produces a rising on the temperature, losing its main advantage, which is the no heat emission. This statement is in agreement with the research in which the epithelial cells of the retinal and the bronchial epithelial cells were exposed to different types of lamps, such as: incandescent lamps, halogen lamps, warm and cool LEDs lamps. The results presented the cool LED lamps caused a slight significant genotoxic damage after 4 hours of exposure to the photopolymerizer, being equivalent to the present research in which was used the cool blue light LED and was observed genotoxic damage in 10 min of exposure to the curing light (Silva, 2014).

The non-ionizing electromagnetic radiation of LED is absorbed by the skin and deeper levels of the body, dissipating profundly. It may cause temperature increase not perceived by the natural thermal sensors located on the surface. The internally heating generated depends on the time exposure, the field strength, the tissue thickness, and sometimes it cannot be compensated by the organism, causing biological effects (Santos, 2006).

In a study that used the Transmission Electron Microscopy(TEM), was observed that only the mitochondria of gingival fibroblasts was damaged by blue LED curing light in more than 5 minutes in a dental treatment, increasing the cytotoxic effect of reactive oxygen species(ROS) in soft tissues of the mouth, and that light is richer in radiation when the wavelengths is shorter than 500 nm, inducing to higher cytotoxicity (Brasil, 2001).

The use of photopolymerizable equipments have been increased significantly in recent years in professional dental practice, due to the elevated number of studies about its optimization, powers more adequate and times of necessary operation for each procedure. However, relative studies to occupational risks from these techniques are still scarce, making this field poor in specific legislation and literature. The Unhealthy Activities and Operations (NR-15) instituted by the Ministry of Labor and Employment, establishes as unhealthy activities those that expose workers to non-ionizing radiations, but do not specify maximum exposure limits (Costa & Paz, 2006).

The Directors' Collegiate Resolution (RDC-185) classifies into 4 categories the risk of medical and dental equipment, and the current models of photopolymerizer are part of risk category II, being of medium risk for individuals involved with these equipments (Costa & Paz, 2006).

All the occupational risks of dental care can be minimized by the using of personal and collective protection equipment, in compliance with Regulatory Norm(NR-32) that establishes basic guidelines for the protection implementation for the safety and health of workers in health services, adopting health promotion and prevention measures, such as the correct use of the equipment, following the ergonomics law and adequate environment (Brasil, 2005).

5. Conclusion

In view of the results obtained, taking into consideration the exposure time used in this research, it's possible to conclude that the light emitted by the LED photopolymerizer presented genotoxic effects in a period of 10 minutes. In the experimental groups were not observed genotoxic effects in periods of 40 sec and 7-and-a-half-minutes to the LED photopolymerizer, suggesting that times are safe for the dental surgeon and your assistant during clinical care.

For future projects, it is valid to test different induction times and different wavelengths, with other light curing devices being tested, which should still have the same practical effectiveness when used in dental offices, but do not harm the professional.

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