

Effect of β -Carotene patch application on micronucleus formation of gingival epithelial cells exposed to panoramic radiography, an *in vivo* study

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Abstract

Objective: Previous studies have indicated that the number of micronucleus, a marker of DNA damage, is increased in gingival mucosa after a panoramic examination. β -Carotene is the lipid-soluble antioxidant substance and expected to prevent the oxidative reaction by improves the junctional communication and reduce the risk from DNA damage. The objective of this study was to monitor the changes of micronucleus during panoramic examination by applying β -Carotene Patch to rabbit gingiva.

Methods: A total of 18 New Zealand rabbits were divided into 2 groups equally. Group I was the control group that was given a panoramic examination without applying β -Carotene Patch. Group II was the test group that applied β -Carotene Patch before the panoramic examination. Micronucleus samples were taken from rabbit's gingival mucosa then stained by the method of modified Feulgen-Rosenbeck. The results were analyzed using one-way ANOVA ($P < 0.05$).

Results: The number of micronucleus in Group I was significantly higher than those in the Group II ($P = 0.001$).

Conclusion: β -Carotene Patch may reduce the number of micronucleus formation of rabbit's gingival epithelial cells exposed to panoramic radiography.

Keyword: β -Carotene; Patch; Micronucleus; Panoramic Examination; Gingival Mucosa

1. Introduction

Previous studies have shown that X-ray radiation from dental radiographs using panoramic technique causes genotoxic effects by increasing the number of micronucleus in epithelial cells of the gingival and buccal mucosa ^{1,2,3}. Micronucleus is one of the earliest signs of DNA damage and related to the risk of cancer. The number of micronucleus directly related to the radiation dose, i.e., when there is a repetition of exposure so that twice exposures occurred in a day, the number of micronucleus will also be increased accordingly ⁴. Micronucleus detection is believed as a reliable method to evaluate chromosomal damage on exfoliated cells ⁵. The key advantage of micronucleus detection is the relative ease of scoring, limited cost and person-time required.

Beta carotene, or often referred to as β -carotene, is one of the antioxidant agents which has been known to have a role as an anticancer ⁶. The action mechanism of β -carotene in preventing carcinogenesis is in that it has a double bond that can scavenge free radicals and act as a facilitator of communication between gap junctions ⁷. Gap junctions composed

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by transmembrane proteins is called connexin and one of them is Connexin-43 (Cx-43). The Cx-43 protein is a major protein constituent of gap junctions that facilitate communication between gap junctions ⁸.

Local application of drug through the oral cavity gives the advantage of lowering the dose of medication when compared with systemic administration ⁹. To get a successful local treatment on oral mucosa, drug delivery systems must be designed in such a way that it can survive in the target area and avoid the possibility of drug dissolution¹⁰. In a form of mucoadhesive patches, drug absorption can be directly localized to the area where the patch is placed. In this form, the patch has mucoadhesive strength so it will not be detached by the movement of the tongue¹¹. Rabbit as an experimental animal has a character that its turn-over period of gingival mucosa is similar to humans ranged between 10-12 days ¹². This gives the possibility to employ the rabbit to study the effectiveness of mucoadhesive patches. The aim of this study was to monitor the changes of micronucleus during panoramic examination by applying β -Carotene Patch to rabbit gingiva.

2. Materials and Methods

2.1. Subjects and Panoramic exposure

This was an experimental study using a simple randomized sampling design. Prior to the commencement of the research, the Ethical Clearance was obtained from the Ethical Committee of the Faculty of Dentistry Universitas Gadjah Mada-(No.287/KKEP/FKG-UGM/EC/2012 and No.662/KKEP/FKG-UGM/EC/2014). The formulation of β -carotene gingival mucoadhesive patches was made using formulas HPMC E-15, CMC-Na, Propylene Glycol, β -carotene stock solution and distilled water ¹³.

The sample included in this study were 18 male rabbits of New Zealand groove which have weights range from 2-2,5 kg and age around 6 months. They were equally divided into 2 group, i.e., 9 rabbits in each group. The rabbits in group I were given a panoramic examination without the application of the β -carotene patch, while in the group II the rabbits were applied with the β -carotene patch in a moment before panoramic examination and keep the patch until detached by itself (Figure 1). The panoramic examination was performed with the Yoshida Panoura panoramic radiography machine at the specifications of 80 kVp, 8 mA, 12 second, which gives an effective dose of 47 μ Sv ¹⁴.

2.2. Micronucleus test in gingival mucosa cells

Micronucleus count was measured prior to the panoramic radiographic examination and subsequently on days 3, 6, and 9 after the procedure. Samples were collected by swabbing the anterior gingival mucosa using a cervical brush and then smeared onto a clean glass slide containing two drops of 0.09% NaCl solution. The slides were prepared and stained using the Feulgen-Rossenbeck technique according to Cerqueira's protocol. Cells were fixed in a methanol-acetic acid solution (3:1), briefly treated with 5 M hydrochloric acid at room temperature for 15 minutes, stained with Schiff's reagent for 90 minutes, and counterstained with 1% Fast Green for one minute. For each slide, at least 1000 cells were examined through blinded analysis. Micronuclei were observed under a light microscope equipped with an OptiLab Viewer® (Optilab Inc., USA) at 400 \times magnification at the Histology Laboratory, Faculty of Medicine, Universitas Gadjah Mada. Micronuclei were identified based on the following criteria:

- Round or oval shape with smooth borders,
- Size ranging between one-fifth to one-third of the main nucleus,
- Located close to but clearly separate from the main nucleus,
- Feulgen-positive,
- Similar chromatin staining characteristics to the main nucleus, and
- Not overlapping with any other cell ^{1, 13, 16}.

Only cells with intact cytoplasm were included in the count (see Figure 2). A single experienced researcher, blinded to the sample identity, conducted the histological assessments. Changes in micronucleus frequency were statistically analyzed using one-way ANOVA followed by Tukey's HSD post hoc test. A p-value of less than 0.05 was considered statistically significant.



Figure 1a Application of β -carotene patch on the gingival mucosa of Rabbit before panoramic exposure; 1b. The procedure of taking a panoramic radiograph of the rabbit

3. Results and Discussion

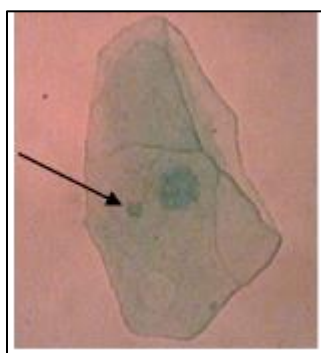


Figure 2 Micronucleus image stained using Schiff's reagent and counterstained using Fast green (light microscope x400)

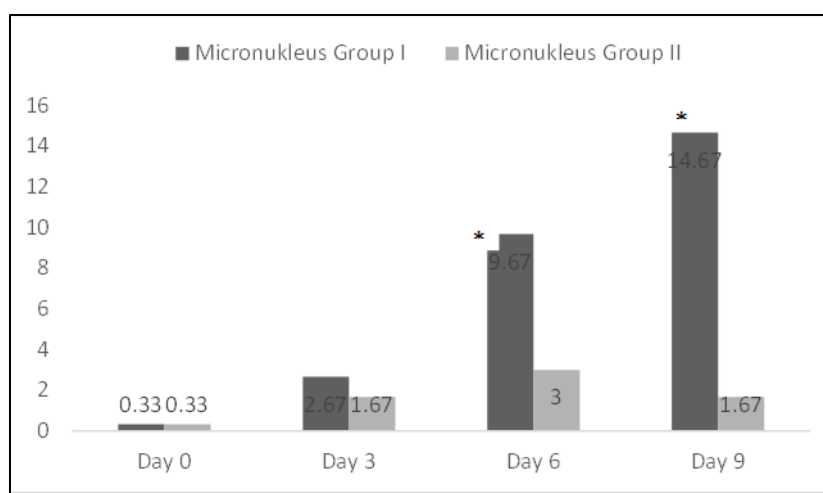


Figure 3 Mean frequency of the micronucleus from Group I (Data were taken from previous research¹³) and Group II at Day 0, Day 3, Day 6 and Day 9, respectively. *indicates significant differences between the two groups. There was a significant difference between group I and group II ($p=0.001$) for the observed frequency of micronucleus at day 6 ($p=0.029$) and day 9 ($p=0.004$)

Panoramic radiography is often used for diagnosis of dental arch and tooth disease¹³. X-rays that result from panoramic radiography has a potential to induce gene mutation and chromosomal aberration despite it only has a small dose of radiation^{1,13,14}. This is also supported by the present study that in the control group the frequency of micronucleus is increased from 2.67‰ at day 3 to 14.67‰ on day 9 (Fig. 3). This result showed that Rabbit as an experimental animal has a similar character to human's gingival mucosa to prove micronucleus increasing¹⁴.

Figure 3. also shows that by the use of β -carotene patch the frequency of micronucleus was just increased from 1.67‰ at day 3 to 3‰ at day 6 and decreased down to 1.67‰ at day 9, which is much lower than the control groups at the respective days. The difference between the groups was significant. This indicates that the β -carotene patch has an effect on protecting gingival cells from chromosomal damage induced by X-ray radiation. The possible explanation may be that β -carotene patch restrains the oxidation reaction by breaking the chain reaction of oxidation and have a function as a chain-breaking oxidation reaction ^{7,16}. Zhang ⁸ explained that carotenoids including β -carotene have the capability to enhance gap junction communication which was characterized by increased expression of the gene connexin-43 (Cx-43).

4. Conclusion

The conclusion of this present study is that application of β -Carotene patch in the gingiva of rabbits during panoramic radiography may reduce the formation of micronucleus cells. Further study in human beings is needed.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest with the data contained in the manuscript.

Statement of ethical approval

Ethical Approval performed by Committee of the Faculty of Dentistry Universitas Gadjah Mada-(No.287/KKEP/FGK-UGM/EC/2012 and No.662/KKEP/FGK-UGM/EC/2014)

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