

TOXICITY EVALUATION STUDY OF CHLORHEXIDINE USED IN DENTAL TREATMENT



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ABSTRACT

The aim of this study is the biological evaluation of chlorhexidine used in periodontal disease and disinfection of root canals. This compound is a broad spectrum antibacterial agent and is currently used as a local antiseptic. At low concentrations it has bacteriostatic effect and at high concentrations it has bactericidal effect. At low concentrations cell death occurred through apoptosis, and at higher concentrations, chlorhexidine induced necrosis.

Key words: chlorhexidine, topical application, cytotoxic effect; non-invasive skin measurements

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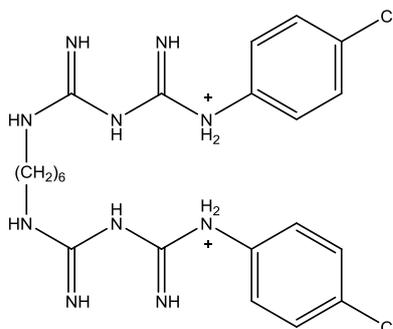
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INTRODUCTION

Chlorhexidine is a cationic synthetic molecule that contains in his structure 2 cycles of 4-chloro- and 2 biguanide groups united by a central chain of hexamethylene (Figure 1). This compound is known as a broad

spectrum antibacterial agent and currently is used as a local antiseptic [1,2]. At low concentrations has bacteriostatic effect and bactericidal at high concentrations [3].



Clorhexidina

Figure 1. Chemical structure of chlorhexidine

As a therapeutic recommendation, chlorhexidine is used to reduce the formation of dental plaque, gingivitis and disinfection of root canals. After administration, chlorhexidine is absorbed at oral mucosa and gastrointestinal level. It is metabolized in the liver and it's eliminated in the urine [4].

Regarding the toxicity of this compound in vitro and in vivo exist a considerable number of studies that shows an increased interest of researchers in this subject, but the toxic mechanism of action is incompletely understood. In a study carried out on a fibroblasts cell line isolated from mouse (L929), they were exposed to different concentrations of chlorhexidine (from 0.000025% to 0.016% by weight) for 24 h. At low concentrations (up to 0.002%) occurred cell death through apoptosis, and at higher concentrations, chlorhexidine induced fibroblasts necrosis. The results of this study indicated that the mechanism of action may involve the endoplasmic reticulum [5].

Giannelli M and his collaborators proposed checking the effects of different concentrations of

chlorhexidine digluconate (form that is found in most dental products) on the viability of cell lines osteoblasts, fibroblasts and endothelial cells and mechanism of action involved. The results have indicated proapoptotic effect of dozo- and tempo-dependent solution especially if osteoblast effect associated with disruption of mitochondrial function, increasing intracellular calcium ions and oxidative stress [3].

Chlorhexidine had showed a cytotoxic effect on cells isolated from Chinese hamster ovary. At low concentrations, chlorhexidine took proapoptotic effect and necrotic effect at high concentrations [1].

Salim and his co-authors have demonstrated its efficacy as an antifungal agent against *Candida* spp species [6].

The potential toxic effect of chlorhexidine has been studied on different types of normal cell lines, which are found in oral cavity, evaluating the toxicity of this compound in vivo after 8 days by oral gavage / 1 time / day of 3 ml solution chlorhexidine digluconate 0.12% on Wistar rats. The results indicated an

increase of defects at DNA level of leukocyte and kidney cells, they represent potential targets of toxic action of chlorhexidine digluconate [4].

So far no attention was paid to the effects of chlorhexidine solution in

the skin, where the solution comes into contact with skin. The objective of this study was to verify the effects of chlorhexidine gluconate solution (2%) on physiological parameters in mice skin after topical application SKH 1.

MATERIAL AND METHODS

SKH1 mice were obtained from Charles River Germany, female, 12 weeks. SKH1 mice were divided in 5 groups (4 mice/group): for each group mice were topically exposed to active agent, 1 application/day during 5 days. The solutions of chlorhexidine digluconat 2% from PPH CERKAMED.

All experimental procedures performed in this experiment were performed according to the Directive 2010/63/EU regarding the animal protection.

Non-invasive skin measurements

Determination of melanin and erythema values, markers with an important role in evaluating lesions in the skin were obtained with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. The measurements of erythema were obtained by means of the MPA5

Mexameter® MX 18 probe, as quantitative results regarding erythema (haemoglobin) subjected to toxicological evolution. The units for erythema and melanin were determined by a spectrophotometer evaluation.

The haemoglobin values for erythema were measured using 2 wavelengths: 560nm (green) and 660 nm (red) and for melanin also at two wavelengths: 660 nm (red) and 880 nm (infrared) [7-10].

Statistical analysis

Data were analyzed using paired Student's *t* tests or One-way Anova followed by Bonferroni's post-tests were used to determine the statistical difference between experimental and control groups; *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS AND DISCUSSIONS

This research was conducted on SKH1 mice in order to evaluate the effects of chlorhexidine digluconate 2%. The compound was topically applied on the dorsal side of the mice and were measured physiological skin

parameters with the help of a non-invasive techniques.

The first evaluation, macroscopic evaluation indicated the presence of both redness and skin dryness in the mice treated with the solution.

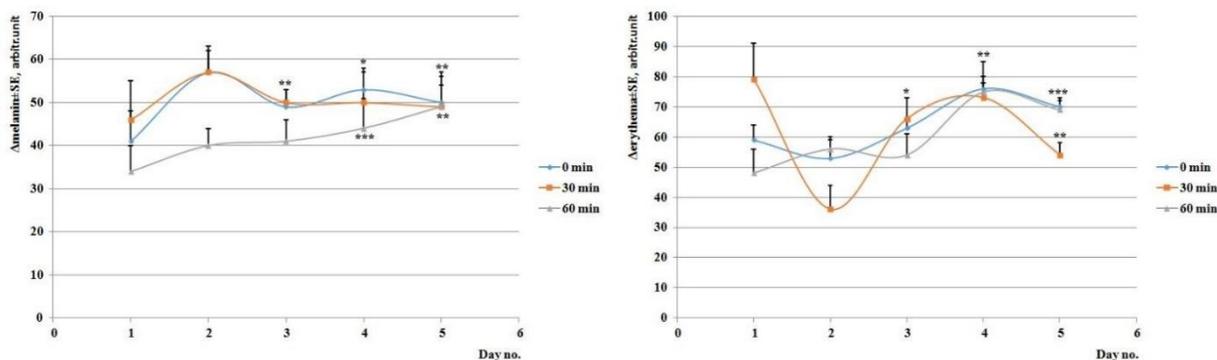


Figure 2. Melanin and erythema measurements prior to application, 30 minutes after application, 60 minutes respectively after application over the course of 5 days

Melanin and erythema (hemoglobin) indices are the indicators that quantify the intensity of pigmentation. The results showed a higher variation of melanin after immediate application of chlorhexidine digluconate as compared to the control

group as can be observed in the figure 2. The same conclusion can be drawn regarding the assessment of erythema, higher values at 30 minutes after application, which persists until the last day of the experiment.

CONCLUSIONS

At these concentration chlorhexidine digluconate lead to minor changes in the skin which persist for a long time. Future study, involving

use in various concentrations of chlorhexidine digluconate able to determine the value of which can exert harmful effects, need to be made.

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