Toxicological Activity of Some Compounds with Application on Dentistry Field

Experimental study

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The aim of present study was the biological evaluation of some compounds used in dentistry application like chlorhexidine, zinc chloride, eugenol, citric acid, and trichloroacetic acid. The compounds were topically applied on the dorsal side of the mice and were measured physiological skin parameters by means of a non-invasive techniques, mexametry in order to evaluate the values of erythema.

Keywords: chlorhexidine, zinc chloride, eugenol

In dental practice are used a number of chemical compounds with different effects, some of them even toxic. We investigate in this study the biological evaluation of five compounds commonly used in dentistry: chlorhexidine, eugenol, citric acid, zinc chloride and trichloroacetic acid.

Chlorhexidine (CHX) is a drug widely used as therapeutic agent both in medicine and dentistry with a toxic effect *in vitro* and/or *in vivo* and the direct application in the oral cavity can delay and alter wound healing [1,2]. In dentistry is used for decreasing plaque formation, gingivitis controlling and root canal disinfection [3], as endodontic irrigant, and local antiseptic. It is also used in products for dental hygiene like sprays, toothpaste, mouthwash, gels, and chewing gums in different concentrations and formulations [2]. Various studies demonstrated the cytotoxic effect of CHX in different cell lines and we can mention here murine fibroblast cell lines, human gingival fibroblast, human periodontal ligament cells, human alveolar bone cells etc [2]. In topical use is active at low concentrations and have activity against *Candida* spp. because it can be retained in the oral cavity for long time [4]. CHX is absorbed by the oral mucosa and the gastrointestinal tract and the mechanism of action of this compound involves different interactions with external cell components and the cytoplasmic membrane [5].

Eugenol (E), 4-allyl-2-methoxyphenol, is a natural compound with phenolic structure and have the antiviral, antibacterial, anticoagulation, antioxidant, anti-inflammatories activities [6,7]. In dentistry field is used in combination with zinc oxide for indirect pulp capping, temporary fillings and root canal sealers and it is included in of periodontal dressings, impression materials and endodontic medications. [8, 9]. It is a topical antiseptic and possess anti-inflammatory and analgesic properties. In humans orally administered eugenol are rapidly absorbed from the gastrointestinal tract and extracted by the liver, where take place conjugation, a phase II reaction, and excreted in the urine. The cytotoxicity of eugenol is determined by the growth or viability of cells.

Citric acid (CA) is a natural compound found usually in the nature and it is completely safe for both human health and environment. It is used in dental practice for the removal of the smear layer which appeared during mechanical root canal treatment. For dental treatment it is recommended to use the solution of 30%-40% concentration.

Zinc chloride (ZC)

Zinc is well known that is a fundamental ion that controls cell growth, is involved in bone metabolism and has an effect in dentine matrix formation but the mechanisms involved in this process are not entirely understood. Dentifrices with 2% zinc citrate have been used in the treatment of poor gingival health, because the anti-inflammatory and antimicrobial properties. [10, 11]. In dental materials is used for cements, amalgams, denture adhesives or fixatives, and is also added to toothpastes and mouth rinses [11]. Zinc chloride is used as dentine desensitizer for hypersensitive teeth [12].

Trichloroacetic acid (TA) has various applications in industry, but it is also used in medicine, as a hemostatic and/or cauterizing chemical agent in dentistry.

Experimental part

Materials and methods

The solutions of eugenol, zinc chloride 30% and trichloroacetic acid 15% were purchased from S.C. Lucstar Prod SRL, and the solution of citric acid 40% and chlorhexidine diglucanat 2% from PPH CERKAMED.

SKH1 mice were obtained from Charles River Germany, female, 8 weeks. SKH1 mice were divided in 5 groups (4 mice/group): for each group mice was topically exposed to chemical agent, 1 application/day during 5 days.

Non-invasive skin measurements

All the non-invasive measurements on mice skin were carried out 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) subject to toxicological evolution. The units for erythema were determined by a spectrophotometer evaluation.

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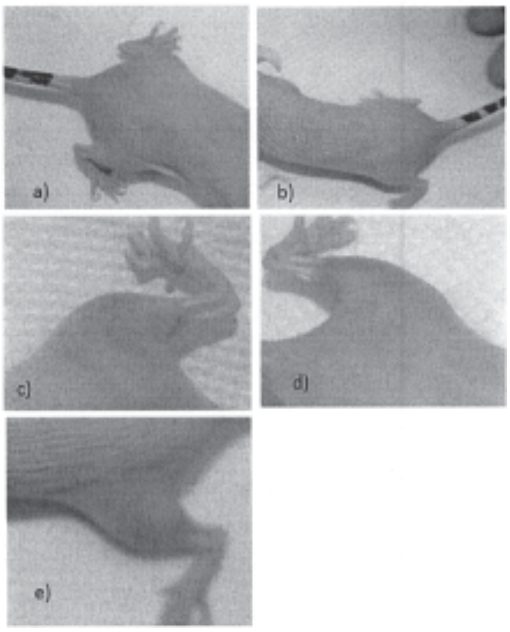

Fig. 1. Toxicological aspect of SKH1 mice after topical exposure to: (a) chlorhexidine solution, (b) citric acid 40% solution, (c) eugenol solution, (d) trichloroacetic acid solution and (e) zinc chloride solution

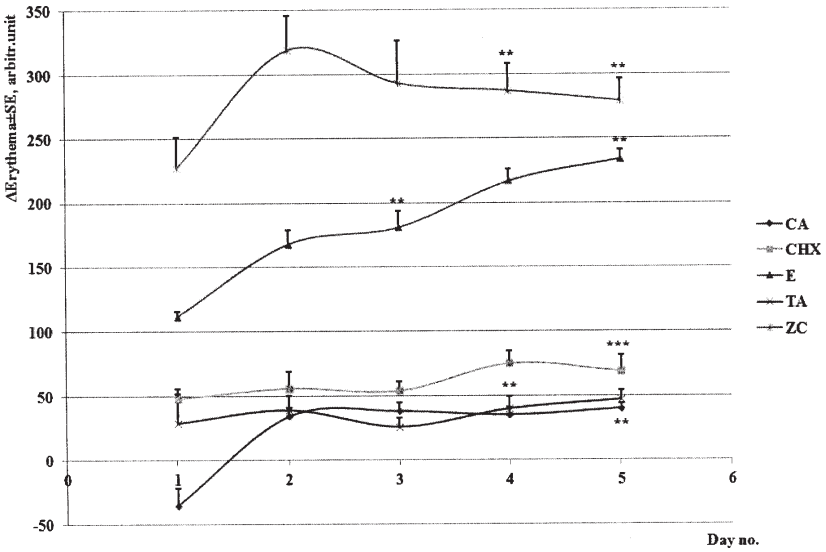

Fig. 2. Dental samples local erythema evaluation

The haemoglobin values for erytema were measured using 2 wavelengths: 560 and 660 nm [13-17]. We used their general units obtained by Mexameter soft evaluation and not the index as value. The applied area was 5 mm diameter for 20 s.

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

Macroscopic aspect of the dentistry compounds-induced lesions

This study was developed on SKH1 mice in order to evaluate the effects of different compounds used in dentristry field. The compounds were topically applied on the dorsal side of the mice and were measured physiological skin parameters by means of a non-invasive techniques, mexametry.

Macroscopic evaluation indicated the presence of redness and skin dryness especially in the groups treated with zinc chloride and eugenol (fig. 1).

Our results showed a higher degree of erythema after application of eugenol and zinc chloride as compared to the other three compounds, chlorhexidine, citric acid and trichloroacetic acid (fig. 2). The lowest values of erythema were observed in the group that received citric acid.

The present study indicated that series of usual compounds with a high relevance on fields like dental medicine determine important toxicological consequences. These data are relevant in any pharmacotoxicological evaluation. Dental applications are relevant in safety studies and these imposed a strong monitoring for this type of products.

Conclusions

Our preliminary results showed that topical application of the compounds led to cutaneous signs of toxicity, erythema values were higher after administration of zinc chloride and eugenol as compared to the other compounds.

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