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LETTER TO THE EDITOR

Cytogenetic damage induced by toothpastes: what is the best way to evaluate the harmful effects on oral mucosa cells?

Sir,

I read the manuscript of Tadin et al. [1], recently accepted for publication in the Acta Odontologica Scandinavica and titled 'In vivo evaluation of fluoride and sodium lauryl sulphate in toothpaste on buccal epithelial cell toxicity' with much interest. In this article, the author has demonstrated that some dentifrices containing fluoride and sodium lauryl sulphate are able to induce cytotoxicity on buccal mucosa cells, as assessed by micronucleus assay *in vivo*. In fact, a recent comprehensive literature review was published by our research team that concluded that fluoride is able to induce cell death *in vitro* and *in vivo* [2]. However, this suggests thinking through of the matter.

In this article, there was an increase in the frequency of some metanuclear changes, such as pyknosis, nuclear buds and karyorrhexis following toothpaste exposure. Nevertheless, Giemsa was used for staining slides. It is important to mention that the Giemsa method is not recommended when evaluating the micronucleus assay on buccal mucosa cells since it is not specific for nucleic acids [3]. Therefore, the precise identification of nuclear changes is quite complicated since other cell structures, as for example, keratohyalin granules or bacteria, are stained as well [4]. This leads inevitably to false-positive results. Another question refers to the total number of cells evaluated. According to the Micronucleus Assay Expert Group, it is necessary to evaluate a minimum of 2000 oral cells per volunteer [3]. In this study, a total of only 1000 cells were recorded. The approach is very important because the occurrence of micronucleus is scarce in the oral mucosa. In addition, the micronucleus frequency presented in Tables 2 and 3 decreased following toothpaste exposure, even if it was not statistically significant. It is important to stress that the micronucleus frequency decreases when cytotoxicity is increased, because micronuclei are lost due to the cell death. Taken together, to increase the total number of cells would significantly improve the power analysis of the assay.

In Table 3, the results from pyknosis show zero values in many time points established in the experimental design. How is the occurrence possible inasmuch as the event represents the normal process of epithelial differentiation? Finally, it was established that only buccal cells would be evaluated in the study (the information was described in material and methods section) . However, it was related to Tables 2 and 3 that 'average cytogenetic damage score/1000 gingival epithelial cells'. Did the authors evaluate buccal and gingival cells simultaneously? This requires further clarification.

We believe that these comments would be useful for better understanding of the relevant study investigating cytogenetic damage on buccal mucosa cells of volunteers continuously exposed to toothpastes containing fluoride and sodium lauryl sulphate.

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Disclosure statement

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