The aim of this study was to evaluate the protective effects of different concentrations of vitamin E alpha-tocopherol (\(\alpha\)-T) isomer against the toxicity of hydrogen peroxide (H\(_2\)O\(_2\)) on dental pulp cells. The cells (MDPC-23) were seeded in 96-well plates for 72 hours, followed by treatment with 1, 3, 5, or 10 mM \(\alpha\)-T for 60 minutes. They were then exposed or not to H\(_2\)O\(_2\) for 30 minutes. In positive and negative control groups, the cells were exposed to culture medium with or without H\(_2\)O\(_2\) (0.018%), respectively. Cell viability was evaluated by MTT assay (Kruskal-Wallis and Mann-Whitney tests; \(\alpha = 5\)%). Significant reduction of cell viability (58.5%) was observed in positive control compared with the negative control. Cells pretreated with \(\alpha\)-T at 1, 3, 5, and 10 mM concentrations and exposed to H\(_2\)O\(_2\) had their viability decreased by 43%, 32%, 25%, and 27.5%, respectively. These values were significantly lower than those observed in the positive control, thereby showing a protective effect of \(\alpha\)-T against the H\(_2\)O\(_2\) toxicity. Overall, the vitamin E \(\alpha\)-T isomer protected the immortalized MDPC-23 pulp cells against the toxic effects of H\(_2\)O\(_2\). The most effective cell protection was provided by 5 and 10 mM concentrations of \(\alpha\)-T.

1. Introduction

Hydrogen peroxide (H\(_2\)O\(_2\)) is a thermally instable chemical agent with high oxidative power, which dissociates into free radicals and other reactive oxygen species (ROS), such as hydroxyl radicals (OH\(^-\)), singlet oxygen (O\(^2^-\)), and superoxide anion (O\(_2^+\)) [1]. This molecule has been widely used in dentistry to treat discolored teeth, because of its capability to oxidize the complex organic molecules of the dental structure that respond for the darker coloration of the teeth [2]. However, these highly oxidative molecules can diffuse through mineralized tooth structures, such as enamel and dentin, to reach the subjacent pulp tissue, a specialized connective tissue responsible for maintaining the tooth viability [3, 4]. The contact of the pulp cells with ROS results in oxidative stress generation, mainly because of the imbalance between the amount of ROS and endogenous antioxidants [1]. This oxidative stress damages the cell membrane and causes cell viability reduction, extracellular matrix degradation, inflammatory tissue reaction, and even pulp pal necrosis [3–5].

The treatment of dental pulp cells with antioxidants has been proposed in order to prevent the oxidative damage from components leached by dental materials and bleaching gels, which are capable of diffusing across mineralized tissues of teeth [6, 7]. Vitamin E (VE) has a recognized anti-inflammatory and antioxidant activity in different cell
2. Materials and Methods

The H$_2$O$_2$ concentration capable of reducing the cell viability by approximately 50% (IC-50) was determined. For this purpose, solutions containing decreasing H$_2$O$_2$ concentrations were prepared (0.035%, 0.018%, 0.009%, and 0.045%) in serum-free DMEM (Dulbecco's Modified Eagle's Medium; Sigma Aldrich Corp., St. Louis, MO, USA). Then, odontoblast-like MDPC-23 cells were seeded in DMEM supplemented with 10% fetal bovine serum (FBS; Gibco Co., Grand Island, NY, USA) and antibiotics (IU/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L glutamine; Gibco Co.), in 96-well plates (1 x 10$^4$ cells/well) (Costar Corp., Cambridge, MA, USA) during 72 h at 37°C and 5% CO$_2$. After that, the DMEM was aspirated and 100 µL of the H$_2$O$_2$ solutions were applied on the cells during 30 minutes. Cell viability was evaluated by the cytochemical demonstration of the succinic dehydrogenase (SDH) enzyme using the methyl tetrazolium (MTT) assay (Gibco Co.) [3, 4]. The absorbance values of the groups (570 nm) were transformed into percentages of cell viability, considering the negative control group (DMEM) as having 100% of cell viability. The 0.018% H$_2$O$_2$ concentration resulted in 59% of cell viability reduction and was selected to evaluate the α-T protective effect against H$_2$O$_2$ aggression.

In order to evaluate the protective effect of α-T against H$_2$O$_2$ toxicity, four decreasing concentrations of this molecule (1, 3, 5, and 10 mM) were prepared by diluting a stock α-T solution (Sigma Chemical Co.) in DMEM with 5% dimethyl sulfoxide (DMSO). In this way, experimental groups were formed according to the treatment of the MDPC-23 cells with different α-T concentrations followed by exposition or not of the cells to a 0.018% H$_2$O$_2$ solution for 30 minutes. To evaluate α-T toxicity (α-T+ H$_2$O$_2$−), the α-T solutions were applied on cultured cells for 60 minutes; to evaluate α-T protective effect against H$_2$O$_2$ aggression, the solutions were applied for 60 minutes and then aspirated, followed by H$_2$O$_2$ application for 30 minutes (α-T+ H$_2$O$_2$+). In negative control group, DMEM containing 5% DMSO was applied (α-T− H$_2$O$_2$−) on the MDPC-23 cells. In positive control group, 0.018% H$_2$O$_2$ was applied on the cultured cells for 30 minutes. After treatments, the MTT assay was performed and percentages of cell viability for each experimental group were determined. Data were subjected Kruskal-Wallis complemented by the Mann-Whitney test. The significance level was set at 5% and the following null hypotheses were established: (1) H$_2$O$_2$ does not cause toxic effects to odontoblast-like cells; (2) α-T cannot eliminate or at least reduce the oxidative effects of H$_2$O$_2$. Three independent experiments were performed at different times to demonstrate the reproducibility of data, and, in each appointment, a total of six replicates (n = 6) were used for each group.

3. Results

Table 1 shows the results for the H$_2$O$_2$ IC-50. The experimental groups used to assess the protective role of α-T against cell toxicity mediated by H$_2$O$_2$ are summarized in Table 2. Cell viability data obtained after cell treatment with α-T followed or not by exposure to H$_2$O$_2$ are shown in Table 3. Considering the negative control group (G1) as having 100% of cell viability, there was a 58.5% decrease in the positive control group (G2) that was lower than that observed in the experimental groups (P < 0.05). The cell viability reduction in groups G3, G4, G5, and G6, in which the MDPC-23 cells were treated with different concentrations of α-T, was 6%, 13%, 10%, and 14%, respectively. Despite being considered discrete, the cell viability reduction for G4, G5, and G6 was
the cytotoxicity of different dental products and the immortalized pulp cell line has widely been used to evaluate oxidative effects of this unstable chemical agent to the biological components of dental products capable of diffusing through enamel and dentin [11]. In addition, for over a decade, this cell structure [14]. Antioxidants such as α-T reduced oxidative reactions generated during the inflammation [17–20]. The authors showed that this kind of vitamin can block nitric oxide synthase (iNOS), COX-2 expression, and the NF-κB signaling pathway in cultured monocytes stimulated by E. coli LPS. Additionally, VE was capable of inhibiting the synthesis of PGE2 and inflammatory cytokines, such as TNF-α, IL-4, and IL-8. Therefore, one can consider that VE has a broad therapeutic potential. The present investigation revealed that cells exposed only to H₂O₂ (G2) presented a 58.5% reduction in cell viability. The toxic effect of H₂O₂ was also reported in previous studies in which the authors evaluated the trans-enamel and trans-dentinal cytotoxicity of high concentrations of H₂O₂ on odontoblast-like cells [3, 4]. On the other hand, the treatment of MDPC-23 cells with different concentrations of α-T prior to their exposition to H₂O₂ increased the cell viability by 16–33.5%.

Despite the important protective effect, α-T alone caused a slight cell viability reduction in those groups in which the cells were not exposed to H₂O₂ (G3 to G6). It was shown that 1 mM α-T concentration was statistically similar to the control (G1). On the other hand, 3, 5, and 10 mM α-T concentrations were significantly different from G1. These data suggest that an increase of the α-T concentration...
available to the cells might cause a prooxidant action of this VE isomer, resulting in reduction in the viability of the treated cells. Some studies have demonstrated the prooxidant action of α-T at high concentrations or in the presence of heavy metals or peroxides [21–23]. These findings could explain the results observed in those groups in which the MDPC-23 cells were exposed only to α-T (G3 to G6). However, while a slight prooxidant action of α-T was observed (6–14% cell viability reduction), this molecule was capable of minimizing the oxidant effect caused by H₂O₂ on cultured MDPC-23 cells (G7 to G10). The most relevant protective effects were obtained with 5 mM (G9) and 10 mM (G10) α-T concentrations, in which 33.5 and 31% of cell viability recovery were observed, respectively. Since no significant difference was found between G9 and G10, it may be suggested that the best α-T concentration for pretreatment of odontoblast-like cells would be 5 mM. This is not only because of the protective effect of this molecule against the H₂O₂ cell damage but also due to its slight toxicity (G5–10% cell viability reduction).

Overall, this in vitro study demonstrated the potential of α-T as an antioxidant agent because this VE isomer was capable of protecting pulp cells against the harmful effects of H₂O₂, which is the main active component of tooth bleaching gels. Although the present laboratory-based results cannot be directly extrapolated to clinical situation, the original data obtained under the tested experimental conditions are promising.

5. Conclusion

It can be concluded that previous exposition of odontoblast-like MDPC-23 pulp cells to VE α-T isomer protects this cell line against the toxic effects generated by hydrogen peroxide in vitro. These data can drive further in vivo studies with the purpose of establishing specific therapies capable of preventing or at least minimizing the pulpal damage caused by tooth bleaching techniques widely used in dentistry. This may avoid the postbleaching tooth sensitivity, making this esthetic clinical procedure safer and more comfortable to the patients.

Conflict of Interests

The authors have no conflict of interests.

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