The aim of this study was to evaluate the protective effects of different concentrations of vitamin E alpha-tocopherol (α-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 α-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; α = 5%). Significant reduction of cell viability (58.5%) was observed in positive control compared with the negative control. Cells pretreated with α-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 α-T against the H$_2$O$_2$ toxicity. Overall, the vitamin E α-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 α-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 pulpal 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
lineages, such as fibroblast, osteoblasts, and neurons [8]. This kind of vitamin is composed of a blend of tocopherols and tocotrienols; however, the antioxidant action of VE is mediated by the alpha-tocopherol (α-T) isomer [9]. The α-T is capable of stabilizing cell membrane against reactive oxygen species (ROS) produced during normal cellular metabolic activities, preventing the chain propagation from the oxidative stress [10]. The protective activity of this molecule against the oxidative damage related to different conditions, as atherosclerosis, diabetes, Alzheimer, and Parkinson diseases, has been widely described [8]. In view of this, it was hypothesized that the VE antioxidant property may also protect pulp cells against the oxidative toxic effects caused by components leached by dental bleaching gels. Therefore, the aim of this study was to evaluate the protective effects of different concentrations of VE α-T isomer against the toxicity of H₂O₂ applied on the immortalized odontoblast-like MDPC-23 cell line.

2. Materials and Methods

The H₂O₂ concentration capable of reducing the cell viability by approximately 50% (IC-50) was determined. For such purpose, solutions containing decreasing H₂O₂ concentrations were prepared (0.035%, 0.018%, 0.009%, and 0.0045%) 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 10⁴ cells/well) (Costar Corp., Cambridge, MA, USA) during 72 h at 37°C and 5% CO₂. After that, the DMEM was aspirated and 100 μL of the H₂O₂ 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% H₂O₂ concentration resulted in 59% of cell viability reduction and was selected to evaluate the α-T protective effect against H₂O₂ aggression.

In order to evaluate the protective effect of α-T against H₂O₂ 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% H₂O₂ solution for 30 minutes. To evaluate α-T toxicity (α-T+ H₂O₂−), the α-T solutions were applied on cultured cells for 60 minutes; to evaluate α-T protective effect against H₂O₂ aggression, the solutions were applied for 60 minutes and then aspirated, followed by H₂O₂ application for 30 minutes (α-T+ H₂O₂+). In negative control group, DMEM containing 5% DMSO was applied (α-T− H₂O₂−) on the MDPC-23 cells. In positive control group, 0.018% H₂O₂ 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) H₂O₂ does not cause toxic effects to odontoblast-like cells; (2) α-T cannot eliminate or at least reduce the oxidative effects of H₂O₂. 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 H₂O₂ IC-50. The experimental groups used to assess the protective role of α-T against cell toxicity mediated by H₂O₂ are summarized in Table 2. Cell viability data obtained after cell treatment with α-T followed or not by exposure to H₂O₂ 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>H₂O₂ concentration</th>
<th>Cell viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>(α-T− H₂O₂−)</td>
<td>0.018% H₂O₂</td>
<td>5</td>
</tr>
<tr>
<td>G2</td>
<td>(α-T− H₂O₂+)</td>
<td>0.009% H₂O₂</td>
<td>41</td>
</tr>
<tr>
<td>G3</td>
<td>(1mM+ H₂O₂−)</td>
<td>0.0045% H₂O₂</td>
<td>41</td>
</tr>
<tr>
<td>G4</td>
<td>(3mM+ H₂O₂−)</td>
<td></td>
<td>77</td>
</tr>
<tr>
<td>G5</td>
<td>(5mM+ H₂O₂−)</td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>G6</td>
<td>(10mM+ H₂O₂−)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G7</td>
<td>(1mM+ H₂O₂+)</td>
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<tr>
<td>G8</td>
<td>(3mM+ H₂O₂+)</td>
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<tr>
<td>G9</td>
<td>(5mM+ H₂O₂+)</td>
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<td></td>
</tr>
<tr>
<td>G10</td>
<td>(10mM+ H₂O₂+)</td>
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</table>

Table 2: Control and experimental groups (n = 6) formed according to the treatment of the MDPC-23 cells with different alpha-tocopherol (α-T) concentrations followed by exposure or not to hydrogen peroxide (H₂O₂).
The cytotoxicity of different dental products and the immortalized pulp cell line has widely been used to evaluate investigations [9, 10]. A recent study demonstrated that the oxidative effects of this unstable chemical agent to the H2O2, cytotoxicity observed in G7, G8, G9, and G10 was significantly higher when compared to the positive control group (G2) regardless of the α-T concentration (P < 0.05). G8, G9, and G10 presented the highest values of cell viability recovery, with no significant difference among them (P > 0.05) (Table 3, rows). G9 and G10, which did not show significant difference when the cells were treated or not with H2O2 (P > 0.05) (Table 3, columns), presented the best results for cell viability recovery.

Based on the fact that H2O2 caused toxic effects to the cultured odontoblast-like cells and that α-T reduced the oxidative effects of this unstable chemical agent to the immortalized pulp cell line, both null hypotheses presented in this study were rejected.

### 4. Discussion

In spite of being very popular in dental offices, vital tooth bleaching has been associated with postoperative sensitivity and pulpal damage [3–5]. In view of this, different therapies have been suggested to minimize these adverse effects, including pretreatment with antioxidant agents to reduce the oxidative stress generated by bleaching gel components to the pulp cells [7]. In the present study, the biological activity of VE α-T isomer against the toxic effects of H2O2 to MDPC-23 cells was evaluated. This specific kind of pulp cell, which presents odontoblast phenotype, was used in this study because in mammalian teeth odontoblasts are organized in a monolayer to underlie the dentinal tissue. Therefore, odontoblasts are the first pulp cells to be reached by components of dental products capable of diffusing through enamel and dentin [11]. In addition, for over a decade, this cell structure [14] has been described that α-T is the compound responsible for the orthoposition of its methyl group, compared with the other VE isomers [15]. Therefore, α-T can prevent oxidative stress propagation and stabilize the cell membrane, thus preventing the disruption of the amphipathic balance of this cell structure [14]. Antioxidants such as α-T can stop free radicals by donating one of their electrons to the free radical. However, α-T does not become a new free radical because it remains stable before and after donating the electron, which characterizes its antioxidant action [14]. It has also been shown that VE can prevent diseases such as atherosclerosis as well as cardiovascular and inflammatory disorders [16]. Some researchers have reported that VE is directly involved in the maintenance of the balance of 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-enzyme 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 1mM α-T concentration was statistically similar to the control (G1). On the other hand, 3, 5, and 10mM 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 H$_2$O$_2$ 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 H$_2$O$_2$ 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 
H$_2$O$_2$, 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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