SUMMARY

Tooth bleaching procedures have become very popular since their first introduction in dental clinical practice. There are concerns about the effects of exposure of oral tissues to bleaching agents. The purpose of the present study was to determine the cytotoxic effect of 2 bleaching agents (sodium perborate and carbamide peroxide) against 2 fibroblastic cell lines: BHK21/C13 baby hamster kidney fibroblasts and RPC-C2A rat pulp cells were used for the experiment. The cells were grown as monolayer cultures at 37°C in an atmosphere containing 5% CO₂ in air, and 100% relative humidity. Cells were plated in multiwell plates and serial dilutions of the bleaching agents were prepared and placed in contact with cell cultures. The anti-proliferative effect was determined after 24 and 48 hours of exposure by means of the colorimetric sulphorodamine B assay, in reference to controls.

BHK21/C13 and RPC-C2A reacted to the bleaching agents with different intensity. Cell proliferation was reduced compared to the controls after 24 and 48 hours of exposure in both cell lines. The cytotoxic effect was concentration- and time-dependent. Sodium perborate was the most potent material tested.

Keywords: Cytotoxicity; Sodium Perborate; Carbamide Peroxide

Introduction

Tooth discoloration is an important concern in aesthetic dentistry. There are several reasons why teeth may change colour, including food, drinks, tobacco, poor oral hygiene, diseases, medications, dental materials, age, genetics, environment, dental trauma, endodontic treatment, systemic diseases etc.1-4.

Tooth bleaching techniques were developed a century ago and all of them involved the process of oxidation. Generally, bleaching includes 2 types of techniques: vital and non-vital. There are 2 main methods of vital tooth bleaching. One involves the application of an oxidizing agent for a short period of time and may include activation of the process by heat or light. The other method involves using a specially prepared mouth guard to hold the oxidizing agent next to the teeth for few hours a day, usually during night, for 1 to 3 weeks.

Non-vital tooth bleaching techniques include mainly the thermocatalytic method5 and the walking bleach method6. In the thermocatalytic method, heat is used to activate the oxidizing agent placed in the pulp chamber. In the walking bleach technique, the bleaching agent is sealed in the pulp chamber for 3-7 days and thereafter it is replaced regularly until a satisfactory result is obtained (usually 2-3 times)7.

The most commonly used bleaching agents are hydrogen peroxide and sodium perborate, either used alone or in combination. More recently carbamide peroxide has also been recommended. Carbamide peroxide is mainly proposed for vital tooth bleaching at concentrations ranging from 10% to 35%. Sodium perborate alone or in combination with hydrogen peroxide is the main medicament in non-vital tooth bleaching techniques8-10.

Although generally positive results have been reported concerning the whitening ability of the bleaching agents, rather few studies have addressed their possible undesirable effects on living tissues11-14. The risk of bleaching agents to be a threat to the adjacent tissues is correlated to several factors, such as the used amount and concentration, the diffusion through dental tissues, the contact to the gingival tissues.

The biological risk from bleaching agents can be estimated by tests in vitro. In vitro methods are routinely...
used in toxicity testing, safety assessment, and risk evaluation. Cell cultures can be used for screening the cytotoxicity of materials. The purpose of the present study was to evaluate the cytotoxic effect of sodium perborate and carbamide peroxide using 2 fibroblastic cell lines.

Material and Methods

Sodium perborate and carbamide peroxide were purchased from Sigma Aldrich Co (USA). 2 established cell lines were used: BHK21/C13 (baby hamster kidney fibroblasts) and RPC-C2A (rat pulp cells). BHK21/C13 were obtained from ICRF (London, UK) and RPC-C2A cells were a generous offer from Prof. S. Kasugai (Tokyo, Japan). Cells were grown as monolayer cultures in T-75 flasks (Corning Costar), sub-cultured twice a week at 37°C in an atmosphere containing 5% CO₂ in air and 100% relative humidity, and maintained at a low passage number (5-20). The culture medium was Dubelcco’s modified Eagle’s medium (DMEM, Gibco, Glasgow, UK), supplemented with 10% foetal bovine serum (FBS, Gibco, Glasgow, UK), 100 IU/ml penicillin and 100 μg/ml streptomycin.

Adherent cells at a logarithmic growth phase were detached by the addition of 2-3 ml of a 0.05% trypsin (Gibco Brl, 1:250) - 0.02% EDTA mixture and incubation for 2-5 min at 37°C. Cells were plated (5,000 cells in 100 μl of culture medium per well) in 96-well flat-bottomed micro-titre plates (Corning Costar). Micro-plates were left for 24 h at 37°C to allow cells attach to the bottom of the wells and resume exponential growth. 100 μl of serial dilutions of the bleaching agents (diluted in the culture medium) were added on the cells (final volume in each well 200 μl) after 24 h. 6 replicate wells for each concentration were used. Negative control wells containing the same volume of complete medium, were included in each experiment. Cell growth was evaluated 24 and 48 h later by means of the SRB assay. All experiments were repeated at least twice.

The SRB assay was carried out as previously described¹⁵ and modified by Papazisis et al¹⁶. In brief, 70 μl 0.4% (w/v) sulforhodamine B (SRB, Sigma) in 1% acetic acid solution were added to each well and left at room temperature for 20 min. SRB was removed and the plates washed 5 times with 1% acetic acid before air drying. Bound SRB was solubilized with 200 μl 10 mM un-buffered Tris-base solution (Sigma) and plates were left on a plate shaker for at least 10 min. Absorbance was read in a 96-well plate reader (Anthos-2001, Anthos labteck instruments, A-5022, Salzburg) at 492 nm subtracting the background measurement at 620 nm. The test optical density (OD) value was defined as the absorbance of each individual well, minus the blank value (“blank” is the mean optical density of the background control wells, n = 8). Mean values and CV from 6 replicate wells were calculated automatically. Dose-response curves were plotted (values expressed as percentage of control optical density). The data were analyzed by ANOVA and the Student-Newman-Keul test (p<0.05).

Results

![Graph](image1)

Figure 1. Effect of carbamide peroxide and sodium perborate on BHK21/C13 cells after (a) 24 hours and (b) 48 hours of exposure. Each point and error bar represents mean ± SD of 6 replicate wells.

![Graph](image2)

Figure 2. Effect of carbamide peroxide and sodium perborate on RPC-C2A cells after (a) 24 hours and (b) 48 hours of exposure. Each point and error bar represents mean ± SD of 6 replicate wells.
The results are shown in figure 1 for BHK21/C13 and in figure 2 for RPC-C2A cells. BHK21/C13 and RPC-C2A cells reacted to the bleaching agents with different intensity and BHK21/C13 cells were more sensitive than RPC-C2A. Cell number was significantly reduced compared to controls after 24 and 48 hours of exposure in both cell lines (p<0.05). The cytotoxic effect was concentration- and time-dependent. Sodium perborate was the most potent material tested.

Discussion

In this study both bleaching agents tested suppressed the proliferation of BHK21/C13 and RPC-C2A cells as analyzed by the SRB assay, which is a well established method for material testing. The method is based on the measurement of cellular protein content. Sulphorhodamine-B (SRB) stains vital cells and the amount of dye taken up is measured in a spectrophotometer.

The mechanism of action of sodium perborate and carbamide peroxide is based on the production of hydrogen peroxide. Hydrogen peroxide is a reactive oxidizing agent, along with superoxide (O2−), hydroxyl (HO), peroxyl (ROO) and alkoxyl (RO) radicals. Oxygen radicals are a potential source of cell damage through causing DNA strand breaks, genotoxicity, and cytotoxicity.

Woolverton et al compared 2 carbamide peroxide oxygenating agents with 7 widely used dental products in 1.929 cells and found that both agents were no more toxic than the other materials. In another study, carbamide peroxide was less cytotoxic than hydrogen peroxide although both materials exerted cytotoxic effect to fibroblasts. Today, carbamide peroxide concentrations of either 35% or 22% may be used for external tooth bleaching. However, to avoid hazardous side effects (tooth sensitivity, mucosal irritation, alteration of enamel surface) concentrations higher than 10% should be avoided.

Although recently an intracoronal bleaching method with carbamide peroxide has been proposed, sodium perborate and hydrogen peroxide are still the most common materials used in clinical practice.

External cervical resorption of endodontically treated teeth has been reported after intracoronal bleaching. One possible mechanism for this effect is that the bleaching agent reaches the periodontal tissues through dentinal tubules and causes an inflammatory reaction.

Asflorad et al evaluated the biocompatibility of sodium perborate and 30% hydrogen peroxide using the analysis of adherence capacity and morphology of macrophages. They showed that hydrogen peroxide caused irreversible cellular damage while sodium perborate caused neither morphological nor functional alteration in macrophages. Kinomoto et al examined the cytotoxicity of internal bleaching agents on human periodontal ligament cells in vitro. The least cytotoxic material after 24h was sodium perborate and the most toxic the mixture of sodium perborate and hydrogen peroxide. However, after 48 hours the cytotoxicity of sodium perborate increased and it was more cytotoxic than hydrogen peroxide. The results of our study showed that both bleaching agents tested were cytotoxic compared to controls, and sodium perborate was the most potent material.

It is clear that it is not possible to extrapolate the in vitro results to in vivo conditions since various mechanisms exist in human tissues protecting them from damage. On the other hand, the bleaching agents remain in direct contact with living tissues for prolonged time thus increasing the risk of undesirable biological effects.

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References


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