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Effect of oxybenzone on PGE_2-production in vitro and on plaque and gingivitis in vivo

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Abstract

Objectives: To study the effect of oxybenzone on prostaglandin E_2 (PGE_2) production in cell culture and to evaluate the effect of an oxybenzone-containing dentifrice on plaque and gingivitis in a 6-week clinical trial.

Material and Methods: Human embryo palatal mesenchyme (HEPM) cells were used for testing the inhibition of IL-1β-stimulated PGE_2-production in vitro by different concentrations of oxybenzone. For the in vivo study, a total of 66 individuals with a Quigley & Hein plaque index of at least 1.5 and an Ainamo & Bay gingival index of at least 0.2 were included in a double-blind clinical trial with two cells and a parallel design. Two compositions of fluoride dentifrice were used, one with the addition of 0.5% oxybenzone, and one without. Plaque and gingival index were obtained at three time points: (1) at baseline, (2) after 3 weeks, and (3) after 6 weeks.

Results: A dose-dependent inhibition of PGE_2-production was found in the HEPM cell culture following oxybenzone exposure. In the clinical trial, a 25% reduction of gingival index was observed in the oxybenzone group ($p<0.001$) after 6 weeks as compared with 2% for the placebo group.

Conclusions: These findings indicate that PGE_2-production is reduced by oxybenzone in vitro and that the use of oxybenzone in a dentifrice reduces gingivitis in vivo.

Key words: cell culture; gingivitis; oxybenzone; PGE_2; plaque; salivary microflora; toothpaste

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Oxybenzone, or benzophenone-3, is a monomethyoxylated derivate of 2-hydroxybenzophenone. It is an ultraviolet-absorbing compound that occurs naturally in flower pigments (French 1992). Oxybenzone has been used in industry and medicine for more than 30 years, for example as a sunscreen lip-protection for individuals at a high risk for squamous cell carcinoma and recurrent herpes labialis (Lundeen et al. 1985). In 1983, the Cosmetic Ingredient Review Expert Panel (CIR) concluded that oxybenzone is safe for topical application to humans as cosmetics.

A large number of phenolic compounds exhibit both antibacterial and anti-inflammatory activities. It is well known that triclosan inhibits the production of inflammatory mediators, such as prostaglandin E_2 (PGE_2) (Gaffar et al. 1995, Modéer et al. 1996), and plaque formation (Garcia-Godoy et al. 1990, Cubells et al. 1991, Deasy et al. 1991, Lindhe et al. 1993). Other phenol compounds, such as oxybenzone, may have similar anti-inflammatory and anti-plaque properties as triclosan. It is therefore possible that oxybenzone may influence plaque and gingivitis formations in humans. The aim of the present study was to test the effect of oxybenzone in vitro on PGE_2-production in cell culture and to evaluate the effect of an oxybenzone-containing dentifrice on plaque and gingivitis in vivo.

Material and Methods

Cell culture

Human embryo palatal mesenchyme cells (HEPM, fibroblast-like cells, ATCC #1486) were used for testing the inhibition of IL-1β-stimulated PGE_2-production. These cells were grown in high glucose Dulbecco's modified Eagle's medium (DMEM, Gibco/BRL, Cheshire, UK) containing 10% heat-inactivated foetal bovine serum (FBS, Gibco/BRL). Cells were grown at 37°C with 100% humidity and 10% CO_2. Prior to analyses, cells were subcultured onto 24-well plates and allowed to become 70–90% confluent. On day 1 of the experiment, cells were washed three times with serum-free

media containing 0.5% bovine serum albumin (BSA), followed by replacement with the same media that also contained 1 ng/ml of IL-1β with or without oxybenzone. As a negative control, cells were exposed to neither IL-1β nor oxybenzone. As a positive control, cells were exposed to IL-1β but not oxybenzone. After an exposure time of 24 h, the media were removed, the pH adjusted to 3.5 with HCl, and finally assayed for PGE_2-production using a PerSeptive Diagnostics (Cambridge, MA, USA) TiterFluor PGE_2 EIA kit. Normal growth media were placed back onto the cells after two washings, and the cell viability was tested using MTT assay (Boehringer Mannheim Biochemica Cell Proliferation Kit I, Indianapolis, IN, USA). IL-1β (Boehringer Mannheim Biochemica) at 1.0 ng/ml was used to stimulate the production of PGE_2 and 15 different concentrations of oxybenzone (50–0.01 μM) were used for testing inhibition. Duplicates were run for each concentration.

Clinical trial

One hundred and twenty students and staff members at the University of Kristianstad, Sweden were screened for the presence of plaque and gingivitis. Those having an initial plaque index of at least 1.5 and a gingival index of at least 0.2, recorded as described below, were invited to participate. Other inclusion criteria were good general health, a minimum of 20 uncrowned permanent teeth (excluding third molars) and an age between 18 and 65 years. Subjects with orthodontic bands, removable dentures, advanced periodontal disease, more than five carious lesions and known history of allergy to components of the dentifrice, were excluded from the study. Subjects who, 1 month prior to the study or that during the course of the study, used antibiotics were also excluded.

Of the 120 individuals, totally 66 fulfilled the inclusion criteria. The study was carried out double-blind with two cells and a parallel design. The individuals were stratified according to plaque and gingivitis levels in order to balance the two groups. One and the same person (author L.J.) carried out all clinical recordings at three occasions: (1) at baseline, (2) after 3 weeks, and (3) after 6 weeks. The study protocol was approved by the Ethics Committee at Göteborg University and each individual signed an informed consent form before the study.

Two compositions of fluoride dentifrice were used with the same basic formula, one with the addition of 0.5% oxybenzone, and one without (placebo). They were packed in identical and numbered tubes. The code was not broken until all data had been analysed. The participants were instructed to use the assigned dentifrice twice daily (after breakfast and immediately before going to bed) according to the slurry rinsing technique described by Renvert & Birkhed (1995).

Plaque was disclosed using topically applied erythrosin (Rondell Röd, Nordenta, Enköping, Sweden). The modified Quigley & Hein (1962) plaque index was used (Turesky et al. 1970). The buccal and lingual surfaces of all teeth except third molars were examined. A mean plaque index (PI) was calculated by adding the score for all tooth surfaces and dividing this score by the number of surfaces examined.

The gingival bleeding index according to Ainamo & Bay (1975) was used. A CPITN probe (Ainamo et al. 1982) was inserted into the gingival sulcus to a depth of 0.5 mm. It was held at an angle of 60° to the long axis of the tooth and moved around the tooth, stroking the soft tissue walls of the facial, mesial and lingual surfaces. Bleeding occurring within 30 s was recorded. A mean gingival index (GI) was calculated in the same way as for PI.

Comparisons between the two groups in the clinical trial were carried out with an unpaired *t*-test and within the groups with a paired *t*-test. All tests were two-sided at the 5% significant level. The statistical package SPSS 8.0 was used.

Results

Cell culture

Oxybenzone was tested for its ability to inhibit PGE_2-production in cell culture. At the same time, any damage to the cells used for the test was noted. No decrease in cell viability was noted up to 50 μM. A dose-dependent inhibition of stimulated PGE_2-production was found (Fig. 1). A 50% inhibition (IC_{50}) was demonstrated at 0.6 μM (Fig. 2).

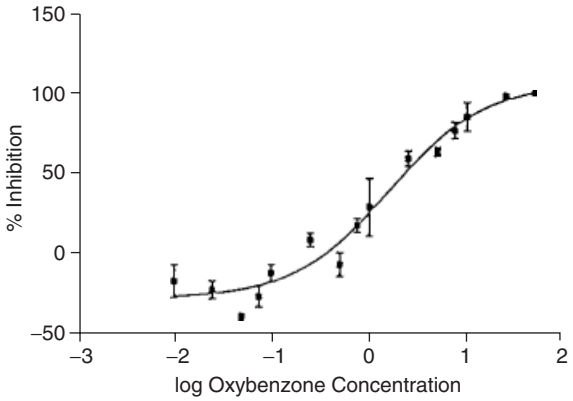

Fig. 1. Dose-dependent inhibition of PGE_2-production.

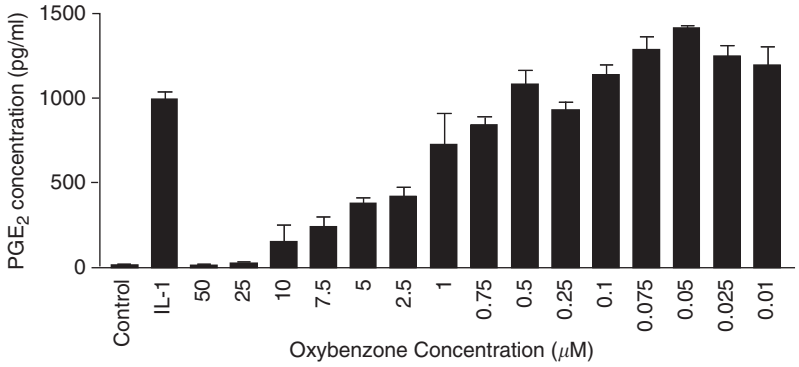

Fig. 2. PGE_2 concentration as related to oxybenzone concentration.

Clinical trial

The results are presented as mean values in Tables 1 and 2. There are 31 individuals in the oxybenzone group and 33 in the placebo group. Thus, there was a drop-out of two of the 66 individuals. These individuals used antibiotics during the course of the study.

PI was reduced in both groups, 18% in the oxybenzone group and 16% in the placebo group ($p<0.001$) (Table 1). The reduction in the oxybenzone group was most pronounced at 6 weeks, in contrast to the control group. There were no statistically significant differences between the two groups.

After 6 weeks, a 25% reduction of GI was observed in the oxybenzone group ($p<0.001$), compared with 2% in the placebo group (Table 2). The reduction in the oxybenzone group was most pronounced at 6 weeks, in contrast to the control group. Comparing the two groups with each other, both the reduction from baseline to 3 weeks and from baseline to 6 weeks were more pronounced in the oxybenzone group compared with the control group ($p<0.01$ and $p<0.001$, respectively) (data not shown).

Discussion

The results from the 6-week clinical study indicate that unsupervised use of a fluoride dentifrice containing 0.5% oxybenzone reduces the GI. There seems to be a time-dependent effect since the difference was more pronounced at the 6-week registration. When evaluating the change in PI, a reduction was also found in the control group both at 3 and 6 weeks. This is a common finding in toothbrushing studies and can be attributed to the so-called Hawthorne phenomenon. This effect may mask the efficacy of anti-plaque formulations in the dentifrice. On the other hand, a clear reduction of GI was found in the oxybenzone group, while only a minor reduction in GI was found in the placebo group. PI was similarly reduced in both groups, whereas GI was reduced in the oxybenzone group only, indicating that oxybenzone has an anti-inflammatory effect.

Several studies have shown that triclosan, which has a similar structure as oxybenzone, has an anti-inflammatory effect (Waaler et al. 1993, Barkvoll & Rölla 1994). Triclosan has also been shown to reduce the degree of gingival inflammation beyond what could be expected by its plaque-reducing capacity (Saxton & van der Quderaa 1989, Lindhe et al. 1993). A conceivable explanation for this is that the formation of the pro-inflammatory mediator prostaglandin (PGE_2) is inhibited. Coleman et al. (1993) reported that triclosan reduces the PGE_2-formation in gingival fibroblasts challenged with sodium lauryl sulphate (SLS) and Modéer et al. (1996) reported that triclosan inhibits the formation of PGE_2 in gingival fibroblasts challenged with IL-1β or tumour necrosis factor α (TNFα). TNFα and IL-1β bind to the cell surface receptors of resident fibroblasts, initiating signals to synthesize and secrete matrix metalloproteinases and PGE_2. Matrix metalloproteinases mediate the destruction of the extracellular matrix of the gingiva and the periodontal ligament and PGE_2 mediates alveolar bone destruction (Page et al. 1997). The synthesis of prostaglandins is mediated by the release of arachidonic acid from phospholipids in the cell membrane by the enzyme phospholipase A_2 and converted to prostanoids by cyclooxygenase, a key enzyme in the prostaglandin pathway (DeWitt 1991). In the present in vitro cell culture experiment, oxybenzone was found to reduce the production of PGE_2 induced by IL-1β. The structure of oxybenzone is similar to other phenolics with dual activity. Based on the evaluation of the structures of compounds used in personal care products, oxybenzone appears to possess the silent structural features associated with cyclooxygenase inhibition. The reduction of PGE_2-production may be one possible explanation for the reduction in GI demonstrated after a 6-week use of the oxybenzone-containing dentifrice.

In the present study, oxybenzone reduced gingivitis but had no effect on plaque formation. This suggests indirectly that the anti-inflammatory effect may be a key factor in reducing gingival inflammation. Key studies to assess the retention of oxybenzone in the plaque and penetration through gingival tissue of oxybenzone following topical application are in process to ascertain the use of this phenolic compound in oral products improving gingival health. Nevertheless, the results from the present laboratory and clinical investigations indicate that agents such as oxybenzone, which modify host response to bacterial plaque on teeth, can be useful in preventive dentistry.

Table 1. Plaque index (PI) at baseline and after 3 and 6 weeks

Toothpaste	*n*	Baseline	3 weeks	6 weeks	Reduction (baseline–6 weeks)
oxybenzone	31	$1.73 \pm 0.20^{***}$	$1.51 \pm 0.31^{*}$	1.41 ± 0.31	18%
placebo	33	$1.73 \pm 0.20^{***}$	$1.45 \pm 0.28^{***}$	1.46 ± 0.33	16%

Reduction is given as % between baseline and 6 weeks.
$^{*}p<0.05$, $^{***}p<0.001$.

Table 2. Gingival index (GI) at baseline and after 3 and 6 weeks

Toothpaste	*n*	Baseline	3 weeks	6 weeks	Reduction (baseline–6 weeks)
oxybenzone	31	$0.47 \pm 0.15^{***}$	$0.37 \pm 0.13^{*}$	$0.35 \pm 0.14^{***}$	25%
placebo	33	$0.42 \pm 0.10^{**}$	$0.35 \pm 0.14^{*}$	0.41 ± 0.12	2%

Reduction is given as % between baseline and 6 weeks.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

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