DNA-Damaging Effects of Dental Bleaching Agents

K. L. Pligina, I. A. Rodina, T. V. Shevchenko, E. S. Bekchanova, V. P. Tikhonov, and N. P. Sirota*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 1, pp. 65-69, 2012
Original article submitted September 13, 2010

We studied DNA-damaging effects of dental bleaching systems containing hydrogen peroxide and/or carbamide peroxide by the "comet assay" (alkaline version). Dental bleaching systems in a hydrogen peroxide concentration range from 0.03 to 30 mM produced a genotoxic effect on isolated HeLa cells *in vitro* comparable with the effects of pharmacopoeial hydrogen peroxide or urea peroxide. Catalase protected the cells against products containing hydrogen peroxide and had no effect on the genotoxicity of samples containing carbamide peroxide.

Key Words: *comet assay; DNA damage; hydrogen peroxide; carbamide peroxide; catalase*

Teeth whitening technologies have a leading position among conservative methods of aesthetic dental treatment. Chemical teeth bleaching with commercial whitening systems containing H_2O_2 and/or carbamide peroxide (urea peroxide) is one of the most popular dental procedures. These compounds penetrate the enamel and dentin through organic matrix located between the inorganic crystals of dental hard tissue and cause oxidative cleavage of the pigment due to the formation of the reactive oxygen species (ROS). However, some studies of the safety of tooth bleaching agents demonstrated such unwanted adverse effects as demineralization of tooth enamel and desquamation of oral epithelium [6,7]. It is believed that H_2O_2, either directly included in bleaching agent or formed from carbamide peroxide, can cause these phenomena [3]. Is also known that ROS induce a wide spectrum of genotoxic effects in various model systems including base modification and DNA breaks, potential sources of mutagenesis and carcinogenesis [5,12].

Until recently, registration of the DNA-damaging effects in living cells was hampered by the lack of adequate methods.

Testing Laboratory of Biological Activity and Toxicological Safety, Moscow Factory of Ecological Engineering and Ecological Nutrition "DIOD"; *Laboratory of Radiation Molecular Biology, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia. ***Address for correspondence:*** kirapligina@yandex.ru. K. L. Pligina

Recent studies has shown that single-cell gel electrophoresis (comet assay) [1] is a promising method for measuring DNA damage caused by ROS [9,10,11]. Alkaline version of this assay allows estimating mainly the yield of DNA single-strand breaks and alkaline-labile sites including apurinic bases [8].

Here we evaluated DNA-damaging effects of commercial systems used in dentistry for chemical teeth whitening using inoculated HeLa cell culture *in vitro*.

MATERIAL AND METHODS

Cells from inoculated culture of human cervical carcinoma (HeLa) were used as the test object (cell culture collection of human, D. I. Ivanovsky Institute of Virology RAMS).

Cells were cultured at 37°C and 5% CO_2 in plastic flasks in DMEM (PAA) containing 0.3 mg/ml L-glutamine, 10% fetal cattle serum (Cibro BRL), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. After attaining confluence, the cells were washed two times with Ca^{2+},Mg^{2+}-free phosphate-buffered saline (PBS) and trypsinized in 0.05% trypsin for 5 min. The cell suspension was washed three times with cold EDTA/PBS (PBS with 1 mM EDTA) by centrifugation at 400*g* for 5 min. To prepare micropreparations, the cell suspension was diluted to a concentration of 10^6 cells/ml and immobilized on agarose-gel surface (sandwich technique). To this end, a layer of cell suspension mixed in

0007-4888/12/1531-0057

equal parts with 1% low-melting agarose was applied on a glass slide pre-coated with 1% universal agarose. After solidification of the agarose, the third layer of 0.5% low-melting agarose was applied [2].

Pharmacopoeial carbamide peroxide (Tatkhimpharmpreparaty) and H_2O_2 (Galeno Farm) were used as the reference samples. Aqueous extracts of the samples (whitening gels) were dissolved in PBS. The samples were adjusted to H_2O_2 concentrations of 0.03-30 mM.

Aqueous extracts of the test samples were applied on the micropreparations and incubated for 10 min at 25°C.

For evaluation of the contribution of exogenous peroxides in DNA damage, bovine erythrocyte catalase was introduced into agarose gel simultaneously with the cells (280 U/ml; Sigma). PBS was used as the control.

Cells embedded in agarose on slides were lysed in a solution containing 2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris-HCl, 1% sodium sarcosinate, pH 10, and 1% Triton X-100 for 1 h at 4°C. Then the cells were treated with a alkali (0.3 M NaOH, 0.001 M EDTA at pH>13) for 20 min at 4°C. This was followed by electrophoresis in a fresh portion of alkaline solution at electric field strength 2 V/cm and current 260-270 mA for 20 min at 4°C. After electrophoresis, the slides were washed with distilled water and stained with EtBr solution (2 mg/ml) in PBS for at least 1 h. Stained DNA was analyzed under a fluorescent microscope. Digital images (30-35 cells on the slide) were saved and processed using special software. Percentage of tail DNA (%TDNA) was used as an measure of DNA damage [2]. Statistical analysis was performed using Student's *t* test ($p<0.05$).

Aqueous extracts of the following commercial systems for teeth whitening: Yotuel 30% (Spain), Yotuel 16% (Spain), Aquafresh (Ireland), Day White (USA), Colgate (USA), were prepared by suspending an aliquot of the gel in PBS.

RESULTS

Initially, we assessed the level of spontaneous DNA damage in single HeLa cells under normal conditions and under the influence of samples of H_2O_2 and carbamide peroxide. The mean value of DNA damage in the control groups was 1.3±0.3% TDNA. H_2O_2 in a concentration range from 0.03 to 30 mM increased the level of DNA-damage in HeLa cells (Fig. 1). The indicator of DNA damage increased in a dose-dependent manner: from 21.9±1.7 to 55.0±2.7% with increasing the concentration from 0.03 to 30 mM. The damaging effect of carbamide peroxide on cell genome was more potent (23.7±1.8 and 60.4±1.8%, respectively, Fig. 1), but did not differ significantly ($p=0.532$ and $p=0.131$, respectively) from the effect of H_2O_2 in the same concentrations.

According to manufacturers' information, all samples of multicomponent systems for chemical teeth bleaching contained high concentrations of H_2O_2 and/or carbamide peroxide (Table 1).

Our experiments showed that all aqueous extracts of bleaching gels with H_2O_2 concentration ranged from 0.03 to 30 mM provided genotoxic effect on isolated HeLa cells *in vitro* (Fig. 2). The level of DNA breaks significantly and dose-dependently increased and this effect was comparable with the effects of the reference samples H_2O_2 and carbamide peroxide (Fig. 1, 2).

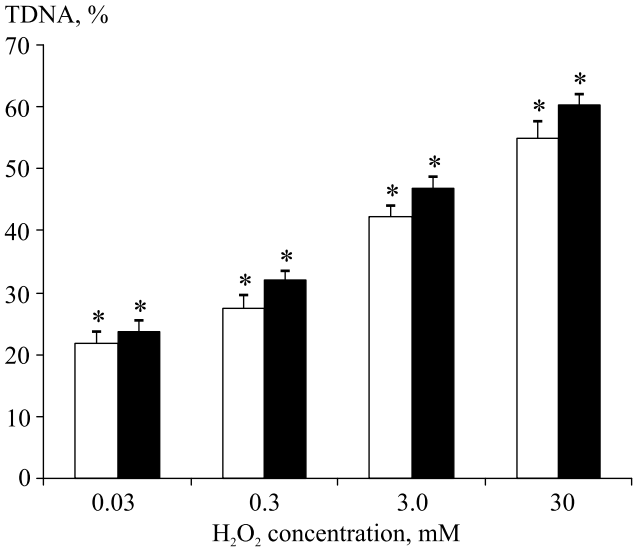

Fig. 1. Level of DNA damage in HeLa cells after exposure to H_2O_2 (light bars) and carbamide peroxide (dark bars). ***Here and on Fig. 2:*** **p*<0.001 compared with controls.

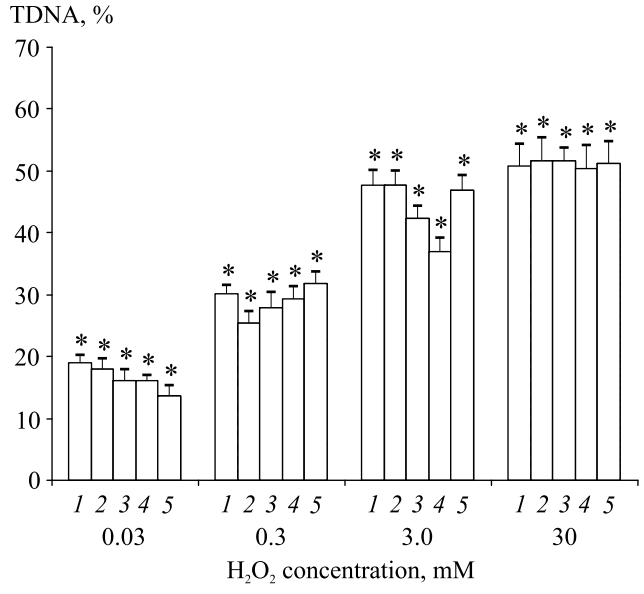

Fig. 2. Level of DNA damage in HeLa cells after exposure to aqueous extracts of teeth whitening gels. *1*) Yotuel 30%; *2*) Yotuel 16%; *3*) Aquafresh; *4*) Day White; *5*) Colgate.

Aqueous extracts of whitening gels in the lowest concentration of 0.03 mM increased the levels of DNA-damage by on average 12.8±0.7 times in comparison with the control. These data are comparable to the results of experiments with pharmacopoeial H_2O_2 (Fig. 1) suggesting that DNA-damaging action of aqueous extracts of whitening gels is related to exogenous H_2O_2.

For evaluation of the direct contribution of exogenous H_2O_2 into the DNA-damaging effect of the test chemical bleaching agents containing carbamide peroxide, experiments were conducted in the presence of catalase, an enzyme specifically degrading H_2O_2.

The enzyme present in agarose gel together with the cells in a concentration of 280 U/ml protected the cells from DNA-damage induced by 0.3 mM exogenous H_2O_2. There was no DNA-damaging effect of H_2O_2 after exposure of cells with catalase (Fig. 3).

Evaluation of H_2O_2 and carbamide peroxide effects on cells embedded in agarose gel containing catalase showed that DNA-damaging effects of both pharmacopoeial H_2O_2 in concentrations of 0.03 mM (1.3±0.2% TDNA) and 0.3 mM (2.1±0.3% TDNA) and aqueous extracts of commercial systems for teeth whitening including only H_2O_2 as active ingredient were completely inhibited by catalase in agarose gel. However, the enzyme had no protective effect when exposed to pharmacopoeial carbamide peroxide (Table 2).

A similar picture was observed for aqueous extracts of commercial bleaching gels. Catalase in the applied concentration showed no protective action against the agents containing carbamide peroxide (Yotuel 30%, Yotuel 16%, and Aquafresh) and 2-fold reduced the levels of DNA breaks caused by whitening system (Day White) containing H_2O_2 and carb-

TABLE 1. Content of Active Ingredients in Commercial Tooth Whitening Systems

Name of sample	Active ingredient	Content of active ingredient
Yotuel 30%	Carbamide peroxide	30%
Yotuel 16%	Carbamide peroxide	16%
Aquafresh	Carbamide peroxide	11.2%
Day White	Hydrogen peroxide Carbamide peroxide	7.5%
Colgate	Hydrogen peroxide	5.9%

Note. Data on the content of active ingredient are presented according to manufacturer's label.

TABLE 2. DNA Damage in HeLa Cells after Exposure to H_2O_2, Carbamide Peroxide, and Aqueous Extracts of Teeth Whitening Gels in the Presence of Catalase and without Enzyme in Agarose Gel ($M\pm m$)

Name of sample	Concentration of H_2O_2, mM	Without catalase	In the presence of catalase
		TDNA, %	TDNA, %
Control		1.3±0.3	1.3±0.3
H_2O_2	0.3	26.7±1.7	2.1±0.3*
Pharmacopoeiall sample	0.03	18.8±1.2	1.3±0.2*
Carbamide peroxide	0.3	29.5±2.1	24.1±1.9
Pharmacopoeiall sample	0.03	19.7±1.9	16.6±1.3
Yotuel 30%	0.3	30.2±2.9	25.7±2.8
Carbamide peroxide 30%	0.03	20.1±1.1	18.7±1.8
Yotuel 16%	0.3	27.6±1.1	26.8±2.8
Carbamide peroxide 16%	0.03	17.9±2.3	15.4±1.4
Aquafresh	0.3	29.5±2.8	29.9±2.2
Carbamide peroxide 11,2%	0.03	16.1±1.4	13.6±1.7
Day White	0.3	29.3±2.7	14.7±1.3*
H_2O_2 7.5% Carbamide peroxide	0.03	18.2±1.7	7.91±1.4*
Colgate	0.3	26.2±1.6	1.3±0.3*
H_2O_2 5.9%	0.03	13.7±1.6	1.4±0.3*

Note. *$p<0.001$ in comparison with %TDNA of cells incubated without catalase.

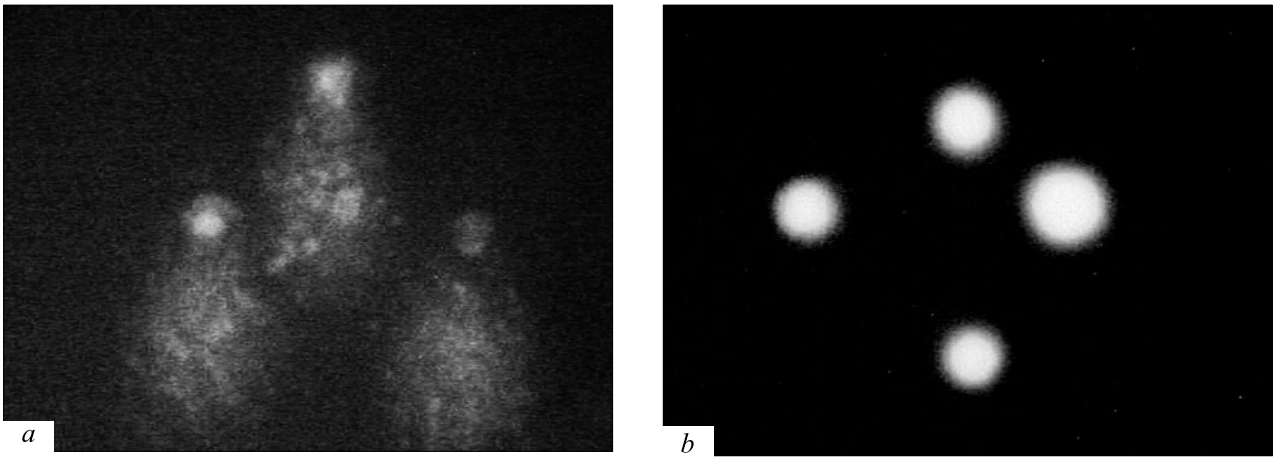

Fig. 3. Nucleotides of HeLa cells. Staining with ethidium bromide, ×300. *a*) after exposure to 0.3 mM H_2O_2; *b*) after exposure to 0.3 mM H_2O_2 in the presence of catalase (280 U/ml).

amide peroxide. DNA damage when exposed to this sample was 14.7±1.3% in concentration of 0.3 mM and 7.91±1.4% in concentration of 0.3 mM (Table 2). Catalase completely abolished DNA-damage to cells induced by H_2O_2 in Colgate aqueous extract in concentrations of 0.3 and 0.03 mM (Table 2).

Thus, commercial whitening systems containing H_2O_2 or carbamide peroxide induced DNA damage in HeLa cells *in vitro*. The damaging effects of the extracts of whitening gels are comparable with the effects of pharmacopoeial H_2O_2 or carbamide peroxide. Catalase protected the cells against products containing hydrogen peroxide and had no effect on the genotoxicity of samples containing carbamide peroxide.

REFERENCES

1. A. D. Durnev, A. K. Zhanataev, and E. A. Anisina, *Application of the Alkaline Gel Electrophoresis of Isolated Cells to Assess the Genotoxic Properties of Natural and Synthetic Compounds. Methodical recommendations* [in Russian], Moscow (2006).
2. N. P. Sirota, A. Ya. Podlutsky, and A. I. Gaziyev, *Radiobiologia*, **31**, Issue 5, 722-727 (1991).
3. *Encyclopedia of Chemistry*, Ed. I. L. Knunyants [in Russian], Moscow (1992), Vol. 3.
4. N. R. Asad and A. C. Leitao, *J. Bacteriol.*, **173**, No. 8, 2562-2568 (1991).
5. P. Daroui, S. D. Desai, T. K. Li, *et al*., *J. Biol. Chem*., **279**, No. 15, 14 587-14 594 (2004).
6. R. A. Floyd, *J. Am. Dent. Assoc*., **128**, Suppl., 37S-40S (1997).
7. Y. Li, *Compend. Contin. Educ. Dent*., **19**, No. 8, 783-786 (1998).
8. P. L. Olive, *Int. J. Radiat. Biol*., **75**, No. 4, 395-405 (1999).
9. D. A. Ribeiro, M. E. Marques, and D. M. Salvadori, *Braz. Oral. Res*., **20**, No. 1, 47-51 (2006).
10. D. A. Ribeiro, A. P. Bazo, C. A. da Silva Franchi, *et al*., *J. Periodontal. Res*., **39**, No. 5, 358-361 (2004).
11. D. A. Ribeiro, M. E. Marques, and D. M. Salvadori, *J. Endod*., **30**, No. 8, 593-596 (2004).
12. R. R. Tice, E. Agurell, D. Anderson, et al., *Environ. Mol. Mutagen*, **35**, No. 3, 206-221 (2000).
13. S. L. Zouain-Ferreira, T. R. Zouain-Ferreira, C. R. da Silva, *et al*., *Cell. Mol. Biol. (Noisy-le-grand)*, **48**, No. 5, 521-524 (2002).