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The polysaccharide from *Tamarindus indica* (TS-polysaccharide) protects cultured corneal-derived cells (SIRC cells) from ultraviolet rays

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Abstract

The aim of this work was to investigate the possible protective effect of a new viscosising agent, TS-polysaccharide, on corneal-derived cells (SIRC) exposed to ultraviolet-B rays. To verify this, SIRC cells were first exposed, in the absence or in the presence of TS-polysaccharide (1% w/v), for 9 s at the UV-B source and then post-incubated for 45 min at 37°C. After this period the hydrogen peroxide (H_2O_2) accumulated in the medium and the concentration of 8-hydroxy-2′-deoxy-guanosine (8-OHdG) in cell DNA was measured. In addition, the amount of ^{3}H-methyl-thymidine incorporated in cellular DNA was evaluated after 18 h from irradiation. Our results show that cells exposed to UV-B rays accumulate H_2O_2, and have higher levels of 8OHdG and a lower amount of ^{3}H-methyl-thymidine incorporated in DNA than control cells. In the presence of TS-polysaccharide, the H_2O_2 and 8-OHdG accumulation, and the ^{3}H-methyl-thymidine incorporation were significantly reduced with respect to the values measured in cells exposed in the absence of the polysaccharide. We propose a protective role of the polysaccharide in reducing UV-B derived DNA damage to eye cells. This finding could be of some clinical importance when the polysaccharide is used as a delivery system for ophthalmic preparations.

Introduction

Many studies report on the dangerous effects induced by ultraviolet rays (UV) to mammalian cells (Black 1987). Of the UV wavelengths, the UV-B range is the more harmful, producing specific damage to cellular DNA (Matsumoto et al 1991). Because of the ozone reduction, produced by atmospheric pollution, the concentration of potentially dangerous UV rays that reach the earth has increased progressively, constituting an insult and a risk factor of importance for mammal cells. This is of particular interest for skin and eye cells which are physiologically subjected to continuous light exposure.

It is well established that UV-derived damage to tissues or cells is due to an increase in the production of reactive oxygen species (ROS), which are responsible for the typical DNA oxidation and damage after UV exposure (Halliwell & Aruoma 1991). DNA damage stimulates signal transduction pathways resulting in cell repair and then cell survival or cell death (Cerruti 1985; Coates et al 1995; Troll et al 1985). ROS produce inflammatory intracellular cascades (Liao et al 1994) responsible for gene activation (Tyrrell 1996), changes in cell metabolism (Young et al 1999), proteins (Guptasarma et al 1992) and DNA oxidation (Yamamoto et al 1992) and are held responsible for being co-factor or initiators of ocular pathologies (Spector 1990). Luckily, eye cells are extremely organised in terms of antioxidant defences (Russell & Johnson 1996), which are rapidly recruited to counteract the attack by ROS on DNA, a mechanism which is not very efficient in skin. Notwithstanding this, increase in intracellular ROS is deemed responsible for the onset of some eye pathologies (Riley 1988; Winkler et al 1999). An increase in ROS content of the eye tissues, resulting from a reduction of oxygen concentration at the eye surface, is also a consequence of prolonged contact lens use (Cejkova et al 1998), a bad habit that can induce corneal cell damage.

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Notwithstanding this, the free radicals derived from UV irradiation of corneal cells produce activation of other ROS-generating sources, such as xanthine oxidase activity and inhibition of superoxide dismutase, both responsible for the increase of hydrogen peroxide (H_2O_2) content of cells following UV irradiation (Babizhayev & Costa 1994; Shimmura et al 1996).

Different strategies can be followed to reduce the intracellular concentration of ROS, thus limiting the oxidative stress generated by UV exposure, or by other trauma lesions. Among them, the administration of antioxidant or scavenging compounds are of particular usefulness. These compounds, by different mechanisms of action, exert protective effects reducing the concentration of cellular ROS and their impact with cells. In this respect, the use of antioxidants (Ciuffi et al 1999), lactoferrin (Shimmura et al 1996) and other iron chelators (Cai et al 1998; Balla et al 1992), offers a possible approach to limit ROS-induced cell damage. However, the bioavailability of xenobiotics in eye fluids is often the rate-limiting step in the pharmacological treatment of eye pathologies. To increase the pharmacokinetic features of drugs, they are usually delivered into eye cells by the use of high-viscosity solutions (artificial tears). The high viscosity of these solutions is achieved by adding natural, semi-synthetic or synthetic polymers (Dick et al 1999). Among these, many polysaccharides of natural origin are used as drug-delivery systems for ophthalmic preparations. Their presence prolongs the time of contact of the drug with the cell surface, increasing its absorption.

However, polysaccharides cannot be considered as just additives for ophthalmic preparations. They can have their own pharmacological profile, helping drug treatment of eye diseases. Thus, their cell tolerability and possible interaction with drugs should be checked carefully, so that compounds devoid of cell toxicity can be chosen and, possibly, the drug's therapeutic index can be increased. Among the polysaccharides, hyaluronic acid and carboxymethylcellulose represent two of the most used in ophthalmic preparations for their tolerability for eye cells. In addition to this, hyaluronic acid presents some effects as a promoter of cell adhesion and growth (Burgalassi et al 2000), and its protective role against ROS damage to corneal endothelium and iris has been documented (Artola et al 1993a, b). This allows characterisation of polysaccharide features and clarification of the beneficial interaction between the vehicle and the drug delivered.

The use of the polysaccharide from *Tamarindus indica* (TS-polysaccharide) gum as a viscosising agent for ophthalmic preparations has been documented recently. This has been studied in terms of cell viability (Raimondi et al 2000) and promotion of corneal-wound healing (Burgalassi et al 2000).

To assess the possible role of TS-polysaccharide as a protective agent against UV (in particular, UV-B) damage to eye cells, we studied the effect of TS-polysaccharide addition to rabbit corneal-derived cells (SIRC cells) exposed to a UV-B source. In particular, we evaluated the accumulation of hydrogen peroxide in cell medium and the cellular DNA oxidation, by measuring the concentration of the early marker, 8-hydroxydeoxyguanosine (8-OHdG) and the incorporation of ^{3}H-methyl-thymidine in the DNA after a short-term exposure of SIRC cells to UV-B rays and whether these parameters were changed by pre-treating the cells with the TS-polysaccharide.

Materials and Methods

Rabbit corneal cells (SIRC) were obtained from the European Cell Culture Collection (ECACC, Salisbury, UK). MEM-HBSS medium, glutamine, antibiotics, fetal calf serum, trypsin-EDTA and horseradish peroxidase were all from Sigma (St Louis, MO). Homovanillic acid was from Merck (Darmstadt, Germany). ^{3}H-methyl-thymidine (38 mCi mmol^{-1}) was from Amersham.

TS-polysaccharide was kindly supplied by Farmigea S.p.A. (Pisa, Italy)

Chemicals

A fraction of natural polysaccharide (TS-polysaccharide, Glyloid 3S, Dainippon Pharmaceutical Co. Ltd, Osaka, Japan) was purified by dispersion in water at 80 °C and centrifugation for 30 min at 6000 rev min^{-1}. The settled cake was discarded, and the polymer was recovered from the supernatant by freeze-drying. All other chemicals and solvents were of analytical grade.

UV source

UV generating lamp: Vilber Lourmat Lamp, Cedex, France, wavelength range set at 280–350 nm and peak at 312 nm; 2.2 mW cm^{-2}.

Cell culture

SIRC cells were cultured in MEM-HBSS (containing 1% non essential amino acids). The medium contained glutamine, penicillin, streptomycin and 10% heat-inactivated fetal calf serum (culture medium). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Subsequent cell passages were performed using trypsin (0.25%)/EDTA (0.02%) solutions.

Exposure of cells to UV rays

Cells (80–90% of confluence) in culture medium devoid of serum were exposed for 9 s to the UV-B source (20 mJ cm^{-2}) at room temperature. After that time, they were usually kept for 45 min (unless otherwise stated; post-incubation time) at 37 °C in an atmosphere of 95% air and 5% CO_2. Control cells, not irradiated, were kept for 9 s under natural light and then post-incubated at the same conditions as for UV-treated cells.

TS-polysaccharide treatment

TS-polysaccharide (1% w/v) was prepared in culture medium and added to cell monolayer 30 min before UV exposure.

Measurement of H_2O_2

Hydrogen peroxide accumulation in cell medium following UV exposure of cells was measured according to Matsumoto et al (1982). Just before UV irradiation, cell medium was added (homovanillic acid (0.2 mg mL^{-1}) and peroxidase (100 U mL^{-1})) and cells were treated as described above for UV irradiation. After 45 min of post-incubation, the medium was collected and 2 mL of 0.1 M NaOH was added. The peroxide content, measured fluorimetrically using a Shimadzu spectrofluorimeter ($\lambda_{ecc} = 323$ nm, $\lambda_{em} = 426$ nm), was calculated from a calibration curve generated by using known concentrations (2, 5, 10, 20 nmol) of hydrogen peroxide. Results are expressed as nmol of H_2O_2 produced by 10^5 cells in 45 min (nmol/10^5 cells/45 min).

8-OHdG assay

The cells were thawed at room temperature and the isolation of cell DNA was obtained using a previously published method (Lodovici et al 2000), with few modifications. Briefly, cells (about 5×10^5) were diluted with 1 mL of 10 mM Tris-HCl buffer pH 8.0 containing EDTA (10 mM), NaCl (10 mM) and sodium dodecyl sulfate (SDS; 0.5%) and incubated at 37 °C for 1 h with 15 μL of RNAse (20 μg mL^{-1}); the samples were then incubated at 37 °C for 3 h with 35 μL of proteinase K (100 μg mL^{-1}) under oxygen-free conditions by insufflating argon. After incubation, the mixture was extracted with 1 mL of chloroform–isoamyl alcohol (10:2 v/v) in the presence of 0.2 volumes of 10 M ammonium acetate and DNA was precipitated from the aqueous phase, dried and solubilized in 100 μL of 20 mM acetate buffer pH 5.3 and denatured at 90 °C for 3 min. The extracted DNA was supplemented with 10 μL of P1 nuclease (10 IU) and incubated at 37 °C for 60 min under argon in the dark. The mixture was then digested for 1 h at 37 °C with 5 μL alkaline phosphatase (5 IU) in the presence of 20 μL of 0.4 M Tris-HCl buffer pH 8.8. All these procedures were done under argon and all samples were protected from light with an aluminium foil. The hydrolysed mixture was filtered by Micropure-EZ enzymes remover (Amicon) and 80 μL were injected into the HPLC. The nucleosides were separated by C18 reverse-phase column (Supelco, 5 μm, i.d. 0.46 × 25 cm). The eluting solution was 50 mM KH_2PO_4 pH 5.5 containing 8% methanol at a flow rate 1 mL min^{-1}. The 8-OHdG and 2-deoxyguanosine (2dG) were detected using an ESA Coulochem II electrochemical detector in line with a UV detector as reported previously (Lodovici et al 2000). The levels of 8-OHdG were expressed as ratio of 8-OHdG and 2dG/10^5 cells.

To prevent oxidative stress as an artefact in the measurement of 8-OhdG levels, we followed the European Standard Committee on Oxidative DNA Damage (ESCODD) Protocol (ESCODD 2002).

^{3}H-methyl-thymidine incorporation in cellular DNA

After UV-B exposure, cells were put back in the incubator for a further 30, 45 or 60 min at 37 °C (5% CO_2 and 95% air). Appropriate controls were performed for each post-incubation time.

After each period, cells were rinsed with saline solution and fresh medium containing 10% FCS and ^{3}H-methyl-thymidine (0.5 μCi mL^{-1}) was added and cells were further incubated for 18 h at the same conditions described for cell culturing. Cell medium was then removed and nucleic acids were first precipitated by adding trichloroacetic acid (10%) and then solubilised by using NaOH (3 M). The radioactivity present in the alkaline phase was counted using a β-counter machine. Results are expressed as mean ± s.e. (counts min^{-1}/10^5 cells or as a percentage of the radioactivity found in irradiated over that found in control cells).

Protein content

The protein content of samples was estimated using the micro BCA (Pierce) methods (based on the use of the biocinchoninic acid, a stable and specific reagent for Cu^{2+}, which in turn reacts with proteins, according to the biuret reaction). A calibration curve, using known amounts of bovine serum albumin as standard protein, was produced to estimate the protein content of samples.

Statistical analysis

Kruskal-Wallis test or Student's *t*-test were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). A value of $P < 0.05$ was considered significant.

Results

^{3}H-methyl-thymidine incorporation in irradiated cells

Cells exposed to UV rays had a lower amount of radioactivity incorporated in their DNA than control cells. The decrease in the radioactivity recovered was dependent on the post-incubation time (Figure 1).

The presence of TS-polysaccharide increases ^{3}H-methyl-thymidine incorporation in the DNA of irradiated cells

SIRC cells pre-treated with TS-polysaccharide, exposed for 9 s to UV-B and post-incubated for 45 min had a higher ^{3}H-methyl-thymidine incorporation in their DNA with respect to that which occurred in irradiated cells (Figure 1).

Accumulation of H_2O_2 in medium of UV-B irradiated cells

In SIRC-cell medium after irradiation, a micromolar concentration of hydrogen peroxide was recovered; the same compound was undetectable in control cells that had not been irradiated (Table 1).

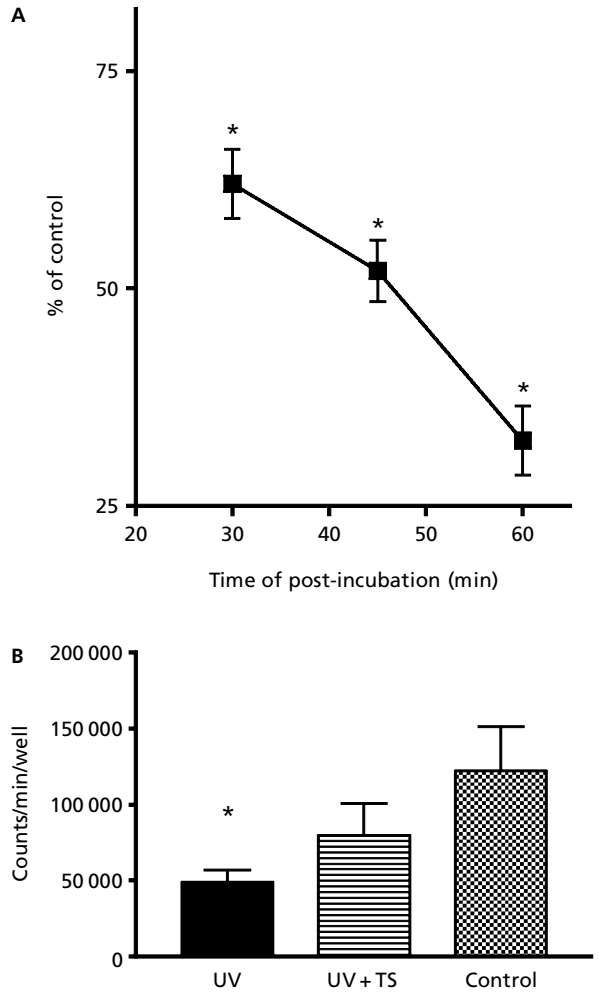

Figure 1 The effect of TS-polysaccharide on DNA synthesis of SIRC cells exposed to UV-B. A. SIRC cells were exposed for 9 s to UV rays and then post-incubated in irradiation medium for 30, 45 or 60 min. ^{3}H-methyl-thymidine incorporation in DNA was evaluated 18 h later as described in Methods. Appropriate controls were run in parallel, keeping cells at the same condition as for UV-exposed cells. Results are expressed as the % of radioactivity recovered in irradiated cells over that found in the corresponding control ones. Results are the mean ± s.e. of 4 different experiments run in triplicate. *$P < 0.01$, compared with the respective value in control cells (Student's *t*-test). B. Cells exposed for 9 s in the presence of TS-polysaccharide (UV + TS) and then post-incubated for 45 min at 37 °C showed an increase in ^{3}H-methyl-thymidine incorporation over the radioactivity found in irradiated cells without the polysaccharide (UV). Results are expressed as counts min^{-1} recovered per well and they represent the mean ± s.e. of 6 different experiments run in triplicate. *$P = 0.0020$, compared with control (Kruskal-Wallis test, Gaussian, the medians vary significantly, $P < 0.05$).

The effect of TS-polysaccharide presence on H_2O_2

In the presence of TS-polysaccharide, a significantly lower concentration of H_2O_2 than that recovered in irradiated cells not pre-treated with the polysaccharide was measured (Table 1).

Table 1 The effect of TS-polysaccharide on H_2O_2 content of SIRC cells exposed to UV-B irradiation.

	H_2O_2 (nmol/10^5 cells/45 min)
Control	Not measurable
UV	7.70 ± 0.08
UV + TS-polysaccharide	4.8 ± 0.1*

H_2O_2 concentration was measured as described in Methods. Results are the means ± s.e. of 4 different experiments run in duplicate. *$P < 0.05$, compared with UV-exposed cells, Student's *t*-test.

UV irradiation produces a significant increase of 8-OHdG levels in cellular DNA

A significant increase in 8-OHdG levels was found in UV-exposed cells over control cells (Figure 2).

TS-polysaccharide reduces the 8-OHdG concentration in irradiated cells

The presence of TS-polysaccharide (1% w/v) reduced 8-OHdG concentrations recovered in UV-exposed cells. In the presence of the polysaccharide, the levels of 8-OHdG were significantly lower than those found in cells exposed to UV in the absence of the polysaccharide (Figure 2).

Cell number and protein content in irradiated cells

Protein content was not significantly different among control (0.399 ± 0.021 mg mL^{-1}) and irradiated cells (0.44 ± 0.147 mg mL^{-1}) in the absence or in the presence of TS-polysaccharide. Again, the number of cells recovered after trypsinisation from UV-irradiated wells

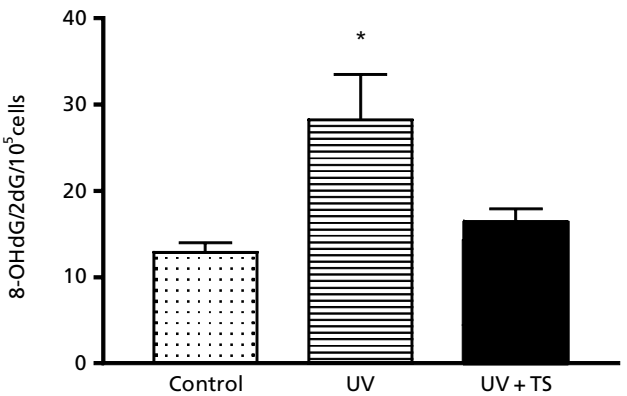

Figure 2 8-OHdG levels in irradiated SIRC cells: the effect of TS-polysaccharide. 8-OHdG concentration recovered in the DNA obtained from non-irradiated cells or irradiated cells in the absence (UV) or in the presence of TS-polysaccharide (UV + TS-polysaccharide) was evaluated as described in Methods. Results are the means ± s.e. of 4 different experiments. *$P = 0.0081$, compared with control (Kruskal-Wallis test, the median values vary significantly, $P < 0.05$).

($4.8 \pm 1.1 \times 10^5$) was not significantly different from that counted from control wells ($7.1 \pm 1.6 \times 10^5$). No release of lactate dehydrogenase (LDH) activity was measured in control or in irradiated cells after 45 min of post-incubation.

Discussion

Eyes are exposed to UV-B (290–320 nm) at different concentrations, these rays being normal components of sunlight, but they also can be occasionally exposed to H_2O_2, a compound used for contact lens cleaning and which is present in the eye fluid of the elderly and in some pathologies (Ramachandran et al 1991). Both UV and H_2O_2 exposure produces typical oxidative stress, which can play a significant role in damage to eye cells, as already described for skin (Black 1987).

We have verified that a corneal-derived cell line (SIRC cells) presents a lower DNA duplication when briefly exposed (9 s) to UV-B rays at an intensity similar to that reported by Lerman (1980) for solar irradiation at sea level (about 2–5 mW cm^{-2} for the ultraviolet region), by measuring the incorporation of ^{3}H-methyl-thymidine in their DNA. The decrease of the labelled DNA recovered in irradiated cells was dependent on their post-incubation period, suggesting that, notwithstanding the brief exposure time chosen, irreversible damage to DNA occurred.

From this we decided to study whether this effect was mediated through an attack on cellular DNA by ROS and whether a natural polysaccharide (TS-polysaccharide), used as viscosising agent, could help in reducing the impact of this radical attack. All our experiments were run after 45 min of post-incubation.

It is already known that UV-B exposure of isolated eye cells activates H_2O_2-generating enzymes. In fact, it has already been shown that UV light produces pyrimidine dimers (Grossman et al 1988), DNA strand breaks (Cadet et al 1986; Audic & Giancomoni 1993), DNA-protein cross-links (Kodaina et al 1984; Matsunaga et al 1991) and base modifications mediated by oxygen-radical attack. In particular, the formation of 8-OHdG is held to be an early and accurate marker of oxidation damage to DNA (Yamamoto et al 1992; Lodovici et al 2002).

Under these conditions, we found that SIRC cells, irradiated according to our schedule, accumulated H_2O_2, thus confirming that H_2O_2 can be regarded as a co-factor, an intermediate step, in UV-B generating oxidative attack directed towards DNA. In addition, under our conditions, cells did not release intracellular materials, confirming the absence of damage to cell membranes. Moreover, the fact that we found almost the same amount of cells after irradiation suggests that cell death is not responsible for the decreased ^{3}H-methyl-thymidine incorporation in irradiated cells.

We have shown that SIRC cells exposed to UV-B rays have a consistent increase in the amount of 8-OHdG in their DNA, confirming the selectivity of the damage induced under our experimental conditions.

Having ascertained that, under our conditions, UV-B exposure produced oxidative damage to cell DNA and a decrease in DNA duplication, we then evaluated whether these conditions could be prevented by using TS-polysaccharide, a natural compound which produces highly viscous aqueous solutions.

We verified that TS-polysaccharide pre-treatment reduced DNA oxidation (Figure 2) and increased DNA duplication (Figure 1B). Moreover, the protective role of the polysaccharide in the medium of the irradiated cells was also confirmed by the H_2O_2 levels recovered in irradiated cells. The protection exerted by TS-polysaccharide on the levels of 8-OHdG and ^{3}H-methyl-thymidine confirms what is already known on the protective role of natural polysaccharides against ROS attack on mammal cells.

From our results, we can confirm that UV-B rays produce oxidative radical attack on SIRC cell DNA and, because of the protection exerted by polysaccharide, we propose the use of highly viscous compounds to limit the UV-B rays-dependent DNA damage. The presence of the TS-polysaccharide in the irradiation medium is a requisite to afford this protection. In fact, no variation of the parameters studied was evident when the TS-polysaccharide was added just after UV-B exposure (data not shown).

We have demonstrated that this high-molecular-weight polysaccharide, likely offering a physical shield to rays because of its bioadhesive features, protects cells from the hurtful effect, on DNA, of UV-B radiation at an intensity similar to that to which man is exposed. The extension of our results to the treatment of human pathology is plausible, being that the polysaccharide is very well tolerated by human eye. In this respect, the occasional protection against UV attack produced by ophthalmic preparations containing the polysaccharide could be an accessory feature of such preparations.

References

Artola, A., Alio, J. L., Bellot, J. L., Ruiz, J. M. (1993a) Protective properties of viscoelastic substances (sodium hyaluronate and 2% hydroxymethylcellulose) against experimental free radical damage to the corneal endothelium. *Cornea* **12**: 109–114

Artola, A., Alio, J. L., Bellot, J. L., Ruiz, J. M. (1993b) Lipid peroxidation in the iris and its protection by means of viscoelastic substances (sodium hyaluronate and hydroxypropylmethylcellulose). *Ophthalmic Res.* **25**: 172–176

Audic, A., Giancomoni, P. U. (1993) DNA nicking by ultraviolet radiation is enhanced in the presence of iron and of oxygen. *Photochem. Photobiol.* **57**: SW512

Babizhayev, M. A., Costa, E. B. (1994) Lipid peroxide and reactive oxygen species generating systems of the crystalline lens. *Biochem. Biophys Acta* **22**: 326–337

Balla, G., Jacob, H. S., Balla, J., Rosenberg, M., Nath, K., Apple, F., Eaton, J. W., Vercellotti, G. M. J. (1992) Ferritin: a cytoprotective antioxidant strategem of endothelium. *J. Biol. Chem.* **267**: 18148–18153

Black, H. S. (1987) Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. *Photochem. Photobiol.* **46**: 213–221

Burgalassi, S., Raimondi, L., Pirisino, R., Banchelli, G., Boldrini, E., Saettone, M. F. (2000) Effect of xyloglucan (tamarind seed polysaccharide) on conjunctival cell adhesion

to laminin and on corneal epithelium wound healing. *Eur. J. Ophthalmol.* **10**: 71–76

Cadet J., Berger, M., Decarroz, C., Wagner, J., Van Lier E., Ginot, Y. M., Vigny, P. (1986) Photosensitized reactions of nucleic acids. *Biochimie* **68**: 813–834

Cai, C. X., Birk, D. E., Linsenmayer, T. F. (1998) Nuclear ferritin protects DNA from UV damage in corneal epithelial cells. *Mol. Biol. Cell* **9**: 1037–1051

Cejkova, J., Labsky, J., Vacik, J. (1998) Reactive oxygen species (ROS) generated by xanthine oxidase in the corneal epithelium and their potential participation in the damage of the corneal epithelium after prolonged use of contact lenses in rabbits. *Acta Histochem.* **100**: 171–184

Cerutti, P. A. (1985) Prooxidant states and tumor promotion. *Science* **227**: 375–381

Ciuffi, M., Neri, S., Franchi-Micheli, S., Failli, P., Zilletti, L., Moncelli, M. R. (1999) Protective effect of pirenoxine and U74389F on induced lipid peroxidation in mammalian lenses. An in vitro, ex vivo and in vivo study. *Exp. Eye Res.* **68**: 347–359

Coates, P. J., Save, V., Ansari, E., and Hall, P. A. (1995) Demonstration of DNA damage/repair in individual cells using in situ end labelling association of p53 with sites of DNA damage. *J. Pathol.* **176**: 19–26

Dick, B., Schwenn, O., Pfeiffer, N. (1999) Classification of viscoelastic substances for ophthalmologic surgery. *Ophthalmologie* **96**: 193–211

ESCODD (2002) Inter-laboratory validation of procedures for measuring 8-oxo-7,8-dihydroguanine/8-oxo-7,8-dihydro-2′-deoxyguanosine in DNA. *Free Radic. Res.* **36**: 239–245

Grossman, L., Caron, P. F. L., Mazur, J., Oh, E. X. (1988) Repair of DNA containing pyrimidine dimers. *FASEB J.* **2**: 2696–2701

Guptasarma, P., Balasubramanian, D., Matsugo, S., Saito, I. (1992) Hydroxyl radical mediated damage to proteins, with special reference to the crystallins. *Biochemistry* **31**: 4296–4303

Halliwell, B., Aruoma, L. (1991) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.* **281**: 9–19

Kodaina, K., Ishikawa, T., Takayama, S. (1984) Dose response, wavelength dependence, and time course of ultraviolet radiation-induced unscheduled DNA synthesis in mouse skin in vivo. *Cancer Res.* **44**: 2150–2154

Lerman, S. (1980) *Radiant energy and the eye*. Macmillan, New York

Liao, F., Andalibi, A., Qiao, J. H., Allayee, H., Fogelman, A. M., Lusis, A. J. (1994) Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. *J. Clin. Invest.* **94**: 877–884

Lodovici, M., Casalini, C., Cariaggi, R., Michelucci, L., Dolara, P. (2000) Levels of 8-hydroxydeoxyguanosine as a marker of DNA damage in human leukocytes. *Free Radic. Biol. Med.* **28**: 13–17

Lodovici, M., Raimondi, L., Guglielmi F., Gemignani, S., Dolara, P. (2002) Protection against ultraviolet B-induced oxidative DNA damage in rabbit corneal-derived cells (SIRC) by 4-coumaric acid. *Toxicology*. **184**: 141–147

Matsumoto, T., Furuta, T., Nimura, Y., Suzuki, O. (1982) Increased sensitivity of the fluorimetric method of Snyder and Handley for oxidase assay. *Biochem. Pharmacol.* **31**: 2207–2210

Matsumoto, K., Sugiyama, M., Ogum, R. (1991) Non-dimer DNA damage in Chinese hamster V-79 cells exposed to ultraviolet-B light. *Photochem. Photobiol.* **54**: 389–392

Matsunaga, T., Hieda, K., and Nikaido, O. (1991) Wavelength dependent formation of thymine dimers and (6-4) photoproducts in DNA by monochromatic ultraviolet light ranging from 150 to 365 nm. *Photochem. Photobiol.* **54**: 403–410

Ramachandran, S., Morris, S.M., Devamanoharan, P., Henein, M., Varma, S. D. (1991) Radio-isotopic determination of hydrogen peroxide in aqueous humor and urine. *Exp. Eye Res.* **53**: 503–506

Raimondi, L., Sgromo, L., Banchelli, G., Corti, I., Pirisino, R., Boldrini, E. (2000) A new viscosity enhancer for ophthalmic preparations devoid of toxicity for human conjunctiva cells. *J. Cutan. Ocul. Toxicol.* **19**: 31–42

Riley, M. V. (1988) Response to the corneal endothelium to oxidative stress. In: Cavamagh, H. D. (ed.) *The cornea. Transactions of the World Congress on the Cornea III.* Raven Press, New York, pp 211–216

Russell, P., Johnson, D. H. (1996) Enzymes protective of oxidative damage present in all decades of life in the trabecular meshwork, as detected by two-dimensional gel electrophoresis protein maps. *J. Glaucoma* **5**: 317–324

Shimmura, S., Suematsu, M., Shimoyama, M., Tsubota, K., Oguchi, Y., Ishimura, Y. (1996) Subthreshold UV radiation-induced peroxide formation in cultured corneal epithelial cells: the protective effects of lactoferrin. *Exp. Eye Res.* **63**: 519–526

Spector, A. (1990) Oxidation and aspects of ocular pathology. *CLAO J.* **16** (Suppl. 1): S8–S10

Troll, S. W., Wiesner, R. (1985) The role of oxygen radicals as a possible mechanism of tumor promotion. *Annu. Rev. Pharmacol. Toxicol.* **25**: 509–528

Tyrrell, R. M. (1996) UV activation of mammalian stress proteins. *EXS* **77**: 255–271

Winkler, B. S., Boulton, M. E., Gottsch, J. D., Sternbergl, P. (1999) Oxidative damage and age-related macular degeneration. *Mol. Vis.* **5**: 32–43

Yamamoto, F., Kasai, H., Bessho, T., Chung, M. H., Ohtsuka, E., Hori, T., Nishimura, S. (1992) Ubiquitous presence in mammalian cells of enzymatic activity specifically cleaving 8-hydroxyguanine-containing DNA. *Jpn. J. Cancer Res.* **83**: 351–357

Young, L. H., Russell, R. R., Yin, R., Caplan, M. J., Ren, J., Bergeron, R. (1999) Regulation of myocardial glucose uptake and transport during ischemia and energetic stress. *Am. J. Cardiol.* **83**: 25H–30H