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Anti-oxidative capacity of various artificial tear preparations

Received: 8 August 2000
Revised: 31 October 2000
Accepted: 8 November 2000
Published online: 31 March 2001
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Abstract *Background:* Increased UV radiation and ozone exposure may cause “dry eyes of environmental origin”, if the normal anti-oxidative capacity of the tear film can no longer cope with the oxidative stress. The use of artificial tears with an adequate anti-oxidative effect may be beneficial in the treatment of dry eyes caused by environmental factors. *Methods:* The anti-oxidative capacity of various commercial artificial tear preparations was determined with a modified TRAP procedure. The two preparations with the strongest anti-oxidative effect were then examined for their protective effects against UV or ozone exposure in a hyaluronate model. *Results:* Of 19 artificial tear preparations tested, only 6 showed strong to moderate anti-oxidative effects. All others were at best weakly anti-oxidative or had no anti-oxidative effect at all.

Some of them even acted as oxidants. Although the two most strongly anti-oxidative preparations performed somewhat differently on UV and ozone exposure, they were both found to be highly protective against these important oxidative stress factors. *Conclusions:* The anti-oxidative capacity of artificial tear preparations varies widely. While some are strong anti-oxidants, others are less active or even act as oxidants. If the carefully elicited history of patients with dry eyes suggests that noxious environmental factors may be causally involved, artificial tears which are not just lubricants or contain wetting agents, but act as anti-oxidants, should be chosen for treatment from the many commercially available preparations. Such an etiology-oriented concept would probably improve the success rate of treatment for dry eyes.

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Introduction

The ozone layer overlies the earth at an altitude of about 20–50 km and restricts the intensity of solar UV radiation reaching the Earth's surface. There can be no doubt that the ozone layer is continuing to become increasingly thinner [3, 4, 24, 31]. In fact, it has become so thin above several continents that talk of an “ozone hole” would seem legitimate. The protective function of the ozone layer is increasingly lost as it becomes thinner so that more UV radiation reaches the earth.

On the Earth's surface the increased UV radiation interacts with air borne pollutants. As a result, the above-

ground ozone concentration increases and photochemical smog is generated. This may be harmful to the eyes [10, 19].

The human eye is a unique organ in as much as it is continually exposed to the effects of radiation, atmospheric oxygen, environmental chemicals or pollutants and physical factors without any appreciable protection [18]. Airborne pollutants like ozone, exhaust gases, smoke and increased UV radiation are among the many factors underlying the development of the dry eye syndrome [2, 28]. All of these factors contribute to the generation of reactive oxygen species (ROS) or to the presence of various types of free radicals in the tear film.

i.e., to oxidative stress [20,21]. Oxidative stress destroys proteins, mucous components and lipids contained in the tear film so that the tear film ultimately loses its stability. Precorneal tear film break-up causes the characteristic symptoms of the dry eye syndrome [9, 15, 16, 18, 19].

If the eye is exposed to excessive oxidative stress, the scavengers normally present in the tear film, e.g., cysteine, ascorbic acid, GSH (reduced glutathione), uric acid, tyrosine, catalase and peroxidase, are apparently no longer capable of preventing damage [5, 7, 8, 13, 17, 25]. This raises the question of whether the effects of oxidative stress can be antagonized by anti-oxidative eye drops, e.g., artificial tears. We studied the anti-oxidative capacity of various commercial artificial tears.

Materials and methods

From among the many commercially available artificial tears, the following 19 preparations were randomly selected (in alphabetical order): Artelac, Artelac-EDO, BDO 7 (Bor-Dexpanthenol-Okuzell), Chinoline-Derivat (substance A), Corneregel fluid, HJM (Halla-Jod-M), HJV (Halla-Jod-V) (substance B), Hyalodrop, Intra lipid, Iodinated Brine Solution, LipoNit Spray, Lipotears, Lipotears Gel, Oculoteet fluid, Protagent, Protagent SE, Siccaprotect, Thilotears Gel, Vidisic Gel. These preparations were sent to the Institute of Analytic Chemistry at Johannes Kepler University in Linz for analysis. Their total anti-oxidative capacity was determined with a modified TRAP procedure as follows. Instructions as reported in the literature [1, 26]: A quantity of 475 μ l of a 100-mmol phosphate buffer with a pH of 7.4 (in oxygen-saturated physiologic saline) is mixed with 50 μ l of a 400-mmol 2,2-azobis(2-amidino-propan) hydrochloride (ABAP) solution (prepared in 100 mmol phosphate buffer at a pH of 7.4 in physiologic saline). Then 50 μ l of 10-mmol luminol solution in 20-mmol sodium tetraborate is added. The resultant solution is heated to 37°C in a luminometer and its luminescence is measured every 30 s. After 25 runs, 20 μ l of the sample is added and the luminescence is again determined every 30 s or 25 times.

As preliminary tests with these instructions failed to produce acceptable results, the procedure was modified in order to assess the anti-oxidative effects of the samples tested. Using the modified procedure, semiquantitative tests were performed as follows: A quantity of 230 μ l of the luminol solution was mixed with 25 μ l of the ABAP solution in a microtiter plate. Measurements were made with the luminometer as described above. After 25 runs, 25 μ l of the sample was added.

The anti-oxidative capacity was rated semiquantitatively using the following scale: +4 (very strong anti-oxidative effect) to -3 (strong oxidative effect). (Table 1).

In addition, two of the preparations (substance A and substance B) were independently examined for their ozone- or UV-blocking action at the laboratory of the University Eye Clinic in Graz. For these tests (in each case five measurements), hyaluronate was dissolved in physiologic saline at a concentration of 1 mg/ml to produce a viscous solution. Its viscosity is shown as control (in centistokes, St) in the illustrations. When exposed to ozone or UV radiation in a quartz-glass container, the solution rapidly becomes less viscous. This is indicative of destruction of the hyaluronate molecules [22]

Table 1 Rating scale

Score	Effect
4	Very strong anti-oxidative effect
5	Strong anti-oxidative effect
6	Moderate anti-oxidative effect
7	Minor anti-oxidative effect
0	Very weak (virtually no) anti-oxidative effect
-1	Weak oxidative effect
-2	Oxidative effect
-3	Strong oxidative effect

Table 2 Anti-oxidative capacity of various artificial tear preparations. Only preparations with a very strong to minor anti-oxidative effect are listed. All other preparations tested showed a very weak or no anti-oxidative effect or were even weakly oxidative

Preparation	Anti-oxidative effect	pH
HJV (substance B)	Very strong (4)	6.79
Chinolin Derivata (substance A)	Strong (3)	6.71
Artelac EDO	Moderate (2)	7.20
BDO 7	Moderate (2)	7.49
HJM	Moderate (2)	7.10
Protagent SE	Minor (1)	6.34

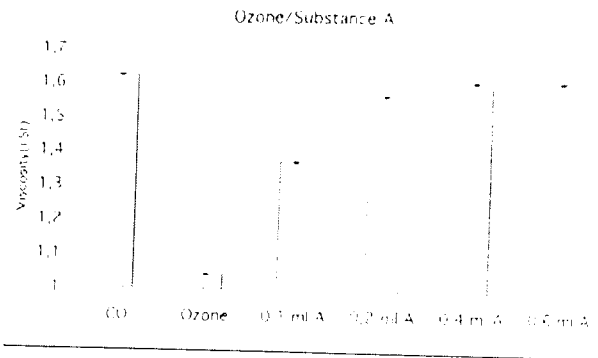


Fig. 1 Ozone - Substance A. CO Hyaluronate in physiologic NaCl (1 mg/ml) = control; Ozone hyaluronate in physiologic NaCl (1 mg/ml) after ozone exposure (10 ml with 20 μ g O₃/ml); 0.1 ml A, 0.2 ml A, 0.4 ml A, 0.6 ml A the amount of substance A added to 20 ml hyaluronate in physiologic NaCl (1 mg/ml) followed by ozone exposure (10 ml with 20 μ g O₃/ml)

Results

If either substance A or substance B is added to the hyaluronate solution before exposure to ozone or UV radiation, destruction by ozone or UV radiation is prevented, as seen from the viscosity diagrams. To develop its full protective effect substance A is needed in higher dosage than substance B. In our test system substance B offered

Table 3 Active agents of the artificial tear preparations above

Preparation	Active agent
HJV (Substance B) (Hallajod V)	Acidum boricum Natrium tetraboricum Kalium jodatum Natrium jodatum Kalium bromatum Natrium ascorbicum Calcium pantothenicum Rutinum Aneurinum hydrochlor. Riboflavinum Retinolpalmitat Tocopherolacetat Methylum-p-hydroxy benzoicum Propylum-p-hydroxy benzoicum
Chinolin Derivata (substance A)	8-Hydroxy-1- methylchinolinium-methyl sulfat
Artelae EDO	Methylhydroxypropylcellulosum, without preservative
BDO 7	Acidum boricum Natrium tetraboricum Dexpanthenolum Hypromellosum
HJM (Hallajod M)	Acidum boricum Natrium tetraboricum Kalium jodatum Natrium jodatum Natrium ascorbicum Hypromellosum Thiomersal
Protagent SE	Polyvidon {Poly (1-vinylpyrrolidin-2-on)} without preservative

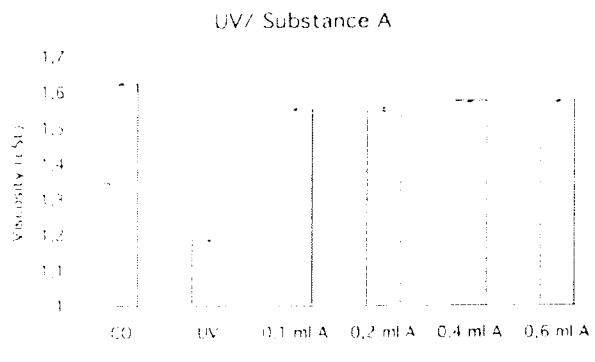


Fig. 2 UV – Substance A. *CO* Hyaluronate in physiologic NaCl (1 mg/ml) = control; *UV* hyaluronate in physiologic NaCl (1 mg/ml) after UV exposure (15 min); *0.1 ml A*, *0.2 ml A*, *0.4 ml A*, *0.6 ml A* the amount of substance A added to 20 ml hyaluronate in physiologic NaCl (1 mg/ml) followed by 15 min UV exposure

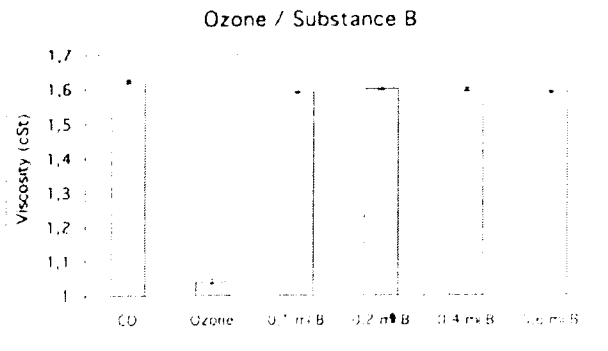


Fig. 3 Ozone – Substance B. *CO* Hyaluronate in physiologic NaCl (1 mg/ml) = control; *Ozone* hyaluronate in physiologic NaCl (1 mg/ml) after ozone exposure (10 ml with 20 µg O₃/ml); *0.1 ml B*, *0.2 ml B*, *0.4 ml B*, *0.6 ml B* the amount of substance B added to 20 ml hyaluronate in physiologic NaCl (1 mg/ml) followed by ozone exposure (10 ml with 20 µg O₃/ml)

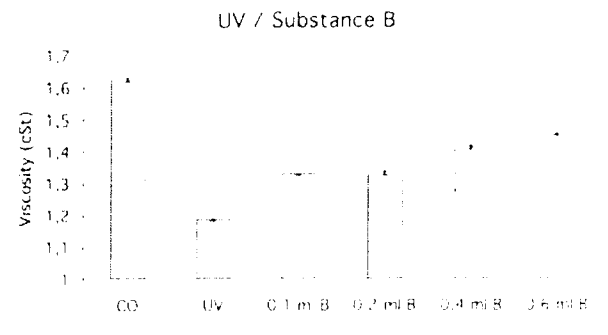


Fig. 4 UV – Substance B. *CO* Hyaluronate in physiologic NaCl (1 mg/ml) = control; *UV* hyaluronate in physiologic NaCl (1 mg/ml) after UV exposure (15 min); *0.1 ml B*, *0.2 ml B*, *0.4 ml B*, *0.6 ml B* the amount of substance B added to 20 ml hyaluronate in physiologic NaCl (1 mg/ml) followed by 15 min UV exposure

almost total protection from ozone on addition of no more than 0.1 ml of the complex preparation.

The reverse was seen on exposure to UV radiation: While substance A was highly effective at an addition of 0.1 ml, 0.6 ml of substance B was needed to produce an appreciable protective effect – which was, however, inferior to that of substance A.

Table 2 lists the anti-oxidative artificial tear preparations in the order of their anti-oxidative effect as well as their pH [14]. The active agents of these preparations are listed in Table 3.

The protective effects of the two most strongly anti-oxidative preparations, substance A and substance B, during ozone or UV exposure are shown in the illustrations (Figs. 1, 2, 3, 4). These indicate that substance B per

formed less well on UV than on ozone exposure. As in the general experiment, however, both substances had a pronounced anti-oxidative effect in the hyaluronate model.

Discussion

At least in some patients, the dry eye syndrome is likely to be caused by environmental factors [18, 19, 20]. Together with an increased above-ground concentration of ozone and more intense UV radiation due to thinning of the protective ozone layer, airborne pollutants generate what is known as photochemical smog. The resultant oxidative stress may be toxic for the components of the tear film. If the anti-oxidative mechanisms normally present in the precorneal tear film are inadequate, the tear film becomes unstable as its components are destroyed. This causes dry eye symptoms.

The administration of artificial tears containing an anti-oxidant (radical scavenger) may help to prevent the damage provoked by oxidative stress by supporting the natural defense mechanisms of the eyes. To act as scavengers, eye drops must contain certain substances, i.e., anti-oxidants. The UV absorption of the tear film itself is

inadequate for this purpose [11, 12]. Commercial UV blockers also failed to ensure physical UV absorption at normal thickness, although they were strongly anti-oxidative biochemically in our experiments [6].

Our studies once more confirmed the efficacy of preparations containing iodine (HJV,HJM). Iodine is known to be an anti-oxidant and radical scavenger [27, 29, 30]. The anti-oxidative effect of Iodinated Bad Hall Eye Drops was confirmed both with the modified TRAP procedure and the hyaluronic acid model. This may well be one of the reasons why iodine ophthalmio-iontophoresis as practiced in Bad Hall Austria, is beneficial for patients with dry eyes [15, 16, 21, 23].

If the carefully elicited history of patients with dry eyes suggests that noxious environmental factors, including ozone-generating devices in offices, may be causally involved, artificial tears which are not only lubricants but contain anti-oxidants should be chosen for treatment from the many commercially available preparations. The use of such eye drops would probably increase the success rate of treatment for dry eyes.

To develop treatment modalities tailored to specific causes of the dry eye syndrome, further studies would appear warranted.

References

1. Aejmelaeus RT, Holm P, Kaukinen U, Metsa-Ketela TJA, Laippala P, Hervoonen ALJ, Alho HER (1997) Age-related changes in the peroxyl radical scavenging capacity of human plasma. *Free Radic Biol Med* 23:69-75
2. Avunduk AM, Avunduk MC, Evirgen O, Yardimer S, Tastan H, Güven C, Cetinkaya K (1997) Histopathological and ultrastructural examination of the rat conjunctiva after exposure to tobacco smoke. *Ophthalmologica* 211:296-300
3. Blumthaler M (1993) Solar UV measurements. In: Tevini M (ed) UV-B radiation and ozone depletion. Lewis, Boca Raton, Fla, pp 71-94
4. Blumthaler M (1996) UV monitoring in Austria and Switzerland: past, present and future. In: Diffey BL (ed) Measurement and trends of terrestrial UVE radiation in Europe. OEME, Milan, pp 31-40
5. Crouch RK, Goletz P, Snyder A, Coles WH (1991) Antioxidant enzymes in human tears. *J Ocul Pharmacol* 7:253-258
6. Daxer A, Blumthaler M, Schreder J, Ettl A (1998) Effectiveness of eye drops protective against ultraviolet radiation. *Ophthalmic Res* 30:286-290
7. Gogia R, Richer SP, Rose RC (1998) Tear fluid content of electrochemically active components including water soluble antioxidants. *Current Eye Res* 17:257-263
8. Hofmann H, Schmut O (1980) The inability of superoxide dismutase to inhibit the depolymerization of hyaluronic acid by ferrous ions and ascorbate. *Graefe's Arch Clin Exp Ophthalmol* 14:181-185
9. Horwath J, Schmut O, Farr Ch, Faulborn J (1995) Behandlung des trockenen Auges mit Hyaluronat-Augentropfen. *Spektrum Augenheilkd* 9:215-217
10. Longstreth J, de Gruijl FR, Kripke ML, Abseck S, Arnold E, Slaper HL, Velders G, Takizawa Y, van der Leun JC (1998) Health risks. *J Photochem Photobiol B Biol* 46:20-39
11. Lopez Bernal D, Ubels JL (1993) Artificial tear composition and promotion of recovery of the damage corneal epithelium. *Cornea* 17:115-120
12. Michalos P, Avila EN, Florakis GJ, Hersh PS (1994) Do human tears absorb ultraviolet light? *CLAO J* 20:192-193
13. Modis Jr L, Marshall GE, Lee WR (1998) Distribution of antioxidant enzymes in the normal aged human conjunctiva: an immunocytochemical study. *Graefe's Arch Clin Exp Ophthalmol* 236:86-90
14. Pitz S, Haber M, Pfeiffer N (1998) Beobachtungen über den pH-Wert verschiedener Tränenersatzmittel. *Klin Monatsbl Augenheilkd* 213:123-124
15. Rieger G, Winkler R, Stoiser E (1997) Zur Wirkungsdauer balneotherapeutischer Maßnahmen bei Patienten mit Beschwerden des „trockenen Auges“. *Spektrum Augenheilkd* 11:255-257
16. Rieger G, Winkler R, Stoiser E, Landerl J (1997) Zur subjektiven Befindlichkeit von Patienten mit Beschwerden des „trockenen Auges“ vor und nach Absolvierung von Jodkurbehandlungen in Bad Hall. *Spektrum Augenheilkd* 11:66-71
17. Rose RC, Richer SP, Bode AM (1998) Ocular oxidants and antioxidant protection. *Proc Soc Exp Biol Med* 217:397-407
18. Schmut O (1994) Pathologische Veränderungen des vorderen Augenabschnitts durch Umwelteinflüsse. *Vitaminspur* 9:119-121
19. Schmut O, Gruber E, El Shabrawi Y, Faulborn J (1994) Destruction of human tear proteins by ozone. *Free Radic Biol Med* 17:165-169
20. Schmut O, Nassiri Ansari A, Faulborn J (1994) Degradation of hyaluronate by the concerted action of ozone and sunlight. *Ophthalmic Res* 26:340-343

21. Schmut O, Rieger G, Faulborn J, Winkler R, Horwath J (1998) Iodid schützt Tränen vor der Zerstörung durch Ozon und UV-Licht. *Spektrum Augenheilkd* 12:190–192
22. Schmut O, Faulborn J, Trummer G (1999) Quantifizierung der Schädigung von Bindehaut- und Hornhautzellkulturen durch UV-Licht mit Hilfe des CASY (Zellanalyse)-Systems. *Ophthalmologie* 96:375–381
23. Schmut O, Rieger G, Faulborn J, Winkler R (2000) Iodid schützt Bindehautzellen vor der Schädigung durch UV-Licht. *Spektrum Augenheilkd* 14:214–217
24. Stick C, Harms V, Pielke L (1997) Auf den Menschen beziehbar Messungen der ultravioletten Sonnenstrahlung. *Phys Rehab Kur Med* 7:55–59
25. Stolze HH, Becker J (1993) Die Hornhaut – bradytroph und abwehrschwach? Entgiftung UV-induzierter toxischer Sauerstoffradikale. *Contactologia* 15D:143–145
26. Uotila JT, Kirkkola AL, Rorarius M, Tuimala RJ, Metsä-Ketelä T (1994) The total peroxy radical-trapping ability of plasma and cerebrospinal fluid in normal and preeclamptic parturients. *Free Radic Biol Med* 16:581–590
27. Venturi S, Donati FM, Vaenturi M, Venturi A, Grossi L, Guidi A (2000) Role of iodine in evolution and carcinogenesis of thyroid, breast and stomach. *Adv Clin Pathol* 4:11–17
28. Versura P, Profazio V, Cellini M, Torreggiani A, Caramazza R (1999) Eye discomfort and air pollution. *Ophthalmologica* 213:103–109
29. Winkler R, Moser M (1992) Jodid Ein potentielles Antioxidans und Sauerstoffradikalfänger und seine Rolle bei Peroxidase-Reaktionen. *Vitaminspur* 7:124–134
30. Winkler R, Moser M, Buchberger W (1989) Die Wirksamkeit von Jodid als Sauerstoff-Radikalfänger. *Wiss Z Humboldt Univ Berl R Med* 38:76–79
31. World Meteorological Organization (1994) Scientific assessment of ozone depletion. Report no. 37