Free Radical Content of Drugs Used in Ophthalmology

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ABSTRACT

To evaluate the presence of free radicals in various drugs used in ophthalmology. Twenty five samples of drugs that are used by intracameral or intravitreal route, eleven samples of drugs used as fortified eye drop, thirty samples of topical commercial preparations that are used as long term, and eight samples of autologous serum were analyzed for total free radical presence using a kit for total oxidant status. The highest free radical concentration was in Moxifloxacin (Vigamox) in intracameral group, clindamycin in intravitreal group, brinzolamide in antiglaucoma group. Free radicals are present in drugs used in ophthalmology. The surgeon should consider the risk of oxidative damage in the presence of diseased eye.

Key words: *Free radical, oxidant status, drugs, ophthalmology*

Göz Hastalıklarında Kullanılan İlaçların Serbest Radikal İçerikleri

ÖZET

Oftalmolojide kullanılan ilaçlarda bulunan serbest radikallerin varlığını incelemek. İntrakameral ve intravitreal kullanılan 25 ilaç örneği, fortifiye damla olarak kullanılan 11 ilaç örneği, uzun süreli kullanılan 30 göz damlası örneği ve farklı yaş ve cins hastadan alınan 8 otolog serum örneği serbest radikal varlığını analiz etmek için total oksidan status kiti kullanıldı. En yüksek serbest radikal konsantrasyonu intrakameral grupta moksifloxacinde (Vigamox), intravitreal grupta klindamisinde, antiglokom grubunda brinzolamidde ölçüldü. Oftalmolojide kullanılan ilaçlarda serbest radikaller bulunmaktadır. Cerrah hastalıklı göz varlığında oksidatif doku hasarı riskini göz önünde bulundurmalıdır.

Key words: *Serbest radikal, oksidatif durum, ilaçlar, oftalmoloji*

INTRODUCTION

The most important targets of free radicals are unit membranes, e.g. plasma, mitochondrial, rough endoplasmic reticulum, and nuclear membranes. Free radicals initiate toxic reactions at the plasma membrane and lead to cellular edema or necrosis (1-3). The unsaturated bonds of cholesterol and fatty acids in the membranes can readily react with free radicals and undergo peroxidation. This process can become autocatalytic after initiation and will yield lipid peroxides (2,3). Lipid peroxides have a sufficient lifetime, which means that they can migrate and damage other cellular components, including DNA, apart from the membranes (4). In a recent study, free radicals were detected in significant amount in agents used for intracameral injections after cataract surgery (5). Numbers of off label agents that are used in intravitreal and intracameral route are increasing continuously. Free radicals in these agents may cause undetectable damage to corneal endothelium, trabecular meshwork, and retina.

Ophthalmic eye drops contain preservative to prevent bacterial and fungal growth secondary to contamination from the patient. Common preservatives are benzalko-

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nium chloride, chlorobutanol, parahydroxbenzoates, and polysorbate. Drugs itself and preservative may act as free radicals and cause peroxidation of polyunsaturated lipids in cell membranes (6). Chronic use of ophthalmic eye drops such as antiglaucoma medications causes dry eye and failed trabeculectomy bleb (7).

Many off labeled fortified eye drops that are prepared from intravenous formulation are used for treatment of fungal and bacterial keratitis and endophthalmitis in which there is a diseased and impending perforated cornea. Fortified eye drops are used in very high concentration and in a frequent regimen such as hourly or half an hour. Frequent and long time use of fortified eye drops containing high level of free radicals may induce peroxidation of corneal epithelium and keratocyte cell membrane in the presence of diseased cornea with altered epithelium and Bowmans membrane. Thus, these drugs may induce corneal melting and perforation while eradicating the infecting organisms (8). Knowing the amount of free radicals in fortified eye drops will guide ophthalmologist to choose the least free radical containing agents.

In this study, we aimed to measure the amount of free radicals in agents used in intracameral and intravitreal injections, in fortified eye drops, in autologous serums, and in long term used topical commercial preparations.

MATERIALS AND METHODS

In our study, totally 74 samples of agents were analyzed for total oxidant and antioxidant capacity. 0.2 mL of each samples were used for the analysis.

Intracameral or intravitreal agents

Balanced salt solution (Albosol), balanced electrolyte solution (Isolyte S, Ezcacıbası), normal saline solution (Ezcacıbası), Lactated Ringer's solution (Ezcacıbası), adrenalin (25 µg/0.1 ml, Adrenalin, Drogsan), carbachol (10 µg/0.1ml, Miostat, Alcon), levobupivacain (500 µg/0.1ml,Chirocaine, Abbott), Lidocain, (100 µg/0.1 ml, Jetmonal 2%, Adeka), vancomycin HCL DBL (1 mg/0.1 ml, Vankomisin HCL DBL, Orna), vancomycin HCL (1 mg/0.1 ml, Edicin, Sandoz), moxifloxacin (250 µg/0.05 ml, Vigamox, Alcon), moxifloxacin (240 µg/0.15 ml, Avelox, Bayer), cefuroxime (1 mg/0.1 ml and 3 mg/0.1 ml, Zinnat, GSK), cefuroxime (1 mg/0.1 ml and 3 mg/0.1 ml Multisef, Mustafa Nevzat), cefotaxime (1 mg/0.1 ml, Betaksim, Mustafa Nevzat), ceftazidime (2.25 mg/0.1 ml, Zidim, Tum Ekip), clindamycin (1 mg/0.1 ml, Cleocin, Pfizer), clindamycin (1 mg/0.1 ml, Clin, IE Ulagay), dexamethazone (400 µg/0.1 ml, Onadron, Bilim), voriconazole (100 µg/0.1 ml Vfend, Pfizer), fluconazole (100 µg/0.1ml, Triflucan, Pfizer), fluconazole (100 µg/0.1ml, Lumen, Mustafa Nevzat), bevacizumab (1.25 mg/0.05 ml, Altuzan, Roche) (Table 1).

Fortified eye drops

Clindamycin (150mg/1ml, Cleocin, Pfizer), clindamycin (150mg/1ml, Clin, IE Ulagay), vancomycin HCL DBL (25mg/ml, Vankomisin HCL DBL, Orna), vancomycin HCL (25mg/ml, Edicin, Sandoz), vancomycin HCL (25mg/ml, Vankomisin HCL, Meditera), cefazoline (50mg/ml, Sefazol, Mustafa Nevzat), gentamycin (20mg/ml, Genta, İE Ulagay), amikacin (50mg/ml, Amikozit, Zentiva), voriconazole (10 mg/ml Vfend, Pfizer), fluconazole (2mg/ml, Triflucan, Pfizer), fluconazole (2mg/ml, Lumen, Mustafa Nevzat) (Table 2).

Commercial eye drops

Anti-glaucoma drugs: Brimonidin tartrate 0.15% (Alphagan P, Allergan), brimonidine tartrate 0.2% (Alphagan, Allergan), bimatoprost 0.3 mg/ml (Lumigan, Allergan), latanoprost 0.005% (Xalatan, Pfizer), latanoprost 0.005% (Glokoprost, Cenovapharma), travoprost 0.004% (Travatan, Alcon), bimatoprost 0.3 mg/ml+ Timolol 5mg/ml (Ganfort, Allergan), latanoprost 0.005%+ Timolol 5mg/ml (Xalacom, Pfizer), brimonidine tartrat 0.2%+ Timolol 5mg/ml (Combigan, Allergan), betoxolol 0.5% (Betoptic, Alcon), timolol 5mg/ml (Timolol POS, Ursapharm) (Table 3).

Drugs used for treatment of dry eye

Polividon (Oculotect, Novartis), Polyvinyl alcohol (Siccaprotect, Ursapharm), Polyvinyl alcohol (Refresh single dose, Allergan), Polyvinylprolidon (Protagent, Alcon), Dekstran 70 (Tears Naturale 2, Alcon), Polyethylen glycol (Systane, Alcon), Sodium hyaluronate (Hyolocomod, Ursapharm), Hypromellose (Artelac advanced, B and L), N acetyl cystein (Brunac, Bio-Gen) (Table 3).

Antiallergic drugs

Sodium cromoglicate 2% (Allergocrom, Ursapharm), Sodium cromoglycate 2% (Allergocomod, Ursapharm), Ketotifen 0.25 mg/ml (Zaditen, Novartis), Olopatadin 0.1% (Patanol, Alcon), Emadastin 0.05% (Emadine, Alcon), Epinastin 0.5mg/ml (Relestat, Allergan), Lodoksamide 0.1%(Thilomide, Alcon) (Table 3).

***Table 1**. Presence of free radicals (TOS) and antioxidant status (TAS) in agents used in intravitreal and intracameral injections.*

Product	Concentration	Brand name/Producer	TAS μmol Trolox equivalent/L	TOS mmol/L
Balanced salt solution		Aquamed	0.0546	1.4813
Balanced electrolyte solution		Isolyte S, Ezcacıbaşı	0.0347	1.18
Normal saline solution		Ezcacıbaşı	0.0381	0.6617
Lactated Ringer's solution		Ezcacıbaşı	0.0353	0.8878
Adrenalin	25 μg/0.1 ml	Adrenalin, Drogsan	1.4731	0.7853
Carbachol	10 μg/0.1 ml	Miostat, Alcon	0.0389	0.2277
Levobupivacain	500 μg/0.1 ml	Chirocaine, Abbott	0.0376	0.6445
Lidocain	100 μg/0.1 ml	Jetmonal 2%, Adeka	0.0705	0.9155
Vancomycin HCL DBL	1 mg/0.1 ml	Vankomisin HCL DBL, Orna	1.2003	3.4672
Vancomycin HCL	1 mg/0.1 ml	Edicin, Sandoz	1.1089	2.2356
Moxifloxacin	250 μg/0.05 ml	Vigamox, Alcon	0.0969	24.0867
Moxifloxacin	240 μg/0.15 ml	Avelox, Bayer	0.0333	0.9988
Cefuroxime	1 mg/0.1 ml	Zinnat, GSK	0.5086	1.0028
Cefuroxime	3 mg/0.1 ml	Zinnat, GSK	1.0723	4.2747
Cefuroxime	1 mg/0.1 ml	Multisef, Mustafa Nevzat	0.5341	1.208
Cefuroxime	3 mg/0.1 ml	Multisef, Mustafa Nevzat	1.1056	3.923
Cefotaxime	1 mg/0.1 ml	Betaksim,Mustafa Nevzat	1.2073	1.2645
Ceftazidime	2.25 mg/0.1 ml	Zidim, Tüm Ekip	1.2041	1.104
Clindamycin	1 mg/0.1 ml	Cleocin, Pfizer	0.1177	11.7482
Clindamycin	1 mg/0.1 ml	Clin, İE Ulagay	0.1209	9.5673
Dexametazone	400 μg/0.1 ml	Onadron, Bilim	1.4742	0.1
Voriconazole	100 μg/0.1 ml	Vfend, Pfizer	0.0235	1.8439
Fluconazole	100 μg/0.1 ml	Triflucan, Pfizer	0.1156	1.3538
Fluconazole	100 μg/0.1 ml	Lumen, Mustafa Nevzat	0.0393	1.3074
Bevacizumab	1.25 mg/0.05 ml	Altuzan, Roche	0.4133	3.0919

Nonsteroid Anti-inflamatory drugs

Ketorolac 0.4% (Acular LS, Allergan), Diclofenac sodium 1mg/ml (Inflased, Bilim), Diclofenac sodium 1mg/ml (Voltaren Ophta, Novartis) (Table 3).

Autologous serum

Autologous serum from eight patients from different sex and age without any known disease were also analyzed (Table 4).

Total antioxidant status (TAS)

TAS levels were measured using commercially available kits (Rel Assay). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) radical cation by antioxidants. The commercial kit work colorimetric method is based on 660 nm absorbance. The results were expressed as mmol Trolox equivalent/L.

Total oxidant status (TOS)

TOS levels were measured using commercially available kits (Rel Assay). In this method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L).

RESULTS

Intracameral or intravitreal agents

Mean TOS and TAS values of these 25 agents were 3.17±5.15 mmol/L (range 0.10 to 24.09) and 0.48±0.54 μmol Trolox equivalent/L (range 0.02 to 1.47) respectively. Moxifloxacin had the highest concentration of free radicals (24.09 mmol/L) (Table 1).

***Table 2**. Presence of free radicals (TOS) and antioxidant status (TAS) in agents used in fortified eye drops.*

Product	Concentration	Brand name/Producer	TAS µmol Trolox equivalent/L	TOS mmol/L
Clindamycin	150 mg/ml	Cleocin, Pfizer	0.9145	157.6516
Clindamycin	150 mg/ml	Clin, İE Ulagay	0.924	169.1327
Vancomycin HCL DBL	25 mg/ml	Vankomisin HCL DBL, Orna	1.2083	1.5724
Vancomycin hcl	25 mg/ml	Edicin, Sandoz	1.3549	2.7166
Vancomycin HCL	25 mg/ml	Vankomisin HCL, Meditera	1.2867	3.2176
Cefazoline	50 mg/ml	Sefazol,Mustafa Nevzat	1.4699	5.4295
Gentamicin	20 mg/ml	Genta, İE Ulagay	1.4791	<0.1
Amikacin	50 mg/ml	Amikozit,Zentiva	1.4762	0.7179
Voriconazole	10 mg/ml	Vfend, Pfizer	0.0723	2.6091
Fluconazole	2 mg/ml	Triflucan, Pfizer	0.0345	10.9083
Fluconazole	2 mg/ml	Lumen, Mustafa Nevzat	0.0386	38.0616

Fortified eye drops

Mean TOS and TAS values of these 11 agents were 35.77±64.03 mmol/L (range 0.71 to 169.13) and 0.85±0.62 µmol Trolox equivalent/L (range 0.03 to 1.47) respectively (Table 2).

Commercial eye drops

Anti-glaucoma drugs: Mean TOS and TAS values of these 11 agents were 4.73± 8.07 mmol/L (range 0.17 to 24.72) and 0.04±0.03 µmol Trolox equivalent/L (range 0.00 to 0.09) respectively (Table 3).

Drugs used for treatment of dry eye: Mean TOS and TAS values of these 9 agents were 4.90±8.08 mmol/L (range 0.17 to 23.31) and 0.27±0.53 µmol Trolox equivalent/L (range 0.0031to 1.48) respectively (Table 3).

Antiallergic drugs: Mean TOS and TAS values of these 7 agents were 6.80±9.36 mmol/L (range 0.06 to 24.28) and 0.16±0.23 µmol Trolox equivalent/L (range 0.00 to 0.65) respectively (Table 3).

Nonsteroid Anti-inflamatory drugs: Mean TOS and TAS values of these 3 agents were 9.86±8.02 mmol/L (range 0.90 to 16.35) and 0.28±0.40 µmol Trolox equivalent/L (range 0.04 to 0.74) respectively (Table 3).

Autologous serum: Mean TOS and TAS values of 8 samples were 6.68±1.33 mmol/L (range 5.12 to 9.29) and 0.69±0.07 µmol Trolox equivalent/L (range 0.57 to 0.78) respectively (Table 4).

DISCUSSION

The numbers of off-label drug used in intracameral and intravitreal injections are continuously increasing. Intracameral agents include anaesthetic agents for iris anesthesia, antibiotics such as cefuroxime, moxifloxacin and vancomycin for endophthalmitis prophylaxis after cataract surgery, adrenalin for pupillary dilatation, carbachol for miosis, trypan blue for capsule staining, triamcinolone for identifying vitreous strands. Intravitreal agents that used in treatments are vancomycin and ceftazidim for bacterial endophthalmitis, voriconazole and amphotericin B for fungal endophthalmitis, clindamycin for toxoplasma retinitis, dexamethazone and triamcinolone for retinal and vitreous inflammation. Most of these drugs are prepared in the operation theatre and accuracy of concentration may not be obtained.

Corneal endothelium is very sensitive to any form of toxic exposure. Recently, studies have been published with regard to the oxidative damage of intracameral agents. Borazan et al. (9) documented the induction of apoptosis by intracameral preservative free lidocaine hydrochloride 2%, ropivacaine1%, and levobupivacaine 0.75% in rabbit corneal endothelium. Similarly, Demir NM et al. (10) showed immediate toxicity of levobupivacaine 0.5% on rabbit corneal endothelium and oxidative damage of lidocaine hydrochloride 2% on corneal and lens tissue. In another similar study, Ozlem YT et al (11) reported that cefuroxime made an oxidative damage on corneal tissue while vancomycin had no harmful effect. Intracameral injection of these antibiotics after removal of OVD which acts as free radical scavengers cause the corneal endothelium open to oxidative damage. Additionally, free radicals in intracameral agents may cause toxic anterior syndrome (12). In this study, we found that moxifloxacin from eye drop had the highest free radical content (Table 1). TAS levels were similar in this group.

***Table 3**. Presence of free radicals (TOS) and antioxidant status (TAS) in long term used commercial eye drops.*

Group	Product	Brand name	TAS μmol Trolox equivalent/L	TOS mmol/L
Anti-glaucoma	Brimonidine tartrate 0.15%	Alphagan P	0.0084	24.7292
	Brimonidine tartrate 0.2%	Alphagan	0.0092	14065
	Bimatoprost 0.3 mg/ml	Lumigan	0.0007	1.7471
	Latanoprost 0.005%	Glucoprost	0.0919	0.3582
	Travoprost 0.004%	Travatan	0.0487	15.0622
	Latanoprost 0.005%	Xalatan	0.0946	0.1700
	Bimatoprost 0.3 mg/ml +Timolol 5mg/ml	Ganfort	0.0376	0.3371
	Latanoprost 0.005% +Timolol 5mg/ml	Xalacom	0.0857	0.5612
	Brimonidine tartrate 0.2% +Timolol 5mg/ml	Combigan	0.0178	0.1800
	Betoxolol 0.5%	Betoptic	0.0139	7.3520
	Timolol 5mg/ml	Timolol POS	0.0084	0.1900
Drugs used for treatment of dry eye		Polyvidon Oculotect	0.8811	23.3142
	Polyvinyl alcohol	Siccaprotect	0.0031	0.1765
	Polyvinyl alcohol	Refresh tek doz	0.0142	0.6688
	Polyvinyl prolidon	Protagent	0.0417	3.0601
	Dextran 70 1mg/ml	Tears Naturale 2	0.0069	0.9441
	Polyethylene glycol 0.4%	Systane	0.0223	0.2029
	Sodium hyaluronate 1 mg/ml	Hyolocomod	0.0031	2.1212
	Hypromellose 3.2 mg/ml	Artelac advanced	0.0126	0.1800
	N-Acetyl Cysteine 5%	Brunac	1.4806	13.4527
Anti-allergic	Sodium cromoglicate 2%	Allergocrom	0.0469	24.2870
	Sodium cromoglicate 2%	Allergo-comod	0.2408	0.1800
	Ketotifen 0.25 mg/ml	Zaditen	0.6459	0.0618
	Olopatadin 0.1%	Patanol	0.0020	0.1871
	Emadastin 0.05%	Emadine	0.0005	0.1288
	Epinastin 0.5 mg/ml	Relestat	0.1492	11.2574
	Lodoxamide 0.1%	Thilomide	0.0322	11.5468
Anti-inflammatory	Ketorolac 0.4%	Acular LS	0.0527	16.3505
	Diclofenac sodium 1mg/ml	Inflased	0.0365	12.3462
	Diclofenac sodium 1mg/ml	Voltaren Ophta	0.7433	0.9018

Non-toxic doses of many agents that are used in intravitreal injections were determined in various studies. (13). Clearance of an intravitreal drug takes longer time than intracameral drugs. Superior complexity of retinal anatomy is another factor that puts the retina to undetermined toxicities. In this study, among the intravitreal agents, clindamycin had the highest free radical content. TAS levels were similar in this group (Table 1).

Fortified eye drops contain high concentration of antibiotics or antifungal agents. It was shown that fortified antibiotic eyedrops had negative influence on corneal epithelial wound healing (14). Free radicals may have more damage on diseased corneal cells in the presence of keratitis. Additionally, poradic toxicity case reports secondary to fortified eye drops usage were reported (8,15,16) Fortified eye drop contains a higher amount of free radicals. Information about free radical content will help physician to choose the least free radical containing agents. In our study, we found that clindamycin had the highest free radical concentration (Table 2). TAS levels were similar in this group.

Table 4: Presence of free radicals (TOS) and antioxidant status (TAS) in autologous serum

Patient no	Sex	Age	TAS μmol Trolox equivalent/L	TOS mmol/L
1	F	9	0.6503	5.7192
2	M	5	0.5726	5.9342
3	M	16	0.7393	7.4538
4	F	14	0.7512	6.2385
5	F	39	0.7789	6.1474
6	M	33	0.6786	9.2903
7	F	52	0.6530	5.1162
8	M	69	0.7562	7.5376

The normal ocular tear film contains protective components such as lactoferrin, aldehyte dehydrogenase, superoxide dismutase, and glutathione which are suboptimal in eyes with ocular surface disease or tear film deficiency (17,18). Usage of multiple topical preparations may cause free radical related damage. Ocular surface disorders may develop secondary to preservative related oxidative injury in long term treated glaucoma patients (7). It was shown that, preservative free preparations have less cytotoxic effects than preserved preparations (19). The highest free content agents in the eye drop group were (Table 3); Brimonidine tartrate 0.15% (Alphagan P) and Travoprost 0.004% (Travatan) in anti-glaucoma drugs, Polyvidon (Oculotect) and N-acetyl-cystein 5% in drugs used for treatment of dry eye group, Sodium cromoglycate 2% (Allergocrom) in anti-allergic eye drop group, Ketorolac 0.4% (Acular LS) in anti-inflammatory group. TAS levels were similar in this group. Autologous serum eye drops are prepared from patients own blood and used for treatment of dry eye (20). In our study we found that TOS and TAS levels were similar for different aged patients (Table 4).

In conclusion, we have shown that free radicals are present in agents used in intravitreal and intracameral injections, in long term used commercial eye drops, in fortified eye drops, and in autologous serum eye drops. The physician should use these drugs carefully by considering the amount of free radical content. More studies should be done to further investigate the free radical content of agents used in ophthalmology.

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