Influence of Coenzyme Q10 on Ocular Surface Microbiata

Koenzim Q10'un Oküler Yüzey Mikrobiyatasına Etkisi

Tolga KOCATÜRK[1], Murat TELLİ[2], Berna KORKMAZGİL[2], Mehmet BİLGEN[3]

ABSTRACT

Purpose: To evaluate the effects of Coenzyme Q10 on the proliferation of the most common ocular surface bacteria; *S. aureus, P. aeruginosa* and fungus; *C. albicans*

Material and methods: Experiments were performed in-vitro with total 15 glass tubes of each filled with 1.5 ml of fluid media were prepared. The tubes were then divided equally into 3 groups to culture the bacteria *staphylococcus (S.) aureus, pseudomonas (P.) aeruginosa* and fungus *candida (C.) albicans*. In a set of 5 tubes, colony of each microorganism was thus produced. In each group, Tube (numbered 0) was established as a control. The remaining Tubes 1-4 were respectively added with 1 to 4 drops of CoQ10 containing eye drops. The tubes were then evaluated at 0, 1, 2, 4, 5 and 24 hours. The proliferation of microorganism in a liquid medium resulted in turbidity from which microbial concentration was estimated using McFarland barium sulphate turbidity standard. Measurements were performed on the spectrophotometer as McFarland standard and interpreted through the changes in reproduction. For each group, the estimates of the average numbers of the corresponding microorganisms subjected to the four different strengths of CoQ10 were recorded and statistically compared to those in the control group.

Results: In all the groups, the concentrations of the microorganisms (S. aureus, P. aeruginosa and C. albicans) increased with time. However, exposure to CoQ10 containing eye drops did not significantly alter the microorganismal growth as the measurements from the CoQ10-instilled tubes remained comparable to those of the controls.

Conclusion: CoQ10 under in-vitro conditions did not induce proliferation of S. aureus, P. aeruginosa and C. albicans.

Key words: ocular microbiata, coenzyme Q, ocular surface.

ÖZ

Amaç: Koenzim Q10'un (CoQ10), oküler yüzeyde sık görülen bakteriler olan *stafilokok (S.) aureus, psödomonas (P.) aeruginosa* ve mantarların (*C. albicans)* üremeleri üzerine etkisinin değerlendirilmesi

Gereç ve yöntem: Deney, herbiri 1,5 ml'lik sıvı içeren, 15 adet cam tüp ile in-vitro olarak gerçekleştirildi. Tüpler 3 gruba ayrıldı ve *S. aureus, P. aeruginosa* ve *C. albicans* kültürleri oluşturuldu. Her bir grupta yer alan 5 adet tüpte mikroorganizma kolonileri üretildi. Her grupta, kontrol grubu olarak ayrılan tüp 0 olarak numaralandırıldı. Geri kalan 4 tüpe sırasıyla 1 ile 4 damla CoQ10 içeren damla eklendi. Tüpler 0, 1, 2, 3, 4, 5, ve 24. saatlerde değerlendirildi. Sıvı besiyerindeki mikroorganizmaların proliferasyonu sonucu, mikroorganima konsantrasyonu artışı nedeniyle oluşan bulanıklık mikrarı McFarland baryum sülfat bulanıklık standard kullanılarak değerlendirildi. Ölçümler McFarland standardına göre spektrofotometre ile üreme değişiklikleri gözönüne alınarak yorumlandı. Her bir grup için, 4 farklı doz içeren CoQ10 tüplerindeki bulanıklık ölçümlerine karşılık gelen mikroorganizma sayıları kaydedildi ve istatistiksel olarak kontrol grubu ile karşılaştırıldı.

Bulgular: Tüm gruplarda, mikrorganizma *(S. aureus, P. aeruginosa* ve *C. albicans)* konsantrasyonları zaman ile arttı. Ancak, CoQ10 içeren damla uygulanan tüplerde, kontrol grubu ile karşılaştırıldığında, mikroorganizmaların çoğalmalarında, istatistiksel olarak anlamlı derecede bir değişiklik oluşturmamıştır.

Sonuç: İn vitro koşullarda CoQ10, *S. aureus, P. aeruginosa* ve *C. albicans'*ın proliferasyonunu arttırmadı.

Anahtar kelimeler: oküler mikrobiyata, koenzim Q, oküler yüzey.

1- Doç. Dr., Adnan Menderes Üniversitesi Tıp Fakültesi Hastanesi, Göz Hast. AD., Aydın, Türkiye

2- Doç. Dr., Adnan Menderes Üniversitesi Tıp Fakültesi Hastanesi, Mikrobiyoloji Anabilim Dalı, Aydın, Türkiye

2- Prof. Dr., Adnan Menderes Üniversitesi Tıp Fakültesi Hastanesi, Biyofizik Anabilim Dalı, Aydın, Türkiye

Geliş Tarihi - Received: 13.09.2019
Kabul Tarihi - Accepted: 24.09.2019
Glo-Kat 2019; 14: 171-174

Yazışma Adresi / Correspondence Adress:
Tolga KOCATÜRK
Adnan Menderes Üniversitesi Tıp Fakültesi Hastanesi, Göz Hast. AD., Aydın, Türkiye

Phone: +90 256 444 1256
E-mail: tolgakocaturk@gmail.com

INTRODUCTION

Past research has focused on characterizing the broad and various microbial communities of ocular surface.[1-4] Advanced molecular tools were utilized for the comprehensive and precise identification of the ocular microbiata based on 16S rRNA sequencing. Under normal circumstances, microbial composition prevents the proliferation of pathogenic species, as it is immunologically protective. Thus alterations in the homeostatic microbiome are linked to ophthalmic pathologies. Such conditions are clinically intervened by endogenous and exogenous processes. However, some interventions aim for the treatment and prevention of the ophthalmic diseases, and others for the improvement of the recovery from eye injuries. But in either case, the underlying microbial spectrum may simultaneously be disturbed. Therefore, it is critically important to carefully craft the interventional strategy for successfully achieving the targeted therapeutic intent while maintaining the microbial balance undisturbed.

Ubiquinone (CoQ10, a 10-chain form Coenzyme Q) has exogenously been applied for the treatment of ophthalmic diseases or eye injuries.[5,6] It is commonly found in all cells in nature from bacterias to mammals and used in energy production in humans and microorganisms. It was shown that CoQ10 therapy improves corneal wound healing by accelerating the corneal healing especially in neurotrophic keratitis[5], blocks apoptosis of corneal fibroblast[4], and reduces corneal edema by increasing corneal endothelial pump function.[6] It prevents retinal ganglion cell death due to oxidative stress and post-ischemia-related injury in glaucoma.[7,8,9] It protects fruit flies against bacterial and fungal infections but increases virus amplification and reduces resistance to viral infections.[10] It affects gut microbiota when taken orally. However, how exogenous CoQ10 would affect the individual elements of the ocular microbiata is still unknown and needs further exploration. This study was initiated to address this need. In particular, how CoQ10 delivery influence the most common ocular surface bacteria; *staphylococcus (S.) aureus, pseudomonas (P.) aeruginosa* and fungus; *candida (C.) albicans* is investigated experimentally. With in-vitro studies, normal reproduction lines of these microorganisms were compared when CoQ10 was administered at different doses using McFarland turbidity standard and sensitive spectrophotometric measurements.

MATERIALS AND METHODS

The study was approved by the institutional ethics committee (No: 2018/091). Experiments were performed in-vitro with the standard strains of bacteria *S. aureus, P. aeruginosa* and fungus; *C. albicans*. Total 15 glass tubes of each filled with 1.5 ml of fluid media were prepared. The tubes were then divided equally into 3 groups to culture the three microorganisms (in Mueller-Hinton broth). In a set of 5 tubes, colony of each microorganism was thus produced (Figure 1). In each group, Tube (numbered 0) was established as a control. The remaining Tubes 1-4 were respectively added with 1 to 4 drops (about 50 to 200 µl) of Visudrop solution (CoQ10, Vitamin E-TPGS, Hypromellose, Visufarma S.p.A-ViaCanino, 21-00191 Roma, Italy). The tubes were then evaluated at 0, 1, 3, 2, 4, 5 and 24 hours. The proliferation of microorganism in a liquid medium resulted in turbidity from which microbial concentration was estimated using McFarland barium sulphate turbidity standard.[11,12] The presence of $1.5x10^8$ cfu / mL (typically between $1.0x10^8$ and $2.0x10^8$ cfu/mL) bacteria in suspension is referred as 0.5 McFarland standard. While determining the increase in microbial density, it was initiated from this level, at the zero point of time. This amount exhibits an absorbance of 0.08-0.10 at 625 nm on the spectrophotometer.[13] Subsequent measurements were continued and interpreted through the changes in reproduction level (Figure 2). For each

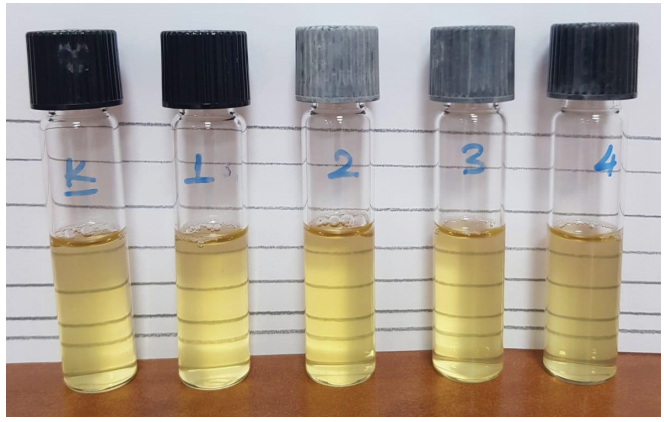

Figure 1. *Mueller-Hinton broth containing tubes at the zero point of time. Tube 0 was assigned as control. The remaining Tubes 1-4 were respectively added with 1 to 4 drops CoQ10 containing eye drop.*

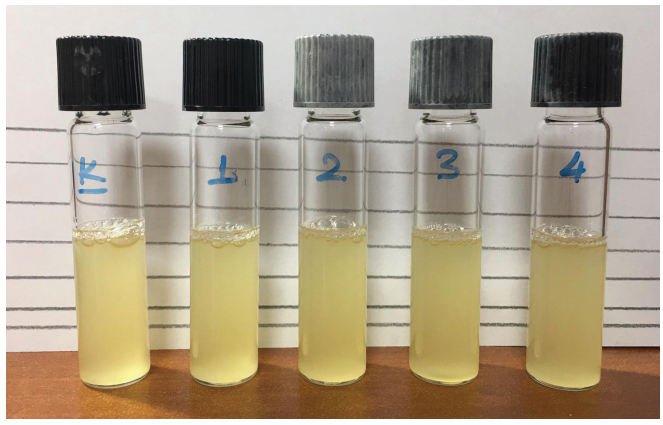

Figure 2. *The tubes at 24 hours.*

group, the estimates of the numbers of the corresponding microorganism subjected to the four different strengths of CoQ10 were measured three times and the average of the three was recorded for comparison with that of the control group.

RESULTS

The measured McFarland values regarding the counts of the microorganisms examined under in-vitro conditions are listed in Table 1. In all groups, the concentrations of the microorganisms increased with time. However, visudrop did not significantly alter the growths of *S. aureus, P. aeruginosa* and *C. albicans* as the measurements from the visudrop-instilled tubes with1 to 4 drops remained comparable to those of the controls.

DISCUSSION

CoQ10 is the main ingredient of Visudrop eye drop, which is increasingly used in ophthalmology; in corneal epithelial and wound healing[5] and in treating corneal edema.[6] In-vitro and in-vivo studies demonstrated that it inhibits the excimer laser induced corneal keratocyt apoptosis better than other antioxidants.[14] It blocks apoptosis of corneal fibroblast by blocking caspase-2.[4] In two cases of corneal edema, known as Kearns-Sayre Syndrome, CoQ10 improves corneal health by increasing the corneal endothelial pump function.[6] These effects are explained by the actions of inhibiting corneal apoptosis and accelerating electron transport in the electron transport chain in corneal cells. It is found in biological membranes especially in the complexes I (NADH-ubiquinone oxidoreductase), II (succinate-ubiquinone oxidoreductase) and III (ubiquinone-cytochrome oxidoreductase) of mitochondria which act as an electron transport medium.[15] CoQ10 accelerates corneal healing especially in neurotrophic keratitis.[5] Its unique antioxidant effect enhances bioenergetic capacity by acting as an electron carrier between the complexes in the mitochondrial respiratory cycle. Its antioxidant effect accumulates reactive free oxygen radicals in the environment, regulating NFkappaB1-dependent gene expression. Its

Table 1. *McFarland turbidity values in the tubes.*

Microorganism	Hours	Control	1st tube	2nd tube	3rd tube	4th tube
S. aureus (ATCC25923)						
	0	0.57	0.50	0.49	0.51	0.55
	1	0.60	0.59	0.64	0.58	0.62
	2	0.70	0.78	0.86	0.71	0.77
	3	0.83	1.03	0.97	0.97	0.98
	4	0.98	1.18	1.25	1.09	1.14
	5	1.22	1.34	1.85	1.26	1.28
	24	3.46	3.83	2.04	3.00	2.85
P. aeruginosa (ATCC49619)						
	0	0.53	0.51	0.51	0.55	0.50
	1	0.59	0.60	0.54	0.56	0.48
	2	0.54	0.66	0.55	0.57	0.58
	3	0.75	0.85	0.85	0.73	0.70
	4	0.79	0.95	0.76	0.73	0.67
	5	0.88	0.94	0.88	0.82	0.83
	24	2.76	2.33	2.77	1.66	2.04
C. albicans (ATCC24433)						
	0	0.52	0.51	0.53	0.43	0.52
	1	0.58	0.52	0.58	0.45	0.58
	2	0.59	0.56	0.61	0.48	0.48
	3	0.60	0.57	0.62	0.52	0.52
	4	0.61	0.59	0.62	0.54	0.52
	5	0.64	0.60	0.62	0.57	0.53
	24	1.83	1.47	1.48	1.15	1.10

anti-inflammatory effect helps the regeneration of corneal subbasal nerve plexus. Its anti-apoptotic function inhibits the apoptotic signal transduction by affecting the permeability of pores in mitochondrial membranes.

This study was initiated to uncover if exposing CoQ10 to ocular surface would alter its microbial balance. The bacteria; *S. aureus, P. aeruginosa* and fungus; *C. albicans* are the common elements of the unique ophthalmic microbial flora. According to the data in Table 1, CoQ10 delivered in Visudrop solution under in-vitro conditions surprisingly did not induce proliferation of these organisms, despite its wide and universal domain of effect on cellular energy metabolism. This may be due to the fact that these microorganisms use different forms of CoQ in their energy production. For example, Saccharomyces cerevisiae uses CoQ6, Escherichia coli uses CoQ8 and Caenorhabditis elegans uses CoQ9 chains. Nevertheless, it overall appears that the benefits of CoQ10 surpass the detrimental effects, especially in corneal healing of keratitis infected by these microorganisms as CoQ10 remains benign to their population balance. However, how CoQ10 interacts with viruses remains to be seen in further studies.

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