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RESEARCH ARTICLE

Corneal endothelial changes in long-term cannabinoid users

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Abstract

Purpose: The aim of this study was at evaluating the effects of long-term cannabis use on the corneal endothelial cells with the specular microscopy.
Methods: The study enrolled 28 eyes of 28 patients diagnosed with cannabinoid use disorder. The cannabinoid group was selected among patients who had been using the substance for three days or more per week over the past one year. Thirty-two eyes of 32 age- and sex-matched healthy individuals enrolled as control group in the study. Corneal endothelial cell density (CD), coefficient of variation (CV) and hexagonal cell ratio (HEX) values were analyzed by specular microscopy.
Results: The mean CD was 2900 ± 211 cells/mm^2 in the cannabinoid group and 3097 ± 214 cells/mm^2 in the control group ($p < 0.01$). There was a significant decrease in cannabinoid group. The mean CV was 29 ± 7 and 27 ± 4 in the cannabinoid and control groups, respectively ($p > 0.05$). No significant difference was present between the cannabinoid and the control groups in terms of mean CV value. The mean HEX was 52 ± 5% in the cannabinoid group and 53 ± 10% in the control group ($p > 0.05$). There was not a significant difference between the cannabinoid and the control groups in terms of mean HEX value.
Conclusion: A significant decrease in CD was found in cannabinoid users compared the control group.

Keywords

Cannabinoids, cornea, endothelial cells, specular microscopy, toxicity

History

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Introduction

Cannabis (marijuana) is the most commonly used illegal substance both nationally and internationally. About 180.6 million people have been reported to use cannabis in their lifetime worldwide[1]. The active ingredient responsible for the pharmacologic effects of cannabis is the delta-9 isomer of tetrahydrocannabinol (THC) (Δ9-THC)[2]. Cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2) are the main cannabinoid receptors. CB1 receptors are located in the presynaptic region of the central and peripheral neurons, and regulate neurotransmitter secretion. CB1 receptors are also present in the heart, smooth muscle, and endothelial cells and help to regulate blood flow and blood pressure[3]. CB2 receptors are located in immune and hemopoetic cells[4]. In the anterior segment of the eye, CB1 cannabinoid receptors are located in the corneal epithelium, corneal endothelium, trabecular meshwork, Schlemm's canal, ciliary body and sphincter papillae[5]. Cannabinoids have been reported to reduce intraocular pressure (IOP) by increasing aqueous outflow[6]. Both cannabinoids obtained from plants and synthetic cannabinoids have been shown to potentially have a neuroprotective effect in glaucoma treatment[7]. Given the profit/loss ratio in systemic use, medical use remains limited due to various side effects as well as especially addictive effects[8]. According to our knowledge, the effects of cannabis use on corneal endothelium have not been examined before.

In this study, we aimed at evaluating the corneal endothelium of cannabis users by specular microscopy and to determine possible effects.

Materials and methods

This study was conducted at the Ophthalmology Department of the Inonu University Faculty of Medicine. The study was planned in accordance with the Helsinki Declaration and permission of the local Ethics Committee was received (Reference number: 2017-22). The patients provided their written informed consent. One eye of each patient was included in the study. The cannabinoid group consisted of 28 eyes of 28 patients who were diagnosed with cannabinoid use disorder according to the Diagnostic and Statistical Manuel of Mental Disorders V (DSM-V) diagnosis criteria in the psychiatric clinic. The cannabinoid group was selected among patients who had been using the substance for three days or more per week over the past one year. The patients in cannabinoid group have not any systemic disease, and they

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have not used other additional drugs. The control group consisted of 32 eyes of 32 age- and sex-matched individuals. All patients in both groups underwent a standard eye examination by the same ophthalmologist. This examination included spherical equivalent (SE), best-corrected visual acuity (BCVA; by Snellen charts), biomicroscopic cornea and anterior segment evaluations, fundus examination, and an IOP measurement (Goldmann applanation tonometry). Patients with a corneal disorder, glaucoma, uveitis disease, patients who had experienced eye trauma or eye surgery, and patients who were using any systemic or topical drug were excluded from the study. Corneal endothelial measurements were performed using specular microscopy (Konan Medical Inc., Nishinomiya, Japan). The central corneal thickness (CCT) measurements were also taken at the same time. Corneal endothelial cell density (CD), coefficient of variation (CV) and hexagonal cell ratio (HEX) values were used for endothelial cell investigations.

Statistical analysis

The IBM SPSS Statistics software, version 22.0 for Windows (Microsoft Inc., Chicago, IL, USA), was used for statistical analyses. The independent-samples t-test was used for the comparison of the study and control groups. A p level of ?0.05 was accepted as statistically significant.

Results

The median age was 26 (21–35) years in the cannabinoid group and 25 (20–35) years in the control group ($p > 0.05$). The SE was -0.50 ± 0.50 diopters (D) in the cannabinoid group and -0.75 ± 0.50 D in the control group ($p > 0.05$). The BCVA was 20/20 in all subjects. The mean IOP was 15.6 ± 1.4 mmHg in the cannabinoid group and 15.8 ± 1.6 mmHg in the control group ($p > 0.05$). No significant differences were present between the cannabinoid and control groups in terms of BCVA, SE and IOP.

The CD, CV, HEX and CCT data for both groups are shown in Table 1. The mean CD was 2900 ± 211 cells/mm^2 in the cannabinoid group and 3097 ± 214 cells/mm^2 in the control group ($p < 0.01$). There was a significant decrease in cannabinoid group. The mean CV was 29 ± 7 and 27 ± 4 in the cannabinoid and control groups, respectively ($p > 0.05$). No significant difference was present between the cannabinoid and the control groups in terms of mean CV value. The mean HEX was $52 \pm 5\%$ in the cannabinoid group and $53 \pm 10\%$ in the control group ($p > 0.05$). There was not a significant difference between the cannabinoid and the control groups in terms of mean HEX value (Figures 1 and 2). The mean CCT was 548 ± 47 μm and 565 ± 33 μm in the cannabinoid and control groups, respectively ($p > 0.05$). No significant difference was present between the cannabinoid and the control groups in terms of mean CCT value.

Table 1. Specular microscopy results and statistical analysis between groups.

Variables	Cannabinoid group (mean ± SD)	Control group (mean ± SD)	p
CD (cells/mm^2)	2900 ± 211	3097 ± 214	<0.01*
CV	29 ± 7	27 ± 4	>0.05
HEX (%)	52 ± 5	53 ± 10	>0.05
CCT (μm)	548 ± 47	565 ± 33	>0.05

CD: corneal endothelial cell density; CV: coefficient of variation; HEX: hexagonal cell ratio; CCT: central corneal thickness.
*Statistically significant.

Discussion

Corneal endothelial cells maintain corneal hydration by pumping aqueous humor out of the cornea to maintain corneal transparency[9]. Corneal endothelium is one of the anterior segment structures in which CB1 cannabinoid receptors are abundantly located. It is suggested that CB1 receptor activation inhibits corneal endothelial mechanisms for removing aqueous humor from the cornea[5]. It is reported that cannabinoid use may cause corneal opacification, accommodative changes, photophobia, and alterations of vision[10–12].

Aging, intraocular surgery, trauma, glaucoma, smoking and alcohol decrease the number of endothelial cells and cause morphological changes in the endothelial cell layer[13–15]. Endothelial cell analysis is important to evaluate corneal function and viability. Noncontact specular microscopy is a noninvasive method which that provides morphological analysis of the corneal endothelial cells[16].

In our study, a significant decrease in CD was found in cannabinoid users compared the control group. CD shows the endothelial cell count per mm^2. Decrease in CD of cannabinoid users may due to endothelial cell death. Therefore, we linked the loss of endothelial cells to possible cannabinoids toxicity. We could not evaluate cell death *in vivo* because of our clinic conditions. Our findings suggest that the prolonged use of cannabis may cause corneal endothelial toxicity. The mechanism underlying such corneal endothelial toxicity is unknown. There are many studies about the effect of cannabinoids. Some of that suggest cannabinoids have toxic, and another of that report protective effects. Wolff et al.[17,18] reported that cannabinoids cause mitochondrial dysfunction and increase the generation of reactive oxygen species (ROS) and so induce oxidative stress in brain. Hao et al.[19]. suggested that cannabinoids improve mitochondrial function and decrease oxidative/nitrative stress in heart. We think that these different results originate from the receptors of cannabinoids in the body. There are studies that support our view. The activation of CB1 receptors has been shown to have toxic effect by amplifying oxidative stress, mitogen-activated protein kinase (MAPK) activation and cell death, and the subsequent inflammatory response[20]. The inactivation of CB1 receptors has been shown to have protective effect[21]. On the other hand, studies reported that when CB2 receptors activated protective effect became marked by the anti-inflammatory actions[22,23]. In light of these studies we can say that when CB1 receptors abundantly available in any tissue the toxic effects of cannabinoids might occur. While CB1 receptors are located in the anterior segment and corneal endothelium abundantly, CB2 receptors have been found only in the retina and

trabecular meshwork[5,24–27]. Therefore, decrease of CD in our study group may due to CB1 receptors in the corneal endothelium and oxidative stress.

We did not find a significant change in CV and HEX although significant decrease in CD in cannabinoid users. The endothelial cells have not mitotic activity in vivo at a rate sufficient to replace dead or injured cells and that results in a gradual decrease in CD throughout life[28]. When the decrease in CD occurs the endothelium is recovered by cellular migration and enlargement[29]. CV and HEX are used to describe these changes. According to our CV and HEX results we can say that cellular migration and enlargement are prevented due to cannabinoid toxicity to corneal endothelial cells. There are limited reports about cannabinoid toxicity of the cornea. In a study corneal opacification was shown in primates given high dose THC[30]. In another study reported that topically used THC resulted in corneal opacification in some cat corneas[10].

In our study, we did not find a significant difference in CCT values between cannabinoid users and healthy subjects. When CD decrease under 400–500 cells/mm^2 the pumping activity of the endothelium deteriorates. Endothelial dysfunction results in increase of the corneal thickness[31]. We believe that the CCT did not change because CD of cannabis users in our study was not under critical number. Therefore, it did not lead to a clinically significant result like corneal edema. Our study consisted of patients who used cannabinoid for only one year. However, if cannabinoid use continues in these patients, loss of CD at this rate may fall below the critical threshold after the years and may lead to clinically significant consequences. Also, an intraocular surgery that will be performed in later ages may lead to easier endothelial decompensation. Studies with patients who have been using cannabinoid for a longer time can give more information on this issue.

In conclusion we showed that the corneal endothelial cells decreased but cell morphology did not change in long-term cannabinoid users. Studies on a larger number of subjects, together with histochemical examinations of oxidative stress markers and research on the effects at the cellular level, will

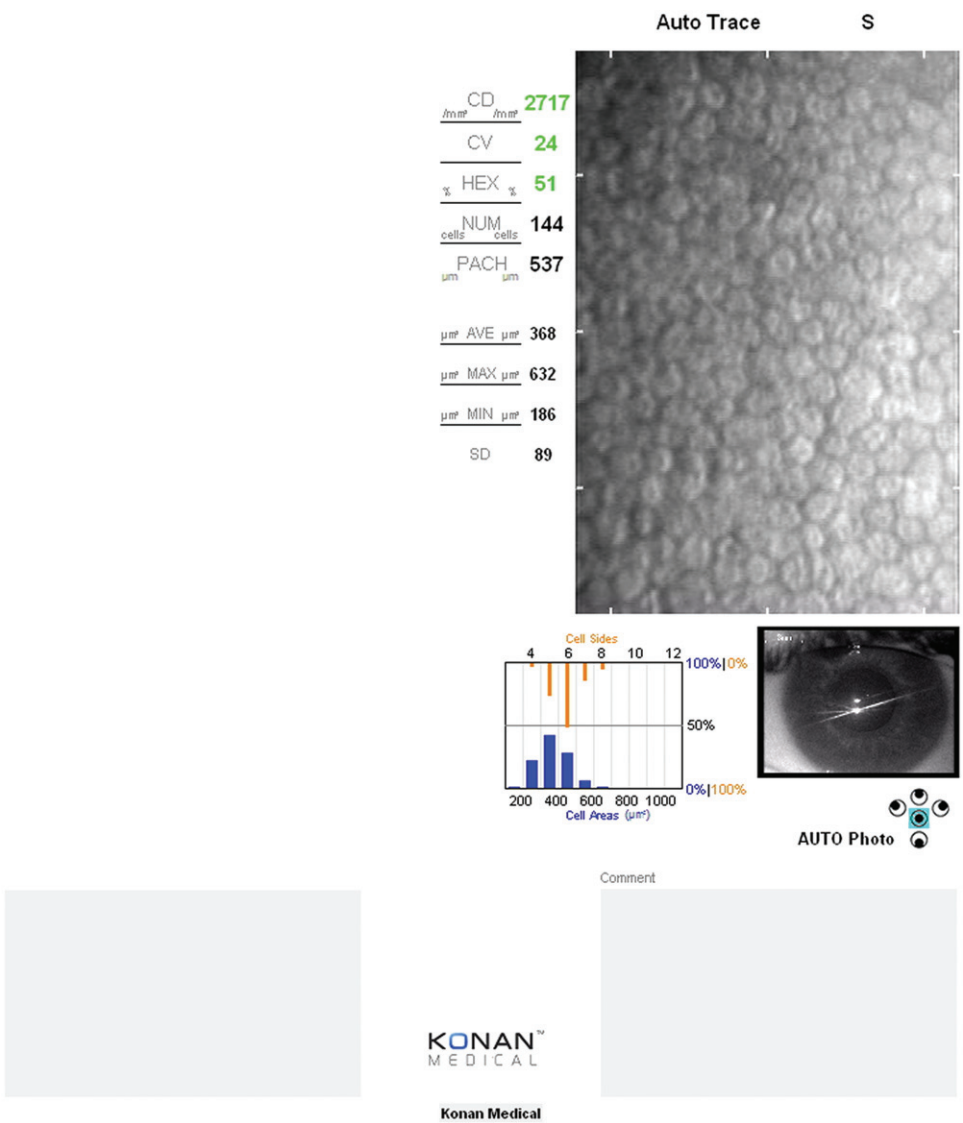

Figure 1. Specular microscopy image shows the endothelial layer of a cannabinoid user.

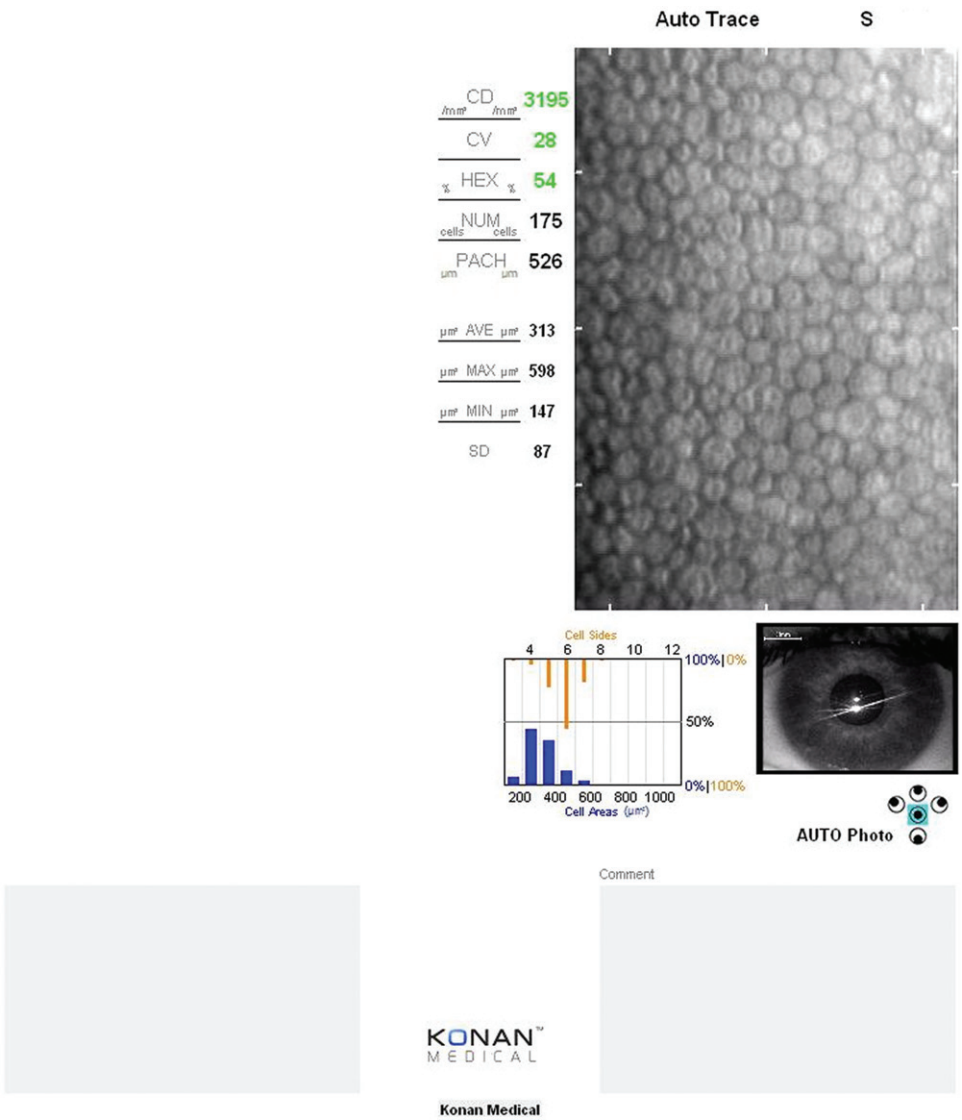

Figure 2. Endothelial layer of a healthy subject.

help to further elucidate the effects of cannabinoids on the corneal endothelium.

Declaration of interest

No potential conflict of interest was reported by the authors.

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