Density of corneal endothelial cells in eyes of dogs using specular microscopy

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Abstract

The aim of this study was to examine the endothelial surface and to perform a morphometric analysis of the corneal endothelial cells in normal eyes of dogs using specular microscopy. Morphometric analysis with regard mean cell area and cell density was performed. Both eyes of ten mixed-breed, males and females, with 6 years of age, weighing about 15 kg euthanatized for reasons unrelated to this study were evaluated. Eyes were examined to determine that they did not have visible ocular disease and transported to the laboratory in moist chamber. Using a contact specular microscope the corneal endothelium was examined. Three images of the central corneal endothelium of each eye were obtained. The mean cell area and the cell density of the corneal endothelial cells were obtained using software for corneal endothelium analysis and density measurement. The mean cell area was 395 ± 36 μm^2 and the endothelial cell density was 2555 ± 240 cells/mm^2. The present work demonstrates that the normal corneal endothelium of dog is similar to those described in human.

Key-words: Corneal Endothelium. Specular Microscopy. Dog.

Introduction

The normal endothelium is a single layer of polygonal cells covering the posterior surface of the cornea[1]. The barrier function and the active fluid pump of the corneal endothelium are important to maintain the normal thickness and transparency of the cornea[2]. It is well established that the corneal endothelial cell count decreases with age [3,4,5,6,7]. Furthemore, cell loss occur in many conditions including corneal dystrophies, keratoconus, glaucoma, and surgical trauma involving opening of the anterior chamber of the eye[8,9,10,11,12]. In the most species when endothelial cell loss occur the remaining cells enlarge and spread out to cover the posterior corneal surface, thus decreasing the cell density[12, 13]. A minimun density of endothlial cells is necessary for the maintenance of normal corneal transparency. In humans, corneal decompensation occurs with endothelial densities from 300 to 500 cells per milimeters square[2,14].

Currently, specular microscopy, vital staining and scanning electron microscopy (SEM) are used and accepted methods to obtain endothelial morphometric data[3,6,7,15,16,17]. The former method has become a standard technique to determine

endothelial cell density and to evaluate the effects of medications, chemicals and surgical procedures on the endothelium[4,6,8,13,16,18,19].

Examination of the corneal endothelium is important due to the growing number of intraocular and corneal procedures and this layer can be compromised by a number of disease of the cornea[18]. Furthermore, has been suggested that the knowledge of the surface structure of the corneal endothelium may assist our understanding of this tissue and its evalutionary development[3]. To quantify endothelial cell size two equivalent parameters have been used. They are the mean cell area, express in units of μm^2 per cell, and the cell density express in units of cells per mm^2. The density of normal corneal endothelium has been documented in cats, monkeys, and other animal species[3,6,10,16,17,-18,20]. However there are few reports of endothelial cell count in dogs[4,7,19]. The purpose of this study was to examine the posterior surface and determine the values of the mean cell area and density of corneal endothelial cells in normal eyes of dogs using specular microscopy.

Materials and Methods

Twenty eyes from ten mixed-breed dogs with 6 years of age, males or females and weighing 15 kg were studied. The dogs were euthanatized for reasons unrelated to this study. All procedures were performed in compliance with the Association for Research in Vision and Ophthalmology statement on the use of animals in ophthalmic and vision research. The anterior segment of each eye was examined using a transilluminator and portable slit lamp and those with evidence of ocular disease were excluded. Eyes were enucleated immediately after dogs were euthanatized and transported to the laboratory in moist chamber containing physiologic saline. Studies of these corneas were initiated within 1 h of enucleation. Eyes were mounted on an eyeball holder and examined using a contact specular microscope (Bio-Optics, model LSM-2100C) with software for corneal endothelium analysis and density measurement. Specular microscopy was performed on all eyes in orders to determine the conditions of the corneal endothelium and those with evidence of corneal alteration were excluded. All analysis was carried out by the same investigator (F.C.A.). Three images of central corneal endothelium of each eye were chosen for analysis. A minimum of 100 well defined endothelial cells obtained from each frame was analyzed. The corneal endothelial cell analysis was done with a variable frame method. The mean cell area and the cell density were obtained from both eyes. Statistical data analysis was conducted using the Tukey test. Values of $p < 0,01$ were considered significant.

Results

The specular microscopy showed that normal dog corneal endothelium was characterized by a continuos monolayer of polygonal cells of uniform size and shape (Figura 1 and 2). The mean cell area was $395 \pm 36\ \mu m^2$ and the endothelial cell density was 2555 ± 240 cells per mm^2. The parameters evaluated did not differ significantly between the right and the left eye from the same dog.

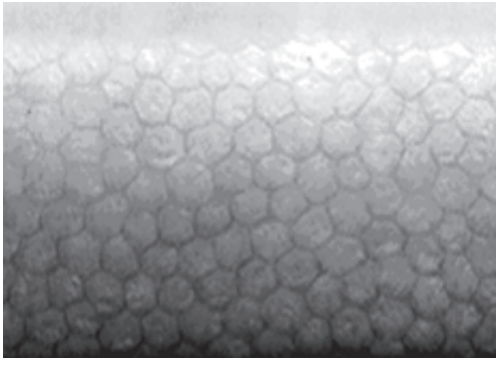

Figure 1 - Contact specular micrograph of the normal corneal endothelium of the right eye of a mixed-breed dog with 6 years of age. The endothelium has a mean cell density of 2535 cells/mm^2

Discussion

The thickness and transparency of the cornea are maintained by the barrier function and active fluid pump of the corneal endothelium[1,2]. In the most vertebrates the normal corneal endothelium is formed by a mosaic like pattern of homogenous polygonal cells arranged regularly on innermost layer of the cornea[1,3,18,20]. Similarly, in the current study, contact specular microscopy shows that normal corneal endothelium of dogs consists of a continuous monolayer of polygonal cells of uniform size and shape.

Specular microscopy has become a standard technique to determine endothelial cell density and cell morphology[18,20,21,22,23]. The specular microscopy may be either a contact microscope, in which th front of the objective lens touches the cornea, or a non contact microscope, in which there is an air space between the front of the objective lens and the cornea[22]. In our study contact specular microscope allows observatrion of the corneal endothelium in whole globe. Thus corneas with endothelial disease were discarted and only healthy eyes were used in this study.

In this study, the endothelial cell count was taken only in the central cornea. Previous studies performed in healthy corneas of humans and dogs reported that the endothelial cell count in the central cornea is representative of all regions of the cornea[4,7,21,22].

The methods used to morphometric analysis of specular images are fixed frame analysis and variable frame analysis[5]. The variable frame method used in our study eliminates the problem of counting fractional cells along the boundary and provided a more accurate determination of evaluated parameters than the fixed frame analysis. The parameters to quantify endothelial cell size are normally the mean cell area and cell density[3,5,7,18,20]. Previous reports using specular microscopy of corneas of adult dogs have demonstrated that cell densities are about 2500 cells per milimeters square[4,19]. These findings were similar to those observed in our analysis. Rodrigues[7] using SEM, reported cell density ranging from 3.666 to 17.122 cells per millimeters square in dogs of different ages. These values are higher than those found in our study. Despite their considerable use, the effects of preparation of corneas for SEM have been described. Schutten and Van Horn[24], studying corneal endothelial cell of rabbit eyes, found an average of tissue shrinkage of 29,7% when they measured the same corneal endothelial cells before fixation and after processing for SEM. Hence, direct comparisons can not be made between values obtained from SEM and specular microscopy.

The decline in the corneal endothelial cell density with age is documentated for some mammalian species including cats, dogs, rabbits, and humans[4,5,7,13,17,19]. In the most of species, the endothelium low regenerative ability is compensated by the growing in size of endothelial cells with a reduction of the endothelial cell density[2,9,13]. Concerning the parameters evaluated no statistically significant difference was observed between the left and the right eyes from the same dog. It is well established that endothelial cell densities of both healthy eyes of the same patient are fairly constant[10,18,19]. Our results showed that the contact specular microscopy can be used to obtain useful endothelial morphometric data in enucleated

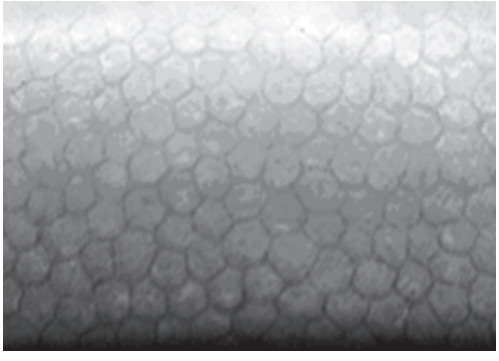

Figure 2 - Contact specular micrograph of the normal corneal endothelium of the left eye of the same dog showed in Fig. 1. The endothelium has a mean cell density of 2498 cells/mm^2

eyes of dogs. Previous reports have demonstrated that use specualr microscope to determine endothelial cell density is a practical way to evaluate corneal endothelium for eyes that have been harvested for tissue banks or corneal transplantation[5,18].

Furthermore, knowledge of the endothelialcell count before surgical procedures involving opening of theanterior chamber is useful for better defining the riskof endothelial decompensation.

The results of this study indicate that the normal corneal endothelium of dog is similar to those described in human.

Densidade das células do endotélio corneano em olhos de cães à microscopia especular

Resumo

Objetivou-se examinar a superfície posterior do endotélio corneano e realizar análise morfométrica das células endoteliais da córnea de olhos normais de cães à microscopia especular. Procedeu-se à morfometria avaliando-se a área celular média e a densidade endotelial. Ambos os olhos de dez cães, sem raça definida, machos ou fêmeas, com 6 anos de idade, com peso médio de 15 kg, eutanasiados por razões não relacionadas a este estudo foram estudados. Os olhos foram avaliados para determinar as condições de higidez e transportados ao laboratório em câmara úmida. Valendo-se de microscópio especular de contato o endotélio corneano foi examinado. Obteve-se 3 imagens da região central de cada córnea. Realizou-se a análise morfométrica valendo-se de software adequado para determinação da área celular média e da densidade endotelial. A área celular média foi 395 $\pm$ 36 μm^2 e a densidade endotelial 2555 $\pm$ 240 células/mm^2. O presente estudo demonstrou que o endotélio de bulbos oculares normais de cães é similar ao endotélio de indivíduos da espécie humana.

Palavras-chave:
Endotélio.
Corneano.
Microscopia.
Especular.
Cão.

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