Evaluation of peculiarities of the acetylcholinesterase-positive nerve plexus and its length in the cornea

Vidmantas Lasys, Edvinas Stanevičius, Gintaras Zamokas

Department of Anatomy and Histology,
Department of Internal Diseases, Lithuanian Veterinary Academy, Lithuania

Key words: acetylcholinesterase-positive nerve plexus, dog’s cornea.

Summary. The objective of this study was to evaluate age-related peculiarities of acetylcholinesterase-positive nerve plexus and its length in the dog cornea.

Material and methods. Nine mixed breed dogs (male and female) of 1.8–20 kg weight were used. They were divided into three age groups: young (until 1 year), adult (1–7 years) and old (8 and more years). Acetylcholinesterase-positive nerve plexus was identified using acetylcholinesterase method of M. J. Karnowsky and L. A. Roots (1964) modified by D. H. Pauza et al. (1996). The length of nerve bundles was measured by mm in 1 mm² of cornea.

Results and conclusions. Branching thick, medium and thin nerve bundles form acetylcholinesterase-positive nerve plexus in dog cornea. The average of the length of nerve bundles between the left and the right corneas was similar in different age groups (p>0.05). The length of acetylcholinesterase-positive nerve bundles in 1 mm² of the cornea in the group of adult dogs (10.32±0.11 mm) was higher than in the groups of young (9.42±0.02 mm) and old dogs (7.75±0.14 mm) (p<0.001).

Introduction

The cornea is one of eyeball’s structures, which carries out important optical functions. The cornea has peculiar anatomical structure and innervation. The mammalian cornea is one of the most innervated parts of the body surface (1–3). The greatest number of sensory nerve fibers originating from trigeminal ganglion was observed in the corneal nerve plexus (4, 5).

The enzyme acetylcholinesterase (AChE) performs an important function in the metabolism of neurotransmitter acetylcholine (ACh). Because AChE hydrolyses ACh rapidly, there are no direct histochemical methods for determining the localization of this neurotransmitter. The localization of AChE in nerve structures is determined by identification of acetylcholinesterase or cholinacetyltransferase, which assists in synthesis of ACh (6).

Using the AChE-histochemical reaction for identification of nerve plexus, the fine granules of copper thiocohline locate themselves along nerve bundles and provide optimal uniformity to the smaller nerves and terminals (7). N. Ishida et al. (8) maintains that the product of this reaction is stable and such preparations are suitable for the quantitative evaluation of nerve plexus. This reaction is not specific because acetylcholinesterase and butyrylcholinesterase are distributed in the other tissues (9). Therefore nerves determined by AChE-histochemical reaction are named as AChE-positive nerves.

This reaction is better to perform in whole-mount preparations than in sections because it is possible to analyze nerve plexus in three dimensions (10).

By this method (8, 11, 12) it was determined that trunks of AChE-positive nerve plexus were located evenly in rat cornea (2–4 trunks in each quadrant). Nerves that run from limbus to central part branched and conjoined. They were varicosed in some places. T. Tervo and A. Palkama (13) noticed AChE-positive nerves in the stroma and epithelium of rabbit’s cornea. According to these researches the thickest nerve bundles are located in limbal part.

J. L. Jacot et al. (7) measured the length of AChE-positive nerve bundles in the rat cornea. But they did not investigate age-related changes of the length in nerve plexus.

Information about researches of length of dog corneal nerve plexus elements was not found in the literature.

The aim of this study was to evaluate age-related peculiarities of the acetylcholinesterase-positive nerve plexus and its length in the dog cornea.
Materials and methods

Nine mixed breed dogs (male and female) of 1.8–20 kg weight were used. They were divided into three age groups: young (until 1 year), adult (1–7 years) and old (8 and more years).

All dogs were pre-treated with chlorpromazine (50 mg/kg i. m.) and deeply anaesthetized with an overdose of sodium pentobarbital (100 mg/kg i. v.).

Scientific research was carried out according to “Law of Lithuanian Republic on animal care, keeping and using” No. 8-500 and according to law statements such as Lithuanian Republic orders of veterinary service concerning veterinary requirement of breeding, care and transportation of laboratory animals (December 31, 1998, No. 4-361) and concerning usage of laboratory animals for scientific experiments (January 18, 1999, No. 4-16).

Removed eyes were washed in 0.1 M phosphate buffer (pH 7.4). Later eyes were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer with 18% sucrose for 30 min. Corneas were isolated by circumferential cut leaving 2–3 mm scleral ring surrounding the cornea.

Every cornea was divided into upper, nasal, lower and temporal quadrants (Fig. 1). Then the corneas were rinsed with 0.1 M phosphate buffer for 18 hours at 4°C. AChE-positive nerve plexus was identified using acetylcholinesterase method of M. J. Karnowsky and L. A. Roots (1964) (9) modified by D. H. Pauza et al. (1996) (10). Histochemical demonstration of AChE-positive reactivity was performed on whole-mounts preparations. Chemical composition of the incubation medium (pH 5.6) was (in mM): sodium acetate buffer, 60; acetylthiocholine iodide, 2; sodium citrate, 15; CuSO$_4$, 3; K$_3$Fe(CN)$_6$, 0.5; Triton-X 100, up to 1%. From time to time during incubation the staining of nerve structures was controlled by a light microscope.

Later corneas were dehydrated in graded alcohol steps and then placed in xylene for 10 min. Corneas were mounted epithelium upwards and cover slipped on glass slides using balsam.

Each corneal quadrant was divided into central, pericentral and limbal parts for computerized morphometric evaluation of length of nerve bundles (Fig. 2). Length of nerve bundles was measured by mm in 1 mm$^2$ of cornea. Camera (SPOT RT Slider Digital by Diagnostic Instruments Inc., St., Sterling Heights, Michigan, USA), program Image Pro Plus (version 4.1 by Media Cybernetics, Silver Spring, Maryland, USA), microscope (Leica DMRXA, by Leica Microsystems AG, Ernst-Leitz-Strasse 17-37, 35578 Wetzlar, Germany), were used for computerized quantitative estimation of the length of nerve bundles.

Results of length of AChE-positive nerve plexus elements were processed using Student- Gaset’s criteria (14, 15).

Results

Dog’s cornea is innervated by 12–15 thick of AChE-positive nerve bundles, which are distributed evenly round limbus. Some thick nerve bundles, which come to peripheral part of cornea branch into 2 nerve bundles of equal thickness and running to the central part they split into various nerve bundles. Other thick nerve bundles run almost straight to the center and split into medium and thin nerve bundles. Thick nerve bundles run along intermediate stromal layer from limbus to the center.

Having branched from of AChE-positive thick, medium and thin nerve bundles are distributed into two hardly separated interlocated layers. These nerve bundles split and repeatedly join. Medium and thin nerve bundles
bundles are dominating in superficial stromal layer of cornea both in central and pericentral parts of cornea. Part of medium nerve bundles split from thick nerve bundles and branch coming back to the limbal part of cornea. Medium and thin nerve bundles run from limbus to peripheral part but they don’t reach the central part of cornea. These nerve bundles are distributed among thick nerve bundles. Thin and medium nerve bundles run along superficial stromal layer and innervated peripheral part of cornea.

Thin nerve bundles run to epithelium and are abundantly anastomosed with one another. Some thin nerve bundles end in conic enlargements. Average of the length of nerve bundles between left and right corneas was similar in different age groups (p>0.05). Average of the length of nerve bundles of young dogs was in right (9.4±0.19 mm) and left (9.42±0.03 mm) corneas; of adult dog respectively (10.39±0.19 mm) and (10.25±0.17 mm) (p>0.05); old (7.53±0.24 mm) and (7.96±0.06 mm) (p>0.05). We added average of the length of nerve bundles in right and left corneas. The length of acetylcholinesterase-positive nerve bundles in the 1 mm² of the cornea in the group of adult dogs (10.32±0.11 mm) was higher than in the groups of young (9.42±0.02 mm) and old dogs (7.75±0.14 mm) (p<0.001).

Discussion

Structures of AChE-positive nerve plexus were described in rat cornea by N. Ishida et al. (8) and T. Tervo (11, 12), in rabbit cornea by T. Tervo and A. Palkama (13), in guinea pig cornea by F. A. Moustafa et al. (16).

We have not found information about dog corneal AChE-positive nerve plexus elements in literature. We determined that 12–15 thick AChE-positive nerve bundles, which distributed evenly around limbus, formed nerve plexus of dog cornea. Some thick nerve bundles, which came to peripheral part of cornea, branched into 2 nerve bundles of equal thickness and run to the central part and split into nerve bundles of various thicknesses. Other thick nerve bundles run almost straight to the center of cornea and split into medium and thin nerve bundles.

These data coincide with N. Ishida’s et al. (8), T. Tervo’s (11, 12), T. Tervo’s and A. Palkama’s (13) results. N. Ishida et al. (8), T. Tervo (11, 12) wrote that AChE-positive nerve trunks were distributed evenly in all quadrants of rat cornea (2–4 nerves in each). Nerves run from limbus to central part; they branch and rejoin. T. Tervo and A. Palkama (13) noticed that the thickest AChE-positive nerve bundles were in the limbal part of rabbit cornea.

In this study we noticed that having branched from of AChE-positive thick, medium and thin nerve bundles were distributed into two hardly separated interlocated layers. These nerve bundles split and joined repeatedly. Medium and thin nerve bundles dominated in superficial stromal layer of cornea in central and pericentral parts. Part of medium nerve bundles split from thick
nerve bundles branching well came back to limbal part of cornea. Medium and thin nerve bundles run from limbus to peripheral part but they don’t reach central part of cornea. These nerve bundles distributed among thick nerve bundles. Thin and medium nerve bundles run along superficial stromal layer and innervated peripheral part of cornea.

Our results were coincident with N. Ishida’s et al. (8) data. They noticed that thin nerve bundles came out of plexus and formed short terminal branches, which went up vertically and diagonally to the epithelial layer in the rat cornea. Nerves run from limbus to central part, they split and repeatedly join one with another. T. Tervo with co-workers (17) investigated that AChE-positive nerve bundles of human cornea split into thinner branches and formed basal epithelial nerve plexus. Branches of this plexus formed terminal branches among epithelial cells.

T. Tervo and A. Palkama (13) determined that AChE-positive nerves of rabbit cornea are located in stroma but not in the epithelial layer. It differs from Ishida’s et al. (8), T. Tervo’s et al. (17) and our results, which showed that thin AChE-positive nerve bundles innervate human, dog and rat corneal epithelial layer.

J. L. Jacot et al. (7) measured average of the length of nerve bundles (5.52±1.31 mm/mm²) in the rat cornea. But these authors did not compare the average of the length of nerve plexus between left and right corneas in age groups. Results of our study showed that the average of the length of nerve bundles between left and right corneas was similar in different age groups (p>0.05). When dogs grow up the length of AChE-positive nerve bundles increases, when dogs get older it decreases (p<0.001).

We had no possibility to compare our results with the results of other investigations because we have not found any data in literature.

Conclusions

1. Branching thick, medium and thin nerve bundles form acetylcholinesterase-positive nerve plexus in dog cornea.

2. Average length of nerve bundles between left and right corneas was similar in different age groups (p>0.05).

3. The length of acetylcholinesterase-positive nerve bundles in the 1 mm² of the cornea in the group of adult dogs (10.32±0.11 mm) was higher than in the groups of young (9.42±0.02 mm) and old dogs (7.75±0.14 mm) (p<0.001).
Ragenos acetylcholinesterazės pozityvaus nervinio rezginio ypatybės ir jo ilgio įvertinimas

Vidmantas Lasys, Edvinas Stanevičius, Gintaras Zamokas

Lietuvos veterinarijos akademijos Anatomijos ir histologijos katedra, 1Vidaus ligų katedra

Raktažodžiai: acetylcholinesterazė pozityvus nervinis rezginys, šuns ragenas.

Santrauka. Darbo tikslas. Įvertinti įvairaus amžiaus šunų ragenos acetylcholinesterazės pozityvaus nervinio rezginio ypatybės ir jo nervinių pluostų ilgi.


Rezultatai ir išvados. Šuns ragenos acetylcholinesterazė pozityvų nervinių rezginį formuoja besiūkajantys stūri, vidutinio storio ir ploni nerviniai pluoštai. Skirtingų amžiaus grupių šunų nervinių pluostų ilgio vidurkis tarp kairės ir dešinės ragenų buvo panašus (p>0,05). Suaugusių šunų ragenos 1 mm² acetylcholinesterazė pozityvių nervinių pluostų ilgis (10,32±0,11 mm) buvo didesnis negu jaunų (9,42±0,02 mm) ir senų šunų (7,75±0,14 mm) (p<0,001).

Adresas susirašinėjimui: V. Lasys, LVA Anatomių ir histologijos katedra, Tilžės 18, 3022 Kaunas El. paštas: vidmantas.lasys@lva.lt

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