Appliance of digital ultrasonic technique in canine cornea investigations

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Introduction

Ultrasound examination is widely used in ophthalmology. As an imaging modality, ultrasound has many benefits. Diagnostic ultrasound is a no ionizing form of energy that has no known health risk [Green, 1996]. Ocular ultrasonography is valuable diagnostic tool because it allows evaluation of the interior of the eye, which may be obscured from direct visualization by any disease that causes ocular opacity [Nyland, Mattoon, 1995].

The principal methods used in ultrasonic diagnostic are based on a reflection principle, i.e. acoustic energy sent into the body and the reflected energy received and evaluated at the point of the transmission [Krestel, 1990]. Piezoelectric crystals generate ultrasound waves of 5-50 MHz. Short pulses of 2 or 3 cycles are sent from transducer into the eye. These pulses propagate through the tissues of an eye with the speed that proportional to the elasticity and inversely proportional to density of the eye.
Acoustic parameters of biologic tissues are described by velocity and attenuation coefficient. The attenuation coefficient is approximately proportional to a frequency – high frequency components of echoes are attenuated more than the lower frequency components [Sugata, Murakaami et al., 1992]. Ultrasound pulses are attenuated as the result of absorption and scattering as it propagates through the tissue [Tabandeh, Wilkins et al., 2000].

The cornea is the most powerful refracting surface of the eye and the most sensitive part of eye protecting mechanisms. Unlike other connective tissues the cornea is transparent and anything that alters the underlying structure of cornea, such as swelling, will affect the mechanical and optical properties and the function of the eye. When the corneal stroma swells it loses its transparency, because of the increased light scattering, which can be caused by a non-uniform distribution of the water and disruption of the collagen packing [Huang, Meek, 1999].

Aim of the study was to adapt a digital ultrasonic technique for corneal thickness investigation in dogs and evaluate corneal thickness changes after eyeball enucleation.

Materials and methods

Eight mixed breed dogs were used in our study. The principles of laboratory animal care (NIH publication No. 86-23, revised 1985) and the specific national laws on protection of animals were followed. We examined three 10-15-days-old puppies, two 20-days-old puppies and three adult dogs. The cornea of puppies and two adult dogs were measured in the central point in vivo adapting the digital ultrasonic technique. The cornea of one adult dog was measured in five points (central, peripheral superior, peripheral inferior, peripheral nasal and peripheral temporal) in vivo, just after death, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 24 hours after eyeball enucleation. Peripheral points were measured 1 mm from limbus. The eyeball was kept in wet camera; cornea was irrigated by physiological solution to protect from drying.

Thickness of the cornea was measured using ultrasonic pachymeter, signals of the cornea fixed byte digital oscillograph Tektronix TDS 220 and stored in a computer as nbk-type files of the "Wave star" program. The analysis of these signals can be done using "Matlab" programming package (Fig. 1).

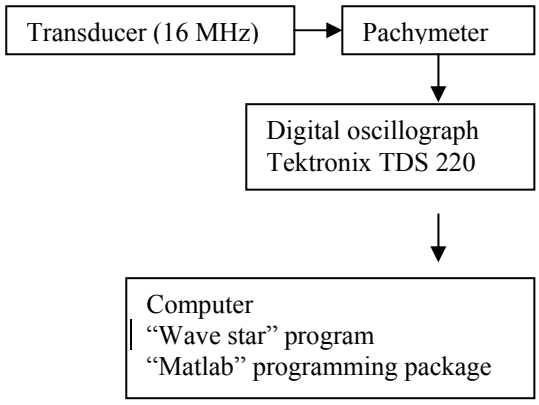
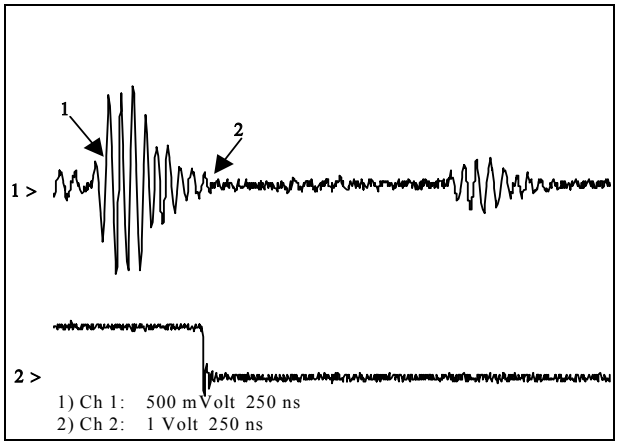


Fig. 1. **The scheme of ophthlamological digital ultrasonic technique**

Results

Measuring of canine cornea using the digital ultrasonic equipment showed the central cornea thickness in 10-15-days-old puppies was 636.33±18.15 μm in the right eye and 686±49.12 μm in the left eye (average± standard deviation). In 20-days-old puppies the central corneal thickness was 524± 31 μm in the right eye and 528± 10 μm in the left eye; in adult dogs the central cornea thickness was 602.67± 85.68 μm in the right eye and 589.33± 80.13 μm in the left eye. The signals of the central point of the dogs' cornea showed in Fig. 2. There were no clear signal from the posterior surface of the cornea (corneal endothelium) in 10-15-days-old puppies as well as in 20-days-old puppies and adult dogs this signal appeared very clear.

The cornea measurements, made at the five points of the cornea in vivo and after eye ball enucleation, showed that in the central point of the cornea thickness changed from 699 to 704 μm in the right eye and from 680 to 843 μm in the left eye; in the peripheral inferior point of the cornea thickness changed respectively from 706 to



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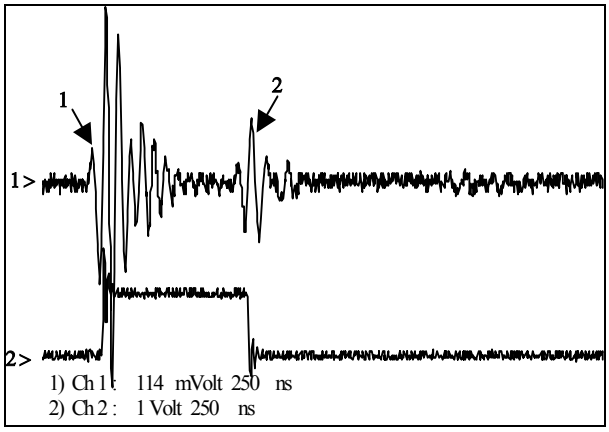

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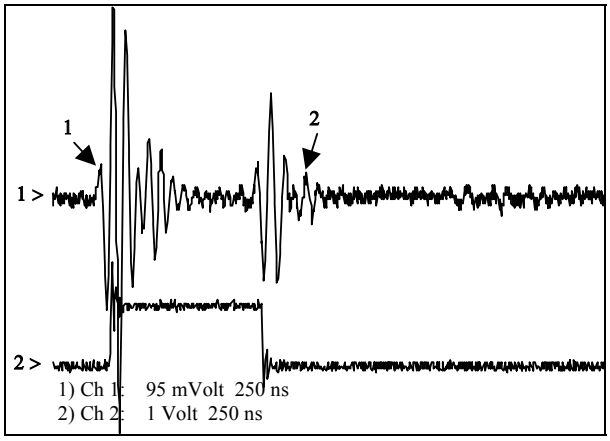

c

Fig. 2. **Corneal signal, fixed by the oscilograph. a – 10-15-days-old puppies, b – 20-days-old puppies, c – adult dogs cornea. 1 – signal from anterior surface of cornea (epithelium), 2 – signal from posterior surface of cornea (endothelium)**

727 μm and 714 to 740 μm; in the peripheral nasal point of the cornea the thickness changed from 703 to 734 μm in the right eye and from 704 to 760 μm in the left eye; respectively in the peripheral superior point of the cornea thickness changed from 702 to 740 μm and from 681 to 789 μm; in the peripheral temporal point of the cornea thickness changed from 693 to 722 μm in the right eye and from 698 to 788 μm in the left eye (Table 1). The graphic oscilograms of the cornea showed that 1-4 hour corneal signals amplitude changed marginally (Fig.3). Later the amplitude decreased. 5 hours after eyeball enucleation the secondary reflection appeared in the oscilograms. The low-key secondary reflection is shown in the oscilogram of the peripheral temporal point (Fig. 3).

Discussions

The cornea and sclera together form the outermost covering of the eye and withstand both the internal and external force of the eye to maintain the shape of the eyeball and to protect the contents from mechanical injury anything that alters the underlying structure of the cornea, such as swelling, will affect the mechanical and optical properties and affect the function of the eye [Huang, Meek, 1999]. Ultrasonography is a relative simple and quick examination to perform and creates little stress for a patient. It allows evaluating the internal structures of the eye when cornea or anterior chamber is opaque and direct visualization with ophthalmoscope is not possible [Schiffer, Rantanen et al., 1982]. The aim of this study was to adapt the digital ultrasonic technique for investigation of corneal thickness in the dog and to evaluate corneal thickness changes after eyeball enucleation.

Corneal thickness measurements, using the digital ultrasonic technique, showed that the thickest cornea was in 10-15-day-old puppies, but there were no clear signal from the posterior surface of the cornea [corneal endothelium] in 10-15-days-old puppies as well as in 20-days-old puppies. In adult dogs this signal appeared very clear. The lens of young dogs is very close to the posterior surface of the cornea and this may be reason of such an appearance.

Cornea is composed of five layers such as anterior epithelium, sub-epithelial basement membrane, substantia propria (stroma), Descemet's membrane and posterior epithelium (corneal endothelium) [Dellmann, 1998]. According to current models, corneal transparency at a given wavelength depends on certain structural parameters of a corneal stroma such as fibrils diameters, the density of fibril packing, the position of each fibril relative to its neighbors, and the refractive indices of the collagen and the interfibrillar matrix, and changes in one or more of these factors may be sufficient to increase light scattering [Huang, Meek, 1999; Clark, 2001].

Preservation of cornea tissue for transplantation has been well documented in human medicine. Most experimental studies have used rabbits, cats, and other animals. The increase on corneal thickness plays a significant role in cornea transplantation [Lindstrom, Kaufman et al., 1992; Van Horn, Sendele et al., 1977]. The primary function of corneal endothelium is to nourish and hydrate the cornea. It was reported that impaired endothelial function was responsible for diffusion of water into corneal stroma [Warning, Bourne, 1982]. Due to the linear relationship between hydration and corneal thickness, the pachymetry provides information about the hydration status of the cornea [Mishima, 1982]]. Our study

Table 1. **Corneal thickness of adult dog in vivo and after eyeball enucleation, µm**

Time of measuring	Right eye					Left eye				
	c	pi	pn	ps	pt	c	pi	pn	ps	pt
In vivo	699	706	703	702	693	680	714	704	681	698
Just a.e.	628	629	632	664	652	576	628	621	623	605
1 h a.e.	605	632	607	634	629	569	576	581	582	594
2 h a.e.	629	641	653	644	664	651	526	616	644	606
3 h a.e.	596	618	618	624	637	624	639	619	630	634
4 h a.e.	608	654	626	615	661	624	646	625	618	660
5 h a.e.	623	663	635	647	661	611	651	605	628	650
6 h a.e.	638	650	655	644	651	614	655	609	636	655
7 h a.e.	635	670	680	646	634	634	669	611	619	649
8 h a.e.	636	653	631	678	667	632	654	609	641	654
9 h a.e.	630	600	603	641	613	696	641	633	694	630
10 h a.e.	704	727	734	740	722	608	740	666	645	656
24 h a.e.	628	652	658	696	680	843	722	760	789	788

e. – after enucleation; c – central; pi – peripheral inferior, pn – peripheral nasal; ps – peripheral superior, pt – peripheral temporal points of the cornea

of the cornea thickness changes after eyeball enucleation showed the increasing of corneal thickness in all points of the cornea in both eyes. The graphic oscilograms of the cornea showed that 1-4 hour corneal signals amplitude changed marginally. Later the amplitude decreased. 5 hours after eyeball enucleation the secondary reflection appeared in the oscilograms. The low-key secondary reflection showed in the oscilogram of the peripheral temporal point. The increase in the corneal thickness is attributable to uptake of fluid, particularly on the stroma [Arndt, Reese, Kostlin, 2001]. Secondary reflection appeared in corneal oscilograms may show the changes of corneal stroma ultra structure. When cornea swells increased the diameters of collagen fibrils, changed the packing of the fibrils. But the large portion of water goes not into interfibrilar space, but into another space, such as lakes [region devoid of collagen fibrils]. The lakes would act as light –scattering centers and these may cause scattering of sufficient magnitude to account for the cloudiness of the stroma [Huang, Meek, 1999; Meek, 2001]. So, we suggest that the corneal tissue can be taken for preservation till the 5th hour after the dog's death. The ultrasonic investigation can be used as very informative and inexpensive method to evaluate cornea suitability for transplantation.

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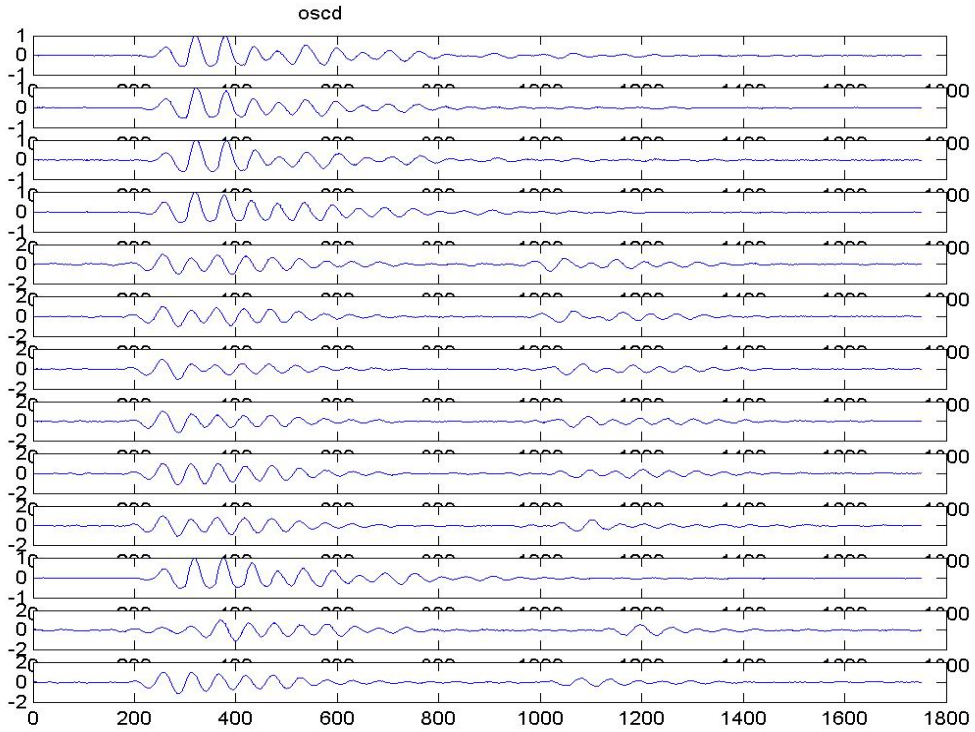

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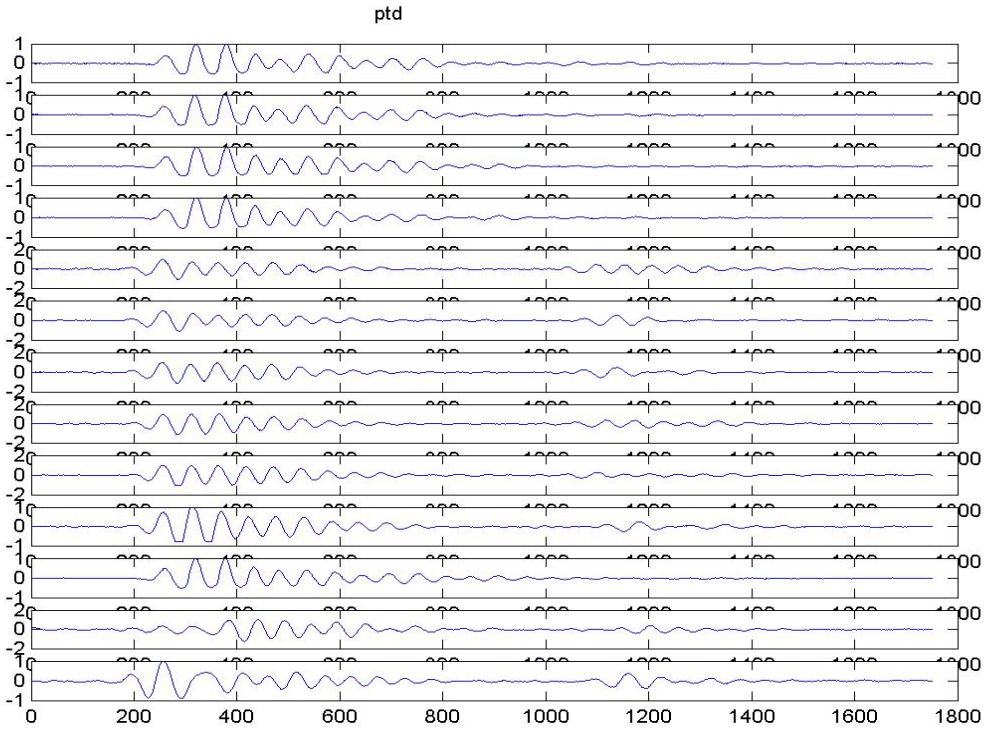

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Fig.3. **Corneal oscilograms of the right eye. A – central point of the cornea; B – peripheral temporal point of the cornea**

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Skaitmeninių ultragarsinių metodų taikymas šunų akių ragenai tirti

Reziumė

Tyrimo tikslas buvo pritaikyti skaitmeninę ultragarsinę aparatūrą šunų akių ragenai tirti ir ragenos storio pokyčiams po akies obuolio enukleacijos įvertinti. Buvo tiriami 8 neveisliniai šunys: 10-15 dienų amžiaus, 20 dienų amžiaus šuniukai ir suaugę šunys. Naudojant ultragarsinę skaitmeninę aparatūrą penkių jaunų šuniukų ir dviejų suaugusių šunų ragenos storis buvo išmatuotas viename – centriniame – ragenos taške in vivo, vėliau vieno suaugusio šuns ragenos storis buvo išmatuotas penkiuose taškuose in vivo, iš karto ir praėjus 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ir 24 val. po akies obuolio enukleacijos.

Skaitmenine ultragarsine aparatūra įvertinus šunų ragenas, nustatyta, kad ragenos signalų amplitudė 1-4 valandą po enukleacijos mažai kinta. 5-ą valandą po enukleacijos atsirandantis antrinis oscilogramos atspindys rodo ragenoje vykstančius biocheminius pokyčius. Šis informatyvus ir finansiškai optimalus metodas gali būti naudojamas ragenos būklei vertinti konservuojant ir persodinant.

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