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HISTOCHEMICAL AND MORPHOMETRIC STUDIES OF CONNECTIVE TISSUE FIBRES IN CANINE PARANAL SINUS

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Summary

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The aim of the present investigation was to determine the localization, histochemical reactivity and the dimensions of connective tissue fibres in the wall of canine paranal sinus (PS) as well as to determine the dimensions of elastic and collagen fibres. The stroma was composed mainly by collagen fibres (CF). The thicker CF were situated in the subglandular connective tissue between the apocrine glands and sinus musculature (SGS) whereas those located in the connective tissue between the sinus epithelium and apocrine glands (SES) were statistically significantly thinner ($P<0.01$). CF with a various thickness were observed, that decreased in the direction of the epimysium of the external anal sphincter (ES) to the endomysium. The reticular fibres (RF) were assembled immediately under the multilayer squamous sinus epithelium. They were located both around the apocrine tubules and among the tubules. RF embedded sebaceous glands, skeletal muscle cells and smooth muscle cells. Elastic fibres (EF) located in SGC and ICT were thicker and longer than those in SEC. The EF in SES were thinner and shorter compared to those in SGS. Histochemically, a various degree of reactivity of CF and EF in the wall of the paranal sinus, was observed.

Key words: connective tissue fibres, dog, perianal sinus

INTRODUCTION

As known, collagen is a fibrillar glycoprotein and is the primary matrix protein comprising 25 % of body proteins in multicell animals. The most abundant collagen type is type I, that accounts for about 90 % of the total collagen content. It prevails in the skin, bones, fascia and the sclera. Collagen type II is composing cartilage, type III – the wall of blood vessels, the skin, the spleen and lymph nodes, type IV – the basal lamina. Collagens type I, II and III form fibres while that of type IV – a net. The amount and the orientation of fibres are variable and depend on the localization and their specific function in a given organ (Bacha & Wood, 1990; Dellmann & Eurell, 1998).

Reticular fibres are individual collagen fibrils (collagen type III), covered with proteoglycans and glycoproteins. These fibres could be identified by impregnation with silver and that is why they are also called argirophilic. RF that form a three-dimensional network around the capillaries, the muscle fibres, nerves, adipose cells, hepatocytes and that served as scaffold for supporting cells in the endocrine, lymphatic and haematopoietic organs, are observed. They are an integral part of the sub-basal lamina that connects lamina

densa to the subepithelial connective tissue (Bacha & Wood, 1990; Dellmann & Eurell, 1998).

It is well known that elastic fibres are an important structural component of the wall of large blood vessels (particularly arteries), the dermis and the subcutaneous connective tissue, the urinary bladder, the prostate gland, the lungs and tubular respiratory organs, the nuchal ligament, the epiglottis, the vocal folds, the heart (Woodburne, 1961; Ross & Bornestein, 1969; Cliff, 1971; Ross, 1973; Bock, 1977; Klein & Bock, 1983; Bock & Stockinger, 1984; Dellmann & Eurell, 1998, Zhang *et al.*, 2003). They are composed of amorphic substance and filaments. The amorphic substance is formed by the protein elastin and is situated in the centre of the elastin fibre. The filaments (microfibrils) made of fibrillin, are located into and around the amorphic elastin matter. They serve as matrix and spatial landmark in the morphogenesis of EF (Fahrenbach *et al.*, 1966; Bock & Stockinger, 1984; Bressan *et al.,* 1993). EF are encountered as single, branching and anastomizing fibres or form elastic lamellae (similarly to blood vessels' wall) (Ross & Bornestein, 1969; Dellmann & Eurell, 1998). Collagen fibrils counteract mechanical stress due to their strength, resistance, while elastic fibrils – by means of their flexibility and elasticity (Dellmann & Eurell, 1998).

The data from investigations on the structural features of connective tissue fibres in the paranal sinus wall in the dog are few. Salazar *et al.* (1996) have observed the localization of EF in the subepithelial connective tissue, in the sinus wall without studying the excretory duct. Other authors have studied the localization of the three types of connective tissue wall in feline paranal sinus, but do not provide data about their dimensions (Greer & Colhoun, 1966). They neither offer any explanation for the existing relationship between the structural elements of the stroma and the PS function.

Because of the lack of available research on connective tissue fibres in canine paranal sinuses, the present study was aimed at performing a histochemical investigation of collagen, reticular and elastic fibres as well as of some morphometric parameters with regard to throwing light on the function of paranal sinus and the pathogenesis of diseases affecting this organ.

MATERIALS AND METHODS

The paranal sinuses of 4 male and 4 female mixed-breed dogs at the age of 2 months to 15 years, were investigated. The specimens were obtained from the PS wall, fixed in Bouin's liquid and 10% formalin for 48 hours, dehydrated in ascending ethanol series, cleared with xylene and embedded in paraffin. Cross sections of 5 μm were stained by means of different histochemical techniques for visualization of the three types of connective tissue fibres. The Elastica Van Gieson Staining Kit (Merck, Germany) was used to stain and differentiate collagen fibres (in red), elastic fibres (in black), muscle tissue (in yellow), and nuclei (in black-brown). The presence of elastic fibres (in red-brown) was also detected by means of Taenzer-Unna's orcein staining (Sigma, Germany). The Azan-Trichrome 001802/L kit (Bio Optica, Milano, Italy) was utilized for histochemical detection of collagen (in blue), muscle tissue (in red). Reticular fibres (in black) were detected by methenamine sliver staining (Methenamine silver plating kit acc. to Gomori, Merck KGaA, Darmstadt, Germany). The

length and thickness of fibres were determined on a microscope OLYMPUS BX 40 using the analysis programme of Soft Imaging System GmbH. The statistical analysis of data was performed with the non-parametric Mann-Whitney test (StatMost for Windows).

RESULTS

The stroma was mainly composed of CF (Fig. 1), intersecting under various angles in the stroma, some of them forming bundles of fibres. Table 1 shows that thicker fibres were located near the musculature of the outlet duct (SGC). A network of CF was observed in the epimysium with fibre thickness of 10.01±0.47 μm, thinner in the perimysium and the thinnest in the endomysium. Among the individual smooth-muscle cells, a network of fine CF was situated. The histochemical study showed a moderate and well expressed reactivity of CF in the subepithelial connective tissue of the outlet duct (SEC), the subglandular connective tissue in the periphery of the outlet duct (SGC), the subepithelial connective tissue between the sinus epithelium and the apocrine glands (SES) and the subglandular connective tissue between apocrine glands and musculature (SGS) where these fibres were oriented in various directions forming larger bundles. In the interstitial tissue between apocrine

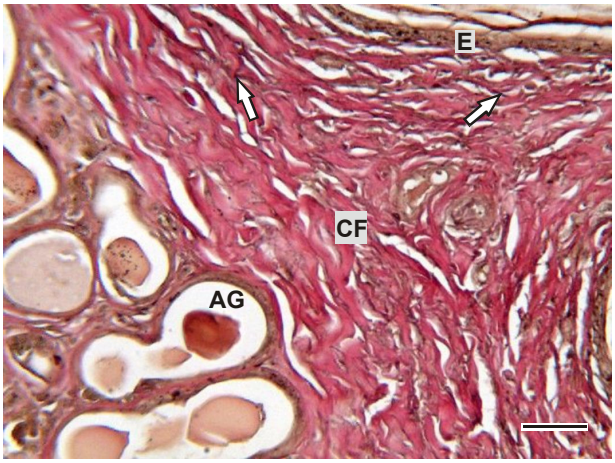

Fig. 1. Collagen fibres (CF) with a various thickness between the lining epithelium and apocrine glands (AG), as well as around glandular tubules with fine elastic fibrils among (arrows): E – sinus epithelium; Elastica Van Gieson staining kit. Bar = 70 μm.

Table 1. Thickness (μm) of collagen fibres in the paranal sinus in the dog

	SEC	SGC	SES	SGS
Mean±SEM	6.75±0.60	9.53±0.32 **	7.07±0.44	10.06±0.37 **
Range	4.29–11.55	7.90–11.04	5.38–10.56	6.93–11.88

SEC – subepithelial connective tissue of the outlet duct; SGC – subglandular connective tissue in the periphery of the outlet duct; SES – subepithelial connective tissue between the sinus epithelium and the apocrine glands; SGS – subglandular connective tissue between apocrine glands and musculature. ** P< 0.01 of SEC vs SGC, and SES vs SGS.

Table 2. Histochemical reactivity of collagen and elastic fibres in the paranal sinus in the dog

Parameter	SEC	SGC	SES	SGS	ICT
Degree of histochemical reactivity of collagen fibres	++/+++	++/+++	++/+++	++/+++	+/++
Degree of histochemical reactivity of elastic fibres	+/++	+++	+/++	+++	+/++

SEC – subepithelial connective tissue of the outlet duct; SGC – subglandular connective tissue in the periphery of the outlet duct; SES – subepithelial connective tissue between the sinus epithelium and the apocrine glands; SGS – subglandular connective tissue between apocrine glands and musculature. ICT – interstitial connective tissue; + weak reactivity; ++ moderate reactivity; +++ good reactivity.

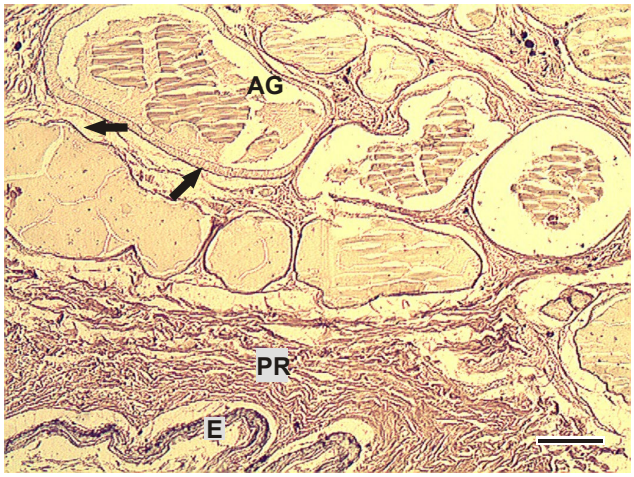

Fig. 2. Reticular fibres (arrows), located around the apocrine glands (AG): E – sinus epithelium, PR – propria; methenamine silver staining; bar = 70 μm .

glands tubules (ICT), a weak to moderate reactivity was observed (Table 2).

RF were concentrated near the multilayer squamous epithelium. They outlined the epithelial basal membrane. This type of fibres was observed around the apocrine tubules (Fig. 2), as well as around the sebaceous glands. A fine RF net was formed around the individual muscle fibres, the peri- and epimysium of the external anal sphincter. A similar net was also present in the intracellular space among the smooth muscle cells.

The dimensions of EF (Table 3) varied within a broad range: the fibres in SEC had a thickness of 0.71 ± 0.09 μm, length of 14.95 ± 0.70 μm, and the more distant EF located in SGC and ICT were thicker and longer compared to those in SEC. The EF, located in SES (Fig. 1 and 3), were thinner and shorter compared to those in SGS (Fig. 4). The histochemical study for detection of EF showed a weak to moderate reactivity in SEC, SES and ICT, whereas the reactivity of fibres in SGC and SGS was well expressed (Table 2). In the present study, a higher EF concentration per one observation field (magnifica-

tion ×400) was noticed in SGS (n=214) and SGC (n=282) compared to SES (n=197) and SEC (n=257), i.e. the EF in the outlet duct were more numerous than those in the sinus wall.

The basal membrane of the lining epithelium of the sinus reacted negatively to staining for EF. Also, wave-shaped convoluted EF were mainly observed in the epi- and the perimysium and less frequently, in the endomysium of ES (external anal sphincter), oriented in various directions.

DISCUSSION

In the present study, data about the dimensions and localization of collagen and elastic fibres in the canine PS wall are reported for the first time. It is acknowledged that collagen type III – a component of RF, is situated near the epithelium as it plays a supporting role. This type is mostly encountered during the embryonic stage of life (Dellmann & Eurell, 1998, Epstein, 1974). The histochemical studies on collagen in human skin (Epstein, 1974) showed that with age, colla-

Table 3. Size of elastic fibres in the perianal sinus of the dog

Parameter	SEC	SGC	SES	SGS
Thickness, µm				
Mean ± SEM	0.71±0.09	1.26±0.03 **	0.58±0.03	0.90±0.13 *
Range	0.41–1.01	0.51–2.01	0.42–0.74	0.49–1.31
Length, µm				
Mean ± SEM	14.95±0.70	27.53±1.37 **	14.41±0.36	25.91±1.28 **
Range	12.89–17.48	22.80–33.48	13.28–16.35	21.37–30.34

SEC – subepithelial connective tissue of the outlet duct; SGC – subglandular connective tissue in the periphery of the outlet duct; SES – subepithelial connective tissue between the sinus epithelium and the apocrine glands; SGS – subglandular connective tissue between apocrine glands and musculature. * P< 0.05, of SES vs SGS; ** P< 0.01 of SEC vs SGC and SES vs SGS.

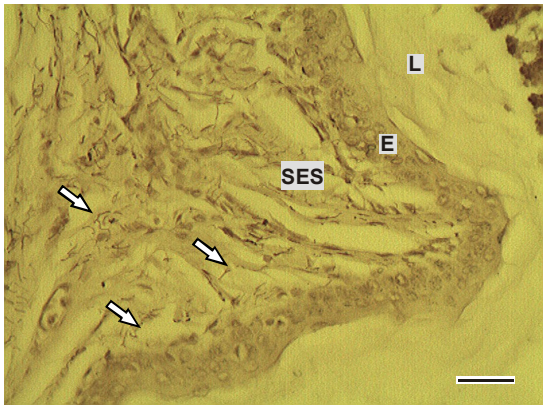

Fig. 3. Elastic fibres (arrows), located in both the subepithelial connective tissue (SES) between the sinus epithelium and the apocrine glands and around glandular tubules: L – sinus lumen, E – sinus epithelium; orcein staining, bar = 20 µm.

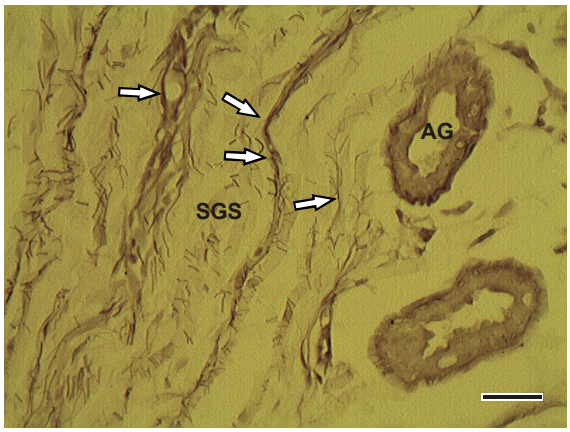

Fig. 4. Elastic fibres (arrows) located in the subglandular connective tissue between apocrine glands and musculature (SGS): AG – apocrine gland; orcein staining, bar = 20 μm.

gen type III was gradually replaced by type I and thus, it could be assumed that most probably it prevailed in canine sinuses. The prevalence of CF in the sinus stroma compared to the lower incidence of EF and RF was also observed by Greer & Colhoun (1966) in the cat. The thickness of fibres was statistically significantly lower ($P<0.01$) in the subepithelial connective tissue compared to connective tissue between apocrine glands and the musculature. These results correspond to data of other authors having studied skin CF forming thick bundles in the reticular dermis (Young *et al.*, 2006). The CF in the epimysium of the external anal sphincter were considerably thicker ($P<0.01$) compared to those in the perimysium. The CF in the perimysium were statistically significantly thicker than CF of the endomysium ($P<0.05$). The triple helical structure of collagen type I molecules makes them nonelastic. This way, CF could counteract stretching and torsion. They form bundles of fibres, intersecting at various angles similarly to those in the skin and organ capsules. Thus, an adaptation to changes in organ's dimensions or in muscles' diameter is acquired (Dellmann & Eurell, 1998). Therefore, CF give strength and resistance to the PS wall.

The histochemical reactivity of the basal membrane for collagen allows us to suppose that it probably referred to collagen type IV that is known to be a part of its composition and that produces a structural support and a filter barrier (Dellmann & Eurell, 1998).

The mutation of the gene that codes collagen production results primarily in reduced resistance to breakdown in supporting tissues to abnormal tissue looseness or susceptibility to damage, resulting in the development of various skin diseases (Young *et al.*, 2006). This fact makes us believe that the alterations in collagen-coding gene could be important for disorders of the paranal sinuses.

The localization of RF, immediately under the multilayer squamous epithelium, mainly around apocrine tubules and at a lesser extent around the sebaceous glands, determines the supporting function of these stromal fibres. These observations of ours confirm the investigations of authors about the formation of a fine RF

network around capillaries, smooth muscle cells, adipose cells, hepatocytes and play a role in scaffold-building in endocrine, lymphatic and haematopoietic organs (Greer & Colhoun, 1966; Bacha & Wood, 1990; Dellmann & Eurell, 1998). They outline the basal membrane of the lining and glandular epithelia, because, as shown by electron microscopy, they are a component of the sub-basal lamina, that connects lamina densa to the subepithelial connective tissue (Dellmann & Eurell, 1998). This lamina permits stretching or shortening of the epithelium of organs with variable size. RF were observed as fine fibrils, thinner than collagen fibrils, with moderate to well expressed histochemical reactivity. This result is explained with the property of collagen fibrils to gather in bundles, thus forming a CF of a diameter larger than that of RF (Dellmann & Eurell, 1998).

In our view, the localization of elastic fibres in the PS wall was similar to that observed in the wall of the urinary bladder and the derma of the skin. Cotta–Pereira *et al.* (1976) have observed variations in the diameter of elastic fibres in human skin. The dimensions in this study (thickness between 0.41 and 2.01 μm) are close to the dimensions of EF – thickness from 0.2 to 5.0 μm, reported in the loose connective organ tissue (Dellmann & Eurell, 1998). Their diameter, as well as their density, increased from the lining sinus epithelium to the musculature, similarly to those observed in the urinary bladder and the skin (Woodburne, 1961, Bacha & Wood, 1990). The thickness of fibres was statistically significantly lower in SES than in SGS ($P<0.05$). In the region of the PS outlet duct, the thickness of CF also exhibited significant differences between SEC and SGC ($P<0.01$). It was established that EF that form elastic bonds, are with a larger diameter – up to 12 μm (Dellmann & Eurell, 1998). A higher EF density in the SGS was observed in cats (Greer & Colhoun, 1966). These authors observed a higher EF density in the outlet duct than in the PS wall. These facts are also confirmed in our study – more EF per observation field (at magnification ×400) in SGS and SGC than in SES and SEC, as well as more fibres in the outlet duct than in PS wall. Along with the observed higher dimensions of EF in the outlet duct than in the sinus wall ($P<0.05$), this information suggested that the fibres played a role in the reduction and increasing in the lumen of the outlet duct and thus, in the retention or release of sinus secretion. Salazar *et al.* (1996) determined the localization of EF in SES, without describing the prevalence of EF in SGS. The authors have not investigated the outlet duct of the PS. We believe that EF do not act independently, but synergically with the musculature, as shown by Woodburne (1961) in the urinary bladder. When the musculature is active, EF are stretched, allowing enlargement of the lumen of the outlet duct with simultaneous shortening in length and this way, the discharge of accumulated secretion is facilitated. Therefore, we hypothesize that the impairment of this mechanism could result in retention of secretion in the sinus lumen, predisposing to development of various pathologies as described by some authors (Baker, 1962, Duijkeren, 1995).

On the other side, when the musculature is not active, EF are activated and contract the lumen of PS outlet duct. In our opinion, the impairment of this synchronicity in the action of musculature and EF, could be responsible for a permanent discharge of secretion from the PS.

Through this first investigation on the dimensions of elastic and collagen fibres

in the PS wall, and on the basis of their localization in the wall of the urinary bladder, we presumed a role of this fibre type in both the filling of sinuses and in the elimination of accumulated secretion, and in the pathogenesis of diseases of this organ.

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