MORPHOMETRIC STUDY OF ACINI IN PAROTID GLAND IN SOME MAMMALS

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Abstract. The structure of major salivary glands in animals sometimes differs according to the diet the animals have and the enviroment they live in. We did not find any information regarding the size of the acini among species, therefore we conducted morphometric investigations on the parotid gland's acini in four mammal species (guinea pig, mouse, rabbit and chinchilla). We harvested the parotid glands and histologically processed them in order to later capture images and assess them with AmScope program (for measuring and counting the acini). The morphometric results revealed that, on the same section area, the number of acini is highest in mouse, followed by chinchilla, rabbit and guinea pigs. The largest acini in the parotid gland are found in guinea pig, followed by rabbits, mice and the smallest in chinchilla. Regarding the polymorphism in acinar size, guinea pig has the most polymorphic ones, followed by the rabbit, chinchilla and mouse. No statistically significant differences were found among mouse and chinchilla parotid gland acini sizes, but the rest of the species presented statistically significant differences among the size of their parotid gland acini.

Keywords: acini, morphometry, parotid, rodents.

INTRODUCTION

In mammals, the salivary glands are connected to the digestive tract and they divide into two categories: minor and major salivary glands. Major salivary glands are more or less voluminous, depending on the species (Treuting and Dintzis, 2012). The secretory units are represented by the acini. They have a different structure depending on the salivary gland. Some of them are formed out of serous or mucous cells, others are mixed (Tandler, 1993; Wolfgang 2003; Miclăuș, 2012).

The product secreted by the acini drains in the intralobular and interlobular ducts, then the larger collecting ducts, which open into the oral cavity. It is known that histology, histochemistry and morphometry of the salivary glands present a great diversity among mammals and sometimes intraspecific differences appear, depending on gender (Cangussu *et al.*, 2002; Lentle *et al*., 2002).

The morphological differences observed in this study may be associated with differences in diet and environment. Salivary glands in mammals are diversified structures. In rodents, the secretory activity is mainly located in acini (Cangussu *et al*., 2002). Saliva has an important function in early digestion, cleans and protects the teeth and oral mucosa. Specialty literature shows that there is a wide variety in the structure of the major salivary glands in animals, even within the same species, but in different strains (Cangussu *et al*., 2002; Lentle *et al*., 2002).

Specialty literature states that the acini of the parotid gland are serous in all species, but there is no data showing whether these acini are identical, similar or may be different in shape, size or secretory activity. In this context, we considered it appropriate to conduct morphometric investigations on the parotid gland's acini in four mammal species.

MATERIALS AND METHODS

For this study, we used one of each animal: guinea pig (*Cavia porcellus*), mouse (*Mus musculus*), rabbit (*Oryctolagus cuniculus*) and chinchilla (*Chinchilla laniger*), all males. The animals were euthanized after exposure to inhalational anesthesia (Isoflurane). Parotid glands were harvested for histological and morphometric investigations. The samples were fixed in 10% formalin, dehydrated in ethyl alcohol (70°, 95°, absolute), clarified with n-butanol and embedded in paraffin. The 5μm thickness sections were stained with hematoxylin-eosin and examined under a light microscope (Olympus BX41) equipped with a digital camera to capture images (Olympus E330). The images were processed with Adobe Photoshop CS2 software. We took a 1699509.677 μm^2 surface into study, using AmScope program for measuring and counting the acini. The data was analyzed with GraphPad Prism 6 software. We determined the minimum, maximum, mean, standard error of mean and standard deviation. We calculated the percentage occupied by the acini, the difference in area which was occupied by other structural elements (excretory ducts, other types of acini, connective tissue, blood vessels).

RESULTS AND DISCUSSIONS

In mice, the parotid gland has the classic histo-architecture: lobulated gland, comprising connective tissue and parenchyma. The capsule has medium thickness and relatively fine trabeculae, but the body is subdivided into polymorphic lobules. The parenchyma consists of relatively small acini, presenting the morphological features of serous acini (Fig. 1). In rabbits, the acini of the parotid are all of the same type, with a general aspect of seromucous acini. Acini present an obvious polymorphism, and the cytoplasm of the acinar cells has a vacuolar appearance (Fig. 2). In guinea pigs, the general aspect of the parotid gland is comparable to the other studied species, namely the acini are all the same type, polymorphic and with different sizes. However, in this species, the acini are significantly larger than the acini of the species mentioned above. Acinar cells contain markedly vacuolar cytoplasm, with nuclei (spherical shape) placed in the basal pole (Fig. 3).

In chinchilla, the general aspect of the parotid gland is more similar to the one present in mouse. Acini are polymorphic and have different sizes, comparable with those of the mouse, but obviously smaller than in other studied species. The cytoplasm of acinar cells is acidophilic, with a predominantly granular aspect and at very small extent, vacuolated (Fig. 4).

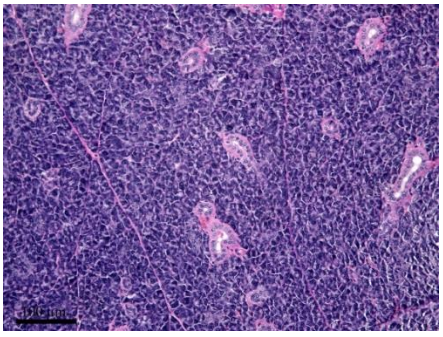

Fig. 1
Mouse parotid gland (H-E)

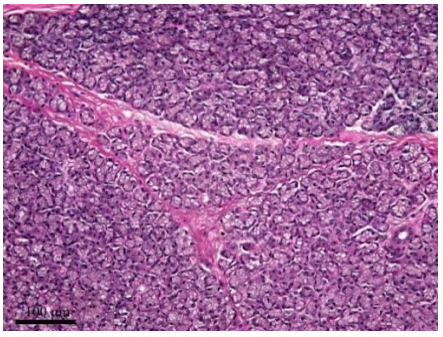

Fig. 2
Rabbit parotid gland (H-E)

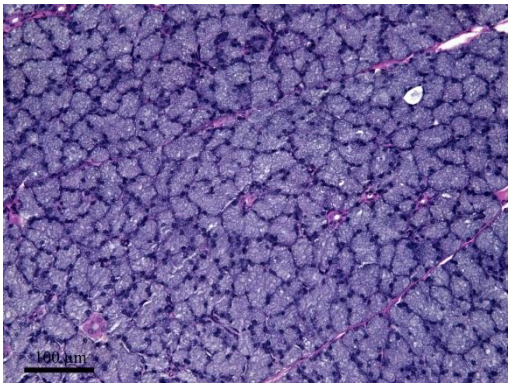

Fig. 3
Guinea pig parotid gland (H-E)

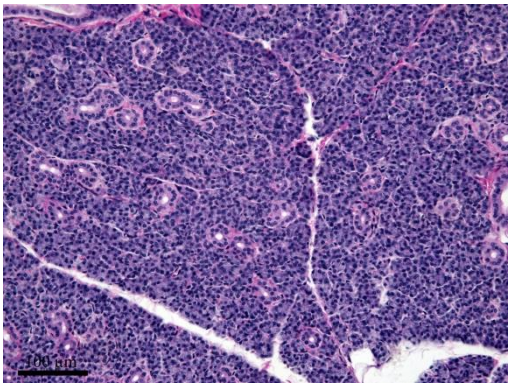

Fig. 4
Chinchilla parotid gland (H-E)

The histological assessment of the parotid glands revealed both similarities and differences regarding the components of the parenchyma in the studied animals.

The similarities refer to the pronounced polymorphism of the parotid's acini in all studied species. Differences are related to the size of the acini and the type of cells. Their size is similar among some species and different among others. The smallest acini were identified in mouse and chinchilla parotid glands, with insignificant differences between the acinar size in the two species. The parotid gland in rabbit presents larger acini than the one in previously mentioned species. The largest parotid acini were identified in guinea pig. The appearance of the acinar cells is also similar in mouse, chinchilla, presenting morphological characteristics of serous cells, which entitles us to believe that at least morphologically, parotid gland's acini are serous in these species. The only acini which, in addition to the classic granular appearance, present a very discreet vacuolar aspect, are the ones in chinchilla. In rabbits, the cytoplasm of acinar cells has a granulo-vacuolar aspect, which leads us to consider that the acini in this species are seromucous (AL-SAFFAR and SIMAWY, 2014). In guinea pig, besides the fact that the acini of the parotid are, as we mentioned before, the largest among the studied species, they present cells whose cytoplasm have a pronounced vacuolar aspect. These aspect suggests that in terms of size and aspect of the cytoplasm, the acini are comparable with the mucous ones.

The morphometric results revealed that, on the same section area, the number of acini is different from one species to another. The highest number of acini/studied area was found in mouse (803), followed by chinchilla (769), rabbit (720) and the lowest in guinea pigs (266) (Table 1).

Table 1

Number of acini/studied surface (1699509.677 μm^2)

	Cavia porcellus	*Oryctolagus cuniculus*	*Chinchilla lanigera*	*Mus musculus*
N=	266	720	769	803
Media	639.5			

Dividing the total acinar surface to the whole studied surface (1699509.677 μm^2), we noticed that the highest values were recorded in guinea pigs (1544532 μm^2 = 90.88%) followed by rabbit (1302917.97 μm^2 = 76.66%), mouse (1181138.59 μm^2 = 69.50%) and the smallest values were recorded in chinchilla (1118218.78 μm^2 = 65.80%).

Table 2

Dimensions of the acini. Min – minimum; Max – maximum; $\bar{x}$ - mean; SD – standard deviation; SEM – standard error of mean; CV - Coefficient of variation

Picture surface 1699509.677 µm²				
Species	*Cavia porcellus*	*Oryctolagus cuniculus*	*Chinchilla lanigera*	*Mus musculus*
Min (µm²)	453.1	319.4	245.1	345.9
Max (µm²)	21243	5251	3047	3115
$\bar{x}$ (µm²)	5807	1810	1454	1471
SD (µm²)	3237	725.2	465.6	464.8
SEM (µm²)	198.5	27.02	16.79	16.4
CV (%)	55.75	40.07	32.02	31.60

In order to determine the size and polymorphism of the acini, we measured their section surface, and observed that there are differences between the species studied by us. Taking into account the average of the surfaces, we obtained the highest value in guinea pigs (5807 µm²), followed by rabbits (1810 µm²), mice (1471 µm²) and the smallest value in chinchilla (1454 µm²) (Table 2). Based on these values, we can state that the largest acini in the parotid gland are found in guinea pig and the smallest in chinchilla. Dividing the average acini area from guinea pig to that of other species, we find that it is more than 3.20 times larger than the one in rabbit, 3.94 than the one in mice and 3.99 than the one in chinchilla.

The coefficient of variation has highest value in guinea pig with 55.75%, then in rabbit - 40.07%, chinchilla - 32.02% and mouse - 31.60%. The smaller the coefficient of variation is, the less polymorphic the acini are. We can assert that acini in guinea pigs are the most polymorphic in sizes, followed by rabbit, chinchilla and the less polymorphic ones are encountered in mouse (Table 2).

Table 3

Results of the statistical analysis for the studied species

Multiple comparison test	Statistical significance
Rabbit vs. Guinea pig	****
Chinchilla vs. Guinea pig	****
Mouse vs. Guinea pig	****
Chinchilla vs. Rabbit	****
Mouse vs. Rabbit	****
Mouse vs. Chinchilla	ns

We used the One-Way ANOVA test to analyze the results of the statistical calculations. There were no statistically significant differences between the size of the acini in mouse and chinchilla parotid glands in multiple comparison test. Upon comparison of the size of the parotid acini in guinea pig with the ones in the rest of the species, we found that there were highly statistically significant differences among all of them ($p<0.0001$). We also obtained the same results by comparing the size of the acini in rabbit with the ones in chinchilla and mouse (Table 3).

These results highlight some differences in the size of the parotid gland acini in the studied species.

CONCLUSIONS

The investigation highlights the fact that the parotid gland of the species taken into study presents highly polymorphic acini. Among the studied species there are certain differences, with the most polymorphic acini present in guinea pig and the less polymorphic ones in mouse. Regarding the size, the largest acini are present in guinea pig and the smallest ones in chinchilla and mouse. The largest average acinar surface is encountered in guinea pig, followed by the one in rabbit, mouse and chinchilla. The parotid gland in mouse proved to have the highest number of acini/surface taken into study and the one in guinea pigs the fewest.

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