Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 10
Sofia • 2005

Distribution and Morphometric Characteristics of Mast Cells in the Kidney of Domestic Swine

*A. Vodenicharov**, *M. Gulubova***, *T. Vlaykova****, *G. Kostadinov**

* *Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora (TUSZ)*
** *Department of General and Clinical Pathology, Faculty of Medicine, TUSZ*
*** *Department of Chemistry and Biochemistry, Faculty of Medicine, TUSZ*

The distribution and morphometric characteristics of mast cells in pig kidney were studied using the histochemical staining with toluidine blue (TB) and alcian blue/safranin (AS). It is found that the mast cells are located in all part of the kidney, and their number per mm^2 in the cortex is significantly smaller than that in the medulla of the kidney independently of the methods of staining ($p<0.0001$, ANOVA test). However, the mast cells in the medulla have significantly smaller length compared to the cells in the cortex, independently of staining procedure ($p=0.005$, ANOVA test), whereas the width are commensurable. In addition, the comparative analyses show that the staining with AS detects significantly more mast cells than that with TB ($p<0.0001$, Paired t-test), and the values of the length of the mast cells stained with AS are significantly bigger than the values obtained after TB staining ($p<0.0005$ ANOVA test). The values of the width are comparable. The results of our current investigation are in the line of those in similar studies on domestic pig and other animals. On the basis of our findings we discuss the role of the mast cells in the kidney of domestic pig.

Key words: Mast cells, kidney, domestic pig, localization, morphometry.

Introduction

Although the mast cells are extensively studied in human and experimental animals, there is only very limited information about them in domestic animals as the data for mast cells in pigs are extremely scanty. Such data are reported in the studies of some investigators [1, 18] which concern the characteristics of mast cells in intestine mucose of pigs after helminth invasion. X u et. al., [24] observed variable density and distinct difference in number and size of mast cells in some organs of pigs with different age. Earlier we reported data for the presence of mast cells in the media of kidney artery and vein and in its valves [20, 21, 25], as well as in the wall of proximal tubule of kidney in pigs [22].

The presence of mast cells in the kidney of mammals is also very poorly investigated. In the studies on heart and kidney of guinea-pig and man, it was shown that the mast cells have similar localizations in kidney of the both species. They were found in intersticium of the cortex and in the outer medulla, in perivascular connec-

tive tissue of proximal and distal tubules, as well as in vicinity of the Bowman's capsule [9]. According to the authors, the mast cells observed in heart and kidney of the both species showed certain resemblance, however, they were morphologically and histochemically distinct from peritoneal mast cells of rats.

For quantitative measurements of the density of mast cells, it has been ascertained that in addition to the fixative, some influence is exerted by the staining method. The results of quite a number of research groups have shown that the observed cells are more numerous when the Carnoy's liquid is used compared to the fixation with neutral formalin [6, 7, 8, 14, 15, 16, 17]. Moreover, after comparative staining with alcian blue/safranin (AS) and toluidine blue (TB), the number of mast cells labelled with AS was higher [2, 10, 12, 24].

The insufficiency of data for the localization, number and morphometrical characteristics of mast cells in kidney of domestic pig motivated us to carry out the current study. The aim of this investigation is to clear up the type and organ specificity of mast cells in domestic swine.

Material and Methods

Tissue materials was obtained from 12 pigs of both genders (6 castrated male, 2 noncastrated male, and 4 castrated female), with age from 8 to 10 months. The pigs were slaughtered in town's abattoir, Stara Zagora. The kidneys were obtained immediately after slaughtering of the animals and small pieces — 5 mm^3 from different areas of the cortex and medulla — were fixed in Carnoy's liquid. Six of the kidneys were preliminary perfused with Carnoy's liquid, after which the tissue samples were fixed with the same fixative. Fixation was performed for 2 to 4 hours depending on the size of the specimens, followed by routine process for paraffin-embedding: rinsing in water, dehydration in grading ethanol chain, clearing in xylene and embedding in paraffin. The 5-7 μm sections were made and were stained either with 0.1% solution of toluidine blue in McIlvane buffer (pH 3) [19] or with a solution of alcian blue/safranin, pH 1.42 [5].

The mast cells were counted in the cortex and medulla of the kidney using the light microscope Zièss with an ocular-micrometer and the results were presented as mast cells/mm^2. Morphometrical measurements of the mast cells (length/width) were carried out only on well outlined cells with nuclei using an ocular-micrometer as well. There were studied 200 fields with 1360 mast cells. 779 of them were stained with alcian blue and 581 with toluidine blue.

The data were statistically analysed using the StatView™ package for Windows, v.4.43 (Abacus Concepts. Berkeley CA, USA). Basic descriptive statistics were used to calculate mean values and the standard deviations (SD). The ANOVA test was utilized to evaluate the significance of differences of data between two independent groups of specimens, whereas Paired *t*-test was applied for analyzing the differences between the data of dependent groups. When $p<0.05$, the differences were considered to be statistically significant.

Results

The light microscopic observations showed that mast cells in examined structures were distributed unequally: the density of mast cells in the medulla of kidney was significantly higher than in cortex independently of staining procedure ($p<0.0001$, ANOVA test) (Fig. 1).

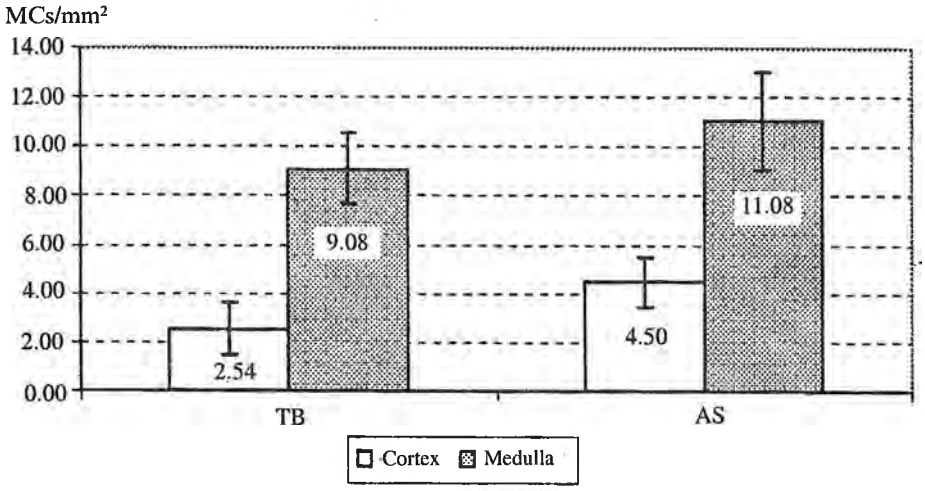

Fig. 1. The density of mast cells in cortex and medulla of pig kidney stained with alcian blue/ safranin (AS) and toluidine blue (TB). The data are presented as mean of MCs/mm² ± SD

The mast cells in cortex stained with the both methods were observed in perivascular connective tissue, between the tubules of nephron and the collective tubules, as well as in vicinity of their basal membrane. Single mast cells were also seen between the epithelial cells of the tubules and inside of the glomerules. The latter localization was detected predominantly in glomerules of the juxtamedullary nephrons (Fig. 2).

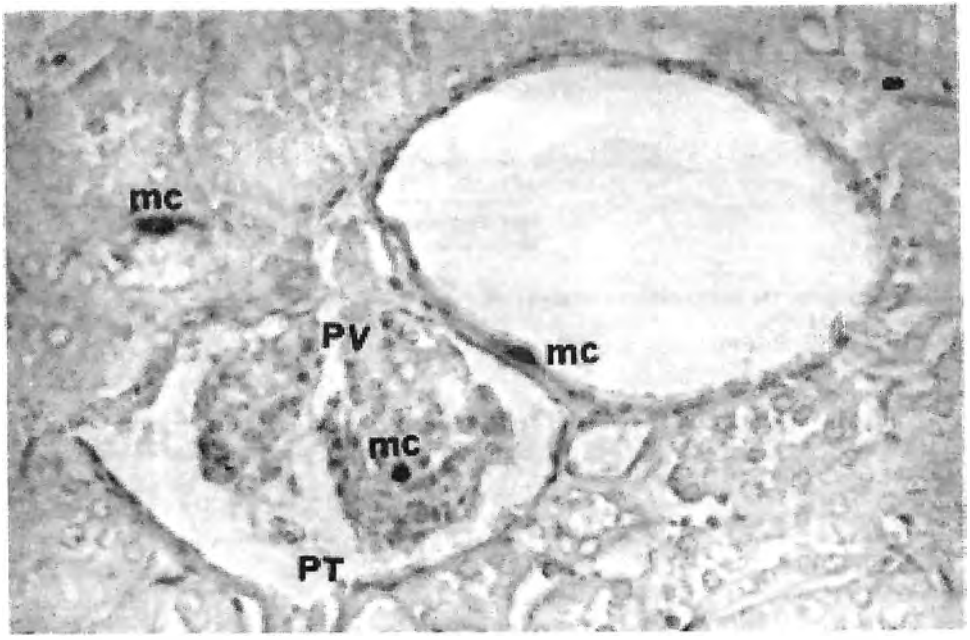

Fig. 2. Mast cells (mc) in the juxtamedullar glomerul, wall of venula and proximal tubule: PV — Polus vascularis and Polus tubularis of the glomerulus. Under PT the beginning part of proximal tubule is seen (× 100)

In the medulla, the mast cells had no predilection localization, although there was also a tendency for an uneven distribution. Mast cells were observed around all structures of kidney medulla. As it was in the cortex, mast cells were found in propinquity to the basal membrane of epithelial cells of the tubules and in close proximity to the wall of straight vessels.

Mast cells seen both in the cortex and in medulla showed positive reaction for biogenic amine when they were stained with AS and well-expressed g-metachromasia when stained with TB. All other cells were orthochromatic. The shape of prevalent part of the observed mast cells was mainly ovoid. Rarely the cells were with cylindrical (cigarette-like) and spindly shape. The latter two types of cells were found predominantly near to the adventitia of blood vessels.

The morphometric analyses showed that the mast cells in the cortex were with significantly bigger length of the sections compared to the cells in the medulla, independently of the staining procedure ($p<0.0001$ for TB staining, and $p=0.005$ for AS staining, ANOVA test) (Fig. 3). However, the width was commensurable, independently on the staining type ($p=0.638$ for TB, and $p=0.727$ for AS, ANOVA test) (Fig. 3).

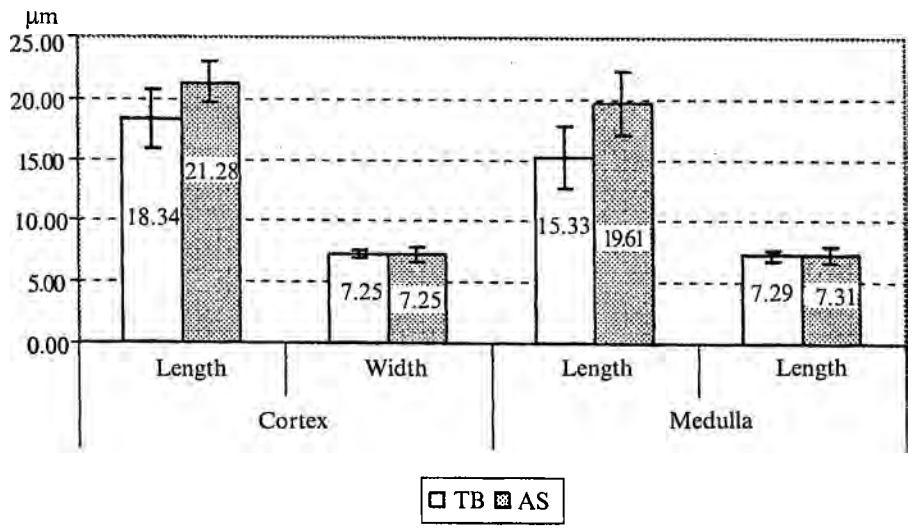

Fig. 3. Results of the morphometric analyses on the length and width of the mast cells sections located in the cortex or in the medulla of the kidney and stained with either alcian blue/safranin (AS) or with toluidine blue (TB). The data are presented as mean values in mm ± SD

The comparative analyses of the both staining procedures showed that the staining with AS detected significantly more mast cells than the other type of procedure, with TB ($p<0.0001$, Paired t-test) (Fig. 1). The mean number of TB-labelled mast cells per mm^2 in the cortex was 2.54 ± 1.073, whereas this was 4.5 ± 1.074 when mast cells were stained with AS ($p<0.0001$, Paired t-test). Analogous results were obtained for the medulla: 9.08 ± 1.426 TB-stained mast cells/mm^2 vs. 11.08 ± 1.978 AS-stained mast cells/mm^2 $p<0.0001$, Paired t-test).

The size of the mast cells (length and width of the sections) also presented higher values after AS staining compared that after TB staining (Fig. 3). In the cortex, the mean of the length of AS-stained mast cells was significantly higher (21.28 ±

1.7 μm) than TB-stained cells (18.0 ± 2.5 μm, $p<0.0001$, ANOVA test). The width of the cells with both days were commensurable (7.25 ± 0.6 μm for AS and 7.25 ± 0.3 μm for TB, $p=0.957$, ANOVA test). Respectively, the mean value of the length of AS-labeled mast cells in the medulla was 19.61 ± 2.6 μm, which was significantly higher in comparison to the mean value of 15.33 ± 2.6 μm corresponding to the length of TB-stained mast cells ($p<0.0001$, ANOVA test). The width of the cells in the medulla stained with the both types of procedure was again comparable (7.31 ± 0.7 μm for AS and и 7.29 ±0.4 μm for TB, $p=0.927$, ANOVA test).

Discussion

The results of the current study showed that the mast cells in kidney of pigs are localized unequally. Analogous unequal distribution was earlier reported in the ureter's wall of domestic pig [23], but the number of mast cells of mm^2 in the present study was less in comparison to that found in the ureter. Moreover, in the porcine kidney it was found out that number of the mast cells stained with AS is higher than that of the cells stained with TB, as the difference was statistically significant. Our data confirmed those reported by other research groups [2, 10, 12, 14, 24], which were focused on the study of other domestic and experimental animals. The difference of staining quality may depend on variable maturity of mast cells, by the influence of tissue surrounding these cells [3], and the presence of different proteoglycans in the mast cells granules [2, 11, 12, 13].

An interesting observation is the difference in the size between the mast cells localized in the cortex and in the medulla. It makes impression that after staining with AS, the length of the cells has statistically higher values than that of cells stained with TB. Differently, the width of the cells with the both types of staining was commeasurable, and no significant differences were obtained. Similar results were reported for cattle mast cells [4] and mast cells in mucosal and submucosal layer in porcine intestine [24]. Recently, we also observed similar differences in the size of mast cells in porcine ureter, where the biggest cell were found in the middle shell [23]. That bigger size of mast cells stained with AS may explain with a large content of biogenic amines and better capacity of Carnoy's liquide to fix mast cell granules and their contents, respectively. In the other part, the size of mast cells may also depend on their function [10].

In conclusion, the presence of mast cells in porcine kidney suggest that they may take an important role in physiological and pathological conditions of that organ. Further immunocytochemical investigation allow more knowledge about detailed function of porcine renal mast cells.

References

1. Ashraf, M., J. Urban, T. Lee. Characterization of isolated porcine intestinal mucosal mast cells following infection with *Ascaria suum*. — Veterinary Parasitology, **29**, 1988, 143-158.
2. Barret, K., F. Pearce. Heterogeneity of mast cells. — In: Histamine and Histamine Antagonist, Vol. 97 (Ed. B. Uvnäs), Berlin, Springer-Verlag, Berlin-Heidelberg, 1991, p. 93-116.
3. Beil, W., M. Schulz, U. Wefelmeyer. Mast cell granule composition and tissue location — a close correlation. — Histology and Histopathology, **15**, 2000, 937-946.
4. Chen, W., M. Alley, B. Mankteлow, P. Slack. Mast cells in bovine lower respiratory tract: morphology, density and distribution. — British Veterinary Journal, **146**, 1990, 425-436.
5. Csaba, G. Alcian blue — Safranin method for mast cells. — In: Theory and Practice of Histological Techniques, (Eds. Y. D. Bancroft, A. Steven), New York Livingstone, 1990, p. 639.

6. Enerbäck, L. Mast cells in rat gastrointestinal mucosa. 1. Effect of fixation. — Acta pathologica et Microbiologica Scandinavica, **66**, 1966a, 289-302.
7. Enerbäck, L. Mast cells in rat gastrointestinal mucosa. 2. Dyebinding and metachromatic propertirs. — Acta pathologica et Microbiologica Scandinavica, **66**, 1966b, 303-312.
8. Frangogiannis, N., A. Burns, L. Michael, M. Entman. Histochemical and morphological characteristics of canine cardiac mast cells. — Histochemistry Journal, **31**, 1999, 221-229.
9. Ghanem, N., E. Assem, K. Leung, F. Pearce. Cardiac and renal mast cells: morphology, distribution, fixation and staining properties in guinea pig and preliminary comparison with human. — Agents and Action, **23**, 1988, 223-226.
10. Harris, W., J. Marshal, S. Yamashiro, N. Shaku. Mast cells in the bovine trachea: Staining characteristics, dispersion techniques and response to secretagogues. — Canadian Journal of Veterinary Research, **63**, 1999, 5-12.
11. Hill, P., R. Martin. A review of mast cell biology. — Veterinary Dermatology, **9**, 1998, 145-166.
12 Hunt, T., A. Campbell, C. Robinson, S. Holgate. Structural and secretory characteristics of bovine lung and skin mast cells: evidence for the existence of heterogeneity. — Clinical and Experimental Allergy, **21**, 1991, 173-182.
13. Jolly, S., J. Detilleux, F. Coignoul, D. Desmecht. Enzyme-histochemical detection of a chymase-like proteinase within bovine mucosal and connective tissue mast cells. — Journal of Comparative Pathology, **122**, 2000, 155-162.
14. Küther, K., L. Audige, P. Kube, M. Welle. Bovine mast cells — distribution, density, heterogeneity, and influence of fixation techniques. — Cell and Tissue Research, **293**, 1998, 111-119.
15. Kube, P., L. Audigé, K. Küther, M. Welle. Distribution, density and heterogeneity of canine mast cells anf influence of fixation techniques. — Histochemistry and Cell Biology, **110**, 2, 1998, 129-135.
16. Marshall, J., G. Ford, E. Bell. Formalin sensivity and differential staining of mast cells in human dermis. — British Journal of Dermatology, **117**, 1987, 29-36.
17. Michaloudi, H., G. Papadopoulus. Mast cells in the sheep, hedgehog and rat forebrain. — Journal of Anatomy, **195**, 1999, 577-586.
18. Pabst, P., B. Beil. Mast cell heterogeneity in the small intestine of normal, gnotobiotic and parasitized pigs. — International Archive Allergology, **88**, 1988, 363-366.
19. Pearce, A. Histochemistry, 2nd ed., London, J. & A. Churchill, Ltd., 1960, p. 692.
20. Vodenicharov, A., P. Cinuchanov. Morphological studies of the valves of the kidney vein in domestic swine. — Anatomia, Histologia, Embryologia, **24**, 1995,a 159-163.
21. Vodenicharov, A., P. Cinuchanov. Microscopic and ultrastructural studies of the renal artery in domestic swine. — Anatomia, Histologia, Embryologia, **24**, 1995b, 237-240.
22. Vodenicharov, A., Ch. Chouchkov. Morphological study of mast cells localization in the wall of the proximal tubule in the domestic swine kidney. — Anatomia, Histologia, Embryologia, **28**, 1999, 85-88.
23. Vodenicharov, A., R. Leiser, M. Gulubova, T. Vlaykova. Morphological and immunocytochemical investigations on mast cells in porcine ureter. — Anatomia, Histologia and Embryologia, 2004, (in press).
24. Xu, L., M. Carr, A. Bland, A. Hall. Histochemistry and morphology of porcine mast cells. — Histochemistry Journal, **25**, 1993, 516-522.
25. Воденичаров, А. Върху структурата на бъбречната вена при домашната свиня. — Ветеринарна медицина, 1, Suppl., 1995, 12-15.