Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Histological Study of the Adrenal Gland of African White Rhinoceros

Ke-mei Peng[§]*, Hui Song[§], Hua-zhen Liu, Jian-bin Zhang[1], Zhi-qiang Lu, Zhi-wei Liu and Yin-xue Liu

College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, PR China
*Corresponding author: km.peng@yahoo.com; pengkm@mail.hzau.edu.cn

ARTICLE HISTORY

Received: July 01, 2011
Revised: November 21, 2011
Accepted: December 31, 2011

Key words:
Adrenal gland
African White Rhinoceros
Histology

ABSTRACT

The microstructures of adrenal gland of white rhinoceros (*Ceratotherium simum*) were observed by light microscopy. The results showed that the surface envelope of adrenal gland was covered with developed connective tissues whose average thickness was up to 210 μm. A large number of sinusoids were distributed between the neighboring cells. The zona glomerulosa of adrenal cortex was thin and composed of columnar and cubic cells. The columnar cells were close to the edge and the cubic cells were found in the deep zone. The two kinds of cells were arranged in irregular groups. The thickness of zona fasiculata was about 3 times as much as that of zona glomerulosa, and the boundary was not obvious. Cells were arranged in irregular cords. Some small lipid droplets were in the shape of small vacuolation, and distributed in the cytoplasm. The cells of zona reticularis were adjacent to medulla with which they formed a jagged boundary. Cells were in network or in enclose acini. Medullae were darkly stained and clearly distinct from the cortex. Cells were large with elliptical nuclei whose nucleoli were significant. Cytoplasm was basophilic and stained to give blue violet appearance. A small amount of sympathetic ganglion cells were distributed in the medulla. The above results suggested that the adrenal gland structures of African white rhinoceros compared with other animals have similarities. . The rich sinusoids in capsule suggested that its blood supply was abundant, full of metabolism and endocrine activity.

To Cite This Article: Peng KM, H Song, HZ Liu, JB Zhang, ZQ Lu, ZW Liu and YX Liu, 2012. Histological study of the adrenal gland of African white rhinoceros. Pak Vet J, 32(3): 394-397.

INTRODUCTION

There exist five kinds of rhinoceros in the world, namely, black, white, Sumatran, Indian and Sunda rhinoceros. Of all these, white rhinoceros are the most valuable which live in the dry and open grasslands of African continent. They have the largest body configuration, since the weight of adult white rhinoceros can reach up to 6 tons**.** Due to illegal and arbitrary killing and capturing in the past few years, the number of African white rhinoceros has dwindled sharply. It is difficult for people to see them in the widest African grasslands except in zoological gardens. Many parts of white rhinoceros, like the horn, fur, flesh, blood, bone and internal organs etc, have great economic values, especially horns which not only can be made into the knife holders of traditional Arabian curved knives, but also serve as a notable precious materia medica from time immemorial. With the effects like cardiotonic, clearing heat, detoxification and hemostasis, they also have algefacient activity to detoxify and clear heat. But there is some misunderstanding in the foreign countries that leads people to consider cornu rhinoceri as a love potion invigorating and strengthening yang. Consequently, the black market trades run wild. Owing to the transition of natural environment and the effect of human activities, especially arbitrary arrest and indiscriminate hunting, overexploitation and utilization of wild animal resources have reduced serious and even to the point of extinction. Due to continuous and sharp reduction in the number of white rhinoceros, the white rhinoceros are listed as one among the most endangered species (Wei, 2009).

Adrenal gland is one of the important cryptorrhetic organs in animal body. With a complicated structure, it can secrete many kinds of hormones and execute various physiological functions. Studies showed that adrenal gland has different characteristics in disparate species. At present, the studies of adrenal glands in mammals include rat (Coupland, 1965), water buffalo (Luo, 2000), baboon (Fadhil

[§]These authors contributed equally to this study.

and Stephen, 1991) and dolphin (Clark *et al*., 2005) etc. Extensive studies have been accomplished in mammals to rule out the regulation of growth and function of the adrenal gland (Mataoui-Mazari *et al*., 2011; Chan *et al*., 2011) and the signaling pathway of melanocortin receptor mediated adrenocortical hormone secretion (Sebag and Hinkle, 2010 Webb and Clark 2010 Agulleiro *et al*., 2010). But as one of the world's endangered animal specie, the relative research data on the African white rhinoceros is lacking and there is no study report about the histological characterization of adrenal gland of rhinoceros. By observing and studying the histological microstructure characteristics of African white rhinoceros, the author has offered a reliable morphology foundation for further studies on rhinoceros' adrenal gland endocrine, the physiology, biochemistry and pathology of matter metabolic and veterinary clinical medicine.

MATERIALS AND METHODS

The adrenal glands of two adult African white rhinoceros (impending death) were used in the present study. The two African white rhinoceros, one male and another female, were kept in The Rhinoceros Research Center of Sanya in Hainan Province of China, geography coordinate north latitude: 18°14', East longitude: 109°31'. After being deeply anesthetized, the adrenal glands were immediately removed from the abdominal cavity.

The tissues of adrenal gland of the African white rhinoceros were preliminarily fixed by 10% neutral formalin phosphate-buffered solution (0.1 mol/L, pH 7.4), incised into a size of about 5mm×5mm×3mm, and then fixed for 4 days in the same tissue fixation solution. Four μm thickness paraffin serial sections were made by Leica Microtome (RM2245, Germany) in accordance with standard procedures, and stained with hematoxylin-eosin (H.E.). The sections were observed, measured and subjected to photomicrography by using Olympus light microscope (BX51+DP72, Japan).

RESULTS

Anatomical structure: African white rhinoceros had a couple of adrenal glands of brunneus color that were located nearby the renal anterior medialis margin. The surface of adrenal was wrapped with connective tissue velamen and connected with kidney. Connective tissue was extended into parenchyma together with blood vessels and nerves. Adrenal parenchyma was divided into superficial cortex and bathypelagic medulla. Cortex occupied most part of the adrenal, about 80%. Its color was comparatively light (Fig. 1). Medulla had a small size and occupied approx. 20% of the kidney, with a comparatively dark color.

Histological structure

Capsule: The surface of the African white rhinoceros adrenal glands were covered with developed connective tissue capsule whose average thickness was up to 210μm. In the capsule, there were abundant blood vessels, smooth muscle fibers and sporadic nervous ramifications. The connective tissue of capsule penetrated deeply into parenchyma and formed comparatively slimy mesh stent, among which scatter abundant collagen fibers, reticular fibers and blood vessels or venous sinuses were observed (Fig. 1 & 2).

Cortex: It covered about 80% volume of the adrenal gland and had a light color. According to the different arrays of cells, it could be divided into three zones, namely, zona glomerulosa, zona fasciculata and zona reticularis in ectoentad order.

Zona glomerulosa: This zone was situated under velamen. It was very thin and composed of columnar and cuboidal cells. Columnar cells were distributed mainly near the borderline while cuboid cells mainly in bathypelagic. The two kinds of cells had an irregular align style. Connective tissue that contained plenty blood capillaries was distributed among cell groups. The nuclei of the two kinds of cells were round, darkly stained and had a small amount of cytoplasm (Fig. 1 & 2).

Zona fasciculate: This zone was located in the capsule's intercellular layer and was 3 times as thick as zona glomerulosa, and there was no apparent boundary between them. The cells were lined into irregular streak style. Large sinus venosus was between the neighboring cell streaks, and the sinus walls were lining with monolayer applanate endotheliocytes. Cytoplasm contained a lot of tiny lipid droplets in the zona fasciculata cells as well as many small vacuoles formed from dissolved lipid droplets during H & E staining (Fig. 1 & 3).

Zona reticularis: It was situated in the deepest layer of the capsule. In this region, cells and medulla were closely adjacent to each other and form flexuose boundaries. There were two array styles of zona reticularis cells: some cells anastomose with each other and intertwined into reticular formation while the other cells surrounded into irregular vesicular shapes. There was a lot of blood sinusoids distributed between neighboring acini. Cytoplasm of the zona reticularis cells also had a lot of small droplets that formed many little vacuoles (Fig. 1, 4 & 5).

Medulla: The medulla constituted 20% of the whole adrenal gland and had a clear boundary with cortex. Medullary cells were comparatively larger and most of them had a polygonal shape and an elliptical nucleus with distinct nucleolus. Cytoplasm was basophilic and stained lyons blue, thus the boundary between nucleus and cytoplasm was not clear (Fig. 5 & 6). A small amount of sympathetic ganglion cells usually scattered in the medulla in groups of threes and/or fives was also observed. These cells were round or elliptical with distinct nuclei.

DISCUSSION

Comparison of the adrenal glands between African white rhinoceros and other animals: The average thickness of the developed capsule on the surface of African white rhinoceros' adrenal glands could reach 210 μm. It was much larger than those of horses, pigs and sheep; and equivalently 15 times as larger as that of the buffalo (Luo, 2000). Adrenocortical zona glomerulosa of African white rhinoceros was composed of columnar and cuboidal cells. Columnar cells were distributed mainly

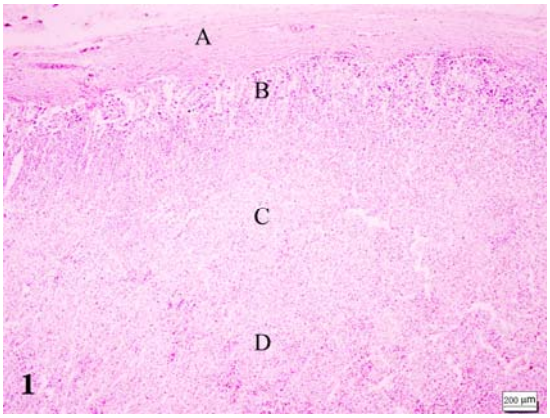

Fig. 1: White rhinoceros adrenal gland (HE staining, 40x), showing the capsule (A), zona glomerulosa (B), zona fasciculate (C), zona reticularis (D).

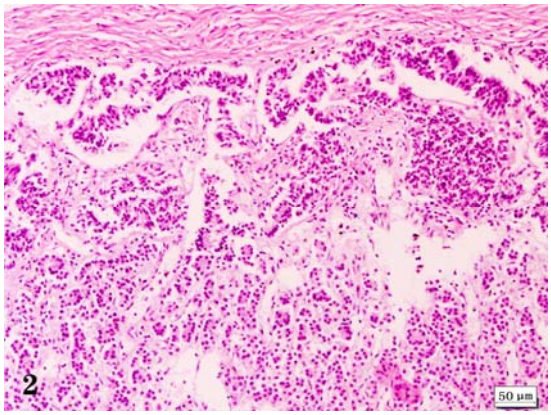

Fig. 2: White rhinoceros zona glomerulosa of adrenal gland (HE staining, 100x), showing the align style of zona glomerulosa cells and smooth muscle in capsule.

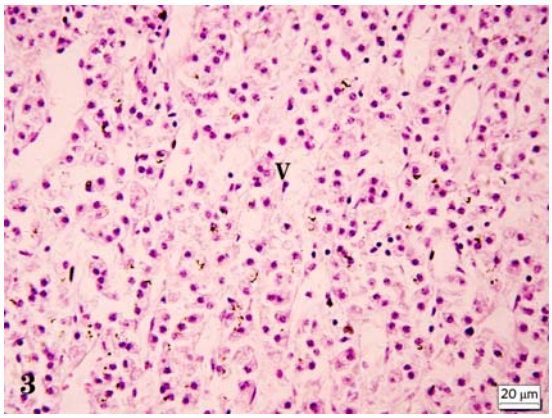

Fig. 3: White rhinoceros zona fasciculata of adrenal gland (HE staining, 200x), showing cell align style and blood sinusoid (V).

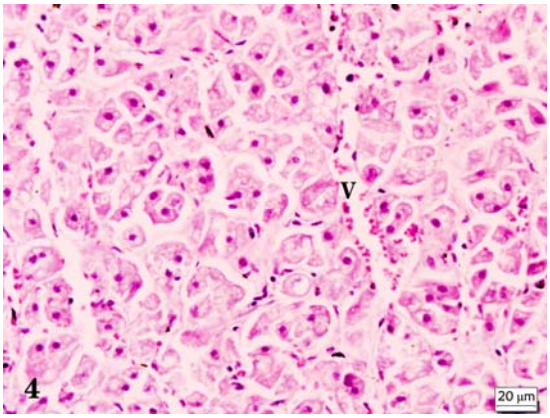

Fig. 4: White rhinoceros zona reticularis of adrenal gland (HE staining, 400x), showing cell shape, align style and blood sinusoid (V).

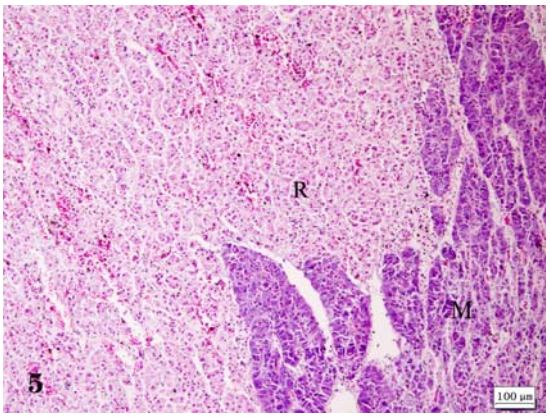

Fig. 5: White rhinoceros adrenal gland (HE staining, 100x), showing the cell align style in the boundary of cortex's zona reticularis (R) and medulla (M).

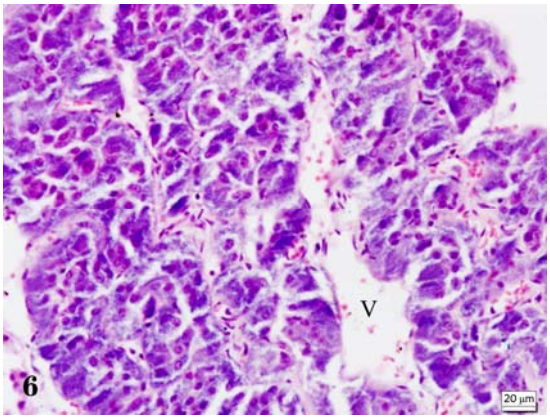

Fig. 6: White rhinoceros adrenal medulla (HE staining, 400x), showing cell shape, align style and blood sinusoid (V).

near the margins while cuboidal cells lied in the deep lamella. Unlike in horses and carnivore where they arrange into regular arcuate (Peng, 2009), these two kind of cells align irregularly similar to the style in pigs. The thickness of zona fasciculata was 3 times greater than that of zona glomerulosa, and the boundary between them was not clear. Cells were arranged into irregular cord shapes and a lot of lipid droplets into tiny vacuole were distributed throughout the cytoplasm similar to domestic animals (Peng, 2009).

The cells of zona reticularis were closely adjacent to medulla and together they formed great flexuose boundaries with ups and downs. This situation has a far cry from the majority of animals, but is similar to pigs. Deeply stained medulla had a very clear boundary with cortex. Cells were comparatively larger with oval shaped nuclei and distinct nucleoli. Cytoplasm was basophilic and stained into lyons blue color. Consequently, the boundary between cytoplasm and nucleus is not clear. The above results significantly differed from domestic animals

and most other animals' results. May be, it has something to do with the variety of species, but it requires further studies.

Relationship between tissue structures and functions of adrenal cortex and medulla: As an important endocrine organ, adrenal gland has lots of physiologic functions in various kinds of vital movements like creaturely metabolism, growth and development, etc. Adrenal cortex secretes 3 kinds of hormones including mineral corticoids secreted by zona glomerulosa which regulate water and electrolyte metabolism; glucocorticoid secreted by zona fasciculata promote saccharide and protein's metabolism, in addition to certain immunological activities by which they express anti-inflammatory, immunosuppressive properties and are crucial for defending the host against infectious insults (Padgett and Glaser 2003; Zacharowski *et al.,* 2006). Sex hormones including dehydroepiandrosterone and andro-stenedione secreted by zona reticularis, can serve as substrates for conversion into testosterone and estradiol in the periphery to promote gonadogenesis and the formation of secondary sexual characters (Keegan and Hammer, 2002). Adrenal medulla secretes adrenalin and noradrenalin. The former enhances cardiac excitability and causes systole to speed up and strengthen, and the latter contracts arteriolar all over the body, and elevates blood pressure (Peng, 2009). Adrenal cortex and medulla differ greatly in embryogenesis, structure and function. Cortex is traceable from mesoblastema, but medulla originates from neural crest. In different species, ages and organisms of different functional status, the proportions of cortex and medulla are different, but the two are closely correlated in function due to the blood supply of adrenal (Mesiano and Jaffe, 1997). The large amounts of blood vessels or blood sinusoids in African white rhinoceros' adrenal glands indicated that blood supply was abundant; and metabolism and secretory activities were very vigorous. Adrenal artery was subdivided into arteriole after capsule entry. Most of the arterioles expand into blood sinusoids that go through the cortex into medulla while only a few arterioles get into medulla directly and connect with blood sinusoid in the medulla. Since most of the blood in adrenal glands passes cortex first before it reaches medulla, medullary blood contains abundant cortical hormones. Among the hormones, glucocorticoid can activate N-methyltransferase of medulla cells, and make noradrenalin take off methyl group and change into adrenalin (Cheng *et al*., 2003). Judging from this, we can see that adrenal cortex has great effect on the hormonogenesis of medulla cells. Blood coverage in first in medulla in small veins, then into medium-sized veins, and after departure from portal, turn into venae suprarrenales that take all kinds of adrenal glands' hormones from postcaval vein into the heart, and further into corresponding target organs.

Acknowledgement: We would like to thank Miss YIN Wei of School of Liberal Arts and Foreign Language in Tianjin University for her valuable comments on the manuscript. This work was supported by National Natural Science Foundation of China No. 30972152 and No. 39970547; and Specialized Research Fund for the Doctoral Program of China Higher Education No. 200805040023.

REFERENCES

Agulleiro MJ, S Roy, E Sanchez, S Puchol, N Gallo-Payet and JM Cerda-Reverter, 2010. Role of melanocortin receptor accessory proteins in the function of zebrafish melanocortin receptor type 2. Mol Cell Endocrinol, 320: 145-152.

Chan LF, LA Metherell and AJ Clark, 2011. Effects of melanocortins on adrenal gland physiology. Eur J Pharmacol, 660: 171-180.

Cheng LZ, YF Wang and CP Zong, 2003. Histology and Embryology. Shanghai Science and Technology Literature Press, Shanghai, China, pp: 173-186.

Clark LS, DC Pfeiffer1 and DF Cowan, 2005. Morphology and histology of the Atlantic bottlenose dolphin (*Tursiops truncatus*) adrenal gland with emphasis on the medulla. Anat Histol Embryol, 34: 132-140.

Coupland RE, 1965. Electron microscopic observations on the structure of the rat adrenal medulla. J Anat, 99: 231-254.

Fadhil AL and WC Stephen, 1991. Microscopic anatomy of the baboon (*Papio hamadryas*) adrenal Medulla. J Anat, 178: 213-221.

Mataoui-Mazari, H, A Zaina, K Farida and M Antoine, 2011. Identification, cloning and regulation of cDNA encoding Aldo–Keto Reductase 1B7 in the adrenal gland of two saharan rodents *Meriones libycus* (Libyan jird) and *Gerbillus gerbillus* (gerbil). Gen Comp Endocrinol, 174: 292-300.

Keegan CE and GD Hammer, 2002. Recent insights into organogenesis of the adrenal cortex. Trends Endocrinol Metab, 13: 200-208.

Luo K, 2000. Organic Histology of Chinese Buffalo. Ruiyu Press,Taiwan, China, pp: 77-100.

Mesiano S and RB Jaffe, 1997. Developmental and functional biology of the primate fetal adrenal cortex. Endocrinol Rev, 18: 378-403.

Padgett DA and R Glaser, 2003. How stress influences the immune response. Trends Immunol, 24: 444-448.

Peng KM, 2009. Animal Histology and Embryology. High Education Press Beijing, China, pp: 119-129.

Sebag JA and PM Hinkle, 2010. Regulation of G protein-coupled receptor signaling: specific dominant-negative effects of melanocortin 2 receptor accessory protein 2. Sci Signal. 3: ra28.

Webb TR and AJ Clark, 2010. Minireview: the melanocortin 2 receptor accessory proteins. Mol Endocrinol. 24: 475-484.

Wei M, 2009. The protected rare animal in the world-Rhinoceros. Scientific Showplace, 3: 4. (In Chinese)

Zacharowski K, PA Zacharowski, A Koch, A Baban, N Tran, R Berkels, C Papewalis, K Schulze-Osthoff, P Knuefermann, U Zahringer, RR Schumann, V Rettori, SM McCann and SR Bornstein, 2006. Toll-like receptor 4 plays a crucial role in the immune-adrenal response to systemic inflammatory response syndrome. Proc Natl Acad Sci USA, 103: 6392-6397.