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Comparative Histology of the Malayan Pangolin Kidneys in Normal and Dehydration Condition

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ABSTRACT

The kidney of Malayan Pangolins *(Manis javanica)* in normal and dehydrated conditions were collected for a histological study. The microscopic morphology of normal kidney was similar to those of other mammals, except the prominence of proximal straight tubules. The tubules were lined by high columnar epithelial cells which were higher than in proximal convoluted tubules. The kidney of dehydrated pangolin showed intense acidophilic staining in proximal straight tubules. The lumen of affected tubules was narrower due to swollen tubular epithelial cells. Certain tubular cells were mildly vacuolated. These appearances were the signs of degenerative cell. Since the proximal straight tubules needed more blood supply than the other due to high metabolic activity, it was likely that the proximal straight tubules were more prone to hypoxia than other kinds of renal tubules when the animal was in starvation and dehydration.

Key words: Malayan pangolin kidney, dehydration, starvation, proximal straight tubule, histology

INTRODUCTION

Malayan Pangolin *(Manis javanica)* is a species of pangolins found in Thailand and other countries in Southeast Asia such as Philippines, Indonesia, Myanmar, Cambodia, Laos, and Vietnam. Currently, the Malayan Pangolin is an endangered species which has been listed on Appendix II of the CITES. This mammal is less than 1 meter long and its back and tail are covered with large, overlapping, horny scales. Its belly and chest are covered with soft hair. It protects itself by hiding from predator, rolling up when threatened and injecting the bad smelling liquid from its anal gland. All species of pangolin has poor vision and hearing. It locates its prey by scent. The natural foods of this insectivore are ants and termites taken from nests on trees or on the ground where are also its habitats. From the harmless behavior, it is intensively hunted for skin, meat and scales, which are believed to be sources of medicines for relieving fever and curing skin diseases (Humphrey and Bain,1990; Nowak, 1999).

The gross and histological structures of Malayan Pangolin have been studied in some organs (Nisa *et al.*, 2005). However, its urinary tracts have not been studied. Because of its medicinal properties, Malayan pangolins are frequently smuggled for sale in some restaurants. Once the smuggler was captured, pangolins will be quarantined that may cause starvation and dehydration. The kidney which is known to regulate body fluid and electrolyte balance is

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affected by these conditions. 20% of cardiac output is pushed through the glomerular barrier in renal corpuscle to form the filtrate. As the filtrate passes along the renal tubule, 99% is reabsorbed back into the blood stream, whereas 1% drips from the papillary ducts as urine (Henrikson,1998). The nephron consisting of renal corpuscle and renal tubules is the main part of kidney which is responsible for this function. Renal corpuscle acts in urine filtration mainly influenced by water consumption and juxtaglomerular system. The general mechanism of water and sodium reabsorption is dominantly in renal tubules, especially in proximal tubules, influenced by several factors. In addition, collecting tubule is also involved in this regulation (Rennke *et al*., 2007). In dehydrated condition, histological morphology of these structures is trended to be changed, either by physiologically or pathologically. The histological study of kidney of Malayan pangolin in normal compare to dehydration was the objective of this work to clarify this hypothesis.

MATERIALS AND METHODS

The kidney samples were collected from fully mature Malayan pangolin carcasses which were seized from smuggler and quarantined at Khao Pratub-Chang wildlife breeding center. Two were dead after arriving at the center and two were starved for at least 7 days since they rejected any feeding which was not their natural food. All samples were fixed in Carnoy's fixative. The tissues were embedded in paraffin, cut at 5 µm thickness and stained with Hematoxylin and Eosin (H&E) according to standard histological methods (Luna, 1960). The microscopic structures of kidney were observed under light microscope and photographs were taken.

RESULT

The microscopic appearance of normal Malayan pangolin kidney showed the cortex, which was underneath dense connective tissue capsule, and the medulla. The renal cortex was divided into cortical labyrinths and medullary rays. The cortical labyrinth contained renal corpuscles surrounded by proximal and distal convoluted tubules. These tubules were lined by simple cuboidal epithelium. However, the proximal tubular cells were stained with more eosinophilic and the lumen of proximal tubule was narrower caused by cellular microvilli at the apical border (brush border). The macula densa was found as the packed epithelial cells on one site of distal tubule adjacent to afferent arteriole near the vascular pole of renal corpuscle. The medullary ray of cortex was composed of proximal and distal straight tubules and collecting tubules were in parallel arrangement. The epithelial lining of distal straight tubules resembled distal convoluted tubules but proximal straight tubular cells became columnar (Figure 1). The cells of collecting tubules were cuboidal which were lower than distal tubule. The renal medulla also presented profiles of tubular structure consisting of distal straight tubules, thin segments and collecting tubules. The proximal straight tubule was absent in medulla. The thin segments were lined by low cuboidal or simple squamous epithelium. From outer to inner medulla, distal straight tubule disappeared and collecting tubules were taller and became the papillary ducts which left the medulla into minor calyx. The boundary between renal cortex and medulla was located by arcuate vessels.

Dehydrated Malayan pangolin kidney showed similar microscopic appearance to the normal one. The renal corpuscle and convoluted tubules were in normal condition although the macula densa was not clearly seen. However, there were some lesions confined to the medullary ray. The predominant lesion was within the cytoplasm of proximal straight tubular epithelial cells. The affected cells were swollen and their cytoplasm was hypereosinophilic and mildly vacuolated. The

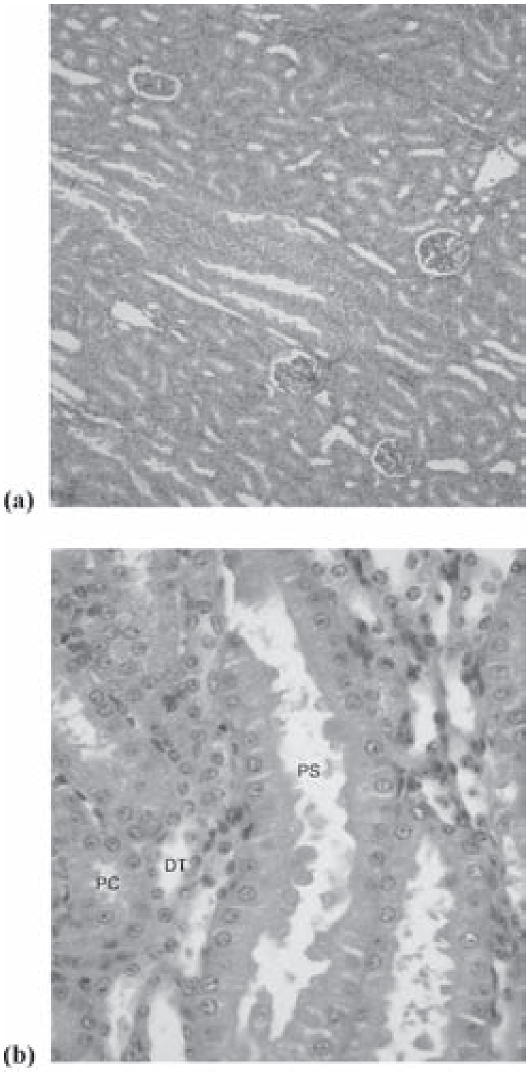


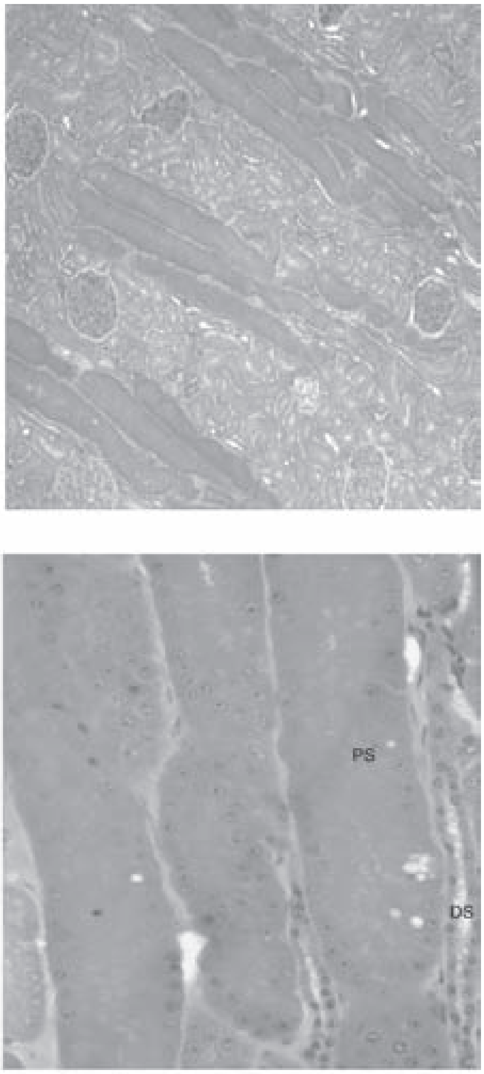
Figure 1 The microscopic appearance of normal Malayan pangolin kidney
(a) Cortical labyrinth and medullary ray in renal cortex (20x)
(b) Proximal and distal tubules in medullary ray. The proximal straight tubular epithelial cells were high columnar where as proximal convoluted tubular cells were cuboidal (40x)
(PS = proximal straight tubule; PC = proximal convoluted tubule; DT = distal tubule).

lumen of the affected tubule was thus narrower. There was no significant microscopic change of the nuclei of the affected cells. Although the lesions were observed in kidney of dehydrated pangolin, the renal medulla was not affected by this condition (Figure2).

DISCUSSION

The microscopic features of cortical labyrinth and renal medulla of normal Malayan Pangolin kidney were similar to those of other mammals. The difference was obviously seen at the site of medullary rays especially the thickening of proximal straight tubular epithelial lining. In most species, the proximal convoluted tubule is actively functioned. More than fifty percents of water and sodium are reabsorbed here (Rennke *et al*., 2007). The proximal straight tubular cells are usually less developed than convoluted tubular cells. They are mostly shorter and contain less and shorter microvilli (Henrikson, 1998). In contrast, the proximal straight tubule of normal Malayan pangolin kidney in this study was lined by simple columnar epithelium with prominent brush border. This evidence indicated that the proximal straight tubule of pangolin kidney was more active than the convoluted tubule. In addition, the macula densa was clearly found. This meant the juxtaglomerular apparatus was also a system to regulate body blood pressure and blood volume that entered the afferent arteriole as in other mammals.

In dehydrated Malayan pangolin, the kidney showed microscopic lesion predominately in proximal straight tubule. The swelling of tubular epithelial cells and their vacuolated cytoplasm indicated that proximal straight tubules of dehydrated pangolin were being degenerated. The hypereosinophilic cytoplasm might be due to mitochondria enlargement which was also the degenerative sign. These pathological changes have been reported in renal tubules of other



(a)

(b)

Figure 2 The microscopic appearance of dehydrated Malayan pangolin kidney
(a) Cortical labyrinth and medullary ray in renal cortex: The straight tubules in medullary ray showed intensely eosinophilic staining (20x)
(b) Proximal and distal straight tubules in medullary ray: The lumen of proximal straight tubule was narrower and epithelial cells were swelling, hypereosinophilic and mildly vacuolated
(PS = proximal straight tubule; DS = distal straight tubule) (40x).

mammals attributed to hypoxia, toxins or dehydration (Berdjis, 1978; Anderson *et al.*, 1997). However, the lesions were mostly found in proximal convoluted tubular epithelial lining (Kumar *et al.*, 2007). According to histological study in normal pangolin kidney, proximal straight tubule was more active than the convoluted tubule. This might be why the degenerative lesions were first observed in proximal straight tubule rather than convoluted tubule. As known in renal physiology, the blood from renal artery was finally entered afferent arterioles and then filtered through glomeruli. After filtration, efferent arterioles from glomeruli located in the superficial and middle cortex contributed to peritubular capillary networks of cortical labyrinth and medullary rays. Meanwhile, efferent arterioles from juxtamedullary glomeruli supplied the entire medulla. Starvation and dehydration caused hemoconcentration leading to the decreased renal filtration rate at glomerulus. The concentrated blood in turn supplied the renal tubules in cortex and medulla. This condition induced the function of juxtaglomerular system to enhance renal tubular reabsorption especially at proximal straight tubules which showed high metabolic activity. While they needed more energy to compensate this condition, the nutrient and oxygen in blood were insufficient. This caused hypoxic renal tissues and proximal straight tubules were prone to be the most affected. Consequently, the degenerative changes were found in this site prior to other components.

CONCLUSION

The histology of normal Malayan pangolin kidney was similar to other mammals', except in proximal straight tubules. This finding suggested that proximal straight tubules had higher metabolic activity than proximal convoluted tubules. In dehydrated animal, decreased renal blood supply caused tissue hypoxia predominantly in proximal straight tubules which required more nourishment. This condition led to localized ischemia and degeneration of susceptible tubular cells. However, the affected tubular cells were been necrotic at the time of animal death.

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