Renal Microvasculature in Lyle's Flying Fox (*Pteropus lylei*)

Sirinush Sricharoenvej, Ph.D., Passara Lanlua, Ph.D., Piyanee Sriya, M.Sc.

Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: The purpose of this study was to elucidate the renal microvasculature of Lyle's flying fox.

Methods: The kidneys of twelve adult Lyle's flying foxes of both sexes were processed by using vascular corrosion cast technique combined with SEM.

Results: It was found that arcuate arteries at the corticomedullary junctions give off several interlobular arteries, which run perpendicularly into the renal cortex. The interlobular artery branches into two sets of vessels. Firstly, aglomerular arteriole divides into capsular and peritubular capillary plexus without forming glomeruli. Secondly, an afferent arteriole, a branch of the interlobular artery, breaks into the glomerular capillary plexus that gathers to form a single efferent arteriole. An efferent arteriole gives rise to a peritubular capillary plexus and vasa recta. A peritubular capillary forms a plexus among renal tubules. Vasa recta are straight vessels that run parallel to Henle's loops and collecting ducts in the outer medulla. In addition, vasa recta form U-shaped loops in the inner medulla. Moreover, the fenestrated type of capillaries is observed. It was found that high numbers of the fenestration were seen in the glomerular capillary plexus and venous limbs of vasa recta in the outer medulla. In contrast, fewer knobs were presented in the loops of vasa recta in the inner medulla and peritubular capillary plexus. Both peritubular capillary plexus and vasa recta collect the blood into interlobular and arcuate veins.

Conclusion: In this investigation, the aglomerular arteriole might be an important shunting of the blood, while this animal alters the position immediately. With the advantages of this technique, fenestrated capillaries are demonstrated which are related to the functions of each tubule. Moreover, the microvascular patterns of the kidney in this animal are similar to that in human. Therefore, it is a suitable model for renal microvascular investigation.

Keywords: Kidneys; Lyle's flying fox; Microvascularization; SEM

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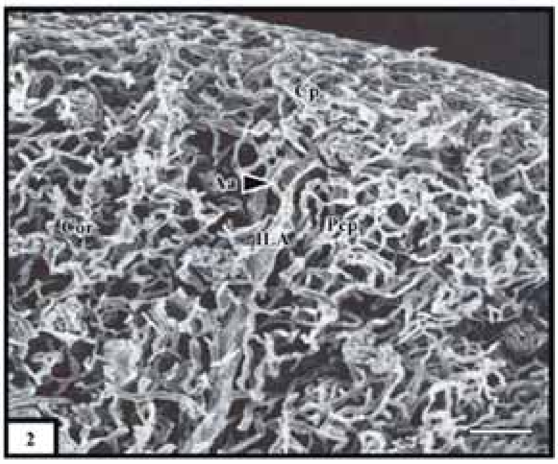
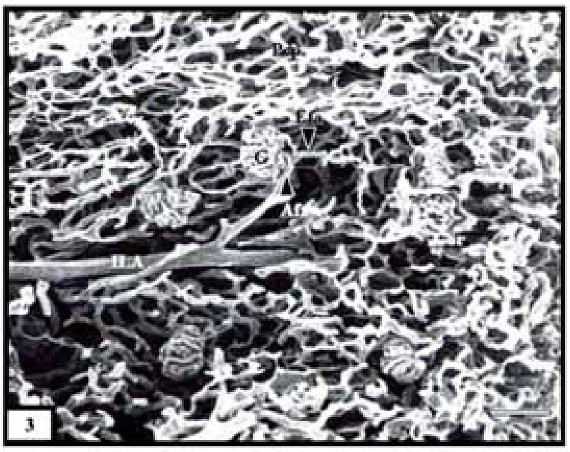
The kidneys are highly vascular organs which play an importance role in maintenance homeostasis and excretion of metabolic waste products. In addition, other functions of this organ are syntheses and secretions of two hormones: erythropoietin and renin. Besides, the kidneys are also involved in the process of vitamin D hydroxylation, turning it into its active form.[1,2] Accordingly, the normal physiological functions of the kidney, such as glomerular filtration, the control of blood pressure and the countercurrent exchange are maintained by the sufficient blood supply. Therefore, new knowledge, concerning to renal vasculature and microvasculature, is required to understand these specific functions of the kidney.

Many techniques and/or methods have been developed to examine renal blood supply in various animals. With various applications of dye injections, it was demonstrated that the system only exhibits the main blood supply of human,[3] opossum, dog, cat, rabbit, and guinea-pig.[4] After an Indian ink injection, the kidneys of a rat[5] were processed by serial sections of a histological technique. By using these two techniques, renal vasculature was presented in two dimensions. The three-dimensional configuration of renal blood vessels was achieved by an intravascular injection of silicone rubber in a dog.[6] However, these mentioned injected media can neither flow into the capillary level nor be kept in place after the renal tissue digestion. In addition, specimens can be viewed under a stereomicroscope, but not a scanning electron microscope (SEM). To overcome these disadvantages, a casting media, Batson's plastic mixture, was extensively developed to withstand the heat of the electron beam.[7] By using this plastic mixture in the vascular corrosion cast technique with SEM, the vascular replica clearly shows the distributions of the blood supply in three dimensions, the whole system of the blood circulation, and types of capillary such as continuous, fenestrated and sinusoidal capillaries. With these advantages of the technique, it was applied in a detailed study of vascular supply of kidneys in the rat,[8] macaque monkey,[9] black bear,[10] and common tree shrew.[11]

Although the studies of blood supply in the kidneys of various animals had been done, the report

Correspondence to: Sirinush Sricharoenvej
E-mail: sissc@mahidol.ac.th





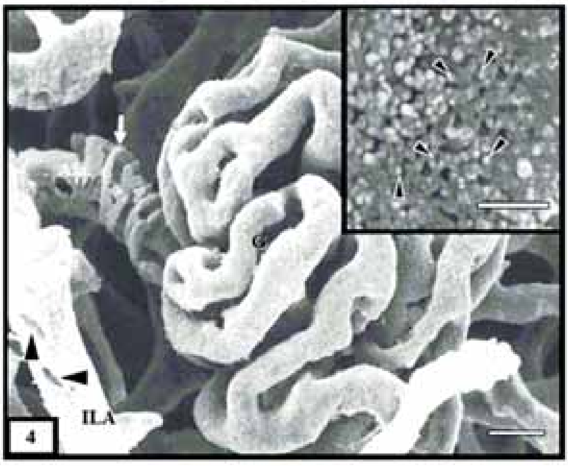


Fig 1. SEM micrograph of the renal vascular cast in the Lyle's flying fox showing the different arrangement of the blood vessels in the cortex (Cor) and medulla (Med). At the corticomedullary junction (CM), the arcuate artery (AA) branches into interlobular artery (ILA) that gives rise to two sets of branching; aglomerular (Aa) and afferent arterioles (Afa). Moreover, the efferent arteriole (Efa) at the CM continues to be the vasa recta (VR). Peritubular capillary plexus (Pcp), Interlobular vein (ILV) and arcuate vein (AV). Bar = 200 μm.

Fig 2. SEM micrograph of the renal vascular cast in the outer cortex (Cor). The interlobular artery (ILA) ramifies into aglomerular arteriole (Aa) that breaks into peritubular capillary (Pcp) and capsular plexuses (Cp). Bar = 100 μm.

Fig 3. SEM micrograph of the vascular cast in the inner cortex (Cor). The second branch of the interlobular artery (ILA) is an afferent arteriole (Afa) that divides into glomerular capillary plexus (G). This plexus gathers to only one efferent arteriole (Efa) that branches again into the peritubular capillary plexus (Pcp). Bar = 100 μm.

Fig 4. SEM micrograph of the vascular cast of the glomerular capillary plexus (G). The interlobular artery (ILA) branches into afferent arteriole (Afa) that divides into the G. Endothelial nuclear imprint (arrowheads) and undigested smooth muscle cells (an arrow) are observed. Bar = 10 μm. Inset: High magnification of the glomerular capillary cast shows numerous knobs on its surface, presenting the fenestration, (arrowheads). Bar = 1 μm.

concerning to the fenestrations of the renal blood vessels, was obscured and limited. Additionally, the data about the renal microvasculature in Lyle's flying fox *(Pteropus lylei)* has not been illustrated. Although many scientists have studied this creature in several aspects,[12] there is no information about the renal microvasculature. Therefore, it is very interesting to use vascular corrosion cast technique in conjunction with SEM to study the renal microvasculature in this mammal.

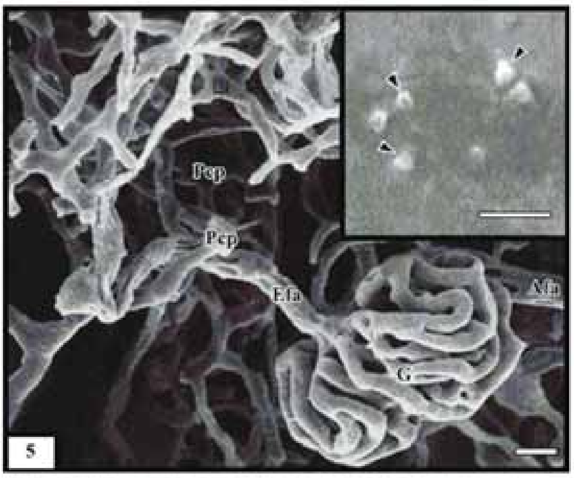
MATERIALS AND METHODS

Twelve adult Lyle's flying foxes (*Pteropus lylei*) of both sexes, weighed between 350-450 g, were used. After each animal was anesthetized by halothane inhalation, Batson's no.17 plastic mixture injection and the preparation of the renal microvascular casts for the study with SEM were done according to the process that has been previously described by Sricharoenvej and her coworkers.[13]

RESULTS

By using the vascular corrosion cast technique in conjunction with SEM, the renal vascular cast was clearly elucidated. At the low magnification, the kidney of Lyle's flying fox was shown to be a highly vascularized organ. It was revealed that the arcuate artery branches into interlobular arteries at the corticomedullary junction (Fig 1). Then, the interlobular arteries run perpendicularly through the renal cortex before they give off two branches: aglomerular and afferent arterioles.

Firstly, aglomerular arterioles branches into capsular and peritubular capillary plexuses that supply the capsule



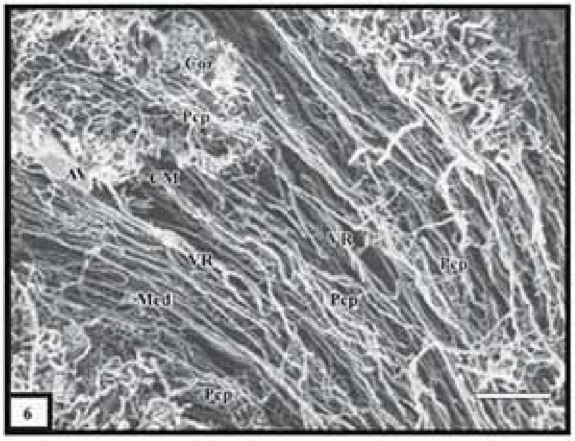
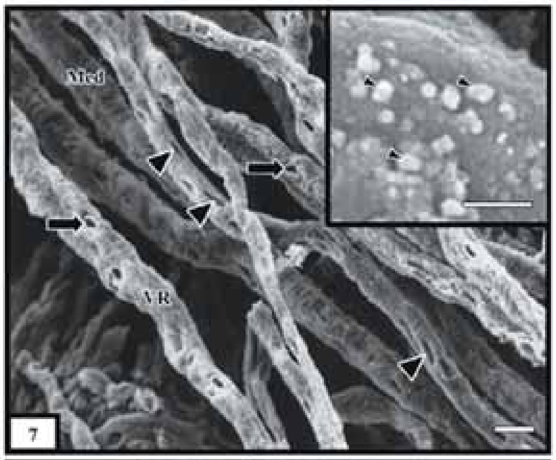


Fig 5. The afferent arteriole (Afa) breaks into the glomerular capillary plexus (G) that gathers into the efferent arteriole (Efa). Then, the efferent arteriole divides into the peritubular capillary plexus (Pcp). Bar = 10 μm. Inset: High magnification of the peritubular capillary plexus illustrates the knobs on its surface that represent the fenestrated capillary. Bar = 1 μm.

Fig 6. The peritubular capillary plexus (Pcp) is found in both cortex (Cor) and medulla (Med). The density of the Pcp in the Cor is denser than that in the Med. At the corticomedullary junction (CM), both Pcp and vasa recta (VR) collect into the arcuate vein (AV). Bar = 200 μm.



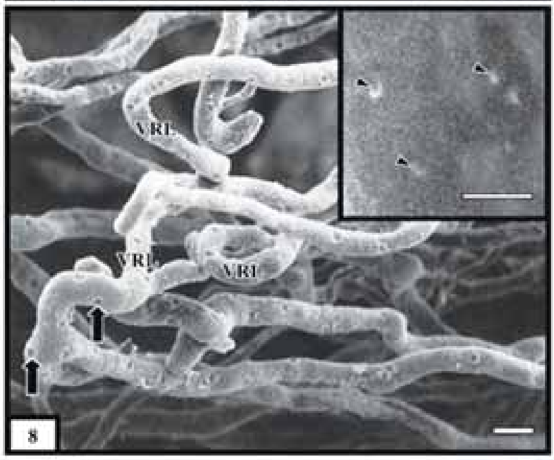


Fig 7. The descending arterial (arrowheads) and ascending venous limbs (arrows) of vasa recta (VR) in the outer medulla (Med) arrange in the parallel fashion along the renal tubules in this area. Bar = 50 μm. Inset: High magnification of the venous limb of vasa recta in the outer medulla presents many knobs of fenestrations (arrowheads). Bar = 1 μm.

Fig 8. The loops of the vasa recta (VRL) are found in the inner zone of the medulla, and there are endothelial nuclear imprints (arrows) on its surface. Bar = 50 μm. Inset: High magnification of the loop of the vasa recta shows the knobs (arrowheads) of fenestrations on its surface. Bar = 1 μm.

and outer cortex, respectively (Figs 1-2). Secondly, afferent arterioles are branches of the interlobular artery (Figs 1, 3). Each afferent arteriole divides to form glomerular capillary plexus (Figs 3-5). Then, the capillaries in the glomerulus gather to form a single efferent arteriole (Figs 1, 3, 5). It was found that the diameter of the efferent arteriole is smaller than that of the afferent arteriole. The efferent arteriole brakes up into two sets of vessels: peritubular capillary plexus and vasa recta (Figs 1, 3, 5). Firstly, the peritubular capillary plexus was observed in both cortex and medulla. Furthermore, the arrangement of this plexus in the cortex is denser than that in the medulla (Figs 1, 6). Lastly, the vasa recta run parallel to the ducts of the medulla (Figs 1, 6). After the efferent arteriole has given off these vessels at the corticomedullary junction, they descend into the outer medulla, turn back, and ascend in the inner medulla like U- shaped loops. Moreover, various lengths of these loops were observed in the medulla. It was noticed that the smaller descending parts were arterial limbs, whereas the larger ascending ones were venous limbs (Figs 7-8). Then, these two sets of vessels collect into either an interlobular or an arcuate vein (Figs 1, 6).

At the high magnification, the fenestrated capillary was found in the kidney of Lyle's flying fox. This type of capillary has pores on its wall. After the Batson's plastic mixture was injected into the lumen of a fenestrated capillary, the injected media leaked out through the pores. Therefore, they were seen as round regular knobs on the surface of the vascular cast after tissue digestion. Moreover, the fenestrated capillary was observed in the glomeruli, peritubular capillary plexus, and the ascending venous limbs of vasa recta in the outer and inner medulla (insets of Figs 4-5, 7-8). The numbers of the fenestration are high in the glomeruli and the ascending venous limbs of vasa recta at the outer medulla, whereas they decrease in the peritubular capillary plexus and the loops of vasa recta in the inner medulla.

DISCUSSION

Concerning to the microvasculature of the kidney, the interlobular artery normally gives off several afferent arterioles. Each afferent arteriole breaks into glomerular capillary plexus that collects into only one efferent arteriole. This type of capillary arrangement has been observed in bat, human,[1] rat,[5,14] dog,[6] cat,[15] and monkey.[16] However, there are multiple afferent and efferent arterioles in the kidneys of aged people.[17] The diameter of the afferent arteriole is larger than that of the efferent one. Normally, the afferent arterioles are resistance vessels, so the blood pressure in these vessels is higher than that in the efferent one. The high blood pressure in the afferent arterioles enables the filtration to occur in the glomeruli. Additionally, the blood pressure in the efferent arterioles has to be sufficient to carry the blood to the peritubular capillary plexus.[2] Not only that the efferent arterioles in this species of bats branch into peritubular capillary plexus but also vasa recta that are the same pattern as in human,[1,2] rat,[5] dog,[6] black bear,[10] common tree shrew,[11] cat,[15] and monkey.[16] In the renal cortex, the capillary casts of the peritubular capillary plexus arrange in network fashion, whereas the most of blood vessels in the renal medulla run in parallel formation. So that, it can be expected that the density and the pattern of renal vascular supply are similar to the arrangements of renal tubules and collecting ducts.[1]

Furthermore, it can be demonstrated that at the high magnification of the SEM, there are knob-liked fenestrations on the wall of the renal vascular cast. There are numerous and regular distributed knobs on the wall of glomerular capillary plexus in this animal. In addition, the fenestrated type of the capillary in the glomerulus are observed by using transmission electron microscopy in human,[1,2] dog,[6] black bear,[10] monkey[16] and SEM in common tree shrew.[11] The fenestration in the glomeruli associates with selective ultrafiltration. In this study, it was shown that the capillary in the peritubular capillary plexus is also of the fenestrated type. The amount of the fenestration in this plexus is lower than that in the glomeruli. To maintain its normal functions, the fenestration on the peritubular capillary plexus involves in exchanges of water and solutes between interstitial fluids and the capillary plexus.[1,2,17] The fenestration was also observed on the ascending venous limbs of vasa recta. Interestingly, the number of the fenestration on the ascending venous limbs of vasa recta is denser in the outer medulla, and gradually decreases toward the loops of vasa recta in the inner medulla. In the vasa recta, the osmolarity of tubular fluid at the outer medulla is lower, whereas that at the inner medulla is higher.[2,11] Additionally, the proximity and large contacted surfaces between the descending arterial and ascending venous limbs of the vasa recta are to facilitate rapid movement of diffused substances between these two limbs. Thus, these characteristics are important in countering current exchanges of urine concentration.[1,2]

In addition, not only that the interlobular artery gives rise to the afferent arterioles, but it also gives rise to the aglomerular arterioles. These aglomerular arterioles are also found in the kidneys of common tree shrew[11] and rat.[18] The aglomerular arterioles branch into peritubular capillary network, which directly supplies the tubules of the renal cortex. In this bat, these shunts of aglomerular arterioles might supply sufficient blood to the kidneys, when this animal abruptly changes the position such as hanging upside down on trees to flying and vice versa. Moreover, some investigators suggested that aglomerular arterioles might support shunting mechanism in hypotensive state.[11,18] In this mammal, the venous blood of peritubular capillary plexus and vasa recta drains into either an interlobular or an arcuate vein. In addition, this arrangement is similar to that of human,[1] rat,[5] dog,[6] black bear,[10] common tree shrew,[11] cat,[15] guinea pig,[19] and pig.[20] On the other hand, the venous drainage pattern of the kidneys in Lyle's flying fox differs from that in the reptile, the system of which is important in tubular secretion, especially uric acid.[21,22]

CONCLUSION

In this investigation, the microcirculation of the kidney in Lyle's flying foxes is demonstrated by using vascular corrosion cast technique combined with SEM. An arcuate artery gives off an interlobular artery that is further divided into two branches: aglomerular and afferent arterioles. The first branch is a shunting vessel that supplies the capsule and tubules in the outer cortex. The second branch ramifies into a glomerular capillary plexus that collects into one efferent arteriole. The last arteriole brakes out into a peritubular capillary plexus and vasa recta that finally collects into interlobular and arcuate veins. Furthermore, the fenestrated capillaries were observed in the glomeruli, ascending venous limbs of vasa recta and peritubular capillary plexus. The numbers of the fenestration are related to the functions of the renal tubules. Moreover, there are similarities of the microvascular patterns in the kidneys between this species and human.

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บทคัดย่อ

การศึกษาโครงหลอดเลือดโดยละเอียดของไตในค้างคาวแม่ไก่

ศิรินุช ศรีเจริญเวช ปร.ด., ภัสรา ลานเหลือ ปร.ด., ปิยาณี ศรียา วท.ม.
ภาควิชากายวิภาคศาสตร์, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กทม. 10700, ประเทศไทย.

วัตถุประสงค์: เพื่อศึกษาระบบไหลเวียนเลือดอย่างละเอียดของไตในค้างคาวแม่ไก่
วิธีการ: สัตว์ทดลองทั้งสองเพศ จำนวน 12 ตัวใช้ในการศึกษาโครงหลอดเลือดอย่างละเอียดโดยเทคนิค vascular corrosion cast ร่วมกับกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด
ผลการศึกษา: Arcuate artery ที่บริเวณ corticomedullary junction จะให้แขนงเป็น interlobular artery หลายเส้นทอดตัวตั้งฉากไปยังเนื้อไตส่วนนอก (renal cortex) โดย interlobular artery จะแตกแขนงออกเป็นหลอดเลือดสองชุด ชุดแรกคือ aglomerular arteriole ซึ่งแตกแขนงให้เป็น capsular และ peritubular capillary plexusesในบริเวณ outer cortex ชุดที่สองคือ afferent arteriole ซึ่งแตกแขนงต่อไปเป็น glomerular capillary plexus ก่อนที่จะรวบรวมออกเป็น efferent arteriole 1 เส้น แล้วจึงแตกแขนงให้ peritubular capillary plexus และ vasa recta โดยส่วน peritubular capillary plexus จัดเรียงตัวเป็นร่างแหไปเลี้ยงส่วนของ renal tubule ในขณะที่ vasa recta จัดเรียงตัวขนานกับ Henle's loop และ collecting duct ลงมาในส่วนของ outer medulla และวกกลับที่ inner medulla ยิ่งไปกว่านั้นยังพบว่าหลอดเลือดฝอยในไตของค้างคาวแม่ไก่นั้นส่วนใหญ่เป็นชนิด fenestrated capillary ซึ่งพบมากที่ glomerular capillary plexus และ venous limb ของ vasa recta บริเวณ outer medulla โดยจำนวนของ fenestration จะพบน้อยลงที่ loop ของ vasa recta บริเวณ inner medulla และ peritubular capillary plexus หลังจากนั้นเลือดจากทั้ง peritubular capillary plexus และ vasa recta ถูกรวบรวมเข้าสู่ interlobular และ arcuate veins
สรุป: จากการศึกษาในครั้งนี้พบว่า aglomerular arteriole น่าจะมีความสำคัญในกระบวนการ shunting ของเลือดในไตในสภาวะที่สัตว์เปลี่ยนอิริยาบถอย่างรวดเร็ว และด้วยจุดเด่นของเทคนิค vascular corrosion cast ร่วมกับ SEM นี้สามารถแสดงให้เห็น fenestration บนหลอดเลือดฝอย ซึ่งสัมพันธ์กับหน้าที่ของท่อบริเวณนั้น ๆ นอกจากนี้ลักษณะของการจัดเรียงตัวของหลอดเลือดยังคล้ายคลึงกับคนมาก ดังนั้นสัตว์ชนิดนี้จึงเหมาะสมที่จะเป็นสัตว์ทดลองในการศึกษา microcirculation ของไต