Adrenal Microvasculature in the Lylei's Flying Fox (*Pteropus lylei*)

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ABSTRACT

Objective: The purpose of this study was to elucidate the microvasculature of the adrenal glands in the Lylei's flying fox.
Methods: The adrenal glands of the Lylei's flying foxes were processed in the histological technique and vascular corrosion cast technique combined with the SEM.
Results: Upon reaching the gland, the adrenal arteries divided into the cortical and medullary arteries. Firstly, the cortical arteries gave off subcapsular and true cortical capillary plexuses. Few loop cortical arteries were observed. At the corticomedullary junction, true cortical capillary plexus formed two groups, large peripheral venous radicles and sinusoidal medullary capillary plexus. Secondly, the medullary arteries supplied the inner cortex and medulla as true medullary capillary plexus. Therefore, the medullary capillary plexus composed of branches from cortical and medullary arteries. The medullary capillary plexus became a tributary of deep venous radicle. Both peripheral and deep venous radicles drained into the collecting, central, and adrenal veins, respectively. Furthermore, some medullary capillary plexus directly drained into the central vein without gathering into the collecting veins.
Conclusion: Not only the microvascular connections in the cortex and medulla, but also several channels of the venous drainage were found in the glands of this animal model. Especially, the direct connections between the medullary capillary plexus and the central vein have not been demonstrated in other animal models. These direct routes may supply the sufficient blood to this organ, when the animal suddenly alters the positions. These findings also support the internal control of the cortex over the medulla. In addition, the pattern of adrenal microvascularization in this animal is similar to that in human. So that, this mammal is a suitable model for microvascular investigation.

Keywords: Adrenal glands; Lylei's flying fox; Microvascularization; SEM

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One of an important endocrine gland is the adrenal gland. Its parenchyma can be divided into cortex and medulla that differ in their embryonic origins, types of secretion, and functions.[1,2] The functions of this outer adrenal cortex are secretions of several steroid hormones; as well as the inner adrenal medulla secretes catecholamines. In addition, the cortex and medulla are derived from mesoderm and neural crest cells, respectively.[3] Even though these two parts of the gland are embryologically different, their functions are related.[4] In fact, it has been accepted that glucocorticoids, synthesized in the adrenal cortex, control the adrenaline biosynthesis in the medulla. This cortical control over the medulla is mediated by blood vessels.[1,2] Moreover, the normal functions of the adrenal glands are maintained by its blood supply. As a result, it is very important to study the microcirculation in this gland that connects endocrine and nervous systems.[1,2,5] Furthermore, the vascular arrangement of the gland has been intensively studied in various animals by two and three dimensional techniques.[6-11] One of the animal models, which has not been investigated in this organ, is the Lylei's flying fox. This animal, which is classified in Megachiroptera, can abruptly change its positions such as hanging upside down on tree to flying. Consequently, the microvascular system of this animal is interesting. In addition, the detailed vascular connections between adrenal cortex and the medulla have been a subject of controversy.[12,13] Therefore, the adrenal microvasculature had been elucidated in this study.

MATERIALS AND METHODS

Fifteen adult Lylei's flying foxes of both sexes, weighing between 300-500 g, were used. The animals were divided into two groups. The first group, 3 animals,

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was processed for the histological study of the adrenal glands. Under halothane anesthesia, the thoracic cage of each animal was cut to expose the heart. Then, 0.05 ml of heparin (Leo 5,000 iu/ml) was immediately injected into the left ventricle. After the perfusion with 0.9% NaCl solution, 500 ml of Bouin's solution was perfused to preserve the tissues. Then, the tissues were processed in the conventional histological technique, sectioned at 6 μm thick and stained with hematoxylin and eosin. The second group, 12 animals, was used for the study of microvasculature in the adrenal glands by processing vascular corrosion cast technique combined with a scanning electron microscope (SEM). After 0.9% NaCl perfusion, Batson's no. 17 plastic mixture was injected into the ascending aorta. The adrenal glands were removed and corroded in 40% KOH solution at room temperature for 4-7 days with daily change of the solution. Then, the corroded specimens were carefully rinsed in tap water and gently washed in the distilled water to remove the remaining tissues. The adrenal vascular casts were left air-dried at room temperature, dissected under a stereomicroscope before coating with gold/ palladium and then examined under the SEM at the accelerating voltage of 15 kV.

RESULTS

Histological observation of the adrenal glands

Each gland was surrounded by a thin capsule of dense fibrous connective tissue. The parenchyma consisted of outer adrenal cortex and inner adrenal medulla. The adrenal cortex can be divided into three distinct layers, zona glomerulosa, zona fasciculata, and zona reticularis. At the corticomedullary junction, sinusoidal capillaries were found, which were called peripheral venous radicles. The adrenal medulla contained irregular networks of cell cords. Among these cords, the continuous and sinusoidal capillaries were presented as true and sinusoidal medullary capillary plexuses, respectively. The collecting veins became tributaries of one large central vein (Fig 1a).

Microvasculature of the adrenal glands

After the adrenal vascular casts were observed under the SEM, it was shown that several adrenal arteries deeply penetrated into the gland. Then, these arteries branched into cortical and medullary arteries (Fig 1b). Each cortical artery initially gave off several branches that anastomosed with the other branches to form subcapsular capillary plexus. It consisted of a single layer of free branching and anastomosing capillaries investing the whole gland (Fig 2). Then, this plexus deeply penetrated into the adrenal cortex to form true cortical capillary plexus, which supplied three zones of the parenchyma: zona glomerulosa, zona fasciculata and zona reticularis (Fig 2). Few cortical arteries penetrated into the zona glomerulosa and upper part of zona fasciculata, and then these arteries curved back to subcapsular capillary plexus. These arteries were called loop cortical arteries or arterial loops (Fig 3a). At the corticomedullary junction, true cortical capillary plexus formed two groups. The first group of this true cortical capillary plexus converged into large sinusoidal capillaries as peripheral venous radicles that situated at the peripheral part of the medulla (Figs 1a, 1b, 3b). The second group of the capillary plexus passed the corticomedullary junction to go deeply into the adrenal medulla. Then, the smaller sinusoidal capillaries formed sinusoidal medullary capillary plexus (Fig 3b).

The second branch of the adrenal artery was medullary artery. The medullary artery traversed into the adrenal medulla and gave off small branches to supply the inner cortex, especially the zona reticularis. Furthermore, these arteries divided into continuous capillaries in the medulla that formed true medullary capillary plexus (Figs 1b, 2, 3b). Therefore, the medullary capillary plexus consisted of sinusoidal and true medullary capillary plexuses. Then, these plexuses became tributaries of the deep venous radicle. Both peripheral and deep venous radicles drained into the collecting and central veins, respectively. Moreover, some medullary capillary plexuses directly drained into the central vein without gathering into the collecting veins (Figs 1a, 3b). It should be noted that the adrenal medulla received not only the venous blood from the adrenal cortex through the vessels at the corticomedullary junction, but also the arterial blood directly from the medullary arteries. After that the central vein emerged from the gland, it was called an adrenal vein (Fig 1b). At the high magnification, the fenestrations were observed on the adrenal vascular corrosion casts. The appearance of the fenestration looked like regular-sized knobs or blebs on the surface of the casts.[11] In addition, this characteristic was found in the capillaries of all layers in the parenchyma of the adrenal cortex. The amount of the fene stration in the zona reticularis was less than those in zona glomerulosa and zona fasciculata (Fig 2a-c).

DISCUSSION

With the advantages of the injected plastic replica combined with the SEM, the three dimensional arrangement of the adrenal microvasculature is clearly shown. The adrenal arteries give off cortical and medullary arteries, which have been demonstrated in human,[3-5] cat,[7] monkey,[8] dog,[9] rat,[10] and common tree shrew.[11] Then, these cortical arteries branch into subcapsular capillary plexus,

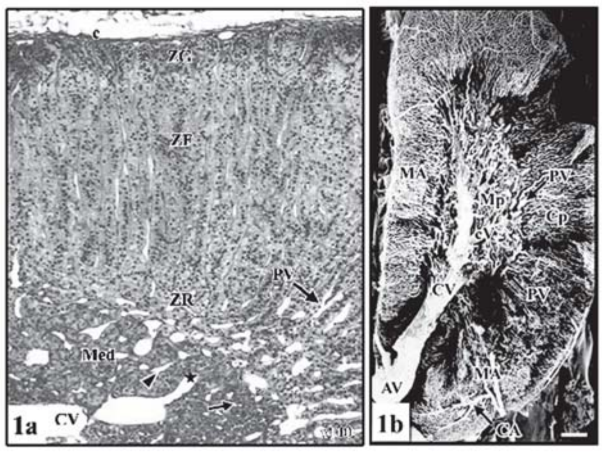

Fig 1. 1a: Light micrograph shows three layers of adrenal cortex: zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR). At the junction of cortex and medulla (Med), the peripheral venous radicle (PV) is observed. In the medulla, there are two types of capillaries: sinusoidal medullary capillary plexus (an arrowhead) and true medullary capillary plexus (an arrow). Capsule (c), collecting vein (★), central vein (CV) . x100 1b: SEM micrograph of the adrenal vascular cast after fine dissection. The adrenal artery divides into cortical (CA) and medullary (MA) arteries. The CA ramifies into cortical capillary plexus (Cp) that collects into peripheral venous radicle (PV). Medullary capillary plexus (Mp), collecting vein (cV), central vein (CV) and adrenal vein (AV). Bar = 250 μm.

which is similar to those in cat,[7] monkey,[8] dog,[9] rat,[10] and common tree shrew.[11] In contrast, the cortical arteries in human[3-5] and dog[9] branch into capsular capillary plexus. Both subcapsular and capsular capillary plexuses supply the capsules of the adrenal glands. It is likely that the thickness of the capsule may effect on the positions of its capillary. For example, if the thickness of the capsule is thin, the capillaries situate in the subcapsular space, called subcapsular capillary plexus. On the other hand, the capillaries can penetrate and stay in the thick capsule as capsular capillary plexus like those in human[3-5] and dog.[9] Moreover, the cortical arteries send off loop cortical artery or arterial loop, which is also found in the other mammals such as human,[3-5] cat,[7] monkey,[8] dog,[9] and rat.[10] This arterial loop deeply penetrates into zona glomerulosa and zona fasciculata. At the second layer, this loop turns back to the subcapsular capillary plexus. It has been investigated that the cells in the zona fasciculata are the most vulnerable layer for insufficient blood supply. Thus, the arterial loops might be an additional arterial source to supply this zone.

The patterns of the cortical capillary plexus in this animal are similar to those in human,[3-5] cat,[7] monkey,[8] dog,[9] rat[10] and common tree shrew.[11] Furthermore, the cortical capillary arrangement corresponds to the patterns of cells in three layers of the adrenal cortex. For example, the glomus-shaped cells are found in the zona glomerulosa, and the form of the capillaries in this layer is round. In the zona fasciculata, both cells and capillaries are aligned in the parallel straight cord. The arrangement of the capillaries in the zona reticularis is the same irregular characteristic as the cells among them. The capillaries of the adrenal cortex in the Lylei's flying fox are fenestrated type, which are like those in the common tree shrew.[11] The fenestrations on the vascular cast surface are regular-sized knob or bleb like. The fenestrations are found in the zona glomerulosa, zona fasciculata, and zona reticularis. The amounts of fenestrations in the zona glomerulosa and zona fasciculata are much more than that in the zona reticularis. This characteristic might relate to their functions that these outer two zones produce and secrete the essential hormones for metabolism in a large amount.[3] After the hormonal secretion, these substances pass the fenestrations of the capillaries to blood circulation.

The cortical capillary plexus supplies all three layers of the adrenal cortex, and then this plexus passes to supply the adrenal medulla. The level of the oxygen in the cortical capillary plexus is low, when the plexus reaches the inner cortex and adrenal medulla. Consequently, the medullary artery with high oxygenated blood deeply penetrates from the adrenal cortex to supply the adrenal medulla. These features are similar to what have been described in the human,[3-5] rabbit,[6] cat,[7] monkey,[8] dog,[9] rat[10] and common tree shrew.[11] However, this study also clearly presents that the medullary artery gives off small branches to nourish the inner cortex as the same pattern as that in the common tree shrew.[11] In the present study, it has been confirmed that the adrenal medulla receives not only sinusoidal medullary capillary plexus passing from the adrenal cortex, but also continuous type of true medullary capillary plexus from medullary arteries. Such ordinary capillaries have also been reported in the rat,[10] human fetus,[3,4] and common tree shrew,[11] but not in the rabbit and cat.[6,7] It is likely that the major role of the medullary artery is to give off continuous true medullary capillary plexus contributing oxygenated blood to the inner zone of the adrenal cortex and to the adrenal me-

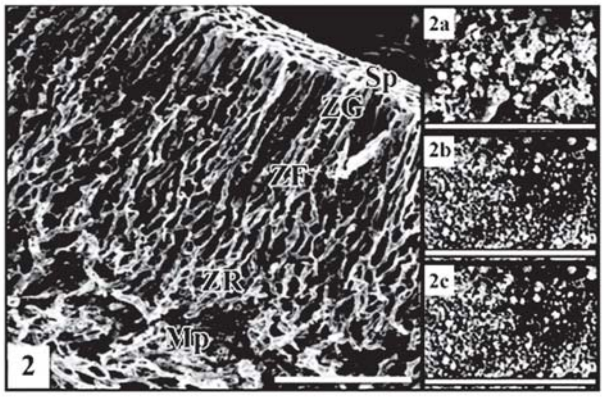

Fig 2. SEM micrograph illustrates vascular patterns in three layers of adrenal cortex: zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR) and medullary capillary plexus (Mp). Subcapsular capillary plexus (Sp). Bar = 500 μm. Inset; 2a: High magnification of fenestrated capillary in the zona glomerulosa presents rough surface with blebs or knobs. Bar = 5 μm. Inset; 2b: High magnification of fenestrated capillary in the zona fasciculate. Bar = 5 μm. Inset; 2c: High magnification of fenestrated capillary in the zona reticularis. Bar = 5 μm.

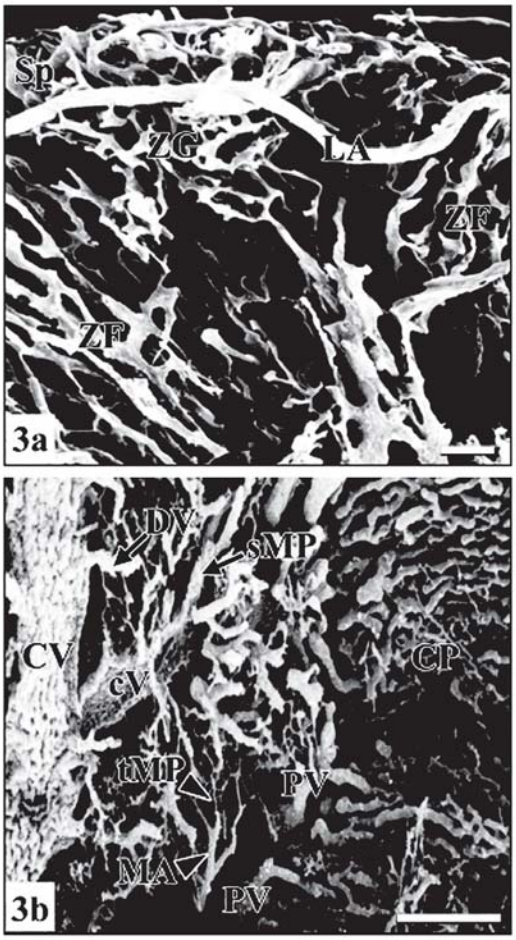

Fig 3. 3a: SEM micrograph shows loop cortical artery (LA) that passes zona glomerulosa (ZG) and zona fasciculata (ZF). This artery turns back to ZG. Subcapsular capillary plexus (Sp). Bar = 100 μm. 3b: SEM micrograph presents the cortical capillary plexus (Cp) that drains into peripheral venous radicle (PV). Both sinusoidal (sMP) and true (tMP) medullary plexuses collect into either collecting vein (cV), central vein (CV), or deep venous radicle (DV). Bar = 500 μm.

dulla. On the other hand, the venous blood in the sinusoidal medullary capillary plexus from the adrenal cortex carries the steroid hormones to influence on the adrenal medulla. Indeed, many investigators have demonstrated that the cortical hormones mediate the synthesis and release of catecholamines in the adrenal medulla.[1,2] It is noteworthy that there is dual blood supply of the adrenal cells, especially in the inner cortex and medulla as mentioned above.

It is clearly shown in this study that some part of true cortical capillary plexus converges into large sinusoidal capillaries as peripheral venous radicles. The deep venous radicles collect blood from sinusoidal medullary capillary plexus of true cortical capillary plexus and true medullary capillary plexus. Both peripheral and deep venous radicles drain into the collecting veins that are tributaries of the central vein. Interestingly, some parts of the medullary capillary plexus directly drain into the central vein. This phenomenon has never been mentioned before in the human,[3-5] rabbit,[6] cat,[7] rhesus monkey,[8] dog,[9] rat[10] and common tree shrew.[11] This characteristic might support enough channels for the adrenaline secretion into the blood circulation. It might be related to the Lylei's flying fox's behavior that is changes its positions abruptly.

CONCLUSION

In summary, the microvasculature of the adrenal gland in the Lylei's flying fox has been investigated in this study. The adrenal arteries branch into cortical and medullary arteries. The cortical arteries send off subcapsular capillary plexus, true cortical capillary plexus and loop cortical arteries. The fenestrated capillaries in the true cortical capillary plexus converge into large sinusoidal capillaries as peripheral venous radicles and smaller sinusoidal capillaries of medullary capillary plexus. Additionally, the medullary arteries give off true medullary capillary plexus. Then, both sinusoidal and true medullary capillary plexuses collect into the deep venous radicles. Both peripheral and deep venous radicles drain into the collecting veins. These veins finally empty into a central vein that emerges from the gland as an adrenal vein. Occasionally, both medullary capillary plexuses directly drain into the central vein which has not been elucidated in the previous reports. It is concluded that there is microvascular connections of cortex and medulla that relate to its functions. Moreover, the adrenal microvascular patterns of the Lylei's flying fox are similar to those of human.

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บทคัดย่อ

การศึกษาโครงหลอดเลือดโดยละเอียดของต่อมหมวกไตในค้างคาวแม่ไก่

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วัตถุประสงค์: เพื่อศึกษาระบบไหลเวียนเลือดอย่างละเอียดของต่อมหมวกไตในค้างคาวแม่ไก่

วิธีการ: สัตว์ทดลองแบ่งออกเป็น 2 กลุ่ม โดยกลุ่มแรกจำนวน 3 ตัว ใช้ในการศึกษาทางจุลกายวิภาคศาสตร์ กลุ่มที่ 2 จำนวน 12 ตัวใช้ในการศึกษาโครงหลอดเลือดอย่างละเอียดโดยเทคนิค vascular corrosion cast ร่วมกับกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด

ผลการศึกษา: แขนงของ adrenal artery ถูกแบ่งออกเป็น 2 กลุ่ม คือ cortical และ medullary arteries โดยที่ cortical artery แตกแขนงให้ subcapsular capillary plexus เพื่อไปเลี้ยง capsule ของต่อมหมวกไต และให้แขนงต่อไปเป็น true cortical capillary plexus เพื่อเลี้ยง parenchyma ในชั้น cortex และพบว่า cortical artery บางส่วนให้แขนงเป็น loop cortical artery เพื่อเลี้ยง cortex ชั้นนอก หลังจากนั้น true cortical capillary plexus จะแบ่งออกเป็น 2 กลุ่ม เมื่อมาถึงบริเวณ corticomedullary junction กลุ่มแรกรวบรวมเป็น peripheral venous radicles ในขณะที่กลุ่มที่สอง ผ่านเข้าชั้น medulla และแตกแขนงให้ sinusoidal medullary capillary plexus เพื่อไปเลี้ยงส่วนของ medulla ส่วน medullary artery จะทอดผ่านชั้น cortex เข้าสู่ชั้น medulla โดยตรง และให้แขนงเลี้ยงบริเวณ inner cortex ก่อนที่จะผ่านมาในชั้น medulla และแตกแขนงเป็น true medullary capillary plexus ซึ่งทั้ง true และ sinusoidal medullary capillary plexus ต่างก็รวมเป็น deep venous radicles ในที่สุดทั้ง peripheral และ deep venous radicles รวบรวมเทลงสู่ collecting, central และ adrenal veins ตามลำดับ นอกจากนี้อาจพบ medullary capillary plexus เทลงสู่ central vein โดยตรง

สรุป: จากการศึกษาในครั้งนี้พบว่ามี microvascular connection ระหว่างชั้น cortex และชั้น medulla ซึ่งสนับสนุนการศึกษาที่รายงานว่ามี internal control ของชั้น cortex ต่อชั้น medulla เช่นเดียวกับที่พบในคน และยังพบว่ามี venous drainage หลายช่องทางในต่อมหมวกไตของค้างคาวแม่ไก่ การค้นพบในครั้งนี้ น่าจะสนับสนุนเกี่ยวกับพฤติกรรมของสัตว์ชนิดนี้ที่สามารถเปลี่ยนแปลงอิริยาบถอย่างรวดเร็ว