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Re-Examination of Blood Vessels of Rat Tail Using Angiographic and Histological Methods

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Abstract : Although the tail vessels are frequently used for serial blood sampling in rat, the definition of its vasculature remains in dispute. Herein, we investigated the number of blood vessels in Sprague-Dawley rat tail using angiographic and histological methods. Our results showed rat tail has one dorsal vein, two lateral veins, one ventral artery and one ventral vein. In the conclusion, when deciding which site is best for collecting blood, it is critical to consider the structure and features of the blood vessels to be used. This study will also be helpful for investigators to understand the structure of blood vessels in rat tail.

Key words **:** angiographic, rat, tail, vessels.

Introduction

Blood values are used as important indicators in various scientific researches including pharmacology, physiology and oncology. Blood has been collected from various sites of laboratory animals including the tail vessels, retro-orbital sinus, caudal vena cava, aorta, heart and etc (6,11,12). Among these, tail vessels and orbital sinus are frequently used for serial blood sampling. However, retro-orbital puncture has been shown to be stressful to the animals because of the necessity of anesthesia and the perpetual danger of blindness caused by insufficiently skilled experimenters (4). For these reasons, blood collection from tail vessels in rats is frequently employed, which involved tail vein incision, amputation of the tail tip and tail vein puncture (3,5). Different methods at the same sampling site, particularly the tail, might produce different results in the oral glucose tolerance test (2). Significant differences between arterial and venous blood has been reported in hematological and biochemical analysis (8-12). A study comparing the blood of the orbital sinus to that of the abdominal aorta in rats indicated substantial differences in white blood cells as well as glucose and cholesterol (8). In the present study, we attempted to analyze the number of blood vessels within the rat tail via angiographic and histological methods. We evaluated the possibility that blood collected from the tail artery could contain a mixture of arterial and venous blood.

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Materials and Methods

Animals and housing

Twelve male Sprague-Dawley rats (SD) were purchased from NARA Bio. (Seoul, Korea). Twelve-week-old rats were used in this study. The animals were bred at the barrier facility of College of Veterinary Medicine at Konkuk University and were housed on woodchip bedding (Sani-chip®, Harlan TEKLAD, Madison, USA) with a light-dark cycle of 12:12 h (08:00 to 20:00 h). The room temperature was maintained at 22 ± 2°C with a relative humidity of 50 ± 10%. The animals were fed a sterilized pelleted diet (2918C, Harlan TEKLAD, Madison, USA) and had an access to autoclaved water through drinking bottles *ad libitum*. All procedures in this study were carried out with the approval of the Konkuk University Institutional Animal Care and Use Committee.

Angiographic images

Four rats were anesthetized by intra-peritoneal injection of tribromoethanol (300 mg/kg, Sigma-Aldrich). After inspecting the adequate depth of anesthesia in terms of the absence of a withdrawal reflex, the abdominal aorta and the caudal vena cava were exposed. Saline was perfused for the protection of blood clots via the abdominal aorta. The perfusion was stopped when colorless saline drained from the caudal vena cava. 10 ml contrast medium (1 ml/min, Omnipaque, Nycomed Imaging AS, Oslo, Norway) was then injected via the abdominal aorta. The tail was observed in fluoroscopy (DF-151SB, Medison co., Seoul, Korea) at 44 kvp and 1.8 mas. Radiographic images of the tail were taken with an X-ray machine (REX 525RF, Listem co, Incheon, Korea) at 42 kvp and 3 mas.

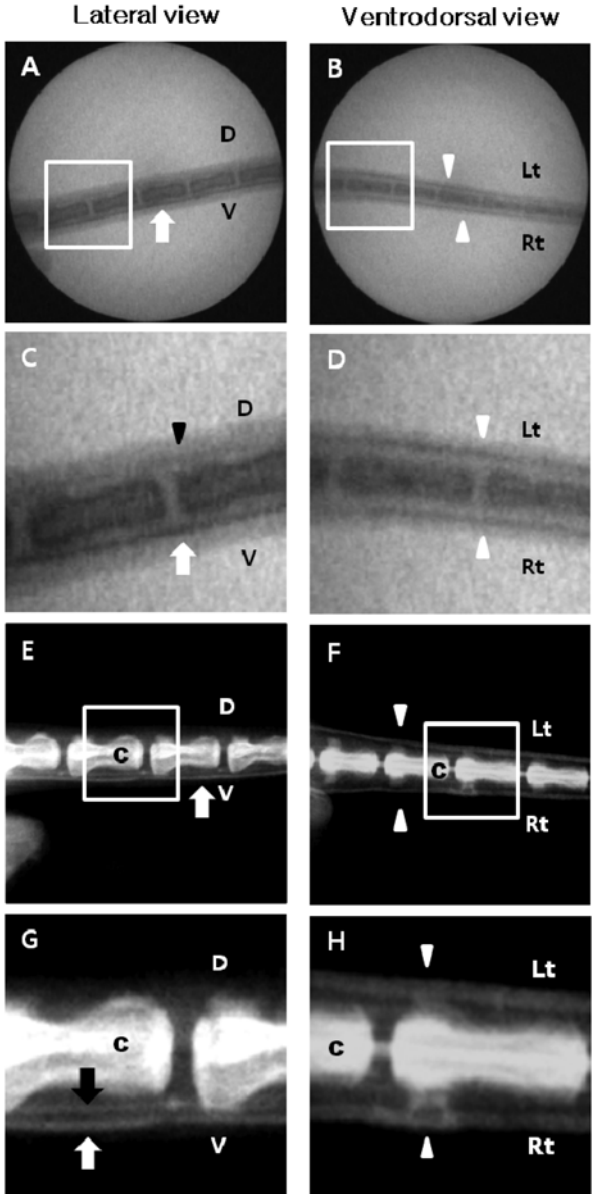

Fig 1. Angiographic images of rat tail. Fluoroscopic images (A-D) and radiographic images (E-H). (C, D, G, H) are the enlarged views of boxed areas on (A, B, E, F), respectively. In the ventral portion of tail on lateral views (E, G), two radiopaque linear stripes, ventral artery (white arrow) and ventral vein (black arrow), are seen. In the ventrodorsal view (B, D, F, H), lateral vein (white arrowhead) is observed on bilateral portions. D, dorsal; V, ventral; Lt, left; Rt, right; c, coccygeal vertebrae; arrow, artery; white arrowhead, lateral vein; black arrowhead, dorsal vein.

Histological analysis

Eight rats were perfused with 10% neutral buffered formalin in the abdominal aorta, as mentioned above. The tails were then cut into five segments of equal length, post-fixed in 10% neutral buffered formalin for 24 h and decalcified in 4% aqueous formic acid mixture. After embedding in paraffin, the specimens were transversally cut into 5 μm-thick. Transverse sections were stained with hematoxylin and eosin in order to identify the tail structure. The sections were examined by an experienced histologist.

For detection of vasculature, the immunohistochemical analysis was performed using an avidin-biotinylated complex system (Vectastain Elite mouse IgG ABC kits, Vector Labs., Burlingame, CA, USA). Mouse monoclonal antibody (1:1000, Millipore, MA, USA) for alpha smooth muscle actin was used as primary antibody. Briefly, paraffin sections were deparaffined, hydrated, treated with proteinase K (20 μg/ml, BioBasic inc, Ontario, Canada) and incubated for 20 mins at 37°C to retrieve the antigen. The sections were treated with 3% hydrogen peroxide in methanol and incubated for 1h at RT with 1.5% anti-horse serum (Vectastain Elite ABC-kit) in PBS including 5% bovine serum albumin (BSA). The sections were then incubated with primary antibodies in PBS including 5% BSA overnight at 4°C. After washing in PBS, the sections were incubated with biotinylated secondary antibody (Vectastain Elite ABC-kit) for 60 min and then with avidin–biotin peroxidase complex for 30 min at room temperature. Sections were visualized with peroxidase substrate kits (DAB, Vector Labs., Burlingame, CA, USA) and counterstained with hematoxylin and dehydrated in ethanol. After clearing in xylene, sections were examined under a microscope (BX51, Olympus, Tokyo, Japan) and analyzed using a computerized image analyzer (MetaMorph 7.5, Molecular Devices, Downingtown, PA, USA).

Results

We investigated the number of dominantly vascular streams that existed within the tails of the SD rats using angiographic methods. In the fluoroscopic images, lateral views (Fig 1A, C) showed a dominantly radiopaque line as a ventral artery at the ventral portion that was oriented toward the tip of the tails. A faint radiopaque line as a dorsal vein at the dorsal portion that was directed back to the body was also observed. However, in the radiographic images, two radiopaque lines as a ventral artery and a ventral vein were detected in the ventral portion of the lateral view (Fig 1E, G). Due to the difference in resolution and the response of the dominant radiopaque line in the fluoroscopic images, the faint stream in the ventral portion was covered into the dominant stream. In the ventrodorsal images of the radiography (Fig 1B, D, F, H), two dominant radiopaque lines (white arrowhead) representing two lateral veins were running bilaterally. Of particular note were fine lines, as arterioles, oriented upward from the ventral radiopaque line on the lateral and ventrodorsal view of the radiography. (data not shown).

Histological analysis was used to further detect the anatomical structure of the tail and its blood vessel. As shown in Fig 2, the rat tail was shown to consist of skin, coccygeal vertebrae, ligaments, tendon and musculus coccygeus. The tendons and the muscles were surrounded by a thick fascia and formed a bundle. Four bundles were reported within the rat tail

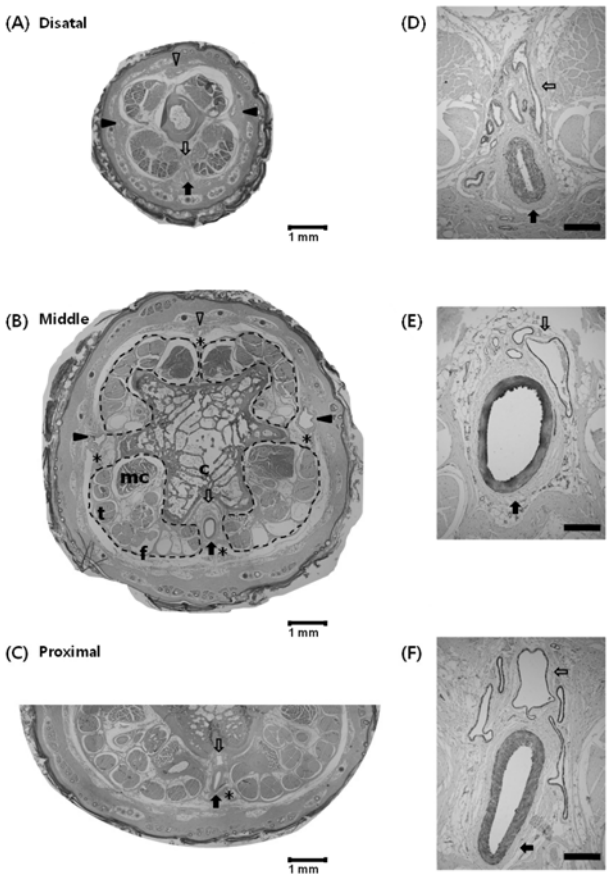

Fig 2. Histological analysis of rat tail. Transverse sections of tails were stained with H&E (A-C) and immunohistochemistry (D-F). (D-F) are the enlarged ventral portion of each segments (A-C). Black arrow, ventral artery; white arrow, ventral vein; closed arrowhead, lateral vein; open arrowhead, dorsal vein; c, coccygeal vertebrae; t, tendon; mc, musculus coccygeus; f, fascia; dotted circles, bundle; *, groove; bars in (D-F) = 500 μm.

and most blood vessels were shown to be nestled between the bundles.

In the immunohistochemical analysis (Fig 2), the enlarged ventral groove, the space between the bundles, was observed the ventral vein dipped deeper than the ventral artery. The luminal diameters of the lateral veins were broader than those of the ventral and dorsal veins. Of these, the dorsal vein was the smallest. According to the transverse section, all dominant veins may have several branches, venules. Also, arterioles and venules were often observed at the each groove.

Discussion

Even though the rat tail has proven to be an excellent blood sampling site, the structure of arteries and veins in the rat tail is still not clearly defined. In the present study, we tried to define the vessels structure as well the anatomical features of the SD rat tail. Our radiographic results described a ventral artery running from the proximal to the distal portion of the SD rat tail. Many upward arterioles were branched out from ventral artery (data not shown). In the histological analysis, we report the presence of four bundles consisting of disconnected tendons and muscles, and four grooves within the rat tail. We also observed a vein in each groove, particularly a ventral vein in the ventral groove. According to the location of the cut, a wide vein or several small venules were observed.

Collecting blood from the mouse and rat tail has several benefits in terms of the broad vascular distribution and serial collection. For these reasons, many investigators have used the rat tail. Some studies have proposed alternative methods for repeating blood collection, such as tail incision and tail amputation because of minimal restraint and low plasma corticosterone level (1,3,5,14). However, recent studies showed that different methods of blood collection and sites of incision may induce different results in pharmacokinetic and physiological studies (2,7). Furthermore, the results of the present study indicate the chance for arterial and venous blood to mix, depending on the location of incision and amputation.

Staszyk and colleges asserted the structure of tail vessels in Wistar rats comprised of one dominant ventral artery and three dominant veins (13). However, our results showed the ventral vein in SD rats was wider than the dorsal vein, which was observed through all sections. The difference may be caused by strain difference or their ignoring a ventral vein accompanied near a ventral artery. Thus we now explain the vessel structure in the SD rat tail based on the main stream and branches, arterioles and venules. As the angiographic and histological analysis demonstrated (Figs 1, 2), the SD rat tail contained one dorsal vein, two lateral veins, one ventral artery and one ventral vein. Moreover, there was a branching out of arterioles and venules from these blood vessels for the purpose of supplying various tissues with nourishments. In the present study, we concluded one ventral artery exists for supplying the tail with blood, which goes out into four dominant veins. Also, the arterioles and venules play critical roles in the supply and withdrawal of blood to the peripheral tissues of the tail.

In conclusion, our results also imply that bleeding from a tail incision or tail amputation could result in the mixing of arterial and venous blood. Therefore, when deciding which site is best for collecting blood, it is critical to consider the structure and features of the blood vessels to be used. The results of the present study are valuable in furthering the understanding of the structure of blood vessels within the rat tail.

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혈관조형술과 조직학적 방법을 이용한 랫드 꼬리의 혈관구조 규명

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요 약 : 랫드 꼬리는 연속적인 채혈을 위해서 주로 이용되는 채혈부위로 꼬리 절개법, 꼬리 절단법, 꼬리 천자 등의 방법을 통해 채혈이 이루어지고 있다. 하지만, 랫드 꼬리에 대한 혈관 구조는 아직 잘 밝혀져 있지 않다. 그래서 본 연구에서는 독성학이나 약리학에서 가장 많이 이용되는 폐쇄군인 Sprague-Dawley 랫드 꼬리의 혈관구조 규명하기 위해 혈관조형술과 면역조직학적인 방법을 실시하였다. 그 결과 SD 랫드 꼬리는 한 개의 등쪽 정맥과 두개의 외측정맥, 한 쌍의 배쪽 정맥과 동맥이 존재했다. 그러므로, 본 연구는 SD 랫드 꼬리를 이용해서 채혈을 하고자 하는 연구자들이 꼬리의 혈관구조를 이해하고, 그 연구 목적에 맞게 채혈부위와 방법을 선택하는데 도움이 될 것으로 생각된다.

주요어 : 혈관조형술, 랫드, 꼬리, 혈관.