Application of Digital Subtraction Angiography in Canine Hindlimb Arteriography

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Research article

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Abstract

BACKGROUND: Research in the field of lower extremity vascular disease has increased in popularity over the years. In order to properly characterize and validate the effectiveness of interventions, in vivo experimentation in large animals is required. Thus, it is necessary to find a method to detect the shape and density of blood vessels in the lower extremities, which can evaluate and verify the effectiveness of the treatment measures, and has high feasibility and repeatability. This experiment is to characterize the influencing factors and determine both the feasibility and overall value of digital subtraction angiography in lower limb arteriography in a canine animal model.

METHODS: Six Beagle dogs were immobilized on a working bed after administration of anesthesia. Percutaneous access was achieved via the femoral artery using an indwelling needle. A bolus of contrast agent was injected into the access site and digital subtraction angiography bolus chase technology was used to collect contrast images for analysis of this method.At the end of our procedure, the Beagles was anesthetized,veterinary injected excess potassium chloride.After confirming the loss of vital signs of beagles, the carcasses were handed over to the animal experimental center of Xinjiang Medical University,handled by qualified institutions.

RESULT: The final arteriographic images of the hind limbs from all six canines were clear, and the branches of small vessels could be distinguished without any visible artifacts.

CONCLUSION: These results suggest that arteriography using digital subtraction angiography can clearly reveal the shape and density of blood vessels in canine animal models. This method has great potential to significantly improve research related to limb ischemia, due to its simple and reproducible results.

Background

Peripheral arterial disease, which occurs in the extremities, has great influence on limb function and quality of life. In recent years, arterial disease in the lower extremities has been increasing significantly, partly due to the increased incidence rate of diabetes. One common clinical manifestation of a diabetic patient with peripheral artery disease is the formation of a foot ulcer, which is an important risk factor for amputation and death of diabetic patients[1].At present, the main principle of diagnosis and treatment of peripheral artery disease is to reconstruct the peripheral artery circulation, but most of the treatment protocols are still in the stage of research and exploration. The use of animal models are an essential link in medical research to study the pathogenesis of a disease and to optimize treatment plans[2]. Animal models simulating acute and chronic ischemia of the lower limbs are established through ligation, cutting and embolization of the arteries. These methods are becoming of great interest to the research community, both locally and internationally, in order to explore the best diagnosis and treatment approaches for peripheral arterial diseases[3].Due to operational feasibility and experimental costs, the most widely used animal models are rabbits and rats, despite the size of their blood vessels and skeletons being significantly different from that of humans. Furthermore, there are many limitations in simulation studies, especially in the current study of Ilizarov bone transfer technology for the treatment of peripheral artery disease.These simulation studies, however, provide an ideal platform to observe and carry out research in this field.

The examination and evaluation of angiogenesis are the keys to success when establishing a hindlimb ischemia model for the study of peripheral arterial disease in canine models. Digital subtraction angiography (DSA), which is the "gold standard" for clinical diagnosis of peripheral vascular disease and cerebral vascular disease, clearly visualizes vascular morphology and density, and is also the first choice and main basis for clinical diagnosis of peripheral vascular disease, assessment of vascular residual degree and detection of a treatment's effect[4]. DSA is also the most direct and reliable method for the detection of peripheral blood vessels.This paper discusses the feasibility and influencing factors of DSA in the study of large animal model, by analyzing its application value, in order to provide the theoretical basis and operational reference for this type of *in vivo* study.

1 Methods

1.1 Design

To establish Angiographic imaging of canine animal models.

1.2 Time and place

This study was completed in the intervention room of the Sixth Affiliated Hospital of Xinjiang Medical University from October 2018 to May 2019.

1.3 Materials

1.3.1 Experimental animals

Six Beagles are selected by veterinarians and are single blind to the experimenters.Six 12-month-old female Beagles were selected with a body mass of 15.0-16.0 kg, an average of (15.7 ± 0.6) kg, a body length of (55 ± 2) cm, and a tibia length of (11.0 ± 0.8) cm. All canines were healthy and equal body size , and were provided by the animal experimental center of Xinjiang Medical University. The license number was syxk (New) 2018-0002. This study has been authorized by the animal experimental ethics committee of Xinjiang Medical University.And there are some beagle details:

1. Beagles are raised in the Animal Experimental Center of Xinjiang Medical University, one experimental dog and one canine room, which are constructed from cement.
2. Dog room has a window, indoor heating, warm winter and cool summer, indoor cleaning twice a day.Drinking water is tap water and feed is dog food, which is distributed uniformly by the breeders.

3. Regarding animal welfare, physiological welfare, beagle dogs are satisfied before and after the experiment and given daily diet; environmental welfare, dog room has a window, indoor heating, warm winter and cool summer, indoor cleaning twice a day; health welfare, veterinarians regularly check the body; behavioral and psychological welfare, daily regular walking of dogs, to comfort and accompany.At the end of the experiment, they were euthanized.

1.3.2 Equipment

Siemens axiom artis MP multi-functional angiography machine, 30 ml syringe, 18G intravenous indwelling needle (1.3 mm × 30 mm, Suzhou Linhua company) and lipanol injection (300 g / L, Beijing Beilu company) were used as a contrast agent; 30 ml of lipanol and physiologic saline were extracted using a syringe with a ratio of 2:1 and connected to the tail-end interface of an indwelling needle.

1.4 Method

1.4.1 Preoperative preparation

Before digital subtraction angiography, a 12-hour fasting period was observed for all canines to prevent the risk of life-threatening risks (e.g., aspiration) during the operation. The region of the canine used for operation was prepped using an electric pet shaver.

1.4.2 Anesthesia and posture

Shutai (France Vick Co., Ltd., batch No.: 6smm 2017.12) and simvastatin II (silazine hydrochloride injection, Shengda animal drug Co., Ltd., Dunhua City, Jilin Province, batch No.: 20180401), which are commonly used in canines for their short induction time and high level of safety, were selected as anesthetics. 0.5ml of each anesthetic was loaded according to their prescribed 1:1 ratio, and simultaneously injected into the thick solid region of the canine leg muscle. During the operation, according to the needs of the canine, it was placed on the working bed after successful administration of the anesthesia.Use sterile bandage to fix tongue to avoid suffocation caused by tongue drop,heart rate and respiration are also measured.

1.4.3 Operation method

Before inspection, remove any objects that may affect the movement of the C-arm and the working bed, and determine the starting and ending points of the moving track of the working bed. Two researchers, wearing lead clothes and other protective measures, were separated on either side of the working bed.

The pulsating femoral artery on the upper surface of the inner thigh was identified by touch, and punctured through the skin using a 18 gauge catheter. After the successful puncture, the needle was fixed in position using tape. One researcher fixed the hind limb of the experimental dog with both hands to prevent shaking of the limb during radiography from affecting the image acquisition quality. The contrast agent was manually injected (at an average rate of 3 ml/s) by another researcher at the beginning of image acquisition. In the control room, an experienced imaging technologist controlled the working bed and imaging exposure according to the flow of contrast agent in the posterior limb blood vessels. The sequence subtraction image was carefully observed and recorded with a total exposure of 15 s. When the image acquisition was not satisfactory, contrast agent was administered again with the same protocol. After the end of the angiography, the indwelling needle was removed and the puncture point was pressed for 15 minutes to avoid internal bleeding. See Figure 1 for the location of the puncture point and contrast agent injection.At the end of our procedure, the Beagles was anesthetized,veterinary injected excess concentrated potassium chloride.After confirming the loss of vital signs of beagles, the carcasses were handed over to the animal experimental center of Xinjiang Medical University,handled by qualified institutions.

1.5 Main observation indicators

The collected images are classified as excellent, good and bad. Excellent: clear image, no artifacts, clear display of small blood vessels; good: clear image, few artifacts, poor display of small blood vessels; poor: blurred image, obvious artifacts, only main blood vessels can be identified.

Finally, the image quality was graded by a deputy chief physician and an attending physician in the intervention room.

2 Results

2.1 Quantitative analysis of experimental animals

Six beagles were included in the result analysis without shedding.

2.2 Image acquisition results

For all 6 canines, 12 arteriography image sets were acquired. The image of the first canine was poor. Because of the initial operation, the contrast agent was not diluted, which led to the shaking of the canine’s legs, resulting in unclear images of the blood vessels. After diluting the contrast agent and strengthening the fixation in the operation of the hind limbs, the angiography of 12 hind limbs of all 6 experimental dogs obtained were of high image quality.

2.3 Effect of animal operation and prognosis

The average operation time of each canine was 20 minutes, no anesthetic was added; six experimental canines recovered normally within 30 minutes after operation, resulting in no deaths. There were slight swelling and subcutaneous hemorrhage at the puncture point of 2 canines, which was caused by insufficient pressing time, and the swelling was relieved one week later; the energy of the canines recovered after normal eating on the second day, and there was no functional and morphological abnormality such as limping in the hind limbs. Because of the need of follow-up study, the canines in this group had arteriography performed again at 1 week, 2 weeks and 1 month after the initial operation. The image acquisition is shown in Figure 2.

3 Discussion

3.1 Advantages of beagles as animal models

Animal models are an indispensable in medical research, especially for operations with trauma or uncertain safety, which therefore cannot be directly carried out on the human body in clinical research. Thus, using an animal model to carry out preliminary research to verify safety and efficacy is the best choice[5]. The Beagle breed is the only standard experimental canine recognized in the world because of its gentle temperament, stable genetic gene profile, repeatability of anatomic structures, and stable physiological index. Compared with rats and other small experimental animals, the Beagle canine is closer to human in the research model of skeletal and blood vessel structure, which is critical for comparison of research results[6].

In the study of scoliosis, Hou et al.[7] observed that the Beagle animal model was close to clinical cases, and obtained typical scoliosis performance when establishing a scoliosis model, which completely achieved the purpose of simulating clinical scoliosis, and the modeling operation was simple, the success rate was high, and the damage to the accessory structure around the spine was minimal.

Li et al.[8] used the Beagle canine model in the study of hip joint stress by fixing one side of the forelimb, and then increasing the load of the hind limb of the canine in order to simulate the load-bearing walking state of the human. Three-dimensional gait analysis was conducted to show that the changes of the mechanical parameters of the hind limb of the Beagle conform to the biomechanical changes of the human hip joint. The study highlights that the Beagle animal model is used to study the related diseases of the human hip joint.

Kim et al.[9-10] studied a new type of internal fixation materials, and found that the bone shape and specification of Beagles are close to the human body, and therefore can be used to test standard size screw and steel plates. Furthermore, they found that the Beagle has a muscle strength load that can reach that of a human, and approximates well the stress simulation and stability.

In the research field of osteonecrosis of the femoral head and revision of the hip joint, Omoto et al.[11-12] put forward that the hip joint and surrounding tissue of beagles are suitable for human body simulation. They claim the modeling operation is simple, the success rate is high, the imaging examination results are clear, the local bone mineralization rate after operation is similar, and the biological consistency is good. In a follow-up osteotomy experiment in this study, Beagles served as a more satisfactory bone model than small animals, so as to better recapitulate simulation and visualization of the operation.

The author's opinion is that compared with sheep, orangutan and other animals, beagles are the best animal model for this field of research, particularly for better representing operation procedures and mimicking skeletal, nervous and vascular structures.

3.2 Operation and interference factors of DSA

DSA is a combination of angiography and computer technology. It refers to the contrast agent scanning and developing of X-ray, by digital processing, which removes unnecessary tissues from the image, and finally presents a clear vascular morphology. At present, DSA technology mainly includes step-by-step technology and segmentation technology. The step-by-step technology realizes the acquisition of a complete image and a dynamic image by controlling the movement and exposure of the working bed, while the segmentation technology is to inject the contrast agent and collect the image iteratively; the step-by-step Technology has the advantages of low contrast agent consumption and short exposure duration[13]. The technique used in this study is step-by-step technique for DSA.

DSA is regarded as the "gold standard" for the diagnosis of vascular diseases internationally, especially in the diagnosis and treatment of peripheral arterial diseases, such as diabetic foot and lower extremity arteritis. Accurate angiography of arterial circulation is an important basis for diagnosis, reconstruction of blood circulation and prognosis [14-16].

Yoshinori et al. [17] performed DSA of the hepatic artery for the diagnosis and treatment of liver tumor diseases. Due to its good imaging quality, we can clearly observe the morphology of the hepatic artery and its branches, the morphology and number of proliferative vessels in a tumor, which provides a more intuitive treatment support for tumor tissue disconnection and final resection.

Blagojevic et al. [18-20] highlighted the clinical diagnosis, treatment and research of lower extremity artery disease that DSA technology can accurately reflect its blood circulation and define the location of disease occurrence and accumulated tissue area. These capabilities play an indispensable role in the formulation of treatment plans, even in the case of amputation, since DSA can provide a more reliable reference standard, thus reducing the amount of amputation, allowing as much limb function to be preserved as possible.

In the study of animal models, McCollough et al. [21] performed contrast examination on beagles' hearts by injecting contrast agent into the central vein. According to the density of the contrast agent before and after the same cycle and the volume difference, the volume and ejection fraction of the ventricles can be quantitatively measured. The author pointed out that DSA technology has the advantages of objectivity, simple operation and repeatability.

Anesthesia and contrast agents are the two most important factors affecting the final image quality of DSA.[22-23] When DSA is performed, it is required that the examinee keep absolutely static relative to the working bed. Good anesthesia can play a role of sedation and analgesia, and ensure smooth operation. A large dose of anesthesia will lead to deep inhibition or even death of the canine, so that normal and effective observation images cannot be obtained. However, a shallow degree of anesthesia will lead to shaking of the canine due to pain stimulation during operation, resulting in mobility artifacts affecting the image quality.[24] In this study, 1 ml of intramuscular anesthesia with 1:1 ratio of Shutai and shumianxin II was used. The degree of anesthesia was appropriate, and the effective time of anesthesia was about 40 min, which could meet the smooth operation.[25]

The influence of contrast agent on the image quality of DSA mainly includes the concentration, dose and flow rate. Too high of a concentration will cause shaking and artifacts due to the stimulation of blood vessels in canines; too low concentration will cause poor development of small blood vessels.[26] In this study, the contrast agent used was lipanol injection (300 g / L). During the initial operation, the contrast agent was not diluted, resulting in the irritant shaking of the hind limbs of the canine during the injection, and the quality of the collected image was therefore poor. Afterwards, the concentration was diluted by a ratio of 2:1 between lipanol and normal saline, and there was no obvious shaking in the operation of the canine, and the collected image also met the experimental requirements. In addition, proper flow rate is also an important factor to ensure the image quality. For example, if the flow rate of contrast medium is too slow, it will be diluted by the high-speed arterial blood in the artery; and if the flow rate is too fast, it will cause huge pressure on the blood vessel, and even cause the rupture of the blood vessel. In this study, 30 ml syringe was used for manual injection, and the diluted 30 ml contrast agent was pushed into the artery at a constant speed within 10 seconds, with an average flow rate of about 3 ml/s. During the operation, the flow of contrast agent in the artery was tracked and captured well, and the quality of the collected image was satisfactory.

In this study, a 18 G vein indwelling needle instead of a catheter can directly puncture the femoral artery through the skin, which avoids the process of crossing the iliac artery in the routine operation. This approach has minimal stimulation and damage to the blood vessel, is convenient to operate, and saves a lot in cost; but in the process of puncture,The first beagle shook when administered with the bolus of contrast agent, resulting in a poor quality angiographic image. To obtain high quality images, the canine limbs were fixed in position and a diluted concentration of the contrast agent was used. We should master the puncture tool, adjust the concentration of contrast agent and control the injection speed of contrast agent to avoid possible massive bleeding and Beagle tremor. It should be noted that there is a risk that the success of the experiment will be affected by the accidental removal of the needle. The image obtained by this method shows the shape of blood vessels clearly, the display of small blood vessels is good, and the image quality completely meets the requirements of research and design. In addition, all dogs in this study had three repeated arteriography over a two month period, with good repeatability. At the same time, some shortcomings in this experiment can also provide reference for this kind of experimental study.

3.3 Conclusion

Beagle, as the only standard experimental canine breed, provides ideal conditions for disease models and is a very suitable for large animal experiments. The DSA has a clear effect in the angiography of the canine's lower extremity artery. It can display the shape and density of the lower extremity artery very clearly, and the image quality can fully meet the experimental requirements, and recapitulates well the simulation research and operation of similar diseases. Moreover, the DSA technology is simple in operation and repeatable, and thus can be used to simulate the lower extremity artery diseases in canines. The verification of related theories is of great significance. However, appropriate measures should be taken during preoperative preparation, intraoperative anesthesia and immobilization, contrast agent control and image processing to ensure the quality of the final DSA image meets the experimental requirements.

Abbreviations

Digital Subtraction Angiography (DSA)

Declarations

Ethics to Ethics approval and consent to participate:

The experimental scheme was approved by the animal experimental ethics committee of Xinjiang Medical University, approval number: iacuc201181024-01. The experiment was conducted in accordance with the international society of veterinary editors' consensus on authors' guidelines on animal ethics and welfare and local and national regulations.

Availability of data and materials

The experimental data confirmed that DSA can be used in the lower extremity angiography of beagle dogs. On this basis, some difficult to solve lower extremity vascular diseases, such as the use of Ilizarov technology to treat diabetic foot, can be verified by this method.Beagle, as the only standard experimental canine breed, provides ideal conditions for disease models and is a very suitable for large animal experiments.

Conflflict of Interest to Competing Interests:

In this article, digital subtraction angiography (indwelling needle, contrast agent) is used as the treatment intervention method. All the authors of this article declare that they have not received any funding from the above instruments, and there is no conflict of interest in the research and writing process.

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Authors' contributions：

Professor Wang Chengwei is responsible for the overall coordination and guidance of the experiment.Xue Wang and Tiannan Chen are responsible for the overall implementation, evaluation and operation of the experiment. Zhang Shiyong and Wang Jie are responsible for image processing.Xue Wang and Tiannan Chen contributed equally to this experiment.

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Figures

Figure 1

Schematic diagram of arterial puncture and contrast agent injection Note: Figure A shows the location of the puncture point, the position of the upper segment of the femoral artery is superficial, the shape is straight, and the puncture conditions are ideal; figure B shows the injection of contrast agent along the direction of the shape of the blood vessel

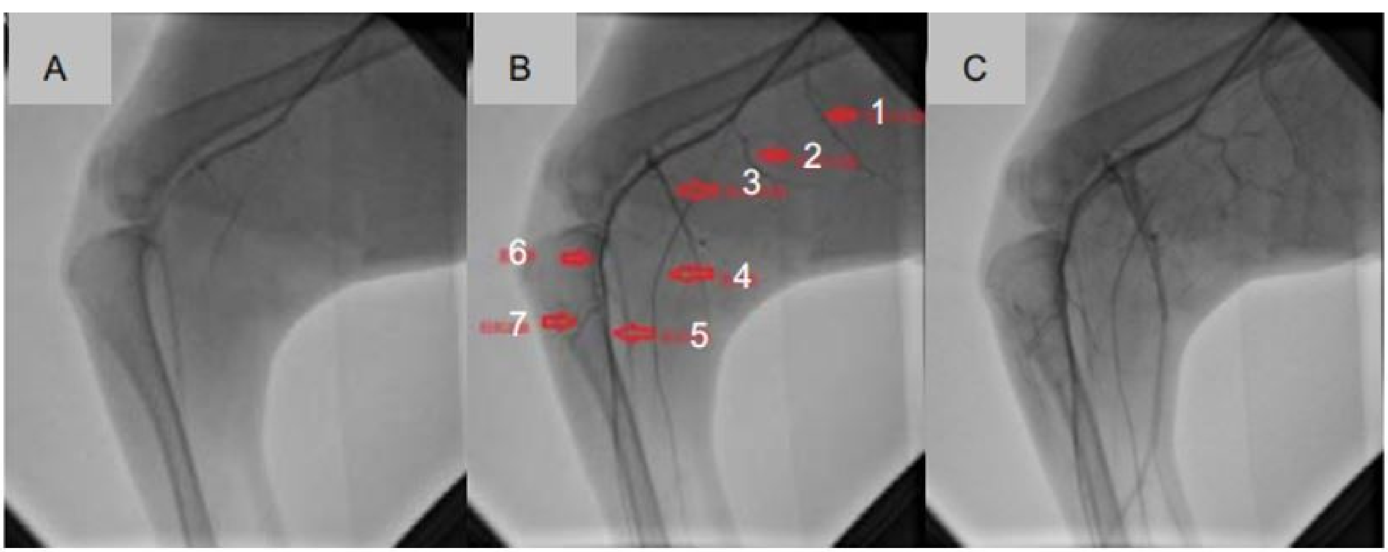

Figure 2

Schematic diagram of angioplerosis developed on the arteriography Note: Figure A shows the contrast agent filling and developing along the artery; Figure B shows the image of the dog's posterior limb artery, with clear display of each branch of blood vessels, 1 is the posterior proximal femoral artery, 2 is the posterior middle femoral artery, 3 is the posterior distal femoral artery, 4 is the saphenous artery, 5 is the posterior tibial artery, 6 is the popliteal artery, 7 is the anterior tibial artery; Figure C shows the simultaneous development of artery and vein, with clear display of local microcirculatory vascular density

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