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Research paper

Three-dimensional alteration of cervical anterior spinal artery and anterior radicular artery in rat model of chronic spinal cord compression by micro-CT

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HIGHLIGHTS

- The 3d variation of cervical anterior spinal artery and anterior radicular artery can be shown by micro-CT after chronic spinal cord compression.
- The most serious neurological damage occurred at the 28th day after chronic compression.
- Neural functional recovery was accompanied by vascular alteration. And this alteration has implications for studying the pathophysiology of cervical myelopathy.

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ABSTRACT

Objective: To investigate the spatial and temporal changes of anterior spinal artery (ASA) and anterior radicular artery (ARA) of chronic compressive spinal cord injury on rat model by three-dimensional micro-CT.
Methods: 48 rats were divided into two groups: sham control group ($n=24$) and compressive spinal cord injury group ($n=24$). A C6 semi-laminectomy was performed in the sham control group, while a water-absorbable polyurethane polymer was implanted into C6 epidural space in the compression group. The Basso Beattie Bresnahan (BBB) score and somatosensory evoked potentials (SEP) were used to evaluate neurological function. Micro-CT scanning was used to investigate the change of ASA and ARA after perfusion at the 1th ($n=6$), 28th ($n=6$), 42th ($n=6$) and 70th ($n=6$) day of post operation. The diameter, angle-off and vascular index (VI) was measured by 3D micro-CT.
Results: In comparison with sham control, BBB score have a significant reduction at the 28th day ($p<0.05$) and abnormal SEP have a significant severity at the 28th day ($p<0.05$). Both of them have a significant improvement at the 70th day compared with that of the 28th day ($p<0.05$). VI shows the amount of microvessels reduced at the 28th day ($p<0.05$) and increased at the 70th day ($p<0.05$). The diameter and angle-off of ASA and ARA also changed significantly at the 28th, 42th, 70th day ($p<0.05$).
Conclusion: There was a significant alteration of cervical anterior spinal artery and anterior radicular artery after chronic cervical spinal cord compression. Alteration of ASA and ARA may affect the vascular density of spinal cord and play an important role in neural functional change of chronic cervical spinal cord compression through 3D micro-CT.

1. Introduction

Chronic cervical spinal cord compression is a common pathological scenario in the process of cervical spondylotic myelopathy. Cervical spondylotic myelopathy (CSM) results from the combination of static and dynamic compression leading to local spinal cord ischemia [3,19]. Nevertheless, the change of blood flow and its mechanism in CSM is undefined. Chronic cervical spinal cord compression is a common pathological scenario accompanied with disruption of microvascular networks. The loss of vessel network integrity leads to blood flow reduction and local hypoxemia [5]. Progressive vascular dysfunction may exacerbate neurological deficits in chronic spinal cord injury pathophysiology. Although previous studies indicate that spinal cord injury is accompanied

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by a subsequent neurovascular repair process such as angiogenesis and compensatory mechanisms [7]. However, it is still largely unknown about this complex process, which involves vascular architectural repair and reorganization in the chronic phase of compressive spinal cord injured.

The ASA and ARA supply the two-thirds of the spinal cord blood [26]. Studies have demonstrated that cervical spinal cord ischemia is the result of altered spinal cord blood supply following ASA or ARA injury [9,15]. Spinal cord ischemia is one of the predominant pathogenic mechanisms of neurological dysfunction due to disruption of vascular structures after compression. Specifically, vascular pathological changes such as vessel wall thickening and hyalinization in the anterior spinal artery have been recognized in patients with cervical spondylotic myelopathy [20]. Based on the hypothesis that the spinal cord would undergo reorganization and modification of the vascular networks after chronic compressive spinal cord injury, we suppose that the architectural repair and reorganization of the two arteries may play a crucial role in chronic cervical spinal cord compression. Investigation of the morphological alteration of cervical ASA and ARA will be helpful to gain further insight into the neurovascular pathology of chronic cervical spinal cord compression.

In this study, we utilized micro-computed tomography (micro-CT) to investigate the spatial and temporal changes of ASA and ARA in compressive cervical cord segment. Micro-CT has been used as a powerful tool to explore 3D vascular structure in many specimens [2,11,16,18,23]. And we envisioned that this technique would shed light on the self-recovery of ASA or ARA in rat model of chronic cervical spinal cord compression by presenting vivid 3D vascular images of normal and compressive specimens in different time-point that with neurological functional change. Thus, provide further evidence of ischemia caused by chronic compression plays a significant role in the development of myelopathy by means of imageology.

2. Materials and methods

2.1. Chronic spinal cord compressive model in rat

All experimental procedures were approved by the Research Ethics Committee of the authors' institutes. A total of 48 adult Sprague-Dawley (SD) rats (280–300 g) were allocated to two groups: control group ($n = 24$) with sham surgery, and compressive group ($n = 24$) with implantation of a water-absorbable polyurethane polymer. All rats were anesthetized with 10% chloral hydrate (3 mL/kg), the C5 lamina was exposed. Then the ligamentum flavum and C5 partial lamina were removed to access the epidural space, and a compression material was implanted into the C6 epidural space on the posterolateral side to induce a compression to the cord after water-absorbtion expand. The sustained-release membrane was made of polyurethane, which was synthesized in the laboratory by isocyanates and polyols (Guangzhou Fischer Chemical Co., Ltd., Guangzhou, China). Each water-absorbable polyurethane polymer was cut to a standard size of 1 mm × 3 mm × 1 mm. The water-absorbable polyurethane polymer did not show any inflammatory reaction or tissue granulation after implantation in previous studies and this model showed a close similarity in characteristic features between the progressive neurology deficits and clinical cervical myelopathy [10]. For rats in the sham control group, the C5 laminae were removed without inserting the compression material. After surgery, the incision was closed by layers with complete hemostasis. All animals were given an Intramuscular injection of Penicillin G (8000 U/100 g, intramuscular injection) to prevent infection during the surgery and housed in cages individually and allowed free access to food and water. Post-operative analgesia was applied to rats with subcutaneous injection of buprenorphine (0.01 mg/kg) 12 hourly for 3 days. After 3 days, analgesia would be applied when the rat was observed any signs of pain or distress. In this study, no rat received analgesia after 3 days.

2.2. Behavior analysis and Electrophysiological evaluations

Effective compression is the basis for our research. In terms of motor function, the Basso Beattie Bresnahan (BBB) score was used to assess the severity of paralysis due to spinal cord compression. For the two groups, BBB scores were evaluated once a day from 24 h to 70th day post-surgery. The double-blind methods were used to evaluate, and the average scores in each group were calculated. In addition, all rats received the SEP test [11,27] before euthanasia at each time point.

2.3. Micro-CT

The spinal cord was subjected to micro-CT scanning after gelatin-lead oxide intracardiac injection. Animals were anesthetized with an overdose of 40 mg/kg of intravenous sodium pentobarbital. An abdominothoracic incision was rapidly performed to expose the heart. An obtuse cannula was inserted into the thoracic aorta via the left ventricle followed by heparinized saline perfusion by adjusting the perfusion height (110 cm H2O) and rate (20 mL/min). Subsequently, the right atrium was sheared for use as a venous drain vent. The application of heparinized saline ensured effective removal of the circulatory blood. Effectual perfusion was characterized by effective decoloration of the liver and the presence of limpid drainage fluid. Thereafter, 10% buffered formalin was perfused with the same perfusion height and rate for temporary vessel network fixation. All perfusion solutions were preheated and warmed to 40 °C. Gelatin-lead oxide mixed liquor (medical gelatin 5 g, lead oxide 100 g, distilled water 100 mL) was continually infused into the aortic cannula by syringe (6–8 mL/min) for 5 min, until the contrast liquid that flowed freely from the right atrium vent and the viscera was completely red. After the perfusion procedure, the infused animal was moved to a refrigerator for preservation at 4 °C overnight to achieve effective casting of the entire vascular system. Entire cervical spinal cords were then carefully harvested and fixed with 4% phosphate buffer liquid in formaldehyde solution for another 24 h. The muscle tissue was carefully removed from the lamina under a microscope, and the specimens were then subjected to micro-CT scanning (ZKKS-MCT-Sharp-II, Guangzhou ZhongkeKaisheng Medical Technology Co., Ltd., Guangzhou, China). Three-dimensional reconstruction figures of each specimen were performed using the 3D-Med 4.3 software analysis system (Guangzhou ZhongkeKaisheng Medical Technology Co., Ltd., Guangzhou, China), and the integral vascular index (VI) of every specimen in each group was calculated.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 16.0 analysis software (SPSS Inc., Chicago, IL, USA). All measurement results were presented as mean ± standard deviation. The parameters and integral VI values measured in micro-CT results of both groups were analyzed by the variance test and independent samples t-test. $p < 0.05$ was considered as statistically significant.