Three-dimensional Micro-structural and Micro-morphologic Analyses of the Microvasculature of the Spinal Cord after Acute Injury in a Rat Model using MicroCT Method

INTRODUCTION:

Acute spinal cord injury (SCI) is a devastating paralytic event accompanied with severe destruction of micro-vascular architecture [1]. It is great importance to study the vascular alteration of the three-dimensional micro-morphology after SCI in order to evaluate the significance of micro-imaging description and investigate the efficacy of drugs therapy [2]. This study was designed to visualize the 3D pathological changes of the micro-vascular conformation in a rat model after SCI and measure the quantitative micro-morphometry data using microCT scanner in combination with silicone rubber perfusion.

METHODS:

Nine 5D adult rats underwent laminotomy and were performed acute SCI model, with 10th thoracic cord exposed and subjected to the Allen's weight impact loading. Respectively, nine SCI rats each were perfused via left ventricle with silicone rubber (Microfil, Flow tech, USA) at day 1, 7 and 14 postoperatively, followed by 4℃ cold preservation overnight. Two normal rats were perfused similarly. Morrow, spinal cord was harvested and scanned by microCT with voxel size of 12.149 x 12.149 x 12.14μm. A three-dimensional image was reconstructed from serial tomograms via filtered back-projection algorithm, then performed globally thresholded to obtain a binarized micro-vascular digital image. Micro-vascular morphometric analysis software package similarly to that used in cancellous bone morphometry was accessed from the commercial MicroCT Facility Company.

RESULTS:

1. Blood supply to local normal spinal cord segment: Compared to the abridged general view shown in Figure 1 A and C (from Anaesthesi)(UK) [4], B and D represented the architecture of microvasculature at 10th thoracic cord. These three-dimensional images clearly showed the spatial distribution of the intramedullary and extramedullary micro-vessels. It was found that the intramedullary blood supply was definitely differentiated into centrifugal sulcal arterial system feeding the motor neurons gathering in the anterior horn, and a centripetal peripheral arterial system providing the partial posterior horn and the white matter. 2. Gross observation of microvasculature alteration after SCI: Structure alteration of the entire micro-vascular network in the SCI model was demonstrated (Figure 2). Before rendering the binarized images, an appropriate threshold was chosen [3]. Figure 2 B (day 1) and C (day 7) showed that the integrity of the basal blood supply architecture was broken, registering as partial blood vessels with discontinuous flow, obstruction, and local blood supply system in disorder. Following day 1, 7 and 14 after SCI, more plenty of tiny and star-like vessels with less connectivity could be visualized by eyes in the central area of spinal cord, especially the gray matter, that may be involving micro-vascular remodeling process (Figure 2D). 3. Demonstration of quantitative change in vascular micromorphometry parameters after SCI: Potential assessment indices including vascular volume fraction, connectivity and vessel separation that were described for vessel morphometry. Respectively, the data in Figure 3 (mean values) showed normal samples (n=2) and the SCI postoperative specimens at day 1 (n=2), 7 (n=4) and 14 (n=3). Results showed that vascular volume fraction was gradually increased with the healing over time after SCI. However, the connectivity and vessel separation were decreased. In the early phase at day 1, vascular volume fraction immediately reduced than that in the normal specimen, then gradually recovered at day 7 and 14 after SCI. However, the connectivity and vessel separation were slow in recovery, which might be explained by a weak compensatory vascular remodeling and reconstruction.

DISCUSSION:

Compared with conventional two-dimensional analysis (e.g. histological section and scanning electron microscope), both the digital imaging and micromorphometry was based on intact 3D microvessels network, that’s recasted via perfusion technique with radiopaque material completely. A spatially, objectively and intuitively impression was given to show the shape, branches, vessel route, connective relationship and distribution in microvascular changes in SCI process. In addition, an appropriate threshold should be cautiously chosen for contrastive morphometric parameters analysis. MicroCT with high resolution successfully illustrated the three-dimensional architecture of the microvasculature tree in normal and injured spinal cord segment, which helped to visualize the pathological changes of the blood vessel micro-morphology after acute SCI in a rat model. These data can be used to develop reliable and quantitative morphometric parameters to investigate the structure-function relationship after SCI and proceed to evaluate the efficacy of drug and rehabilitation therapy and to monitor the progress of such management in experimental animals.