Evaluation of collateral microcirculation induced by limb ischemia using synchrotron radiation microangiography

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Introduction
Therapeutic angiogenesis has emerged as a new therapeutic tool for intractable ischemia using angiogenic genes and endothelial progenitor cells. Therapeutic angiogenesis consists of angiogenesis (small vascular formations of less than 50 um) and arteriogenesis (collateral circulation of less than 200 um). Since conventional angiography cannot identify small vessels of less than 200 um, a new angiographic system using synchrotron radiation (SR) has been established, and has been to identify vessels down to 50 um. With this method, the time course of microcirculation (angiogenesis) for limb ischemia was investigated, since it had not not been evaluated angiographically due to limitations of resolution.

Method
The left femoral artery and its branches were ligated and completely excised in male Wistar rats (n=30). Rats were divided into five groups according to the times from ligation: one day (control), one week, two weeks, four weeks, and eight weeks. SR angiography was performed under the following conditions: photon energy; 33.3 KeV, exposure time; 150 ms, resolution 26 um/pixel, visual field 26 x 26 mm. The development of collateral microcirculation was evaluated by the angiographic score (AGS). A composite of 4 mm² grids printed on a transparent sheet was placed over the median thigh area of each image to calculate the AGS.

Results
The AGS were as follows: 1.3+/−0.3 at 1 day, 1.8+/−0.3 at 1 week, 2.1+/−0.3 at 2 weeks, 2.6+/−0.2 at 4 weeks, and 2.7+/−0.5 at 8 weeks after ligation (1 day vs. 1 week: P < 0.05, 1 day vs. 2 weeks: P < 0.05, 2 vs. 4 weeks: P < 0.05). The formation of collateral microcirculation persisted up to four weeks and no change was observed during eight weeks follow up (Fig-1 A-E).

Conclusion
It was found that the development of collateral microcirculation is completed by up to four weeks in rat’s ischemic limbs. Therapeutic angiogenesis could be visualized by SR angiography.

Fig-1. Microangiography of the rat hindlimb

A. 1 day after ligation
B. 1 week after ligation
C. 2 weeks after ligation
D. 4 weeks after ligation
E. 8 weeks after ligation

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