New Monochromatic Synchrotron Radiation Microangiography System to Measure Intra- and Extracerebral Arteriole Change in the Rat

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ABSTRACT.

BACKGROUND AND PURPOSE: Using a newly developed angiography system, which consists of monochromatic synchrotron radiation (MSR) as an X-ray source with a high-resolution camera system, we aimed at obtaining simultaneous in vivo three-dimensional (3D) cerebral vessel images and making observations of the intra- and extracerebral arterioles in rats during reperfusion after transient forebrain ischemia.

METHODS: The detector features a 7 μm equivalent pixel size projected onto the input area and an input field size of 7.0 mm x 7.0 mm. The changes in the cerebral microvessels were observed continuously before and for 30 min after transient cerebral ischemia with the MSR angiography system.

RESULTS: Micro 3D rotational angiography provided good visibility of the rat intra- and extracerebral arterioles under basal conditions. The diameters of the middle cerebral artery and striate artery significantly increased one min after reperfusion, while the pial arteriole diameter significantly decreased. Thereafter, all of the three-type vessels significantly increased at 10 min after reperfusion.

CONCLUSION: We herein describe and discuss the use of an in vivo experimental model in which changes in transient cerebral ischemia in the rat cerebral microcirculation were clearly depicted with an MSR angiography system. These findings show that an assessment of the cerebral regulation system can thus be performed easily and quantitatively using this method.

Key words ① **synchrotron radiation** ② **transient ischemia** ③ **reperfusion** ④ **pial arteriole** ⑤ **striate artery**

Rats are useful as an experimental small animal model of intracranial diseases including cerebral vascular disorders. Cerebral angiography has been performed with an experimental rat model showing cerebral vascular obstruction and subarachnoid hemorrhage. However, cerebral angiography depicts relatively thick blood vessels, such as the internal carotid artery and middle cerebral artery, but does not show arterioles and striate arteries measuring less than 100 μm in size[1)~5)].

Therefore, the cranial window method has been used to observe microvessels[6),7)]. Pial arterioles

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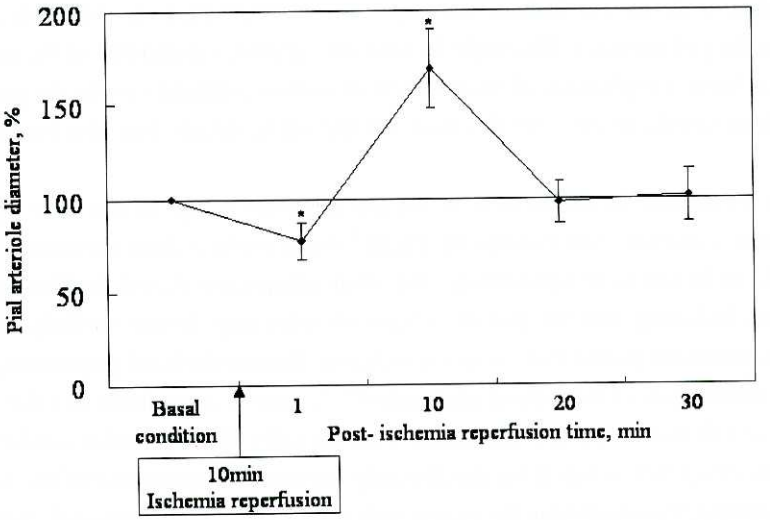

Fig. 4. Changes in pial arteriole diameter expressed as a percent of basal values. Each asterisk (*) shows $p<0.05$ as compared with the basal condition.

since this method has a drawback in that observation of the striate arteries, which are distributed in the cerebrum, is impossible, the parenchymal circulation of the brain must be speculated on from the pial circulation. Confocal laser microscopy, which has been used in recent studies, provides a method for the evaluation of blood vessels in the brain, but the visual depth is limited to the partial brain surface even when using this method[6),7),12)]. Therefore, it is impossible to simultaneously visualize the difference in regulation in blood vessels of various diameters distributed in different regions.

The spatial resolution of conventional medical x-ray imaging is affected by the size of the x-ray source and the source-to-detector distance. In contrast, the MSR angiography system has a spatial resolution of more than 30 μm, which is due to the nearly parallel X-rays emitted from its source. MSR angiography has been suggested as a possible tool for diagnosing cardiovascular disease in microvessels and malignant tumors in various organs[8),9)]. The microvessels are of arteriole size.

One problem is exposure to synchrotron radiation. To minimize this effect, irradiation was blocked during the intervals between the angiography recordings. Six consecutive angiography recordings were conducted every 10 min with normal rats, yielding clear images without any significant changes in the intracranial vascular diameters. Another problem is that vessel diameter is affected by blood pressure. However, in this study, there were no significant changes in blood pressure at the time of angiography.

In the four-vessel occlusion rat model of 15-min ischemia, Pinard *et al*[7)] reported that the diameter change in arterioles of the cortical surface was not significant for 5 min after reperfusion and, thereafter, a significant increase was observed from 5 to 15 min after reperfusion. Their results were not consistent with our findings of pial arteriole diameter change at 1 min after reperfusion. On the contrary, the diameter of the striate arteries in the present study showed a significant increase at 1 min after reperfusions. The striatum nourished by the striate arteries is reported to be more vulnerable than the neocortex perfused by the pial arterioles[13)]. Liachenko *et al* reported that transient hyperperfusion after 12 min of circulatory arrest in rats started from the thalamus and hypothalamus and later shifted to the cortex[14)]. Judging from Liachenko's

report, in addition to the present findings, the striate arteries might be more vulnerable to ischemia and reperfusion than the pial arterioles. This might be caused by hypoxic vulnerability of the striatum following global ischemia. Another explanation might exist with or without collateral vessels: the pial arterioles have abundant collateral vessels on the cortical surface but the striate arteries have few because they are end arteries.

The significant increase in the diameter of the pial arterioles during 10 min after reperfusion in the present study was consistent with findings by Pinard[7] using confocal laser microscopy. Therefore, our ischemia model can be said to be reproducible. The striate arteries also dilated significantly during 10 min after reperfusion, indicating that the pial and striate arterioles may behave similarly except during an immediate post-reperfusion period after 10 min of ischemia. Because the basal ganglia may have 54 - 73% the number of microvessels of the cortical gray matter[15),16)], there is a possibility that the more prolonged ischemia is, the more behavior between the pial and striate arterioles after reperfusion will differ.

Early venous filling, which was defined as the early angiographic appearance of the venous structures from the arterial phase, was observed in the present study during reperfusion. Ohta *et al*[17] reported that there was a significant correlation between the appearance of angiographic early venous filling during intra-arterial reperfusion therapy and post-therapeutic hemorrhagic complications. Although the appearance of early venous filling seems to be a good predictor of hemorrhagic complications after reperfusion therapy, its pathophysiologic basis is incompletely understood. Clear detection of early venous filling in rats with the MRS angiography system could help us clarify its pathophysiologic basis.

CONCLUSION

We herein described an *in vivo* experimental model in which rat 3D cerebral vessels and microcirculation changes in transient global cerebral ischemia were clearly depicted using the MSR angiography system. Our results indicate that an assessment of the cerebral regulation system can be performed both easily and quantitatively using this method.

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