Stroke-prone Spontaneously Hypertensive Rats as an Experimental Model of Malignant Hypertension

I. A Light- and Electron-microscopic Study of the Brain

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Summary. A pathological study of the brain of the stroke-prone spontaneously hypertensive (SHRSP) rats revealed development of fibrinoid necrosis of the wall of the intracerebral arterioles. These arterioles were frequently accompanied by occlusion of the lumen, and occasionally intracerebral hemorrhages and microinfarcts.

The predominant tissue alteration consisted of rarefaction and cyst formation in the white matter, and rarefaction of the neuropil and preserved neurons in the neocortex at the paramedian region of the cerebral hemispheres. Edema fluid was present in and around the lesions. The tissue degeneration can be interpreted to be the sequela of brain edema. Microinfarcts or hemorrhages are only focal lesions, and are assumed to have minor contribution to the brain swelling. Widespread expansion of the extracellular space is assumed to be responsible for the brain swelling.

Overall vascular changes of the brain, kidney, and other organs were consistent with those found in malignant hypertension.

Key words: SHRSP rat — Fibrinoid arteriolar necrosis — Microinfarct — Hemorrhage — Edema

Materials and Methods

The SHRSP rats under investigation were F38–41 generations derived from Dr. Okamoto’s laboratory (Committee on care and use of SHR rats, 1976). They were reproduced by selective inbreeding of offspring with higher blood pressure whose parents showed severe hypertensive vascular changes at autopsy. The rats were fed with stock chow diet produced by Oriental Company containing 0.6% NaCl and tap water ad libitum. Blood pressure and body weight were recorded periodically. Systolic blood pressure was measured without anesthesia by tail phlebography.

Under anesthesia with i.p. amobarbital (100 mg·kg⁻¹), brains of 11 rats with neurological symptoms were perfused with 3.5% formaldehyde in 1/15 M sodium phosphate buffer (pH 7.3) through the heart with a pressure of 180 mm Hg for 30 min. Then the skull was opened and the brain immersed in the same fixative, as previously described (Ogata et al. 1976). Five coronal sections of the brain, inclusive of cerebrum, cerebellum, and brain stem, were embedded in paraffin and cut at 6 μm. Sections were stained with hematoxylin and eosin (H&E), Nissl method, Luxol fast blue-periodic acid-Schiff, azocarmine, elastin van Gieson, axon stain, and phosphotungstic acid hematoxylin (PTAH) as needed.

Seventy-three rats, which showed cerebral lesion at autopsy after spontaneous death, were utilized for the present investigation. The brain was fixed by immersion in 10% formalin after removal. The unfixed ventricles of the heart were weighed to obtain the ratio of heart to body weight (cardiac index).

The brains of four rats with neurological symptoms were fixed by perfusion through the heart, with a dilute initial fixative containing 1.0% formaldehyde and 1.25% glutaraldehyde in 1/15 M cacodylate buffer (pH 7.3) for several minutes followed by a concentrated fixative containing 4% formaldehyde and 5% glutaraldehyde for 20 min. After immersion of the skull in the fixative, brain was removed. Diced brain tissue was post-fixed for 1 h in 2% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in alcohol, and embedded in Araldite. Ultrathin sections of the tissue were stained with lead citrate, and observed with JEM T8 and 100S electron microscopes. The rest of the brain tissue was embedded in paraffin and examined under microscope.

The other organs, such as the kidney, mesentery, testicle, heart, adrenal gland, and lung, were examined microscopically.

Results

The rats showed a rapid rise in blood pressure over 230 mm Hg in males and 210 mm Hg in females of
4–5 months of age and remained at a high level. After 6 months of age, many of them died within a few days to a few months after developing neurological symptoms. These symptoms consisted of reduction of activity, inappropriate behavior, irritability, stupor, jumping, aggressiveness, and absence of normal sniffing. These rats showed swollen head, atrophic testicles in males, weight reduction, and ruffling of the fur. Disturbance of consciousness and respiratory distress were observed in the agonal period.

The average life span of the rats with cerebral lesions which spontaneously died was 11.5 ± 2.6 (mean ± S.D.) months with a range of 5–17.7 months in 52 males, and 14.5 ± 3.8 months with a range of 9–21 months in 21 females. The average age of the rats killed by perfusion was 11.0 ± 2.2 months with a range of 6–18 months in 12 males, and 11.3, 15, and 16 months in three females.

Observation of Formaldehyde-perfused Brain

All of the rats with neurological symptoms showed cerebral lesions. Many rats possessed enlarged and thinned-out skull. Frequently, there was swelling of the brain, and discoloration at the paramedian region of the cerebral hemispheres and the vermis of the cerebellum. There were small circumscribed foci of cortical discoloration containing hematoma (Fig. 1). On section, there were discoloration and multiple cysts in these regions (Fig. 2). Intracerebral hemorrhages were often present in the neocortex (Fig. 3), and less often in the cerebellum and basal ganglia.

Histological examination of the brain revealed multiple cysts in the white matter and neocortex at the paramedian region of the cerebral hemispheres. Cysts were principally observed in the white matter (Fig. 4). Cysts in the neocortex were smaller than those found in the white matter, and occasionally contained trabeculae of astrocytic fibers and blood pigment-laden macrophages. There was a tendency to develop cysts in the neocortex at the deeper layers where myelinated fibers were abundant. Rarefaction of the white matter in these areas was marked, and degeneration of the myelin was present. Though there was rarefaction of the neuropil in the neocortex, the neurons were preserved. However they were smaller than normal and possessed rounded contour. There was disorganization of the lamellar arrangement of the neurons (Fig. 5). In some instances, these lesions contained lakes of PAS-positive material within the tissue (Fig. 6). These changes were frequently accompanied by reactive microglia, macrophages, and reactive astrocytes.

Fibrinoid necrosis of the intracerebral blood vessels was often observed in the neocortical lesions, and less