Cell Density and Cortex Thickness in the Border Zone Surrounding Old Infarcts In The Human Brain

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SUMMARY Six cases of completed ischemic stroke in the middle cerebral artery territory of more than two months' duration were selected for this study of neuropathology. Coronal brain slices of the entire brain were cut for histology and stained with Klüver-Barrera's stain. Neuronal and glial cell density, and cortex thickness were measured at various distances from the border of the infarct. Corresponding counting points in the contralateral hemisphere served as control in all cases. The density of histologically intact neurons was in all cases normal at a distance of 0.5 cm or more from the border of the infarcts. In one half of the cases the border zone between infarcted and normal tissue was less than a few cells in thickness.

This study of old brain infarcts confirms the commonly held view that there is an abrupt transition between infarcted and normal tissue. This observation suggests that the wide zone of low blood flow and metabolism surrounding cerebral infarcts is not caused by selective loss of neurons. Instead, we hypothesize that such change in blood flow and metabolism is the result of neuronal disconnection and cortical deactivation.

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CELL DENSITIES and cortex thickness in the border zone surrounding a chronic ischemic infarct in the human brain were measured. It is a commonly held view that in cases of complete infarction, the transition from infarcted to normal tissue is abrupt. This is also the usual appearance of a chronic infarct on CT. Clinical studies of regional cerebral blood flow in stroke patients have shown evidence of a border zone of low flow widely extending beyond the edges of the lesion as seen on CT. Further, consecutive observations using 133Xe inhalation flow tomography have indicated that the blood flow in this zone of low flow is refractory to microsurgical revascularization. This suggests that blood flow in the border zone around the infarct is low because there is permanently impaired neuronal function rather than sustained ischemia caused by marginal collateral blood flow and low perfusion pressure. In support of this view, experimental studies and clinical-pathological observations have suggested reexistence of a zone of selective neuronal necrosis in the zone of normal appearing tissue around the infarct, a so-called area of incomplete infarction.

This apparent divergence between the traditional view of an abrupt transition from infarcted to normal tissue, and the recently expressed view of incomplete infarction with discrete changes in the perifocal zone, prompted us to conduct the present study. We chose neuronal and glial cell densities and cortex thickness in the perifocal zone as essential parameters to be measured.

Material and Methods

All brain autopsy cases from August 1978 to April 1983 were consecutively searched for cases of major completed stroke in the middle cerebral artery (MCA) territory occurring more than two months before death.

Six cases met these criteria and were selected for study. Cases with a history of bilateral symptoms, and cases with evidence of ischemic damage in the opposite hemisphere were excluded. Infarctions in connection with trauma, neoplasm or intracranial hemorrhage were not included.

After immersion fixation in formalin for at least 14 days, the cerebral hemispheres were separated from the brain stem and cerebellum by transection through the midbrain. The hemispheres were cut coronally at 1 cm intervals. Cerebellum and brain stem were cut horizontally. Routine slices were taken from the frontal and occipital lobes, the striate body, mesencephalon, pons and medulla.

Blocks were taken from the three coronal slices in which the macroscopic lesion was most pronounced in size. Each slice was cut into blocks in a symmetrical manner (fig. 1). The tissue blocks were embedded in paraffin, and 7μm sections were stained with Klüver-Barrera's stain.

At least three gyral apices adjacent to the infarct on each side were identified by the course of the myelin fibers and marked for measurements. If the nearest gyral apex was more than 0.5 cm from the border of the infarct, one or two points were marked for measurements in the interposed sulcus. The corresponding gyri on the contralateral hemisphere were selected for control measurements.

Slides were prepared at the marked positions and numbered in arbitrary sequence and read blindly by one of us (MN). Only nucleolated cells with Nissl substance were defined as neurons. The number of glial cells was found by subtraction of neuron count from total cell count. Endothelial cells were not counted. The numbers of histologically intact neurons and glial cells were counted in columns with a breadth of 0.90 mm measured perpendicularly to the cortical surface by means of an ocular grid and an object micrometer. The total number of neurons and glial cells in one column, and the thickness of the cortex at each gyral apex were measured by moving the grid from the base to the top. The average density of neurons and glial...
cells was indicated arbitrarily by dividing total cell count per column by cortex thickness. The interobserver agreement for these counting procedures was found by counting the same column of cortex 10 times. Expressed as mean and standard deviation, values of 1566 ± 102 for total cell counts, and 488 ± 16 for neuron count were found.

The borderline of the infarct was defined as the outermost point with total demyelination of the white matter subjacent to an area with total loss of neurons. Usually, the cystic space of the infarct was sharply marked.

The width of the borderzone between infarcted and normal tissue was indicated in the following manner. The distances to the counting points were measured in radial direction from the infarct rim. Surface gyri and sulci were projected on the outer surface of the hemisphere and the distance along the surface to the infarct measured (fig. 2). Distance measurements were completed on photos of the brain slides.

The linear shrinkage of 27% ± 4% produced during the histological preparation was incorporated into calculations of all length and density measurements.

**Case Histories**

**Patient 1**

(NP 5405/78). Age of infarct: 18 months. 80 year old man. At the age of 65 the patient had a paresis of the right arm, lasting a few days. At the age of 74 he had an acute myocardial infarction without clinical sequelae. Eighteen months before death he developed a right-sided hemiparesis which persisted until death. 2 days before death he was admitted to hospital with massive ischemia of both legs, and thrombectomy was performed with removal of large thrombi in the iliac arteries. He died of bronchopneumonia on the first postoperative day. Apart from an old infarct in the left hemisphere no other ischemic lesions were observed, and the major cerebral vessels were without occlusions.
Distances from counting points to border of infarct. The counting points are marked on the figure. The distances from the infarction to the counting points are indicated on the line.

**Patient 2**
(NP 5960/79). Age of infarct: 27 months. 71 year old woman. At the age of 69 she developed a left-sided hemiparesis with persistent spastic paresis of the left limbs. Three months before death she was hospitalized with coli-meningitis successfully treated by antibiotics. She regained consciousness, but her condition deteriorated due to renal failure, endocarditis, and bronchopneumonia, leading to death.

The meninges did not show evidence of persisting meningitis at time of death. Ischemic lesions other than the old infarct in the right MCA-territory were not found. The major cerebral vessels were without occlusion.

**Patient 3**
(NP 6321/79). Age of infarct: 3 months. 92 year old man. At the age of 77 he had an acute myocardial infarction without clinical sequelae. Three months prior to death he was hospitalized with acute right hemiplegia. He was awake, but aphasic. He remained in this condition for three months, when he unexpectedly was found dead in his bed. Examination of the brain showed the old infarct, but no other signs of ischemic lesions. The major cerebral vessels were without occlusion.

**Patient 4**
(NP 8279/78). Age of infarct: 36 months. 86 year old woman who for many years had suffered from intermittent claudication. At the age of 83 years she suffered a fracture of the femoral neck, which was treated with osteosynthesis. During the operation she developed right-sided hemiparesis. Four days before death she had an acute myocardial infarction and the paresis progressed during these four days.

The autopsy showed an infarct in the left cerebral hemisphere with old changes in the middle surrounded by recent infarction, and showed thrombosis in the right internal carotid and middle cerebral artery.

**Patient 5**
(NP 8450/82). Age of infarct: 70 days. 62 year old woman with hypertension. She was admitted to the hospital with pneumococcal meningitis. After 4 days she had a grand mal seizure. During endotracheal intubation blood pressure dropped to 70/50 for several minutes. Continuing seizures occurred in the right limbs for two to three days. A right-sided hemiparesis

**Figure 3.** Photomicrograph from the margin of an infarct with sharp demarcation (original magnification ×46). Lower picture shows neuron and glial cell density counted along the cortical surface. The transition between zero and normal density is abrupt.
with mild aphasia persisted until death 70 days later, but clinical signs of global/ischemic damage were present. The cause of death was stridor and cardiac arrest following biopsy of a tracheal polyp during bronchoscopy. Examination of brain and meninges showed no evidence of persisting meningitis. Except for the infarct in the left MCA territory no other ischemic lesions were found. The major cerebral vessels were without occlusion.

**Patient 6**

(NP 8606/82). Age of infarct: 5 months. 71 year old woman. She developed paresis of the left limbs, which persisted until death 5 months later.

An osteosynthesis of the left femoral neck was performed and one week later she suddenly developed pulmonary embolism and died.

Examination of the brain showed the old infarct, but no other signs of ischemic lesions. The major cerebral vessels were without occlusion.

**Results**

**Macroscopic Findings**

An old infarct in the territory of the MCA was found in all cases. The largest extension of the infarct on the outer surface of the hemisphere varied from 2.48 cm to 7.26 cm, median 4.92 cm. In all cases the infarcted cystic areas were demarcated sharply from the surrounding tissue. Enlargement of the ipsilateral lateral ventricle was encountered as a sign of local atrophy (fig. 1).

**Histologic Findings**

The infarcted areas appeared as large or small cavities, containing fluid and a variable number of fat filled phagocytes. Blood vessels were seen in many of the spaces. Hyperplastic and hypertrophic astrocytes were present at the margin of the lesions. The cell bodies were often swollen with an abundance of hyaline eosinophilic cytoplasm. Many astrocytic fibers were noted. Neither blood vessel proliferation for fibrosis was observed in the marginal zone. The infarct was sharply demarcated and the zone of transition from affected to normal tissue did not exceed a width of 2 mm (fig. 3). The subpial margin of the infarct was often composed of cortical tissue with intense gliosis. Cortex positioned as a shell on the deeper part of the infarct often appeared spotted with small rounded areas of destroyed tissue (fig. 4). These small areas were surrounded by a very thin capsule of gliosed cortical tissue.

This spotted appearance indicates that the infarcts, although sharply demarcated, are bordered by an irregular undulating surface bulging in and out. The spotted appearance of bordering deep cortex was more often observed in the posterior than in the anterior watershed.

In patient no. 4 a rim of encrusted ferruginated ganglion cells was observed. In addition, a few cm wide zone with only partial neuron loss was observed in the anterior and in particular in the posterior watershed. This corresponds to the clinical information of a superimposed recent infarct with aggravation of the hemiparesis within 4 days before death.
FIGURE 5. Neuron density. Relationship between neuron density and distance to border of infarct. The infarct is indicated on the x-axis by its zero neuron density. The border zones are marked on the y-axis with the upper zone (the ACA/MCA watershed) placed arbitrarily at the fifth point. Values on both sides of the two zero values are neuron density at increasing distances from the border of the infarct. x-axis intervals in cm, y-axis intervals in 100 neurons per 0.81 mm². o—o neuron density in the hemisphere with infarct. x-x neuron density in the opposite hemisphere.

Quantitative Findings

The density of histologically intact neurons was in all cases (except in patient no. 4) normal at a distance of more than 5 mm from the infarct measured on the outer brain surface (fig. 5). In three patients, a slightly elevated glial cell density was observed in the posterior border of the infarct (fig. 6).

The cortex thickness showed the same picture as the neuron density although with more pronounced individual variation (fig. 7).

A periinfarct zone with gradually decreasing neuronal density was not observed.

Discussion

This study of large chronic ischemic infarcts in the human brain confirms the general view of a fairly abrupt transition from infarcted to normal tissue. Observations in adjacent cortex on the outer surface of the brain indicated transition to normal cell densities and cortex thickness within a distance of 5 mm or less from the infarct. This pattern was constantly observed in five of the six studied brains, with brain no. 4 as the only exception. This patient suffered a recurrent stroke in the same territory four days before death. In this case signs of acute ischemic necrosis with loss of neurons occurred diffusely in a periinfarct zone which extended some centimeters from the chronic infarct. Observations in embedded cortex adjacent to deeper parts of the infarct also revealed a pattern of abrupt transition from infarcted to normal tissue. However, embedded cortex has a rather complex geometry, and a detailed explanation of the changes observed is appropriate. Embedded cortex usually was positioned as a shell on the deeper part of the infarct, and could be followed down in the depth of the sulcus and further on to the neighboring gyrus. The cortex lining the infarct appeared spotted with foci of low or absent neurons interspersed with foci of normal or subnormal neuronal counts. This picture could be traced to the depth of the sulcus and continued some millimeters into the neighboring gyrus before normal cell densities appeared.

In conclusion, we have observed a sharp demarcation of large chronic ischemic infarcts. A periinfarct zone with incomplete loss of neurons was observed, but was narrow and extended only a few millimeters from the deeper as well as the superficial parts of the infarct.

This observation is in accordance with the appearance of a sharp demarcation of chronic ischemic infarcts on CT. This observation cannot explain the wide border zone of low blood flow around ischemic infarcts as visualized by 133Xe blood flow measurements and also by positron emission tomography. As suggested by Phelps et al" disconnection with deactivation of cortex adjacent to the infarct due to the deep
white matter lesion might be the cause of low function, metabolism and blood flow in the perifocal zone. This implies that disconnected and metabolically inactive neurons survive the ischemic injury and remain structurally intact on light microscopy. These properties of the perifocal zone relate to the phenomenon of diascisis often described as a mirror focus of low function, metabolism and blood flow in the contralateral hemisphere. 

Our observations in the human brain correspond well to observations in experimental infarction. In waking primates with permanent MCA occlusion and large infarcts, Jones et al11 described coagulation necrosis surrounded by a rim of inflammatory changes and astrocystosis. Such changes followed permanent ischemia with local flows below 21–23 ml/100 g/min and were accompanied by lasting neurological deficits. This description is in accordance with earlier observations made by Symon and Brierley in a chronic stroke model in baboons.7 They also found a sharp demarcation between the infarct and normal tissue, and in adjacent cortex "short lengths of laminar sclerosis" were sometimes observed. These observations collectively indicate that large infarcts caused by lasting severe ischemia in the MCA territory are sharply demarcated from normal tissue without evidence of a wide perifocal zone of incomplete partial neuronal loss. However, the observations in the human brain and in subhuman primates differ from recent observations in the cat. Mies et al4 and Strong et al3 have shown loss of neurons in a wide zone of grey matter extending to the neighboring gyri around a complete infarct following permanent MCA occlusion. This selective neuronal necrosis occurred in remote areas with only subnormally reduced flows. This points to a secondary type of lesion. We suggest that the long lasting elevations of extracellular potassium concentrations observed by Strong et al12 in gyri adjacent to the infarct may be associated with this type of lesion. Such transient elevations of potassium are a rare phenomenon in the baboon.11 The human brain probably resembles the baboon's in this pathophysiological aspect of focal ischemia. Possibly the cat and other species having a relatively small brain, and a high degree of membrane instability behave differently.

We conclude that large chronic ischemic infarcts in the human or subhuman primate brain display a sharp transition to normal tissue. This does not preclude more incomplete signs of infarction in cases of ischemia of short duration as presented clinically as TIA or minor stroke. In fact the studies by Jones et al11 and Garcia et al14 suggest that the complete sharply demarcated infarct represents only one end-stage of a spectrum of changes governed by the duration and severity of ischemia. If duration of ischemia is short enough, no histological changes are found, and clinical recovery is complete. Ischemia of longer duration, followed by incomplete clinical recovery, was associated with a picture of incomplete infarction with scattered small foci of neuronal necrosis and variable preservation of the neuropil. A similar plethora of changes possibly related to severity and duration of ischemia can be found in humans; the extremes being TIA and major completed stroke. We have studied major completed stroke. A study of TIA and minor strokes is warranted.

References

The Profile Of Recovery From Stroke And Factors Influencing Outcome

Mervi Kotila, M.D., Olli Waltimo, M.D., Marja-Liisa Niemi, M.A., Ritva Laaksonen, M.A., and Maire Lempinen, B.A.

SUMMARY The recovery from stroke of 154 survivors out of 255 stroke patients was analyzed. The outcomes documented were: discharge from hospital, activities of daily living (ADL) and return to work. A clear improvement in neurological and neuropsychological deficits was seen from the acute stage to three months, and this continued to twelve months, but to a lesser degree. 69% and 78% respectively, of the patients were at home three and twelve months after stroke. Independence in ADL increased from 32% acutely to 62% and 68% by three and twelve months, respectively. Of those gainfully employed prior to stroke, 55% had returned to work after twelve months.

As a group, SAH patients seemed to recover better, but, for those that could be age-matched with infarction patients, there was no difference in outcome. Old age, acute stage hemiparesis, impairment of intelligence and memory, visuospatial deficits, nonadequate emotional reactions, and living alone all had a major negative influence on outcome.

This study suggests that neurological and neuropsychological deficits, as well as emotional reactions, influence the outcomes after stroke, and all should be taken into consideration in prognosis.
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