SUMMARY

Nine brain autopsy cases of small old cerebral infarcts were selected for neuropathological studies. Eight of the patients had cortical infarcts, in two cases with extension into the striate body. In one case the infarct involved the striate body only. The density of neurons and glial cells was measured in the coronal and the horizontal planes at various distances from the margin of the infarct. Corresponding counting points in the contralateral hemisphere served as control.

On light microscopy, the infarcted cortex was irregularly shaped, but on serial sections the bulging parts appeared to be cut off from the infarcted tissue ("pseudo-infarct islands"). The zone of transition from infarcted to normal brain tissue was less than a few mm wide. In one patient, tomographic measurements of the cerebral blood flow (CBF) and a CT scan could be compared with the neuropathological findings. In this patient, CBF in the surroundings of the infarct was decreased despite a normal neuronal density. The study supports the traditional view held by pathologists that a sharp transition exists between infarcted and normal brain tissue and suggests that the hypoperfusion zone surrounding the region of complete infarction may be due to mechanisms other than selective loss of neurons.

A CHRONIC BRAIN INFARCT appears sharply demarcated on the CT scan. In contrast, three-dimensional imaging of the cerebral blood flow (CBF) by xenon-133 inhalation1 shows a reduced flow in the region of infarction and, in most cases, a large zone of hypoperfusion around the infarct.2,3 The cause of this hypoperfusion remains uncertain. The tissue is not ischemic in terms of a low perfusion pressure and poor collateral filling, because in most cases the hypoperfusion is resistant to surgical revascularization.4 A state of incomplete infarction, i.e. a selective neuronal cell necrosis with otherwise intact glial tissue and a normal CT appearance, has been suggested.4,5 This explanation has found support from experimental studies,6 but in a recent study of large old infarcts in humans, we found no evidence of selective neuronal death in the surroundings of the infarct.7 This previous neuropathological study showed that the infarcts were sharply demarcated from the normally structured brain. As large infarcts represent complete necrosis of the territory of a major intracerebral artery, the sharp demarcation could represent the transition from one vascular territory to the other.

We therefore felt it was necessary to study small infarcts located within the territory of a major artery. The aim was to ascertain whether evidence could be found of selective neuronal cell death in the surroundings of minor infarcts in explanation of the hypoperfusion observed in the chronic phase.

Material and Methods

Nine brain autopsy cases of minor chronic infaracts were selected for the study. Only cases with a single ischemic infarct and good clinical recovery were selected. Eight patients died a non-cerebral death without clinical evidence of recent cerebral ischemia. One patient died after a brain stem infarct. The patients were selected among the total number of neuroautopsy cases performed at the Institute of Neuropathology of Rigshospitalet from 1979 to 1984.

After immersion fixation in formalin for at least two weeks, 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.

The brain from patient No. 9, in whom neuropathology could be compared with both the CT scan and the xenon-133 tomogram, was cut into horizontal sections to allow this comparison. The sections were placed parallel with the plane through the inferior aspect of the frontal lobe and the groove between the pons and medulla oblongata. A brain-cutting box of plexiglass was constructed for horizontal sectioning of the brain.

The brain was on a support plate which could be lifted to adjust the plane of section according to the planes indicated by the CT and flow measurement. A movable frame mounted with 10 knives (trimming blade, cat. no. 02.055.00.000 Feather) allowed sectioning of the entire brain in 10 mm thick slices.

The coronal slices from patients 1–8 and the horizontal slices from patient No. 9 were cut into blocks at the level where the macroscopic lesion was largest. For the estimations in the horizontal direction in patients 1–8 at least 3 blocks rostral and 3 blocks caudal to the infarct were taken. The blocks were embedded in paraffin, and 7 μm sections were stained by the method of Klüver-Barrera. At least 3 counting points adjacent to the infarct on each side were marked for cell density measurements. Corresponding points on the contralateral hemisphere were selected for control measurements. Slides were prepared at the marked positions, numbered in arbitrary sequence, and the cells were

1. Stroke Vol 17, No 6, 1986
TABLE 1  Summary of Age, Sex, and Major Diseases, Cause of Death, Localization of the Infarct in Main Artery Territory, Cortical and/or Striate Involvement, Artherosclerosis. Finding of Arterial Occlusions, Size of Cortical Surface of the Infarct, Time-lapse between Stroke and Death, and Clinical Recovery in Patients No. 1–9

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Other diseases</th>
<th>Cause of death</th>
<th>Artery territory</th>
<th>Location</th>
<th>Artherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Prostatic cancer</td>
<td>General deterioration (remained conscious)</td>
<td>PCA</td>
<td>+</td>
<td>Severe</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Prostatic cancer</td>
<td>Acute myocardial infarction</td>
<td>MCA</td>
<td>+</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Pulmonary disease</td>
<td>Acute pulmonary embolus</td>
<td>MCA</td>
<td>+</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Pulmonary disease</td>
<td>Acute respiratory failure</td>
<td>MCA</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>Pulmonary disease</td>
<td>Acute myocardial failure</td>
<td>MCA</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Stenosis of the mitral valve</td>
<td>Postoperative cardiogenic failure</td>
<td>MCA</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>F</td>
<td>-</td>
<td>+</td>
<td>Cardiac failure</td>
<td>Not found</td>
<td>MCA</td>
<td>+</td>
<td>Severe</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Cardiac failure</td>
<td>Drowned</td>
<td>MCA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>Brain stem disease</td>
<td>Brain stem infarction</td>
<td>MCA</td>
<td>+</td>
<td>Severe</td>
</tr>
</tbody>
</table>

MCA = middle cerebral artery; PCA = posterior cerebral artery.

counted blindly by one of us (MN). Only nucleolated cells with Nissl substance were defined as neurons. The number of glial cells was found by subtracting the neuronal cell count from the total cell count. Endothelial cells were not counted. The number of histologically intact neurons and glial cells was counted in columns with a width of 0.40 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 point were measured by moving the grid for the base to the top from every counting point. The diameter of neurons and glial cells was determined as the average of 40 cells in each patient by a computerized analyser (Leitz TAS plus). The thickness of the sections was determined as the thickness of folds. The cell numbers were corrected for section thickness and cell diameter by means of Abercromie’s formula. The intra-observer agreement for counting procedures was found by counting the same column of cortex 10 times. Expressed as the mean and standard deviation, values of 1566 ± 102 were found for the total cell count and 488 ± 16 for the neuron count.

The borderline of the infarct was defined as the outermost point with a total loss of neurons. The zone of transition between infarcted and non-infarcted cortex was defined as the distance between zero and normal neuron count. The width of this transition zone was measured in a radial direction from the rim of the infarct.

After sectioning, photographs of the cut surface of the tissue blocks before (in fixed state) and after histological preparation were taken. The lengths of 10 tissue blocks and corresponding sections were compared in each patient. The linear shrinkage of 29% ± 3% (mean and standard variation) produced during the histological preparation was taken into account in all length and density measurements.

The patient’s data are summarized in table 1, but more details on patient 9 will be given. This patient was a 47 year old man, who had been treated for hypertension for 8 years. 23 months prior to death he suffered an attack of right-sided weakness and dyscoordination with hemiparesis. Five months later he had an episode of a day or two with motor aphasia. CT performed after the latter episode showed a small infarct in the left parieto-occipital region. Aortocervical arteriography revealed occlusion of the left internal carotid artery. Measurement of CBF by xenon-133 inhalation and tomography showed extensive areas with decreased flow in the left hemisphere. An extracranial-intracranial (EC-IC) anastomosis was performed. Postoperatively the clinical condition was unchanged with persisting weakness of the right extremities and slight aphasia. Angiography 5 months after shunting showed a patent but narrow anastomosis with sparse filling of the MCA-territory, and the regional CBF was unchanged (fig. 5). Two months later the patient suddenly experienced nausea, vertigo, and vomiting, followed by weakness of both lower limbs and the right arm and inability to talk. He died after 15 hours. This stroke was clinically regarded as an insult in the brain stem.

Autopsy showed a small infarct in the posterior part of the left MCA territory and a small infarct of older age in the anterior part of the left caudate nucleus. In
addition, several small areas of encephalomalacia were found in the pons, only one of recent date. Atherosclerosis of the intracerebral vessels was pronounced, but no occlusions were found. The vessels on the neck were not dissected.

Results

General Comments on the Cases

All the patients had a good clinical recovery following the infarct, and in three patients the ischemic episode had passed without admission to hospital. Only two had permanent arterial occlusion treated in one with an EC-IC bypass. Two patients had hypertension and two had diabetes mellitus. All infarcts were less than 5 cm² measured at the cortical surface. Five were often preserved. In all cases cortical as well as striatal tissue were occasionally observed within a few mm from the border of the infarct in all cases (fig. 2). A tendency to gliosis was evident in cases 1 and 6 (fig. 3). In no instance was a peri-infarct zone with a reduced neuron density observed.

Patient No. 9 deserves a specific comment, as the neuropathological findings could be compared with the CT scan and measurement of the CBF (fig. 4). The finding of a small infarct in the left parieto-occipital region on the CT scan was confirmed by the neuropathological examination; the infarct appeared as a single irregular cavity measuring 1.2–1.4 cm in diameter and containing clear fluid. It was located in the posterior part of the MCA territory. On light microscopy it was as sharply demarcated as described above for the cortical infarcts. The infarct in the caudate nucleus was evident by an enlargement of the frontal horn. CBF tomography showed a low flow in major parts of the left hemisphere extending beyond the regions of the small cortical infarct. The flow in the peri-infarct areas was reduced to 60% of the values in the symmetrical situated regions in the opposite hemisphere.

Histological Findings

The areas of infarction were composed of small cavities traversed by fine trabeculae with only a few remaining cells. Lymphocytes grossly infiltrated the area, but some hypertrophic macrophages were seen. Several blood vessels traversed the spaces. A subpial margin of cortical tissue with intensive gliosis was often preserved. In all cases cortical as well as striatal infarcts were sharply demarcated from the normal tissue. The zone of transition was less than a few cells in thickness in nearly all cases. In 3 out of the 25 sections cutting the zone of transition between the infarct and the bordering tissue disclosed small rounded islands of infarcted tissue. The distance between the islands and the infarct never exceeded more than a few mm. Serial sections revealed that these islands were "periinsulas" since, they could be traced as extensions from the irregular bulging margin of the infarct. The astrocytes in the surrounding tissue were both hypertrophic and hyperplastic. No fibrosis was observed in this area, but many astrocytic fibers were present. Irrespective of their location or of their combination with deep infarcts as in patients No. 6 and 7, the cortical infarcts appeared as described above. In patient No. 8 two separate parts of the cortex lying superjacent to the striate infarct showed cortical necrosis with preservation of a few neurons in the tissue necrosis. As seen from figure 1 the cortical thickness was reduced, but the injury was clearly recognizable on CT despite relative preservation of the intervening white matter.

Quantitative Findings

The neuron counts were in accordance with the histological findings, as the density of the neurons was restored a few mm from the border of the infarct in all cases (fig. 2). A tendency to gliosis was evident in cases 1 and 6 (fig. 3). In no instance was a peri-infarct zone with a reduced neuron density observed.

TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Artery occlusion</th>
<th>Size (cm²)</th>
<th>Age of infarct</th>
<th>Clinical recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td>1.4</td>
<td>1 year</td>
<td>Very rapidly improving</td>
</tr>
<tr>
<td>ICA</td>
<td>1.7</td>
<td>18 months</td>
<td>Improving rapidly</td>
</tr>
</tbody>
</table>

Estimation of a few percent loss of neurons in human material is difficult. The reduced quality innate in any autopsy material leaves the possibility that we have overlooked a minor reduction in the neuron density in the surroundings of the infarct. Furthermore, cellular injury caused by alterations not detectable by...
light microscopy could also explain the state of low function. In addition, the measurements of cell density would tend to be overestimated in case of collapse of the tissue in the peri-infarct zone. Our findings are in agreement with a study by Torvik and Svindland. In 16 stroke cases studied by them in the early phase after onset all had only narrow zones with scattered necrotic neurons. One patient had a 2 cm broad zone, but only in one out of 5 sections. Recently, Metter et al studied a 69 year old man with multiple brain infarcts who died 8 days after being examined by positron emission tomography and (F-18)-fluorodeoxyglucose. Metabolic abnormalities were greater than structural changes in size and extent, and hypometabolism was found in areas with normal neuron density. Lassen et al found extensive areas of incomplete infarction in two cases with deep infarcts on the CT scan and proximal occlusion of the MCA in the acute phase. These patients died of a new stroke in the opposite hemisphere 3 and 34 months respectively after the first incident. Proximal occlusion of the MCA with only deep infarction causes a severe decrease in perfusion in the corresponding cortex. The cortical tissue covering the deep infarct may be considered as an extended infarct border, and in cases with limited collateral capacity this may represent a unique state in which incomplete infarction can be recognized in large areas of the human brain.

**Figure 1.** Coronal brain slide and horizontal CT scans from patient No. 8. A major infarct in the striate body is observed (*). The superjacent cortex shows necrosis (arrows) with relative structural preservation of the intervening white matter. The necrotic parts of the cortex show a marked reduction in cortical thickness. The CT scan (bottom) showed the striate infarct and a decreased density in the areas corresponding to the cortical necrosis.

**Figure 2.** Neuron density in patients 1–8. Relationship between the neuronal density and the distance to the margin of the infarct as measured in the frontal and the horizontal plane. The infarct is indicated on the x-axis by its zero neuron density. Points on both sides of the two zero values are neuron densities at increasing distances from the infarct margin with the frontal or upper point marked first on the x-axis. The counting points were projected on the outer surface of the hemisphere and the distance along the outer surface measured on photo of the brain slices. X-axis intervals in cm, y-axis intervals in 10 neurons per 0.001 mm³. 0-0 neuron density in the hemisphere with infarct. x-x neuron density in the opposite hemisphere.
Figure 3. Glial cell density in patients 1–8. Relationship between glial cell density (y-axis), and the distance to the infarct margin (x-axis). X-axis intervals in cm, y-axis intervals in 10 glial cells per 0.001 mm³. 0-0 glial cell density in the hemisphere with infarct. x-x glial cell density in the opposite hemisphere.

Observations in baboons with permanent MCA occlusion are in agreement with our finding of an abrupt infarct margin in the human brain. Symon and Brierly found that 3 year-old infarcts in the primate brain were sharply demarcated, although short lengths of sclerosis with calcified neurons were sometimes observed in cortex lying adjacent to the infarct. Similarly, in the macaque monkey, permanent MCA occlusion caused total necrosis with relatively sharp margins. In contrast, monkeys that underwent moderate to shortterm ischemia (30 min to four hours) showed multiple lesions with incomplete tissue destruction and with predominant loss of neurons.

In cats, experimental ischemia has not confirmed that cerebral infarcts display an abrupt transition between necrotic and normal tissue. Garcia et al found "red neurons" in the marginally perfused border areas 18 hours after MCA occlusion. Strong et al noted shrunken neurons with dark nuclei a few hours after MCA occlusion. In cats with MCA occlusion of 8 weeks' duration, Mies et al observed reduced neuronal density in a wide peri-infarct zone. Neuronal density was depressed in areas with normal or near-normal levels of CBF. Ongoing studies of focal ischemia in rats in our laboratory indicate that selective neuronal injury takes place in a wide cortical peri-infarct zone. Obviously there is a discrepancy between the results observed in humans and baboons and those observed in smaller experimental animals.

Patients with minor stroke show a small infarct on CT, but on measurement of the CBF in the chronic phase a large low-flow area corresponding to the infarct and peri-infarct regions is a common finding. In most cases, this flow defect cannot be corrected by an EC-IC bypass. This indicates that the reduction of CBF is not caused by a restriction in flow with a reduced perfusion pressure, suggesting that the flow defect is non-ischemic representing more probably regional low function and metabolism. Patient No. 9 was such a case. Despite a patent EC-IC bypass he had an unchanged flow pattern with decreased flow in the infarct and peri-infarct areas. These low flow areas appeared normal on CT and at the subsequent postmortem examination. This suggests that the peri-infarct low flow areas, although of normal structure, probably have a reduced function and metabolism. Deactivation of the cortex due to undercutting of the fibers which pass through the infarcted area could be one reason for
CELL DENSITY AROUND SMALL BRAIN INFARCTS/Nedergaard et al

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the reduced flow. The deactivated neurons survive and remain light microscopically normal — a phenomenon well known from transplantation studies in which the graft remains structurally normal despite "no sprouting". In addition, the integrity of brain function may be compromised by damage to remote cortical areas. The disturbance of function in the surroundings of an infarct, as caused by direct disconnection of neuronal pathways or by disturbed integrity, may be termed peri-infarct diaschisis.

Our histopathological studies support the concept of a sharp transition from infarcted to structurally normal (although not functionally normal) brain tissue in man. This sharp transition may be viewed in connection with the so-called flow thresholds in ischemia, which have been discussed in association with the concept of the ischemic penumbra. In acute ischemia there appears to be a flow threshold of failure of the synaptic transmission and a distinctly lower flow threshold of energy failure with ATP depletion and ion-pump failure causing transmembrane leak of ions and membrane depolarization. The term penumbra describes the zone of intermediate flow between these two thresholds. It appears that the state of energy failure (as well as the state of synaptic transmission failure) is sharply demarcated already in the acute state: it is either present or not, depending on whether blood flow is below or above the threshold value. Assuming a similar flow threshold of infarction, which may be identical with the flow threshold of energy failure, the sharp demarcation of the infarct can be understood. The infarct may well be of an irregular shape depending on local vaso-architecture, but at the microscopic level a sharp demarcation should be expected, reflecting the position of a critical low flow value in the acute state. In our view, the sharp demarcation of the ischemic infarcts is in accordance with the concept of the ischemic penumbra.

References
Restenosis and Occlusion After Carotid Surgery Assessed by Duplex Scanning and Digital Subtraction Angiography

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SUMMARY In a study of 140 patients operated upon with 143 carotid endarterectomies (mean follow-up time 5.2 ± 2.3 years, range 1 month — 9.3 years), vessel morphology was examined with duplex scanning in 113 patients and with digital subtraction angiography (DSA) in 82 patients. The operative mortality was 1.0% on the non-operated side. Fourteen new occlusions (12%) of the operated carotid artery was found and restenosis (>50%) in 13 patients (11.2%). Progression of the atherosclerotic disease in the contralateral non-operated carotid artery was found in 41 patients (37%) including 3 new occlusions. Agreement DSA/DSA was 88% on the operated side and 92% on the non-operated side. New strokes or TIA on the operated side were more common in patients with occlusions or restenosis (p < 0.05), whereas no symptoms were referable to occlusions on the non-operated side. Risk factor analysis revealed an increased risk of atherothrombotic progression on the non-operated side in smokers and those with two or more risk factors. The role of restenosis in the operated carotid artery was higher in females (p < 0.025).

MORPHOLOGICAL AND HAEMODYNAMIC CHANGES in extracranial arteries following endarterectomy have not been extensively investigated, since serial angiography examinations involve a certain risk. The rate of restenosis >50% of diameter reduction/occlusion has previously been investigated predominantly in patients with recurrent neurological symptoms and is reported to amount to 1–5%. Using continuous wave Doppler, the incidence of restenosis >50% and occlusion has been reported to be 36% after a mean observation time of 6 years. A combination of 2-D image and pulsed Doppler, so-called duplex, offers the advantage of providing both anatomical and haemodynamic information and makes possible the detection of stenosis less than 50%. The validity of this method has also been demonstrated in patients after endarterectomy in comparison with postoperative angiogram. With this technique, the incidence of restenosis >50%/occlusion has been reported to be 19% after a mean observation time of 16 months.

A new semi-invasive method, digital subtraction angiography (DSA), has gained wide use during recent years and is still under evaluation although hitherto the reported results are less accurate compared to conventional angiography.

The aim of the present study was to evaluate the frequency of restenosis or occlusion after carotid endarterectomy (CE), the possible correlation between morphological changes and recurrent symptoms and the possible influence of vascular risk factors on these events.

There are few published investigations on the natural course of asymptomatic carotid artery lesions.
Cell density in the border zone around old small human brain infarcts.
M Nedergaard, S Vorstrup and J Astrup

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