Light and Electron Microscopic Study of Lipid Accumulation Along Margins of Experimental Cerebral Infarcts in Rats

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Using light and electron microscopy, we studied the interaction between lipids and host tissue for up to 15 days after experimentally produced cerebral infarcts in 16 rats. A lipid-dense zone was formed along the periphery of the infarcts before a glial response started; a glycogen-rich zone appeared peripheral to the lipid zone. Macrophages and astrocytes then started to proliferate in the lipid and glycogen-rich zones. The cerebral tissue within the lipid zone underwent complete necrosis. Ultrastructurally, lipids were observed in the edematous areas as well as in various types of hematogenous and resident cells. Glycogen granules were present mainly in the astrocytic processes. Macrophages rapidly evolved into foamy macrophages in the central necrotic areas, whereas foamy transformation was not striking in the peripheral, less injured areas. Reactive fibrous astrocytes also contained varying amounts of lipids. The exact biologic significance of the lipid zone in the premacrophagic stage remains unclear; however, since lipids are hydrophobic, they may function as a barrier against edema fluid extension into the adjacent tissue. (Stroke 1988; 19:1544-1549)

It is well known that when tissue necrosis occurs, membrane lipids start to break down without the participation of macrophages, although lipids in cerebral infarcts are derived mostly from macrophagic breakdown of tissue debris. However, to our knowledge, no morphologic description, experimental or clinical, is yet available in the literature with respect to the appearance of lipids in cerebral infarcts before the macrophage response. During a morphologic study of the chronology of experimentally produced cerebral infarcts in rats, we were intrigued by the presence of abundant lipid droplets, which were observed predominantly along the margins of the lesions, during the premacrophagic stage of infarction.

Materials and Methods

We used 16 male Wistar rats weighing 250–350 g and created cerebral infarcts by injecting homologous blood clot emboli into the carotid artery. The rats were anesthetized with ether and killed by transcardiac perfusion with 2% paraformaldehyde and 2% glutaraldehyde 1, 2, 3, 5, 7, 10, or 15 days after infarction, two rats each day. Two unoperated rats killed in the same manner served as controls.

We used hematoxylin and eosin, toluidine blue, or periodic acid-Schiff (PAS) to stain Epon-embedded, semithin sections of dissected brain for light microscopy. Representative areas of the infarcts were postfixed with 1% osmium tetroxide; ultrathin sections were made, mounted on coated copper grids, stained with uranyl acetate and lead citrate, and then examined under an electron microscope.

Results

Practically no lipid droplets or crystalloids were noted anywhere in the rats.

Light Microscopic Findings

Two days after infarction, lipid droplets accumulated along the periphery of the infarcts, forming a distinct lipid zone (Figure 1, left). The droplets were gray to gray-brown and globular and were noted in the edematous neuropil as well as in neutrophils, mononuclear cells, glia, neurons, endothelial cells, and perivascular cells. The cerebral tissue within the lipid zone subsequently underwent complete necrosis. Peripheral to the lipid zone was another zone of PAS-positive granular material (Figure 1, right). The amount of lipids and tissue debris varied from area to area and was less in slightly injured than in severely injured areas.
On Day 3, foamy macrophages appeared in the lipid and PAS-positive zones. The infarcts became delineated from the surrounding edematous areas. Foamy transformation of macrophages was not striking in the peripheral, less injured areas compared with the central necrotic areas, in which macrophages rapidly evolved into foamy macrophages.

On Day 5, reactive fibrous astrocytes became prominent in the infarcts. On Day 7, the infarcts were made up of three concentric rings of reactive cells: foamy macrophages in the center, macrophages in the intermediate ring, and reactive fibrous astrocytes in the outer ring (Figure 2). Lipid droplets were also observed in the reactive fibrous astrocytes.

The osmiophilic property of the lipids became weaker with time, and the droplets were usually pale gray on Day 10. Free, scattered lipid droplets at that time were generally larger than those found earlier. There was often destruction of the pia-glial membrane, and there was a prominent ingrowth of arachnoid cells in the cystic areas. Foamy macrophages and lipid droplets were scattered throughout the subarachnoid space.

Electron Microscopic Findings

Lipids were widely distributed as globules in the edematous-to-necrotic neuropils (Figure 3, top) as well as in infiltrated neutrophils and mononuclear cells, endothelial cells, perivascular cells, glia, and neurons (Figure 3, bottom). The PAS-positive material just peripheral to the lipid zone was found to be glycogen. Glycogen was widely distributed, mainly in the perivascular edematous processes of astrocytes (Figure 4). Glycogen granules were also found in neuronal processes.

Foamy macrophages were filled with many highly variegated phagosomes, lysosomes, dense bodies, and lipids, and dying foamy macrophages, the cell membranes of which had disintegrated and released organelles and lipids, were observed in the infarcts (Figure 5). Reactive fibrous astrocytes also contained varying amounts of lipids (Figure 6). The droplets varied in appearance; some were homogeneously electron dense, some were less dense to lucent, and others had lucent halos or annular vacuolations.
Discussion

Ours is, to our knowledge, the first morphologic demonstration that a lipid zone is formed along the margins in the premacrophagic stage of cerebral infarcts. This accumulation of lipids, which are hydrophobic, seems natural because water content in the margins is much less than in the centers of infarcts.

The rapid evolution of macrophages into foamy macrophages in the central necrotic areas of infarcts appears to be simply related to the large amount of lipids and tissue debris in the centers. Incidentally, the transformation to foamy macrophages is weak and slow in the less injured peripheral areas of infarcts, in which there are much less lipids and tissue debris. The fate of foamy macrophages is debatable, that is, do they return to the circulation or do they die in the infarct? In this respect, since some foamy macrophages showed disintegration of their cell membranes in our study, there is no doubt that some foamy macrophages eventually die in the infarcts.

Although reactive fibrous astrocytes contain some lipid droplets, it seems difficult for their lipidization to be a fatty degenerative process, if only because reactive fibrous astrocytes are regenerative during the repair of infarction. A possible explanation is, therefore, that lipidization of astrocytes is in part the result of their phagocytosis.

It is extremely difficult, if not impossible, to correlate the biochemistry of lipid droplets with their light microscopic and ultrastructural features due to the fact that droplet morphologic appearance depends on various factors such as the method of fixation and tissue preparation and the size, the fatty acid content, and the degree of unsaturation of the fatty acids in the lipids.

The exact biologic significance of the lipid zone remains unclear. It is possible that immediately after infarction, both water and lipids spread out from the infarct; as lipids accumulate along the periphery of the infarct, the passage of water through the lipid layer would be prevented. This suggests that the lipid zone serves as a barrier against further unlimited centrifugal edema fluid extension into the
FIGURE 3. Electron microscopic sections 2 days after cerebral infarction in rats. Top: Lipid droplets (arrows) are present in edematous neuropils. ×6,000. Bottom: Edematous neuron contains lipid droplets (arrows). Nt, neurotubules. ×8,000. Inset: Nt. ×16,000.
FIGURE 4. Electron microscopic sections 2 days after cerebral infarction in rats. Left: Glycogen granules (arrows) found mainly in edematous astrocytic processes. Gf, glial filaments. ×7,000. Inset: Gf. ×11,000. Right: Another field of edematous neuropils shows more glycogen granules (arrows). ×7,000.

FIGURE 5. Electron microscopic sections 10 days after cerebral infarction in rats. Dying foamy macrophages show disintegration of cell membrane (arrows) and release of lipids. ×8,000. Inset: Release of lipids in another dying foamy macrophage. ×15,000.
surrounding, surviving tissue. The glycogen-rich zone may not be formed unless the infarct produces a kind of water-protective barrier within it because the central parts of infarcts contain a lot of water and because glycogen is water soluble.

Our model fulfills the fundamental requirements for experimental cerebral infarction applicable to ultrastructural studies and is presently being applied to the biochemical, metabolic, and physiologic study of lesions. Therefore, we can compare the morphologic changes with biochemical, metabolic, and physiologic data obtained at selected points in time to reconstruct the natural history of infarcts. A pathologic study with concurrent biochemical, metabolic, and physiologic studies of such lipid and glycogen-rich zones in approximately 2-day-old cerebral infarcts may help to clarify the mechanism of rapid localization of infarcts.

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References


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