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Photochemically-induced vascular damage in brain cortex. Transmission and scanning electron microscopy study

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Abstract. Morphological changes of microvessels of cerebral cortex were evaluated in a model of cerebral infarction initiated by a photochemical reaction. Rats were treated with intravenous injection of rose Bengal and irradiated from a halogen lamp source through an intact cranium to precipitate microvascular damage. Investigations in transmission and scanning electron microscopy revealed platelet aggregation on endothelial cells preceded by its early ultrastructural damage. Other typical microscopic features of brain ischaemic injury were present suggesting that the present method may be used as a model for investigating ischaemic brain damage. Since the photochemical activation of the rose Bengal dye results in formation of reactive oxygen species this model may be particularly useful to elucidate the role of free radical-mediated endothelial damage in the formation of microthrombi and blood-brain-barrier integrity.

Key words: cerebral ischaemia, photochemistry, microvascular damage, rat

INTRODUCTION

Research on pathogenesis and mechanisms of cerebral ischaemia relies on the availability of suitable experimental models. Cerebral ischaemia in experimental animals has been most widely induced by ligation of large arteries (Korpaczew et al. 1982, Łazarewicz et al. 1989, Pluta et al. 1991) obliteration of carotid arteries (Kågström et al. 1984), or injection of endothelin (Fuxe et al. 1989). The most important pathophysiological changes in the brain after ischaemia are found in endothelial cells of brain capillaries which demonstrate an increase in the number of microvilli and pitting in its luminal surface (Pluta et al. 1991, Wester et al. 1995). Cerebral ischaemia involves also platelet adhesion and their accumulation on the luminal surface of the vessels (Joseph et al. 1991). Furthermore, permeability of the blood-brain barrier increases which leads to the emergence of perivascular oedema (Pluta et al. 1994).

Watson et al. (1985) have shown that brain ischaemia can be experimentally induced by an intravascular photochemical reaction causing thrombosis in cortical vessels. These authors injected the animals with a dye, rose Bengal, which initiated the thrombotic reaction upon irradiation with laser light trough an intact skull. Here, we report that the photochemical reaction leading to brain ischaemia can also be precipitated with visible light from a non-coherent light source. This would lower the cost of the procedure and lead to a wider acceptance of this non -invasive method as a model of cortical brain ischaemia.

METHODS

Male Wistar rats (150-200 g, age 2-2.5 months) were used. Eight animals were anaesthetized with 325 mg/kg chloral hydrate and injected intravenously with a rose Bengal (Sigma, St Louis, USA) in physiological saline at a dose of 40 mg/kg. Another 8 animals were not injected with the dye solution and used as controls.

The head was immobilized in the stereotactic apparatus, skin was incised in the sagittal plane to the parietal bone.The periosteum was removed and the brains of both experimental and control animals were irradiated through the intact skull over left hemisphere, half distance between the coronal and lambdoid sutures. An air-cooled halogen light bulb of 250 W (Osram) was used as a light source. Light was transmitted to the skull with an optical waive-guide, the ending of which was placed over the preselected part of the bone. The skull-light pipe interface was cooled by water to remove the thermal effect irradiation. After a 30 min irradiation session the animals were housed in standard conditions. Material for microscopic studies were sampled from the cerebral cortex of the left hemisphere 1 and 4 days after irradiation (4 animals in each group). The animals were anaesthetized with ether and perfused with 2.5% glutaraldehyde in cacodylate buffer, as described previously (Lossinsky et al. 1989). The sampled material was processed for scanning and transmission electron microscopy using standard procedures (Pluta et al. 1994, Lossinsky et al. 1995).

RESULTS

In the control material, the capillary vessels remained ultrastructurally normal (Fig. 1 a, b). The endothelial surface was smooth and continuous, occasionally dem-

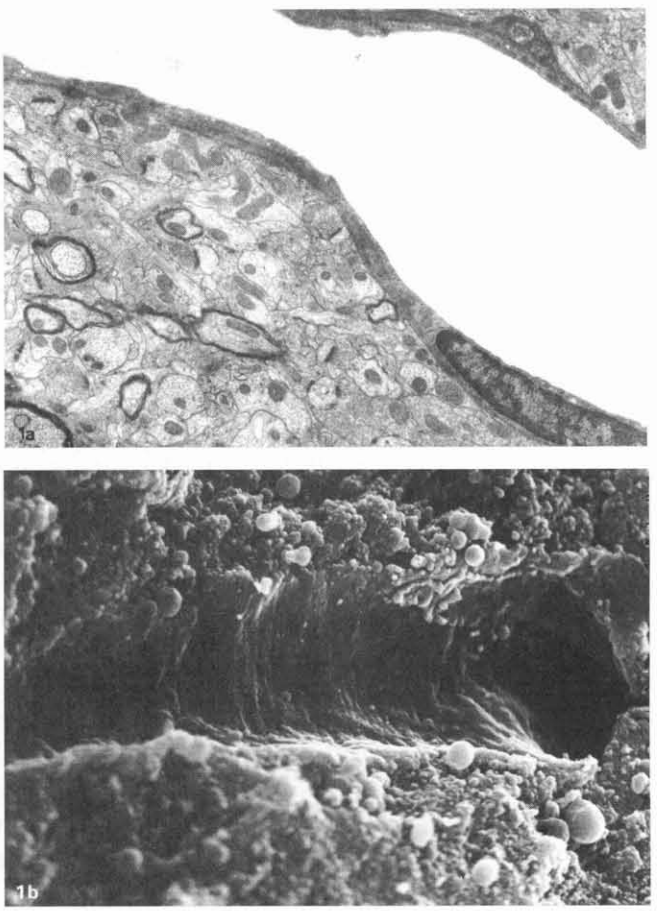


Fig. 1. Control animal. Normal capillary vessels in transmission (a) x 21,000 and scanning (b) electron microscopy. x 10,500.

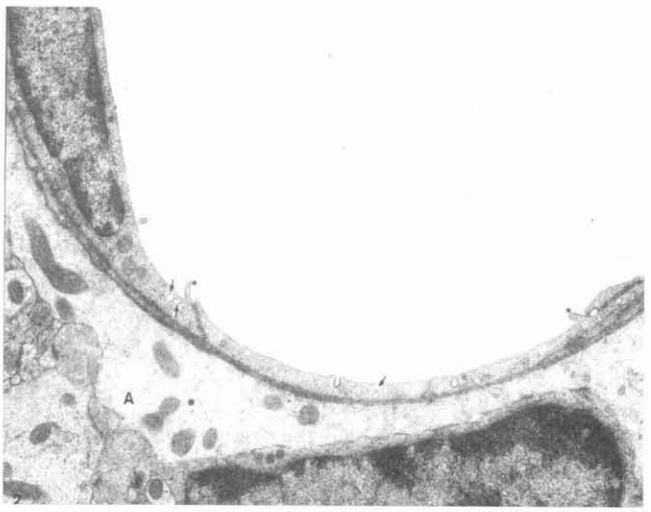


Fig. 2. Experimental animal, 1 day after irradiation. Capillary vessel with elongated inter -endothelial junctions (asterisks) and features of a high pinocytosis activity of endothelial cells (arrows). Note the swollen astrocyte process (A) in the perivascular space. x 18,000.

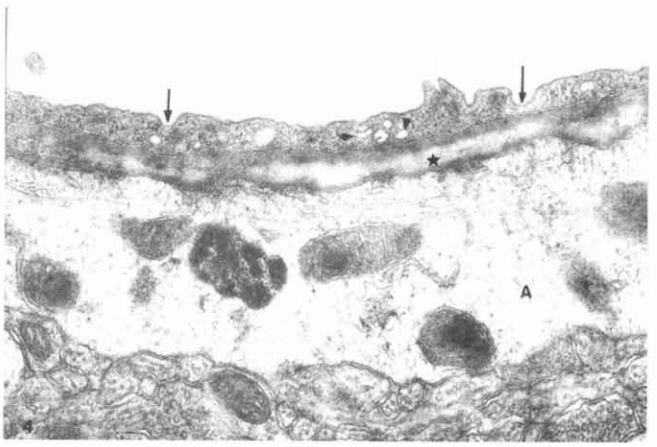


Fig. 4. Experimental animal, 1 day after irradiation. Endothelial cells with deep invaginations approaching basal lamina (arrows). Note numerous cytoplasmic vesicles of different size (arrowheads) and collagen fibre layer (asterisk) under the basement membrane. Note inter-endothelial junction ultrastructurally unchanged and swollen astrocyte (A) in the perivascular region. x 45,000.

onstrating a few microvilli. In contrast, in experimental animals 1 day after irradiation we observed clear- cut ultrastructural changes. The endothelial cells were no longer flattened and their cytoplasm contained multiple microvesicles of various sizes (Fig. 3). Deep invaginations on the endothelial surface were present and the interendothelial junctions were elongated (Figs. 2 and 4). There was an increase in the number of microvilli (Figs. 2 and 3). A few red blood cells were in a close contact with the endothelium (Figs. 3 and 5). In the perivascular region collagen fibres were seen in the vicinity of basal membranes (Fig. 4). Perivascular astrocytes were swollen (Fig. 4). Four days after irradiation ultrastructural alterations were more profound and comprised deep endothelial invaginations, shrinkage and sloughing of endothelial cells and further increase in the number of cytoplasmic microvesicles (Figs. 6-10). Four ultrastructural forms of endothelial cells were recognized: (1) ultrastructurally unchanged cells, (2) cells with electron-lucent cytoplasm containing high numbers of microvesicles of various sizes and with short microvilli, (3) clear cells with electron-lucent cytoplasm and elongated microvilli without pinocytosis activity, (4) electron-dense cells with a high pinocytosis activity (Figs. 6 and 7).

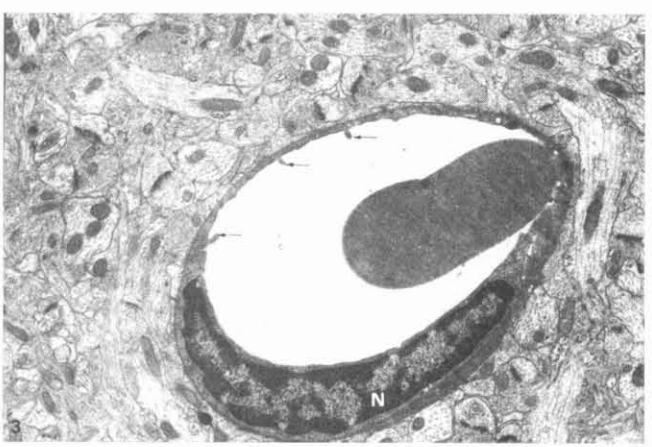


Fig. 3. Experimental animal, 1 day after irradiation. Capillary vessels with adherent red blood cell. Note numerous microvilli protruding towards the lumen (arrows), and nucleus(N) of the endothelial cell. x 15,000.

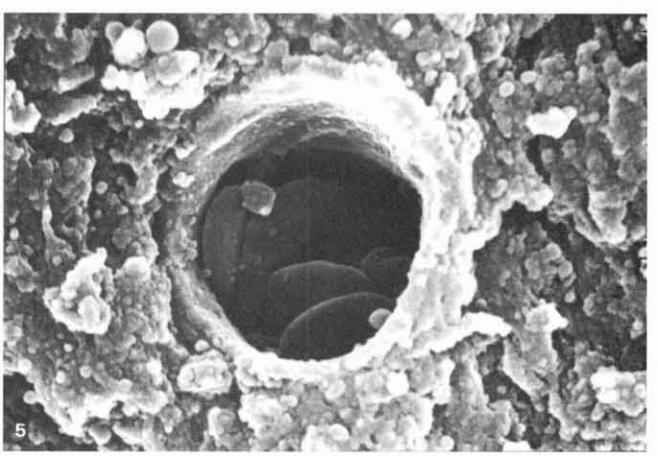


Fig. 5. Experimental animal, 1 day after irradiation. Scanning electron microscopy. Aggregation of red blood cells on the luminal surface of the vessel. x 21,000.

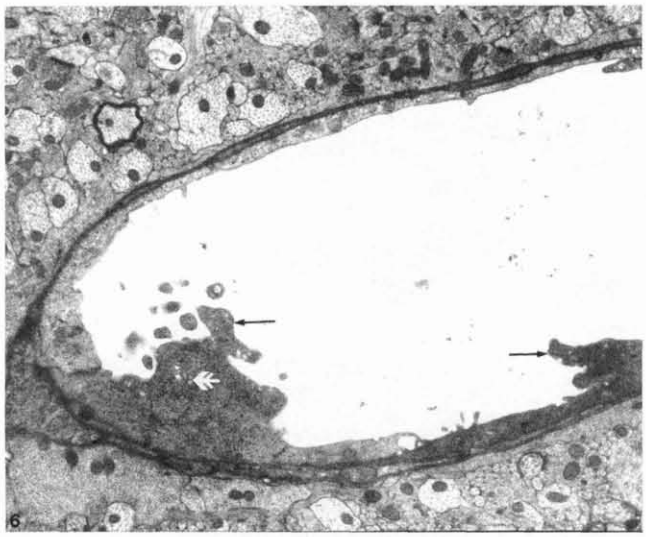


Fig. 6. Experimental animal, 4 days after irradiation. Note flattening of endothelial cells (arrow) and cell shrinking with features of increased pinocytosis activity (double arrows). x 14,000.

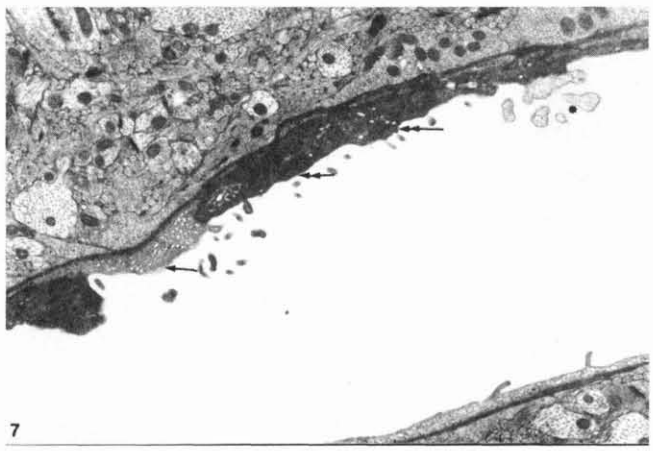
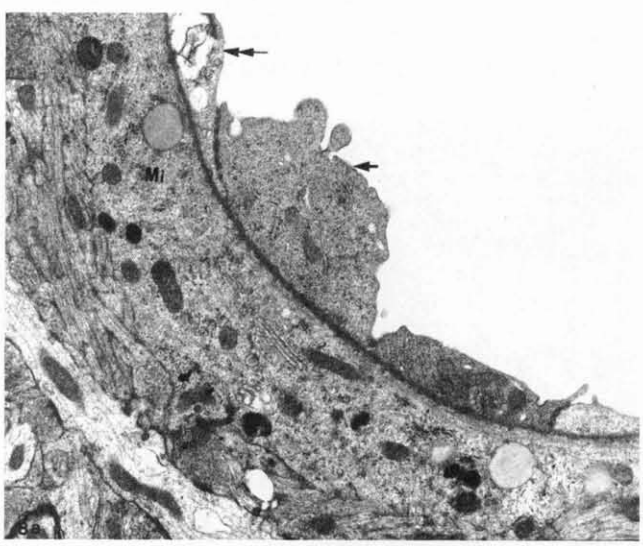


Fig. 7. Experimental animal, 4 days after irradiation. Electron-lucent and electron-dense cells with numerous microvesicles (arrows and double arrows, respectively). On the opposite side note an unchanged cell with elongated microvilli. Note a degranulated platelet (asterisk). x 15,000.

Platelet aggregates and erythrocyte-containing microthrombi were observed on the luminal surface of vessels (Fig. 9). The vessels were surrounded by pericytes and microglial cells (Fig. 8b). Pericytes were enclosed within the basal lamina (Fig. 11). Microglial cells possessed a rich lysosomal apparatus and numerous lipid bodies (Fig. 8a). Denudation of luminal surface of the vessel wall was observed (Fig. 10).

DISCUSSION

The main finding of this study is that a non-coherent visible light produces a typical pattern of brain ischaemia in the animals injected with a rose Bengal dye. Ultrastructural changes were present mainly in the vessels and comprised features of endothelial cell damage and thrombus formation. A similar pattern was observed previously using other models of brain ischaemia (Fischer



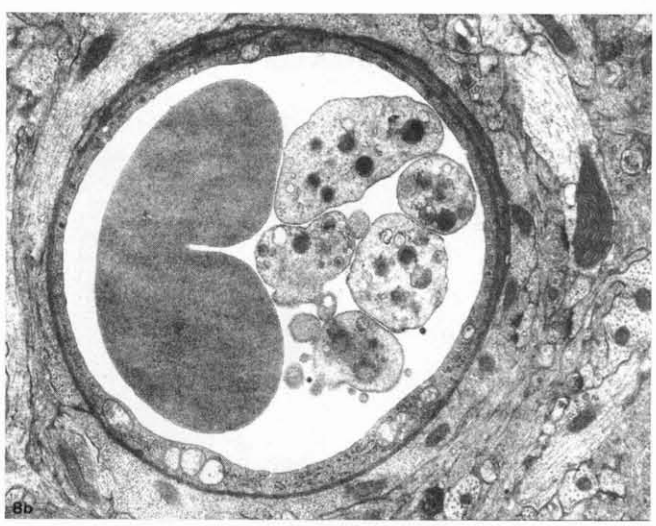


Fig. 8.a, Experimental animal, 4 days after irradiation. Capillary vessel with denuded endothelial cell (arrow) and cells with large vacuoles (double arrows). Note unchanged tight junctions between denuded cells and the lipid bodies in microglial cell (Mi). x 21,000. b, experimental animal, 4 days after irradiation. Platelat aggregates on the luminal surface of the vessel are present. x 18,000.

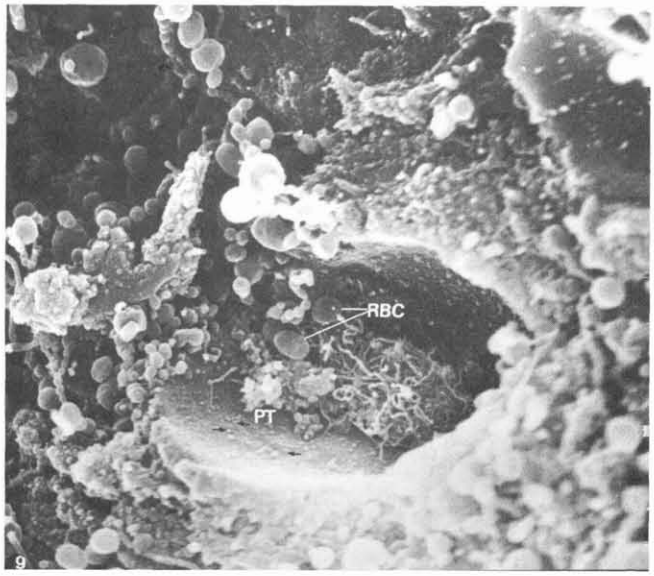


Fig. 9. Experimental animal, 4 days after irradiation. Scanning electron microscopy image showing fibrillar network with red blood cells (RBC) and platelets (PT) in the lumen of vessel. Note numerous endothelial microvilli (arrows). x 10,500.

et al. 1977, Hossmann et al. 1980). Moreover, our data confirmed earlier reports suggesting that both reactive and degenerative changes are present in endothelial cells (Pluta et al. 1994). In a review, Rosenblum (1986) presented evidence that structural endothelial alterations and increased number of microvilli play a role in the formation of microthrombi. It is possible that shortage of oxygen and nutriens during ischaemia is sufficient to

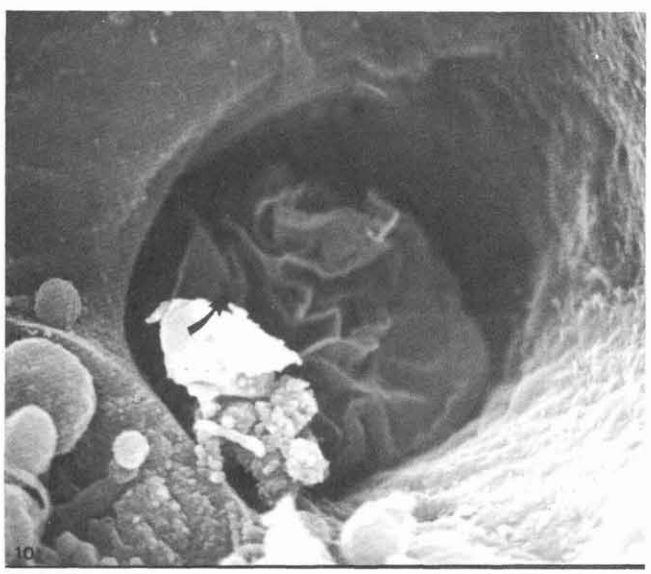


Fig. 10. Experimental animal, 4 days after irradiation. Scanning electron microscopy. Denudation of the luminal surface of vessel wall (arrow). x 17,500.

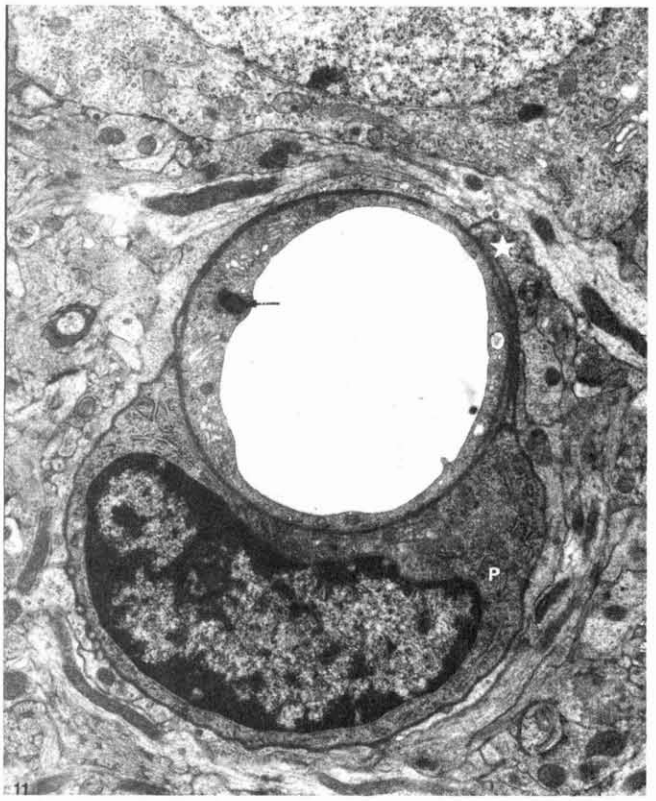


Fig. 11. Experimental animal, 4 days after irradiation. Ultrastructurally unchanged endothelium with centriole (arrow). Note collagen (asterisk) in perivascular region and pericyte (P) x 18,000.

produce metabolic alterations which initiate platelet aggregation (Yao et al. 1996). Pluta et al. (1991) and Hallenbeck et al. (1986) documented clustering of white and red blood cells and platelets immediately after an ischaemic insult. It is significant, since these cells may release proteolytic enzymes and produce free radicals further aggravating the effects of ischaemia (Wedmore et al. 1981). Formation of microthrombi has been thus regarded as an early phenomenon responsible for progression of ischaemic brain damage. Our data are not fully compatible with this scenario. We revealed that ultrastructural alterations were relatively mild 1 day after irradiation and formation of microthrombi was not involved. The damage was significantly aggravated 4 days after irradiation and coincided with thrombi formation. In contrast, vessel ligation produces more immediate thrombotic changes which are observable as soon as 3 min after ischaemia, peaking approximately 6 h after ischaemia, and subsiding slowly during following days (Pluta et al. 1994).

Thus we speculate that light-excited rose Bengal dye molecules induce a direct damage in endothelial cells. Its

is conceivable that free radicals released during irradiation of this dye (Watson et al. 1987) are responsible for initiation of pathologic changes. Singlet oxygen species can initiate peroxidiation of unsaturated fatty acids (Rawls et al. 1970) and proteins on the luminal surface of the cells leading ultimately to an increased platelet adhesion to endothelial cells (Herrmann 1966, Dietrich et al. 1984). It is noteworthy that we observed formation of microthrombi not only in the denuded sites but also directly on the surface of damaged endothelial cells. This confirms the notion of Rosenblum (1986) that exposure of basal lamina is not absolutely required for the formation of thrombi. Our ischaemic model might be thus useful to investigate and elucidate complex relationships between endothelial cell damage and the formation of microthrombi.

Recently the authors of method described previously present a new model which appears to have several advantages. First, it produces an anatomically defined zone at risk which can be precisely controlled and positioned. Second, the zone at risk is accessible to therapeutic strategies. Third, mechanisms involved in the progression of the ring-shaped lesion appear to be relevant to the pathogenesis of thromboembolic stroke with penumbra (Wester et al. 1995).

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