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Flow-dependent versus spreading-like impairment of brain tissue integrity during focal cerebral ischemia and its consequences for neuroprotective strategies

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1. ABSTRACT

Focal cerebral ischemic lesions demonstrate a gradual reduction of blood flow from the rim to the core. Flow reduction induces irreversible damage in the core region, whereas more peripheral tissue, i.e. penumbral tissue, is applicable to therapeutic interventions. Secondary mechanisms for lesion growth involve excitotoxicity, extracellular ion shifts, lactate generation, tissue acidosis, inflammation, spreading depolarization and many other processes. These toxic mediators accumulate in the ischemic core and endanger the still viable rim by diffusion or other spreading-like mechanisms, probably in part largely independent from blood flow. A substantial proportion of hemodynamic penumbral tissue could be demonstrated both in experimental settings and in clinical practice, whereas the precise spatial and temporary contribution of secondary mechanisms is much more difficult to investigate in our patients. Diffusion or spreading-mediated neurotoxicity will affect a small rim around the necrotic lesion. Due to the third power of volumetric analysis this would contribute to a large amount in small experimental lesions, but to a negligible amount of tissue in large clinical lesions and could therefore explain the difference in efficacy of neuroprotective strategies between experimental and clinical setups. Therefore, we discuss the likelihood of direct flow-dependent versus diffusion- or spreading-mediated impairment of endangered tissue in focal cerebral ischemia.

2. INTRODUCTION

There is clear evidence from histological and imaging studies in experimental and clinical trials that focal ischemic lesions tend to increase over time (1-5). Lesion growth may be associated with poor neurological outcome. Thus, understanding the underlying pathomechanisms may lead to further treatment strategies.

3. THE PENUMBRA CONCEPT

Under physiological conditions brain perfusion is highly regulated and controlled to ensure sufficient oxygen supply. However, if blood supply is blocked by a thrombus or embolus, the regulatory capacity may be exceeded, so that reduction of oxygen delivery induces functional and structural deficits.

In the late 70´s Astrup *et al.* (6,7) first defined detrimental thresholds for blood supply under conditions of cerebral ischemia. They precisely established critical thresholds of cerebral blood flow (CBF) to induce impairment of normal brain function as measured by suppression of electrical activity, and to induce structural damage as measured by loss of ion homeostasis. The brain regions with suppressed function but maintained structural integrity, bordering the already infarcted core, were entitled “penumbra” according to the half-shadow resulting from partially blocked light sources (7). The threshold of cerebral blood flow, below which

neuronal function is affected, was found to be 20-22 ml $100g^{-1}$ min^{-1} in the monkey, 30-35 ml $100g^{-1}$ min^{-1} in cats and rats and 14-20 ml $100g^{-1}$ min^{-1} in humans (8-10). For better interspecies comparison reduction of flow between 20 and 40% of normal values are usually used to hemodynamically characterize the penumbra (11).

The potential of the penumbra to completely recover structurally and functionally has attracted much attention due to its therapeutic implications. Numerous vasoactive drugs have been tested to improve hemodynamics and blood flow in the penumbra to achieve a better stroke outcome. Most of these attempts, however, have been disappointing (12-14).

Flow changes induce several biochemical and metabolic alterations which influence the cell fate at the infarct rim and which can be alternatively addressed by therapeutic interventions:

Excitotoxicity, changes in glucose consumption, extracellular ion shifts, lactate generation, tissue acidosis, microcirculatory flow disturbance, inflammation, spreading depolarization and other molecular responses (8,15-17).

4. FLOW-ASSOCIATED VERSUS FLOW-INDEPENDENT, SPREADING-LIKE IMPAIRMENT OF ENDANGERED TISSUE

For all or most of the above-mentioned factors certain threshold values have been identified (18). However, biochemical and metabolic alterations are not restricted to the place of their origin. Indeed, there is good reason to believe that dramatic biochemical changes occurring in the infarcted tissue may harm adjacent tissue. Extensive neurotoxic factors accumulating within the interstitial space of the infarction may easily diffuse to the neighboring still viable infarct rim and endanger tissue within brain regions in which blood flow is quite above threshold values to induce hypoxic or ischemic brain damage (19).

5. CONSEQUENCES OF SPREADING-LIKE IMPAIRMENT FOR PENUMBRA EVOLUTION IN DIFFERENT SPECIES

Whereas tissue with hemodynamically characterized penumbral flow patterns covers a substantial portion of the final stroke volume and seems to be comparable between rodents and humans in terms of relative size, biochemical or metabolic alterations may exert their effects on both, the directly underlying tissue and the bordering tissue by spread of these toxic factors. The latter will act only for a certain distance from the necrotic tissue. Although scientific data is scarce, especially for human stroke, it may be hypothesized that this distance amounts to a few millimeters and may not differ between small and large species. Due to the third power of volumetric analysis a rim of some millimeters around an ischemic lesion in a rat or mouse would contribute significantly to the final stroke volume, but would be negligible in larger stroke volumes as in humans.

Therefore, we would like to discuss - for some classical mechanisms of lesion growth - the likelihood for direct flow-dependent versus spreading-mediated impairment of endangered tissue in ischemic lesions.

6. SECONDARY MECHANISMS OF LESION GROWTH

6.1. Excitotoxicity

The predominant excitatory neurotransmitter glutamate is ubiquitously present throughout the central nervous system. With normal neuronal activity it is released from presynaptic vesicles and removed by rapid re-uptake mechanisms from the synaptic cleft. If blood supply and thereby substrate delivery to the brain tissue is blocked, the energetic requirements to maintain ion gradients and membrane potential are lost. Consequently, neuronal and glial cells depolarize and excitatory amino acids are released into the interstitial space. Simultaneously, the re-uptake for glutamate is down-regulated resulting in concentrations of 180 µM in the penumbra region (20). Glutamate concentrations in the ischemic core may considerably exceed this level (21,22). In cell culture exposure to 100 µM glutamate is sufficient to induce notable neurotoxicity (23).

Animal models of local exogenous glutamate application into cerebral tissue have shown reproducible excitotoxic lesions at quite a distance from the point of application (23,24). This implies that local build-up of glutamate does not harm tissue integrity solely at its direct origin but also impairs bordering tissue by means of diffusion.

Further evidence concerning blood flow-independent, spreading-like neurotoxicity induced by glutamate is provided by observations, that the final infarct volume is positively correlated to glutamate release during ischemia (20) and that glutamate receptor antagonists have been shown to dramatically reduce infarct size without altering cerebral blood flow (25,26).

6.2. Extracellular ion shift

Activation of specific glutamate receptors induces overload of intracellular ions, especially Ca^{2+}, Na^{+} and Cl^{-}, while K^{+} and H^{+} are released (17,27). Due to these ion shifts extracellular K^{+} increases to levels above 50 mmol/l in ischemic tissue, where blood flow is reduced below a threshold of 10 to 15 ml $100g^{-1}$ min^{-1} (28,29). In bordering tissue with CBF values clearly above this threshold K^{+} levels range between 5 to 10 mmol/l and in more distant normally perfused peri-penumbral tissue still elevated K^{+} values are detectable (28-30). The elevated K^{+} values in tissue clearly above CBF threshold levels to induce ion shifts indicate spread of ions by means of diffusion.

The hazardous effect of elevated K^{+} ions is still not clear, whereas some authors postulate involvement in infarct expansion and apoptotic cell damage (30,31), other findings argue for alleviation of the ischemic damage (19). The most important potentially hazardous pathway of ion

shift might be the induction of spreading depolarizations (see below) (32).

6.3. Lactacidosis and alkalosis

Anaerobic metabolism leads to lactate overload in the ischemic core. Lactate diffuses within the interstitial space and together with H^+ causes severe acidosis, which endangers cell viability. Bioluminescence and fluoroscopic studies have shown widespread lactate and acidosis distribution over the affected hemisphere in rats with focal ischemia (33,34). Interestingly, while the infarct core is consistently acidic, the penumbra can also demonstrate foci of alkalosis indicating severe disruption of pH homeostasis. Alkalosis in the penumbra is probably a marker of bad tissue fate (33).

6.4. Glucose utilization/blood flow uncoupling

As for lactate and pH, glucose utilization can be investigated with high spatial resolution over the entire hemisphere. Under normal conditions glucose metabolism is closely coupled to CBF in order to cope with the increased metabolic demand (35). Double-labeled autoradiograms for local cerebral glucose utilization have shown significant uncoupling of flow and metabolism after ischemia (36-38) with significant reduction of blood flow despite near normal utilization of glucose. This mismatch between energy demand and delivery is unlikely to be tolerated for extended periods. Widespread uncoupling over the entire ipsilateral cortex has been documented in association with peri-infarct depolarizations (see below) (39).

6.5. Other mechanisms

Apart from the above-mentioned acute biochemical and metabolic changes a considerable number of molecular responses involving inflammation, generation of free radicals, apoptosis, upregulation of immediate-early genes, up- or down-regulation of specific proteins may influence the final infarct volume (17,39). All of these mainly delayed secondary mechanisms are induced in still viable peri-infarct tissue. However, studies addressing the exact spatial and temporal evolution in relationship to the infarct rim and to the hemodynamic penumbra are scarce or not feasible.

6.6. Peri-infarct depolarization

Cortical spreading depression or peri-infarct depolarizations occur spontaneously in the ischemic brain and are thought to be the electrophysiological correlate to spreading depression of electrical activity originally described by Leao in 1944 (40).

In order to cope with the significant ion shifts present during cortical spreading depression, the increased energy demand of ATP-dependent ion pumps is associated with a reactive hyperperfusion (41,42). Therefore, cortical spreading depression does not induce morphological damage in healthy brain tissue. If, however, CBF is compromised as under conditions of focal ischemia the oxygen delivery is not sufficient to restore the energy demand. Consequently, cortical spreading depressions induce transient periods of tissue hypoxia (43,44). Additionally, inverse coupling of perfusion to peri-infarct depolarizations is well documented and may further aggravate tissue hypooxygenation in focal ischemic lesions (45,46). Hypooxygenation together with significant ion changes (28,29,47) may further exacerbate tissue damage. Indeed, there is a strong correlation between the occurrence of peri-infarct depolarizations and expansion of infarctions (32,48,49).

Together with recent findings, which demonstrated that spontaneous cortical spreading depressions and peri-infarct depolarization also affect the injured human brain (50,51), these events are particularly important, as cortical spreading depolarizations or peri-infarct depolarizations are not limited to the malperfused tissue in focal cerebral ischemia but rather propagate over the entire hemisphere (45,52). Therefore, in contrast to diffusion-mediated spread of toxic mediators, peri-infarct depolarizations may affect viable tissue at quite a distance to the ischemic lesion. This could contribute significantly to infarct expansion in large and small ischemic lesion.

7. LIFE SPAN OF FACTORS INFLUENCING BORDERING TISSUE

There is increasing evidence that the penumbra as defined by hemodynamic measures largely turns into infarction within a few hours (11,53-55). The observation that thrombolytic therapy is only effective during the first few hours proves this finding clinically (56-58). Recently, the “diffusion and perfusion imaging evaluation for understanding stroke evolution (DEFUSE) study” was conducted to extend the time period for thrombolysis from 3 to 6 hours in selected patients and used diffusion/perfusion mismatch on magnetic resonance imaging, which is a surrogate for the viable hemodynamic penumbra. In this study, viable hypoperfused tissue was found in only 48% of patients measured during the first 6 hours.

In contrast to the hemodynamic penumbra, secondary mechanisms influence the cell fate in the stroke´s rim during the acute phase (excitotoxicity, ion shifts, lactacidosis) but are also documented to influence the lesion growth for extended time periods. These delayed secondary mechanisms like inflammation, apoptosis, generation of free-radical species, etc. modulate lesion size for up to several days (17).

8. IMAGING THE PENUMBRA

Whereas a substantial portion of penumbral tissue, as defined hemodynamically, can be detected by different techniques in experimental and clinical settings (53,59,60), simultaneous imaging of tissues viability reveals only a small rim of viable tissue displaying hemodynamic penumbral flow patterns even early after stroke onset (10).

Imaging secondary mechanisms for infarct growth is difficult or impossible in the human brain. The best marker to assess the relevance of delayed secondary mechanisms may be the delayed increase (beyond 8-12

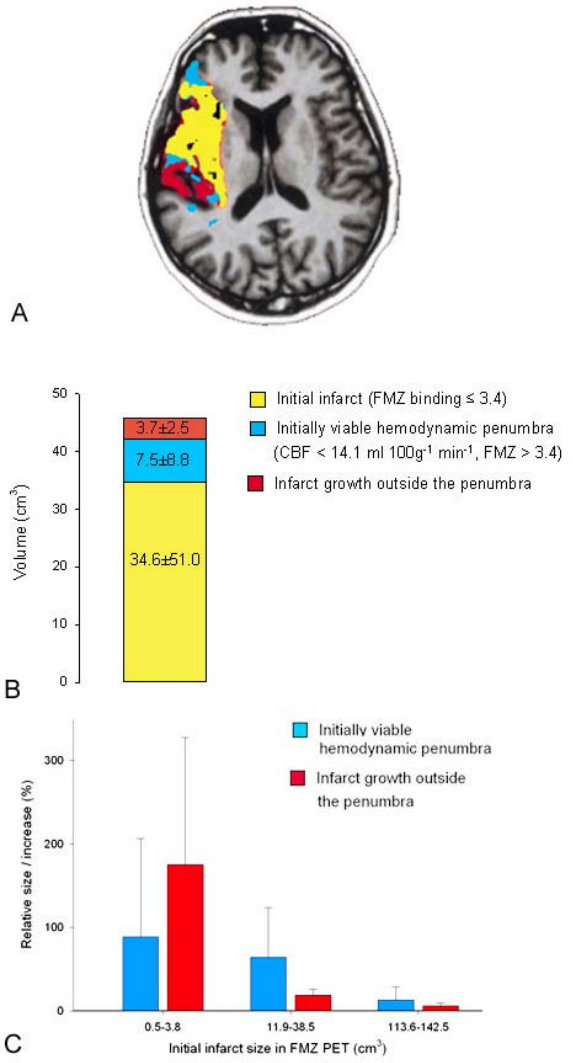

Figure 1. The figure is adapted with permission from (10). Panel A depicts an illustrative flumazenil and CBF PET 454 min after stroke onset in a 52 year-old patient. The yellow inset corresponds to areas of flumazenil (FMZ) binding ≤ 3.4 indicating irreversible tissue damage and the blue inset depicts viable hemodynamic penumbra defined by CBF < 14.1 ml $100g^{-1}$ min^{-1} outside the area with FMZ ≤ 3.4. The secondary infarct growth in areas outside the hemodynamic penumbra was investigated by late magnetic resonance imaging scans and is depicted in red. Panel B shows mean values ± standard deviation of the 10 patients investigated between 2 and 12 hours after stroke onset. The size of the initially viable hemodynamic penumbra and the infarct growth that occurs outside of the hemodynamic penumbra is given in lesions of differing size in panel C: In small ischemic lesions the relative volume of the hemodynamic penumbra and the infarct growth is rather large, while the volumes are practically negligible in large lesions.

hours after stroke onset) of the ischemic volume. This increase is well documented in experimental settings (1-3,61). In humans several reports demonstrated lesion evolution by magnetic resonance imaging (4,5,62), but often address rather early blood flow-coupled lesion evolution than the delayed time interval. Furthermore, impressive relative growth has been reported in small ischemic lesions. In absolute values, however, this growth represents a negligible increase in stoke volume, which is unlikely to affect our patients´ outcome. For example, Baird *et al.* (4) reported lesion growth of 68% assessed by repeated magnetic resonance imaging in 28 stroke patients. Detailed analysis revealed that this increase was due to significant enlargements of rather small lesions during the early phase of infarct expansion / maturation. Although such a small absolute increase in stroke volume can be clinically relevant in the most eloquent areas of the cerebrum, like the brain stem, it would probably not have any impact in the vast majority of strokes.

The most striking work supporting penumbra-independent infarct growth due to secondary mechanism in humans probably is the study reported by Heiss *et al.* (10). They have analyzed the early hemodynamic penumbra by CBF PET in relation to viable tissue determined by flumazenil PET. Additionally, secondary growth of the lesion outside the hemodynamic penumbra was addressed by follow-up magnetic resonance imaging. Again a substantial relative proportion of infarct growth and hemodynamic penumbra was found. This impressive relative increase, however, was mainly due to the small initial lesion size. In larger strokes, the difference was minimal, as the difference in absolute volume units overall was small (Figure 1).

9. PERSPECTIVE

Evolution of ischemic lesions involves both hemodynamic changes and multiple secondary mechanisms with a rather complex spatial and temporal interplay. Whereas the spatial and temporal profile of a hemodynamic penumbra has been shown in humans and animals, secondary mechanisms influencing the cell fate at the rim of infarction are much more difficult to precisely investigate in humans. However, animal studies provide evidence that toxic mediators accumulating in the infarct core may diffuse to still viable tissue and jeopardize peri-infarct regions. These mechanisms may influence bordering viable tissue during the acute as well as during the delayed period of infarct growth. In volumetric analysis of small animal brains diffusion-mediated impairment contributes to large “relative” volumes in small lesions. In human stroke, however, the “relative” infarct growth due to diffusion of secondary involved toxic mediators may be rather small.

In contrast to diffusion-mediated injury, spreading depression events can involve the entire hemisphere. Peri-infarct depolarizations may therefore contribute notably to lesion evolution even in human stroke. In view of recent findings demonstrating these depolarizations in the injured human brain, these events should be investigated in correlation to infarct growth in human stroke victims.

Overall, this personal analysis of the literature on the evolution of stroke easily explains the discrepant efficacy of neuroprotective strategies between animals and humans. This discussion may pave the way for clinically more effective future strategies.

10. REFERENCES

1. J. Woitzik, U. C. Schneider, C. Thome, H. Schroeck and L. Schilling: Comparison of different intravascular thread occlusion models for experimental stroke in rats. *J Neurosci Methods* 151, 224-231 (2006)
2. J. Hofmeijer, W. B. Veldhuis, J. Schepers, K. Nicolay, L. J. Kappelle, P. R. Bar and H. B. van der Worp: The time course of ischemic damage and cerebral perfusion in a rat model of space-occupying cerebral infarction. *Brain Res* 1013, 74-82 (2004)
3. W. R. Schabitz, H. Schade, S. Heiland, R. Kollmar, J. Bardutzky, N. Henninger, H. Muller, U. Carl, S. Toyokuni, C. Sommer and S. Schwab: Neuroprotection by hyperbaric oxygenation after experimental focal cerebral ischemia monitored by mri. *Stroke* 35, 1175-1179 (2004)
4. A. E. Baird, A. Benfield, G. Schlaug, B. Siewert, K. O. Lovblad, R. R. Edelman and S. Warach: Enlargement of human cerebral ischemic lesion volumes measured by diffusion-weighted magnetic resonance imaging. *Ann Neurol* 41, 581-589 (1997)
5. J. O. Karonen, R. L. Vanninen, Y. Liu, L. Ostergaard, J. T. Kuikka, J. Nuutinen, E. J. Vanninen, P. L. Partanen, P. A. Vainio, K. Korhonen, J. Perkio, R. Roivainen, J. Sivenius and H. J. Aronen: Combined diffusion and perfusion mri with correlation to single-photon emission ct in acute ischemic stroke. Ischemic penumbra predicts infarct growth. *Stroke* 30, 1583-1590 (1999)
6. J. Astrup, L. Symon, N. M. Branston and N. A. Lassen: Cortical evoked potential and extracellular k+ and h+ at critical levels of brain ischemia. *Stroke* 8, 51-57 (1977)
7. J. Astrup, B. K. Siesjo and L. Symon: Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke* 12, 723-725. (1981)
8. T. Back: Pathophysiology of the ischemic penumbra--revision of a concept. *Cell Mol Neurobiol* 18, 621-638 (1998)
9. J. C. Baron: Perfusion thresholds in human cerebral ischemia: Historical perspective and therapeutic implications. *Cerebrovasc Dis* 11 Suppl 1, 2-8. (2001)
10. W. D. Heiss, L. W. Kracht, A. Thiel, M. Grond and G. Pawlik: Penumbral probability thresholds of cortical flumazenil binding and blood flow predicting tissue outcome in patients with cerebral ischaemia. *Brain* 124, 20-29 (2001)
11. M. D. Ginsberg: Adventures in the pathophysiology of brain ischemia: Penumbra, gene expression, neuroprotection: The 2002 thomas willis lecture. *Stroke* 34, 214-223. (2003)
12. K. A. Hossmann: Treatment of experimental cerebral ischemia. *J Cereb Blood Flow Metab* 2, 275-297 (1982)
13. R. I. Lindley and C. P. Warlow: Clinical trials in cerebrovascular disease. *Curr Opin Neurol Neurosurg* 5, 58-62 (1992)
14. C. Y. Hsu, J. W. Norris, E. L. Hogan, P. Bladin, H. B. Dinsdale, F. M. Yatsu, M. P. Earnest, P. Scheinberg, L. R. Caplan and H. R. Karp: Pentoxifylline in acute nonhemorrhagic stroke. A randomized, placebo-controlled double-blind trial. *Stroke* 19, 716-722 (1988)
15. F. R. Sharp, A. Lu, Y. Tang and D. E. Millhorn: Multiple molecular penumbras after focal cerebral ischemia. *J Cereb Blood Flow Metab* 20, 1011-1032. (2000)
16. R. Hata, K. Maeda, D. Hermann, G. Mies and K. A. Hossmann: Evolution of brain infarction after transient focal cerebral ischemia in mice. *J Cereb Blood Flow Metab* 20, 937-946. (2000)
17. U. Dirnagl, C. Iadecola and M. A. Moskowitz: Pathobiology of ischaemic stroke: An integrated view. *Trends Neurosci* 22, 391-397. (1999)
18. K. A. Hossmann: Viability thresholds and the penumbra of focal ischemia. *Ann Neurol* 36, 557-565 (1994)
19. H. Yao, Y. Shu, J. Wang, B. C. Brinkman, G. G. Haddad, E. Busch, B. Schmitz, K. Kohno and K. A. Hossmann: Factors influencing cell fate in the infarct rim. *J Neurochem* 100, 1224-1233 (2007)
20. K. Takagi, M. D. Ginsberg, M. Y. Globus, W. D. Dietrich, E. Martinez, S. Kraydieh and R. Busto: Changes in amino acid neurotransmitters and cerebral blood flow in the ischemic penumbral region following middle cerebral artery occlusion in the rat: Correlation with histopathology. *J Cereb Blood Flow Metab* 13, 575-585 (1993)
21. L. P. Caragine, H. K. Park, F. G. Diaz and J. W. Phillis: Real-time measurement of ischemia-evoked glutamate release in the cerebral cortex of four and eleven vessel rat occlusion models. *Brain Res* 793, 255-264 (1998)
22. C. Berger, W. R. Schabitz, D. Georgiadis, T. Steiner, A. Aschoff and S. Schwab: Effects of hypothermia on excitatory amino acids and metabolism in stroke patients: A microdialysis study. Stroke 33, 519-524 (2002)
23. D. W. Choi, M. Maulucci-Gedde and A. R. Kriegstein: Glutamate neurotoxicity in cortical cell culture. J Neurosci 7, 357-368 (1987)
24. H. Landolt, H. Fujisawa, D. I. Graham, W. L. Maxwell and R. Bullock: Reproducible peracute glutamate-induced focal lesions of the normal rat brain using microdialysis. J Clin Neurosci 5, 193-202 (1998)
25. B. Meldrum: Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission. Cerebrovasc Brain Metab Rev 2, 27-57 (1990)
26. C. K. Park, D. G. Nehls, G. M. Teasdale and J. McCulloch: Effect of the nmda antagonist mk-801 on local cerebral blood flow in focal cerebral ischaemia in the rat. J Cereb Blood Flow Metab 9, 617-622 (1989)
27. A. J. Hansen: Effect of anoxia on ion distribution in the brain. Physiol Rev 65, 101-148 (1985)
28. A. J. Strong, G. S. Venables and G. Gibson: The cortical ischaemic penumbra associated with occlusion of the middle cerebral artery in the cat: 1. Topography of changes in blood flow, potassium ion activity, and eeg. J Cereb Blood Flow Metab 3, 86-96 (1983)
29. N. M. Branston, A. J. Strong and L. Symon: Extracellular potassium activity, evoked potential and tissue blood flow. Relationships during progressive ischaemia in baboon cerebral cortex. J Neurol Sci 32, 305-321 (1977)

30. T. J. Sick, Z. C. Feng and M. Rosenthal: Spatial stability of extracellular potassium ion and blood flow distribution in rat cerebral cortex after permanent middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 18, 1114-1120 (1998)
31. C. D. Bortner and J. A. Cidlowski: The role of apoptotic volume decrease and ionic homeostasis in the activation and repression of apoptosis. *Pflugers Arch* 448, 313-318 (2004)
32. E. Busch, M. L. Gyngell, M. Eis, M. Hoehn-Berlage and K. A. Hossmann: Potassium-induced cortical spreading depressions during focal cerebral ischemia in rats: Contribution to lesion growth assessed by diffusion-weighted nmr and biochemical imaging. *J Cereb Blood Flow Metab* 16, 1090-1099 (1996)
33. T. Back, M. Hoehn, G. Mies, E. Busch, B. Schmitz, K. Kohno and K. A. Hossmann: Penumbral tissue alkalosis in focal cerebral ischemia: Relationship to energy metabolism, blood flow, and steady potential. *Ann Neurol* 47, 485-492 (2000)
34. T. Back, M. Hoehn-Berlage, K. Kohno and K. A. Hossmann: Diffusion nuclear magnetic resonance imaging in experimental stroke. Correlation with cerebral metabolites. *Stroke* 25, 494-500 (1994)
35. M. D. Ginsberg, W. D. Dietrich and R. Busto: Coupled forebrain increases of local cerebral glucose utilization and blood flow during physiologic stimulation of a somatosensory pathway in the rat: Demonstration by double-label autoradiography. *Neurology* 37, 11-19 (1987)
36. W. Paschen, G. Mies and K. A. Hossmann: Threshold relationship between cerebral blood flow, glucose utilization, and energy metabolites during development of stroke in gerbils. *Exp Neurol* 117, 325-333 (1992)
37. M. Nedergaard, A. Gjedde and N. H. Diemer: Focal ischemia of the rat brain: Autoradiographic determination of cerebral glucose utilization, glucose content, and blood flow. *J Cereb Blood Flow Metab* 6, 414-424 (1986)
38. K. A. Hossmann, G. Mies, W. Paschen, L. Csiba, W. Bodsch, J. R. Rapin, M. Le Poncin-Lafitte and K. Takahashi: Multiparametric imaging of blood flow and metabolism after middle cerebral artery occlusion in cats. *J Cereb Blood Flow Metab* 5, 97-107 (1985)
39. T. Back, W. Zhao and M. D. Ginsberg: Three-dimensional image analysis of brain glucose metabolism-blood flow uncoupling and its electrophysiological correlates in the acute ischemic penumbra following middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 15, 566-577 (1995)
40. A. Leao: Spreading depression of activity in cerebral cortex. *J Neurophysiol* 7, 359-390 (1944)
41. M. Lauritzen: Regional cerebral blood flow during cortical spreading depression in rat brain: Increased reactive hyperperfusion in low-flow states. *Acta Neurol Scand* 75, 1-8 (1987)
42. A. J. Hansen, B. Quistorff and A. Gjedde: Relationship between local changes in cortical blood flow and extracellular k+ during spreading depression. *Acta Physiol Scand* 109, 1-6 (1980)
43. T. Wolf, U. Lindauer, U. Reuter, T. Back, A. Villringer, K. Einhaupl and U. Dirnagl: Noninvasive near infrared spectroscopy monitoring of regional cerebral blood oxygenation changes during peri-infarct depolarizations in focal cerebral ischemia in the rat. *J Cereb Blood Flow Metab* 17, 950-954 (1997)
44. T. Back, K. Kohno and K. A. Hossmann: Cortical negative dc deflections following middle cerebral artery occlusion and kcl-induced spreading depression: Effect on blood flow, tissue oxygenation, and electroencephalogram. *J Cereb Blood Flow Metab* 14, 12-19 (1994)
45. A. J. Strong, P. J. Anderson, H. R. Watts, D. J. Virley, A. Lloyd, E. A. Irving, T. Nagafuji, M. Ninomiya, H. Nakamura, A. K. Dunn and R. Graf: Peri-infarct depolarizations lead to loss of perfusion in ischaemic gyrencephalic cerebral cortex. *Brain* 130, 995-1008 (2007)
46. H. K. Shin, A. K. Dunn, P. B. Jones, D. A. Boas, M. A. Moskowitz and C. Ayata: Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. *J Cereb Blood Flow Metab* 26, 1018-1030 (2006)
47. K. Ohta, R. Graf, G. Rosner and W. D. Heiss: Calcium ion transients in peri-infarct depolarizations may deteriorate ion homeostasis and expand infarction in focal cerebral ischemia in cats. *Stroke* 32, 535-543 (2001)
48. G. Mies, T. Iijima and K. A. Hossmann: Correlation between peri-infarct dc shifts and ischaemic neuronal damage in rat. *Neuroreport* 4, 709-711 (1993)
49. T. Iijima, G. Mies and K. A. Hossmann: Repeated negative dc deflections in rat cortex following middle cerebral artery occlusion are abolished by mk-801: Effect on volume of ischemic injury. *J Cereb Blood Flow Metab* 12, 727-733 (1992)
50. A. J. Strong, M. Fabricius, M. G. Boutelle, S. J. Hibbins, S. E. Hopwood, R. Jones, M. C. Parkin and M. Lauritzen: Spreading and synchronous depressions of cortical activity in acutely injured human brain. *Stroke* 33, 2738-2743 (2002)
51. J. P. Dreier, J. Woitzik, M. Fabricius, R. Bhatia, S. Major, C. Drenckhahn, T. N. Lehmann, A. Sarrafzadeh, L. Willumsen, J. A. Hartings, O. W. Sakowitz, J. H. Seemann, A. Thieme, M. Lauritzen and A. J. Strong: Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations. *Brain* 129, 3224-3237 (2006)
52. Y. Hasegawa, L. L. Latour, J. E. Formato, C. H. Sotak and M. Fisher: Spreading waves of a reduced diffusion coefficient of water in normal and ischemic rat brain. *J Cereb Blood Flow Metab* 15, 179-187 (1995)
53. W. D. Heiss, R. Graf, K. Wienhard, J. Lottgen, R. Saito, T. Fujita, G. Rosner and R. Wagner: Dynamic penumbra demonstrated by sequential multitracer pet after middle cerebral artery occlusion in cats. *J Cereb Blood Flow Metab* 14, 892-902. (1994)
54. N. A. Lassen: Pathophysiology of brain ischemia as it relates to the therapy of acute ischemic stroke. *Clin Neuropharmacol* 13 Suppl 3, S1-8 (1990)
55. W. D. Heiss, J. Sobesky and V. Hesselmann: Identifying thresholds for penumbra and irreversible tissue damage. *Stroke* 35, 2671-2674 (2004)
56. V. Caso and W. Hacke: The very acute stroke treatment: Fibrinolysis and after. *Clin Exp Hypertens* 24, 595-602 (2002)
57. W. D. Heiss: Stroke--acute interventions. *J Neural Transm Suppl* 37-57 (2002)
58. B. Kaplan, S. Brint, J. Tanabe, M. Jacewicz, X. J. Wang and W. Pulsinelli: Temporal thresholds for neocortical

infarction in rats subjected to reversible focal cerebral ischemia. *Stroke* 22, 1032-1039. (1991)
59. A. M. Kaufmann, A. D. Firlik, M. B. Fukui, L. R. Wechsler, C. A. Jungries and H. Yonas: Ischemic core and penumbra in human stroke. *Stroke* 30, 93-99. (1999)
60. G. Marchal, V. Beaudouin, P. Rioux, V. de la Sayette, F. Le Doze, F. Viader, J. M. Derlon and J. C. Baron: Prolonged persistence of substantial volumes of potentially viable brain tissue after stroke: A correlative pet-ct study with voxel-based data analysis. *Stroke* 27, 599-606 (1996)
61. J. Woitzik and L. Schilling: A new method for superselective middle cerebral artery infusion in the rat. *J Neurosurg* 106, 872-878 (2007)
62. P. W. Schaefer, G. J. Hunter, J. He, L. M. Hamberg, A. G. Sorensen, L. H. Schwamm, W. J. Koroshetz and R. G. Gonzalez: Predicting cerebral ischemic infarct volume with diffusion and perfusion mr imaging. *AJNR Am J Neuroradiol* 23, 1785-1794 (2002)

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