Therapeutic Approaches to the Treatment of Acute Ischemic Stroke

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The traditional therapeutic goals in the treatment of acute ischemic stroke have been confined to stabilization of the general medical state, prevention of systemic medical complications, and efforts to prevent acute recurrence or progression of stroke (1). By contrast, in this era of rapid medical advances in understanding the pathophysiology of ischemic stroke, we should now be concerned with several novel therapeutic possibilities: (a) restoration or enhancement of tissue perfusion; (b) neuroprotective strategies to limit the injurious metabolic consequences of tissue ischemia; and (c) new means to facilitate stroke rehabilitation. In this chapter, I briefly consider clot thrombolysis in acute ischemic stroke but concentrate mainly on novel neuroprotective strategies—an area which I have recently reviewed in detail (2).

THRESHOLDS OF ISCHEMIC INJURY

Recently, Hossmann has considered this topic in detail (3). In understanding focal ischemic injury, it is important to recognize that irreversible histological injury is a function of not only the degree of decrement of local cerebral blood flow (ICBF) but, of equal importance, its duration. For example, Heiss and Rosner (4) have shown a sharp curvilinear relationship between residual flow and the duration of ischemia needed to produce irreversible injury: even short periods of near-total ischemia are injurious, whereas more moderate ischemia, lying above critical thresholds, may be sustained for long periods without producing injury.

REPERFUSION OF THE ISCHEMIC BRAIN

We have recently explored local patterns of tissue reperfusion in a model of proximal temporary clip-occlusion of the middle cerebral artery of anesthetized rats (5). After 30 minutes of middle cerebral artery occlusion, ICBF autoradiograms clearly show the core zone of severe ischemia surrounded by a less ischemic penumbra zone. When the middle cerebral artery occlusion is released, ICBF studies 15
minutes later reveal an initial failure to establish total reperfusion, with persistent moderately severe ICBF deficits in the previously ischemic neocortical as well as subcortical areas. Within 2 hours, reperfusion is complete (5). These observations underscore the fact that even when a short period of vascular occlusion is abruptly reversed, a residual microcirculatory impairment may impede the restoration of full reperfusion for an additional time period during which the brain remains at least partially ischemic.

Previous therapeutic efforts to enhance tissue perfusion in ischemic stroke have included the use of putative vasodilators such as papaverine, as well as hemodilution approaches (6), although these have not met with consistent success. The current approach to therapeutic reperfusion involves thrombolysis induced by recombinant tissue plasminogen activator (rt-PA) (7), or the more traditional agents, streptokinase or urokinase. A continuation of the NIH Multicenter Trial of rt-PA in acute ischemic stroke in the United States is designed to enter patients within a narrow time window (0–90 minutes or 90–180 minutes) following stroke onset—a window necessarily narrow if one is to achieve success and avoid complicating intracerebral hematomas, but one which demands the marshalling of skilled community, paramedical and medical resources to facilitate the rapid admission of the patient to hospital.

THERAPEUTIC WINDOW FOR REPERFUSION IN FOCAL ISCHEMIA

This and related issues have been reviewed in great detail recently (8). In the experimental animal, the therapeutic window for reperfusion may be studied by examining the duration of temporary ischemia needed to produce maximum infarction. In numerous studies (e.g., 9,10), the rather uniform finding has been that middle cerebral artery occlusion of 3–4 hrs followed by reperfusion leads to cortical and subcortical infarcts fully as large as those produced by permanent vascular occlusion. Studies in the cat (4) and the primate (11) are consistent with this finding and lead to the general working hypothesis that efforts at therapeutic reperfusion must be initiated within 3–4 hrs of the onset of brain ischemia if tissue rescue is to be achieved.

NEUROPROTECTION VIA GLUTAMATE ANTAGONISM

Choi (12) has used the metaphor of a “death funnel,” in which a variety of different insults may activate a multitude of specific injury mechanisms which, however, converge into a final common pathway involving membrane failure, activation of catabolic enzymes, free radical reactions, and cytoskeletal failure—all mediated directly or indirectly by inappropriate increases in free intracellular calcium and eventuating in cell death. Glutamate, the most ubiquitous excitatory neurotransmitter in the central nervous sytem, plays a crucial deleterious role in the injury setting. Glutamate neurotoxicity has been abundantly confirmed in cell culture and in vivo studies (12–14).
Glutamate may act in this regard through the N-methyl-D-aspartate (NMDA) receptor-ion channel complex, through non-NMDA (AMPA/kainate) receptors or through metabotropic receptors. The NMDA receptor-linked ion channel is a complex ionophore; this channel normally is blocked by endogenous magnesium ion in a voltage-dependent manner. In addition to a transmitter recognition site, this complex also embodies a glycine modulatory site and other sites sensitive to zinc, polyamines, etc. (15). In focal ischemia, glutamate is released from cells into the extracellular space in a massive, sustained manner in the ischemic core (16–18), whereas in the ischemic penumbra (where cerebral blood flow is in the range of 20–35% of control), substantial glutamate release also occurs, but reuptake mechanisms appear to be active so that levels fall during prolonged ischemia (19).

On the basis of a multitude of studies (see ref. 20 for review), both noncompetitive NMDA antagonists (e.g., MK-801) and competitive NMDA antagonists (e.g., CGS 19755), as well as non-NMDA antagonists (e.g., 21) are dramatically effective in reducing infarct volume, up to approximately 50%, when given before ischemia or within the first hour or so following onset of focal vascular occlusion. Development of MK-801 for clinical use has been halted, presumably because of its production of phencyclidine-like behavioral side effects (22). However, other agents, such as CGS 19755, are in multicenter clinical trials at present. Over the next several years, it will be surely established whether or not clinical efficacy is possible with these agents.

THERAPEUTIC RELEVANCE OF THE ISCHEMIC PENUMBRA

The focal ischemic penumbra was initially defined on the basis of animal studies as a zone, slightly peripheral to the core zone of most dense ischemia, in which electrical silence was observed but terminal anoxic depolarization of neurons was not present (23). The concept of the penumbra has since been extended to refer to a zone of blood flow decrement (e.g., 20–40% of control), slightly above the level of severe ischemia present in the ischemic core (approximately 0–15% of control) which is usually associated with necrosis. The ischemic penumbra is now recognized as a dynamic entity, both temporally and spatially (24).

We have recently been able to carry out a precise assessment of ICBF and local cerebral glucose utilization (lCMRgl) in the ischemic penumbra of a rat distal MCA occlusion model (25), using advanced three-dimensional (3D) autoradiographic image processing strategies developed in our laboratory (26,27). These techniques permit the construction of averaged 3D autoradiographic data sets derived from multiple replicate animal studies (25). In such a manner, by studying matched rat series for ICBF and lCMRgl at 1.5 hrs after middle cerebral artery occlusion, the penumbra (defined as having a flow of 20–40% of control) is seen clearly to surround the ischemic core in its central portions, and it is particularly concentrated at the anterior and posterior poles of the ischemic cortical lesion. Within penumbral pixels, there is a striking preservation of local glucose metabolic rate, on average at near normal levels, with individual animals showing occasional foci of apparent hyper-
metabolism. Most importantly, the ratio of IC\textsubscript{MRgl}/IC\textsubscript{BF} in penumbral pixels is markedly elevated (normal value, 58 μmol/100/ml; penumbral range, 108–490, mean 234 ± 100), thus representing up to 10-fold elevation of the coupling ratio (25). These data are consistent with the misery perfusion and enhanced oxygen extraction fraction observed in human PET studies of acute ischemic stroke.

The basis of the inappropriate maintenance of metabolic activity in the face of diminished perfusion—a condition imposing severe metabolic stress on the tissue—appears attributable to the recurrent ischemic depolarizations observed in the early ischemic penumbra both in our study (25) and those from other laboratories (28,29). Other laboratories have shown that tissue freeze-trapped during episodes of recurrent depolarization exhibits partial depletion of energy metabolites (30). If one induces extra numbers of ischemic depolarizations by electrical stimulation, we have shown that this results in statistically significant increases in numbers of ischemic neurons (31). There is a strong linear relationship between the integrated amplitude of DC penumbral shifts and total ischemic injury (31).

The volume of the initial ischemic penumbra in our study (25) is somewhat greater than 50% of the total ischemic lesion—a percentage remarkably similar to the percentage of tissue salvage achieved with glutamate antagonists and other neuroprotective strategies. Iijima et al. (28) have shown that MK-801 diminishes peri-infarct depolarizations and, pari passu, reduces infarct volume. Other workers have shown that over a range of core body temperatures of 30–40°C, numbers of ischemic depolarizations and infarct volume are tightly correlated, with both diminishing markedly at hypothermic levels (30°C), and both markedly increased by mild-to-moderate hyperthermia (40°C) (29). Yao et al. (32) in our laboratory have shown that the initial ischemic core already has well advanced cytoskeletal proteolysis (spectrin breakdown) by 2 hrs after middle cerebral artery occlusion, and that by 3.5 hrs, the ischemic penumbra has deteriorated in its metabolic rate and has begun to show significant cytoskeletal proteolytic changes as well. This underscores the narrow therapeutic window for neuroprotection of focal ischemia.

**DELETERIOUS EFFECT OF RAISED PLASMA GLUCOSE**

When blood glucose levels become raised, brain glucose levels follow course. When focal ischemia is imposed on a brain with increased glucose stores, the ischemia-induced anaerobic glycolysis results in much greater lactate accumulation in the brain than would result in normoglycemic animals. As tissue lactate accumulates, Kraig et al. (33) have reported an abrupt acidotic pH transition (average tissue pH approximately 6.2). These and other observations have prompted these investigators to conclude that, under ischemic conditions, astrocytes are capable of sequestering hydrogen ion by virtue of their large buffering capacity, but when that buffering capacity is exceeded, tissue pH may then fall to critically injurious levels, precipitating frank infarction (34,35).

Numerous studies in experimental ischemic stroke have shown that hyperglycemia present at the onset of focal vascular occlusion results in much larger infarctions and more pronounced brain edema than is the case with normoglycemia (e.g., 36).
In the clinic, it is probably important to avoid hyperglycemia in patients with acute ischemic stroke and therefore to minimize the intravenous infusion of dextrose, particularly during the first hours following stroke.

**OXYGEN RADICAL ANTAGONISM**

Strong evidence now supports the existence of oxygen radical production in focal ischemia. In our laboratory (37), intracerebral microdialysis studies using the salicylate trapping method for detection of hydroxyl radical activity in the extracellular space have shown significant increases in hydroxyl radical adducts (2,3- and 2,5-dihydroxybenzoic acid), both during the period of vascular occlusion (2 hrs) and somewhat more prominently during the early reperfusion period. Interestingly, there appears to be an inverse correlation between the extent of hydroxyl radical production and the degree of CBF restitution during the recirculation period—that is, less complete degrees of postischemic recirculation may favor the enhanced production of hydroxyl radicals in the ischemic penumbra.

Very convincing evidence of the deleterious role of oxygen radicals comes from studies in transgenic mice overexpressing the human gene for CuZn-superoxide dismutase. In transient middle cerebral artery occlusion in these animals, smaller infarcts are produced than in their nontransgenic counterparts (38). Still other evidence of oxygen radical participation comes from studies of the nitric oxide/nitric oxide synthase systems in the brain. Here again, transgenic animals have proven useful: knockout mutant mice for the neuronal form of NOS showed smaller infarcts following middle cerebral artery occlusion than did nontransgenic rats, suggesting a deleterious role of neuronal nitric oxide synthase (NOS) in ischemia (39). By contrast, vascular NOS activity may be of benefit in brain ischemia by promoting collateral vasodilation. These and other studies of nitric oxide have been exhaustively reviewed recently (40). These studies thus provide a strong conceptual framework for a possible role of oxygen radical scavengers in ameliorating focal ischemic injury, particularly in the setting of ischemia followed by reperfusion (as, e.g., would occur with therapeutic thrombolysis). This rationale is bolstered by studies suggesting that excitatory amino acids and free radical production may reciprocally enhance one another through deleterious feedback mechanism, so that both avenues of therapeutic approach seem feasible (41).

**MILD-TO-MODERATE BRAIN HYPOTHERMIA IS MARKEDLY NEUROPROTECTIVE**

Many exhaustive reviews of this subject have been published (2,42). Reduction of brain temperature by just 3°C during transient global ischemia, for example, completely protects the vulnerable CA1 neurons of the hippocampus from ischemic injury, and is also neuroprotective in the striatum (43). A striking effect of mild hypothermia is to suppress completely the release of glutamate into the extracellular
space during ischemia (44). Hypothermia instituted immediately after global ischemia initially appeared to protect neurons permanently (45), but a more recent study shows rather that irreversible neuronal injury is not ultimately prevented but rather is markedly retarded (46). Further studies from our group have shown that when postischemic brain hypothermia is combined with delayed MK-801 treatment, this combination confers chronic permanent neuroprotection (47). It is clear from our own studies (48) and extensive studies from other laboratories (49) that mild hypothermia, particularly in the setting of transient middle cerebral artery occlusion, is capable of markedly reducing infarct volume, particularly when applied early after ischemia. The therapeutic window of hypothermia in this setting may extend to 2–4 hrs; these studies have been exhaustively reviewed (8). Multicenter trials of therapeutic hypothermia are under way for acute head injury, but to date a similar effort has not been made to study therapeutic hypothermia in stroke—an area in urgent need of study.

MARKEDLY INJURIOUS EFFECT OF SMALL DEGREES OF BRAIN HYPERTHERMIA

It is abundantly clear from studies in our laboratory that even small degrees of brain temperature elevation (39°C) are markedly injurious, promoting massive opening of the blood–brain barrier (BBB) and allowing irreversible neuropathologic changes to develop in areas normally immune to these changes following global ischemia (50,51). In temporary focal ischemia, hyperthermia markedly enlarges infarct volume (48). Thus, it is absolutely incumbent on clinicians treating patients with acute ischemic stroke to avoid temperature elevations in the early post-stroke period by vigorous institution of antipyretic measures, including mild body cooling, together with specific efforts to combat infections as early as possible.

CONCLUSION

As recently summarized (2), the mechanistic factors involved in ischemic injury are multitudinous. Thus, therapeutic intervention is possible, in theory, at any number of mechanistic points. There may, in fact, be no single best therapeutic neuroprotective strategy, but rather, many strategies may be equally effective. In addition, it is possible that combinations of neuroprotectants may show synergistic therapeutic efficacy. Possibilities might include: NMDA plus non-NMDA antagonism; the combination of glutamate antagonists plus oxygen radical scavengers; glutamate antagonists plus calcium channel antagonists; or glutamate antagonists plus an agonist or antagonist of another receptor (e.g., glutamate antagonist plus GABA agonist). In addition, hypothermia might be combined, with advantage, with other neuroprotectant strategies or with thrombolytic therapy. These possibilities await further exploration.
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REFERENCES


DISCUSSION

**Dr. Hossmann:** I am, of course, very sympathetic with your concept that peri-infarct spreading depression adds to the injury. This confirms perfectly the data we have from our own laboratory. What I would like to challenge, however, is your interpretation of an increase of glucose utilization in the penumbra as an indicator of hypermetabolism. I am not sure that this is true. A couple of years ago, we did threshold determinations—in other words, plotting blood flow versus glucose utilization at declining flow values—and we found that, at a flow value of about 35 ml/g/min, you get an increase in glucose utilization before glucose utilization at lower levels begins to decline. That is the range where the oxygen supply to the tissue becomes limiting, and what happens is that the glucose is used anaerobically—not all the glucose but part of it—and the energy yield of the metabolic activity is exactly the same—the ATP during this phase is perfectly stable. So what you do is to compensate for part of the loss of the energy yield from oxidative glucose utilization by metabolizing an excess of glucose anaerobically, so there is not an increased metabolic activity, just a higher rate of glucose utilization to obtain the same amount of ATP.

**Dr. Ginsberg:** I think there is no disagreement between our observations for the most part. The deoxyglucose method does not distinguish between aerobic and anaerobic glucose utilization. It is clear that the glucose utilization becomes anaerobic as oxygen delivery becomes compromised, and with that we are in complete agreement. When one does a true double label study in the same animal, one can show that at flows around the threshold you mentioned—cerebral blood flow values of about 35—40% of normal—one begins to see tissue areas in which the consumption of glucose may become increased. In this study, though, one does not necessarily have to see absolutely elevated glucose consumption. The glucose consumption can be normal or near normal or only slightly depressed, but the point is that it is markedly inappropriate for the amount of blood flow; that is to say, much more glucose is being utilized than should be utilized given the amount of blood flow in the tissue. I think where we disagree is on the energetic consequences of all this. It has been shown very clearly that if you freeze tissue during ischemic depolarization, the ATP is not normal, but rather it is depleted, or is beginning to be depleted, and that is exactly what you would expect because the ionic dislocations produced by ischemic depolarizations, or spreading depression if you will, are really enormous, and the reconstitution of those ion gradients requires a great deal of ATP. ATP production by anaerobic glycolysis is of course only about one-sixteenth of the production by aerobic glycolysis. Thus, although glucose is turning over, it is only producing a very small fraction of the ATP that it usually can. I think the essence of this is that there is a true metabolic stress—the tissue can’t keep up energetically after a point, and then secondary injury mechanisms occur.