Commentary

A radical approach to stroke therapy

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Stroke is a major cause of death and disability throughout the developed world. Cerebrovascular disease ranks third after cancer and heart disease as a cause of death in the European Union and the U.S. The economic and social burdens of stroke are not consequences of mortality; they are imposed by the large majority of stroke patients who survive but are physically and mentally disabled by stroke-induced brain damage. In the U.S., less than 2% of stroke patients benefit from access to early thrombolysis, which removes the primary cause of blood flow reduction. However, no treatment is presently available that protects brain tissue from the multiple neurochemical cascades initiated by both ischemia and reperfusion, and it is these cascades that ultimately cause irreversible brain damage.

Over the last decade, remarkable insight has been gained into the neurochemical mechanisms that contribute to ischemic brain damage, and there is compelling evidence that oxidative damage plays an important role. Two papers in this issue of PNAS test the therapeutic potential of reducing oxidative damage in animal models of ischemic brain damage (1, 2). However, the two studies use quite different conceptual and pharmacological approaches. The study of Huang *et al.* (1) is primarily concerned with the anti-ischemic efficacy of the long-recognized antioxidant ascorbic acid and uses a prodrug treatment strategy with dehydroascorbic acid to deliver ascorbic acid to the central nervous system. The study of Namura *et al.* (2) focuses on establishing the participation of specific mitogen-activated protein kinases and, after characterizing their involvement in ischemia, examines whether pharmacological inhibition of the kinases alters the outcome after ischemia. By using pharmacological agents that act at quite different points in the oxidative damage cascade (Fig. 1), both studies report success of the treatment strategies in reducing the extent of ischemic damage.

Huang *et al.* (1) have focused on the classic antioxidant ascorbic acid (vitamin C) and have used the oxidized form, dehydroascorbic acid (DHA), which readily crosses the blood–brain barrier, to augment endogenous brain ascorbic acid levels by up to 2 mM. However, in the brain, ascorbic acid levels are heterogeneous and highly compartmentalized. In the rat, normal neuronal, glial, and cerebrospinal fluid levels of ascorbic acid are ≈10 mM, 1 mM, and 0.5 mM, respectively (3). The antioxidant effects of ascorbic acid at all of these sites within the brain could contribute to the efficacy of this agent in ischemia. Brain ascorbic acid levels are highly dynamic (although under rigorous homeostatic control), display circadian variations, and, importantly, decline markedly and rapidly in the intracellular compartment at the onset of ischemia (3). Any therapeutic use of DHA in stroke patients will need to ensure that adequate levels of ascorbic acid can be directed to the appropriate subcellular sites of oxidative damage at crucial time points after the onset of ischemia, particularly as the most important cellular site of action of ascorbic acid in the brain is not known. Although systemic ascorbic acid was ineffective in mice (2), ischemic brain damage can be markedly reduced by i.v. ascorbic acid in various animal models of ischemia, including those in nonhuman primates (4, 5). These data suggest that the crucial sites of action of ascorbic acid may be located outside the brain, e.g., in endothelial cells. The spin trap agent, NXY059, which has poor brain penetration, produces large reductions in ischemic brain damage in animals (6) and is well tolerated in human patients after stroke (7). The crucial issues for drugs such as DHA and NXY059 remain those of establishing appropriate dosing regimens after stroke onset.

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The strategy followed by Namura *et al.* (2) focuses on a well-defined cell signaling mechanism that is activated by reactive oxygen and nitrogen species; extracellular signal regulated kinases (ERK1/2), which are activated by MEK1/2 (mitogen-activated protein kinases/ERK1/2). During ischemia, ERK1/2 are dephosphorylated, and there is a significant increase in ERK1/2 phosphorylation during reperfusion after forebrain ischemia. Neurons and oligodendrocytes at the margin of a focal ischemic lesion display increased MEK1/2, indicating that this signaling pathway is activated after ischemia and reperfusion *in vivo* (8). If MEK1/2 is inhibited by the novel agent, U0126, the extent of brain damage is reduced after either forebrain or focal ischemia (2). MEK1/2 are activated *in vitro* by various factors (glutamate, interleukin-1, and tumor necrosis factor), which are elevated in the brain by ischemia and inhibitors of MEK1/2 or drugs that prevent phosphorylation of MEK1 protect neuronal cell cultures from oxidative damage. Mitogen-activated protein kinases are an attractive target for drug development because of their multiplicity of actions, which influence not only cell survival and apoptosis but also inflammatory mechanisms (9). The development of drugs such as U0126, which are systemically active and display selectivity of action (e.g., p38 MAPK, JNK, and $p70^{s6}$ kinase are not affected *in vitro*), suggests that intracellular signaling may provide a new therapeutic approach to reducing oxidative damage during postischemic reperfusion.

There is already compelling evidence that a variety of pharmacological and genetic strategies that attenuate oxidative damage also reduce ischemic brain damage (10). Oxidative damage in ischemia is an exceedingly complex therapeutic target (Fig. 1). Free radicals are a diverse group

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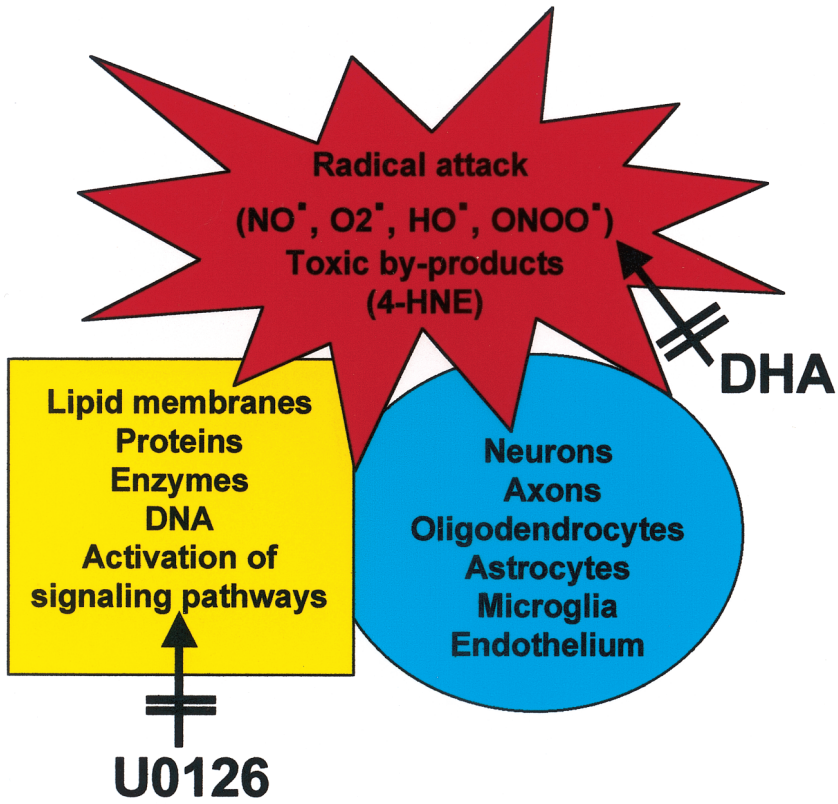

Fig. 1. Oxidative damage is mediated through attack by multiple radicals with differing reactivity, path length, and half-life. Radical attack takes place at many different sites within the brain, and there is interplay between mechanisms. For example, peroxidation of lipid membranes generates toxic aldehydes such as 4-hydroxynonenal (4-HNE), which themselves damage a variety of ion channel, transporter, and cytoskeletal proteins. Free radicals also activate specific cell signaling pathways, such as MEK/ERK, which contribute to damage. The promiscuity of radical attack means that all cellular types and elements in the brain are susceptible to oxidative damage, although the mechanisms mediating this damage differ. For example, DNA damage occurs in the nucleus, whereas lipid peroxidation and damage to structural proteins mediate damage in axons and myelin. Cell signaling-mediated damage will occur only in locations where the pathways are located. The pharmacological agents used by Huang *et al.* (1) and Namura *et al.* (2) target distinct points in the oxidative damage cascade, but both reduce ischemic brain damage.

of agents, are generated at diverse sites, and have diverse reactive distances in tissue. In addition, toxic by-products such as peroxynitrite or 4-hydroxynonenal are generated during oxidative damage, and these also contribute to evolving brain injury. Oxidative damage does not occur in isolation but participates in the complex interplay between excitotoxicity, apoptosis, and inflammation in ischemia and reperfusion (9, 10).

A huge number of pharmacological agents targeted at neurotransmitter receptors and ion channels have been shown to reduce ischemic brain damage in animal models (11). However, no clear evidence of clinical improvement with any neuroprotective drug has yet been obtained in clinical trials of human stroke. Numerous human trials of neuroprotective drugs have not advanced to a definitive assessment of clinical efficacy in a Phase 3 trial, and those that have (Aptiganel, Selfotel, Clomethiazole, Elipordil, D-CPPene, Lubeluzole, Gavestinel, BMS 204352) were suspended before completion of patient recruitment or failed to provide compelling evidence of clinical efficacy (12). The failure to translate mechanistic insight into ischemic damage to effective clinical treatment is due to multiple factors. Definition of the effective dose in clinical trials has been a recurring issue in anti-ischemic drug development because of concerns about adverse effects and, for some agents (e.g., Gavestinel), about kinetics across the blood–brain barrier (13). Both of these issues are pertinent to targeting oxidative mechanisms. The adverse effects of MEK1/2 inhibition remain to be established, and the dose setting with DHA will be difficult because of the tissue compartmentalization of ascorbic acid. In animal models, neuroprotective efficacy is confined to the ischemic penumbra with its special neurochemical characteristics (11, 14). The anatomical and temporal extent of the ischemic penumbra in stroke patients is neither as consistent nor as large as in animals, even when assessed with similar technology, for example, diffusion-weighted imaging (15). There has been concern that the patient entry window in clinical trials (typically within 6 h of stroke onset) has been too long in view of the few agents with proven efficacy in animal models at this time point. The successful recombinant tissue plasminogen activator thrombolysis trial in human stroke had an entry window of 3 h (16). The long therapeutic window displayed by DHA, U0126 (1, 2), and other antioxidants (6), with protection when treatment is initiated at 3 h after onset of ischemia, is encouraging.

The failure of drugs such as *N*-methyl-D-aspartate (NMDA) antagonists to protect cerebral white matter from ischemic damage may have contributed to the absence of functional improvement in clinical trials of these agents, despite their proven ability to protect neuronal perikarya in animal models (17, 18). Unlike NMDA receptor antagonists, brain-penetrating drugs with antioxidant affects protect not only neuronal perikarya but also axons and oligodendrocytes as well as improving neurological outcome after focal ischemia in rats (19). The view that oxidative damage is a crucial target for therapeutic intervention is further supported by evidence that oxidative stress and lipid peroxidation by-products are toxic to axons and oligodendrocytes as well as to neuronal perikarya (20, 21). The nonselective nature of oxidative damage means that it has the potential to involve all cellular types and cellular components of normally functioning brain tissue: neuronal perikarya, axons, oligodendrocytes, astrocytes, microglia, and endothelial cells (Fig. 1). By attenuating oxidative damage, protection of all cellular elements rather than selective neuronal protection may be possible. ERK1/2 is activated in oligodendrocytes after cerebral ischemia (8), and ERK activation is involved in oxidative damage to cultured oligodendrocytes (22). However, it is not clear whether activation of this signaling pathway is involved in ischemic damage to other glia, axons, or endothelial cells. In contrast, the widespread distribution of ascorbic acid in the central nervous system suggests that the nonselective approach of DHA treatment to scavenge free radicals could have the potential to protect all cellular types and elements.

Anti-ischemic drug development is at a crossroads. In excess of $1 billion has already been expended in evaluating neuroprotective drugs targeted at neurotransmitter receptors or ion channels in stroke patients, without success. A re-evaluation

of appropriate pharmacological and cellular targets for therapeutic intervention is taking place. Pharmacological modification of oxidative damage is one of the most promising avenues. If mechanistic insight is to be translated into effective therapy, the advances that have been made in both preclinical evaluation of drug efficacy (17, 23) and clinical trial design (including the use of magnetic resonance imaging technology) (24) need to be harnessed.

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