—Review—

Brain Protection Therapy in Acute Cerebral Infarction

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Abstract

Many drugs for cerebral infarction that were shown to be effective in animal experiments have shown negative results in human clinical trials. For this reason, a completely new approach is needed to develop brain protection therapies against cerebral infarction. Brain protection therapies can be categorized into 3 types: 1) lengthening the therapeutic time window for thrombolytic therapy, 2) reducing the side effects of thrombolytic therapy, and 3) brain protection drug therapy for patients with contraindications for thrombolytic therapy (including combination therapy). Here, we show our recent results of brain protection therapy. First, combination therapy with 2 effective drugs was tried, and time-lag administration was performed. Combination therapy was effective and lengthened the therapeutic time window. Next, a completely new approach to improve cerebral ischemic damage, namely, H_2 gas inhalation therapy, was tried. This therapy was also effective, even in the ischemic core.
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Key words: penumbra, free radical scavenger, FK506, FNK, H_2 gas, combination therapy

Introduction

An estimated 730,000 and 1,500,000 initial strokes occur each year in the United States and Japan, respectively, and stroke is the third leading cause of death in many countries, including Japan[1]. Moreover, about 40% of bedridden elderly inpatients in Japan are bedridden because of stroke[2], which makes stroke the leading reason medical expenses are increasing. Recently, with progress in new therapeutic approaches, a worldwide campaign was started to educate patients about very early stages of stroke (the "Brain Attack" campaign), and numerous new drugs have also been put through clinical trials. Little success has been had to date in the treatment of stroke; however, no trials have examined combination therapy with more than 2 effective drugs. Exploration of an approach to treatment using a combination of several carefully selected neuroprotective drugs with different time courses[3] is greatly needed. Various therapeutic trials have been performed, such as using a recombinant tissue plasminogen activator (rt-PA), neuroprotective drugs, and mechanical thromboembolectomy. In 2005, rt-PA was approved in Japan for treating cerebral infarction in the very acute phase (i.e., less than 3 hours after onset). However, only about 2.5% of patients with cerebral infarction have benefited from rt-PA[4]. Therefore, most patients with cerebral

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infarction still receive conventional therapy, and a new and effective treatment, "brain protection therapy," is urgently required.

Definition of Brain Protection Therapy

Brain protection therapy can be categorized into 3 types: 1) lengthening the therapeutic time window of thrombolytic therapy (including rt-PA), 2) reducing the side effects of thrombolytic therapy (e.g., reducing hemorrhagic transformation), and 3) brain protection drug therapy for patients with contraindications for thrombolytic therapy (for most patients with cerebral infarction).

Mechanisms of Tissue Damage in Cerebral Infarction

Ischemic cell damage in cerebral infarction differs between the core (dense ischemic region) and the penumbra (peri-infarct area) and is affected by the duration of the ischemic period and the presence of cerebral blood reflow. In the core, acute depletion of energy and an imbalance of ionic homeostasis occur, and cell swelling and disruption of the cell membrane (necrosis) is the final state. In the penumbra, programmed cell death due to certain signals (apoptosis, autophagy) probably subsequently occurs. At the very start of the cerebral infarction (**Fig. 1**), the core area may be extremely small, and penumbra may be large. Therefore, rapid recanalization by any method is extremely important. In such cases, the core may disappear, or the increase of the core area can be stopped. In such cases, the tissue (cells) in the penumbra area must be treated, perhaps much more easily than cells in the core area. At present, we are unfortunately unable to treat tissue in the core area, except the early recanalized area. On the other hand, if recanalization is too late, or if the residual cerebral blood flow of the core is too low, the recanalization therapy, including thrombolytic therapy, will have a higher risk of hemorrhagic transformation or reperfusion injury due to free-radical formation. In such cases, brain protection therapy with free-radical scavengers is appropriate. A free radical scavenger, edaravone, is usually used in Japan, and the prognosis after combination therapy with tPA is reported to be better, although this result was obtained through retrospective analysis[4].

In patients for whom thrombolytic therapy is not appropriate, the area of cerebral edema enlarges until about 5 days after the onset of infarction. According to the area of edema and several other factors, cerebral blood flow in the penumbra will gradually decreases, the former penumbra area changes into a deep ischemic area (core), and a new penumbra region appears around the new core area. Thus, cerebral infarction may expand. Anti-edema therapy with glycerol is usually chosen in Japan and is often effective; however, evidence for an improved prognosis is lacking, except for a reduction in the death rate within 14 days after onset[5]. When cerebral infarction cannot be detected with computed tomography (6 to 12 hours after onset), cerebral edema is thought to be mostly cytotoxic edema, and a large penumbra area is thought to exist. During this period, we might be able to apply a new brain protection therapy. To this end, blocking the cell-death mechanisms is extremely important.

When cerebral ischemia occurs, the cell membrane is depolarized because of the depletion of energy, and voltage-dependent ion channels, including calcium channels, and glutamate receptors are activated; in addition, various signal transduction systems leading to cell death (e.g., activation of lipases, calcineurin, and disturbance of mitochondria) or cell survival are switched on. We show the simplified cell death cascade in **Figure 2**, according to the existence of compounds, most of which have been tested in human clinical trials.

Neuroprotective Drugs

Magnesium is thought to inhibit voltage-dependent calcium channels and N-methyl-D-aspartate receptors, and clinical trials are ongoing. DP-b99 is a membrane-activated chelator for Ca^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{2+} which is now undergoing phase III clinical trials. Free-radical scavengers are the most promising drugs because various free radicals,

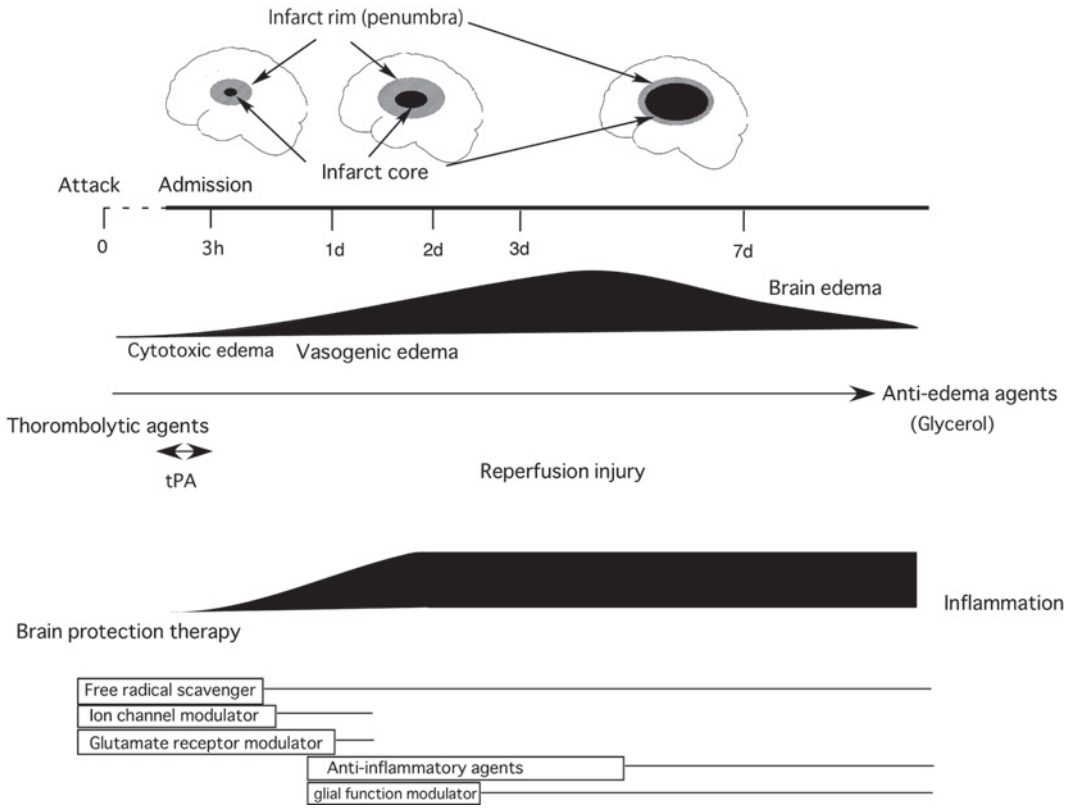

Fig. 1 Mechanisms of ischemic damage in cerebral infarction and the strategy for treatment
Because cells in the ischemic core still cannot be rescued with neuroprotective drugs, our objective is to protect against cell death in the ischemic rim (penumbra). At the very early phase of cerebral infarction, the ischemic core may be extremely small and the penumbra may be large. Therefore, if less than 3 hours has passed since the onset of infaction, thrombolytic therapy with tissue plasminogen activator (tPA) is possible. If vascular reperfusion occurs after thrombolytic therapy, the expansion of the core area may be inhibited. At that time, to reduce reperfusion injury, brain protection therapy may be useful, especially that with a free-radical scavenger. If thrombolytic therapy is not appropriate, the ischemic core area gradually increases, and the penumbra expands around it. Twelve to 24 hours after the onset of infarction, various inflammatory cytokines are released, and secondary cell damage occurs in the penumbra. The peak of brain edema is usually about 5 days after the onset of infarction, and around that time, ischemic tissue damage is largely completed. Also around this time, therapy with drugs, such as that with anti-inflammatory or anti-glial activation effects, is considered to be a brain protection therapy.

including hydroxyl radicals, are generated owing to the reperfusion (reoxygenation) and because these radicals attack membrane phospholipids, enzymes, proteins, and DNA. These attacks induce a chain reaction of oxidization. Most researchers accept that free radicals are deeply involved in neuronal cell death in cerebral infarction. However, NXY-059, a free-radical scavenger, does not appear to be protective in patients with stroke. Although NXY-059 has been protective in animal experiments, in a large clinical trial, NXY-059 initially appeared to reduce disability after stroke (Stroke-Acute Ischemic NXY Treatment [SAINT] I trial), but this effect could not be reproduced (SAINT II trial)[6]. Another feature of NXY-059 is the potential to decrease symptomatic intracerebral hemorrhage after treatment with tPA; however, the results did not reach statistical significance. Although there are many interpretations for why NXY-059 was not protective in human trials, possible reasons are the short treatment period (72 hours) and the enrollment of patients who had received t-PA. In contrast to NXY-059 in the SAINT study, edaravone (MCI-186) was injected twice daily for 14 days, and patients who had been treated with t-PA were not included in the clinical trial. Edaravone has been shown to be

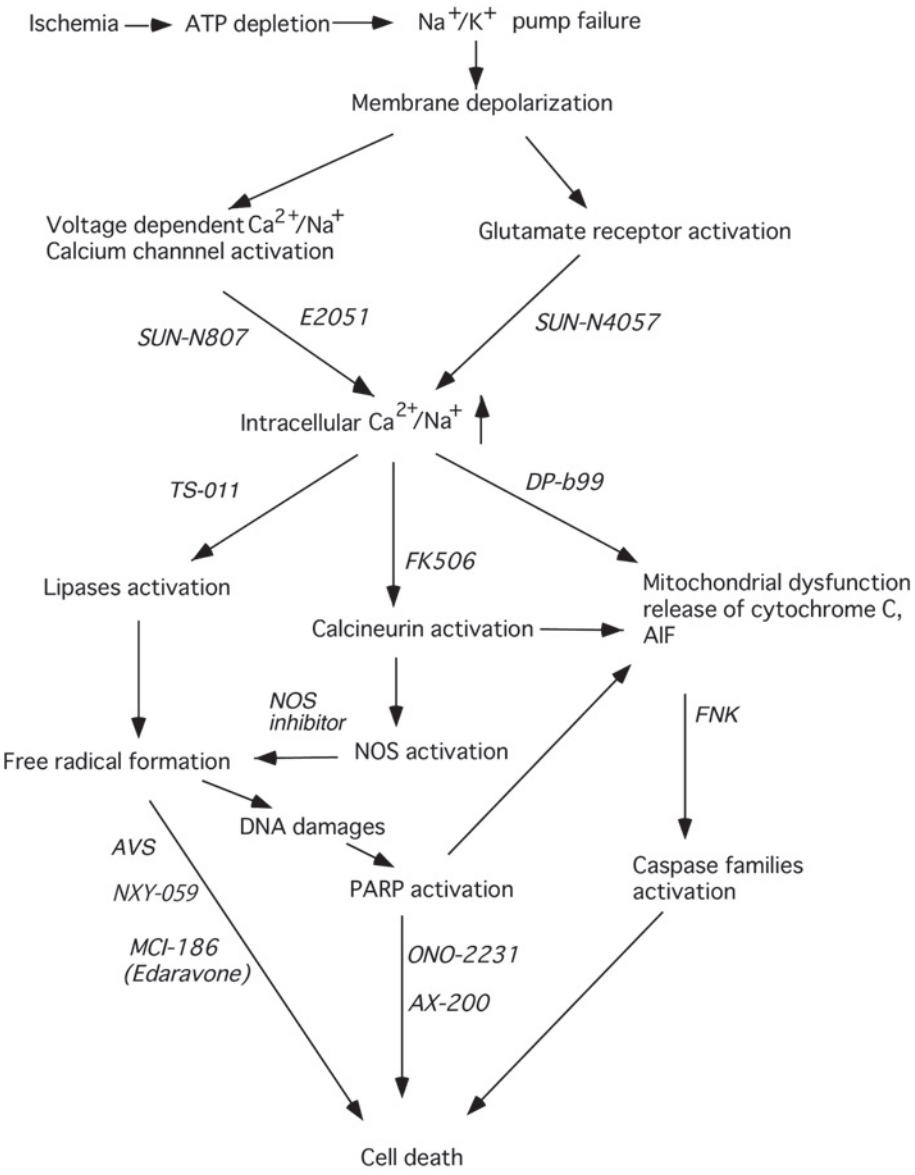

Fig. 2 Simplified cell death cascade, according to the existence of compounds, most of which have been tested in human clinical trials
After ischemia, oxygen and glucose are depleted, and membrane Na^+/K^+ pumps fail; in addition, through voltage-dependent ion channels and glutamate receptor-activated channels, intracellular calcium concentrations increase. Subsequently, various pathways, including lipase activation, calcineurin activation, and mitochondrial dysfunction, are activated. Through these pathways, nitric oxide (NO) or free radicals are produced, which further damage mitochondrial and nuclear functions, leading to cell death. Various drugs that can inhibit the pathways are shown in italics (most of these drugs have already been tested in humans; however, in human clinical trials negative results were obtained for most drugs, except for MCI-186 [edaravone]).

protective[7] in acute stroke and is now commonly used in stroke therapy in Japan.

Several new compounds are proven to be protective against cerebral ischemia. FK506 is an immunosuppressive drug used for transplantation and to treat myasthenia gravis. Since FK506 was found to have neuroprotective effects[8], many animal experimental studies, including ours, have examined it[9–11]; however, human stroke trial was stopped in phase II. ONO-2506 inhibits S-100β and nerve growth factor β from astrocytes and inhibits secondary expansion of the infarct area in the

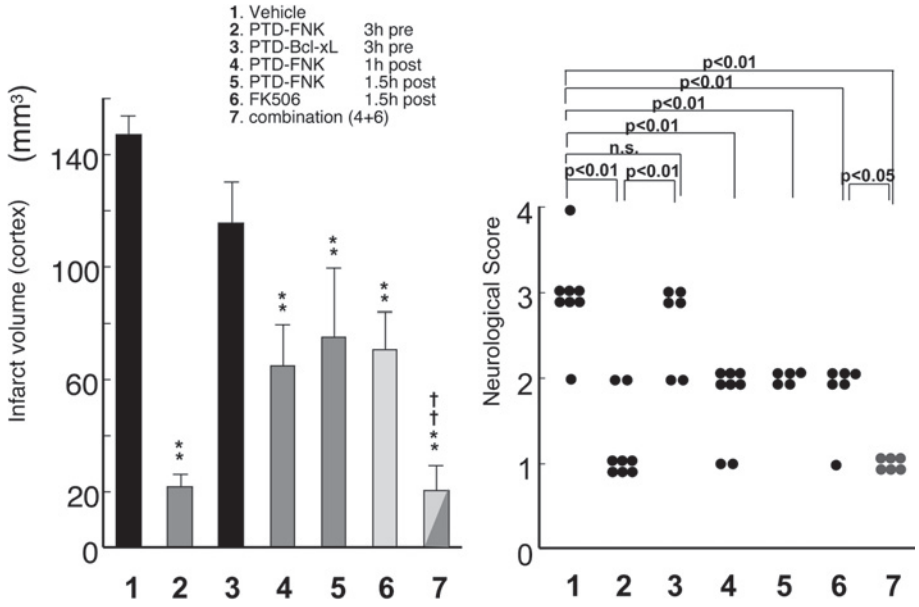

Fig. 3 Protein transduction therapy for cerebral infarction in rat

Left column: PTD-FNK administration 3 hours before ischemia (column 2) produced a marked reduction in cortical infarct volume to 20%. Administration of PTD-FNK 1 to 1.5 hours after ischemia (columns 4 and 5) produced a 50% reduction, which was similar to that produced by FK506 treatment (column 6). Original PTD-Bcl-xL administration did not reduce infarct volume significantly (column 3). The combination therapy of PTD-FNK (after 1 hour) and FK506 (after 1.5 hours) (column 7) produced a marked reduction in infarct volume, which was similar to that produced by pretreatment with PTD-FNK (column 2).

Right column: Neurological symptoms were evaluated under similar conditions as for the left column. Neurological deficits were scored on a scale of 0 to 5 as follows: 0, no neurological deficit; 1, failure to extend the right forepaw fully; 2, circling to the right; 3, falling to the right; 4, unable to walk spontaneously; and 5, death. The combination therapy (column 7) produced the greatest improvement in neurological symptoms.

subacute phase of stroke[12]; it is now undergoing phase III trial in Japan.

A New Concept of Brain Protection Therapy

For gene therapy and stem-cell transplantation therapy, many steps should be taken before clinical trials, except for bone marrow cell transplantation therapy for cerebral infarction. Basic experiments, including our studies[13,14], in animals have shown promising results, and clinical trials have already been started for bone marrow cell transplantation therapy for patients with stroke in Japan[15]. Many clinical trials with drugs that were protective in animal experiments failed to show protection in human patients. Possible reasons of this failure are that animal models do not precisely mimic human stroke and that treatment involved a single administration of the drug. Combination therapy will be necessary for clinically useful treatment, which should be considered according to the chronological pathophysiological changes after the onset of stroke. A strong need exists for combination therapy that could lengthen the therapeutic time window of effective drugs and allow them to work synergistically. Another possibility is a completely new approach to protect against ischemic cell death in cerebral infarction. Here, we show our recent results of trials using a rat focal ischemia model.

The delivery of therapeutic materials into the brain is severely limited by the blood-brain barrier. Gene therapy might be used to overcome this problem. However, a considerable time is needed to deliver a gene of interest into the brain and to express a sufficient amount of therapeutic protein; therefore, gene therapy might not be suitable for

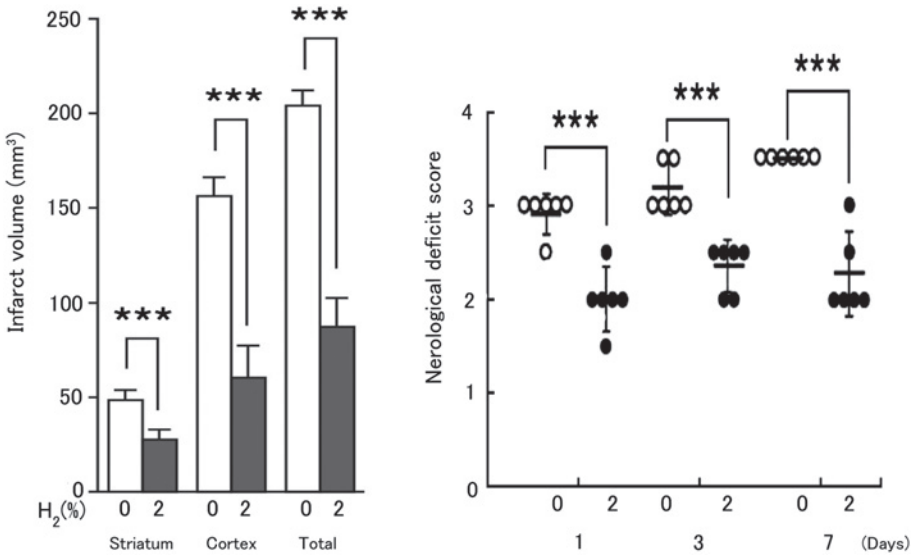

Fig. 4 Effects of H_2 gas inhalation in a rat model of transient focal ischemia
Left column: When 2% H_2 gas was inhaled during and after ischemia (30 minutes), the cerebral infarct volumes were significantly reduced in the striatum and cortex.
Right column: The change in neurological deficit score showed significantly better recovery in 2% H_2 inhalation groups.

emergency treatments. As an alternative, delivering therapeutic proteins directly to ischemic brain tissue through the use of a protein transduction domain (PTD) has been proposed[16,17]. An antiapoptotic member of the Bcl-2 family, Bcl-xL, would be a good candidate for protein therapeutics[18,19]. To enhance its cytoprotective activity, we constructed a powerful artificial cytoprotective protein, FNK, from Bcl-xL by means of site-directed mutagenesis of 3 amino acid residues (Y22F, Q26N, and R165K) and fused a PTD to its 5' end[20,21]. On the other hand, postischemic administration of FK506 mitigates the brain injury following permanent cerebral ischemia, and many studies have confirmed the neuroprotective effect of FK506 in various experimental models, including ours[9,22]. Therefore, we explored the potential of a therapy combining PTD-FNK and FK506 and varied the timing of drug administration. Striking protective effects were obtained with a time-lag combination therapy[11].

Figure 3 shows the effects of PTD-FNK and FK506 combination therapy on cerebral infarct volume and neurological symptoms. PTD-FNK alone showed protective effects until administration 1.5 hours after the start of reperfusion (3 hours after the onset of ischemia). The protective effect was maximal when PTD-FNK was given 1 hour after the onset of ischemia and FK506 was given 30 minutes later (**Fig. 3**, column 7). In addition, although PTD-FNK alone did not show a significant protective effect when administered 3 hours after reperfusion, the protective effect became significant if PTD-FNK was combined with FK506. The combination therapy lengthened the therapeutic time window of PTD-FNK.

Figure 4 shows the results of a completely new approach. There had been no studies of gases for protecting against cerebral infarction until our previous work[23]. As a potential antioxidant H_2 gas has several advantages: it effectively neutralizes ・OH in living cells and, unlike most known antioxidants, which cannot successfully target organelles[24], it can penetrate biomembranes and diffuse into the cytosol, mitochondria, and nucleus. Despite the moderate reducing activity of H_2, its rapid gaseous diffusion might make it highly effective for reducing cytotoxic radicals. Thus, H_2 markedly decreased oxidative stress and suppressed brain injury caused by ischemia and reperfusion. Effects observed after 2% H_2 inhalation after transient focal ischemia included reduced infarct volume, improved neurological symptoms, increased body temperature, and increased body weight.

Concluding Remarks

After tPA was approved for clinical use, the therapeutic strategy for cerebral infarction rapidly changed. Recently, with the approval of a mechanical thromboembolectomy device, the strategy for treating cerebral infarction has again changed markedly. However, because of the limited time the device can be used after onset (approximately 8 hours) and the need for a skilled physician, most patients with cerebral infarction remain unable to receive this therapy. Therefore, a new effective therapy that can be used for most patients with cerebral infarction is needed. Recently, clinical studies of either tPA or mechanical thromboembolectomy have been highly valued in the field of stroke research; however, a new brain protection therapy is also urgently needed. To this end, basic research should be performed to extensively study the mechanisms of ischemic cell death, and new animal experiments should be performed to evaluate newly developed drugs and new concepts of therapy.

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