Pathophysiology and treatment of cerebral ischemia

Shinji Nagahiro*, Masaaki Uno*, Koichi Sato*, Satoshi Goto†, Motohiro Morioka†, and Yukitaka Ushio†

*Department of Neurological Surgery, The University of Tokushima School of Medicine, Tokushima, Japan ; and †Department of Neurosurgery, Kumamoto University Medical School, Kumamoto, Japan

Abstract : This article describes the pathophysiology of, and treatment strategy for, cerebral ischemia. It is useful to think of an ischemic lesion as a densely ischemic core surrounded by better perfused "penumbra" tissue that is silent electrically but remains viable. Reperfusion plays an important role in the pathophysiology of cerebral ischemia. Magnetic resonance imaging (MRI) and histological studies in rat focal ischemia models using transient middle cerebral artery (MCA) occlusion indicate that reperfusion after an ischemic episode of 2- to 3-hour duration does not result in reduction of the size of the infarct. Brief occlusion of the MCA produces a characteristic, cell-type specific injury in the striatum where medium-sized spinous projection neurons are selectively lost ; this injury is accompanied by gliosis. Transient forebrain ischemia leads to delayed death of the CA1 neurons in the hippocampus. Immunohistochemical and biochemical investigations of Ca^{2+}/calmodulin-dependent protein kinase II(CaM kinase II) and protein phosphatase (calcineurin) after transient forebrain ischemia demonstrated that the activity of CaM kinase II was decreased in the CA1 region of the hippocampus early (6-12 hours) after ischemia. However, calcineurin was preserved in the CA1 region until 1.5 days after the ischemic insult and then lost ; a subsequent increase in the morphological degeneration of neurons was observed. We hypothesized that an imbalance of Ca^{2+}/calmodulin dependent protein phosphorylation-dephosphorylation may be involved in delayed neuronal death after ischemia. In the treatment of acute ischemic stroke, immediate recanalization of the occluded artery, using systemic or local thrombolysis, is optimal for restoring the blood flow and rescuing the ischemic brain from complete infarction. However, the window of therapeutic effectiveness is very narrow. The development of effective neuroprotection methods and the establishment of reliable imaging modalities for an early and accurate diagnosis of the extent and degree of the ischemia are imperative. J. Med. Invest. 45 : 57-70, 1998

INTRODUCTION

Stroke was the leading cause of death 30 years ago when hypertensive intracerebral hemorrhage was the most common cause of stroke deaths. At present, stroke is the second leading cause of death in Japan ; it ranks behind only cancer deaths. The death rate from stroke was around 120 per 100,000 in 1996, almost the same as that of heart disease (Fig.1). The decline in the death rate from stroke over the last 20 years has been attributed to improved control of hypertension and advances in the management of cerebral hemorrhage in the acute stage. However, the incidence of cerebral infarction resulting in disability in the survivors of ischemic stroke appears to be on the increase, at a time when the proportion of elderly individuals is rapidly increasing in Japan. Of all types of stroke, the incidence of hemorrhagic cerebrovascular disease, including subarachnoid
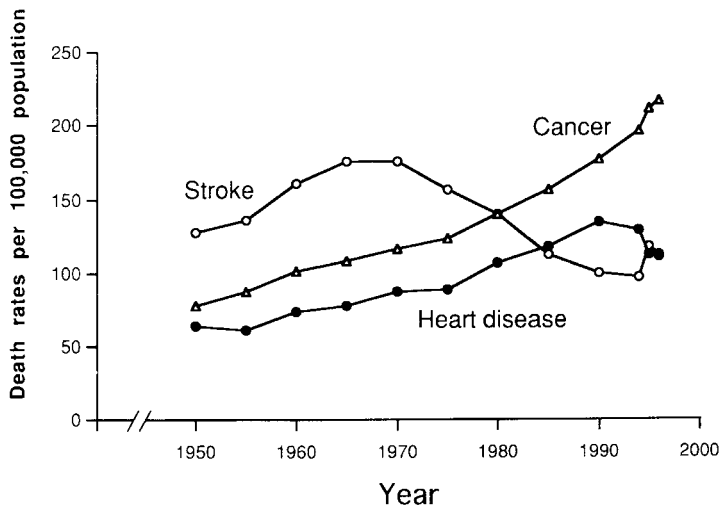
Fig.1. Trends in death rates for three major diseases in Japan, 1950-1996.

hemorrhage and intracerebral hemorrhage, is about 20-25% ; the incidence of ischemic stroke is 75-80%. The increase in the number of individuals with mental and physical handicaps after stroke presents considerable problems in terms of quality of life and socioeconomic costs.

Despite recent medical and surgical advances, e.g. antiplatelet agents, anticoagulant therapy, carotid endarterectomy, bypass surgery and endovascular treatment, the general approach to preventing acute ischemic brain damage resulting in irreversible cerebral infarction remains inadequate. The term "brain attack" has come into use to denote the similarity of stroke to myocardial infarction (heart attack) with respect to the need to receive immediate medical attention.

This review article describes the pathophysiology, and treatment strategy for, acute cerebral ischemia. The discussion is based on the results of our experimental studies (1-20) and a review of the literature.

PATHOPYSIOLOGY OF CEREBRAL ISCHEMIA

Cerebral metabolism and blood flow

Neuronal function and cerebral metabolism are critically dependent on a sufficient oxygen and glucose supply and the production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). An adequate cerebral blood flow (CBF) is essential for maintaining this critical supply of oxygen and glucose. The CBF is regulated by the metabolic demands of the brain to adjust to its functional needs (flow-metabolism coupling). The brain requires more oxygen and glucose on a weight basis than do the other organs. In human

adults, the brain weighs approximatelyl 1400g and represents only about 2 % of the total body weight. However, it uses about 25 % of total body oxygen and glucose per minute. Since the brain is unable to store energy, any interruption of the blood supply easily and quickly results in neuronal dysfunction and neuronal damage.

Focal cerebral ischemia and penumbra

Focal cerebral ischemia is most often brought about by interruption of the blood supply to a part of the brain. Embolic or atherothrombotic occlusion of the cerebral artery is a primary event that occasionally progresses to cerebral infarction in humans.

Focal ischemia due to middle cerebral artery (MCA) occlusion encompasses a densely is chemic core of tissue where CBF decreases markedly and a marginally better perfused area, the so-called "ischemic penumbra" (21,22) (Fig.2). Perfusion of the penumbral tissue is dependent on the degree of collateral circulation coming from the anterior- or the posterior cerebral artery. CBF is normally about 50 ml/100 g/min. Electrical activity in cerebral tissue ceases at flow rates below 16 to 18 ml/100 g/ min (23). This degree of ischemia represents a threshold for neuronal and electrical dysfunction. Although silent electrically, cerebral tissue in the penumbral area remains viable and the ion pump mechanism remains functional until CBF falls below 10-12 ml/100 g/minm (22, 23) (Fig.3). If profound ischemia lasts for a certain time, there is consequent depletion of ATP and failure of the membrane ion pump, involving the efflux of cellular potassium and the intracellular influx of calcium, sodium, chloride and water. This disturbance results in lactate acidosis and membrane depolarization and leads to irreversible degeneration of the neurons and glia cells in the ischemic core (pan-necrosis or complete infarction).

In the penumbra as well as in the ischemic core, neuronal viability is time dependent ; that is, the more profound the degree of ischemia, the less time before the establishment of irreversible damage (24).

Reperfusion

Reperfusion is essential for saving the ischemic brain tissue from irreversible infarction. If occlusion of the artery is corrected immediately and reperfusion is successful, the ischemic tissue at risk of irreversible infarction and the tissue in the penumbra can re-
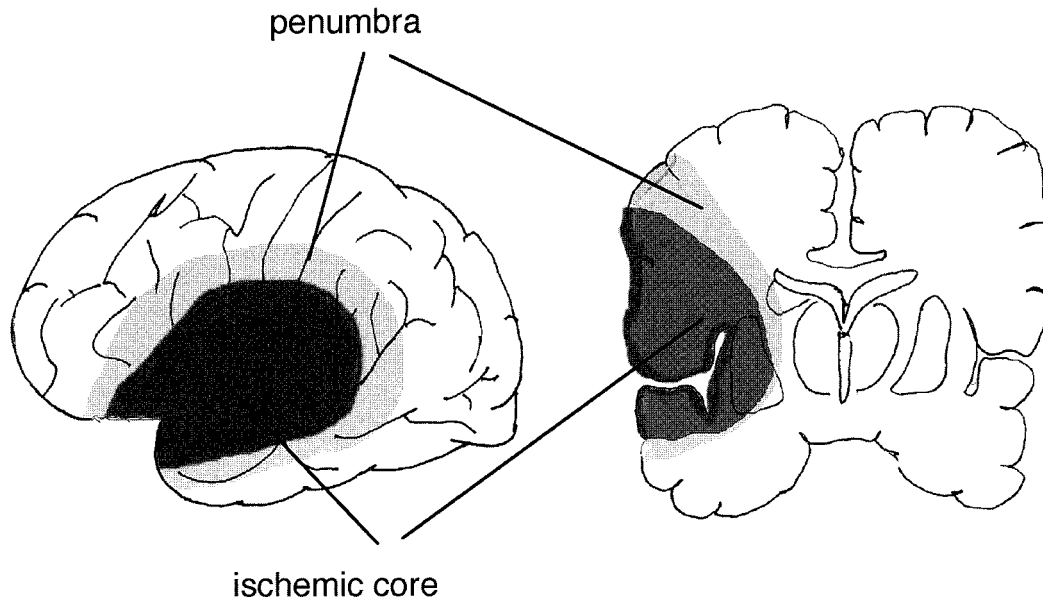
penumbra

ischemic core

Fig. 2. A stroke lesion with MCA occlusion, consisting of a densely ischemic core (black area) surrounded by better perfused penumbral tissue.

cover completely (Fig.4). However, if reperfusion occurs after the establishment of moderate cerebral ischemia, incomplete infarction or selective neuronal death may result (25). The degree and extent of the resulting infarction or neuronal damage depend on the duration and degree of the ischemic insult (Fig.5). On the other hand, reperfusion can be harmful to the ischemic brain if it is performed very late after the insult. Reperfusion injury to the ischemic brain, e.g. aggravation of vasogenic edema or hemorrhagic infarction, may be the result of reactive hyperemia with loss of vasoreactivity, disruption of the blood-brain barrier (BBB), free radical- and toxic oxygen production, the release of inflammatory mediators, and leukocyte adhesion to endothelial cells in ischemic brain tissue (Fig.4).

To elucidate the relationship between the timing of reperfusion and the degree of ischemic brain damage, we subjected rats to transient MCA occlusion and studied the sequential and regional changes of ischemic edema and BBB permeability, using magnetic resonance imaging (MRI) with Gd-DTPA enhancement (5, 10, 16). In addition, we performed behavioral and immunohistochemical studies to determine the pathophysiological mechanism underlying ischemic injury of the cerebral cortex and the striatonigral system (2-4, 6-9, 11-14).
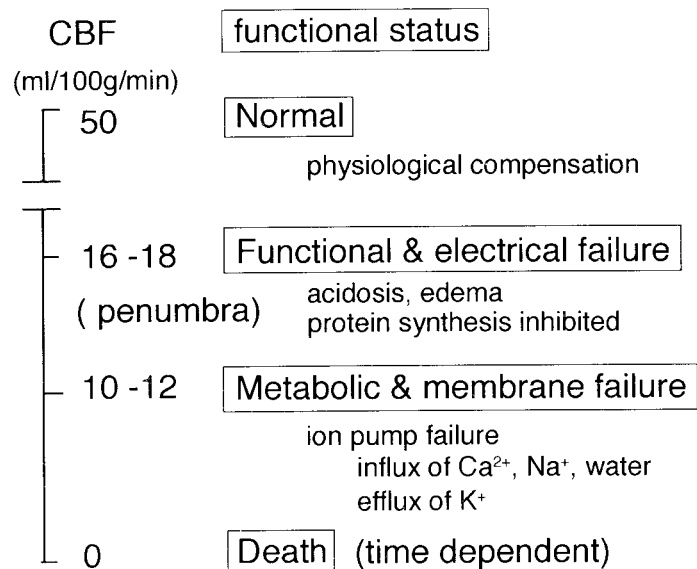
CBF (ml/100g/min)

| functional status |

50 — | Normal |

physiological compensation

16 -18 (penumbra) | Functional & electrical failure |

acidosis, edema
protein synthesis inhibited

10 -12 | Metabolic & membrane failure |

ion pump failure
   influx of Ca^{2+}, Na^+, water
   efflux of K^+

0 — | Death | (time dependent)

Fig.3. Ischemic thresholds for functional and electrical failure, and for metabolic and membrane failure. The diagram is modified from Astrup (22).

Experimental focal ischemia and MRI studies

We used a rat model of reversible focal ischemia. The rats were subjected to unilateral occlusion of the MCA using a method of intraluminal vascular occlusion that mimicks human embolic stroke (26-28). Briefly, a nylon thread was introduced into the right internal carotid artery of Wistar rats weighing 240-270 g so that the tip of the thread reached the proximal segment of the anterior cerebral artery and obliterated the origin of the MCA. MCA blood flow was restored by removing the thread embolus
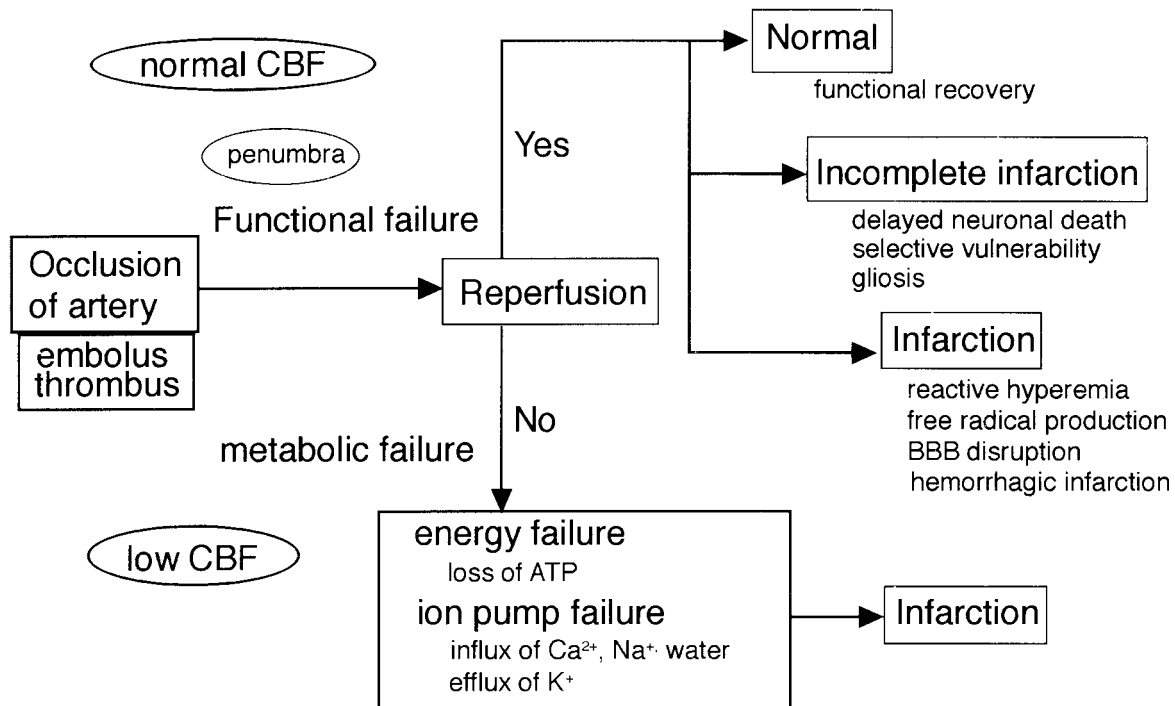
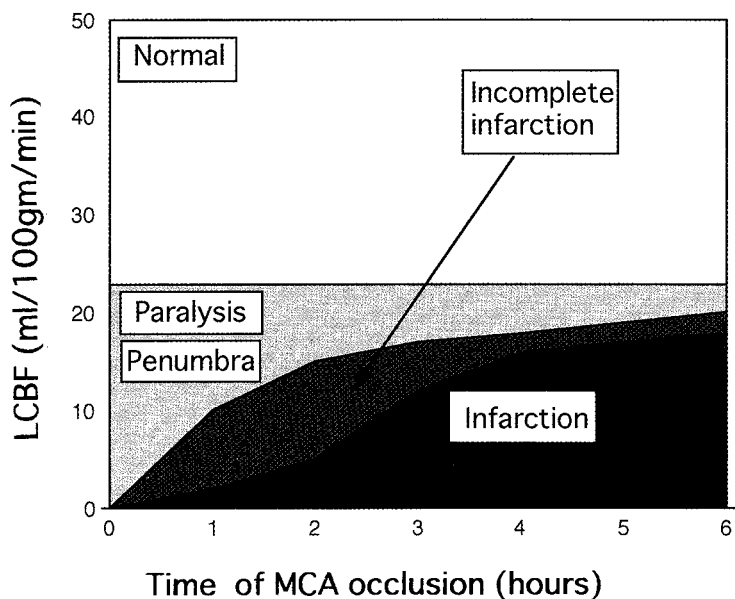Fig.4. Diagram illustrating the mechanisms of cerebral ischemic damage and the effects of reperfusion.

Fig.5. Graph showing the time-intensity relationship of ischemia and degree of tissue damage. The continuum from normal condition to incomplete infarction and complete infarction is illustrated. Even profound ischemia is reversible if its duration is very brief. The curves are modified from Jones et al. (24) and Garcia et al. (25).

15 or 30 minutes, or and 1, 2, 3, or 5 hours after occlusion. Paresis of the left limb and circling movement to the left were observed prior to restoration of the blood flow.

MRI was obtained 6 hours, 24 hours (day 1), and 7 days (day 7) after the onset of ischemia. In all rats that had been subjected to 1-5 hours of ischemia, T2-weighted images obtained at 24 hours post-insult demonstrated high-signal intensity areas representing ischemic edema in the lateral striatum and/or the cerebral cortex. These areas were larger and detected earlier in rats subjected to longer-lasting ischemia (Fig.6). Sequential MRI revealed that the size of the high-signal intensity area on T2-weighted images was largest at day 1 in all rats that had been subjected to 1-5 hours of ischemia. Even brief isc hemia (15 to 30 minutes) resulted in transient T2 high-signal intensity areas in the dorsolateral part of the striatum at day 1.

On Gd-DTPA-enhanced MRI, parenchymal enhancement of the striatum and the cerebral cortex on the ischemic side, due to BBB disruption, was seen following reperfusion after 1 to 5 hours of ischemia. This was detected earlier in rats subjected to longer periods of ischemia (16). Enhancement of the lateral ventricle due to disruption of the blood-cerebrospinal fluid barrier at the choroid plexus appeared in the early reperfusion stage (6 hours and day 1) in rats subjected to 15-minute- to 5-hour ischemia (6). The exact mechanisms and the
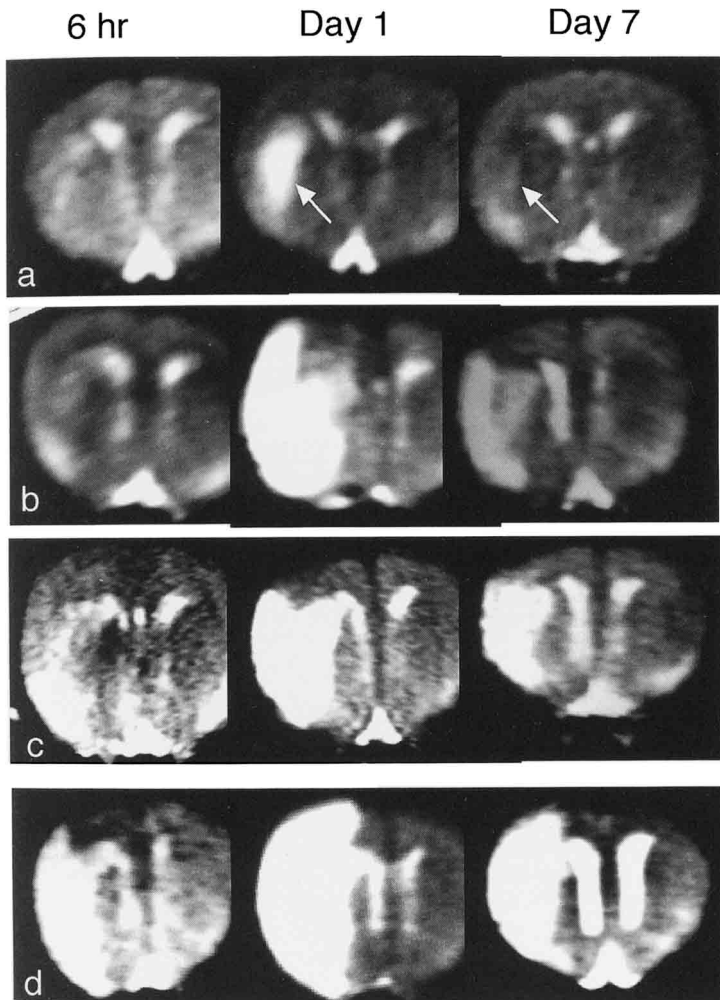
Fig.6. Sequential T2-weighted images obtained at 6 hours (left), day 1 (middle) and day 7 (right) in rats subjected to 1-hour, 2-hour, 3-hour and 5-hour ischemia. The high-intensity areas in the striatum (arrows) and cortex are larger and detected earlier as the duration of the ischemia increases.

significance of the finding that blood-cerebrospinal fluid permeability transiently increased after transient ischemia remain unclear. The increased permeability of the BBB and the blood-cerebrospinal fluid barrier may exacerbate the progress of brain edema following reperfusion after transient ischemia.

Immunohistochemical studies

Immunohistochemical studies were performed on 10- or 20 μm thick vibratome sections using antibodies to calcineurin (CaN), Ca^{2+}/calmodulin-dependent protein kinase II (CaM II), parvalbumin, choline acetyltransferase, glutamic acid decarboxylase (GAD), 72-kD heat shock protein (HSP 72), and glial fibrillary acidic protein (GFAP). Calcineurin, a Ca^{2+}/calmodulin dependent protein phosphatase, was used as a marker for striatal projection neurons (2, 6, 11). HSP72 was used as a marker for stress response

in the neurons to ischemic insult (8, 29).

Histologically, the striatal lesion in rats subjected to brief (15 to 30 minutes) ischemia showed a characteristic, cell type-specific injury ; a marked reduction in medium-sized spinous neurons expressing CaN, and a selective sparing of parvalbumin-positive medium-sized aspiny neurons and choline acetyltransferase-positive giant neurons (2). This cell-type specific injury was accompanied by marked gliosis showing strong GFAP immunolabeling (Fig.7). Scattered or laminar damaged neurons with reactive gliosis were observed in the cortex at 3 to 28 days after short-term MCA occlusion ; this was called "slowly progressive neuronal damage" by Nakano et al. (25).

In the ipsilateral cortex of the rats subjected to 30 minutes ischemia, strong HSP72 immunoreactivity appeared in the lower supragranular layer and laminas IV and V in the acute phase. There was an increase in GAD-immunoreactivity in lamina IV at 3 to 14 days after MCA occlusion (8). GAD is a limiting enzyme of γ-aminobutyric acid (GABA) synthetase, and is enriched in the GABAergic neurons. The enhanced expression of GAD in the ipsilateral cortex may reflect some adaptive functional changes in GABA transmission with slowly progressing cortical ischemic damage. The neuronal injury accompanied by gliosis may also correspond to a state of incomplete infarction.

In rats subjected to 1-hour MCA occlusion, incomplete infarct areas in the cortex and striatum were encompassed by a small pan-necrotic area (complete infarction) in the center of the lesion (Fig 7 c, d). In rats subjected to 2-hour ischemia, there was massive, extensive ischemic damage in the striatum and the cerebral cortex. Almost all striatal cells were degenerated on the affected side. Total tissue necrosis was seen in the striatum and the cerebral cortex of the MCA territory in all rats that had been subjected to 3 - 5-hour ischemia.

Ischemic damage and timing of reperfusion

The extent and the size of the infarctions and the incomplete infarctions detected by MRI and histological studies in rats subjected to various ischemia durations are schematically summarized in Figure 8. Reperfusion earlier than 1 hour after the onset of ischemia significantly reduced the size of the infarc-
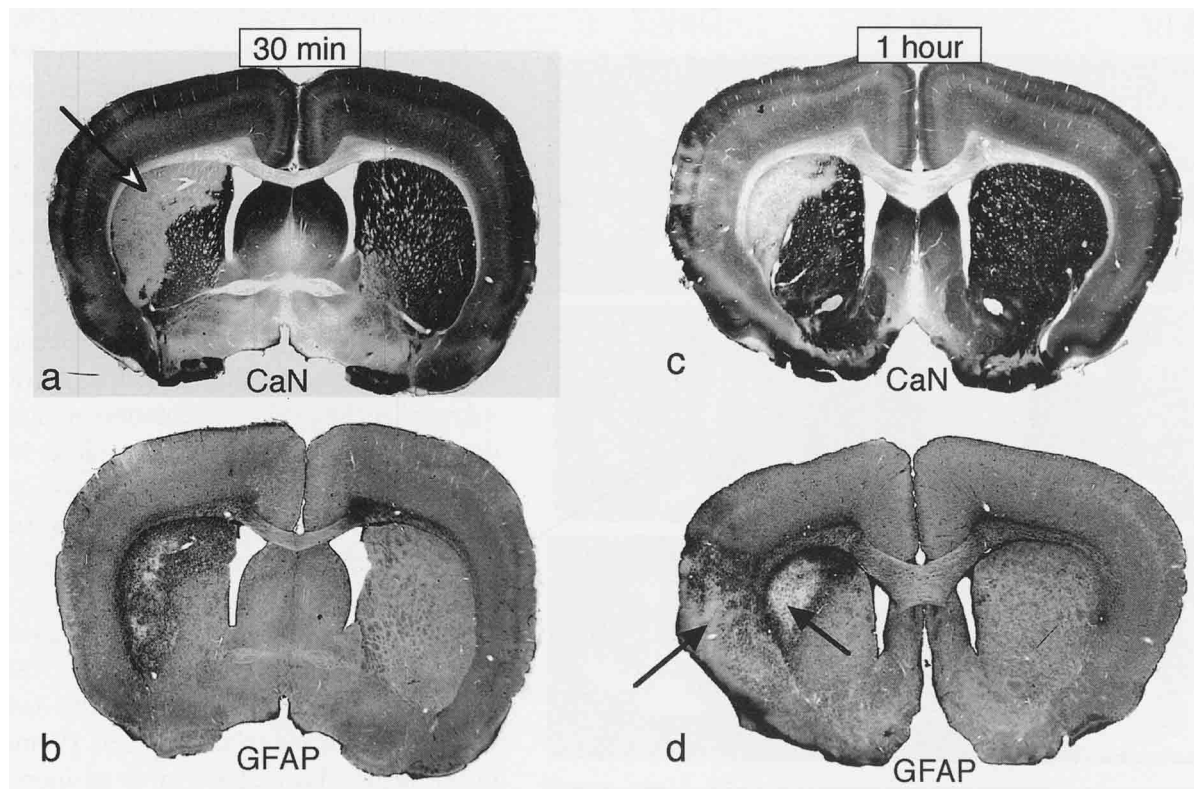
Fig.7. Coronal sections from rats subjected to 30-minute (a,b) and 1-hour (c,d) ischemia were stained immunohistochemically for calcineurin (a,c) and GFAP (b,d). Calcineurin-immunoreactivity is markedly reduced in the striatum (open arrow, a) and is accompanied by strong gliosis (b). This is indicative of incomplete infarction in a rat subjected to 30-minute ischemia. Complete infarct areas surrounded by gliosis are noted in the cortex and striatum (arrows, d) of a rat subjected to 1-hour ischemia.

tion area, although incomplete infarction with selective neuronal loss and gliosis was frequently seen in the dorsolateral part of the striatum. Functionally, all rats subjected to 15- to 30-minute ischemia recovered from paresis of the contralateral limbs within a few hours after restoration of the MCA blood flow. The hemiparesis persisted for more than one day, but disappeared by day 7, in all rats subjected to 1-hour ischemia.

However, reperfusion after ischemia lasting longer than 2 or 3 hours may not be beneficial. In the 3- and 5-hour ischemia groups there was no significant difference in the size of complete infarction area in the striatum and the entire neocortex of the MCA. However, the size and degree of the ischemic lesions varied among rats subjected to 2-hour ischemia.

Ito *et al.* (30) measured the water content in the ischemic brain of Mongolian gerbils after restoration of CBF following temporary ischemia. They found that recirculation after less than 1 hour of ischemia markedly reduced the degree of brain edema, but recirculation performed after more than 3 hours of ischemia greatly exacerbated brain edema. Mamezawa *et al.* (31) investigated ischemic brain damage in rats subjected to MCA occlusion of various duration.

They found that reperfusion after 2 hours of ischemia failed to salvage penumbral tissue. These results suggest that embolic stroke patients with proximal MCA occlusion should receive fibrinolytic therapy within 2-3 hours after the onset. Recirculation later than 3 hours after onset may not only be useless, but may actually be harmful. The poor collateral circulation in the discussed experimental reversible focal ischemia models may result in more severe and extensive ischemic damage than would be expected in larger animals or humans.

Striatonigral involvement following ischemia

The substantia nigra plays a key role in basal ganglia function. The substantia nigra pars compacta sends dopaminergic fibers mainly to the striatum. The substantia nigra pars reticulata (SNr) receives numerous afferent fibers that contain GABA originating from the striatum. As was noted above, a group of medium-sized spiny neurons expressing CaN immunoreactivity were characteristically lost in the dorsolateral part of the striatum. These neurons in this area project to the SNr neurons. Therefore, massive striatal ischemic lesion produced by MCA occlusion lasting longer than 2 hours resulted
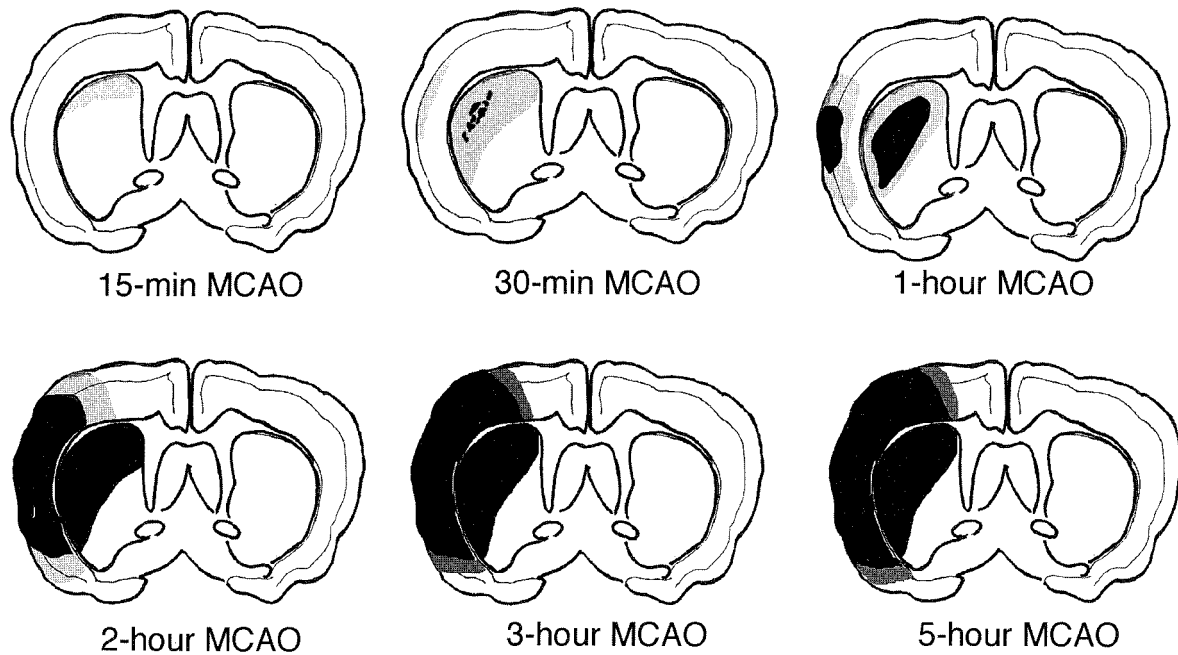
Fig.8. Schematic drawing of the coronal sections of rat brains. Incomplete infarction (shaded area) and complete infarction (black area) resulting from ischemia of varying duration are shown.

in a marked reduction of CaN immunolabeling of the SNr with degeneration of the SNr neurons and gliosis. Notably, the substantia nigra was a distant, non-ischemic area. In humans and in experimental animals, anterograde trans-synaptic degeneration occurs in the substantia nigra following the establishment of striatal or striatopallidal lesions. Tamura et al. (32) demonstrated prolonged hyperemia, hypermetabolism, a decrease in the GABA content, and neuronal loss and gliosis in the substantia nigra following permanent MCA occlusion. The trans-neuronal regression of nigral neurons is thought to result from hyperexcitation of the cells due to a loss of inhibitory GABAergic inputs (deafferentation) (2, 29, 32).

Massive striatal ischemic injury produced by 2-hour MCA occlusion induced the expression of HSP72 (29) and an increase in the synthesis of growth-associated protein-43 in the SNr (9, 12). Neuronal induction of HSP occurs in response to stress, e.g. ischemia/hypoxia, hyperthermia, status epilepticus, and excitotoxin injection. In addition, growth associated protein-43 is synthesized at high level during axonal outgrowth in neural development and during regeneration responses to certain cell injuries (9). These findings suggest that deafferentation of the striatal inputs represents a harmful stress for SNr neurons. The GABAergic agonist mucimor prevents degeneration of SNr neurons following deafferentation (33, 34).

Selective damage of the striatonigral pathway in response to dopamine receptor stimulation produces a motor control abnormality. Contralateral hemiparesis recovered rapidly in rats subjected to brief ischemia and no functional abnormality can be detected without pharmacological stimulation. Rats exhibited ipsiversive rotational behavior elicited by the systemic administration of the dopamine receptor agonist apomorphine in a dose-dependent manner (6). The number of rotations significantly increased as the duration of ischemia increased from 15 to 60 minutes (Fig.9). Even in rats subjected to 15-minutes ischemia, the number of apomorphine-induced ipsiversive rotations increased during the first 3 days, thereafter it gradually decreased until the 30 th day after MCA occlusion (19).

Delayed Selective Neuronal Death

Transient cerebral ischemia leads to delayed and selective degeneration of certain populations of neurons, including hippocampal pyramidal cells (35, 36), striatal medium-sized neurons described above, neocortical neurons, and cerebellar Purkinje cells. The so-called "delayed neuronal death" was first reported and named by Kirino (35) and many studies have been carried out to elucidate the mechanisms underlying selective delayed neuronal death.

Excitotoxic mechanisms triggered by excitatory amino acids such as glutamate are a major factor in
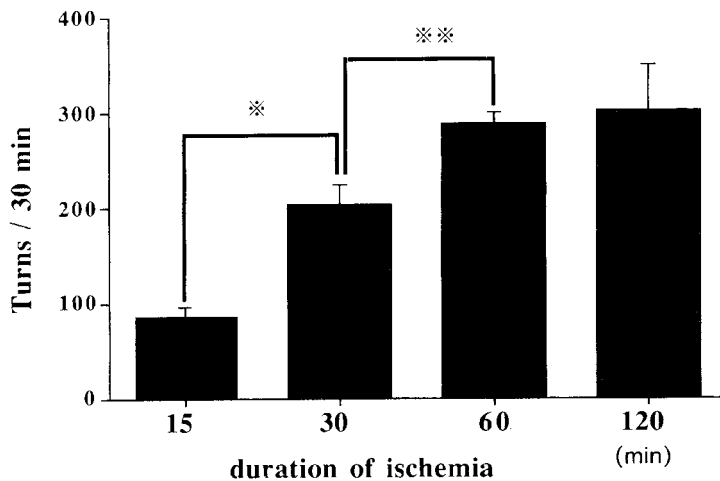
Fig.9. Relationship between apomorphine-induced rotational behavior and duration of ischemia. The total number of ipsilateral turns during a 30-minute period significantly increased with prolongation of ischemia from 15 minutes to 60 minutes. *$p<0.0005$ and **$p<0.005$ in the two-tailed student t-test

ischemic neuronal damage. It has been suggested that glutamate neurotoxicity is mediated by an influx of extracellular Ca^{2+} and the formation of free radicals (37). In ischemia, calcium influx occurs via multiple pathways. The initial entrance of Ca^{2+} through glutamate-receptor gated channel may be augmented by other sources of Ca^{2+} influx or release such as via the Na^+/Ca^{2+} exchanger, membrane leak conductance, or inositol triphosphate receptor-operated channels. The increase of Ca^{2+} is a pivotal event leading to irreversible cell damage during the reperfusion phase and to delayed neuronal death (35, 38, 39, 40). How does an abnormal increase in intracellular Ca^{2+} lead to neuronal death? Why are some neurons more vulnerable than others and why do they die as late as 3 to 4 days after ischemia and reperfusion (35) ? There may be nonphysiological activation of Ca^{2+}-dependent enzymes such as proteases, phospholipases, protein kinases, protein phosphatases, guanyal cyclases, and endonucleases. Proteases such as calpains can break down the neuronal cytoskeleton, resulting in membrane blebing and inhibition of the axonal transport (41). Proteases may exacerbate the deteriorious effects of ATP depletion on the cell skeleton.

Protein kinases such as Protein kinase C or Protein kinase II are enzymes that phosphorylate structural and regulatory proteins, e.g. cell receptors and membrane channels. Calcineurin, as described above, is a neuron-specific $Ca^{2+}/calmodulin$ dependent protein phosphatase, and is abundant in the striatum, hippocampus, substantia nigra and cerebellum. The enzyme may be related to several neuronal actions

by utilizing many functionally important substrates, such as microtubule-associated protein 2, tau factor, tublin, synapsin I and tyrosin hydroxylase (42, 43). Recent slice experiments have shown that CaM kinase II plays a significant role in long-term potentiation (44). Several proteins are common to CaM II and calcineurin as substrates for phosphorylation and dephosphorylation. Both enzyme may be involved complementarily in brain functions by forming the cascade of phosphorylation and dephosphorylation of common substrates (1).

We investigated regional and temporal changes in $Ca^{2+}/calmodulin$-dependent protein kinase II (CaM kinase II) and calcineurin in the hippocampus after transient forebrain ischemia (1). Immunoreactivity and enzyme activity of CaM kinase II decreased in the CA1 region of the hippocampus early (6-12 hours) after ischemia. However, calcineurin was preserved in the CA1 region until 1.5 days post-insult and was then lost with the increase in morphological degeneration of neurons. We hypothesized that an imbalance of $Ca^{2+}/calmodulin$-dependent protein phosphorylation-dephosphorylation may be involved in the observed delayed neuronal death after ischemia. This may also pertain to ischemic injury to the striatum, where, as described above, the medium-sized spiny projection neurons enriched with calcineurin were selectively lost following brief ischemia.

Ischemic tolerance and gene expression

The phenomenon that preceding non-lethal ischemic stress represents a protective effect against CA1 neuronal death of hippocampal neurons following lethal ischemic insults is known as "ischemic tolerance" (45, 46). The expression of a stress gene followed by the production of stress proteins such as HSPs may be involved in the mechanisms of induction of ischemic tolerance (45-47).

The transcription and translation of some groups of genes increases after ischemia. These include immediate early genes, stress genes, and gene encoding growth factors and their receptors. Many immediate early genes such as c-fos, fos-B, c-jun, jun-B, jun-D and Zif/268 are induced by cerebral ischemia (49, 50). The role of gene expression after ischemia is largely unknown at present, but gene expression may reflect processes involved in the death or survival of impaired neurons.

Neurons die after ischemia by necrosis or apoptosis.

Apoptosis is defined as cell death upon characteristic biochemical and morphological changes such as fragmentation of the nucleus and DNA, chromatin condensation at the periphery of the nucleus, formation of apoptotic body, and cell membrane blebing. It is determined by the transcription of specific genes and subsequent protein synthesis (51). Clearly, the predominant process in the ischemic core region is necrotic cell death, while mild cell injury in the penumbral tissue may activate gene expression leading to apoptosis. Mouse over-expression BCL-2, a gene that prevents programmed cell death (53), decreases infarct size after focal cerebral ischemia (54). Several studies demonstrated a DNA ladder or apoptotic body in the hippocampus after transient forebrain ischemia and suggested that delayed neuronal death may actually be apoptosis (55, 56).

TREATMENT OF CEREBRAL ISCHEMIA

Thrombolysis

In the treatment of acute ischemic stroke, immediate recanalization of the occluded artery is the optimal means of restoring blood flow and rescuing the ischemic brain from complete infarction or neuronal death. Intravenous (iv) systemic- or intra-arterial local thrombolytic therapy with urokinase, streptokinase or tissue-plasminogen activator (t-PA) has been used for acute ischemic stroke, however its efficacy has not been established to date. In Europe and the United States, two large trials using the systemic administration of t-PA have been carried out recently. In the European Cooperative Acute Stroke Study (ECASS), multi-center, double-blind placebo-controlled clinical trials were performed to determine the efficacy of iv thrombolysis using t-PA. In that study, patients with acute ischemic stroke were treated within 6 hours of the onset of symptoms. The neurologic recovery at 90 days post-insult was significantly better in t-PA-treated patients. However, the incidence of large parenchymal hemorrhage in that group was also significantly higher than in the other groups (57). The US National Institute of Neurological Disorder and Stroke (NINDS) t-PA Stroke Study Group reported that treatment with iv t-PA within 3 hours of the onset of ischemic stroke improved the clinical outcome (58). Patients treated with t-PA were at least 30% more likely to have no- or minimal disability at 3 months post-insult compared to patients who had received a placebo. However, symptomatic intracerebral hemorrhage occurred in 6.4% of the patients treated with t-PA ; this was the case in only 0.6% of the patients given a placebo (58). Therefore, the US Food and Drug Administration limits the use of systemic t-PA to patients whose treatment can be started within 3 hours of suffering an acute ischemic stroke.

We retrospectively examined the follow-up outcomes of 94 Japanese patients who had undergone intra-arterial local thrombolysis for acute embolic stroke involving major cerebral artery occlusion (59). The clinical outcome was better in patients who had received intra-arterial thrombolysis than in patients who had been treated conservatively. Notably, the outcome was excellent in most of the stroke patients who received thrombolysis therapy within 2 hours after the ictus, followed immediately by complete recanalization. The incidence of poor outcomes increased with time between the insult and recanalization.

Thus, experimental studies described earlier and clinical trials point to the importance of the timing of reperfusion in patients with acute ischemic stroke ; reperfusion should be achieved within 2 or 3 hours of the ictus to spare the ischemic brain from irreversible infarction and from reperfusion injury. As the therapeutic time window for the successful treatment of these patients is very narrow, three critical factors deserve close attention : (1) the establishment of effective brain protection methods during and after the ischemic insult, e.g. hypothermia, and the development of powerful neuroprotective agents, so that the therapeutic time window can be increased and the danger of reperfusion injury can be reduced, (2) the further development of imaging systems to evaluate the extent and degree of ischemia early and precisely so that the indication for thrombolytic therapy can be determined, and (3) the establishment of a dedicated emergency system for stroke patients.

Brain protection

Our current understanding of the pathophysiological mechanisms underlying focal ischemia and of the cellular and molecular mechanisms involved therein suggests that amelioration of ischemic lesions can be obtained by hypothermia and/or agents that reduce calcium influx, prevent cellular acidosis, and suppress the production of free radicals or scavenge those free radicals that have already formed.

In gerbils, mild hypothermia with a brain temperature of 31 and 33℃, prevents delayed neuronal death in the hippocampal CA1 region following

transient forebrain ischemia (60). Similarly, in rats, mild hypothermia reduces the size of the infarct following transient MCA occlusion (61). Just why mild hypothermia appears to be effective in limiting ischemic neuronal damage is not well understood. Each 1℃ reduction in brain temperature reduces the total brain metabolism by approximately 10%. However, even a 30-40% reduction in the cerebral metabolism is thought to be not inadequate for obtaining brain protection. The mechanisms of brain protection by mild hypothermia involve suppression of tissue acidosis (62) and prevention of glutamate release (63), of free radical production (64) and of BBB disruption (65). A pilot study of mild hypothermia in patients with severe cardioembolic stroke revealed a marked reversal of neurological dysfunction in some selected patients (66). However, the indications and the limitations of treatment with hypothermia have not been fully established.

The use of glutamate, calcium antagonists, and free radical scavengers has produced encouraging results in experimental ischemia. However, in human populations, inconsistent and negative results were obtained in several well-controlled studies (67). If the hypothesis proves valid that an imbalance of Ca^{2+}/calmodulin protein phosphorylation-dephophorylation leads to selective delayed neuronal death, calcineurin inhibitors may prevent ischemic neuronal death. The drug FK506, an inhibitor of calcineurin in T-lymphocytes and used as an immunosuppressant, reportedly reduces the severity of ischemic damage in the cortex following MCA occlusion (68). However, further studies are needed to ascertain the appro-priateness of this drug in a clinical setting. At present, there are no safe and effective neuroprotective agents that protect patients with acute ischemic stroke from ischemic damage.

One reason why post-ischemic, systemic administration of neuroprotective agents fails to ameliorate ischemic damage is that occlusion of the cerebral artery prevents the drug from reaching the ischemic tissue in sufficiently high concentration. To overcome this problem, a new approach, retrograde perfusion of the cerebral vein, was developed to deliver cytoprotective agents more efficiently and s electively to ischemic tissues (20, 69, 70). In transient or permanent ischemia models, this approach resulted in a significant reduction

of ischemic damage. While retrograde perfusion of the cerebral vein to achieve targeted drug delivery is still in the experimental stage, clinical application of this method is feasible.

Early diagnosis

The early and accurate diagnosis of stroke has been facilitated by advances in CT scan- and MRI technologies. CT scans are useful for detecting hemorrhagic stroke, i.e. brain hemorrhage or subarachnoid hemorrhage, but they do not depict early ischemic lesions. Conventional MRI techniques are valuable for assessing the extent of the infarct and its location the first 6 to 24 hours after onset. However, within the critical first 3 to 6hours, they do not facilitate assessment of the extent and severity of ischemia. Recent advances in diffusion-weighted MRI technology (DWI), which provides physiological information about the self-diffusion of water,
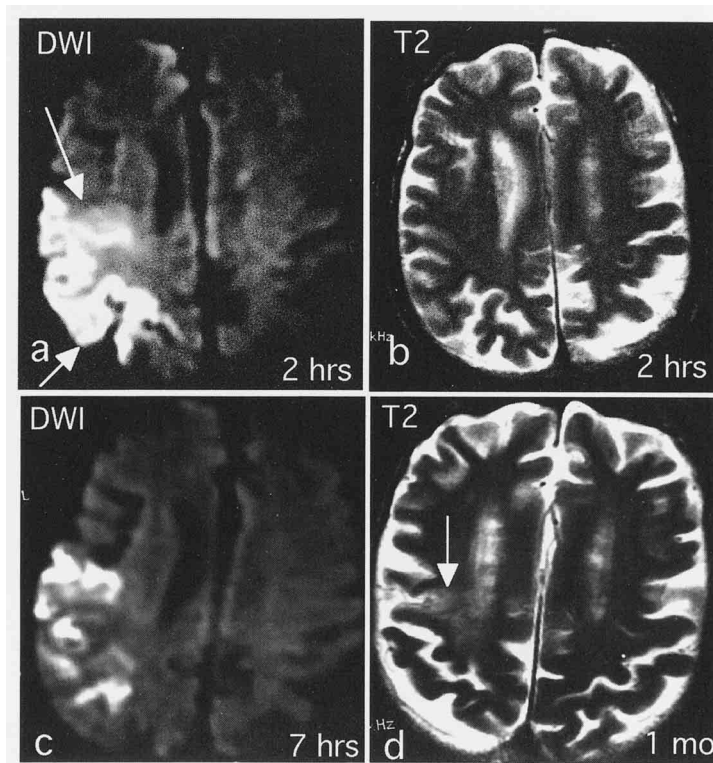

Fig.10. Diffusion weighted image (DWI) and T2-weighted image obtained in an 87-year-old man who experienced the onset of left hemiparesis due to acute embolic occlusion of the right MCA. (a) DWI obtained 2 hours after onset shows a hyperintense lesion in the right motor area and the parietal lobe (arrows). (b) T2-weighted image obtained at the same time shows no definite abnormalities. (c) DWI, obtained after intra-arterial thrombolytic therapy resulting in recanalization of the right MCA and marked improvement of his left hemiparesis, reveals a reduction in the size of the hyperintense area. (d) T2-weighted image obtained one month after the ictus shows a small infarction in the subcortical area (arrow). The hyperintense lesion noted in the first DWI obtained during the superacute phase appears to involve penumbral tissue that was salvaged by the treatment.

make it possible to detect ischemic lesions in the very early phase, i.e. within a few hours after onset (Fig.10). The area of severe ischemic lesions is hyperintense on early DWI because of the shift of water from extracellular to intracellular compartments that results in cytotoxic edema (71). The hyperintense area on DWI in the acute phase has a high probability of later becoming irreversibly infarcted. However, animal experiments indicate that in the very acute phase, the hyperintense area may contain penumbral tissue in which the damage may be reversible (72). At present there is no consensus regarding viability and damage reversibility in the hyperintense area on DWI images in humans. Nevertheless, early examination of patients in the acute phase of ischemic stroke, using DWI, may be essential for identifying patients with an indication for local or systemic thrombolysis. DWI may also be useful for evaluating the prognosis of these patients.

Finally, despite the establishment of sophisticated emergency treatment centers, the concept of "brain attack" is not sufficiently understood by the public. Many patients with focal neurological deficits attributable to ischemic stroke are not seen at a hospital immediately after onset unless their condition manifests as unconsciousness or severe headache. For stroke victims to be seen in an emergency room immediately after onset, a campaign to educate the public is advisable. Also needed is the establishment of a sophisticated emergency system that can deliver the kind of care necessary to reduce mortality and morbidity from stroke. These stroke care units must be staffed by stroke specialists, including neurosurgeons, endovascular surgeons, stroke neurologists, and highly trained support personnel.

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