

Experimental Models and Treatment Trials for Cerebral Infarction

Kazuo ISOZUMI¹⁾ and Yoshio IZUMI²⁾

1) Department of Neurology, Ashikaga Red Cross Hospital

2) Department of Neurology, School of Medicine, Tokai University

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Recent progress in both experimental and clinical studies of cerebral infarction is outlined, and research on delayed neuronal death and ischemic penumbra is described. Development of animal models to study clinical pathophysiology is reviewed, and our focal cerebral ischemia model which has been used for many years is introduced. With elucidation of the pathophysiology of cerebral ischemia, various pharmaceutical agents have appeared recently in the clinical setting and our experimental trials on the treatment of cerebral ischemia are also introduced. From the clinical aspect, practical methods of treatment including antiplatelet therapy are explained. Cerebrovascular dementia and its prevention are also described.

(Key words: cerebral infarction, delayed neuronal death, cerebral ischemia model, neuroprotective agents, cerebrovascular dementia)

INTRODUCTION

In Japan since 1951 the leading cause of death had been cerebrovascular disease, but from 1970 the mortality of cerebrovascular disease started to decline. In 1981 it was surpassed by malignant neoplasms, and in 1985 it was also surpassed by coronary heart disease. Cerebrovascular disease now ranks third as a cause of death in Japan. This change has resulted from prevention and treatment of cerebrovascular disease. However, the number of patients suffering from cerebral infarction is increasing every year. Aging of such patients is another problem and both clinicians and researchers are being required to take measures to cope with this new situation. In this review, cerebral infarction, the clinical importance of which has increased remarkably, is briefly summarized from basic research to clinical practice.

A. Delayed neuronal death

1) What is delayed neuronal death?

There is almost no storage of glucose or oxygen as an energy source in the brain. Therefore, once blood supply to the brain

ceases, brain function stops in a very short time. Actually, if the blood supply completely ceases, the electroencephalogram (EEG) rapidly becomes flat and irreversible damage occurs in the brain within 10 min. Thus, until recently, once cerebral infarction occurred, physicians believed that the ischemic area could never be rescued, and after acute phase life support, the only thing they could do was rehabilitation.

The epoch-making discovery of delayed neuronal death by Kirino replaced this outdated concept. It is known that vulnerability to ischemia of the brain is not uniform. Although glia or vessels are relatively resistant to ischemia, neurons are vulnerable. But this vulnerability differs by region. The third, fifth and sixth layers of the cerebral cortex, the Sommer sector of the hippocampus, and Purkinje cells in the cerebellum are especially vulnerable. In the CA1 subfield of the gerbil hippocampus, an unusual series of changes was noted after ischemia when Mongolian gerbils were subjected to bilateral carotid occlusion for 5 min, the change in the CA1 pyramidal cells was very slow, only becoming apparent by light microscopy 2

days following ischemia. Four days after ischemia, almost all of the pyramidal cells in CA1 were destroyed. These changes in CA1, called "delayed neuronal death," may differ from those thought to be typical of ischemic neuronal damage. It was unlikely that disturbance of the local blood vessels was the cause of these changes (31).

2) Cerebral blood flow (CBF) and metabolism during delayed neuronal death

Delayed neuronal death is a slow degradation of ischemic neurons, and it attracted the attention of many researchers in various studies. Neuropathologically, after 5 min of ischemia no neuronal degeneration was observed within 24 hours. After 48 hours neuronal degeneration began, and within 96 hours (four days) all neurons had completely degenerated. Following release of carotid occlusion, there were two separate openings of the blood-brain barrier. One, occurring shortly after recirculation, was associated with focal hyperemia in the cerebral cortex, hippocampus and basal ganglia; the second opening was observed after several days and was associated with severe neuronal destruction in the CA1 sector. Correlation of quantitative and qualitative regional CBF assays indicated uncoupling between blood flow and glucose metabolism observed in the hippocampus at 10 min after recirculation. EEG recovered after transient ischemia, but this did not guarantee permanent neuronal survival (58).

The levels of high-energy phosphates were restored after 1.5 hours of recirculation, but ATP decreased in the CA1 region at 48 and 96 hours of recirculation. Total adenylates also decreased in the CA1 region at 96 hours, but the normal energy charge in this area indicated that the surviving tissue was metabolically viable. The results clearly indicated that metabolic disturbances persist for long periods of time after ischemia that are compatible with the survival of the animal but that the loss of the CA1 neurons cannot be attributed to a failure in energy metabolism (1).

Since delayed neuronal death progresses gradually, local energy metabolism is maintained and electrical activity of the neurons continues until morphological changes appear. The discovery of delayed neuronal death is extremely important, because it may

be possible to rescue neurons during this surviving period using pharmaceutical or physical methods.

3) Ischemic penumbra

When cerebral infarction occurs, neurons immediately die with irreversible damage due to almost complete cessation of the blood supply in the ischemic core. However, in focal ischemia the tissue in this condition forms a ring around the more densely ischemic center, in which energy failure and ion pump failure develop. In analogy to the half-shaded zone around the center of a complete solar eclipse, this part of the ischemic brain has been termed the "ischemic penumbra" (3). Since this surrounding area retains some blood supply, the brain tissue remains reversible for a fairly long time.

The electrical function of the brain is critically dependent on CBF in the sense that reduction beyond an ischemic threshold of approximately 15 ml/100 gm per minute (approximately 35% of control) leads to complete failure of the electrical function. Also, the massive release of intracellular K^+ is by itself critically dependent on CBF. Thus a dual threshold for neuronal function is present in ischemia; the threshold for release of K^+ is clearly lower than the threshold for complete electrical failure. Further, these findings support the concept of an ischemic penumbra where the neurons remain structurally intact but functionally inactive. Neurons can survive for some time in this state of lethargy (4).

The concept of ischemic penumbra is very easy to understand and is also persuasive. However, the question arises as to how long the state of ischemic penumbra remains reversible. An awake-primate model was developed which permits reversible middle cerebral artery (MCA) occlusion during physiological monitoring. This method eliminates the ischemia-modifying effects of anesthesia, and permits correlation of neurological function with CBF and neuropathology. The model was used to assess the brain's tolerance to focal cerebral ischemia. The MCA was occluded for 15 or 30 minutes, 2 to 3 hours, or permanently. Pathological examination showed microscopic foci of infarction after 15 to 30 minutes of ischemia, moderate to large infarcts after 2 to 3 hours of

ischemia, and in most cases, large infarcts after permanent MCA occlusion. Local CBF appeared to define thresholds for paralysis and infarction. When local flow dropped below about 23 ml/100 gm/min, reversible paralysis occurred, but when local flow fell below 10 to 12 ml/100 gm/min for 2 to 3 hours or below 17 to 18 ml/100 gm/min during permanent occlusion, irreversible local damage was observed (28).

4) Mechanism of delayed neuronal death

The mechanisms by which neurons die after cerebral ischemia and related conditions *in vivo* are unclear, but they are thought to involve voltage-dependent Na^+ channels, glutamate receptors, and nitric oxide (NO) formation because selective inhibition of each provides neuroprotection (57). Evidence from *in vitro* studies suggests that excitotoxic neuronal degeneration can occur by either an acute or delayed mechanism. Studies of the acute mechanism indicate that this process is rapidly triggered by activation of glutamate receptors of either the N-methyl-D-aspartate (NMDA) or non-NMDA subtypes. The non-NMDA agonists include kainate (KA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). The delayed mechanism requires activation of NMDA receptors. Stimulation of non-NMDA receptors does not rapidly trigger delayed neuronal degeneration, or does so only indirectly, via activation of NMDA receptors secondary to glutamate release (51). It appears that Na^+ channels, glutamate receptors, and NO operate interdependently and sequentially to cause neurodegeneration. At the core of the mechanism is a vicious cycle in which NMDA receptor stimulation causes activation of Na^+ channels, leading to glutamate release and further NMDA receptor stimulation. The output of the cycle is persistent production of NO from neuronal sources, and this is responsible for delayed neuronal death (57). To assess the role of NO in cerebral ischemia, the effect of L-arginine, a substrate of NO synthase (NOS), and N^G -nitro-L-arginine (L-NNA), a NOS inhibitor, on neuronal death in the CA1 hippocampal region was investigated. Mongolian gerbils were used in the study. Both carotid arteries were occluded for 4 min to induce forebrain ischemia. L-NNA (100 mg kg^{-1}) significantly ($p < 0.05$)

reduced the occurrence of neuronal death in the lateral CA1 subfield. This result suggests that NO plays an important role in the development of neuronal injury after global ischemia (41). Receptor operated Ca^{2+} channels open after activation of the NMDA receptor, then Ca^{2+} enters the cell and intracellular Ca^{2+} concentration increases. This increase of intracellular Ca^{2+} activates various enzymes including protease, resulting in degradation of structural protein or membrane structure, and consequently in cell death. Cellular damage may be attenuated by the use of agents that block Ca^{2+} channels such as nimodipine, which blocks voltage-sensitive Ca^{2+} channels, and MK-801, which blocks receptor-operated channels. These agents basically owe their beneficial effects to their ability to reduce the accumulation of intracellular Ca^{2+} (14).

5) Apoptosis and nitric oxide

An ischemic insult to the brain evokes cell damage which may progress to cell death. Necrosis exhibits well defined morphological characteristics, and the biochemical and biophysical processes associated with necrosis have been identified. However, another form of cell death exists, apoptosis. Apoptosis plays an important role in the early development of tissues. Cells undergoing apoptosis exhibit very different morphological characteristics and temporal profiles of change from cells undergoing necrosis. Apoptosis has been identified with the internucleosomal fragmentation of DNA. More importantly, apoptosis has been associated with a process of programmed cell death, in which a genetic program is activated which results in the death of the cell (9).

In 1987, one of the endothelium-derived relaxing factors (EDRFs) was found to be NO, a simple and very labile molecule. Since 1990, numerous studies which exclusively employed L-arginine analogues as specific NOS inhibitors have been undertaken to examine the role of NO in the regulation of cerebral circulation. However, some conflicting data have emerged. The few points of consensus among the researchers may be summarized as follows: (1) NO, probably produced in the endothelium, plays an important role in the maintenance of the basal cerebral blood flow, (2) NO is not directly involved in hypoxic vasodilation,

and (3) NO mediates a functional coupling of metabolism and cerebral blood flow in certain types of neural activation. Hypercapnic vasodilation and autoregulatory responses are still the main topics providing conflicting data with substantial areas of controversy (60).

There is increasing evidence that NO, a free radical that can act both as a signaling molecule and a neurotoxin, is involved in the mechanisms of cerebral ischemia. Although early investigations yielded conflicting results, the introduction of more-selective pharmacological tools and the use of molecular approaches for deletion of genes encoding for NOS have provided a better understanding of the role of NO in the mechanisms of ischemic brain damage. NO is protective or destructive depending on the stage of evolution of the ischemic process and on the cellular source of NO. Defining the role of NO in cerebral ischemia provides the rationale for new neuroprotective strategies based on modulation of NO production in the post-ischemic brain (21).

B. Experimental models of cerebral infarction

1) Creation of a model of cerebral ischemia

The best experimental animal model for the study of clinical pathophysiology is thought to be that which mimics clinical conditions as much as possible. There are two categories of experimental cerebral ischemia models: one is the global cerebral ischemia model and the other is the focal cerebral ischemia model. The global cerebral ischemia model is the one in which CBF stops completely for a short time. This model is suitable for the study of delayed neuronal death. However, its pathophysiology is completely different from that of cerebral infarction which we often experience clinically. On the other hand, the focal cerebral ischemia model, in which MCA is occluded, basically resembles the human clinical condition. For this reason various focal cerebral ischemia models have been designed. All of them have advantages and disadvantages with respect to invasiveness, volume of infarction, technique and degree of difficulty. In this review, the models which we have been using will be introduced.

2) Focal cerebral ischemia model of Tamura (59)

A Sprague-Dawley rat is placed in the lateral position, and a curved vertical 2 cm skin incision is made in the midpoint between the left orbit and the external auditory canal. The coronoid process of the mandible and the posterior half of the zygoma are removed. The temporal muscles are retracted downwards and craniectomy is performed using a saline-cooled dental drill at the junction between the medial wall and the roof of the infero-temporal fossa. The dura is opened by means of a fine needle. The MCA runs forwards initially, then turns laterally over the olfactory tract. The arachnoid on either side of the artery is divided by a fine needle, and it is occluded at its cortical branch to the rhinal cortex and the lateral striate arteries. It takes 30 to 40 min from anesthesia to the skin suture. This method is one of the most basic techniques and is widely used. However, the operation is rather invasive and cerebral infarction is not always produced. For this reason we are now using the intraluminal approach.

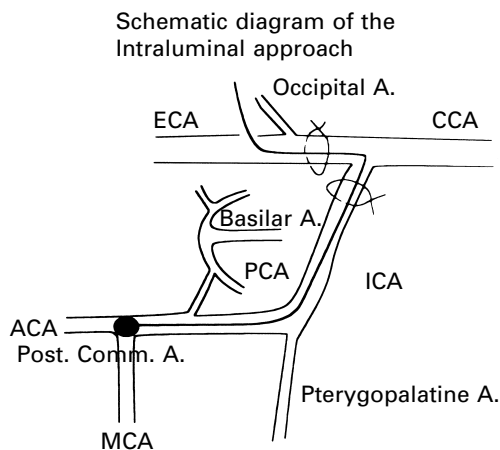


Fig. 1 Schematic diagram of the intraluminal approach with a small thread for MCA occlusion. The nylon suture with its tip rounded by heating occludes the origin of the MCA. The CCA is also ligated. CCA: common carotid artery, ECA: external carotid artery, ICA: internal carotid artery, MCA: middle cerebral artery, ACA: anterior cerebral artery, PCA: posterior cerebral artery, Post. Comm. A.: posterior communicating artery. (Cited from reference (27) with permission.)

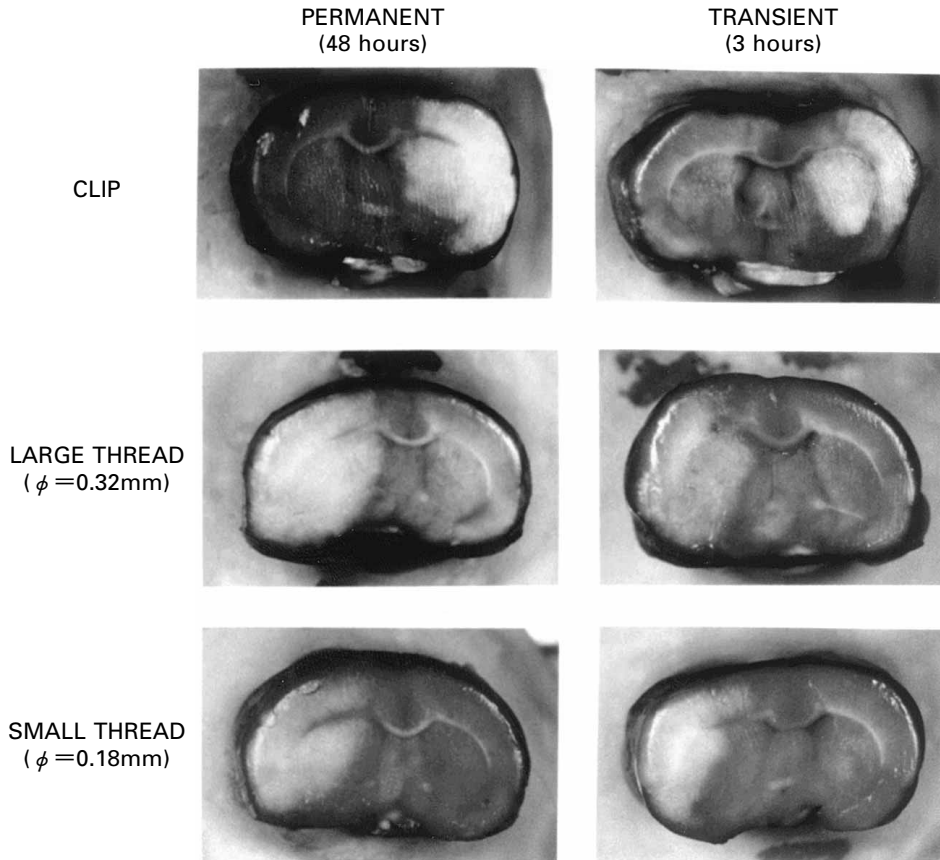


Fig. 2 Coronal sections of rat brains in which transcardiac perfusion was performed with TTC. Colorless region corresponds to the infarct area. (Cited from reference (27) with permission.)

3) Focal cerebral ischemia model prepared with intraluminal approach (27, 34, 40)

Figure 1 is a diagram of the intraluminal approach. A small incision is made on the neck and a nylon intraluminal suture is introduced from the external carotid artery. By way of the internal carotid artery, the MCA is occluded at the bifurcation to the anterior cerebral artery. It takes less than 20 min from anesthesia to the skin suture. There are two kinds of nylon sutures: one is a large diameter 2-0 surgical thread and the other is a small diameter 4-0 thread. This is a simple, relatively non-invasive small-animal model of reversible regional cerebral ischemia.

4) Comparison of two cerebral ischemia models

We have compared the two typical direct and intraluminal approaches to focal cerebral ischemia which appeared elsewhere

(27). Figure 2 shows an actual example of experimental cerebral infarction prepared with these methods. The direct approach by Tamura is shown in the upper part, the intraluminal approach with a large diameter nylon suture is in the middle part, and the intraluminal approach with a small diameter nylon suture is in the lower part. The colorless region indicates the infarct area. In the left column, "permanent" indicates cases with MCA occlusion for 48 hours, while in the right column, "transient" indicates cases with MCA occlusion for only 3 hours following reperfusion by removing the clip or thread. In all groups animals were sacrificed by perfusion with 2% 2, 3, 5-triphenyltetrazolium chloride (TTC) for visualization of the infarct area.

In a comparison of "permanent" and "transient," we found that the infarct area was smaller in the "transient" cases with reperfusion in each group. This reduced

area corresponds to the ischemic penumbra where neurons survive in spite of 3 hour oligemia because of a small residual blood supply after MCA occlusion. This area of ischemic penumbra is a region that could be the target of neuroprotection with a pharmaceutical agent as described later. Comparing the three methods, we found different sizes of infarct area. The largest was in the large diameter thread group, followed by the small diameter thread group, and the clipping group. This difference in infarct area results from the different occlusion position (original or distal) of the MCA and different occluded vessel (MCA only with the clipping method or MCA plus internal and external carotid arteries in the intraluminal approach).

The intraluminal approach has the advantage of a short operating time in comparison with the direct method, and it is possible to operate on many animals in a single day. However, this method has the disadvantage of occasionally inducing subarachnoid hemorrhage because of vessel injury caused by the tip of nylon suture, and the mortality becomes high when the animal is left alive for over 48 hours.

5) Other cerebral ischemia models

The "transorbital approach" of occluding the middle cerebral artery is one of the most commonly applied methods in experimental focal transient ischemia, especially in cats, because no craniectomy is necessary (44, 61). However, this method requires irreversible anesthesia and is not suitable for freely moving animal models. The "microsphere method" has the advantage of an extremely easy operative technique with the injection of 1,000-2,000 microspheres (50 ± 5 microns in diameter) via a tube retrogradely inserted into the external carotid artery in freely moving animals (11). This method creates a microembolism model of cerebral focal ischemia. However, this is an irreversible model and the results may be inconsistent. With the "photothrombotic method" the animals are injected intravenously with a photosensitizing dye, and the distal middle cerebral artery is irradiated with light from an argon laser-activated dye laser to induce thrombotic occlusion. Although special equipment is required, this method has the advantage of allowing the dura to remain

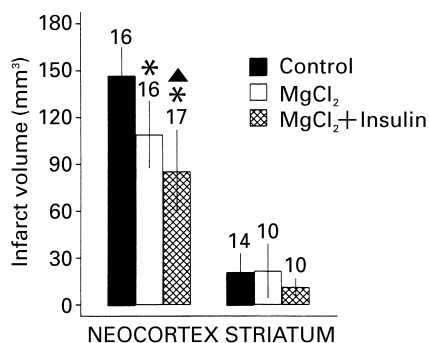
intact, and avoiding mechanical trauma to the brain surface (35).

C. Experimental trials of new treatment

1) Magnesium

Various pharmaceutical agents have been used in an attempt to stop the process resulting in delayed neuronal death. We have applied magnesium (Mg^{2+}) for this purpose. Mg^{2+} is a calcium channel blocker by nature, and also suppresses the release of excitatory amino acids including glutamic acid, dilates cerebral vessels, and suppresses platelet aggregation.

The effects of magnesium, an endogenous inhibitor of calcium entry into neurons, on ischemic brain damage were investigated using a focal cerebral ischemia model in rats (Fig. 3). Infarct volumes were determined by TTC transcardiac perfusion 48 hours after MCA occlusion. The area of ischemic damage was quantified by image analysis in coronal sections taken every 0.5 mm. $MgCl_2$ (1 mmol/kg) was injected intraperitoneally just after MCA occlusion and again 1 hour later. Post-treatment with $MgCl_2$ significantly reduced the cortical



Effects of $MgCl_2$ and $MgCl_2$ +insulin on cortical and striatal infarct volumes in middle cerebral artery (MCA)-occluded rats. $MgCl_2$ group: $MgCl_2$ (1 mmol/kg i.p.) was injected immediately after MCA occlusion and again 60 min later. $MgCl_2$ +insulin group: $MgCl_2$ (1 mmol/kg i.p.)+Actrapid insulin (1 U/kg i.p.) was given immediately after MCA occlusion and $MgCl_2$ (1 mmol/kg i.p.) alone 60 min later. Data are means \pm SD. * $p < 0.05$, significantly different from control group; \blacktriangle $p < 0.05$, significantly different between $MgCl_2$ group and $MgCl_2$ +insulin group.

Fig. 3 Reduction of infarct volume with Mg^{2+} (Cited from reference (26) with permission.)

infarct volume. Compensation for the hyperglycemic effect of MgCl_2 with insulin further reduced the infarct volume in the neocortex. No systemic effects of either treatment could account for the neuroprotection observed (26).

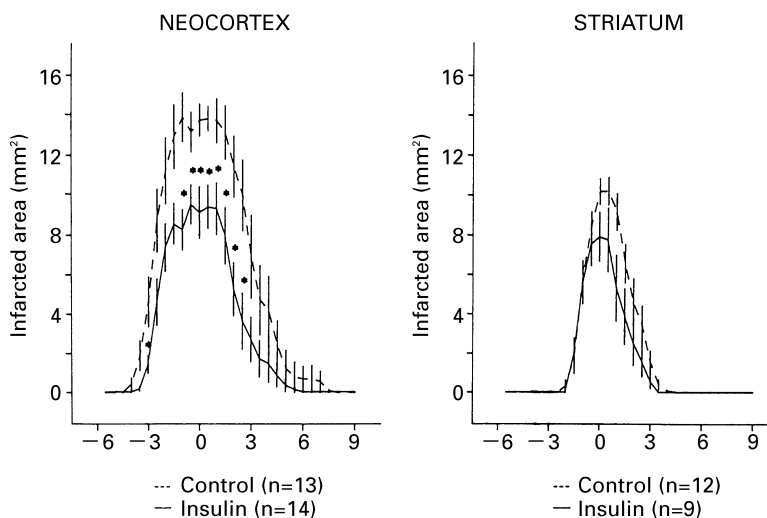
We then investigated the effect of low-dose magnesium in normoglycemia in order to minimize any systemic influence of magnesium. At 48 hours after MCA occlusion, TTC was infused via the heart to stain the healthy tissue. The magnesium-treated group was given MgCl_2 (0.2 mmol/kg) just after MCA occlusion and again at 1.5 and 3 hours later. MgCl_2 treatment did not alter any of the systemic parameters including the blood glucose content as compared with the control group. The total volume of ischemic damage was smaller in the magnesium-treated group than in the control group. MgCl_2 significantly reduced the volume of cortical infarction by 34%, but had no significant effect on the striatum. We concluded that low-dose magnesium exerted a beneficial effect on focal ischemia without any systemic influence (25).

2) Insulin (24)

Hyperglycemia has been considered to

injure the tissue in cerebral ischemia. When excessive glucose is supplied to the ischemic brain tissue as the substrate, cerebral metabolism is accelerated in spite of hypoxia, resulting in acidosis in brain tissue and enlargement of the cerebral infarction. On the other hand, it is known that there are insulin receptors in the brain and insulin itself directly acts on brain tissue.

In order to ascertain whether the neuroprotective effect of insulin on Mg^{2+} administration arose through a blood glucose decrement or the direct effect of insulin on the brain, the influence of insulin on the infarct volume due to MCA occlusion was investigated in rats (Fig. 4). A small dose of insulin (1 unit/kg) was injected i.p. just after MCA occlusion. The infarct areas were measured by planimetry from brains perfused with TTC 48 hours after occlusion. Systemic variables were measured before and at various times after ischemia. The comparison between insulin-treated and control rats provided evidence that insulin significantly reduced the infarct volume due to MCA occlusion. Since insulin minimally and transiently decreased blood glucose, the results suggested that insulin exerts a beneficial effect directly on the central nervous sys-



Effects of intraperitoneal injection of insulin on the area of cortical and striatal damage at various stereotaxic coronal planes relative to the bregma (level 0 mm) in middle cerebral artery (MCA) occluded rats. Insulin group: Actrapid insulin (1 IU/kg i.p.) was given immediately after MCA occlusion. Data are means \pm S.E.M.

Fig. 4 Reduction of infarct volume with insulin. (Cited from reference (24) with permission.)

tem.

According to the literature, insulin causes neuromodulation, acts as a growth factor, inhibits reuptake of GABA, stimulates ATPase, and inhibits platelet aggregation. Through these functions insulin appears to protect ischemic cerebral tissue. Until recently it had been generally believed that once a cerebral vessel was occluded the ischemic lesion could not be rescued and treatment of acute cerebral infarction was supportive therapy only. However, as the pathophysiology of cerebral ischemia was gradually elucidated, experimental or clinical trials of new treatments to minimize infarct volume have been conducted.

3) Other experimental agents

For revascularization of the occluded vessels "t-PA" is the most promising agent and is already approved by FDA for clinical use in the United States (18) although it poses a risk of hemorrhagic events (16, 42). "YM90K" (30), "CGS19755" (54) and "MK-801" (62) are agents for inhibiting the release of excitatory amino acids. "S-312-d" (55) and "nimodipine" (63) are inhibitors of increases in intracellular free calcium. "AVS" (2), "EPC-K1" (6) and "ebselen" (10) are inhibitors of free radical production. N^{ω} -nitro-L-arginine (L-NA), N^{ω} -nitro-L-arginine methyl ester (L-NAME), and N^{ω} -monomethyl-L-arginine (L-NMMA) are NOS inhibitors (52). FK506 (12) and cyclosporin (33) are immunosuppressants. These are examples of promising drugs for clinical use.

D. Practical pharmaceutical treatment of cerebral infarction

1) Onset of cerebral infarction and risk factors

Arteriosclerotic changes of the cerebral vessels are usually present as a background factor in the onset of cerebral thrombosis. This arteriosclerotic change is accelerated by various risk factors such as hypertension, diabetes mellitus, and hyperlipidemia. Cerebral infarction also occurs when other factors induce cerebral thrombosis such as increased platelet function, hyperviscosity of the blood due to elevated hematocrit, and reduction of CBF due to excessive antihypertensive therapy.

Genetic factors play a major role in the

pathogenesis of subarachnoid hemorrhage, and family history of subarachnoid hemorrhage is the strongest independent risk factor for the disease. On the other hand, family history of intracerebral hematoma is not an independent risk factor for hematoma, but it might be a good predictor, which indirectly influences the pathogenesis of intracerebral hematoma via certain hereditary components such as hypertension, and even lifestyle factors such as alcohol consumption. However, genetic factors seem to play a minor part in the pathogenesis of cerebral infarction (32).

Obesity is one of the major risk factors for stroke. Hubert et al., (20), Herman et al. (19), Shinton (56) and Rexrode et al. (49) have shown a positive epidemiological relationship between obesity and stroke. To investigate a possible interrelationship between obesity and the coagulation and fibrinolytic systems, Chan, et al. (8) performed a longitudinal, prospective study, which focused on the evolution of vascular disease risk factors. Hemostatic parameters measured in this study included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, factor VIIIc, factor VIIIc, antithrombin III and plasminogen. Their data demonstrated that all hemostatic parameters show a dose-dependent change with body weight. PT and APTT were shortened with an increase in the body mass index (BMI). Fibrinogen, factor VIIIc, factor VIIIc, plasminogen and antithrombin III increased with higher BMI. This result shows that hemorrheological abnormalities do exist in obese subjects and this thrombophilic phenomenon sheds further light on the study of higher cardiovascular and cerebrovascular mortality and morbidity in the obese.

Metabolic and hemodynamic abnormalities have been separately described in obesity, and weight reduction is known to lead to some improvement in each. Muscelli, et al. (38) simultaneously assessed metabolic and cardiovascular function in normotensive, normotolerant patients with moderate obesity ($BMI = 32.6 \pm 1.1 \text{ kg/m}^2$) before and after weight loss. The obese were insulin resistant, secreted more insulin both in the fasting state and after oral glucose, and had higher resting energy expenditure, systolic and mean blood pressure, stroke volume, and

cardiac output. There was, however, no relationship between the metabolic and hemodynamic abnormalities. After a weight loss of 11 ± 1 kg (approximately 15%), insulin sensitivity improved in proportion to the weight reduction, whereas insulin hypersecretion and high energy expenditure persisted. In contrast, all hemodynamic changes reverted to normal. They concluded that in moderate obesity, the metabolic and cardiovascular abnormalities are largely independent of one another; accordingly, weight loss affects them differentially. Partial weight normalization may provide sufficient cardiovascular and cerebrovascular protection.

The endothelium is the innermost layer of the cerebral vessels. When the endothelium is damaged for some reason, platelet aggregation occurs on the exposed basement membrane of the vessels with exfoliated endothelium (39). At this time, serotonin, ADP, platelet derived growth factor (PDGF), fibroblast growth factor (FGF) are released from the platelets and endothelium. Serotonin contracts vessels and ADP accelerates platelet aggregation, resulting in white thrombus formation. By the action of fibrin, then the thrombus becomes firm and forms what is called red thrombus.

2) Categories of antithrombotic therapy

Judging from the above mechanism, it is clear that there are three categories in antithrombotic therapy. The first is antiplatelet therapy which interferes with adhesion of platelets to injured vessels. The second is anticoagulation therapy using heparin or warfarin which interferes with the formation of red thrombus from white thrombus with a fibrin net. The third is thrombolytic therapy using urokinase or t-PA which directly dissolves the fibrin thrombus. Among these categories, antiplatelet therapy is the most popular method in medical practice.

3) Mechanism of action and usage of aspirin

When some kind of stimulation is applied to the cell, arachidonic acid is released from membrane phospholipid into the cytoplasm. Free arachidonic acid is converted to prostaglandin G_2 (PGG_2) by the action of cyclooxygenase. Aspirin irreversibly blocks the cyclooxygenase. In platelet thromboxan A_2 (TXA_2) is produced from PGG_2 . On the

other hand in the endothelial cells, PGI_2 is produced from PGG_2 by the action of prostacyclin synthase. TXA_2 has strong vascular contraction and platelet aggregation actions, and PGI_2 has a strong potential for vascular dilatation and inhibition of platelet aggregation. These two counteracting substances, TXA_2 and PGI_2 , are biosynthesized originally from the same substrate. However, small amount of aspirin selectively inhibits the synthesis of TXA_2 (13).

A widespread consensus exists in defining a narrow range of recommended daily doses of aspirin, i.e., 75 to 160 mg, for the prevention of myocardial infarction. In contrast, for patients with cerebrovascular disease, a much larger degree of uncertainty persists, with recommendations ranging from 30 to 1,300 mg daily. Until additional information from ongoing trials is available, good clinical practice should dictate the use of the lowest dose of aspirin shown effective in the prevention of stroke, i.e., 75 mg daily (48). In Japan since the commercially available tablet contains 81 mg, this daily dose is the most common at present. This dose rarely causes adverse reactions such as gastrointestinal disorders and bleeding.

4) Mechanism of action of and usage of ticlopidine

The mechanism of action of ticlopidine, another representative antiplatelet agent, is not completely understood. At present it is known that ticlopidine activates adenylate cyclase in the platelets and increases the concentration of cyclic AMP, resulting in inhibition of platelet aggregation (50). The international optimal median daily dose of ticlopidine is 250 mg (5). In Japan since the commercially available tablet contains 100 mg, 200 or 300 mg daily is the most popular regimen at present. This dose occasionally causes adverse reactions including rash and abnormal liver function.

5) Rate of transition from TIA (transient ischemic attack) to cerebral infarction and reduction of stroke incidence by antiplatelet agents

Although the mortality of cerebrovascular disease has markedly decreased, the prevalence or number of patients is increasing in recent years. This means that the severity of stroke is becoming milder and patients are

getting older. Therefore, prevention of recurrence is an important theme in today's treatment strategy of stroke in the chronic stage. The transition rate from TIA to cerebral infarction varies by report, but the mean rate is 30 to 40%. Thus, prevention of TIA becomes prevention of cerebral infarction. Reduction of stroke incidence by antiplatelet agents (aspirin or ticlopidine) is reported to reach 30 to 40%. These antiplatelet agents also prevent other thrombotic diseases including myocardial infarction or thrombosis in limb arteries.

6) Preventive effect on the recurrence of cerebral infarction by anti-platelet agents

The Japanese Investigational Group for Treatment and Prevention of Arteriosclerotic Brain Disease retrospectively studied patients with cerebral infarction, excluding those with cardiogenic cerebral embolism, to determine the effects of antiplatelet agents on the prevention of recurrence and on the incidence of cerebral hemorrhage as an adverse reaction of the drugs. Cerebral infarction recurred in 3.1% of the antiplatelet-treated patients per year, while it occurred in 5.4% per year of the untreated patients. The recurrence rate in those treated with anti-platelet agents was 57.5% of that in those without such agents ($p < 0.01$). Cerebral hemorrhage occurred in 0.5% per year of the antiplatelet-treated patients, while it occurred in 0.6% per year of the untreated patients. These results suggested that antiplatelet agents have preventive effects on the recurrence of cerebral infarction in patients with arteriosclerotic cerebral infarction (43).

When effectiveness was compared between aspirin and ticlopidine, the cumulative recurrence suppression rate was 64% in the aspirin-treated group and 76% in the ticlopidine-treated group. Safety was basically the same between two agents. These results suggested that ticlopidine is superior to aspirin (37).

7) Antiplatelet therapy in the acute stage

Sodium ozagrel, a TXA_2 synthesis inhibitor, is now commonly used in Japan as an antiplatelet agent in the acute stage of cerebral infarction. The clinical usefulness of sodium ozagrel in the acute stage of cerebral thrombosis was investigated in a multicenter double-blind study using a placebo. It

was concluded that sodium ozagrel is effective in the acute stage of cerebral thrombosis, mainly in terms of the improvement of motor paresis without any problems related to safety (45). Sodium ozagrel is considered to decrease platelet aggregation and increase CBF by decreasing TXA_2 , which has a platelet-aggregating and vasoconstricting action (46).

Antiplatelet agents were previously used for prevention of the recurrence of TIA and cerebral infarction. However, since the importance of platelet function in the onset and progression of the acute stage of cerebral infarction has been recognized, they are also being applied in the acute stage of cerebral infarction. Use of aspirin and ticlopidine in the acute stage of cerebral infarction is now under study, and usefulness is expected (7, 23).

8) Heparin

Heparin is a well established anticoagulant. Although heparin has been demonstrated to reduce brain injury after ischemia and reperfusion, its mechanism of action remains unknown. Recent investigations reveal that it can modulate biological processes such as binding to adhesion receptors on endothelial cells and leukocytes. Yanaka et al. (65) hypothesized that heparin's protective effect is closely related to its antileukocyte adherence property. They evaluated the efficacy of sulfated polysaccharides (unfractionated heparin, low-molecular-weight heparin, heparan sulfate, chondroitin sulfate C, and dextran sulfate) on leukocyte accumulation, infarction size, and neurological outcome after transient focal cerebral ischemia in rats subjected to 1 hour of ischemia and 48 hours of reperfusion. The animals receiving unfractionated heparin or dextran sulfate showed a significant reduction in leukocyte accumulation, infarct size, and neurological dysfunction 48 hours after reperfusion ($p < 0.05$) when compared to untreated animals. The animals receiving unfractionated heparin also showed significantly better results than the animals receiving an equivalent anticoagulant dose of low-molecular-weight heparin. These data indicate that heparin's antileukocyte property plays a more important role than its anticoagulant ability in neuronal protection. The relative potency of the sulfat-

ed polysaccharides tested in leukocyte depletion was closely related to their degree of sulfation. Thus, in addition to demonstrating the potential efficacy of heparin as a therapeutic agent for ischemia and reperfusion injury by the prevention of leukocyte accumulation, the results also serve as a basis for studying important cellular and molecular events that contribute to tissue damage.

9) Argatroban

Argatroban, a selective thrombin inhibitor, is the newest clinically available agent for acute cerebral infarction in Japan. The effects of argatroban on microthrombi formation, regional cerebral blood flow (rCBF), infarct areas and neurological deficits were investigated using a rat thrombotic distal middle cerebral artery (dMCA) occlusion model. The rat dMCA was occluded by a platelet-rich thrombus formed after photochemical reaction between rose bengal and green light. One day after dMCA occlusion, the number of microthrombi were counted. In different animals, rCBF was measured using the iodoantipyrine method 1 day after dMCA occlusion. Three days after dMCA occlusion, behavioral tests were performed and the size of the cerebral infarction was determined. In this study, argatroban was administered i.p. by continuous infusion after dMCA occlusion. Argatroban (0.3 mg/h/rat) significantly ($p < 0.05$) decreased the number of microthrombi 1 day after dMCA occlusion. Argatroban (0.1 and 0.3 mg/h/rat) significantly ($p < 0.01$) reversed a decrease in rCBF 1 day after dMCA occlusion. Argatroban (0.3 mg/h/rat) also significantly ($p < 0.01$) reduced the size of the cerebral infarction. Administration of argatroban (0.1 and 0.3 mg/h/rat) resulted in a significant improvement in neurological deficits 3 days after dMCA occlusion ($p < 0.01$ and $p < 0.05$, respectively). It decreased the size of the cerebral infarction and improved neurological deficits in the rat thrombotic dMCA occlusion model. These effects were thought to be due to the improvement of rCBF and to the reduction of secondary thrombus formation after dMCA occlusion (29).

10) Factor Xa inhibitor

The effect of a synthetic low molecular weight factor Xa (FXa) inhibitor, DX9065a,

on thrombosis *in vivo* was examined in a rat animal model using a helium-neon (He-Ne) laser method. DX-9065a administered either intravenously or orally promoted anti-factor Xa activity in a dose dependent manner. Anti Xa activity was maximal immediately after intravenous injection and persisted for approximately 30 minutes. Inhibitory activity was maximal 15 to 30 min after oral administration and persisted for approximately 90 min. Similarly DX-9065a inhibited platelet-rich thrombosis formation in mesenteric arterioles and venules. In these instances inhibition was relatively transient after intravenous injection (10 to 20 minutes), but persisted for more than 3 hours after oral administration. The minimum effective doses of DX-9065a given intravenously and orally were 3.89 mg/kg and 25.9 mg/kg, respectively. The results confirmed that DX-9065a selectively modulates thrombotic mechanisms, and suggested that development of this synthetic FXa antagonist may result in an effective intravenous and oral antithrombotic agent (64).

E. Cerebrovascular dementia and its prevention

1) Classification of cerebrovascular dementia

Approximately 5% of the elderly population (65 or older) is demented in Japan and the number is increasing. Among the demented patients, 80 to 90% suffer from vascular dementia (VD) and senile dementia of the Alzheimer type (SDAT). In Japan the ratio of VD and SDAT is 3:2, i.e., VD is more common than SDAT. Arteriosclerosis of the cerebral vessels has long been mentioned as one of the causes of dementia. However, morbid dementia is not caused directly by cerebrovascular arteriosclerosis which develops with physiological aging. It only arises when organic change occurs in the cerebral parenchyma. Thus, the concept of multi-infarct dementia (MID) has been proposed (17, 53).

However, VD does not only occur due to multiple infarctions in the frontal lobe or thalamus. Figure 5 presents MRI images showing bilateral hyperintense cerebral white matter lesions. These lesions can also be identified in CT scans as a low density area in the deep white matter or periventricular regions although the quality of the

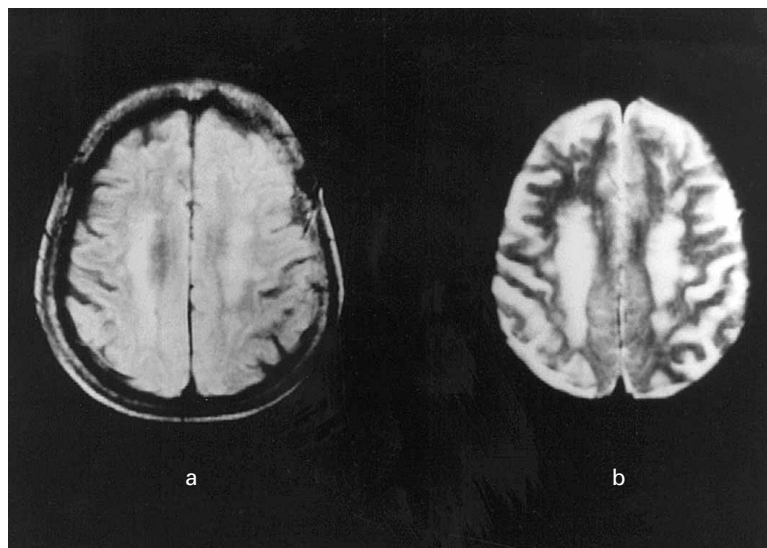


Fig. 5 Hyperintense lesions of cerebral white matter. (a) proton-weighted image, (b) T2-weighted image.

image is inferior. In 1987, Hachinski, et al. named these lesions leuko-araiosis (15), and reported that patients with these lesions have at least mild dementia. On the other hand, progressive vascular leukoencephalopathy, called Binswanger disease, had been described previously (47). Binswanger disease is always associated with severe arteriosclerotic change due to hypertension. Because of this, deep white matter, which is supplied by long penetrating arteries, develops circulation insufficiency, and clinically progressive dementia appears due to ischemic lesions. Recently the term “cerebrovascular dementia of the Binswanger type” has also been used as a diagnosis. These three categories of MID, leuko-araiosis, and Binswanger disease have a common pathology of arteriosclerosis of the penetrating artery. In SDAT primary degeneration occurs in neurons, clinical progression is rapid, and there is almost no means of prevention or treatment. However, in case of VD, prevention or treatment can be achieved to some extent.

2) Circadian blood pressure variation in patients with cerebral infarction

Neurologists have recently shown considerable interest in blood pressure variations. Circadian rhythm, in which blood pressure is high in the daytime and low at night, is present in healthy adults of all ages (22).

However, excessively low nocturnal blood pressure is a major clinical problem. Normally, there is a system of autoregulation in cerebral vessels, by which CBF is kept constant in spite of blood pressure variation. Cerebral vessels dilate when blood pressure decreases and constrict when it increases. However, when this system of autoregulation is impaired due to arteriosclerosis, etc., local CBF decreases when systemic blood pressure decreases, and this could become a trigger of cerebral infarction.

3) Control of hypertension and incidence of cerebral infarction

Which risk factor is most relevant to VD is not necessarily clear. However, Meyer et al. reported that among hypertensive patients with MID, improved cognition and clinical course were correlated with control of systolic blood pressure within upper limits of normal (135 to 150 mmHg), but if systolic blood pressure was reduced below this level, patients with MID deteriorated (36). The optimum range of blood pressure for the aged is an important theme for the future. Physicians should be concerned about excessive falls of blood pressure at night in elderly patients who have underlying arteriosclerosis of cerebral vessels, and the prevention of ischemic change in the brain due to reduction of cerebral perfusion pressure.

CONCLUDING REMARK

Cerebral infarction has been discussed over a wide range from basic research on the pathophysiology to practical pharmaceutical treatment and prevention. In addition, cerebrovascular dementia which will become important in the near future was also outlined.

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