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REVIEW ARTICLE

ANIMAL MODELS OF CEREBRAL ISCHEMIA FOR EVALUATION OF DRUGS

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Abstract : Stroke is a major cause of death and disability worldwide. The resulting burden on the society continues to grow, with increase in the incidence of stroke. Brain attack is a term introduced to describe the acute presentation of stroke, which emphasizes the need for urgent action to remedy the situation. Though a large number of therapeutic agents like thrombolytics, NMDA receptor antagonists, calcium channel blockers and antioxidants, have been used or being evaluated, there remains a large gap between the benefits by these agents and properties an ideal drug for stroke should offer. In recent years much attention is being paid towards the exploration of herbal preparation, antioxidant agents and combination therapies including COX-2 inhibitors in experimental model of stroke. For better evaluation of the drugs and enhancement of their predictability from animal experimentation to clinical settings, it has been realized that the selection of animal models, the parameters to be evaluated should be critically assessed. Focal and global cerebral ischemia represents diseases that are common in the human population. Understanding the mechanisms of injury and neuroprotection in these diseases is important to learn new target sites to treat ischemia. There are many animal models available to investigate injury mechanisms and neuroprotective strategies. In this article we attempted to summarize commonly explored animal models of focal and global cerebral ischemia and evaluate their advantages and limitations.

Key words : cerebral ischemia animal models

INTRODUCTION

Stroke is the second largest cause of mortality worldwide and is surpassesed only by that of heart disease (1). Brain attack is a term increasingly being used to describe the acute presentation of stroke, and to emphasize the need for urgent remedy (2). Except tissue-type plasminogen activator (t-PA), currently no effective therapy exists for the management of acute stroke. Understanding the role of various extrinsic

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and intrinsic pathogenic factors of ischemic damage particularly the biochemical cascade and neurological changes remain unclear and represent a prime objective of ongoing stroke research (3).

Stroke is estimated to be responsible for 9.5% of all deaths. In the United States more than 700,000 new cases of strokes occur annually. Stroke is also the leading cause of disability with an estimated 4 million stroke survivors living with stroke related deficits in US alone. More than 70% of stroke survivors remain vocationally impaired, more than 30 % require help for activities of daily living, and more than 20% walk only with assistance (4, 5).

India is ranked among the countries where the information on stroke is minimal and therefore there is a need to initiate steps to collect data on morbidity and mortality due to stroke in the country as first step towards control measures. The prevalence of stroke is estimated as 203 per 100,000 populations above 20 years amounting to a total estimate of about 1 million cases. The male to female ratio is 1.7. Around 12% of all strokes occur in population below 40 years. The total number of deaths due to stroke is about 102,000, which represents 1.2% of total deaths in the country (6).

The devastating disability after stroke poses a major socio-economic challenge in rehabilitation of stroke survivors. In developed countries like United States alone, health care expenses and lost productivity after stroke was nearly US$ 20 billion (7). Similarly, it is logical to conclude that the cost of treatment poses a major economic burden on health care resources in rehabilitation of the stroke victims in India.

PATHOPHYSIOLOGY

Cerebral ischemia leads to a cascade of pathophysiological processes, which contribute to ischemic cell damage. Among the pathophysiological changes that are postulated to occur as a response to stroke are free radical production, excitotoxicity, disruption of sodium and calcium influx, enzymatic changes, stimulation of the inflammatory process, endothelin (ET) release, activation of platelets and leukocytes, delayed coagulation and endothelial dysfunction. All of these pathophysiological reactions individually and/or collectively contribute to the brain injury following the onset of stroke (8–10) (Fig 1).

The basic cascade of cerebral ischemia

Cerebral ischemia results from decreased or interrupted blood supply leading to reduced availability of glucose and oxygen in the territory of affected vascular bed and thereby causing cellular energy crisis. Shortage of energy interrupts the activity of cellular ions pumps and disturbs the ionic gradient homeostasis. This results in increased release of neurotransmitters particularly glutamate from the presynaptic terminals, within 1–2 min after the onset of ischemia. A massive release of excitatory amino acid activates the glutamate receptors, leading to membrane depolarization and accumulation of free cytosolic calcium by cellular influx at the postsynaptic site (11). The accumulation of

calcium plays a key role in the propagation of the irreversible neuronal damage by activation of series of neurotoxic events such as lipid peroxidation, free radical generation, activation of proteolytic enzymes and pathological gene activation leading to the formation of zone of infarction in the area where blood supply has been interrupted (Fig 1).

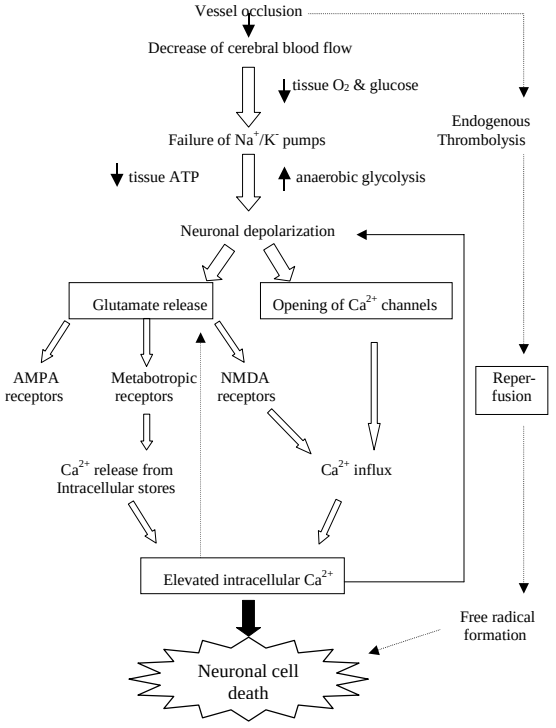

Fig. 1: Diagrammatic representation of events that may lead to cell death after the onset of cerebral ischemia.

STROKE ETIOLOGY

Stroke occurs when blood flow to the brain is interrupted by either a blocked or burst artery, resulting in a sudden decrease in the blood flow to an area of the brain, depriving brain cells of oxygen and other nutrients. Ischemia develops within minutes, forming two zones around the site of thrombosis or embolism (12). Brain cells at the center of ischemic region where the cerebral circulation is completely arrested, irreversible cell damage occurs in several minutes. However, cells in the area surrounding the center, the ischemia is incomplete because of the presence of perfusion from collateral vessels. This region is called penumbra (13), where reduced blood flow falls to the level below the threshold for electrical failure and above the threshold for energy failure. Restoration of cerebral blood flow, even to a sub-optimal level, provides an opportunity for those brain cells to recover and regain functionality (14) (Fig 2).

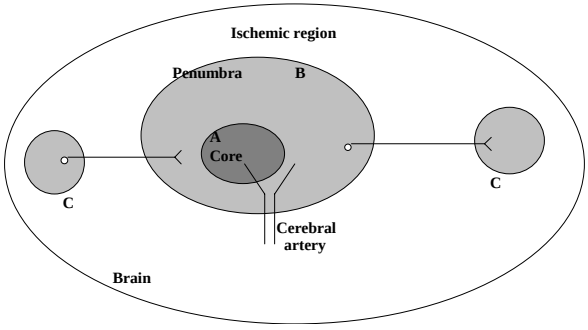

Fig. 2: Diagrammatic representation of (A) ischemic core region (B) penumbra area surrounding ischemic core (C) area connected to ischemic region.

THE CURRENT THERAPY OF STROKE

Present therapeutic approaches to stroke are centered on two distinct approaches, one primarily vascular (reperfusion) and the other primarily neuronal (neuroprotection). Reperfusion has conventionally considered as a very attractive approach since perfusion failure underlying that underlies all ischemic strokes, and the relief of the

initiating event should prevent all consequences of neuronal ischemia. Thrombolytics such as recombinant plasminogen activator (rt- PA) has shown promising results in clinical trials and was approved by FDA in 1996 for limited patients under specific guidelines. However, antithrombotic and antiplatelet drugs have not demonstrated efficacy as acute therapy, although the early use of aspirin appears to produce a reduction in early stroke recurrence.

A wide variety of drugs, which interfere at various points in the ischemic cascade, so-called neuroprotective agents have also been studied but with, mixed successes. Of these, antagonists of voltage gated calcium channels; excitatory amino acid receptor antagonists and scavenger of free radicals have been most extensively studied. Despite proving effective in animal models of acute ischemic stroke these drugs have largely failed to fulfill their promise in clinical trials and currently there is no neuroprotective drug, which is being used clinically. Therefore, presently only rt-PA is the drug used clinically for treatment of stroke. The broad three approaches for stroke are shown in figure 3.

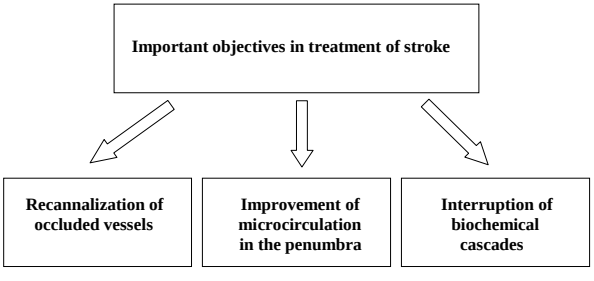

Fig. 3: Different approaches in stroke.

LIMITATIONS OF CURRENT THERAPY

A large number of drugs with varying mechanism of actions have been studied in humans, but as yet, there is no medical treatment approved for the treatment of stroke beyond tissue plasminogen activator, a thrombolytic agent restricted to administration within 3 hours after stroke. Only few patients (5% to 8%) qualify for treatment within this short onset. Aspirin, other antiplatelet, and anticoagulants are used as preventative therapy. None of the currently available neuroprotective agents have demonstrated unequivocal efficacy when administered after stroke in humans although a number of compounds are in development stage. The failure of mechanism-based approach (neuroprotection as well as thrombolytic agents) in stroke treatment have raised lot many queries, which needs to be addressed.

Thrombolytic therapy may lead to a decrease in neuronal damage and improve recovery after acute ischemic stroke. With the higher dose there is reperfusion damage and hemorrhagic transformation are suggested to be responsible for the majority of complications. Lower dose of thrombolytic agent may decrease the risk factor but also decrease its effectiveness. Preliminary evidence suggests that combination of a neuroprotective agent may have additive effect. Further studies on the combination of these agents and dose relationship are however required to test if lowered doses may have beneficial effects.

WHAT WILL BE THE IDEAL DRUG FOR STROKE?

Various agents have been developed for the treatment of stroke, however only a few drugs have shown limited efficacy in the clinical trials. Recently we have shown the protective effect of antioxidant agents like melatonin (15), adenosine (16), resveratrol (17), α-tocopherol (18) and also the combination of antioxidant like melatonin and meloxicam (19) in experimental model of middle cerebral artery occlusion in rats. However an agent, which can improve the blood flow and also beneficial effect on the biochemical cascades will be more useful in the treatment of cerebral ischemia. Also the agents, which relieve the spasm of the neutrient artery and having an antioxidant property, could be an additional important approach to prevent the further damage immediately after the spasm of the affected artery in the brain.

The rational for combination therapy is based on the increasing knowledge of the pathophysiological mechanisms of the ischemic brain damage. Each agent affects only one of the several mechanisms in the ischemic cascade whereas the combination therapy will affect various points in the cascade (20, 21).

With the associated side effects of the western medicine, traditional medicines are gaining lot of importance and are now being studied to find the scientific basis of their therapeutic actions. However Indian herbals that can increase the blood flow and have antioxidant property and also inhibit excitotoxic activity in the brain may have a potential against this disorder.

EXPERIMENTAL MODELS OF CEREBRAL ISCHEMIA

Innumerable invitro and invivo models of cerebral ischemia have been described over the years. The invitro models include cultured neurons with or without synaptic formation, glia and cultured brain slice. However these models can only indicate the level of cytotoxicity of the therapy.

Because living experimental systems (animals) that contain whole elements, neurons, glia, vasculature and cerebrospinal fluid are more close to the human system; therefore significant efforts have been made by the neuroscientist to develop models that mimic closely the physiological and pathophysiological changes associated with stroke.

WHAT WE LOOK FOR IN AN EXPERIMENTAL MODEL OF STROKE

Reflecting the importance of animal ischemia models as a experimental counterparts of stroke in humans, the important conditions that the model should fulfill are given below.

- The production of ischemia should be constant, reliable and reproducible.
- High percentage of infarcts can be produced with the predictable average size.
- Neurological and pathological evaluation should not be too complicated.
- Physiological variations are controllable without much difficulty.

- No barbiturates to be used at the time of arterial occlusion.
- Neuroimaging studies such as autoradiography, MRI etc can be performed economically and easily.
- Long term follow up is possible and resistant to infection
- Economical and easy availability of animals of same species.

CLASSIFICATION OF ANIMAL ISCHEMIA MODELS

A good in vivo animal model of stroke must reproduce the etiology, anatomical, functional and metabolic consequences of human pathology and must also permit the study of anti-ischemic drugs in conditions pertinent to the clinical therapeutics. The major models of stroke available for screening of drugs can be broadly classified into three subgroups as global ischemia, focal ischemia and forebrain ischemia (Fig 4).

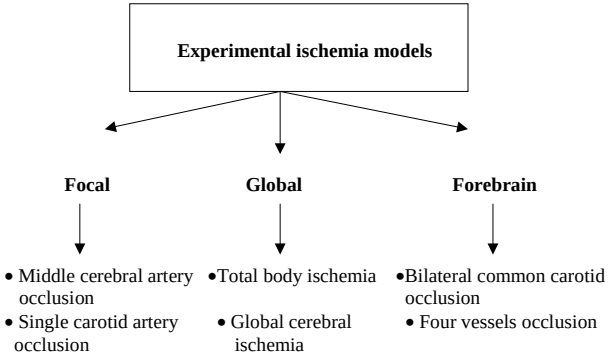

Fig. 4: Diagram representing the major models of cerebral ischemia available for screening of drugs.

MODELS OF GLOBAL ISCHEMIA

In the global ischemia models there is total interruption of blood supply to the brain leading to cerebral necrosis. It can be induced broadly by two ways either by total body ischemia or by global cerebral ischemia (Fig 5).

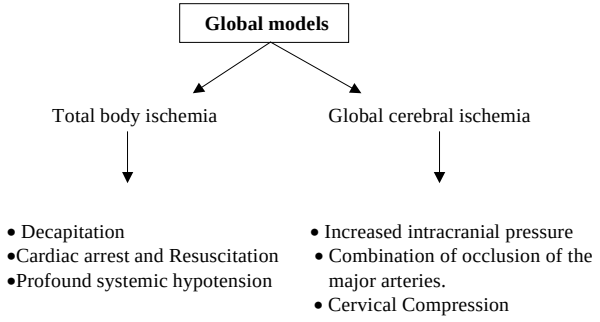

Fig. 5: Diagram represent the classification of models of global ischemia.

I. Total body ischemia: Total body ischemia can be induced by the following methods.

a. Decapitation and cardiac arrest without resuscitation

Decapitation of animals is the easiest way to achieve global cerebral ischemia. However the major disadvantage of this model is that studies involving post ischemic recirculation is impossible. The use of this model is limited for studying morphological and biochemical changes during early stages of ischemia. Studies also reported the involvement of ion channels in anoxic depolarization in a very early phase of global ischemia in a rat model of cardiac arrest induced by intravenous injection of magnesium chloride.

b. Cardiac arrest and resuscitation

This model can be carried out in various animal species like monkeys, dogs, cats and rats to induce complete global ischemia. Cardiac arrest can be induced by injection of potassium chloride; electric shock and the mechanical obstruction of the ascending aorta whereas resuscitation is carried out using artificial ventilation, closed chest cardiac massage, and electrical defibrillation (22–24). It has advantages in the term that it resembles to the clinical setting (mimics cardiac arrest in man), however the exact ischemic duration limits the adoption of this model to specific experiments. As if the time of cardiac arrest increases from 8 min the survival rate becomes low and also the attempts of resuscitation becomes difficult because of brainstem ischemia and involves intensive care including use of various drugs which can interfere with the studies (25).

c. Profound systemic hypotension

It can be induced by pharmacological agents, results in incomplete but almost global cerebral ischemia. The main advantage of this model is the ease of resuscitation of the animal, however possible variability in severity and regional distribution of cerebral ischemia limits the use of this model. Various studies were done to overcome these drawbacks by combining systemic hypotension and hypoxia, using a standard of five minute of isoelectric electroencephalography, observed a protective effect of a rapid acting barbiturate on ischemic damage.

II. Global cerebral ischemia: Global cerebral ischemia can be induced by the following methods:

a. Increased intracranial pressure

Cerebral perfusion pressure is the difference between the arterial blood pressure and the intracranial pressure. When the intracranial pressure is increased more than the systolic arterial pressure it results in a zero cerebral blood flow. It can be carried out in dogs, rabbits and rats. Advantage of this model is the ease of the control of the brain temperature by changing the temperature of the fluid injected. Although in this model, global cerebral ischemia can easily be achieved the primary pathological process is not ischemia but intracranial hypertension (26, 27).

b. Combination of occlusion of the major arteries

It can be performed in the large animals like dogs, cats and rabbits by the surgical occlusion of the major arteries at a time. It can be combination if both common carotid, vertebral, costocervical, mammary, omocervical and axillary artery via left thoracotomy or clamping of the descending aorta, brachiocephalic and left subclavian artery. The major limitation of this model is the involvement of extended intrathoracic or intracranial surgery. Precautions have to be taken while performing the surgery as this can result in myocardial injury.

c. Cervical compression

Occlusion of cervical blood vessels by tourniquet when combined with systemic hypotension results in ischemic damage in the brain. It seems difficult to completely occlude the vertebral and anterior spinal arteries from anatomical point. Cervical compression model should be considered not

a global cerebral ischemia but a forebrain ischemia. However the effect of compression of the various cervical nerves and ganglia on cerebral ischemic injury and postischemic cerebral circulation is unknown.

MODELS OF FOREBRAIN ISCHEMIA

Models of forebrain ischemia were first reported in the early 1980's and have been extensively used for the studies on various aspects of cerebral ischemia. Since the cerebral ischemia in these models is not completely global but restricted to the forebrain, these models do not accurately represent cerebral ischemia encountered in clinical settings in which bilateral forebrain ischemia rarely occurs (Fig 6).

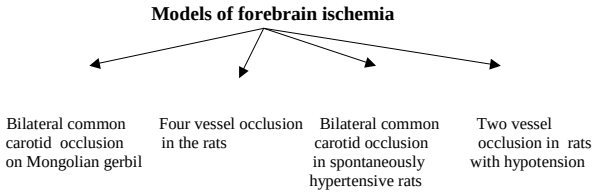

Fig. 6: Diagram represent the classification of models of forebrain ischemia.

a. Bilateral common carotid occlusion on Mongolian gerbil

It is the most popular model because of the relative ease of the surgical technique involved. The absence of posterior communicating arteries in the gerbils, which are necessary to complete the circle if Willis enables forebrain ischemia to be induced by only occluding both common carotid arteries (28). The major limitation of this model is that interanimal variability in the severity of ischemia is very high. Moreover gerbils are known to have a high percentage of post ischemic seizures especially when the duration of ischemia is longer than 15 min. Moreover small size of the gerbils lends difficulties in monitoring of physiological variables.

b. Four-vessel occlusion in the rats

This is also a widely used model since reported by Pulsinelli and Brierley, 1979 (29). It involves difficult surgical procedure. There is a two-stage surgery. On the first experimental day occlusion devices are placed around both common carotid arteries following surgical dissection and then both vertebral arteries are electrocoagulated at the first cervical level following occipital-nuchal dissection. Twenty-four hours later both common carotid arteries are occluded in the awaked rats (30). However a drawback may be inconsistency of the ischemia owing to the difficulty in conforming the complete electro cauterization of thevertebral arteries and the existence of collateral pathways mainly from the anterior spinal artery.

c. Bilateral common carotid artery occlusion in spontaneously hypertensive rats

Bilateral common carotid occlusion does not consistently produce ischemic changes in the brain of normal rats. However bilateral common carotid occlusion in spontaneously hypertensive rats is known to produce consistent ischemic change in the brain. The carotid artery occlusion in this model is permanent and the histopathological changes are seen in the forebrain area. Moreover this model is very important since hypertension is one of the

major risk factors of cerebral ischemia and the occurrence of severe vascular changes secondary to hypertension may be operative in cerebrovascular diseases. The major limitation of this model is the high cost and mortality rate is very high within 24 hours of occlusion (31). Development of hypertension significantly varies depending on the age and sex. Therefore, experimental studies must be performed carefully.

d. Two-vessel occlusion in rats with hypotension

It is a simple method and permits rapid screening. It produces delayed and selective neuronal death. Monitoring of physiological variables can be carried out easily. It has been reported that there was no changes in the energy state in the tissue following ligation of the carotid arteries only, when combined with systemic hypotension there was a severe changes in the energy states. In this model when ischemia was longer, damage was also seen in the caudoputamen and pars reticulata of the substantia nigra. There is deleterious effect of hyperglycemia on ischemic brain injury using this model. The major disadvantage of this model is the alteration in the physiological variables as a result of hypotension.

MODELS OF FOCAL ISCHEMIA

Focal ischemia is the most commonly encountered type of stroke in humans. Among of many causes of cerebral ischemia, occlusion of a single trunk artery particularly the internal carotid or middle cerebral artery is the most frequent. It is different from the global ischemia that it produces a heterogeneous pathology, which includes a necrotic core and a penumbra. In the necrotic core, the area at the center of the ischemic territory, there is pan necrosis in which both neurons and glia die. In the penumbra neurons are at the risk of dying and is the area of therapeutic intervention. Focal ischemia can be divided as permanent or transient.

In the permanent focal ischemia model dense region of ischemic damage (core) is formed and the degenerative changes are seen spread out in a large area from this region. However clinically it is very rare that the cessation of the blood flow occurs permanent in the brain region because in the majority of stroke cases thrombus disintegration and endogenous thromobolysis occurs and the damage is the result of both ischemia and the consequent of reperfusion. Therefore reversible model of focal ischemia is more clinically relevant than the permanent model. Methods for inducing focal ischemia are given below.

I. Photochemical occlusion with craniotomy without dural opening (Photothrombosis model)

This is a permanent model of focal ischemia. After exposing the artery to be occluded a photosensitizing dye, Rose Bengal is administered intravenously with simultaneous laser irradiation (argon laser-activated dye laser operating at 562nm). Advantage of this model is that it is relatively noninvasive (32). However it is not clear whether the thrombus permanently occludes the artery and moreover penumbral region is not seen in this model that have a closer clinical correlate.

Other models in the category include carbon microsphere injection into the

internal carotid artery, injection of platelet aggregates into the common carotid, injection of small blood clots into common carotid (33).

II. Occlusion of middle cerebral artery

Eighty percent of strokes occur in the territory of the anterior circulation and the majority of these effects the territory of the middle cerebral artery (MCA) (34). Therefore this model are the most widely used to study focal ischemia. There are three different types of MCA occlusion.

a. Mechanical or electrical arterial occlusion with craniotomy and dural opening

Proximal MCA occlusion: In this method the coronoid process of the mandible and zygoma is removed and a burr hole opened lateral to the foramen ovale. MCA is identified through the burr hole and occluded at the proximal end. The advantage of the above model is that arterial occlusion is confirmed directly through operative microscope, both temporary and permanent MCA occlusion are possible, mortality rate is low, and any kind of monitoring system is applicable because the rat is fixed on a stereotaxic frame. The disadvantage of the above model are exposure of the brain to air during the craniotomy may alter intracranial pressure and blood brain barrier permeability, clipping or cauterization of the proximal MCA may cause damage to the autonomic nerves around the MCA and auto regulation of CBF may be lost, requires surgical skillfulness under an operating microscope. Moreover occlusion of only the origin of the MCA does not produce consistent infarcts (35).

Distal MCA occlusion with bilateral common carotid arteries occlusion: This model has produced consistent cerebral infarction with relatively non-invasive surgery. The right distal middle cerebral artery and right common carotid artery are ligated and the left common carotid artery is clipped temporarily. A small contralateral infarction is encountered occasionally. This model can be used as a transient focal ischemia model.

b. Intraluminal arterial occlusion without craniotomy

Focal ischemia is the most commonly encountered type of stroke in humans (36). However clinically it is very rare that the cessation of the blood flow occurs permanent in the brain region because in the majority of stroke cases thrombus disintegration and endogenous thrombolysis occurs. The neuronal damage is the result of both ischemia and the consequent of reperfusion.

Koizumi et al 1986 (37) developed a model of MCA occlusion without craniotomy. After then several modifications have been proposed. In this model a midline incision was made and the right common carotid artery, external carotid artery and internal carotid artery were exposed. A 4.0 monofilament nylon thread (Ethicon, Johnson & Johnson) with its tip rounded by heating quickly by bringing it near a flame was used to occlude the middle cerebral artery. The filament was advanced from the external carotid artery into the lumen of the internal carotid artery until a resistance was felt which ensured the occlusion of the origin of middle cerebral artery. The nylon filament was allowed to remain in the place for 2 h after which it

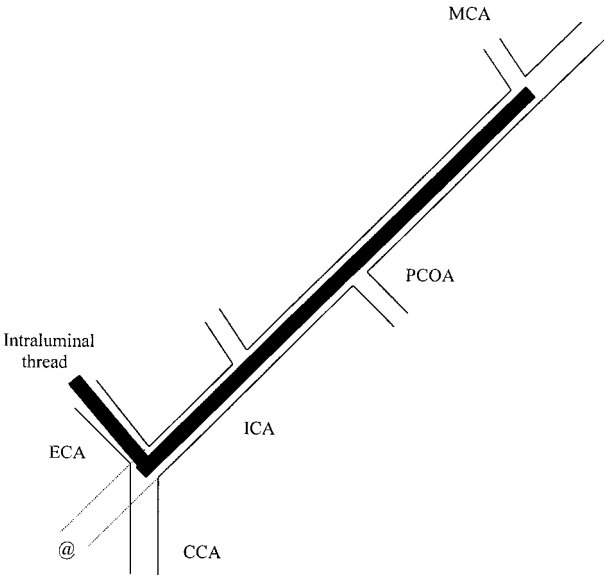

Fig. 7 : Diagram depicts the branches of common carotid artery and the path followed by the intraluminal thread to block the origin of middle cerebral artery (CCA: common carotid artery; ECA: external carotid artery; ICA: internal carotid artery; MCA: middle cerebral artery; PCOA: posterior communicating artery; @: ECA pulled down after making the nick for easy insertion of the thread).

was gently retracted so as to allow the reperfusion of the ischemic region to induce focal ischemia transiently (Fig 7).

The major advantages of this model are firstly the method is simple and secondly the MCA can be occluded and reperfused without craniotomy and for these reasons this model has achieved wide popularity (37). However the major drawback of this model is high mortality in the permanent ischemic models. Therefore reversible model of focal ischemia (middle cerebral artery occlusion model) is clinically more relevant than the permanent occlusion model. In our laboratory we have used this model to evaluate the various neuroprotective agents like melatonin, adenosine, resveratrol, vitamin E etc (15–19).

NEW STROKE MODELS

Transgenic mice models

Delayed neuronal death is also a major cause of the high morbidity and mortality associated with stroke (focal cerebral ischemia). Attention has recently been shifting to mice. This is because transgenic mouse can be developed relatively easy. Using these models one can investigate the efficacy of anti-apoptotic proteins in preventing delayed neuronal death after focal cerebral ischemia in transgenic mice. The major problems that are being faced in the development of transgenic mice models for cerebral ischemia is the variability in the vascular territories in the different species of mice used to generate transgenic mice.

Neonatal hypoxia model

Cerebral hypoxia-ischemia remains a major contributor to perinatal morbidity and mortality. It is estimated that between 0.2 to 0.4% of full-term infants and up to 60% of premature infants experience asphyxiation at or before birth. An established model of neonatal hypoxia/ischemia is being used recently. Ligation of the right common carotid artery and treatment with 8% oxygen produces ipsilateral brain damage (38). Oxygen sensitive genes, apoptosis, and neurological evaluations can investigated using this model.

SPECIAL CARE REQUIRED WHILE PERFORMING THE EXPERIMENTS IN STROKE MODELS

Many factors affecting the extent of ischemic brain damage have been reported

in clinical conditions. Therefore following considerations should be taken care of while carrying out the experiments.

Species: Several animal species are candidate host for both global and focal ischemia models i.e. subhuman primates, cats, dogs, rabbits, rats, gerbils and mice. The focal ischemia models in subhuman primates closely resembles human stroke and extremely valuable for the study of ischemic stroke. However it is currently quiet difficult to use them as a host for experimental models because of economical and ethical reasons. Among the rodents rats are most widely used. This is because pure strains are available, detail anatomical studies have been established, they are inexpensive, easy to handle and intracranial circulation is similar to that of humans. Infarction can be induced consistently and reliably and a large amount of neurochemical data is available on rats. Though the rat models are extensively used however when it comes to introducing a newly developed therapy whose efficacy has been confirmed in the rat model other ischemia models in mid size animals particularly the cat still holds validity.

Age: Stroke usually occurs in humans older than 50-80 years and this corresponds to the ages of 2-3 years in rats. Ischemia lesion has been suggested to be worse in aged rats than in younger animals. Therefore it is clinically relevant to use old animals for the experiments.

Sex: Cerebral ischemia can be induced in male as well as in females but generally male animals are preferred to avoid the possible effects of periodic hormonal changes on the ischemic changes as it has been suggested that estrogen has got neuroprotective effects. In several studies it has been shown that the ischemic damage induced was found more extensive in male than in female rodents. Various studies have shown the sex differences in susceptibility to ischemia were attributed to the collateral circulation or the influence of the gonads on the blood flow and blood pressure. Recently it has been suggested that the estrogen has an antioxidant property.

Temperature: It is well known that the effect of small difference in the brain temperature can affect the extent of ischemic cerebral damage. Postischemic hypothermia may have a protective role on ischemic cerebral damage; temperature management of experimental animals after surgery may also affect the infarction volume. To control the brain temperature during and after ischemia can be done by using heating pads, heated water bath and close chamber with heating fans. Heating lamps can also be used to alter the brain temperature. Therefore brain temperature during the experimental procedure should be controlled (39).

Glucose level: Experimental as well as clinical studies have revealed the aggravating effect of hyperglycemia on ischemic brain damage. It has been reported that an inhibiting effect of hyperglycemia on ischemic brain damage was observed particularly in focal cerebral ischemia model. Overnight fasting is a useful method in experimental animals to maintain the blood glucose level in a narrow range. Therefore, blood glucose level should be

maintained at constant level (40).

Blood pressure: Hypotension during ischemia significantly aggravates ischemia brain damage. Theoretically, blood pressure during cerebral ischemia has no effect on the results of the complete global ischemia without hypotension. Therefore blood pressure should be carefully controlled during the experiments (40).

Anesthetic agents: The anesthetic agents like barbiturates have a proven neuroprotective effect and the use of these agents can affect the outcome of the results. Therefore use of barbiturates should be avoided.

Others: A long duration of surgery/ complicated surgery may result in increased ischemic damage and therefore duration of surgery should be considered while carrying out the experiments.

SEIZURES AFTER ISCHEMIC STROKE

Stroke and epilepsy are two of the commonest serious neurological disorders worldwide. It has been reported that patients who have had a stroke are at increased risk of subsequent seizures compared to people of the same age (41). Stroke (infarction or hemorrhage) is an important cause of epilepsy in adulthood, especially in the elderly. Because of a high incidence and improved survival, poststroke seizures are a great contemporary challenge for physicians (42). Post stroke seizures can occur soon after the onset of ischemia or can be delayed. The overall rate of postischemic stroke epilepsy is approximately 2 % to 4 % (43).

Patients with develop recurrent postischemic stroke seizures generally required pharmacological treatment. The neuroprotective agents which have an protective effect in stroke and epilepsy may be beneficial. Recently we have shown the protective effect of antioxidant agents like melatonin, adenosine, resveratrol and Centella asiatica in different models of epilepsy (44-49). Effective model to study stroke and seizures need to be stabilized.

RESEARCH ON HERBAL DRUGS IN STROKE

Herbal drugs have been extensively studied in stroke therapy. There are many drugs, which have been studied for stroke treatment both in animals as well as in patients. Shengmai san is a traditional Chinese herbal medicine consisting of three herbal components *Panax ginseng, Ophiopogon japonicus,* and *Schisandra Chinenisis* and is being used for treating coronary heart disease. *Shengmai san* in a model of bilateral carotid occlusion when administered directly into the duodenum 2 hours before cerebral ischemia suppressed the TBARS formation, and also prevented the loss of GPx as compared to the control. It was found that Shengmai san also prevented the TBARS levels when administered 45 min after ischemia. In another study Shengmai san (15 g original herbs/kg) treated 2 h before the forebrain ischemia reperfusion inhibited TBARS formation and GPx. Histochemical study of the brain slice using TTC staining revealed that Shengmai san effectively reduced infarct area caused by the cerebral ischemia-reperfusion. These experiments suggest the potential of Shengmai san against cerebral

ischemia reperfusion injury (50, 51).

Zhenxuanyin composed of gastrodia tuber, poria cocos, ligusticum wallichii was tried against 4-O vessel occlusion model in rats. It was administered 3 times a day. 24 hours later 123I-IMP uptake was evaluated in the brain as an index of cerebral blood flow. The results show that 0.3g/kg of Zhenxuanyin increased the cerebral blood flow to the normal (52).

Traditional medicine system 'Ayurveda' have been in existence for thousand of years in India. With the associated side effects of the western medicine, traditional medicines are gaining lot of importance and are now being studied to find the scientific basis of their therapeutic actions.

Although Indian traditional medicines have been extensively studied against various neurological disorders like stress, learning and memory diseases, depression, anxiety however there is scanty experience of the Indian traditional medicine in terms of stroke. Indian herbals that can increase the blood flow/antioxidant property/ antiexcitotoxic activity may have a potential against this disorder.

Recently we have shown the protective effect of herbal preparations like Withania somnifera (53) and Centella asiatica in MCA occlusion model of stroke in rats.

STATUS OF STROKE RESEARCH IN INDIA

The research in the field of stroke in India is relatively new. In recent years number of Institutes/laboratories around the country have engaged themselves in stroke research. Recently research papers of experimental studies on stroke have been published from All India Institute of Medical Sciences, New Delhi; Industrial Toxicology Research Centre (ITRC), Lucknow; Central Drug Research Institute (CDRI), Lucknow; National Institute of Pharmaceutical Education and Research (NIPER), Mohali; National Institute of Mental Health And Neuro Sciences (NIMHANS), Bangalore and Jamia Hamdard, New Delhi. Latest techniques like animal MRI and latest concepts are being used at par with the world. The work so generated is being accepted internationally which can be assessed by the number of papers published in the journals of good impact factor from India. The need of the hour is the collaborative research among different Departments and Institutes to bring India on the world map with regard to stroke research.

CONCLUSION

Studies in experimental ischemia models have contributed vastly in understanding the pathophysiology of stroke. Moreover animal models provide a testing ground for novel compounds before their launching into any clinical trials. Lot many animal models are available at present, however with the failure of neuroprotective drugs in the clinical trials although being efficacious in experimental studies has led to much concern on the validity of these models and also how closely they mimic human conditions. Therefore it is being suggested that animal models should be refined to best reflect the clinical situation.

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