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Experimental Models of Focal Ischemia: from Preference to Parameters

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ABSTRACT

Focal ischemia**,** a ischemia of specific area of the brain, is responsible for many deaths and disabilities across the globe that may be due to clot formation or accidental collapse of arteries in either case treatment is required to treat ischemic insult and further deterioration of neurons in the brain. To know exact pathophysiology and biochemical events it becomes necessary to study those conditions in vivo. Furthermore to check drug moieties for the treatment of ischemic conditions and its prognosis, animal models are required to mimic those conditions artificially. There are many factors on which choice of animal for animal model is depend like age, species and genetics. The species which would be near to humans in evolution hierarchy should exhibit almost similar physiological as well as pathological architecture of vascularity and ischemic condition. But other factors cannot be ignored in this regard like finances, survival and labour intensiveness. During literature survey, out of many available models of ischemia, rodent models are found to be used mostly in preclinical research so this review is a collaborative approach contains methods and types to select animal and animal models along with the mechanisms and assessment of ischemic conditions.

Keywords: Focal Ischemia**,** MCAO, animal models, ischemia, choice of animal models

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INTRODUCTION

Focal brain ischemia is type of ischemia in which specific area of brain is deprived from blood supply because of vessel blockade which lead to neuronal death.[1] This neuronal death give rise to many behavioural and pathological symptoms like memory lose, lose of movements and cognition etc those can be assessed accurately with behavioural and pathological investigations.[2,3] Usually focal brain ischemia is due to clot formation which occlude the MCA in majority of cases, data suggest that ischemia itself is 3rd leading cause of death around the globe with more than 7 lac cases in US alone each year.[4-6] It has been estimated that around 80% of total stroke cases are of ischemic in origin.[5]From 1995 onwards with proven clinical efficacy of tPA in clinical trials make stroke treatable, even prevention from permanent damage can be done with usage of thrombolytics and anti embolytics as soon as possible after stroke[7]but therapy itself cost enough around the word and in US alone it cost more than 50 billion.[8] Advent of animal models of ischemia provides an opportunity to researchers to combat situations arises from stroke by testing various potential drugs before taking them to clinical stage. This review in list a brief bases of animal selection, preference their utilization and rodent models of focal ischemia with the outcome of investigational parameters.

CHOICE OF ANIMALS FOR MODELS BASED ON-

Animal size-

Size of the brain does matter during ischemic evaluation so as the size of the animal during experimental procedure.[9,10] Large animals can be beneficial in many ways because it's easy to monitor all physiological parameters at once on same animal like electroencephalography, blood pressure measurement, gases concentration in blood, blood cells, body fluid and other sample collection more over imaging techniques can be performed easily to study brain like NMR spectroscopy, CT scan and functional imaging.[11] Despite of these advantages large animals have gyrencepahlic brain like humans so they are more close to humans physiology and pathophysiology wise.[12] There are some disadvantages also associated with large size of the animal used like they are difficult to handle, due to invasive surgery mortality rate is high, financial costly and they are labour intensive also.

Small animal size has its own merits and demerits. Small animal species routinely used in laboratories are rodents. Specifically Rat and mice both are easy to handle, less costly than other animals, easy to maintain and study. Added advantage of rodents is that they can be genetically modified and easily reproducible according to desired gene alteration.[13] But beside these some demerits are also there like small animals have lissencephalic brain means they are quite differ in

structure and function wise as compare to humans.[12]

Species Order-

As discuss above the advantages of the sizes of the animals choice can be made wisely according to the species also; species near to humans in evolution hierarchy suppose to have similar anatomical, functional and vascular similarities.[10]

Even dose difference is there in small animals like rodents, require higher dosage as compare to humans in mg/kg body weight bases.[14] However exact selection based on over all analysis which includes: finances, survival rate and labour intensiveness.

Genomics-

Now days with the advent of bioinformatics new genes had been identified responsible for specific functions. With the addition/deletion or altering gene sequence lead to specific feature development resembling human pathology in animals so called genetic models. Rodent species are used usually for genetically modification because of their homogeneity in genes and more reproduction number.[13]

ANIMALS FOR UTILIZATION-

Stroke and ischemia related studies can only be well studied in living and functioning brain in short in vivo studies where due to ischemia multiple factors get triggered leading to neuronal damage as seen in figure-1. But such studies are very difficult to carried out and monitor on humans because of moral and social issues so for exchange animals are used they are socially and institutionally easily acceptable and are used to mimic those conditions artificially so that opportunistic drug moieties can be tested to maximize the beneficial effects of drugs in treating strokes. Furthermore there are similarities in stroke prone rats and humans.[15-17] From the beginning many animals were already used by various researcher and established various animal models few of them are well stabilized and still continued as it is or with little modifications. Animals used in models were dogs, gerbils, rabbit, rats, mice and monkeys.[16,18-22]Out of them most used species is rodent and animal is rat on which various models are present today.

TYPES OF THE RAT MODELS OF FOCAL BRAIN ISCHEMIA

Rat Models of Focal Brain Ischemia

1	Intravascular-	Intraluminal middle cerebral artery (MCA) occlusion
		Thromboembolic model
		Non clotting embolism
		Photochemically induce thrombosis
2	Extravascular-	Electro-cauterization
		Ligation
		Clipping
		Endothelin induced constriction of MCA

Intraluminal Middle Cerebral Artery (MCA) occlusion-

Koizumi et al. first developed intraluminal middle cerebral artery(MCA) occlusion in rat[23] later on many authors introduce variants even mice models were later established.[24] In this model a monofilament is inserted in Internal carotid artery(ICA) through common carotid artery(CCA) when filament reached on MCA bifurcation of ICA it cause blockage of blood flow to MCA,thus lack of blood supply lead to ischemia of that part specifically to caudatoputamen and frontoparietal cortex region along with formation of penumbra.[25,26] Infarct produce is reproducible depends on the time of occlusion and reperfusion.[27]

Many authors used nylon filament to reproduce this model with or without silicon coated tip and succeeded. Detailed procedure can be found in work done by various authors.[28,29]

Thromboembolic Model-

This type of model can be reproduced by injection of fibrin rich clot. However this model is first introduced by Hill et al. in dog later on rat variant of this model were created successfully.[30-32] This model is very much interesting among researcher because of its realistic results of resembling human ischemic stroke that's why this model is used to study various thrombolytics in thrombolytic therapy.[33,34] This should be noticed that physical and chemical properties of the clot are important things and need to be taken care to reproduce this model again because clot should be of specific size (length and diameter) and chemical (fibrin rich).[35] Advantage of this model is that it is capable of reproducible infarcts in MCA region as in intraluminal model and animal exhibit same mechanisms of ischemic stroke as in case of humans.

Non Clotting Embolism Model-

This type of model can be reproduced by injecting embolic compounds in internal carotid artery(ICA) which cause artificial emboli.[36] Out of various emboli's used, embolization due to microspheres is aggressively used and produce severe ischemic damage related to number of emboli used.[37] This model has some disadvantages like multifocal ischemia with low reproducibility. Ischemia produced is permanent which is not similar with many clinical conditions of the human stroke.

Photochemically Induced Thrombosis-

In this model photoactive dye is injected systemically then skull is subjected to radiation.[38]In radiated area, endothelium layer is oxidatively damaged due to altered dye, it leads to platelet aggregation which induce infarct in cortical region.[39] There are many disadvantages associated with this model like breakdown of blood brain barrier and lack of penumbra formation and edema.

Electro-Cauterization

Cauterization is the one of the ancient techniques to stop bleeding by inducing blood clot.[40,41] This technique is modified with electricity in such a manner that it induce blood clot and it was named after. Electricity is used to induce cauterization. First small craniotomy is performed and after excising dura middle cerebral artery (MCA) is exposed and surrounded by metallic forceps which is attach with electric-cauterizer. A small current flow through forceps cause artery cauterization.[42]

Ligation

In this model surgically middle cerebral artery (MCA) is exposed by microsurgical techniques after digging burr hole of 2mm diameter at 1mm rostral to interior junction of zygoma and squamosal bone. Then exposed MCA is occluded by ligation with surgical sutures which stop the blood flow to various regions of cortex of the brain result in ischemia of brain resembling clinical condition of stroke in humans.[43]

Clippping

It is surgical procedure in which a small craniotomy is performed. In this model clip is use to occlude the middle cerebral artery(MCA) in brain distal to lenticulostriate perforators which produce lesion confined to cortex only. Advantage of this model is that it produces more defined and reproducible lesions.[44]

Endothelin Induced Constriction of Middle Cerebral Artery(MCA) -

Endothelin(ET-1) is natural vasoconstrictive peptide already used in various models of focal cerebral ischemia.[45]Advantage of using ET1 includes replacement of invasive surgery. Steriotaxically injection of ET1 near middle cerebral artery(MCA) results in chronic constriction of MCA which restrict blood flow.[46] Ischemic lesions produced are of similar in pattern as seen in MCAO model. Cerebral blood flow (CBF) is restore in less than 30 minutes progressively in dose dependent manner.[47]

PARAMETER ASSESSMENT-

Assessment of experimental outcome of every model depends upon type of study: acute or chronic. Effectiveness of drugs effective in early acute ischemic insult can be checked as soon as after ischemia, however chronic degeneration of neurons takes days to weeks after ischemic insult.

Behaviour Parameters

Behaviour of the animal can be noticed after recovery which would let observer know about the produced neurological deficits due to ischemia like motor control and cognition these two are

easily and effective means of testing neurological status of animal. Instrument used in motor control verification are grip strength, narrow beam walk, rota road and grid walking[48,49] on the other had for cognitive functions Morris water maze can be used.[50]

Pathological Parameters

Pathological damage to neurons can be observed clearly throw histology by using two most commonly used staining dyes: TTC(2,3,5 triphenyltetrazolim chloride) and H&E(haematoxylin-eosin).[51]Out of them TTC is more popular and effective and when used, it get reduced by mitochondrial enzymes and red colour appear in intact brain while damaged area remain colourless.[45]

Combine assessment of neurological and pathological status would be an ideal choice for accuracy in results. However despite of these STAIR commette recommend neurological assessment extended for 1 month after ischemic insult[52]but it is difficult because most of models have survival time 48 hours or less.[53]

DISCUSSION

Animal models (in vivo) are no doubt good alternate to measure clinical outcome of drugs and getting dipper in pathological mechanism involved in stroke prone conditions of the brain regions. However in vitro models are now present to study specific region of brain neurons by making brain slices ex-vivo from decapitated animal to measure ischemic insult and recovery.[54]

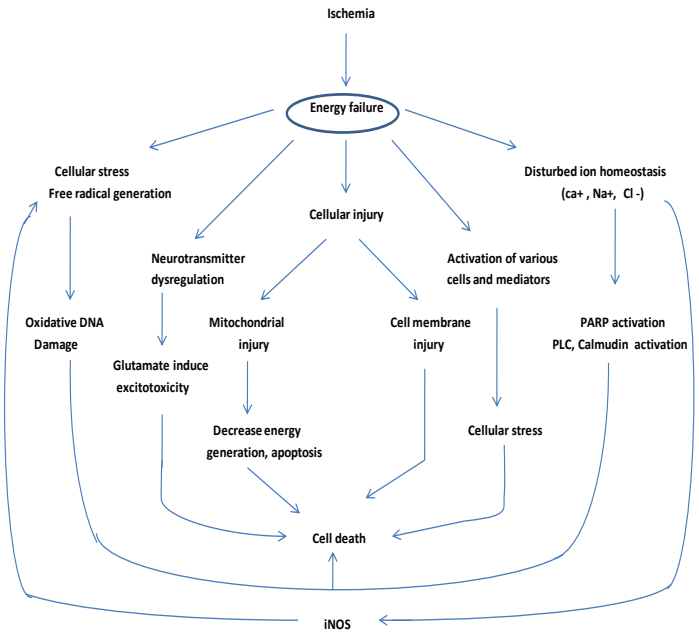

Figure-1. Molecular mechanism involved in the ischemia induced neuronal damage.

But there are no evidences that any of the above model duplicate the exact neurochemical architecture of human ischemic brain. But still those models are sufficient enough to clarify the prognosis of ischemic insult in terms of neurodegenration by various pathways as shown in figure 1.

CONCLUSION

Article provides a review of current rat models present for focal ischemia. Among all animals rodents are found to be more suitable because they are mammalian in origin like human and have almost same brain physiology. With the development of genetic strains chances of getting detailed molecular mechanisms of leading molecules in preclinical testing have increased many folds as compare to normal focal ischemic models however research of decades in rodents had already revealed various hidden pathways and cascades of ischemic insult in neurons and had broaden our knowledge of conditions associated with ischemia. Although at last creating all tiny details of human stroke conditions in animals is very difficult, and almost impossible because of presence of one or more co-existing pathology, most of the time.

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