AGE-RELATED NEURONAL VULNERABILITY TO BRAIN ISCHEMIA: A POTENTIAL TARGET OF GENE THERAPY

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ABSTRACT

Brain infarction is one of the most important age-associated diseases. We have developed aged animal models for brain ischemia, and found the age-related neuronal vulnerability to brain ischemia. Investigation of that mechanism would lead to the effective treatment of brain infarction in the elder population. Recent advancement of gene transfer technique has provided strong tools for the neuronal and vascular biology. We described our recent approaches of gene transfer to blood vessels, including cerebral circulation, using adenoviral vectors. Cerebral blood vessels, atherosclerotic endothelium, and ischemic brain tissue are good targets of gene transfer. Development of these techniques would offer new therapeutic strategies for the age-related neuronal vulnerability and other age-associated diseases.

1. Aged model for ischemic stroke

Stroke is the leading cause of death in the Japanese elderly [1], and aging is known as main risk factors for stroke [2] and vascular dementia [3]. Although aged models for the experimental brain ischemia have been claimed to be important [4], the studies using such models are limited.

Recently, we have developed the experimental model for brain ischemia with aged spontaneously hypertensive rats (SHR), more relevant models for human stroke [5]. Using this model, we demonstrated that the hippocampus in the aged rats was more susceptible to transient cerebral ischemia than that in the adult (Figure 1). Other investigators also reported that embolic brain damages in the aged animals were severer than in the young animals [6]. Our study revealed that reduction of perfusion pressure to the brain was similar between the adult and aged SHR, suggesting that factors other than blood flow may affect the ischemic damages in the aged animals. Therefore, investigation of the mechanism of vulnerability in aged brain would lead to the new therapeutic strategy of brain infarction in the elder populations.

2. Excitotoxicity

Although brain is the most susceptible organ to ischemia, there are more vulnerable cerebral tissues, such as hippocampal pyramidal cells, medium-sized cells in the striatum and pyramidal cell layers in the cortex [7]. Pathophysiology of these selective vulnerability to ischemia has been an important research subject.

One strong hypothesis for the selective neuronal vulnerability is the excitotoxicity theory [8]. Excitatory amino acids, such as glutamate and aspartate, have been proposed to play pivotal roles in neurotoxicity, and increases in extracellular excitatory amino acids during cerebral ischemia were observed in the vulnerable areas [9]. The specific receptors for excitatory amino acids, i.e., N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and metabotropic receptors, locate at those vulnerable areas, and these receptors are reported to play important roles in neuronal death. Hippocampal CA1 subfield is the most energetically studied areas in regard to the selective vulnerability, and is known for its unique response to transient ischemia, called “delayed neuronal death” [10]. In relation to NMDA receptor complex, glycine also plays an important role in the pathophysiology of ischemic cell damage [11]. Furthermore, inhibitory amino acids, e.g., γ-aminobutyric acid (GABA) and taurine, are reported to protect against ischemic damages [12,13].

3. Age-related ischemic vulnerability

As we described in the earlier part, aged brains are more susceptible to ischemia than adult ones [5]. The mechanism of this age-related ischemic vulnerability is still not clear. We examined involvement of extracellular excitatory and inhibitory amino acids in the hippocampus where the vulnerability is most obvious [14]. Interestingly, there were no significant differences in ischemia-induced changes in excitatory amino acids between aged and young animals. In contrast, smaller increases of extracellular taurine during ischemia were observed in the aged animals. Therefore, balances of excitatory and inhibitory amino acids may play a critical role in the age-related vulnerability.
Fig. 1a: Photomicrographs of the CA1 subfield of the hippocampus of aged SHR (21 months old) 7 days after 20 min of transient cerebral ischemia. In the normothermic conditions (a), majority of pyramidal cells revealed shrunken cytoplasm and picnotic nuclei associated with perinuclear vacuolation, which were not observed in the adult (6 months old) rats. Paraffin section with hematoxylin and eosin stain. Magnification, x300.

Fig. 1b: Mild brain hypothermia (3°C reduction) markedly inhibited the degenerative changes in the pyramidal cells (b). Mild brain hypothermia (3°C reduction) markedly inhibited the degenerative changes in the pyramidal cells (b). administered in a lateral position, transgene expression was mainly observed around the middle cerebral artery (Figure 2). Using mice models, we also observed good efficiency of gene transfer to the cerebral blood vessels [25]. Therefore, our perivascular approach via CSF provides efficient gene transfer to cerebral blood vessels.

Mild brain hypothermia is reported to have marked neuroprotection against ischemic insults in the adult animals [15,16]. However, effects of hypothermia on the ischemic damage in the aged animals have not been well clarified. We examined effects of brain hypothermia on the age-related brain damage to ischemia. Even small reduction of brain temperature (by 3°C reduction) during brain ischemia markedly attenuated ischemic damage in the hippocampus (Figure 1). Therefore, mild brain hypothermia may be one of the promising approach for treatment of brain infarction in the elder population, although safety of this approach is an important issue.

4. Gene transfer to blood vessels

Gene transfer is an attractive method for studies of vascular and neuronal biology. Clinical use of this technique, namely gene therapy, may be a promising approach for many disorders [17]. One of the most useful vectors for gene transfer is the recombinant adenovirus [18]. The vector consists of 36 kilo base DNA inside the capsid protein. Because the vector has a wide host rage and great efficiency of gene transfer without relation to malignancy, many clinical trials utilize this vector [19,20].

For gene transfer to blood vessels, intravascular administration of the vector using special catheters is a common approach [21]. In that case, needs of stopping blood flow may be a critical limitation in the cerebral circulation. Therefore, we have examined efficacy of an alternative method, perivascular approach [22,23]. To deliver the foreign gene to cerebral blood vessels, we administered an adenoviral vectors encoding cDNA of bacterial β-galactosidase as a reporter gene into cerebrospinal fluid (CSF) via the cisterna magna [24]. This vector is a replication deficient because the E1 region that is necessary for viral replication is deleted. One day after vector injection, the reporter gene efficiently expressed around the circle of Willis. When the vector was

One of the possible clinical application of this approach is prevention of vasospasm after subarachnoid hemorrhage. Vasospasm occurs several days after the bleeding, and this time course is suitable for expression of transgene. We examine the efficacy of gene transfer to the cerebral blood vessels after subarachnoid hemorrhage in dogs, using the above-mentioned perivascular approach. Two to seven days after injection of the vector, prominent expression of the reporter gene was observed even in the presence of subarachnoid hemorrhage [26]. Transfer of the vasodilating gene using this method was effective in preventing vasospasm in rabbits [27]. Therefore, perivascular approaches to the cerebral blood vessels may be useful in gene therapy for subarachnoid hemorrhages.