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Research report

Behavioral and neuropathological consequences of transient global ischemia in APP/PS1 Alzheimer model mice

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HIGHLIGHTS

- TGI-induced circumscribed neuron loss correlates with spatial memory impairment.
- Neuroinflammation persists in primary lesion sites at least for 5 weeks.
- Young adult APP/PS1 show no increased vulnerability to transient ischemia.

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ABSTRACT

Alzheimer's disease (AD) typically manifests in elderly people with several co-morbidities, especially cardiovascular, whereas transgenic mouse models of this disease usually employ middle-aged animals that have a good general health status. To assess the combined effect of compromised cerebral blood circulation and brain amyloid pathology we induced transient (17 min) global ischemia (TGI) to young adult APPswe/PS1dE9 (APdE9) mice modeling AD amyloid pathology, and assessed the outcome on behavior two weeks and on histopathology five weeks after the ischemic insult. Ischemic injury resulted in reduced motor coordination and impaired spatial learning and memory. Neuropathological examination revealed circumscribed sites of neuronal loss in ischemic mice, including hippocampal CA2, lateral CA3 and medial CA1 pyramidal cell layer, and superficial layers of cortical patches. Notably, Fluoro-Jade staining revealed dying neurons as late as five weeks after the initial insult, and staining for active microglia and astrocytes confirmed the presence of inflammatory reaction. The extent of neuronal loss in CA2 and CA1 correlated significantly with impairment in spatial memory. There was no genotype difference in either behavioral or neuropathological consequences of TGI. However, the post-operative survival of transgenic animals was greatly reduced compared to wild type animals. APdE9 mice at a pre-plaque age appear to be more sensitive than wild-type mice to TGI in terms of post-operative recovery but the surviving APdE9 mice do not display more severe neurological deficits than wild-type mice.

1. Introduction

The most common causes of dementia in the elderly are Alzheimer's disease (AD) and vascular dementia. This division seems to be more or less theoretical as in most pathology reports AD and vascular pathology coincide, with 60–90% of AD patients show cerebrovascular pathology at autopsy [1]. Furthermore, several established risk factors for sporadic AD are vascular, such as hypertension, type 2 diabetes and atherosclerosis [2]. In addition, ischemic stroke, yet another dementia-causing disease among the elderly, may be one contributor in AD pathogenesis. Evidence for this statement includes findings of stroke intensifying the presence and severity of clinical symptoms of AD and increasing AD pathology in the brain [3,4]. Coexistence of stroke and AD occurs more than by chance alone [1] while transient ischemic attacks and stroke episodes also increase the incidence of AD [5]. Some studies have reported that vascular changes in AD brains correlate with cognitive performance [6,7] while the major hallmark of AD, amyloid-β plaque load, does not [8,9]. Moreover, cerebral blood flow has been shown to be reduced in AD patients [10]. Thus it seems that cerebral vascular and blood flow changes have a pivotal role in AD.

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Evidence from AD animal models also points to a significant role of brain ischemia in the AD pathogenesis. After a focal ischemia due to middle cerebral artery occlusion, amyloid precursor protein APP overexpressing mice show larger infarct volumes than wild-type controls [11–13]. In two recent studies mild chronic cerebral hypoperfusion induced spatial learning impairment in APP or APP/PS1 transgenic mice but not in wild-type littermates [14,15]. Repeated hypoxic insults [16] or chronic mild cerebral hypoperfusion [17] have been reported to increase brain Aβ-levels for a prolonged time in APP transgenic mice. The underlying mechanism has been proposed to be increased BACE1 expression due to neuronal energy depletion [18]. On the other hand, reports are less consistent regarding the effect of focal ischemia on amyloid production. One study found a transient increase of amyloid plaques around the infarcted area in 6- to 7-month-old APP/PS1 transgenic mice [19], while another study reported a dramatic clearance of existing plaques around the ischemic area between 7 and 21 days after the lesion [20]. In both reports, however, the final clearance of amyloid deposits was ascribed to activation of macrophages/microglia. Finally, there is evidence that overexpression of Aβ producing mutated APP can impair cerebral blood flow [11,21,22] while overexpression of human APP in the mouse does not [12]. So it seems evident that ischemic incidents can increase amyloid pathology, while amyloid pathology can aggravate the severity of ischemic damage.

One basic problem with the above described ischemia models is that they lack the anatomical specificity of early AD, where most severe neuropathology is largely restricted to the medial temporal lobe structures. However, there is an established model of transient forebrain ischemia by reversible occlusion of common carotid arteries combined with a lowering of mean arterial blood pressure which results in selective lesion in the hippocampus pyramidal cell layer [23,24]. This two-vessel occlusion models acute cessation of cerebral circulation that may accompany serious cardiac arrythmia or acute drop in blood pressure. Our recent study in 9-month-old APPswe/PS1dE9 double transgenic (APdE9) mice demonstrated that when exposed to global brain ischemia (GI) APdE9 mice showed increased neuronal loss in CA2 and CA3 subregions compared to their wild-type controls [13]. Unfortunately, that study did not include a behavioral assessment of GI outcome.

APdE9 mice are widely used in AD research. In these mice amyloid plaques start to develop in the cortex and hippocampus at 4 months of age [25] but memory impairment becomes manifested only close to 12 months of age [26]. Recently, it has also been shown that these mice are prone to epileptic seizures [27] and have neuronal hyperexcitability [28] already at 3 months of age, suggesting that these mice might be more sensitive to excitotoxicity raised by ischemic episode. In addition, the age-related increase in oxidized redox state and decrease in antioxidant GSH defense take place in APP/PS1 mice at an earlier age than in wild-type mice [29], which may seriously compromise their ability to sustain acute ischemia.

To assess the effect of soluble Aβ aggregates without the confounding effect of plaque associated chronic neuroinflammation we induced transient GI on young adult APdE9 mice. Extensive neurological test battery revealed memory and motor impairment in ischemic animals while APdE9 genotype had no contribution. We found severe but selective neuronal loss in CA1-3 fields of the hippocampus and somatomotor cortex, which further correlated with behavioral deficits, but to the same extent in both APdE9 and wild-type mice. However, APdE9 mice were more sensitive to TGI in terms of significantly increased post-operative mortality. Thus the preclinical stage of AD-pathology sensitizes the brain to ischemic brain injury in terms of early survival, but the surviving animals show no additional sensitivity to ischemic damage.

2. Materials and methods

2.1. Animals

Male 3.5-month-old APdE9 mice and their wild-type (WT) littermates were used in the study. The APPswe/PS1dE9 (APdE9) founder mice were obtained from Johns Hopkins University, Baltimore, MD, USA (D. Borchelt and J. Jankowsky, Dept. Pathology) and a colony was established at the University of Kuopio. These mice were generated by co-injection of chimeric mouse/human APPswe (mouse APP695 harboring a human Aβ domain and mutations K595 N and M596L linked to Swedish familial AD pedigrees) and human PS1-dE9 (deletion of exon 9) vectors controlled by independent mouse prion protein promoter elements [30]. This line was originally maintained in a hybrid C3HeJ × C57BL6/J F1 background, but the mice used in the present study were derived from backcrossing to C57BL6/J for 12 generations.

The animals were housed in controlled environment (National Animal Center, Kuopio, Finland, temperature 22 °C, light 7:00–19:00; humidity 50–60%), and food and water were freely available. All behavioral tests were conducted during the light phase (8:00–16:00). The experiments were conducted according to the Council of Europe (Directive 86/609) and Finnish guidelines, and approved by the State Provincial Office of Eastern Finland.

2.2. Surgery

For surgeries the mice were randomly assigned to four groups (WT + SHAM, WT + TGI, APdE9 + SHAM and APdE9 + TGI). Before surgery each mouse was anesthetized with 4–5% halothane (70% N_2O/30% O_2, Nicholas Piramal India Ltd., Mumbai, Maharashtra, India). During surgery halothane was reduced to 1.5–2%. For post-operative analgesia the mice were given carprofen (5 mg/kg, s.c., Rimadyl Vet, Pfizer, Dundee, UK). A midline incision was made in the neck, the common carotid arteries were isolated, and fine silk thread was loosely placed around them. The anesthesia was lowered to 0–1% halothane. With the silk threads arteries were gently raised and atraumatic miniature aneurysm clips were attached to occlude bilaterally the carotid arteries for 17 min. The core temperature was maintained at 36–37 °C throughout the surgery with automated heating pad connected to rectal probe. Control mice were SHAM-operated by the same protocol without the carotid occlusion and anesthesia reduction. For all surgeries the duration was kept constant (30–35 min). Postoperatively the mice were left to recover in a heated 32 °C chamber for 12 h. Since transient GI for 17 min is a drastic operation, for ethical considerations we set the following humane endpoint criteria: >20% weight loss, immobility or clear painful behavior after 12 h of surgery.

2.3. Behavioral testing

After two weeks of post-operative recovery the mice underwent a neurological test battery for three weeks comprising tests for exploratory activity, motor coordination and balance and spatial learning and memory. The order of the tests was the following: spontaneous explorative activity – marble burying – rotarod (week 1); Morris swim task (week 2); rewarded delayed alternation (week 3).

2.3.1. Morris swim task (water maze)

Spatial learning and memory was tested with Morris swim navigation task. The apparatus consisted of a white plastic pool with diameter of 120 cm and of transparent platform (10 cm × 10 cm) submerged 1.0 cm below the water surface. Water temperature was kept at 20 ± 0.5 °C throughout the testing, and a of 8–10 min recovery period in a warmed cage was allowed between all trials.