Clearance of Amyloid- β Protein Deposits in Transgenic Mice following Focal Cerebral Ischemia

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Key Words

Amyloid- β protein • Alzheimer's disease • Ischemia • Clearance • Transgenic mice

Abstract

Background/Aims: There is increased amyloid- β protein precursor (A β PP) expression and amyloid- β protein (A β) production in the brain shortly following cerebral ischemic stroke in rodent models. It has been postulated that this may seed amyloid deposition in the brain. On the other hand, it remains unclear how cerebral ischemia affects preexisting A β deposits in the brain. Here we determine the consequences of focal ischemic stroke on existing A β pathology in Tg-SwDI transgenic mice. **Methods:** At 12 months of age, Tg-SwDI mice were subjected to photo-induced focal cerebral ischemia in one hemisphere. One, 7, or 21 days after lesioning, the amount of deposited A β in the ischemic and control hemispheres was measured using ELISA. Image analysis was used to visualize deposited A β and the presence of microglia/macrophages. **Results:** After 7 days, and further after 21 days, there was a dramatic reduction in the amount of deposited A β and increased presence of microglia/macrophages in the ischemic hemisphere of the mice. **Conclu-**

sions: Focal cerebral ischemia leads to clearance of deposited A β in Tg-SwDI mice starting at 7 days with almost complete removal in the ischemic area by 21 days. The delayed clearance of A β following focal cerebral ischemia may involve the infiltration of activated neuroinflammatory cells.

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The progressive accumulation of amyloid- β protein (A β) in senile plaques and the cerebral vasculature is a prominent feature of Alzheimer's disease (AD) and several related disorders [1]. A β is derived from amyloid- β protein precursor (A β PP) through sequential proteolysis by β - and γ -secretase activities [1]. Situations that can increase expression and/or proteolytic processing of A β PP, including ischemic stroke, have been implicated in promoting A β deposition in the brain and contributing to the development of AD [2].

In animal models, there are numerous reports demonstrating that A β PP expression is increased after an ischemic challenge [3, 4]. Although rats are the most frequently used animal models for ischemic studies, they generally do not develop cerebral A β deposits. Rather, human A β PP transgenic mice that develop age-depen-

Fig. 1. ELISA measurement of total A β levels in Tg-SwDI mice following focal ischemic stroke. Level of total A β peptides in ischemic and contralateral control tissues 1, 7, and 21 days following ischemia. Data presented are the mean $\pm$ SD of 5–8 mice per group per time point. * $p < 0.01$.

dent cerebral deposits of human A β have been useful models to demonstrate increased expression of A β PP, elevated levels of A β peptides, and deposition of A β peptides shortly following the introduced lesion [5]. On the other hand, how ischemic stroke influences preexisting cerebral A β deposits in older transgenic mice is less clear. Tg-SwDI mice are a unique transgenic model that expresses neuronal human cerebral amyloid angiopathy mutant A β PP that produces Dutch (E22Q) and Iowa (D23N) double-mutant A β peptides in the brain [6]. Tg-SwDI mice were shown to develop early-onset cerebral microvascular amyloid and abundant diffuse parenchymal A β deposition [6, 7]. In this study, we investigated how ischemic stroke affects preexisting A β deposits using the aged Tg-SwDI mouse.

Experimental Procedures

We used the photo-induced cerebral ischemic stroke model that introduces a permanent focal lesion in one brain hemisphere as described [8]. This procedure was performed in 12-month-old Tg-SwDI mice that present abundant cerebral A β deposition [7]. Groups of lesioned Tg-SwDI mice ($n = 5$ –8 animals per group) were allowed to survive for 1, 7, or 21 days after which time they were sacrificed, perfused, and their brains collected. The levels of total A β peptides were measured by ELISA of guanidine extracts of the collected tissue samples as described [6, 7]. In parallel studies, the harvested brains were fixed in 70% ethanol, followed by xylene treatment, and 10- μ m-thick coronal tissue sections were prepared. Deposited A β in the brains of the mice was detected by immunolabeling with polyclonal antibody raised to human A β 1–28 and Alexa Fluor 488-conjugated secondary antibody. Infiltrating microglia/macrophages in the brains were detected by immunolabeling with monoclonal antibody 5D4 to keratin sulfate and

Alexa Fluor 594-conjugated secondary antibody. Total A β burden in the ischemic and contralateral control regions was quantified on the same set of systematically sampled A β -immunolabeled sections using NIH image J 1.32 software [7].

Results

As shown in figure 1, 1 day following the ischemic lesion there was no detectable difference in the amount of A β in the ischemic or control hemispheres. However, at 7 days after the ischemic lesion, there was a highly significant ($p < 0.01$) nearly 50% decrease in the amount of extracted A β in the ischemic region. Moreover, 21 days following the ischemic lesion the amount of A β was reduced by nearly 90% in the ischemic region compared to the contralateral control hemisphere. These results obtained by quantitative ELISA were confirmed by image analysis of immunolabeled deposited A β in ischemic and contralateral control regions of brain tissue sections again showing a dramatic reduction in A β levels 7 and 21 days following the ischemic lesion (fig. 2). Concurrent with the loss of deposited A β , there was a marked increase in the presence of infiltrated microglia/macrophages in the ischemic tissue (fig. 2a).

Discussion

The objective of the present study was to determine the effect of permanent focal ischemic stroke on preexisting A β deposits in Tg-SwDI mice, a transgenic model of

Fig. 2. **a** Immunolabeling of A β deposits and microglia/macrophages in ischemic and contralateral control brain tissues. Scale bars = 200 μ m. **b** Quantitative image analysis of A β deposition in Tg-SwDI mice following focal ischemic stroke. Level of A β deposition in ischemic and contralateral control tissues at 1, 7, and 21 days following ischemia. Data presented are the mean $\pm$ SD of 5–8 mice per group per time point. * $p < 0.01$.

early-onset and extensive A β deposition in the brain. In our studies, we found that shortly following ischemic stroke (1 day) there was no discernible effect on the amount of preexisting A β deposits in the ischemic lesion. In other experiments using younger Tg-SwDI mice without preexisting A β deposits, 1 day after induction of a focal ischemic stroke we found an elevation in A β PP expression and small increases in the amount of A β peptides in the ischemic lesion (data not shown). Although this same small effect likely occurs after 1 day in the 12-month-old Tg-SwDI mice used in the present study, the additional amount of accumulated A β would be negligible compared to the extensive amount of preexisting A β deposits observed at this age. However, 7 and 21 days following induction of the ischemic lesion, a dramatic re-

duction in the amount of preexisting A β deposits was observed supported by ELISA measurement of A β peptide levels extracted from the collected tissues and quantitative image analysis of deposited A β in brain tissue sections (fig. 1 and 2, respectively).

Although previous studies have demonstrated an increase in A β PP expression and A β production shortly following ischemic stroke in rodent models, our present results indicate that during later stages of the ischemic lesion there is a marked clearance of preexisting A β deposits. This suggests that in older animals with deposits any initial increase in local A β levels would be overcome by enhanced clearance mechanisms. The robust clearance of preexisting A β deposits following an ischemic lesion might result from increased infiltration and acti-

vation of neuroinflammatory cells such as microglia/macrophages (fig. 2a). This response may lead to enhanced cellular uptake and/or production of extracellular A β -degrading enzymes that can clear the deposited A β peptides.

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