Progressive Increase in Infarct Size, Neuroinflammation, and Cognitive Deficits in the Presence of High Levels of Amyloid

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Background and Purpose—In the elderly, cerebral ischemia (CI) occurs in the presence of high levels of amyloid. Neuroinflammation plays a critical role in the pathophysiology of Alzheimer’s disease and CI. This study examined infarct size, neuroinflammation, and cognitive deficits over time in rat models of Alzheimer’s disease and CI.

Methods—β-amyloid toxicity was modeled using bilateral intracerebroventricular injections of β-amyloid 25 to 35 peptides. CI was modeled using unilateral injections of the potent vasoconstrictor, endothelin-1, into the striatum.

Results—Infarct volumes were higher in the presence of amyloid and compared with the CI model alone. In the CI model alone, the infarct volume was significantly smaller 28 days after surgery compared with 7 days after surgery. However, when Alzheimer’s disease and CI models were combined, the infarct volume was significantly larger 28 days after surgery compared with 7 days after surgery. The neuroinflammation in the region of the infarct was also significantly increased. The Barnes circular platform test showed time-dependent increases in memory and learning deficits in the β-amyloid-treated rats that were even greater when β-amyloid treatment was combined with CI.

Conclusions—CI in the presence of high levels of amyloid results in progressive increases in infarct size, neuroinflammation, and cognitive deficits. (Stroke. 2007;38:3245-3250.)

Key Words: Aβ peptide ■ Alzheimer’s disease ■ memory ■ neuroinflammation ■ rat ■ stroke

A link between Alzheimer’s disease (AD) and cerebrovascular disease has been established. Common risk factors for cerebrovascular disease and AD include hypertension, atherosclerosis, diabetes mellitus, and smoking.1-3 Both share similar clinical symptoms, including cognitive decline, functional deterioration, and changes in patterns of behavior.4-5 Experimental animal models of cerebral ischemia (CI) have demonstrated the presence of amyloid precursor protein in the area of ischemic damage.6-8 Human studies have also indicated that soluble amyloid precursor protein and β-amyloid 1 to 42 (Aβ 1 to 42) accumulates in patients with multiinfarct dementia.6-7,9 AD-related pathology can also influence microvascular integrity. Studies have shown that Aβ peptides can induce endothelial cell degeneration10,11 and may lead to leaky blood vessels. Furthermore, in humans, both silent and clinical infarcts occur in the presence of high levels of amyloid in the aging brain and stroke alters the clinical expression of AD pathology.12 Neuropsychometric tests of cognitive function have shown that patients with autopsy evidence of AD pathology and cerebral infarcts, especially lacunar infarcts in the striatum, show more cognitive impairment than patients with AD pathology alone.12,13

Neuroinflammation is a key component of the pathologies of both CI14-16 and AD.17-19 Common neuroinflammatory events include activation and proliferation of microglia and astrocytes, activation of nuclear transcription factor kappa B, upregulation of inflammatory cytokines such as tumor necrosis factor α and interleukin 1-β, release of prostaglandin E2 under the enzymatic control of cyclooxygenase-2, and release of reactive oxygen and nitrogen species.

Therefore, inducing CI in the presence of high levels of amyloid may increase overall the neuroinflammatory damage in the brain, with progression over time, leading to a general increase in neurodegeneration and greater cognitive deficits. Furthermore, the neuroinflammatory response associated with high levels of amyloid in AD may render the brain vulnerable to ischemic damage leading to progressive increases in infarct size. To examine this possibility, high levels of amyloid (rat AD model) were generated using intracerebroventricular (ICV) bilateral injections of Aβ 25-35 peptide as previously described,20-22 and small striatal infarcts (CI model) was initiated by unilaterally injecting the vasoconstrictor endothelin-1 into the striatum.22,23 The brains were examined for infarct size and pathological and neuroinflammatory changes using histological...
and immunohistochemical staining. Spatial memory and learning changes were tested using the Barnes circular platform test.

**Materials and Methods**

**Surgery**
All experimental procedures were carried out in accordance with the guidelines of the Animal Care and Use Committee of the University of Western Ontario. Male Wistar rats (250 to 300 g) were anesthetized using 40 mg/kg pentobarbital (Somnotol) intraperitoneally. Body temperature was maintained at 37°C. The animals were placed in a David Kopf stereotaxic apparatus with the incisor bar set at 3.3 mm below the interaural line. Small burr holes were made in the parietal bone to allow for the insertion of the injection cannula. The rat model of AD used in this study has been described previously.20–22 Briefly, Aβ 25-35 (50 nmol in 10 µL of saline) (Bachem, Torrance, Calif) or the control peptide Aβ 35-25 (50 nmol in 10 µL of saline) (Bachem) was injected bilaterally into the lateral ventricles through a stainless steel cannula (23 g; anteroposterior: −0.8 mm relative to bregma, medial/lateral: ±1.4 mm relative to bregma, and dorsal/ventral: −4.0 mm below dura).24 To model CI, a single endothelin-1 (Sigma-Aldrich, Oakville, ON) injection (6 pmol in 3 µL saline) was made into the right striatum through a stainless steel cannula (23 g; anteroposterior +0.5 mm, mediolateral −3.0 mm relative to bregma, and dorsal/ventral: −5.0 mm below dura). For rats receiving both ICV Aβ 25-35 injections and striatal endothelin-1 injections, the burr hole for all 3 injections was made at the same time (2 ICV for Aβ, one for endothelin). Before surgery, all peptides were stored in appropriate volume aliquots at −20°C. The Aβ 35-25, Aβ 25-35, or endothelin-1 peptides were loaded into the syringe immediately before injection to minimize time at room temperature. The Aβ 25-35 peptide was injected first into the ventricles followed immediately by the endothelin injection into the striatum. Sham procedures involved all the surgical steps without injections of Aβ 25-35, Aβ 35-25, or endothelin-1. All stereotaxic coordinates were determined based on the atlas of Paxinos and Watson.25 Following Aβ 25-35, Aβ 35-25, or endothelin-1 injections, the cannula was left in situ for 3 minutes and then removed slowly. After wound suture, all rats received 40 mg/kg buprenorphine intramuscularly and were subsequently allowed to recover from surgery 7, 14, or 28 days.

**Infarct Volume Analysis**
All animals were euthanized by an overdose of pentobarbital injection (60 mg/kg) intraperitoneally. Hemispheric areas for both sides of the tissue were measured to account for any brain swelling due to the stroke. Infarct volume was calculated in millimeters squared by multiplying the individual area measurements by the number of sections and by the distance between each section.

**Circular Platform Test**
Memory and spatial learning behavioral skills were assessed using the Barnes circular platform test.27 Rats were assessed using a previous protocol.20 Briefly, the behavioral test was divided into 3 phases: training, testing, and reacquisition phases. The time to reach the escape hole was measured as well as the number of errors (nose poked into wrong hole location). There were 3 recovery periods (7, 14, and 28 days after surgery), after which a single trial was performed with the hole at the initial training location (test). During the reacquisition phase, rats relearned the behavioral task (14 trials) with the hole location rotated by 135°.

**Data Analysis**
Infarct volume measurements were statistically analyzed using analysis of variance and Tukey’s post hoc test with a significance level of P<0.05 (n=10 for each group). For behavioral data, statistical analysis was performed on each individual test and reacquisition trial of the Barnes Circular Platform test data using analysis of variance and Tukey’s post hoc test with a significance level of P<0.05 (n=10 for each surgical group).

**Results**

**Infarct Volume Measurements**
Three markers were used to measure the volume of ischemic damage in the striatum after unilateral striatal endothelin injections or the combination of unilateral striatal endothelin injections and bilateral ICV Aβ 25-35 injections. The Nissl stain thionine was used to measure the volume of infarction; Ox-6 immunostaining was done to measure the volume of activated microglia and inflammation; GFAP immunostaining was done to examine the extent of astrocyte activation (Figures 1 and 2).

Thionine histochemistry indicated no difference in infarct volume between endothelin and combined Aβ–endothelin groups 7 days after surgery (1.65±0.35 and 2.12±0.72 mm³, respectively). However, by 28 days, the infarct volume of the combined Aβ–endothelin group (4.27±0.90 mm³) was significantly larger compared with the 28-day endothelin alone group (0.77±0.16 mm³) and significantly larger than the combined Aβ–endothelin group at 7 days after surgery. In the absence of Aβ 25-35 injections, the endothelin group showed a significant decrease in infarct volume at 28 days compared with 7 days after surgery.

Volume measurements of activated microglia (Figure 2B) were significantly larger in the combined Aβ–endothelin group compared with the endothelin-alone groups at both 7 (18.25±2.86 and 7.62±1.43 mm³, respectively) and 28 days (34.31±4.91 and 4.23±0.67 mm³, respectively) after surgery. Over time, there was a significant decrease in volume of Ox-6 immunostaining in the endothelin alone group and a significant increase in volume of Ox-6 immunostaining in the combined Aβ–endothelin group at 28 days compared with 7 days after surgery.

For the astrocyte activation volume (Figure 2C), there was no difference in the volume of GFAP immunostaining between endothelin and combined Aβ–endothelin surgical groups 7 days after surgery (19.04±3.99 and 23.76±3.47 mm³). At 28 days, the volume of GFAP immunostaining in the combined Aβ–endothelin group (33.45±3.99 mm³) was significantly larger.
compared with the endothelin-alone group (9.53±2.96 mm³) and significantly larger than the combined Aβ–endothelin group at 7 days after surgery. The volume of GFAP immunostaining in the endothelin-alone surgical group was significantly smaller after 28 days compared with 7 days after surgery.

Barnes Circular Platform Test
The Barnes circular platform test is divided into 3 phases: training, testing, and reacquisition. The training phase (trials 1 to 14) occurred before surgical manipulation. The test phase (trial 15) tested the rat’s memory of the original hole location.
and took place 7, 14, or 28 days after surgical manipulation. The reacquisition phase (trials 16 to 29) used a different hole location and tested the rat’s ability to relearn the behavioral test. Two comparisons were made when examining the behavioral data.

Experimental groups yielded similar time score results during the initial training phase (Figure 3). After surgery and recovery, sham, Aβ 35-25 or endothelin-alone groups did not show any change in the time to find the hole for the test phase (trial 15) at any of the recovery times and these groups did not exhibit any differences in time results during the reacquisition phase.

During the testing phase for the Aβ 25-35 group, there was a significant and progressive increase in the time to find the hole for the 3 recovery times of 7, 14, and 28 days after surgery (46.7 ± 11.5, 53.7 ± 5.2, and 72.4 ± 7.8 seconds, respectively) compared with the end of the learning phase. The increase in time to find the hole was significantly higher in the 28-day recovery group compared with the 7- and 14-day recovery groups. During the reacquisition phase, the time scores for trials 16 to 29 were all significantly higher in the 28-day recovery period compared with the 7- and 14-day recovery periods.

To examine differences among the experimental groups 7, 14, and 28 days after surgery, both mean time to find the hole and mean errors were calculated (Figure 4). Data from the 7- and 14-day recovery group were similar; therefore, only the 7-day recovery data are shown (14-day recovery data online). There were no differences among the sham (control), Aβ 35-25, and endothelin groups either at the test phase (trial 15) or during the reacquisition phase (trials 16 to 29) for both time and errors. The Aβ 25-35 group showed significant increases in time and error scores during the test phase (trial 15) compared with sham rats at both 7 and 28 days after surgery. During the reacquisition phase, the Aβ 25-35 surgical group showed significant increases in both time and error scores 7 and 28 days after surgery.

Comparison of differences between the Aβ 25-35 and combined Aβ–endothelin groups indicated that during the test phase,
the combined Aβ–endothelin groups showed significant increases in time scores at 7 and 28 (Figure 4) days after surgery. During the reacquisition phase, the combined Aβ–endothelin groups showed significant increases in both time and error scores compared with the Aβ group 7 days after surgery.

**Discussion**

Data from this study support the hypothesis that CI (endothelin-1 injections) in the presence of high brain levels of amyloid (Aβ 25-35 injections) in the rat results in a significant and progressive increase in infarct size, an exacerbated neuroinflammatory response, and cognitive deficits. This is the first study to examine the progression of infarct size and pathological, neuroinflammatory, and cognitive deficits in combined rat models of AD and CI. Our model of AD, which uses single bilateral injections of Aβ 25-35 into the lateral ventricles, has many advantages. Our previous studies using this model demonstrated the presence of congophilic Aβ deposition; the presence of high levels of endogenous Aβ peptide, amyloid precursor protein, and tau proteins; microgliosis and astrocytosis in cortical and hippocampal regions; and cognitive deficits.\(^9\)\(^{–}\)\(^{31}\) The results from the present study now also demonstrate progressive memory and learning deficits over time.

Other studies have examined the combined effects of AD and CI using different paradigms. Several groups have demonstrated the expression of amyloid precursor protein in neurons and glia after global or focal cerebral ischemia.\(^9\)\(^{–}\)\(^{31}\) One study demonstrated that cerebral ischemia resulted in increase infarct sizes in mice overexpressing mutant amyloid precursor protein compared with wild-type mice.\(^9\)\(^2\) To our knowledge, the present study is the first to examine the combined effects of Aβ 25-35 and CI on infarct volume. Furthermore, this is the first study to correlate increases in infarct volume in combined rat models of AD and CI with increases in neuroinflammatory markers in the region of the infarct.

In the absence of induced high levels of amyloid, the ischemic rats demonstrated a decrease in infarct volume and periinfarct neuroinflammation at 28 days compared with 7 days after striatal endothelin injections. These results suggest that when no additional cellular stress is present, the ischemia-induced scar shrinks and the neuroinflammatory response dissipates over time likely as a result of endogenous brain repair mechanisms in the penumbral region. However, in the presence of high levels of amyloid induced by the Aβ 25-35 injections, there was a large and significant increase in the infarct size over time.

This progressive increase in the infarct size over time in the presence of high amyloid levels was similar to that seen in the measures of cognitive impairment. The Barnes circular platform test is a valuable tool for examining potential spatial memory and learning deficits. By varying the amount of time between the training and testing phase, we were able to show progressive deficits in memory and learning caused by Aβ 25-35 alone. Although the rats with CI alone showed no difference in learning behavior compared with either control groups, the combination of CI with the Aβ 25-35 injections demonstrated increases in both memory and learning compared with the Aβ 25-35 alone and this impairment became progressively greater over time. It is also important to note that rats receiving the inverse Aβ 35-25 peptide showed no behavioral differences from the control (sham) group. In this study, the control group using the inverse Aβ 35-25 peptide in combination with CI was not done. Although it is unlikely that the combination of the benign Aβ 35-25 peptide may interact with the endothelin-1 injections producing a behavioral deficit not seen in either group, one cannot rule out its possibility.

In summary, this demonstration of progressive increases in infarct size, neuroinflammation, and cognitive deficits in the
rat models may in fact be the equivalent of the interactions and the impact of cerebrovascular disease on the clinical expression of AD previously described.12

Disclosures
D.F.C. is a Career Investigator with Heart and Stroke Foundation of Canada. S.N.W is a National Science and Engineering Research Council Scholar.

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