A Mouse Model Characterizing Features of Vascular Dementia With Hippocampal Atrophy

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Background and Purpose—We have previously described effects of chronic cerebral hypoperfusion in mice with bilateral common carotid artery stenosis (BCAS) using microcoils for 30 days. These mice specifically exhibit working memory deficits attributable to frontal-subcortical circuit damage without apparent gray matter changes, indicating similarities with subcortical ischemic vascular dementia. However, as subcortical ischemic vascular dementia progresses over time, the longer-term effects that characterize the mouse model are not known.

Methods—Comprehensive behavioral test batteries and histological examinations were performed in mice subjected to BCAS for up to 8 months. Laser speckle flowmetry and 18F-fluorodeoxyglucose positron emission tomography were performed to assess cerebral blood flow and metabolism at several time points.

Results—At 2 hours after BCAS, cerebral blood flow in the cerebral cortex temporarily decreased to as much as 60% to 70% of the control value but gradually recovered to >80% at 1 to 3 months. At 5 to 6 months after BCAS, reference and working memory were impaired as demonstrated by the Barnes and radial arm maze tests, respectively. Furthermore, 18F-fluorodeoxyglucose positron emission tomography demonstrated that hippocampal glucose utilization was impaired at 6 months after BCAS. Consistent with these behavioral and metabolic abnormalities, histological analyses demonstrated hippocampal atrophy with pyknotic and apoptotic cells at 8 months after BCAS.

Conclusions—These results suggest that the longer-term BCAS model replicates advanced stages of subcortical ischemic vascular dementia when hippocampal neuronal loss becomes significant. (Stroke. 2010;41:1278-1284.)

Key Words: Alzheimer disease ▪ brain atrophy ▪ chronic cerebral hypoperfusion ▪ reference memory ▪ subcortical vascular dementia

Subcortical ischemic vascular dementia (SIVD) is one of the major subtypes of vascular dementia in elderly people and accounts for at least 10% to 20% of all dementia cases in developed countries.1 SIVD is characterized by white matter changes and lacunar infarctions in which cerebral blood flow (CBF) is decreased over an extended period of time because of small vessel changes.1-3 Few studies have explored the molecular mechanisms of SIVD devising chronic cerebral hypoperfusion models that reproduce white matter damage and behavioral changes characteristic of SIVD in rats and gerbils.4-6 However, the rat chronic cerebral hypoperfusion model has significant drawbacks, such as the development of visual impairment and its nonapplicability to genetically engineered mice.7 To address this issue, we have recently developed a mouse model of chronic cerebral hypoperfusion8 that can be produced by placing microcoils bilaterally on the common carotid arteries, resulting in bilateral common carotid artery stenosis (BCAS) for 30 days. The white matter damage was associated with working memory deficits but not with reference memory deficits, suggesting that this method serves as a model of SIVD.9 However, the neurological deficits arising from human SIVD are known to progress over a period of decades. It is therefore apparent that the BCAS model should be applied for a longer period encompassing a significant proportion of life to replicate the condition in humans. Our study therefore examined histological, metabolic, and behavioral changes in older mice after long-term cerebral hypoperfusion by BCAS. This would determine whether the longer-term hypoperfusion replicates advanced stages of SIVD and...
Histological Investigation
At 8 months after BCAS (12 months of age), the mice were euthanized under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneal) by transcardiac perfusion fixation and the brains were removed and processed as previously described. Detailed procedures and antibody information are given in the Supplemental Methods.

Image Analysis and Statistical Analysis
The applications used for the behavioral studies were based on the public domain National Institutes of Health's Image program (available at http://rsb.info.nih.gov/nih-image/) and ImageJ program (http://rsb.info.nih.gov/ij/), which were modified for each test by Tsuyoshi Miyakawa (available through O'Hara & Co, Tokyo, Japan). Statistical analysis was conducted using StatView (SAS Institute). Data were analyzed by 2-way analysis of variance or 2-way repeated measures analysis of variance, unless noted otherwise. Values in the graphs were expressed as mean±SEM. P<0.05 was considered statistically significant.

Results
General Health and CBF
All procedures for BCAS were accomplished within 15 minutes, except for an interval of 30 minutes between operating on the right and left common carotid arteries. By day 3 after the BCAS operation, at least 85% of the mice had survived and, after that period, there was no difference in survival rate between the sham-operated and BCAS-operated mice. In the control group, the mean CBF after the sham operation varied from 96.0% to 103.2%, without any significant changes between time intervals (1 factorial analysis of variance, P>0.2). In contrast, the CBF values decreased significantly from the preoperative baseline after the surgery in the BCAS-operated group. The CBF values temporarily decreased to 62.9±18.5% (mean±SE) at 2 hours after BCAS as compared to the sham group but gradually recovered to 81.7%±4.0% at 1 month, 83.2%±1.8% at 2 months, and 85.0%±8.7% at 3 months (sham, n=5; BCAS, n=6).

18F-FDG Positron Emission Tomography Analysis
The first 5-minute uptake of 18F-FDG in the cerebral cortex decreased to ~70% of the pre-BCAS level at 2 hours after BCAS and recovered to 88% of the pre-BCAS level at 2 months after BCAS (Figure 2A, 2C). The early 18F-FDG uptake scan in the striatum showed a similar temporal profile to that of the cerebral cortex. By contrast, the first 5-minute uptake of 18F-FDG in the hippocampus did not decrease at 2 hours or 2 months after BCAS but decreased at 6 months. The late 18F-FDG uptake scans showed that the glucose uptake of the hippocampus did not decrease by 2 months after BCAS but decreased by 20% at 6 months after BCAS (Figure 2B, 2D).

Behavioral Tests: Working Memory Test
The number of different arm choices in the first 8 entries ranges from 5.3 for a “chance” performance to 8 for a “perfect” performance. Sham-operated mice improved their performance significantly more than did BCAS-operated mice. In contrast, BCAS-operated mice performed significantly more poorly than sham-operated mice on the Barnes maze test (Figure 2D). The Barnes maze test is a spatial memory test and was used to assess the effect of chronic hypoperfusion on hippocampal function. The number of different arm choices in the first 8 entries was significantly lower in BCAS-operated mice than in sham-operated mice (P<0.05). However, the number of different arm choices in the first 8 entries was significantly lower in BCAS-operated mice than in sham-operated mice (P<0.05).
mice with each consecutive training session (Figure 3A). The difference was significant in the 1 to 22 trials ($P<0.0024$; 2-way repeated measures analysis of variance) but not in the 23 to 28 trials ($P=0.1768$). In terms of the number of revisiting (errors), the sham-operated mice improved their performance over the course of training, whereas BCAS-operated mice did not and made significantly more errors than did the sham-operated control (Figure 3B). The difference was significant both in the 1 to 22 trials ($P=0.0098$) and in the 23 to 28 trials ($P=0.0007$).

**Reference Memory Test**

The mice with BCAS exhibited prolonged latency to reach the desired target ($P=0.010$; Figure 4A) and increased number of errors ($P=0.0017$; Figure 4B) compared to the sham-operated mice. The first probe test showed that time spent around each hole did not differ between the BCAS-operated and sham-operated mice ($P=0.6839$; Figure 4C). Consistent with this, there was a significant preference for the target hole vs hole at angle 30° both in the BCAS-operated and sham-operated mice ($P<0.0001$ each; $P<0.0024$ is statistically significant by Bonferroni/Dunn test). The second probe test showed that the time spent around each hole tended to be different between the 2 groups (Figure 4D; $P=0.0520$). There was a significant preference for the target hole in the sham-operated mice ($P<0.0001$), whereas such significant preference was absent in the BCAS-operated mice ($P=0.0089$; $P<0.0024$ is statistically significant). Thus, the probe test suggested that longer-term memory retention was preferably impaired in the BCAS-operated mice.

**Other Behavioral Tests**

The results of other behavioral tests (Figure 1) are described in Supplemental Materials, including Supplemental Figures I and II (locomotion and motor function, respectively; available online at http://stroke.ahajournals.org). Briefly, the BCAS-operated mice exhibited impairment in locomotion and motor function after 3 months.
Histological Findings: Hippocampal Atrophy in Mice With Longer-Term BCAS

At 8 months after BCAS, pyknotic neurons were frequently observed in the cerebral cortex and the hippocampus (Figure 5A). Furthermore, there was significant atrophy in the hippocampus ($P = 0.0064$) but not in the cerebral cortex ($P = 0.8937$) or the corpus callosum ($P = 0.8751$; Figure 5B). The number of fragmented or shrunken nuclei stained for single-stranded DNA increased in the CA1 ($P = 0.0005$) and CA3 sectors of the hippocampus ($P = 0.0086$) but not in the dentate gyrus ($P > 0.05$; Figure 5C). We did not detect amyloid $\beta$ deposits or axonal amyloid precursor protein immunoreactive accumulation in the hippocampus or the cerebral cortex at 8 months after BCAS (data not shown).

Involvement of Cholinergic Fibers

The acetylcholine esterase-positive cholinergic fibers in the external capsule were stained similarly between the sham-operated and BCAS-operated mice, but the cholinergic fibers in the adjacent cortex became disarrayed and fewer, indicating morphological changes in cholinergic fibers (Figure 5D). Densitometric analysis showed a reduction in the cholinergic fibers of the BCAS-operated mice ($n = 6$; % stained area, $20.0\% \pm 12.5\%$) compared to the sham-operated mice ($n = 4$; $41.0\% \pm 4.8\%$). The cell count of choline acetyltransferase-positive cells in the nucleus basalis of Meynert neurons, however, did not differ significantly between the 2 groups but there was a tendency toward a decrease in the BCAS-operated group ($64.9\% \pm 13.2/mm^2$ in the BCAS-operated vs $74.4\% \pm 11.1/mm^2$ in the sham-operated groups; mean $\pm$SE).

Discussion

Histological and behavioral differences between the previous shorter-term BCAS model$^8,9$ and the present longer-term model are summarized in the Table. In the shorter-term model, specific white matter changes were observed without any apparent cerebral cortical or hippocampal changes, which might explain why working memory, but not reference memory, is impaired. By contrast, in the present longer-term BCAS model, not only the white matter changes but also the hippocampal changes (atrophy and cell death) were documented by 8 months after BCAS. Consistent with these histological changes, the series of behavioral batteries demonstrated deficits in both working and reference memory. Thus, mild cerebral ischemia of an insufficient magnitude appears to induce subacute pathologies, which may lead to subsequent changes in the gray matter, including the cerebral cortex and hippocampus.

Laser speckle flowmetry showed that in the cerebral cortex, the CBF decreased at 2 hours after BCAS but recovered by 1 to 3 months. Such temporal profile of CBF was similar to that of the first 5-minute $^{18}$F-FDG uptake in the cerebral cortex. Such a similarity and linear correlation of the early uptake of $^{18}$F-FDG with $^{15}$O-measured blood flow$^{14}$ suggest that the early $^{18}$F-FDG uptake scan can serve as an estimate of CBF. This may also be supported by the substantially different patterns of $^{18}$F-FDG uptake between the early and late $^{18}$F-FDG images. Intriguingly, in the hippocampus, the CBF estimated from the first 5-minute $^{18}$F-FDG uptake did not decrease at 2 months after BCAS probably because the hippocampus is supplied by both anterior and posterior circulations.$^{15}$ However, the hippocampal CBF was found to be decreased at 6 months after BCAS. This temporally correlated with reduced glucose metabolism in the hippocampus and global memory impairment. Given that the shorter-term BCAS mice demonstrate white matter damage without any apparent hippocampal damage at 1 month after BCAS,$^8$ hippocampal degeneration may be secondary to the preceding white matter damage. This may then subsequently contribute to the dementia syndrome, partly overlapping with Alzheimer disease (AD) in their cognitive profiles and histological changes. These findings are intriguing given the widely accepted fact that vascular dementia and AD both increase in prevalence with age, frequently occur concomitantly, and overlap considerably in their symptomatology, pathophysiology, and comorbidity.$^{16}$

Consistent with this notion, patients with AD and SIVD are reported to show a similar pattern of pyramidal neuronal loss and atrophy in the hippocampal CA1 sector.$^{17,18}$ Moreover, most AD patients show amyloid angiopathy$^{19}$ and decreased capillary beds in the cerebral cortex,$^{20}$ thereby leading to chronic cerebral hypoperfusion. These results, as well as those presented here, suggest a bidirectional relationship between AD and SIVD.

By contrast, a recent report from the Honolulu Asia Aging Study suggests that the burden of vascular-type and AD-type
lesions are independent. However, there is a possibility that the studies of vascular dementia contain some proportion of large vessel disease or infarction. Our longer-term BCAS model does not develop overt cerebral infarction despite the global memory deficits. This strengthens the potential significance of chronic noninfarctional hypoperfusion as a cause of the dementia syndrome. The reasoning is also consistent with the hypothesis that hippocampal sclerosis is often accompanied by leukoencephalopathy, and that occult hypoxic-ischemic episodes may represent its pathogenic

Figure 5. Hippocampal atrophy with pyknotic and apoptotic neurons in BCAS-operated mice. Histological examination was performed in mice with BCAS for 8 months (12-month-old). A, Representative images of the deep layer of the frontal cerebral cortex (upper panels) and the CA1 sector of the hippocampus (lower panels) of the sham (left panels) and BCAS-operated mice (right panels). Arrows indicate pyknotic neurons. *P<0.01, scale bars=50 μm. B, Representative hematoxylin and eosin images of the sham-operated (upper left, n=4) and BCAS-operated mice (upper right, n=7), and area of the hippocampus (lower left), cerebral cortex (lower middle), and the corpus callosum (lower right) relative to the brain. Note resultant dilation of the ventricle in the BCAS-operated mice. Scale bars=1 mm. C, Representative images showing fragmented or shrunken nuclei stained with single-stranded DNA (ssDNA) (red) in the hippocampus of the sham-operated (upper left, n=5) and BCAS-operated mice (upper right, n=6), and ssDNA+/DAPI-positive neuronal ratio in the CA1 (lower left) and CA3 (lower middle) sectors and the dentate gyrus (lower right) of the hippocampus. Nuclei are stained with DAPI (blue). *P<0.01, scale bars=50 μm. D, Representative images for acetylcholine esterase of the sham-operated (upper) and BCAS-operated mice (lower). Scale bars=50 μm.

Table. Histological and Behavioral Comparisons Between the Existing Model and the Present Longer-Term Model of BCAS

<table>
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<tr>
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<th>Existing Model</th>
<th>Current Longer-Term Model</th>
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<tbody>
<tr>
<td>Duration of cerebral hypoperfusion</td>
<td>30 days</td>
<td>Up to 8 mo</td>
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<tr>
<td>Timing of BCAS</td>
<td>Age 2–3 mo</td>
<td>Age 4 mo</td>
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<tr>
<td>Working memory deficits</td>
<td>+ at age 3 mo</td>
<td>+ at age 9 mo</td>
</tr>
<tr>
<td>Reference memory deficits</td>
<td>None at age 3 mo</td>
<td>+ at age 10 mo</td>
</tr>
<tr>
<td>Histological changes in the cerebral cortex</td>
<td>Undetectable</td>
<td>Pyknotic neurons</td>
</tr>
<tr>
<td>Histological changes in the hippocampus</td>
<td>Undetectable</td>
<td>Atrophy with pyknotic and apoptotic neurons</td>
</tr>
<tr>
<td>Histological changes in the white matter</td>
<td>Demyelination with oligodendrocytic apoptosis</td>
<td>Demyelination with oligodendrocytic apoptosis</td>
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factors. Thus, chronic “noninfarctional” hypoperfusion may accelerate hippocampal neurodegeneration in a latent manner, as is reproduced in the present mouse model. Nevertheless, amyloid β deposits, which are a pathological hallmark of AD, are not detectable in this model at least up to 8 months after the BCAS operation. This is probably because, in contrast with their human counterpart, murine amyloid β proteins do not have propensity to form oligomers and fibrils. In support of this notion, AD model mice overexpressing the mutant form of human amyloid precursor protein have greater amounts of amyloid β fibrils in their brains after 30 days of cerebral hypoperfusion by BCAS, implying a causal link between vascular and human amyloid β neuropathology.

A limitation of this study is that the effectiveness of the first 5-minute 18F-FDG blood flow analysis needs to be further confirmed. In tumor diagnosis, blood flow estimated from the early uptake of 18F-FDG is linearly correlated with 15O-measured blood flow (the current gold standard method for measuring blood flow in humans). However, because the mean positron range of 15O is far larger than that of 18F (2.5 mm vs 0.6 mm), 15O-water is insufficient to apply to a small animal positron emission tomography scanner in terms of the spatial resolution. In addition, 15O-water is a short-lived tracer with a 2-minute half-life, necessitating an on-site cyclotron. Therefore, 18F-FDG imaging may be practical and feasible to estimate CBF noninvasively in rodent models for the simple first 5-minute, despite the incomplete extraction of 18F-FDG compared to 15O-water. Although appropriate kinetic models of 18F-FDG will be required to separate the flow component and K1 from the metabolic component of uptake, a method of measuring blood flow and metabolism from a single injection of 18F-FDG uptake may be an important addition to functional imaging of rodent disease models with 18F-FDG positron emission tomography.

Another limitation of this study is that the long-term BCAS mice do not have lacunar infarcts, which are a key feature of the advanced stages of SIVD and are often linked with development of motor dysfunction. However, despite absence of lacunar infarcts, the BCAS mice exhibited impaired motor function at 3 months after BCAS, recapitulating one of the key features of SIVD. The fractal–subcortical circuit disruption may have resulted not only in the cognitive impairment but also in the motor dysfunction. Therefore, this longer-term BCAS model will provide evidence linking chronic hypoperfusion and aging-associated neurological deficits such as cognitive and motor impairment. However, this model does not (no current models can) describe all features of SIVD.

In conclusion, we describe a mouse model of longer-term cerebral hypoperfusion that at least partially replicates an advanced stage of SIVD in which hippocampal neuronal loss becomes significant. This mouse model exhibits global memory disturbances, which may help further elucidate the mechanism by which neurodegeneration and dementia progress in the elderly, and enhances strategies to tackle these disorders. Further characterization of our model, particularly in view of the hippocampal circuitry, may help to decipher the substrates associated with impaired memory.

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Disclosures

None.

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