Baseline Cognitive Function, Recurrent Stroke, and Risk of Dementia in Patients With Stroke

Pamela M. Rist, ScD; John Chalmers, MD; Hisatomi Arima, MD; Craig Anderson, MD; Stephen MacMahon, PhD; Mark Woodward, PhD; Tobias Kurth, MD, ScD; Christophe Tzourio, MD, PhD

Background and Purpose—To determine the interrelationships between baseline Mini-Mental State Examination (MMSE) score and risk of overall dementia, post-recurrent stroke dementia, and dementia without recurrent stroke among patients with a history of stroke.

Methods—Prospective cohort study among participants enrolled in the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) for whom baseline MMSE score was available. Baseline MMSE score was divided into 4 categories: 30, 29–27, 26–24, and <24. Participants were followed for incident dementia and recurrent stroke. Logistic regression models were used to examine the association between MMSE score and dementia.

Results—Of the 6080 participants included in this analysis, 2493 had an MMSE score of 30, 1768 had a score of 29–28, 1369 had a score of 26–24, and 450 had a score of <24. Average follow-up time was 3.8 years. There were 407 cases of dementia, 106 of which were preceded by a recurrent stroke. The risk of overall dementia increased with decreasing MMSE score. However, the impact of MMSE score on the risk of dementia without recurrent stroke was much stronger than the impact of MMSE score on the risk of post-recurrent stroke dementia. For those with MMSE score <24, the risk of dementia without recurrent stroke was 47.89 (95% confidence interval, 28.57–80.26), whereas the risk of post-recurrent stroke dementia was only 7.17 (95% confidence interval, 3.70–13.89). Higher MMSE scores were even less strongly associated with the risk of post-recurrent stroke dementia.

Conclusions—Patients with stroke with low MMSE scores are at high risk of dementia over time, even in the absence of a recurrent stroke, and should therefore be followed closely for further cognitive decline. (Stroke. 2013;44:00-00.)

Key Words: cerebrovascular disease ■ cognitive functioning ■ dementia ■ epidemiology
and incident dementia. Using data from this study, we aimed to
determine the interrelationships between baseline MMSE
score and the risk of overall dementia, post-recurrent stroke
dementia, and dementia without the presence of a recurrent
stroke. We hypothesized that recurrent stroke is a strong risk
factor for the development of dementia and cognitive status
before recurrent stroke may modify the impact of recurrent
stroke on the development of dementia.

Methods
Previous studies have described the design of PROGRESS.6,7 In
brief, PROGRESS was a randomized, double-blind, placebo-con-
trolled trial to determine the effectiveness of a blood pressure-
lowering regimen to prevent recurrent stroke and dementia among
6105 participants with previous stroke or transient ischemic attack.
Participants were recruited from 172 collaborating centers in 10
countries from May 1995 to November 1997. To be eligible, partici-
pants needed to have had either a stroke or transient ischemic attack
(but not subarachnoid hemorrhage) within the past 5 years and have
no clear indication for, nor a contraindication to, treatment with
an angiotensin-converting enzyme inhibitor. In addition, dementia was
an exclusion criterion. After a run-in period, participants who toler-
ated and adhered to perindopril therapy were randomly assigned to
continued active treatment or placebo. Randomization was stratified
by study center, age, sex, systolic blood pressure at entry, inclusion
diagnosis, and the intention to begin combination therapy or single
drug therapy.

Cognitive Decline and Dementia Assessment
At baseline, the 6- and 12-month visits, and annually thereafter, par-
cipants completed the MMSE.3 One point was awarded for each
successfully completed item (maximum score of 30); no points were
awarded for any missing item. Baseline MMSE score was divided a
priori into 4 categories: 30 (high MMSE score), 29–28 (medium-high
MMSE score), 27–24 (medium-low MMSE score), and ≤24 (low
MMSE score). In the event that baseline MMSE score was missing
(n=32), baseline MMSE score was imputed using the MMSE score
from the 6-month visit (n=7). Participants for whom baseline MMSE
score could not be imputed were excluded from the analysis (n=25).

Throughout the follow-up, a 2-phase screening and assessment
process was used to diagnose dementia.1 Participants meeting any
of the following criteria were considered to be screened positive for
cerebral stroke dementia: an MMSE score ≤25 at any follow-up visit, a decline in
the MMSE score of ≥14 points between any 2 follow-up visits, an
MMSE score missing for ≥2 scheduled follow-up visits, or a positive
response by the investigator to the question: In your opinion, does this
patient have dementia? All participants who screened positive were
referred to a local specialist experienced in diagnosing dementia. The
specialists were blinded to treatment assignment and to all clinical
data. Participants who screened negative were classified as not hav-
ing dementia.

The local specialist used a checklist based on the criteria for the
diagnosis of dementia as defined in the Diagnostic and Statistical
Manual of Mental Disorder, Fourth Edition.8 The questionnaire in-
cluded systematic questions on the presence of poststroke focal defi-
cits, such as aphasia or motor deficit, and on the presence of more
global problems, such as depressed mood, that could have altered
the diagnosis of dementia. Local specialists were also systematically
asked whether the diagnosis of dementia was reliable. Whenever
possible, the specialist examined the patient. If an interview could
not be conducted, data were sought from all other available sources,
including medical records, interviews with family members, and con-
sultations with other medical professionals. After receiving the infor-
mation from and diagnosis of the local specialist, a 2-person central
Dementia Adjudication Committee confirmed or refuted the diagnosis
and assigned each screen-positive case to 1 of the following 4 catego-
ries: certain dementia, fairly certain (probable) dementia, uncertain
(possible) dementia, or no dementia. No attempt was made to further
classify cases into subtypes of dementia because all participants had
a history of cerebrovascular disease and often had other vascular risk
factors. The main outcome for this analysis was the occurrence of
dementia, either certain dementia or fairly certain dementia.

Stroke Assessment
Recurrent fatal or nonfatal stroke was defined as an acute disturbance
of focal neurological function with symptoms lasting >24 hours (or
resulting in earlier death) thought to be because of either cerebral in-
farction or cerebral hemorrhage.3,9 All suspected strokes and deaths
were first reported by local study investigators and then reviewed by
experts on the central End Point Adjudication Committee. This com-
mittee was provided with a clinical summary of the event and copies
of any available investigation reports (eg, biochemistry, hematology,
radiology, and autopsy findings).11 Strokes were classified as cerebral
hemorrhage, ischemic stroke, or stroke of unknown pathological type.
If dementia occurred before the recurrent stroke event, the person
was classified as dementia without recurrent stroke. If stroke event
occurred before dementia, the person was classified as post-recurrent
stroke dementia.

Statistical Analysis
First, we used logistic regression models to calculate the odds ratio
of recurrent stroke dementia and cognitive status so we used the missing indicator method.
No participant had missing information on age, sex, current alco-
hol consumption, diabetes mellitus status, or systolic blood pressure.
Fewer than 100 people were missing information on height and
smoking and were assigned to the median and past smoker, re-
spectively. More than 100 people had missing information on educa-
tion status so we used the missing indicator method.

All statistical analyses were performed using SAS 9.1.3. All P val-
ues are 2-tailed, and P<0.05 was considered statistically significant.

Results
Of the 6080 participants included in this analysis, 2493 (41.0%)
had an MMSE score of 30, 1768 (29.1%) had an MMSE score
of 29–27, 1369 (22.5%) had an MMSE score of 26–24, and 450
(7.4%) had an MMSE score of <24. Average follow-up time was
3.8 years. There were 407 cases of dementia, 106 of which were
preceded by a recurrent stroke. A total of 709 strokes occurred
either before dementia onset or the end of the follow-up.
Table 1 shows covariates by baseline MMSE categories for our study population. Those with the lowest MMSE scores were older, shorter in stature, had stopped schooling at a younger age, more likely to be female, consumed alcohol less frequently, had higher systolic blood pressure, were more likely to have diabetes mellitus, and were less likely to be current smokers than participants with higher MMSE scores.

The impact of baseline MMSE score on the risk of overall dementia is presented in Table 2. The risk of dementia increased with decreasing MMSE scores. Those with an MMSE score of 28–29 had an RR of dementia of 2.15 (95% confidence interval [CI], 1.43–3.24), whereas those with an MMSE score of <24 had an RR of dementia of 26.81 (95% CI, 18.08–39.76) compared to those with an MMSE score of 30.

The Figure shows the proportion of post-recurrent stroke dementia and dementia without recurrent stroke by MMSE category. Among those with an MMSE score of 30, 50% of those who develop dementia had dementia without recurrent stroke. In contrast, among those with a low MMSE score (<24), nearly all those who develop dementia had dementia without recurrent stroke.

The effect estimates for the association between baseline MMSE score and post-recurrent stroke dementia and between MMSE score and dementia without recurrent stroke can be seen in Table 2. For both outcomes, we observed an increase in the risk of dementia with decreasing MMSE scores. However, the impact of MMSE score on risk of dementia without recurrent stroke was much stronger than the impact of MMSE score on the risk of post-recurrent stroke dementia. For those with an MMSE score <24, the risk of dementia without stroke was 47.89 (95% CI, 28.57–80.26), whereas the risk of post-recurrent stroke dementia was only 7.17 (95% CI, 3.70–13.89). Higher MMSE scores were even less strongly associated with the risk of post-recurrent stroke dementia. The risk of post-recurrent stroke dementia was only 1.32 (95% CI, 0.70–2.49) among those with an MMSE score 29–28, whereas the risk of dementia without stroke was 2.99 (95% CI, 1.73–5.18).

Results stratified by stroke subtype (ischemic versus hemorrhagic; results not shown) were similar to those shown above. Results among those assigned to placebo were similar to those seen for the full cohort (results not shown). Results of

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MMSE Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Age, mean (SE)</td>
<td>61.4 (9.5)</td>
</tr>
<tr>
<td>Female, %</td>
<td>27.8</td>
</tr>
<tr>
<td>Height, mean (SE)</td>
<td>167.3 (8.9)</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
</tr>
<tr>
<td>Never smoked regularly</td>
<td>44.7</td>
</tr>
<tr>
<td>Past smoker</td>
<td>33.8</td>
</tr>
<tr>
<td>Current smoker</td>
<td>21.6</td>
</tr>
<tr>
<td>Current alcohol consumption, %</td>
<td></td>
</tr>
<tr>
<td>&lt;1/week</td>
<td>60.5</td>
</tr>
<tr>
<td>1 to 8 drinks/week</td>
<td>18.0</td>
</tr>
<tr>
<td>≥8 drinks/week</td>
<td>21.5</td>
</tr>
<tr>
<td>Age at which schooling stopped, y</td>
<td></td>
</tr>
<tr>
<td>≤14</td>
<td>22.3</td>
</tr>
<tr>
<td>15–16</td>
<td>20.1</td>
</tr>
<tr>
<td>17–19</td>
<td>26.4</td>
</tr>
<tr>
<td>&gt;19</td>
<td>31.3</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>12.0</td>
</tr>
<tr>
<td>Systolic blood pressure, mean (SE)</td>
<td>145.4 (18.8)</td>
</tr>
<tr>
<td>Type of qualifying event, %</td>
<td></td>
</tr>
<tr>
<td>TIA</td>
<td>22.9</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>64.6</td>
</tr>
<tr>
<td>Hemorrhagic stroke</td>
<td>9.8</td>
</tr>
<tr>
<td>Unknown</td>
<td>2.7</td>
</tr>
<tr>
<td>Medication, %</td>
<td></td>
</tr>
<tr>
<td>Antihypertensive therapy</td>
<td>57.6</td>
</tr>
<tr>
<td>Antiplatelet therapy</td>
<td>71.9</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>7.1</td>
</tr>
<tr>
<td>Lipid-lowering therapy</td>
<td>15.8</td>
</tr>
</tbody>
</table>

PROGRESS indicates Perindopril Protection Against Recurrent Stroke Study; and TIA, transient ischemic attack.
our other sensitivity analyses were also similar to those seen for the full cohort (online-only Data Supplement).

To further explore why baseline MMSE score was not as strongly associated with the risk of post-recurrent stroke dementia, we examined the associations between baseline MMSE and the risk of recurrent stroke as well as between recurrent stroke and the risk of dementia. Baseline MMSE score was not associated with the risk of recurrent stroke (results not shown). However, recurrent stroke was significantly associated with the risk of dementia (RR, 2.93; 95% CI, 2.24–3.82).

Discussion

In this prospective cohort of patients with stroke, baseline MMSE score was strongly associated with the risk of developing dementia. Analyses examining post-recurrent stroke dementia versus dementia without recurrent stroke suggested stronger associations between baseline MMSE score and risk of dementia without recurrent stroke than with post-recurrent stroke dementia.

Previous studies have shown that both lower MMSE scores and stroke predict the risk of subsequent dementia.3−4 However, data on the interrelationships among prestroke MMSE, stroke, and the risk of dementia in one study are sparse, mainly because small numbers of stroke or dementia events prohibits the evaluation of joint effects. Three studies have examined the interrelationships among prestroke cognitive functioning, stroke, and cognitive impairment. Data from the Framingham Heart Study showed that participants who experienced a stroke had significantly lower mean MMSE scores prestroke and poststroke compared to the stroke-free participants.12 The Baltimore Longitudinal Study of Aging found that those with mild cognitive impairment who experience a stroke are at increased risk of developing dementia (odds ratio, 12.4; 95% CI, 1.5–99) compared to those with mild cognitive impairment who do not experience a stroke.13 Another study using data from the Health and Retirement Study found that the rate of cognitive decline was faster among those who later survived a stroke compared to those who remained stroke-free throughout follow-up. Those who died after stroke had even faster rates of decline. After the stroke event, the rate of decline among stroke survivors was similar to their rate of decline before the stroke event.14 Although these studies were able to examine the relationships between cognitive functioning and first stroke, research on the interrelationships among cognitive function, recurrent stroke, and risk of subsequent dementia is sparse. A few studies that assessed cognitive functioning in patients with stroke have shown links between cognitive decline and dementia,15,16 but

Table 2. Multivariate-Adjusted* Relative Risks of Overall Dementia, Post-recurrent stroke Dementia, and Dementia Without Recurrent Stroke by Baseline MMSE Score

<table>
<thead>
<tr>
<th>Baseline MMSE</th>
<th>Crude Incidence Rate (Per 1000 Person-Years)</th>
<th>RR (95% CI)</th>
<th>Crude Incidence Rate (Per 1000 Person-Years)</th>
<th>RR (95% CI)</th>
<th>Crude Incidence Rate (Per 1000 Person-Years)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>2455</td>
<td>3.86</td>
<td>1.00</td>
<td></td>
<td>1.93</td>
<td>1.00</td>
</tr>
<tr>
<td>28–29</td>
<td>1703</td>
<td>9.54</td>
<td>2.15 (1.43–3.24)</td>
<td>3.08</td>
<td>1.32 (0.70–2.49)</td>
<td>4.48</td>
</tr>
<tr>
<td>24–27</td>
<td>1218</td>
<td>28.87</td>
<td>6.59 (4.54–9.55)</td>
<td>44</td>
<td>8.41 (2.10–6.43)</td>
<td>107</td>
</tr>
<tr>
<td>&lt;24</td>
<td>297</td>
<td>105.08</td>
<td>26.81 (18.08–39.76)</td>
<td>22</td>
<td>15.11 (3.70–13.89)</td>
<td>131</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; MMSE, Mini-Mental State Examination; and RR, relative risk.

*Results have been adjusted for age, sex, height, smoking status, current alcohol consumption, educational status, diabetes mellitus, baseline systolic blood pressure, and randomized treatment assignment.
many could not specifically examine the effect of recurrent stroke on the association between cognitive functioning and dementia. One study directly tested the interaction between baseline cognitive status and incident stroke using participants in the Rotterdam Study who were free of dementia or a history of stroke at baseline and found no interactive effect of incident stroke and measures of prestroke cognitive function on the risk of dementia.17

The present study showed that among those who have already experienced a stroke or transient ischemic attack, baseline MMSE score is a strong predictor of the risk of dementia without recurrent stroke. Results from our study suggest that patients with stroke with low MMSE score are already on a trajectory of cognitive decline related to preexisting neurodegenerative lesions or other undetermined factors triggered by the initial stroke event. The stroke event may increase the risk of dementia either because of the direct impact of vascular disease on other neuropathological changes associated with Alzheimer disease or by synergistic effects of Alzheimer neuropathology and vascular neuropathology.18 This has important clinical implications because it demonstrates that for those with low MMSE score, a recurrent stroke is not necessary to develop dementia. This result could seem counterintuitive because in patients with stroke, one would expect a second stroke to result in a greater risk of cognitive decline and vascular dementia. A review article found that the rate of dementia was at least twice as high after recurrent stroke as it was after first stroke and the rate of dementia after recurrent stroke may depend on the number of recurrent strokes.20

An important finding from the main analyses of PROGRESS is that a blood pressure–lowering regimen decreased the risk of recurrent stroke and the risk of post-recurrent stroke dementia.20 In the present study, the associations between baseline MMSE score and post-recurrent stroke dementia were weaker than those seen for the associations between baseline MMSE score and dementia without recurrent stroke. Although baseline MMSE score is highly predictive of dementia in the absence of stroke, the impact of recurrent stroke on the risk of dementia outweighs the impact of baseline MMSE score. Therefore, patients, especially those with high MMSE score who have the lowest risk of dementia without recurrent stroke, should be delivered strong stroke prevention messages. Given the associations seen between blood pressure–lowering drugs and a reduced risk of recurrent stroke, patients with high MMSE score may want to consider a blood pressure–lowering regimen to avoid another stroke and the associated risk of dementia.

One of the main strengths of this study was that unlike previous studies, we had information on MMSE score before recurrent stroke and new diagnoses of dementia after recurrent stroke. Other strengths include the large number of outcome events, which allowed us to assess the interrelationships among baseline MMSE score, recurrent stroke, and risk of dementia. By screening all participants at baseline for dementia, the cohort of individuals in this study were free of preexisting dementia.

Despite the strengths of this study, some weaknesses should be noted. Although this study did have a large number of outcome events, many of our CIs for our effect estimates are wide. In addition, we did not have information on MMSE score before first stroke, which prevented us from determining whether first stroke may be the triggering factor for cognitive decline or whether the patients had already experienced decline before stroke. We took several steps to obtain a consistent diagnosis of dementia across the various centers, including using standardized forms and having a central Dementia Adjudication Committee to minimize heterogeneity. Although it is possible that people with undiagnosed or mild cognitive impairment at baseline may have been included in our study, dementia was an exclusion criterion for entry into the trial, and we used the original trial cohort for these analyses. Although 4 different tools were used to screen for dementia, they were mainly based on MMSE score, which is not sensitive to vascular cognitive impairment. Therefore, it is possible that some cases of dementia may have been missed.

To summarize, these results carry important messages for patients with stroke and their physicians that vary according to the baseline MMSE score of the patient with stroke. Patients with stroke with low MMSE scores are at high risk of dementia over time, even in the absence of a recurrent stroke, and should therefore be followed closely for further cognitive decline.

Sources of Funding
The Perindopril Protection Against Recurrent Stroke Study was funded by grants from Servier (Paris, France), the Health Research Council of New Zealand (Auckland, New Zealand), and the National Health and Medical Research Council of Australia (Canberra, Australia). The study was designed, conducted, analyzed, and interpreted by the investigators independent of all sponsors. Dr Rist was funded by a training grant from the National Institute of Aging (AG-00158) and by the Rose Traveling Fellowship Program in Chronic Disease Epidemiology and Biostatistics at the Harvard School of Public Health.

Disclosures
Dr Chalmers and MacMahon have received lecture fees and research grants from Servier. Dr Arima holds a Future Fellowship from the Australian Research Council. Dr Anderson holds a Senior Principal Research Fellowship from the National Health and Medical Research Council. Dr Woodward has received lecture fees from Servier. Dr Kurth has received funds from Allergan and the American Academy of Neurology for educational lectures. Dr Tzourio has received fees from the ABBOTT Company for participating in scientific committees. The other author has no conflict to report.

References


Baseline Cognitive Function, Recurrent Stroke, and Risk of Dementia in Patients With Stroke
Pamela M. Rist, John Chalmers, Hisatomi Arima, Craig Anderson, Stephen MacMahon, Mark Woodward, Tobias Kurth and Christophe Tzourio

Stroke. published online May 16, 2013;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2013/05/16/STROKEAHA.111.680728

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2013/05/16/STROKEAHA.111.680728.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
ONLINE SUPPLEMENT

Supplemental Methods

Antibodies
Rabbit polyclonal anti-Akt, anti-p-Akt (Ser473), anti-MKK7, anti-p-MKK7 (Ser171, Thr275), anti-p-MLK3 (Thr277, Ser281), monoclonal anti-JNK3 (55A8) and monoclonal anti-β-actin (13E5) antibodies were purchased from Cell Signaling Biotechnology (Boston, MA). Rabbit polyclonal anti-GluK2, anti-MLK3, and mouse monoclonal anti-p-JNKs (Thr 183, Tyr 185, G-7) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). A mouse monoclonal anti-PSD-95 (clone 7E3-1B8) antibody was obtained from Sigma (Saint Louis, MO).

Drug Administration
A selective PI3K inhibitor LY294002 (BioMol, Plymouth Meeting, PA), a specific Akt inhibitor Akt inhibitor IV (Calbiochem) and a potent MEK1 inhibitor PD98059 (Cell Signaling Biotechnology) were used to detect the role of PI3K-Akt and MEK1 signaling in the postconditioning-mediated neuroprotection respectively. LY294002 (100 nmol) or PD98059 (20 nmol) in 10μl DMSO was infused into the rat cerebral ventricle (from the bregma: posterior, 0.8 mm; lateral, 1.5 mm; depth, 3.5 mm) through a stepper-motorized microsyringe (Stoelting, Wood Dale, IL) 20 minutes before ischemia. Akt inhibitor IV (100 nmol) in 10μl DMSO was infused into the rat cerebral ventricle 2 hours after ischemia.

Histological Assessment
Rats were perfusion-fixed with 4% paraformaldehyde under anesthesia after 5 days of reperfusion. Brains were removed and further fixed with the same fixation solution at 4°C overnight. Post-fixed brains were embedded by paraffin and then coronal sections (6μm thick) were prepared using a microtome. The paraffin embedded brain sections were deparaffinized with xylene and rehydrated in a gradient of ethanol, followed by washing with distilled water. The sections were stained with cresyl violet for the assessment of neuronal survival in the hippocampus. The number of surviving hippocampal CA1 neurons per 1 mm length was counted as the neuronal density.

Immunoprecipitation
The hippocampal CA1 regions were isolated after the indicated times of reperfusion and rapidly frozen in liquid nitrogen. Samples were homogenized in ice-old homogenization buffer. The homogenates were centrifuged at 800g/4°C for 10 minutes and the supernatants were collected. Sample proteins were incubated overnight at 4°C with appropriate antibodies diluted in immunoprecipitation buffer. After the addition of protein A/G, the mixture was incubated at 4°C for an additional 2 hours. The bound proteins were collected from Protein A/G by boiling for 5 minutes in Laemmlli sample buffer.
**Immunoblot**
Protein samples were separated by SDS-PAGE and then electrotransferred onto a nitrocellulose membrane. After blocking, the membranes were probed with primary antibodies overnight at 4°C. Detection was carried out by appropriate alkaline phosphatase-conjugated IgG (Sigma) and developed with NBT/BCIP assay kit (Promega).

**Statistical Analysis**
The results are expressed as means±standard deviation (SD). For each type of experiment, data were obtained from at least three independent measurements. Statistical analysis of the results was carried out using one-way analysis of variance (ANOVA) followed by the least significant difference test or Newman-Keul’s test. Differences were considered significant at $P<0.05$.

**Supplemental Figure**

**Figure S1.** LY294002 (LY) or Akt inhibitor IV (AI) has no effect on the neuronal survival in the rat hippocampal CA1 subregion. Nissl staining on neuronal survival after 5 days following drug administration. (a-c) Low-power views of hippocampus sectors. Scale bars = 500 μm. (d-f) High-power views of hippocampal CA1 pyramidal cell layer of (a-c). Scale bars = 50 μm. Neuronal density was counted as numbers of surviving pyramidal neurons per 1mm length. Data are mean ± SD (n=5).

**Supplemental Discussion**
The role of MEK1-ERK1/2 pathway remains controversial, since both activation and inhibition of ERK1/2 are reported to mediate neuronal survival in conditions associated with cerebral ischemia. 2, 3 Although different changes in ERK1/2 phosphorylation after postconditioning have been reported, 4, 5 Pignataro and colleagues found that ERK1/2 may be unrelated to the protective effect of postconditioning after focal ischemia. 4 In this work, our result showed that inhibiting MEK1-ERK1/2 signal by PD98059 didn’t affect protective effect of postconditioning after global ischemia, which is consistent with the study by Pignataro and colleagues.
Considering dual effects of ERK1/2 in ischemic brain damage and controversial data for ERK1/2 phosphorylation in postconditioning, more studies are needed to clarify the role of MEK1-ERK1/2 in the postconditioning neuroprotection.

Supplemental References