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

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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;44:1790-1795.)

Key Words: cerebrovascular disease ▪ cognitive functioning ▪ dementia ▪ epidemiology

Stroke and dementia are 2 of the largest morbidity burdens worldwide.1 Previous research has shown that cognitive status and stroke occurrence are strongly related to the risk of future dementia. Individuals who experience a stroke have double the risk of dementia, including delayed dementia compared to those who do not experience stroke.2 Other studies have also shown that lower scores on tests of cognitive function like the Mini-Mental State Examination (MMSE) are also highly predictive of future risk of dementia.3,4

However, little research has examined whether prestroke cognitive function is still a strong predictor of the risk of dementia after a stroke event because most previous studies have not assessed cognitive function before the stroke event. A review article on the impact of stroke on the risk of dementia concluded that prestroke cognitive decline did not seem to account for the association between stroke and cognitive impairment and did not find evidence for an interaction between incident stroke and prestroke cognition on the risk of dementia.5 However, very few studies have specifically tested this interaction or assessed the impact of baseline cognitive status on the risk of dementia preceded by or not preceded by stroke. Learning more about the associations among baseline cognitive functioning, stroke, and risk of dementia would help determine whether the risk of dementia is driven primarily by the stroke event, which would suggest a vascular pathology, or by the level of cognitive functioning before the stroke event, which would suggest pathologies independent of the stroke event.

The Perindopril Protection Against Recurrent Stroke Study (PROGRESS) was a randomized trial among people with a history of cerebrovascular disease in which a blood pressure regimen was tested against placebo for the secondary prevention of stroke. The study assessed participants’ cognitive function at baseline and followed them for recurrent stroke.
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-controlled 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, participants 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 tolerated 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, participants completed the MMSE.1 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 into 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 dementia: an MMSE score ≤25 at any follow-up visit, a decline in the MMSE score of ≥3 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 having 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 included systematic questions on the presence of poststroke focal deficits, 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 consultations with other medical professionals. After receiving the information 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 categories: 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 infarction or cerebral hemorrhage.7,10 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 committee 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 as a measure for the relative risk (RR) of developing dementia for the 4 categories of the baseline MMSE score using the highest category of MMSE as the reference category. Next, to examine the impact of MMSE score on the joint outcome of recurrent stroke and dementia, we divided incident dementia into post-recurrent stroke dementia and dementia without recurrent stroke. We then used logistic regression models to assess the RR of both dementia types compared to no dementia for each of our MMSE categories.

All models were adjusted for variables that we believed could be potential confounders based on biological mechanisms. These variables were age (continuous), sex, height (continuous), smoking status (never smoked regularly, past, current), current alcohol consumption (currently do not drink more than once/wk, currently drink <8 drinks/wk, currently drink ≥8 drinks/wk), educational status (stopped education by 14, 16, 19, and >19 years of age), diabetes mellitus status (yes/no), and systolic blood pressure (continuous). We also adjusted for randomized treatment assignment.

In secondary analyses, we explored the relationship between baseline MMSE score and recurrent stroke and risk of dementia stratified by the recurrent stroke subtype (ischemic versus hemorrhagic stroke). We additionally performed all analyses using only those randomized to placebo. Finally, we performed separate sensitivity analyses in which we adjusted for baseline Barthel index in addition to our potential confounders, excluded patients who screened positive for dementia at baseline, and excluded patients with MMSE scores <18 at baseline.

No participant had missing information on age, sex, current alcohol consumption, diabetes mellitus status, or baseline systolic blood pressure. Fewer than 100 people had missing information on height and smoking and were assigned to the median and past smoker, respectively. More than 100 people had missing information on education status so we used the missing indicator method.

All statistical analyses were performed using SAS 9.1.3. All P values 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.

Table 1. Baseline Characteristics of Participants in the PROGRESS Trial by Baseline MMSE Score (n=6080)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MMSE Score</th>
</tr>
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<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/wk</td>
<td>60.5</td>
</tr>
<tr>
<td>1 to 8 drinks/wk</td>
<td>18.0</td>
</tr>
<tr>
<td>≥8 drinks/wk</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.2–4 However, data on 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. In addition, those who were cognitively normal at the time of their stroke did not have an increased risk of dementia compared to those without 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>
<thead>
<tr>
<th>Table 2. Multivariate-Adjusted* Relative Risks of Overall Dementia, Post-recurrent stroke Dementia, and Dementia Without Recurrent Stroke by Baseline MMSE Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline MMSE</strong></td>
</tr>
<tr>
<td><strong>No Dementia (n=5673)</strong></td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>28–29</td>
</tr>
<tr>
<td>24–27</td>
</tr>
<tr>
<td>&lt;24</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.

![Figure. Proportion of types of dementia according to baseline Mini-Mental State Examination (MMSE).](http://stroke.ahajournals.org/Downloaded from)
In many cases, particularly among patients with low MMSE, recurrent stroke is a strong predictor of dementia. A recent study conducted in the Rotterdam Study found that recurrent stroke is associated with a greater risk of cognitive decline, even in the absence of dementia. This is consistent with previous findings that recurrent stroke increases the risk 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 neurobiological changes associated with Alzheimer disease or by synergistic effects of Alzheimer neuropathology and vascular neuropathology. This has important clinical implications because it demonstrates that recurrent stroke is not necessary to develop dementia. This result could seem counter-intuitive because in patients with stroke, one would expect a second stroke to result in a greater risk of cognitive decline and vascular dementia. However, 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.

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. 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 is lower due to the 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 first 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 prevents 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 miniimize 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 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.

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Disclosures
Drs 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


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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 Laemmli 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. Although different changes in ERK1/2 phosphorylation after postconditioning have been reported, Pignataro and colleagues found that ERK1/2 may be unrelated to the protective effect of postconditioning after focal ischemia. 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**