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# Baicalin alleviates ischemia-induced memory impairment by inhibiting the phosphorylation of CaMKII in hippocampus

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ABSTRACT

Baicalin has a significant neuroprotective effect in stroke. However, the mechanism remains unclear. This study was to reveal the mechanisms by which baicalin protected hippocampal neurons and improved learning and memory impairment after global cerebral ischemia/reperfusion in gerbil. In the present study, the Morris water maze test showed that baicalin significantly improved learning and memory impairment after global cerebral ischemia/reperfusion in gerbils. Laser scanning confocal fluorescence microscope examination showed that baicalin suppressed OGD-induced augmentation of intracellular calcium concentration. Western blotting analysis indicated that baicalin suppressed ischemia-caused elevated phosphorylation level of CaMKII *in vivo*, in hippocampal neurons in culture, and in SH-SY5Y cells in culture. Western blotting, TUNEL and RNA interference technology were applied to detect effects of baicalin on neuronal apoptosis. We found that baicalin, a CaMKII inhibitor and knocking down the CaMKII prevented OGD-induced apoptosis of hippocampal or SH-SY5Y cells in culture. Therefore, these results suggested that baicalin improves learning and memory impairment induced by global cerebral ischemia/reperfusion in gerbils via attenuating the phosphorylation level of CaMKII and further preventing hippocampal neuronal apoptosis.



## 1. Introduction

Ischemic stroke, a commonly encountered and frequently occurring clinical disease, is closely associated with the development of cognitive impairments (Mori, 2002). The hippocampus is a region of the brain associated with cognitive functions, particularly in learning and memory (Seo et al., 2014). The hippocampus plays a critical role in the creation of new memories as well as in the processing of declarative and spatial memory (Biegler et al., 2001). Cerebral ischemia can induce loss of hippocampal neurons, causing cognitive dysfunction such as learning and memory deficits (Hartman et al., 2005; Tian et al., 2014). Although there are increased demands for treatment of global cerebral ischemia, no effective therapy has been developed to date (Coultrap et al., 2011).

Baicalin is a flavonoid compound isolated from the dried roots of Scutellaria baicalensis Georgi, which exerts anti-apoptotic, anti-oxidant, and anti-inflammatory properties (Cheng et al., 2012; Li et al., 2012; Zhu et al., 2012). But the detailed neuroprotective mechanism of baicalin still needs further clarification. The increase in intracellular calcium concentration ($[Ca^{2+}]_i$) could be involved in many cell activities, such as cell proliferation and apoptosis. Ischemic insults on neurons trigger excessive glutamate release that causes augmentation of $[Ca^{2+}]_i$ resulting in excitotoxicity, $Ca^{2+}$/calmodulin (CaM)-dependent protein kinase II (CaMKII) is a key protein involved in $Ca^{2+}$ signaling (Coultrap et al., 2011). Two independent studies have implicated CaMKII as a promising drug target for post-insult neuroprotection (Ashpole and Hudmon, 2011; Vest et al., 2010). In addition, one of the most important neuroprotective effects of baicalin is carried out by signal pathways such as the MAPK cascades including ERK, JNK and p38 in global ischemia (Dai et al., 2013). CaMKII and ERK-1/2 interact in different biological processes. For example, CaMKII activation by cGMP-dependent protein kinase (PKG) occurred in an ERK-1/2-dependent manner, which indicated that ERK-1/2 is upstream of CaMKII (Zhang et al., 2014). Inhibiting CaMKII with KN-93 or silencing CaMKII attenuated activating factor-induced activation of ERK-1/2 in human soluble B-cells, which suggested that ERK-1/2

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activation was in part through CaMKII-dependence and ERK-1/2 was downstream of CaMKII (Liang et al., 2014). So it is possible that CaMKII is another signaling mechanism underlying the action of baicalin on global cerebral ischemic impairment.

In the present study, we evaluated the effects of baicalin on the spatial learning and memory after global cerebral ischemia/reperfusion in gerbils. We further demonstrated that baicalin exhibited its neuroprotective effects by inhibiting CaMKII phosphorylation (p-CaMKII) in the hippocampus.

## 2. Results

### 2.1. Baicalin improved spatial learning and memory deficits induced by ischemia in vivo

The MWM is the most frequently and widely utilized method for assessing spatial learning and memory in rodents. The escape latency to find the platform in the model group, namely global cerebral ischemia/reperfusion group, was longer than observed in the sham-treated group ($P<0.01$, $n=6$). However, there was a significant reduction of escape latency in the model-baicalin group compared with model group ($P<0.05$, $n=6$) (Fig. 1A). In the probe trials, the number of times the gerbils crossed the place where the hidden platform was previously located was applied to estimate memory performance. The number of passing times of gerbils in the model group was significantly less than sham-treated group gerbils ($P<0.01$, $n=6$). However, the number of times recorded for the model-baicalin group increased compared with the model group ($P<0.01$, $n=6$) (Fig. 1B). In the escape latency and the probe trials, there was no significant difference between sham-treated group and sham-baicalin group ($P>0.05$, $n=6$). Taken together, these data suggest that baicalin could ameliorate spatial learning and memory impairments induced by global cerebral ischemia/reperfusion in gerbils.

### 2.2. Baicalin inhibited OGD-induced increase of $[Ca^{2+}]_i$ in hippocampal neurons

We investigated whether baicalin could affect OGD-induced changes of $[Ca^{2+}]_i$. From fluorescence intensity taken by laser scanning confocal microscope, we found that OGD significantly increased $[Ca^{2+}]_i$ ($P<0.01$, compared with control), and this increase in $[Ca^{2+}]_i$ was inhibited by baicalin ($P<0.01$, contrast to OGD group) (Fig. 2).

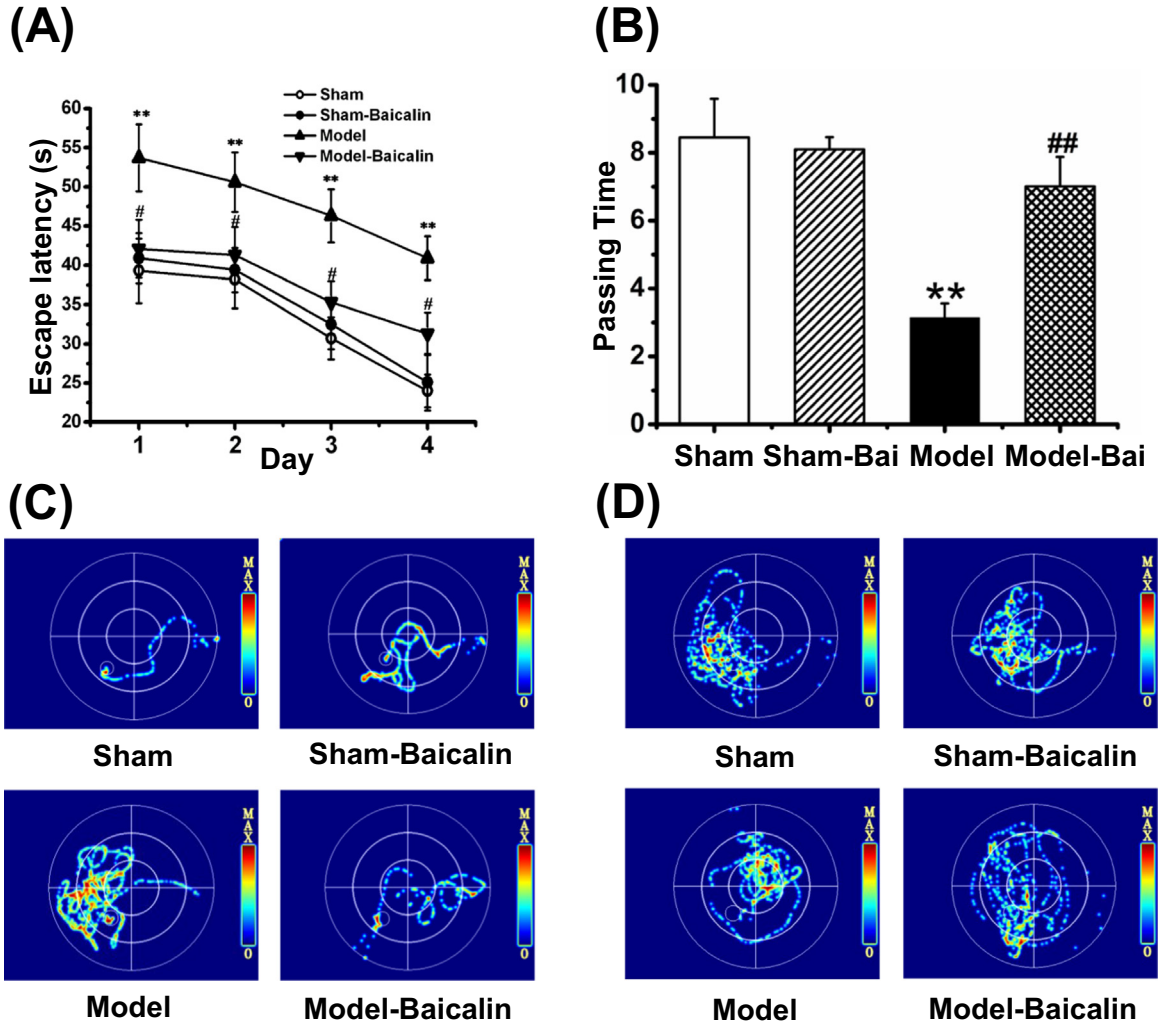


**Fig. 1.** Effect of baicalin (Bai, 100 mg/kg) on spatial learning and memory performance in ischemia/reperfusion gerbils. (A) In the hidden platform test, the daily escape latency to find the platform in every treatment group. (B) In the probe trials, the number of times the gerbils crossed the place where the hidden platform was previously located is shown for every treatment group. (C) The representive locus plot in hidden platform test. (D) The representive locus plot in probe trial. Data were represented as mean ± SEM, $^{**}P<0.01$ versus sham-treated group, $^{\#}P<0.05$ versus model group, $^{\#\#}P<0.01$ versus model group.