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Research report

# Neuroprotective effects of silymarin on ischemia-induced delayed neuronal cell death in rat hippocampus

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ABSTRACT

We examined the effects of silymarin, which was extracted from *Silybum marianum*, on delayed neuronal cell death in the rat hippocampus. Rats were divided into four groups: sham-operated rats (sham group), rats which underwent ischemic surgery (control group), rats which were treated with silymarin before and after ischemic surgery (pre group), and rats which were treated with silymarin after ischemic surgery only (post group). We performed the ischemic surgery by occluding the bilateral carotid arteries for 20 min and sacrificed the rats one week after the surgery. Silymarin was administered orally at 200 mg/kg body weight. Smaller numbers of delayed cell deaths were noted in the rat CA1 region of the pre- and post-groups, and no significant difference was observed between these groups. There were few apoptotic cell deaths in all groups. Compared to the control group, significantly fewer cell deaths by autophagy were found in the pre- and post-group. We concluded that silymarin exerts a preservation effect on delayed neuronal cell death in the rat hippocampus and this effect has nothing to do with the timing of administering of silymarin.



## 1. Introduction

Cerebral infarction is the second most common cause of human death after myocardial infarction and often leaves serious aftereffects which diminish the quality of life in the post-stroke period (WHO, 2014). Treatment for acute cerebral infarction has made remarkable progress in recent years; the therapeutic time window for tissue plasminogen activator has been extended from 3 h to 4.5 h (Jauch et al., 2013) after the occurrence of stroke, and percutaneous revascularization with intravascular clot retrieval devices has contributes to many new opportunities and choices in the treatment of patients. There are several options for treating acute cerebral infarction. However, there are a very limited number of treatment options for complications in the chronic state after ischemic stroke, such as only rehabilitation for motor, speech and cognitive impairment and antithrombotic therapy for the prevention of re-stroke.

More than 47 million people world-wide suffer from dementia (WHO, 2015), among whom vascular dementia resulting from cerebral infarction represents the 2nd most frequent cause next to Alzheimer's disease (Prince et al., 2014). It is known that cerebral blood reduction caused in cerebral infarction often leads to the infliction of permanent damage on memory function (Alexander, 1997), and it has also been suggested that cognitive disorder is strongly associated with delayed neuronal cell death in the CA1 area of the hippocampus (Block, 1999). Prevention of delayed neuronal cell death in this area of the hippocampus is thus essential in the treatment and prophylaxis of vascular dementia.

Silymarin is extracted from the seeds of the milk thistle (*Silybum marianum*) which have been used since the times of ancient Greece. Silymarin has been employed in Europe as a liver protective agent more than 2000 years. In 1986, it was approved as a drug for toxic liver disease and as an adjuvant drug for the chronic hepatitis and cirrhosis by Commission E in Germany. Silymarin is an agent which consists of silibinin (silybin A and silybin B: approximately 50–60%), isosilibinin (isosilybin A and isosilybin B: approximately 5%), silicristin (approximately 20%), silidianin (approximately 10%) and some other minor components. Among these components, silibinin is the primary constituent and the substance of highest activity as an agent for biological tissue protection (Saller et al., 2007). Effects of tissue protection by silymarin have been described for several internal organs such as

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the liver (Oliveira et al., 2001), kidney (Senturk et al., 2008), and heart (Rao and Viswanath, 2007). Especially membrane stabilizing action in liver of silymarin (Valenzuela and Garrido, 1994) and recent reports (Hou et al., 2010; Raza et al., 2011) have indicated protective effects of silymarin in which silymarin diminished the brain tissue damage at the acute phase of cerebral infarction. However, it remains unknown whether such neuroprotective effects of silymarin can help to ameliorates the functional outcome in the chronic phase of ischemic stroke.

In the present study, to clarify whether the neuroprotective effect of silymarin is able to contribute to a favorable functional outcome after ischemic stroke, we examined the time course of histological changes in the hippocampal CA1, and assessed whether the delayed neuronal cell death in the hippocampus is inhibited after silymarin administration using a transient forebrain ischemia model in the rat.

## 2. Results

### 2.1. HE staining

Compared with the sham group, cell densities of CA1 pyramidal cells in the hippocampus of the pre-, post- and control groups were clearly low (Fig. 1), indicating that cell death occurred due to the surgery.

### 2.2. FJB staining (neuronal cell death)

In the post-group (Fig. 2B) and pre-group (Fig. 2A), the numbers of delayed neuronal cell deaths were significantly decreased in comparison to the control group (Fig. 2C) ($P < 0.05$). No delayed neuronal cell death was observed in the sham group (Fig. 2D).

### 2.3. TUNEL staining (apoptosis)

There were a few cell deaths by apoptosis in all the experimental groups, but no in the sham group (Fig. 3D). Several cell deaths by apoptosis were noted in the control group (Fig. 3C). In the post-group (Fig. 3B) and pre-group (Fig. 3A), the numbers of delayed neuronal cell deaths were decreased in comparison to the control group.

### 2.4. MDC staining (autophagy)

Compared to the control group (Fig. 4C), only a few cell death by autophagy were observed in the pre-group (Fig. 4A) and post-groups (Fig. 4B). Specifically, in the post and pre-group, the numbers of cell deaths by autophagy were significantly decreased in comparison to the control group ($P < 0.05$). No cell death by autophagy was evident in the sham group (Fig. 4D).

## 3. Discussion

Silymarin are made up of some substances and, main active component is silibinin. Therefore silibinin can be a substitute for pharmacokinetic parameters of silymarin (Javed et al., 2011). Oral bioavailability of silibinin is known to be 0.73% in rat plasma (Wu et al., 2007).

In this study, we performed HE staining for assessing cell densities, FJB staining for cell death, TUNEL staining for apoptosis, and MDC staining for autophagy. After confirming the cell densities of hippocampus in HE staining, neuronal cell death was confirmed by FJB staining. Moreover, TUNEL and MDC staining was performed to find the cause of cell death. Especially MDC staining does not indicate cell death arose from autophagy but just autophagy. However, we consider positive cell in MDC staining as cell death arose from autophagy because each positive cells in FJB staining and MDC staining are approximately identical.

We revealed the possibility that delayed neuronal cell death can predominantly involve autophagy rather than apoptosis. In the control group, the number of positive cells found on MDC staining was far larger than the number of positive cells found on TUNEL staining. The statistics for TUNEL staining were therefore not analyzed in detail. Recently, Koike et al. (2008) reported that inhibiting autophagy could prevent the cell death of pyramidal cells of the hippocampus by hypoxia. In spite of the preservation effects

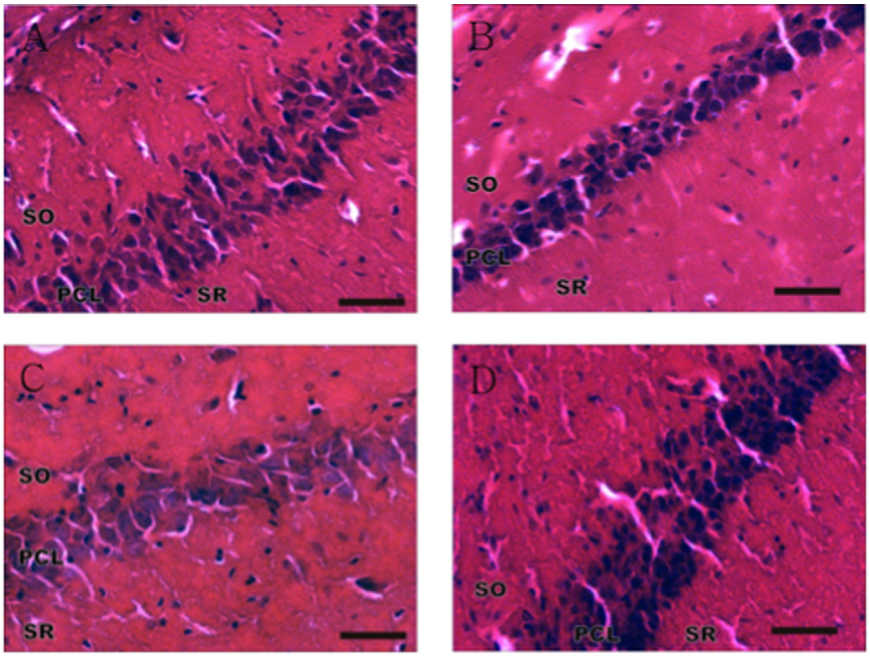


**Fig. 1.** HE staining. Upper left: pre-group (A), lower left: control group (C). Upper right: post-group (B), lower right: sham group (D). The number of decidual cells in the pre- and post-groups was less than that in the control group. (SO: stratum oriens, PCL: pyramidal cell layer, SR: stratum radiatum, black bar: 250 μm).