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BRAIN RESEARCH

Research Report

Potential role of JAK2 in cerebral vasospasm after experimental subarachnoid hemorrhage

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ABSTRACT

The Janus kinase (JAK) proteins are key regulators for transducing signals from the cell surface to the nucleus in response to cytokines to orchestrate the appropriate cellular response. Previous studies have demonstrated that JAK1 is activated in the basilar artery after subarachnoid hemorrhage (SAH), however it has not been investigated whether, and to what degree, JAK2 is induced by SAH and also the role of JAK2 in the pathogenesis of cerebral vasospasm following SAH remains unknown. Experiment 1 aimed to investigate the time-course of the JAK2 activation in the basilar artery after SAH. In Experiment 2, we chose the maximum time point of JAK2 activation and assessed the effect of AG490 (a specific JAK2 inhibitor) on regulation of cerebral vasospasm and endothelial apoptosis. All SAH animals were subjected to injection of autologous blood into cisterna magna twice on day 0 and day 2. As a result, the elevated expression of activated JAK2 was detected in the basilar artery after SAH and peaked on day 3. After AG490 intracisternal administration, the vasospasm was markedly aggravated and the apoptosis index of endothelial cells was also significantly increased in the basilar arteries. Anti-apoptotic genes such as bcl-2 and bcl-xL were down-regulated after the injections of AG490. Our results suggest that JAK2 is activated in the arterial wall after SAH, playing a beneficial role to vasospasm development, possibly through protecting endothelial cells and up-regulating anti-apoptotic genes.

1. Introduction

Cerebral vasospasm is the most common cause of disability and death in patients suffering from aneurysmal subarachnoid hemorrhage (SAH) (Treggiari-Venzi et al., 2001). Treatment of cerebral vasospasm has been considered as a major goal in the management of patients surviving SAH. However, the exact molecular mechanism of cerebral vasospasm still remains obscure, which has hindered the development of effective and specific treatment paradigms for cerebral vasospasm.

The Janus kinase (JAK) family of cytosolic tyrosine kinases, traditionally thought to be coupled to cytokine receptors such as those for the interleukins and interferons, has four members (JAK1, JAK2, JAK3 and TYK2) (Sandberg et al., 2004). In response to ligand binding, these JAK tyrosine kinases associate with, tyrosine-phosphorylate, and activate cytokine receptors. Once

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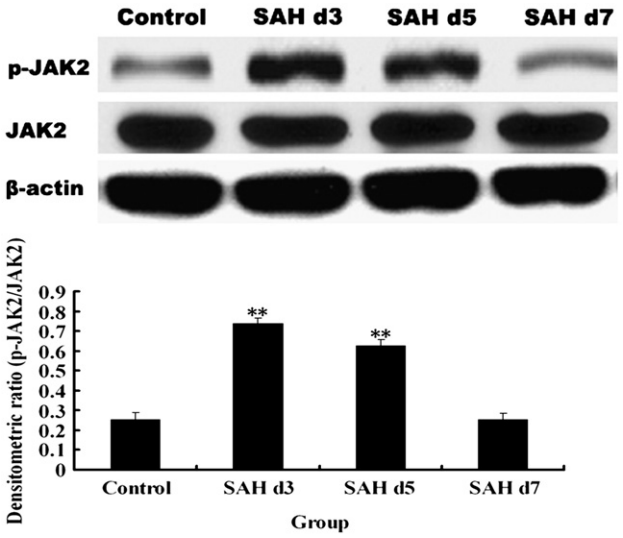

Fig. 1 – Upper panel, Representative autoradiogram of JAK2 activation in basilar arteries. We observed JAK2 at 128 kDa, phospho-JAK2 (Tyr$^{1007/1008}$) at 131 kDa and a loading control β-actin at 42 kDa. It shows that the phosphorylation of JAK2 protein increased after SAH and peaked on day 3. Bottom panel, Quantitative analysis of the Western blot results for JAK2 and phospho-JAK2. It shows that the levels of JAK2 activation in SAH day 3 and 5 groups were significantly higher than that in control. Bars represent the mean ± SD (n = 6, each group). $^{**}P < 0.01$ compared with control group.

activated, JAKs tyrosine phosphorylate and activate other signaling molecules, including the signal transducers and activators of transcription (STAT) family of nuclear transcription factors after binding of STATs to the receptor (Sandberg et al., 2004). Thus, the JAK/STAT pathway is an important link between cell surface receptors and nuclear transcriptional events leading to cell growth. Clinical and experimental studies have shown the increased levels of cytokines in the basilar arterial walls and cerebrospinal fluid (CSF) after SAH (Hendryk et al., 2004; Zhou et al., 2007a). Afterwards, the data from Osuka et al. (2006) suggested that SAH produced the proinflammatory cytokine IL-6 in the CSF, which induced the JAK1 phosphorylation in the basilar artery following experimental SAH. However till now, no study was found in the literature to investigate the time-course and role of JAK2 activation in the pathogenesis of cerebral vasospasm.

Endothelial apoptosis may trigger, aggravate, and maintain cerebral vasospasm, either in the major arteries (Zubkov et al., 2002) or in the penetrating arterioles (Zubkov et al., 2000). Previous research by Negoro et al. (2000) indicated that the JAK2 was activated in myocardium after acute myocardial infarction and administration of JAK2 inhibitor resulted in a significant increase in apoptotic cells of myocardium. Then they concluded that JAK2 played a pivotal role in cytoprotective and anti-apoptotic signaling in acute myocardial infarction. However, none of the previous studies focused on the effect of JAK2 inhibitor on the endothelial apoptosis following SAH. The aim of the current study was to evaluate the changes of basilar arterial JAK2 activation following SAH and determine the potential role of JAK2

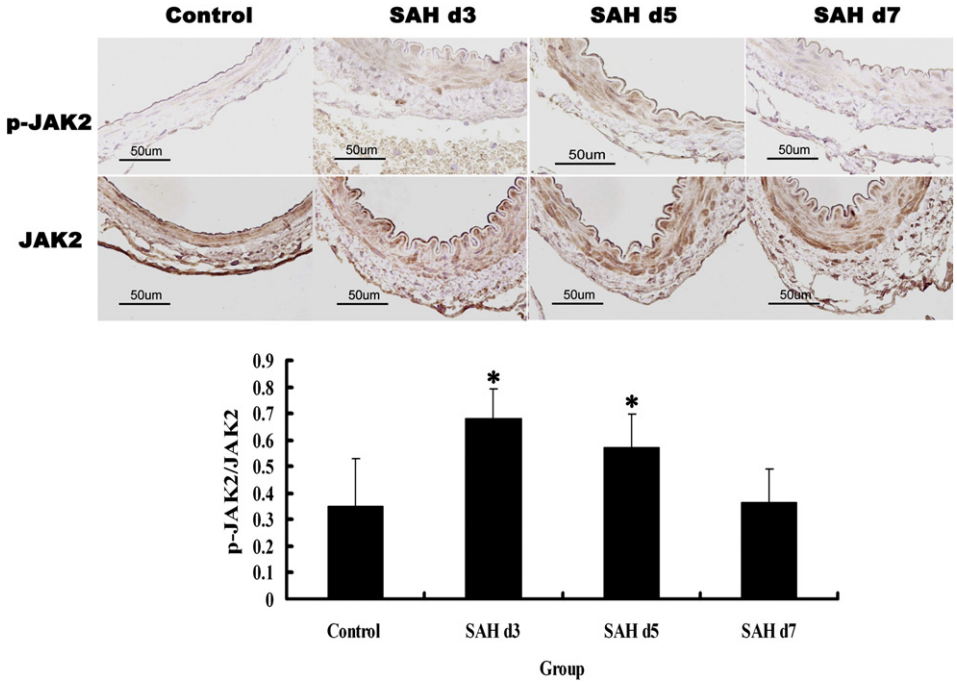

Fig. 2 – Immunohistochemical study of JAK2 and phospho-JAK2 on cross sections of basilar arteries. Upper panel, A few phosphorylated JAK2 positive cells were observed in the control group, which indicates the constitutional activation of JAK2 in the normal basilar arteries of rabbits. Increased phospho-JAK2 positive cells could be found in the basilar arteries of the rabbits in the SAH day 3 and day 5 groups. Bottom panel, Quantitative analysis shows the low ratio of JAK2/phospho-JAK2 in the control group. In contrast, the level of JAK2 activation was increased in the day 3 and day 5 groups. Significant differences were both found between the day 3 or day 5 groups, and the control group. Bars represent the mean ± SD (n = 6, each group). $^{*}P < 0.05$ compared with control group.