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Research Report

Ghrelin alleviates early brain injury after subarachnoid hemorrhage via the PI3K/Akt signaling pathway

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ABSTRACT

Early brain injury (EBI) plays a key role in the pathogenesis of subarachnoid hemorrhage (SAH). Although the neuroprotective effects of ghrelin have been demonstrated in several studies, whether ghrelin reduces EBI after SAH remains unknown. In this study, we hypothesized that treatment with ghrelin would attenuate EBI after SAH, and that this protection would be mediated, at least in part, by activation of the PI3K/Akt signaling pathway. Adult male Sprague-Dawley rats (n=100) were randomly divided into the following groups: control group (n=20), SAH group (n=20), SAH+vehicle group (n=20), SAH+ghrelin group (n=20) and SAH+ghrelin+LY294002 group (n=20). The rats were injected with autologous blood (0.3 mL) into the prechiasmatic cistern to induce SAH. Ghrelin (80 μg/kg, IP), or an equal volume of vehicle, was administered immediately after surgery. The PI3K inhibitor, LY294002, was applied to manipulate the proposed pathway. Mortality, neurological scores, brain edema, cell apoptosis, and the expression of p-Akt, and cleaved caspase-3 proteins were assayed after 24 h SAH. Ghrelin significantly improved neurological function and reduced neuronal apoptosis and brain edema at 24 h after SAH. The level of p-Akt, expressed mainly in neurons, was markedly up-regulated. Additionally, the level of cleaved caspase-3 was decreased by ghrelin treatment. The beneficial effects of ghrelin in SAH rats were partially suppressed by LY294002. These results demonstrate that ghrelin may reduce EBI after SAH, via a mechanism involving the PI3K/Akt signaling pathway.

Abbreviations: SAH, subarachnoid hemorrhage; EBI, early brain injury; CNS, central nervous system; NeuN, neuron specific nuclear protein

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1. Introduction

Subarachnoid hemorrhage (SAH), especially aneurismal SAH, is a deadly cerebrovascular disorder with very high mortality and morbidity rates. SAH accounts for about 5–7% of all strokes despite many therapeutic advances in recent years (Ansar et al., 2011; Sehba et al., 2012). Cerebral vasospasm and early brain injury (EBI) are two major complications that often present in patients suffering from SAH (Tseng et al., 2005). Traditionally, researchers studying SAH primarily focused their efforts on cerebral vasospasm, which was considered the main cause of the poor prognosis of SAH. In order to inhibit vasospasm-induced secondary brain injury, many experimental and clinical studies have been conducted (Golan et al., 2013), but when vasospasm was properly managed, it failed to improve the neurological outcome in patients with SAH (Kramer and Fletcher, 2009). Recently, accumulating studies have suggested that EBI, which occurs within the first 72 h after SAH, seems to be the primary factor in the poor outcome of patients with SAH (Sehba et al., 2012). Therefore, developing new therapeutics for reducing EBI may be essential for improving the neurological outcome in patients with SAH.

Previous studies have shown that neuronal apoptosis is observed in experimental SAH and in clinical patients after SAH (Friedrich et al., 2012; Topkoru et al., 2013; Zhou et al., 2014b). Furthermore, the severity of neuronal apoptosis is indirectly correlated with neurological function, which suggests that apoptosis may be a potential therapeutic target against EBI after SAH (Dai et al., 2014; Friedrich et al., 2012). As a signaling pathway that regulates cell survival, the PI3K/Akt pathway has been implicated in many diseases including several nervous system diseases, such as traumatic brain injury, cerebral ischemia, and spinal cord injury (Noshita et al., 2003; Yu et al., 2005; Zhao et al., 2012). Previous studies have also shown that the PI3K/Akt pathway plays a key regulatory role in apoptosis after SAH (Endo et al., 2006). These observations led us to hypothesize that targeting the PI3K/Akt pathway may attenuate apoptosis during EBI and improve the outcome of patients with SAH.

Ghrelin, a 28-amino acid peptide secreted mainly from the stomach in rats and humans, is an endogenous ligand for growth hormone secretagogue receptor (GHS-R) (Kojima et al., 1999). Ghrelin is released in substantial amounts into the circulation by the stomach (Date et al., 2000). Meanwhile the central nervous system (CNS), kidneys, placenta, pancreas and heart all produce small amounts of ghrelin (Nikolopoulos et al., 2010; Sakata et al., 2002). Ghrelin receptors are found in many neural tissues such as the thalamus, cortex, pituitary, hypothalamus, hippocampus and spinal cord (Lee et al., 2010; Zigman et al., 2006). Furthermore, ghrelin has been found to have neuroprotective effects in several models, for which numerous mechanisms have been postulated. Ghrelin can inhibit the activation of caspase-3 and neuronal death in pilocarpine-induced seizures (Xu et al., 2009); it demonstrates neuroprotection by keeping the balance of oxidants to antioxidants and inhibiting pro-inflammatory mediators in a model of subarachnoid hemorrhage (Ersahin et al., 2010); in addition, studies suggest that the PI3K/Akt pathway may play an important role in the neuroprotective effects of ghrelin against acute brain injury (Lee et al., 2011).

In the current work, we hypothesized that ghrelin may reduce brain edema formation and subsequent apoptosis, with the goal of reducing EBI after SAH, which might involve the PI3K/Akt pathway.

2. Results

2.1. General observations and physiological evaluation

There were no significant differences in body temperature or body weight among all groups during the entire experimental procedure (data not shown). No rats died in the control group ($n=20$), 4/24 rats (16.67%) died in the SAH group, 5/25 rats (20.00%) died in the SAH+vehicle group, 3/23 rats (13.04%) died in the SAH+ghrelin group, and 6/26 rats (23.07%) died in the SAH+ghrelin+LY294002 group. No significant difference was observed for mortality among the operated groups. When rats were sacrificed, subarachnoid blood clots were found on the inferior basal temporal lobes of all rats with experimentally induced SAH groups. Therefore, temporal lobe brain tissue was collected for analysis in this study.

2.2. Effect of ghrelin on neurological deficits

There was significant impairment of behavioral functions in the SAH group compared with the control group at 24 h after SAH ($P<0.01$; Fig. 1), but there was no difference between the SAH and the SAH+vehicle groups ($P>0.05$; Fig. 1). The neurological scores of rats in the ghrelin treated group were significantly higher compared with those of the SAH group; however, LY294002 abolished the beneficial effect of ghrelin on neurobehavioral function ($P<0.05$; Fig. 1).

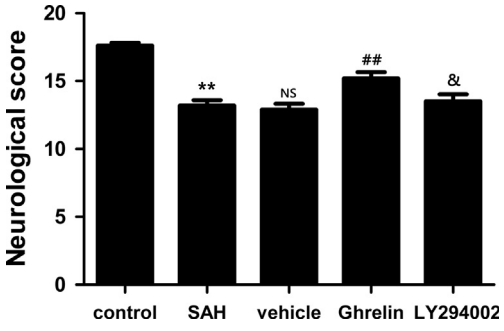

Fig. 1 – Evaluation of neurological function. SAH significantly impaired neurological function at 24 h compared with the control rats. Whereas treatment with ghrelin significantly increased the neurological score compared with the SAH rats. However, Ly294002 abolished the beneficial effect of ghrelin on neurobehavioral function. There was no difference between SAH and SAH+vehicle groups. $^{**}P<0.01$ vs. control group; ns $P>0.05$ vs. SAH group; $^{\#\#}P<0.01$ vs. SAH+vehicle group; $^{\&}P<0.05$ vs. SAH+ghrelin group ($n=5$ per group).