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Research Report

Acute hyperglycemia worsens ischemic stroke-induced brain damage via high mobility group box-1 in rats

Jingyang Huang[a,1], *Baoyi Liu*[b,1], *Chenghui Yang*[a], *Haili Chen*[a], *Dzivor Eunice*[a], *Zhongrui Yuan*[a,*]

[a]*Department of Pathophysiology, School of Medicine, Shandong University, Wenhua Xi Road no. 44, Jinan, Shandong 250012, PR China*

[b]*Department of Respiratory, Qilu Hospital, Shandong University, Jinan, Shandong 250012, PR China*

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ABSTRACT

Hyperglycemia adversely affects the outcome of ischemic stroke. Extracellular HMGB1 plays a role in aggravating brain damage in the postischemic brain. The aim of this study was to determine whether the extracellular HMGB1 is involved in the worsened ischemic damage during hyperglycemic stroke. Male Wistar rats underwent middle cerebral artery occlusion (MCAO) for 90 min with reperfusion. Acute hyperglycemia was induced by an injection of 50% dextrose. Rats received glycyrrhizin, a specific HMGB1 inhibitor, or vehicle. HMGB-1 in cerebrospinal fluid and in brain parenchyma was detected at 2 or 4 h post-reperfusion. Neurological deficits, infarct volume and cerebral edema were assessed 24 h post-MCAO the disruption of blood–brain barrier (BBB) and the expression of tight junction protein Occludin were measured at 4 h post-reperfusion. Hyperglycemia enhanced the early release of HMGB1 from ischemic brain tissue, which was accompanied by increased infarct volume, neurological deficit, cerebral edema and BBB disruption. Glycyrrhizin alleviated the aggravation of infarct volume, neurological deficit, cerebral edema and BBB disruption by decreasing the degradation of tight junction protein Occludin in the ischemic hemisphere of hyperglycemic rats. In conclusion, enhanced early extracellular release of HMGB1 might represent an important mechanism for worsened ischemic damage, particularly early BBB disruption, during hyperglycemic stroke. An HMGB1 inhibitor glycyrrhizin is a potential therapeutic option for hyperglycemic stroke.

Abbreviations: HMGB1, high mobility group box-1; MCAO, middle cerebral artery occlusion; BBB, blood–brain barrier; TTC, 2,3,5-triphenyletetrazolium chloride; CSF, cerebrospinal fluid

*Corresponding author. Fax: +86 531 88382502.
E-mail addresses: zhongruiyuan@sdu.edu.cn, zhongruiyuan@hotmail.com (Z. Yuan).

[1]These authors contributed equally to this work.

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1. Introduction

Ischemic stroke is a leading cause of death worldwide and the commonest cause of long-term disability among adults. Hyperglycemia occurs in 30–60% of patients with acute ischemic stroke, also in individuals without a known history of diabetes, due to a generalized stress reaction and increased levels of glucocorticoids (Kruyt et al., 2010; Luitse et al., 2012; Martini and Kent, 2007). Hyperglycemia during acute stroke is associated with significantly worsened outcome, including larger infarct, more severe edema formation, and a higher risk of mortality, regardless of pre-existing diabetes (Luitse et al., 2012; Pulsinelli et al., 1983; Matchar et al., 1992; Capes et al., 2001). Both diabetic and non-diabetic patients are adversely affected by hyperglycemia, suggesting it is elevated glucose and not diabetic complications that aggravate stroke damage (Luitse et al., 2012; Pulsinelli et al., 1983; Matchar et al., 1992). It is of great significance to clarify the molecular mechanisms involved.

High mobility group box-1 (HMGB1) has been mainly studied in the context of a wide variety of inflammatory conditions in peripheral organs (Klune et al., 2008). Recently, HMGB1 has received particular attention with respect to its pathological role in cerebral ischemia. Numerous studies have reported that HMGB1 is released early after ischemic injury from neurons (Kim et al., 2006; Liu et al., 2007; Kim et al., 2008; Qiu et al., 2008). High levels of serum HMGB1 were observed in patients with stroke compared with healthy control subjects (Muhammad et al., 2008). This early release of HMGB1 into the extracellular space after ischemic injury may contribute to the initial stage of the inflammatory response in the ischemic penumbra (Kim et al., 2006; Liu et al., 2007; Kim et al., 2008; Muhammad et al., 2008; Qiu et al., 2008). Moreover, Zhang et al. reported that extracellular HMGB1 is involved in the disruption of the blood–brain barrier (BBB) during the early phase of ischemic brain injury (Zhang et al., 2011).

It has been established that excitatory amino acids, notably glutamate, play a pivotal role in neuronal death (Benveniste, 1991). Hyperglycemia enhances extracellular glutamate accumulation in rats subjected to forebrain ischemia (Li et al., 2000). Moreover, glutamate induces the release of HMGB1 from neurons, in vitro (Qiu et al., 2008). Based on these observations, we hypothesized that hyperglycemia could worsen brain ischemic damage by enhancing the early extracellular release of HMGB1. In the present study, firstly, we investigated the effect of hyperglycemia on the early release of HMGB1 using a rat model of 90-min middle cerebral artery occlusion (MCAO) with reperfusion. Secondly, we investigated the effect of extracellular HMGB1 on brain ischemic damage and the mechanism involved using an HMGB1 inhibitor glycyrrhizin.

2. Results

2.1. *Hyperglycemia enhances the early release of HMGB1 from ischemic brain tissue*

As shown in Table 1, the blood glucose was signifcantly increased after cerebral ischemia in hyperglycemic rats comparing with normoglycemic rats ($P<0.01$), and further elevated at reperfusion ($P<0.01$).

To assess the extracellular release of HMGB-1 after MCAO, we examined cerebrospinal fluid (CSF) for the presence of HMGB-1 using immunoblot. As shown in Fig. 1A, no detectable HMGB1 band in CSF of normoglycemic rats; however, HMGB-1 band was observed in CSF of hyperglycemic rats, at 2 h after reperfusion following 90 min MCAO. There was weak HMGB1 band in CSF of normoglycemic rats, and stronger HMGB1 band in CSF of hyperglycemic rats, at 4 h after reperfusion. No HMGB-1 was detectable in CSF from sham-operated animals at any above time points. These results indicate that hyperglycemia enhances the early extracellular release of HMGB1 after cerebral ischemia. We ran immunoblots with lysates from contralateral non-ischemic and ispsilateral ischemic rats brain tissues. As shown in Fig. 1B and C, total HMGB-1 did not significantly differ between ischemic and nonischemic tissues from homologous brain regions of normoglycemic and hyperglycemic rats subjected to 90 min MCAO followed by 4 h reperfusion, despite the extracellular release of HMGB1 into CSF. These data suggest the loss of HMGB-1 into the CSF is small relative to the total pool of HMGB-1.

2.2. *HMGB1 contributes to hyperglycemia-enhanced ischemic brain damage*

Using TTC staining, infarct volume was assessed at 24 h post-MCAO. Comparing with the normoglycemic rats, hyperglycemia significantly ($P<0.01$, Fig. 2A) increased infarct volume at 24 h post-MCAO. This was attributable to larger ($P<0.01$) cortical (Fig. 2B), but not subcortical (Fig. 2C), infarct. And, there was also a significant aggravation of neurological deficit in hyperglycemic rats compared with the normoglycemic rats ($p<0.05$, Fig. 2D).

As shown in Fig. 2, inhibiting the activity of HMGB1 using glycyrrhizin, a one of the specific HMGB1 inhibitors, significantly

Table 1 – Alteration of blood glucose.

Glucose (mmol/L)	Basal	After MCAO	At reperfusion
Normoglycemic ($n=20$)	5.03±0.41	9.68±0.76	8.07±1.05
Hyperglycemic ($n=21$)	5.22±0.36	26.67±2.41*	31.63±3.36*

Hyperglycemia was induced by IP injection of dextrose (50%; 6 ml/kg) 30 min before MCAO. Normoglycemic rats received vehicle (0.9% NaCl). Blood samples were withdrawn: before treatment (basal), immediately after MCAO, and at reperfusion, to measure glucose levels. Values are expressed as mean±SD. Data were analyzed for statistical differences with 1-way ANOVA, followed by Tukey–Kramer post-hoc analysis.
* $P<0.01$ vs. normoglycemic group.