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BRAIN RESEARCH

Research Report

A novel conjugate of low-molecular-weight heparin and Cu,Zn-superoxide dismutase: Study on its mechanism in preventing brain reperfusion injury after ischemia in gerbils

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ARTICLE DATA

Article history:
Accepted 31 December 2008
Available online 18 January 2009

Keywords:
Superoxide dismutase
Low-molecular-weight heparin
Conjugate
Cerebral ischemia
Global
Gerbils

ABSTRACT

Low-molecular-weight heparin (LMWH) and Cu,Zn-superoxide dismutase (SOD) were extensively investigated on preventing brain reperfusion injury after ischemia (BRII) in the past few years and both exhibited some advantages as well as limits in practice. To explore whether chemical modification for LMWH and SOD can lead to improved bioactivity, in our present study, we examined the efficacy of LMWH conjugated SOD (LMWH–SOD) in the model of BRII gerbils. Ischemia/reperfusion was performed for 5 min by clamping the bilateral common carotid arteries of gerbils. LMWH–SOD, SOD and the mixture of LMWH and SOD (LMWH+SOD) were administered intravenously to corresponding animals just before ischemia. 24 h after reperfusion, serum malondialdehyde (MDA) content and SOD activity were measured, the expression of intercellular adhesion molecule-1 (ICAM-1) was examined by immunohistochemistry, and the brain sections were processed for Nissl staining and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling. The results showed that LMWH–SOD significantly lowered MDA content ($P<0.001$, versus SOD and LMWH+SOD) and elevated SOD activity ($P<0.05$, versus SOD and LMWH+SOD) in the serum of BRII gerbils. Immunohistochemical results indicated ICAM-1 positive staining was lighter, pyramidal cells of hippocampal CA_1 region were more regular and the changes in cell edema were minor, and apoptosis of hippocampal cells was milder in LMWH–SOD treated animals than in SOD or LMWH+SOD treated animals, untreated BRII animals and sham-operated animals. The results suggest that the novel LMWH–SOD conjugate can inhibit upregulation of ICAM-1 and prevent neuronal cell apoptosis in BRII gerbils, and the LMWH–SOD conjugate has better anti-inflammatory and neuroprotective effects in BRII than native SOD and the mixture of LMWH and SOD.

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doi:10.1016/j.brainres.2008.12.079

1. Introduction

Reperfusion after ischemia often causes inflammatory response and generates an increasing amount of reactive oxygen species (ROS) (Chan, 2001; Maier et al., 2006; Sugawara and Chan, 2003). These two events exacerbate brain reperfusion injury after ischemia (BRII) in turn. Brain ischemia is accompanied by an acute inflammatory response characterized by leukocyte infiltration and development of brain edema (Wang et al., 2007). Additionally, increased leukocyte infiltration in a focal brain ischemia/reperfusion model was accompanied by increased expression of intercellular adhesion molecule-1 (ICAM-1) in cerebral microvessels (Wang et al., 1994). Those facts indicate that the expression of ICAM-1 is a valuable evaluation index for BRII. On the other hand, ROS produced during cerebral ischemia/reperfusion induce lipid peroxidation, protein oxidation and DNA damage, causing both acute and chronic neuronal injury (Wang et al., 2006; Warner et al., 2004). Among these ROS, superoxide anion radical has direct toxic effects on neurons and can initiate a free radical-mediated chain reaction causing neuronal damage such as brain edema, hydrocephalus or cerebral thrombus (Patel et al., 1996).

Several isolated case reports in the literature demonstrated that low-molecular-weight heparin (LMWH), in addition to its anti-thrombin effect, also has moderate immune suppression and anti-inflammation activities (Hermes De Santis et al., 1998; Salas et al., 2000). Moreover, it can affect blood rheology (Cui and Zhang, 1993). These bioactivities make LMWH potentially effective for BRII. In the past few years, some reports showed that Cu,Zn-superoxide dismutase (SOD) can attenuate infarct volume and alleviate hydrocephalus after transient focal or global cerebral ischemia (Noshita et al., 2003; Saito et al., 2003), but its short half-life and poor ability to be transported across the blood brain barrier (BBB) limit its application in clinical practice (Chan et al., 1993). Considering the importance of anti-inflammation during ischemia/reperfusion, we chemically modified SOD with LMWH and obtained a LMWH–SOD conjugate (Zhang et al., 2006) with the anticipation that this novel conjugate could possess unique advantages in BRII therapy.

In our present study, the efficacy of the novel LMWH–SOD conjugate in preventing BRII in gerbils was investigated, including the effects on the serum malondialdehyde (MDA) production and SOD activity, on the expression of ICAM-1 at hippocampal CA1 region, on the damage degree of hippocampus and on the neuronal cell apoptosis.

2. Results

2.1. *Serum MDA content and SOD activity*

After surgical procedure, the number of animals which could be used for the experiment in each group was as follows: (1) 11 in sham-operated group; (2) 11 in ischemia/reperfusion group; (3) 8 in SOD group; (4) 10 in LMWH+SOD group; (5) 10 in LMWH–SOD group. Serum MDA content assay showed that MDA level of LMWH–SOD group was significantly lower than that of the other groups ($P<0.001$, Fig. 1A). Among the five groups, MDA levels of the ischemia/reperfusion group were the highest, suggesting that lipid peroxidation was severe because of ischemia/reperfusion. Multiple comparisons among SOD, LMWH+SOD and LMWH–SOD groups showed that the LMWH–SOD conjugate had superior ability than SOD or LMWH+SOD in preventing MDA production and this ability was also proved by the evidence that the MDA

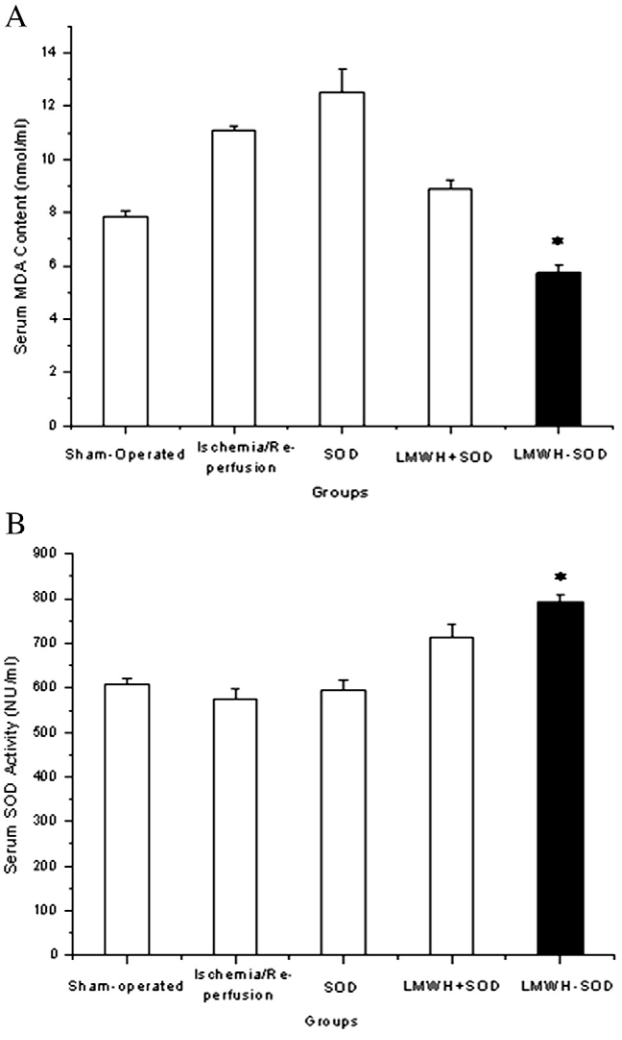

Fig. 1 – Comparison of serum MDA content and SOD activity 24 h after operation. (A) MDA content (mean ± SE) in serum of sham-operated ($n=11$), ischemia/reperfusion ($n=11$), SOD ($n=8$), LMWH+SOD ($n=10$) and LMWH–SOD ($n=10$) groups were compared with each other by one way ANOVA followed by Fisher's post-hoc protected least significant difference test. Asterisk indicates a significant decrease in serum MDA content compared with other groups ($P<0.001$ by Fisher's post-hoc protected least significant difference test). Sham-operated group was the group in which animals underwent the same surgical procedure but did not undergo ischemia/reperfusion. (B) Comparisons of serum SOD activity. Groups and methods of statistical analysis were as same as A. Asterisk indicates a significant increase in serum SOD activity compared with other groups ($P<0.05$ by Fisher's post-hoc protected least significant difference test).