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Research report

Inhibiting the CD38/cADPR pathway protected rats against sepsis associated brain injury

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ABSTRACT

Background: The CD38/cADPR pathway has been found to play roles in various inflammatory conditions. However, whether CD38 plays a protective or detrimental effect in the central nervous system (CNS) is controversial. The aim of this study was to determine the effect of CD38/cADPR pathway in sepsis associated brain injury.

Materials and methods: Male Sprague-Dawley rats were undergone cecal ligation and puncture (CLP) or sham laparotomies. NAD^+, cADPR and CD38 were measured in the hippocampus of septic rats at 0, 6, 12, 24, and 48 h after CLP surgery. Rats were divided into the sham, CLP group, CLP+ CD38 expression lentivirus (CLP+ CD38 LV), CLP+ CD38 interference lentivirus (CLP+ CD38 Ri), CLP+ negative control lentivirus (CLP + NC) and the CLP + 8-Br-cADPR groups. The Western blots of Bcl-2, Bax and iNOS, TUNEL assays, malondialdehyde (MDA) and superoxide dismutase (SOD) assays, transmission electron microscope analysis were performed in the hippocampus of rats.

Results: NAD^+, cADPR and CD38 levels increased significantly in the hippocampus of septic rats as early as 12–24 h after CLP surgery. CD38 knockdown or blocking cADPR with 8-Br-cADPR significantly reduced apoptosis, MDA and SOD activity, iNOS expression and ultrastructural morphology damages in the hippocampus of septic rats.

Conclusions: In this study, we found that the CD38/cADPR pathway was activated in sepsis associated brain injury. Blocking this pathway protected the hippocampus from apoptosis, oxidative stress and ultrastructural morphology damages in septic rats.

1. Introduction

CD38 is a member of the nicotinamide adenine dinucleotide (NAD^+)- glycohydrolase family, which catalyzes both the hydrolysis of NAD^+ and cyclic ADP-ribose (cADPR) to ADP-ribose and the cyclization of NAD^+ to cADPR (Higashida et al., 2007). cADPR is a main second messenger of Ca^{2+}, which activates Ca^{2+} outflow from the endoplasmic reticulum via ryanodine receptors (RyRs) and mobilizes Ca^{2+} influx through the transient receptor potential melastatin 2 (TRPM2) channel on the cell surface (Deshpande et al., 2005; Lee, 2011).

Recently, the CD38/cADPR pathway has attracted much attention for its role in the central nervous system (CNS). Higashida et al. found that CD38 is involved in the regulation of social behavior in mice through regulating oxytocin (Higashida et al., 2007). CD38 is highly expressed in developing brains, and CD38 knockout has been shown to impair the development of astrocytes and oligodendrocytes in mice (Hattori et al., 2017). Cyclic-ADPR is involved in long-term synaptic depression in the hippocampus (Reyes-Harde et al., 1999). However, the specific role of the CD38/cADPR pathway in CNS remains unclear. Some studies reported that CD38 plays a critical role in cell survival (Ma et al., 2012), and is required for neural differentiation by modulating reactive oxygen species (ROS) production (Wei et al., 2015). In contrast, other

Abbreviations: CNS, central nervous system; CLP, cecal ligation and puncture; MDA, malondialdehyde; SOD, superoxide dismutase; NAD^+, nicotinamide adenine dinucleotide; cADPR, cyclic ADP-ribose; RyRs, ryanodine receptors; TRPM2, transient receptor potential melastatin 2 channel; ROS, reactive oxygen species; BSA, bovine serum albumin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; FMN, riboflavin 5′-mono-phosphate; EDTA, ethylene diamine tetraacetie acid; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2 associated X protein; iNOS, indusible nitric oxide synthase; TUNEL, terminal uridine nucleotide end-labeling; TEM, Transmission Electron Microscope.

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studies have reported that CD38 knockdown brain damage in human immunodeficiency virus -1 encephalitis (Banerjee et al., 2008), and improved both histological and neurological outcomes in ischemic brain injury and traumatic brain injury (Long et al., 2017; Levy et al., 2009). The aim of this study was to clarify if the CD38/cADPR pathway plays a protective or detrimental role in sepsis associated brain injury and explore the underlying mechanisms.

2. Results

Dynamic alterations of NAD, CD38, and cADPR levels in the rat hippocampus during sepsis

NAD, CD38, and cADPR levels in the rat hippocampus were measured at 0, 6, 12, 24, and 48 h after CLP. The results show that NAD levels slightly increased as early as 6 h after CLP and peaked at 12 h after CLP ($p = .04$ vs. 0 h), while cADPR levels slightly increased from 12 h after CLP and peaked at 24 h after CLP ($p = .017$ vs. 0 h). Both NAD and cADPR levels had decreased 48 h after CLP (Fig. 1A). CD38 expression was upregulated as early as 6 h after CLP ($p = .011$ vs. 0 h), and had decreased after 48 h when compared to 24 h post CLP ($p = .038$) (Fig. 1B, C). As these changes were most obvious at the post CLP 24 h time-point, we collected tissue samples 24 h after CLP for the following studies.

2.1. Blocking the CD38/cADPR pathway reduced NAD, CD38, and cADPR levels in the hippocampus of septic rats

Intracellular NAD and cADPR levels increased significantly after CLP compared to the Sham group ($p = .005$ and .009, respectively). CD38 knockout significantly reduced cADPR levels compared to the CLP and CLP + NC groups ($p = .009$ and .018, respectively). In the CLP + 8-Br-cADPR groups, the NAD and cADPR levels were significantly diminished compared to the CLP group (Fig. 2A). Western blot analysis showed that CD38 expression was significantly reduced in the CLP + CD38 Ri group compared to the CLP and CLP + NC groups ($p = .004$ and .014, respectively). In the CLP + 8-Br-cADPR (40 nmol) group, CD38 expression was significantly depressed compared to the CLP group ($p = .008$) (Fig. 2B, C).

2.2. Blocking the CD38/cADPR pathway inhibited apoptosis in the hippocampus of septic rats

Western blot analysis showed that the Bcl-2/Bax ratio decreased and the TUNEL assay showed that the TUNEL positivity increased significantly after CLP ($p = .022$ and .000, respectively vs. the Sham group). CD38 knockout increased the Bcl-2/Bax ratio ($p = .022$ vs. the CLP group) but did not significantly decrease the TUNEL positivity. Intraventricular injection of 8-Br-cADPR (20 nmol and 40 nmol) increased the Bcl-2/Bax ratio ($p = .029$ and .048, respectively, vs. the CLP group), and 8-Br-cADPR (40 nmol) decreased the TUNEL positivity ($p = .001$ vs. the CLP group) (Fig. 3).

2.3. Blocking the CD38/cADPR pathway reduced MDA and SOD levels, and iNOS expression in the hippocampus of septic rats

Twenty-four hours after CLP, hippocampal MDA and SOD levels in septic rats significantly increased ($p = .005$ and $p = .038$, respectively, compared to the Sham group). Intraventricular injection of the CD38 interference lentivirus significantly reduced SOD ($p = .03$ vs. the CLP + NC group) and MDA levels ($p = .006$ vs. the CLP + NC group). Intraventricular injection of 8-Br-cADPR significantly reduced both SOD (20 nmol: $p = .021$ vs. the CLP group; 40 nmol: $p = .003$ vs. the CLP group) and MDA levels (20 nmol: $p = .008$ vs. the CLP group; 40 nmol: $p = .011$ vs. the CLP group) (Fig. 4A). Western blot analysis revealed that iNOS expression significantly increased after CLP ($p = .015$ vs. the Sham group), while CD38

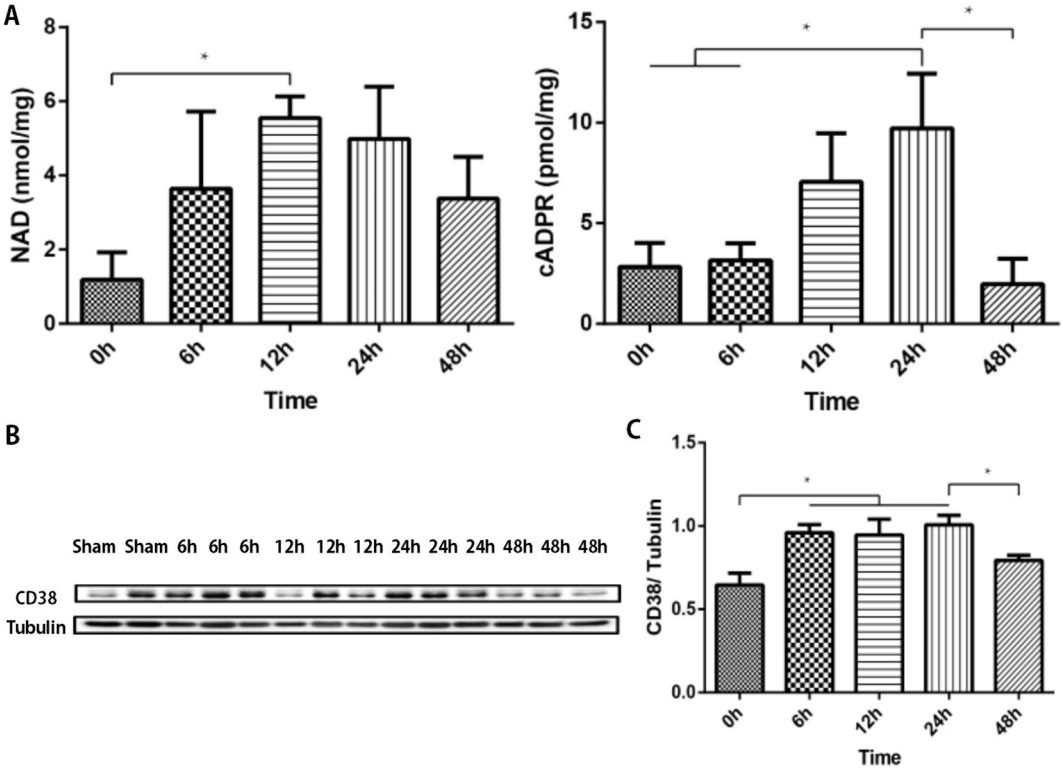

Fig. 1. Intracellular NAD and cADPR levels and CD38 expression in the hippocampus of rats. (A) Intracellular NAD and cADPR levels at different time points after CLP (n = 5). (B) Western blot analysis of CD38 and Tubulin at different time points after CLP. (C) Densitometry of CD38 expression normalized to Tubulin (n = 3).