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Anti-high mobility group box 1 antibody exerts neuroprotection in a rat model of Parkinson's disease

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ABSTRACT

The high mobility group box-1 (HMGB1) exists as an architectural nuclear protein in the normal state, but displays an inflammatory cytokine-like activity in the extracellular space under pathological condition. Inflammation in the pathogenesis of Parkinson's disease (PD) has been documented. In this study, we investigated the involvement of HMGB1 in the pathology and the neuroprotective effects of neutralizing anti-HMGB1 monoclonal antibody (mAb) on an animal model of PD. Adult female Sprague–Dawley rats were initially injected with 6-hydroxydopmaine (6-OHDA, 20 μg/4 μl) into the right striatum, then anti-HMGB1 mAb (1 mg/kg), or control mAb was intravenously administered immediately, at 6 and 24 h after 6-OHDA injection. The treatment with anti-HMGB1 mAb significantly preserved dopaminergic neurons in substantia nigra pars compacta and dopaminergic terminals inherent in the striatum, and attenuated PD behavioral symptoms compared to the control mAb-treated group. HMGB1 was retained in the nucleus of neurons and astrocytes by inhibiting the proinflammation-induced oxidative stress in the anti-HMGB1 mAb-treated group, whereas HMGB1 translocation was observed in neurons at 1 day and astrocytes at 7 days after 6-OHDA injection in the control mAb-treated group. Anti-HMGB1 mAb inhibited the activation of microglia, disruption of blood–brain-barrier (BBB), and the expression of inflammation cytokines such as IL-1β and IL-6. These results suggested that HMGB1 released from neurons and astrocytes was at least partly involved in the mechanism and pathway of degeneration of dopaminergic neurons induced by 6-OHDA exposure. Intravenous administration of anti-HMGB1 mAb stands as a novel therapy for PD possibly acting through the suppression of neuroinflammation and the attenuation of disruption of BBB associated with the disease.

1. Introduction

Parkinson's disease (PD) is a typical neurodegenerative disease that is clinically characterized by muscular rigidity, resting tremor, bradykinesia and postural reflex disorder. Currently, dopamine replacement therapy using L-dopa, dopamine receptor agonists and other agents is established as a gold standard of treatment for PD. Although surgical therapy including subthalamic nucleus or globus pallidus internus stimulation is also performed for PD in advanced stage, these approaches remain palliative at best. Therefore it is necessary to develop therapeutic agents with a different mode of action from the dopamine replacement therapy. The main pathology of PD is a loss of dopamine neurons in the substantia nigra pars compacta (SNc). Despite numerous basic research investigation, the underlying pathology of PD remains not fully understood. The involvement of neuroinflammation in the pathology of PD was reported in clinical settings as well as in experimental disease models. A large number of HLA-DR positive activated microglia were detected within the SNc of PD patients at postmortem (McGeer et al. 1988). Furthermore, a surge in MHC class II (CR3/43) positive microglia was shown to infiltrate the putamen, as well as the SNc of PD patients (Imamura et al. 2003). In an animal model of PD, employing intrastriatal injection of 6-hydroxydopmaine (6-OHDA), activated microglia were identified both in the SNc and striatum (Cicchetti et al. 2002; Depino et al. 2003; He et al. 2001).

High mobility group box 1 (HMGB1) is a highly conserved non-histone nuclear protein and contributes to the architecture of chromatin DNA. HMGB1 also belongs to a damage-associated-molecular-pattern (DAMP) family (Lotze and Tracey 2005; Tsung et al. 2014) and can be passively released from damaged cell to exacerbate inflammation through the receptors including receptor for advanced glycation end

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products (RAGE) and toll-like receptors (TLR2 and TLR4) (Park et al. 2004; Raman et al. 2006; Yang et al. 2006). The release of HMGB1 has been reported in various diseases such as septic shock (Wang et al. 1999; Yang et al. 2004), arthritis (Hamada et al. 2008), acute myocardial infarction (Andrassy et al. 2008) and acute pancreatitis (Sawa et al. 2006; Yasuda et al. 2006). Furthermore, released HMGB1 acts as a neuroinflammatory factor in central nervous system (CNS) disease such as ischemia, traumatic brain injury and spinal nerve ligation (Kawabata et al. 2010; Kim et al. 2006; Liu et al. 2007; Okuma et al. 2012; Zhang et al. 2011). Interestingly, anti-HMGB1 monoclonal antibody (mAb) neutralized the released HMGB1, prevented the inflammation cascade, and afforded a therapeutic effect in experimental model of ischemia and traumatic brain injury (Liu et al. 2007; Okuma et al. 2012; Shichita et al. 2012; Zhang et al. 2011).

In the present study, we explored behaviorally and immunohistochemically neuroprotective effects of intravenous injection of anti-HMGB1 mAb on rat model of PD. Additionally, we examined the mechanisms of action underlying these neuroprotective effects by investigating the inhibition of HMGB1 translocation in neurons and astrocytes, the blockade of activation of microglia, the amelioration of disruption of blood–brain-barrier (BBB) and the suppression of production of inflammatory cytokines.

2. Material and methods

2.1. Animals and animal care

All experimental procedures were conducted in accordance with the Okayama University guidelines for animal experiments and were approved by the University's committee on animal experimentation. Adult female Sprague–Dawley rats, weighing 200 to 250 g, were purchased from SHIMIZU Laboratory Supplies Co., Ltd. and were used for all experiments (n = 101). They were housed two per cage in a temperature- and humidity-controlled room which was maintained on a 12 h light/dark cycle with free access to food and water.

2.2. Surgical procedures

2.2.1. 6-OHDA lesion

All rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and placed in a stereotaxic apparatus (Narishige, Tokyo, Japan). A midline skin incision was made and a hole was then drilled in the skull. After this procedure, 6-OHDA (20 μg, 5 mg/ml, dissolved in 0.9% saline containing 0.2 mg/ml ascorbic acid; Sigma-Aldrich, St. Louis, MO, USA) was injected into the right striatum with a 28-gauge Hamilton Syringe (1.0 mm anterior, 2.5 mm lateral to the bregma, and 5.0 mm ventral to the surface of the brain with the tooth-bar set at −2.5 mm). The injection rate was 1 μl/min. After the injection, the syringe was left in the striatum for an additional 5 min before being retracted slowly (1 mm/min).

2.2.2. Administration of anti-HMGB1 mAb and appropriate controls

Anti-HMGB1 mAb (immunoglobulin, [IgG]2a subclass, 1 mg/kg), or class-matched control mAb (IgG2a) against *Keyhole Limpet* hemocyanin (both previously described; Liu et al. 2007) was intravenously administered three times, immediately, 6 h and 24 h after 6-OHDA injection. Control mAb was confirmed to have no neuroprotective effects on a rat model of PD as preliminary investigation by assessment of motor function and tyrosine hydroxylase immunohistochemistry in the striatum and the SNc (data not shown).

2.2.3. Assessment of motor function

2.2.3.1. Cylinder test. The cylinder test was conducted to assess the degree of forepaw asymmetry. All rats were individually placed in a transparent cylinder (diameter: 20 cm, height: 30 cm) for 3 min and the number of forepaw contacts to the cylinder wall was counted (Schallert et al. 2000). The score of cylinder test in this study was calculated as a contralateral bias, using the following formula: [(the number of contacts with the contralateral limb) − (the number of contacts with the ipsilateral limb) / (the number of total contacts) × 100] (Roof et al. 2001; Shinko et al. 2014).

2.2.3.2. Amphetamine-induced rotation test. All rats were challenged with established drug-induced rotational test, using amphetamine (3.0 mg/kg, i.p. Dainippon Sumitomo Pharma, Japan), at 7 and 14 days after 6-OHDA injection. The rotational behaviors were assessed for 90 min with a video camera. Full 360° turns ipsilateral to the lesion were counted.

2.2.3.3. Fixation and sectioning. All rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.), perfused from the ascending aorta with 200 ml of cold phosphate buffered saline (PBS), followed by 200 ml of 4% paraformaldehyde (PFA) in PBS. Brains were removed and preserved in 4% PFA.

2.2.3.4. Tyrosine hydroxylase (TH) immunohistochemical investigations. The fixed brains harvested at 14 days after 6-OHDA injection were then immersed in 30% sucrose in phosphate buffer (PB) until completely submerged and six series of coronal sections were cut at a thickness of 35 μm with a freezing microtome and stored at −20 °C. Free-floating sections were blocked with 3% hydrogen peroxide in 70% methanol for 8 min. Sections were washed 3 times for 5 min each time in PBS. Sections were then incubated overnight at 4 °C with rabbit anti-TH antibody (1:500; Chemicon, Temecula, CA, USA) with 10% normal horse serum. After several rinses in PBS, sections were incubated for 1 h in biotinylated donkey anti-rabbit IgG (1:500; Jackson Immuno Research Lab, West Grove, PA, USA), then for 30 min in avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA). Subsequently, the sections were treated with 3,4-diaminobenzidine (DAB; Vector) and hydroxygen peroxide, mounted on albumin-coated slides and embedded with cover glass.

2.2.3.5. Fluorescent immunostaining. In order to explore distribution of HMGB1 in neuron, astrocyte and microglia, immunofluorescence for double-staining of HMGB1 with microtubule-associated protein 2 (MAP2, neuron marker), glial fibrillary acid protein (GFAP, astrocyte marker), or ionized calcium binding adaptor molecule 1 (Iba1, microglia marker) was performed, respectively. Fixed brain was embedded in paraffin, and sections of 6 μm thick were used. The slices were soaked in limonene 2 times for 10 min each time, in 100% alcohol 2 times for 5 min each time, in 90% alcohol 2 times for 5 min each time and in distilled water (DW) for 3 min for deparaffinization. The slides were then soaked in 10 mM sodium citrate buffer (pH 6.0) in a heat-resistant container at autoclave unit at 120 °C for 10 min for antigen retrieval. After rinsing in running tap water for 5 min, the slides were washed by 0.025% TBS-triton (TBS-T) 2 times for 5 min each time and incubated with 10% normal goat serum (NGS) containing 1% bovine serum albumin (BSA) in TBS for 2 h at room temperature. And then the slices were incubated with primary antibodies; mouse anti-human HMGB1/HMG-1 antibody (1:100; R&D Systems, Minneapolis, MN, USA), rabbit anti-MAP2 antibody (1:500; Abcam, Cambridge, MA, USA), rabbit anti-GFAP antibody (1:1000; Novus Biologicals, Littleton, CO, USA) and rabbit anti-Iba1 antibody (1:250; Wako Pure Chemical Industries, Osaka, Japan) for 24 h at 4 °C, respectively. Corresponding secondary antibodies; Alexa fluor 555 F(ab′) fragment of goat anti-mouse IgG (H + L) (1:500; Life Technologies, Grand Island, NY, USA) and FITC-conjugated affinity-purified donkey anti-rabbit IgG (H + L) (1:100; Jackson) for 1.5 h at 4 degrees C in a dark chamber after several rinses with TBS-T, followed by 4′,6-diamidino-2-phenylindole (DAPI, 0.5 mg/mL) for 5 min. The sections were then extensively washed with TBS-T and coverslipped.