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Research Report

Valproic acid enhances the effect of bone marrow-derived mononuclear cells in a rat ischemic stroke model

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ABSTRACT

Bone marrow derived mononuclear cell (MNC) transplantation is a potential therapy for ischemic stroke. Here, we hypothesized that valproic acid (VPA) would modulate transplantation effects of MNCs in a rat ischemic stroke model. Male Sprague–Dawley rats were subjected to transient 90 min middle cerebral artery occlusion. Infarct volume, neurological outcome, and immunohistological assessments were performed 7 days after ischemia. MNCs injected 6 or 24 h but not 48 or 72 h after ischemia significantly reduced infarct volume and improved neurological deficits. We then tested whether the therapeutic window of MNC transplantation could be expanded through combination therapy with VPA. MNC transplantation at 48 h combined with VPA injection three times at 47, 53, and 72 h after ischemia significantly ameliorated infarct volume and neurological deficits compared to a vehicle group. Combination therapy reduced the number of myeloperoxidase-positive cells, ionized calcium binding adapter molecule 1-positive cells, tumor necrosis factor-α-positive cells, and von Willebrand factor-positive cells in the ischemic boundary zone. The number of engrafted MNCs that were fluorescently labeled with PKH 26, on day 7, was significantly higher after combination therapy than after that MNC transplantation alone. Our results demonstrated that combination therapy with VPA enhanced the anti-inflammatory and vasculo-protective effects against endothelial damage following ischemia, and increased the survival of transplanted cells, leading to expansion of the therapeutic time window for MNC transplantation. Together, these findings suggest that VPA may be an appropriate partner for cell-based treatment of ischemic stroke.

1. Introduction

Stroke is the third leading cause of death worldwide. It is more likely to leave sequelae than cardiac events are, and requires long-term rehabilitation and care. Stroke is thus also associated with socioeconomic problems such as increased family burden and medical costs. Cell-based treatments represent a new therapeutic approach for stroke. Stem cells from various sources have been evaluated in preclinical models; however, bone marrow derived mononuclear cells

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(MNCs) can be easily obtained from patients, thereby circumventing ethical concerns (Abe et al., 2012; Azizi et al., 1998; Kuroda, 2013). A number of animal studies have suggested that MNCs are a potential treatment for limb ischemia, myocardial infarction, and ischemic stroke (Higashi et al., 2004; Iwase et al., 2005; Yang et al., 2011). MNCs activate endogenous restorative responses in the injured brain by secreting various neurotrophic factors and cytokines (Iihoshi et al., 2004; Kasahara et al., 2013; Yang et al., 2011). Savitz and colleagues reported that intravenous infusion of autologous MNCs within 24–72 h of the onset of ischemic stroke is a safe and feasible therapy, and leads to improved functional outcome (Savitz et al., 2011). Since patients can deteriorate in the first few days after stroke, a strategy to expand the therapeutic time window for clinical application of MNCs is important.

Combination therapies might have additive or synergistic effects in certain experimental and clinical conditions. For example, experimental stroke studies using a combination of therapeutic agents have shown positive effects on infarct size, recovery of neurological functional, and therapeutic time window (Katsura et al., 2008; Leng et al., 2008). Valproic acid (VPA) is a drug that is widely used in the clinical treatment of epilepsy, bipolar mood disorder, and migraine, and has neuroprotective properties when used in cellular and animal models. Previous studies have shown that VPA decreases brain infarct volume and neurologic deficits through inhibition of oxidative stress and inflammation in a rat focal ischemic model (Chen et al., 2007; Jeong et al., 2003; Suda et al., 2013). However, there are no reports to evaluate whether systemic administration of VPA can produce additional improvement when combined with MNC transplantation in ischemic stroke models. The present study aimed to examine whether systemic administration of VPA can enhance the neuroprotective effects of MNCs, thereby expanding the therapeutic time window in a rat ischemic stroke model and, if so, to investigate the mechanisms of neuroprotection.

2. Results

2.1. *Therapeutic time window of MNCs*

Our first step was to examine the therapeutic time window for MNCs in this setting. MNCs injected 6 h or 24 h after middle cerebral artery occlusion (MCAO) significantly reduced the infarct volume and improved neurological deficits compared to the vehicle group ($p<0.05$). However, MNCs were ineffective when administered 48 h or 72 h after MCAO (Fig. 1((A) and (B)). These results indicated that the therapeutic time window of MNCs was up to and including 24 h after MCAO, but was less than 48 h.

2.2. *VPA expands the therapeutic time window of MNCs*

The therapeutic time window for the treatment of MNCs alone was less than 48 h after the onset of ischemia. We then tested whether VPA could expand the therapeutic window of MNC transplantation. MNC transplantation at 48 h combined with single injection of VPA (MNCs+VPA (1) group) had no effect on infarct volume or neurological deficit scores. MNC transplantation at 48 h combined with VPA injection twice at 47 and 53 h (MNCs+VPA (2) group) improved neurological deficit scores, but had no effect on infarct volume. MNC transplantation at 48 h combined with VPA injection three times at 47, 53, and 72 h after ischemia (MNCs+VPA (3) group), significantly reduced the infarct volume and improved neurological deficits compared to vehicle ($p<0.05$; Fig. 2(A) and (B)). VPA alone failed to exert protective effects in this experimental setting. Percentage decrease in body weight in the MNCs+VPA (3) treatment group was significantly lower than that of the control group ($p<0.05$; Fig. 2(C)).

2.3. *Immunohistochemical evaluation*

To elucidate the protective mechanism of combination therapy, we examined myeloperoxidase (MPO), ionized calcium binding adapter molecule 1 (Iba-1), tumor necrosis factor-α (TNF-α), and von Willebrand factor (vWF) staining in the vehicle, MNCs alone, VPA alone, and MNCS+VPA (3) groups. Quantitative analysis demonstrated that the number of Iba1-positive cells in the combination MNCS+VPA (3)-treated group was significantly lower than each monotherapy ($p<0.05$; Fig. 3(B)). The number of MPO-positive cells for MNCs alone and VPA alone was lower than in the vehicle group ($p<0.05$; Fig. 3(D)). Combination MNCS+VPA (3) treatment resulted in the greatest reduction in the number of MPO-positive cells across the four groups ($p<0.05$; Fig. 3(D)).

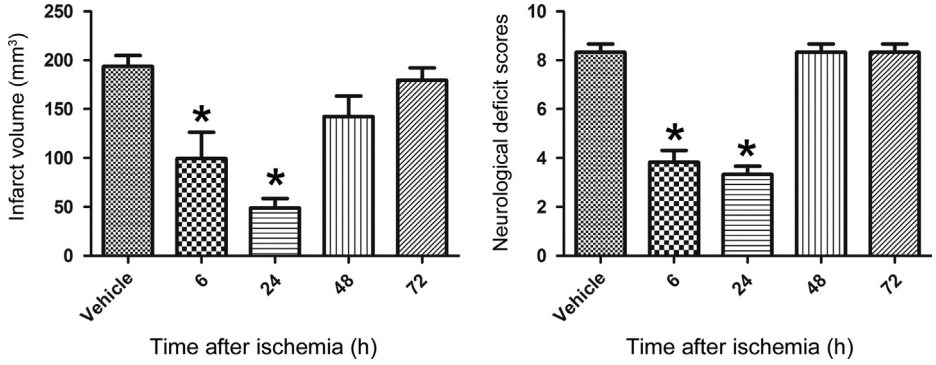

Fig. 1 – Comparison of infarct volume (A) and neurological deficit score (B) at 7 days after ischemia in vehicle-treated and MNCs-treated groups at different time points in rats ($n=6$, each group). Data are the mean±S.D. $^*p<0.05$ compared with vehicle.