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Research report

Electroacupuncture modulates stromal cell-derived factor-1α expression and mobilization of bone marrow endothelial progenitor cells in focal cerebral ischemia/reperfusion model rats

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ABSTRACT

Stromal cell-derived factor-1α(SDF-1α) plays a crucial role in regulating the mobilization, migration and homing of endothelial progenitor cells(EPCs). Electroacupuncture(EA), a modern version of Traditional Chinese Medicine, can improve neurological recovery and angiogenesis in cerebral ischemic area. This study aimed to investigate the effects of electroacupuncture(EA) on the mobilization and migration of bone marrow EPCs and neurological functional recovery in rats model after focal cerebral ischemia/reperfusion and the potentially involved mechanisms. Sprague-Dawley rats received filament occlusion of the right middle cerebral artery for 2 h followed by reperfusion for 12 h, 1d, 2d, 3d, 7d respectively. Rats were randomly divided into sham group, model group and EA group. After 2 h of the reperfusion, EA was given at the "Baihui" (GV 20)/Siguan ("Hegu" (LI 4)/"Taichong" (LR 3)) acupoints in the EA group. Modified neurological severity score (mNSS) was used to assess the neurological functional recovery. EPCs number and SDF-1α level in bone marrow(BM) and peripheral blood(PB) were detected by using fluorescence-activated cell sorting (FACS) analysis and quantitative real time polymerase chain reaction (qRT-PCR) respectively. An mNSS test showed that EA treatment significantly improved the neurological functional outcome. EPCs number in PB and BM were obviously increased in the EA group. After cerebral ischemia, the SDF-1α level was decreased in BM while it was increased in PB, which implied a gradient of SDF-1α among BM and PB after ischemia. It suggested that the forming of SDF-1α concentration gradient can induce the mobilization and homing of EPCs. Eletroacupuncture as a treatment can accelerate and increase the forming of SDF-1α concentration gradient to further induce the mobilization of EPCs and angiogenesis in ischemic brain and improve the neurological function recovery.

1. Introduction

Ischemic cerebrovascular disease is a leading cause of mortality and disability worldwide after heart disease and cancer, which lacks effective therapeutic method (Mozaffarian et al., 2015). Rapid vessel recanalization is a relatively effective therapy, but the short time window makes some patients cannot timely get access to effective treatment (Emberson et al., 2014; Katzan et al., 2000). Although many drugs were confirmed to relieve brain damage and symptoms in animal models, they could not reach similar therapeutic benefit when applied to clinical setting (Rimmele and Thomalla, 2014). Therefore, further investigation of effective treatments and interventions against cerebral ischemia is badly needed (Hennerici et al., 2013). Acupuncture, a traditional Chinese non-drug treatment, which has been used to treat cerebral ischemia for thousands of years, can effectively promote the recovery and angiogenesis after cerebral ischemia. However, its mechanism is still unclear (Lu et al., 2013).

The prognosis of cerebral ischemia mostly depends on the angiogenesis, neurogenesis and the reconstruction of cerebral circulation in ischemic area (Gutierrez-Fernandez et al., 2012). Endothelial progenitor cells (EPCs) hold great potential in treatment of ischemic diseases, including cerebral ischemia (Mund et al., 2009). EPCs mainly exist in bone marrow (BM), which can differentiate into mature endothelial cells (ECs) and promote angiogenesis (Heissig et al., 2002; Urbich and Dimmeler, 2004). Stromal cell-derived factor-1α (SDF-1α) along with its unique receptor chemokine (CXC motif) receptor 4 (CXCR4) can regulate the

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biological characteristics of EPCs (Liekens et al., 2010; Yamaguchi et al., 2003). SDF-1α can promote the proliferation, migration, and inhibit the apoptosis of EPCs (Shao et al., 2008; Zheng et al., 2008). Overexpression of SDF-1α induces EPCs migrating and promoting angiogenesis in ischemia area (Hiasa et al., 2004; Shiba et al., 2009; Zemani et al., 2008). After cerebral ischemia, the SDF-1α level was significantly upregulated in peripheral blood (PB) and ischemic brain (Hill et al., 2004). In addition, the SDF-1α level in peripheral blood was strongly correlated with the circulating number of EPCs in the early stage of ischemic stroke patients (Bogoslovsky et al., 2011).

Electroacupuncture (EA) can definitely promote angiogenesis in ischemic brain (Hennerici et al., 2013; Ma and Luo, 2007; Sun et al., 2012; Wang et al., 2010), and increase the number of $CXCR4^+$EPCs, $VEGFR2^+$EPCs, $VEGFR2^+PECAM^+$ EPCs, $CD31^+VEGFR2^+$EPCs in the PB and BM (Cai et al., 2009; Zhao et al., 2010). We speculated that electroacupuncture promotion of angiogenesis in ischemic brain may be related with the mobilization and migration of EPCs, while its mechanism is still unclear. According to the Experimental Animals Meridians Mapping, the "Baihui"(GV20) acupoints and "Siguan"(Hegu(LI 4)/Taichong(LR 3)) acupoints on the affected side were chosen. This study aimed to explore the mechanism of electroacupuncture promoting angiogenesis after cerebral ischemia, and determine whether it is related with the upregulation of SDF-1α expression and the mobilization of bone marrow EPCs into the ischemic brain.

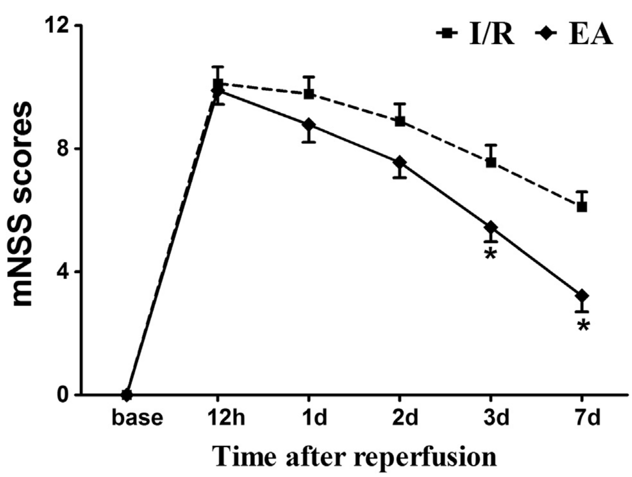

Fig. 1. Modified neurologic severity scores. Rats were treated with or without electroacupuncture (EA) after MCAO/R daily for 7d. mNSS scores were performed before MCAO and 12 h, 1d, 2d, 3d, 7d after MCAO/R. *P < 0.05 versus model group.

2. Results

2.1. Electroacupuncture improves neurological function after focal cerebral ischemia/reperfusion

To determine whether electroacupuncture treatment improves neurological functional recovery, a modified neurologic severity scores tests were performed. After focal MCAO/R, as shown in Fig. 1, rats showed neurological deficit behavior at each time point. Electroacupuncture significantly improved the neurological functional outcome compared with model group at 3d and 7d ($P < 0.01$), while there was no significant functional improvement at 12 h, 1d, 2d. As time went on, the neurological function recovered slightly.

2.2. Electroacupuncture increased the number of $CD34^+VEGFR2^+$EPCs in peripheral blood after focal cerebral ischemia/reperfusion

The number of $CD34^+VEGFR2^+$ EPCs in peripheral blood were quantified by FACS. $CD34^+VEGFR2^+$ EPCs number in the model and EA group were increased compared with that in the sham group ($P < 0.05$). EPCs number in model group began to increase at 12 h ($P < 0.05$), and peaked at 1d ($P < 0.01$) then gradually decreased. While compared with the model group, there was no statistical differences at 12 h in EA group ($P > 0.05$). However, EPCs number in EA group increased and had a significant difference compared with model group from 1d to 7d($P < 0.05$). In the EA group, EPCs number increased from 12 h to 2d and decreased from 3d to 7d in peripheral blood (Fig. 2).

2.3. Electroacupuncture increased the number of $CD34^+VEGFR2^+$ EPCs in bone marrow after focal cerebral ischemia/reperfusion

In the model group, as shown in Fig. 3, EPCs number were significantly upregulated at 12 h, 1d, 2d and peaked at 12 h compared with sham group, while it gradually reduced later ($P < 0.05$). In EA group, EPCs number obviously increased at 1d, 2d compared with model group and peaked at 1d ($P < 0.01$), while there was no significant difference from 3d compared with model group, but it was still higher than sham group ($P < 0.05$).

2.4. Eletroacupuncture promotes SDF-1α expression in the bone marrow and peripheral blood

The mRNA expression of SDF-1α in BM and PB were detected by fluorescent quantitative RT-PCR. Compared with that in model

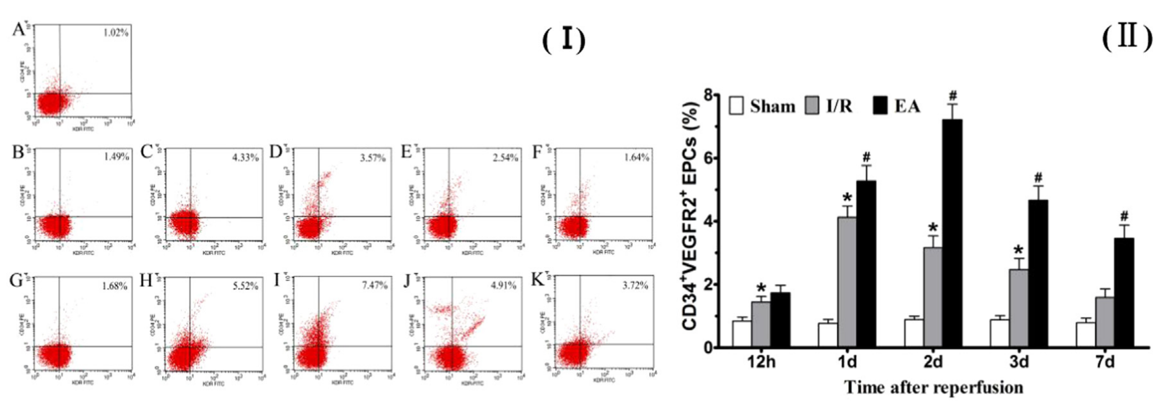

Fig. 2. Quantitative evaluation of endothelial progenitor cells (EPCs) in PB by FACS analyses. (I) Representative FACS data, in which the $CD34^+VEGFR2^+$ cells from the model group (B-F) and EA group (G-K) were judged as EPCs. Analyses of PB were performed at various time points [12 h (B, G), 1d (C, H), 2d (D, I), 3d (E, J) and 7d (F, K) after reperfusion, Sham group (A)]. (II) Comparison number of $CD34^+VEGFR2^+$EPCs in these groups at each time point. *P < 0.05 versus sham group, #P < 0.05 versus model group and sham group.