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Original research

Stem cells from human exfoliated deciduous teeth enhance recovery from focal cerebral ischemia in rats

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ABSTRACT

Purpose: The aim of this study was to investigate the effect of stem cells from human exfoliated deciduous teeth (SHED) after permanent MCAO (pMCAO).
Materials and methods: Adult male Sprague-Dawley rats were subjected to pMCAO. After pMCAO, SHED was transplanted into the brain. Motor function and infarct volume were evaluated. Neurogenesis and vasculogenesis were determined using immunochemical markers.
Results: The SHED group had more positive signals for doublecortin, neurofilament, anti-neuronal nuclei (NeuN) and rat endothelial cell antigen-1 (RECA1) in the peri-infarct area than the PBS group. Migration of doublecortin-positive neural progenitor cells (NPCs) from subventricular zone (SVZ) to the peri-infarct area was observed on day 16. Transplanted SHED merged vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 (SDF-1) positive cells.
Conclusion: SHED promoted migration and differentiation of the endogenous NPCs and induced vasculogenesis, and ameliorated ischemic brain injury after pMCAO in rats.

1. Introduction

Stroke is the third major cause of death worldwide and the most frequent cause of long-term disability in human beings [1]. Recently, the transplantation of bone marrow mononuclear cells achieved clinical efficacy by including angiogenesis in patients with cerebral ischemia [2,3]. However, aspiration of bone marrow to acquire bone marrow mesenchymal stem cells (BMMSCs) is an invasive and painful procedure for the donor. In addition, the number, proliferation, and differentiation potential of BMMSCs decline with increasing age [4]. In contrast, dental pulp stem cells (DPSCs) can be obtained non-invasively from teeth that are generally discarded as medical waste after extraction. DPSCs exhibit highly vasculogenic potential in vitro and promote revascularization in hind limb ischemia [5]. Recently, we reported that the transplanting DPSCs promoted neurogenesis and vasculogenesis in an induced peri-infarct area, and enhanced recovery after middle cerebral artery occlusion (MCAO) in rats [6]. Furthermore, these cells released growth factors, and promoted migration and differentiation of the endogenous neural progenitor cells (NPCs) in subventricular zone (SVZ). Furthermore, the proliferation rate of stem cells from human exfoliated deciduous teeth (SHED) was significantly higher than that of DPSCs and BMMSCs. SHED expressed several growth factors [7]. In the present study, we investigated the effects of SHED on neurogenesis and vasculogenesis in a rat cerebral ischemia model. In addition, we evaluated the effects of permanent MCAO (pMCAO) on the motor dysfunction and infarct volume.

2. Materials and methods

2.1. Harvesting SHED

Human dental pulp tissues were obtained from clinically healthy extracted deciduous teeth from eight patients. The Ethics Committee of Nagoya University approved our experimental protocols. SHED were isolated and cultured as previously described [8,9]. In brief, the pulp was gently removed and digested in a solution of 3 mg/ml collagenase-type I and 4 mg/ml dispase for 1 h at 37 °C. The cells were filtered using 70-μm cell strainers (Falcon; BD Labware, Franklin Lakes, NJ), and cultured in Dulbecco's

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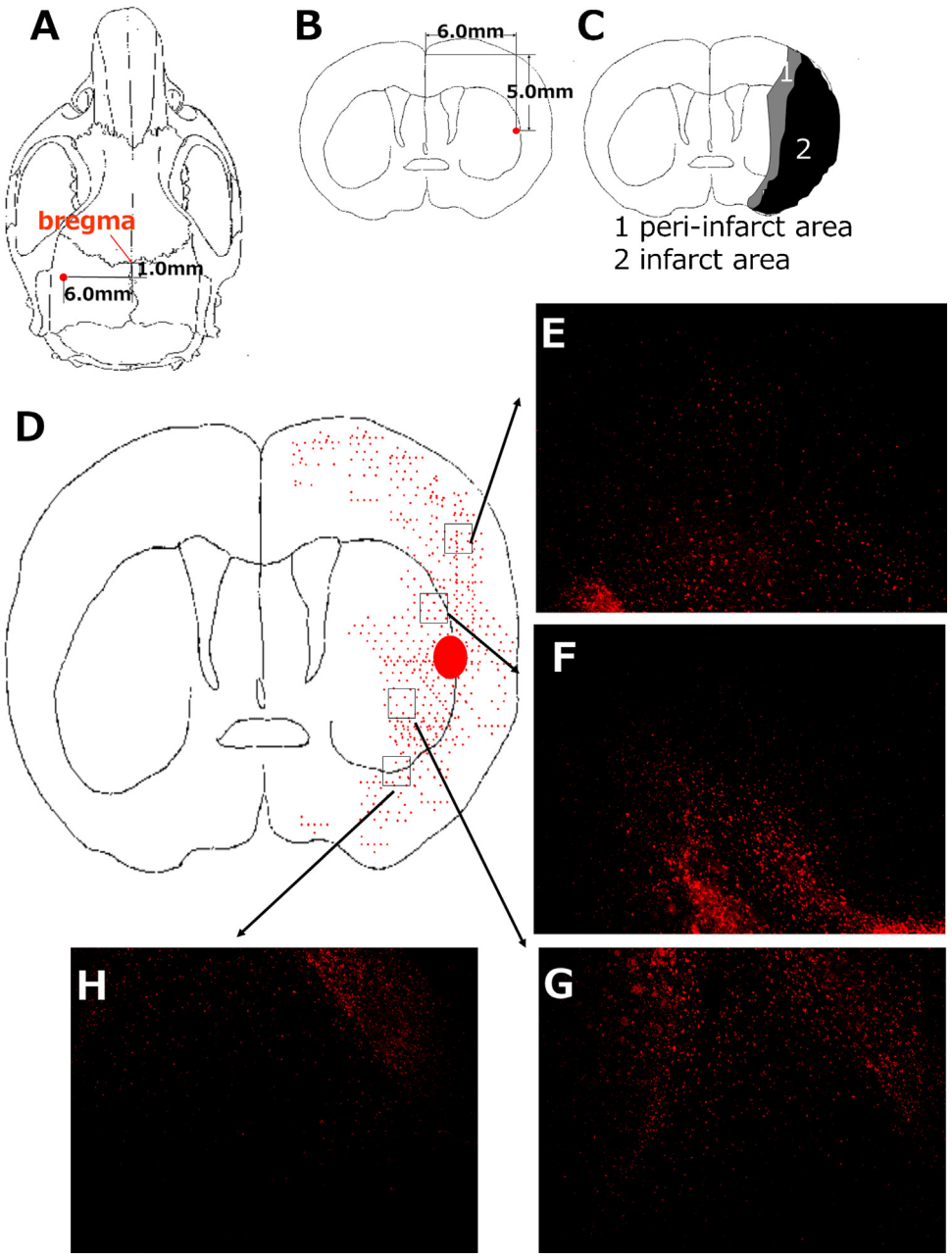

Fig. 1. (A) Overhead and (B) coronal views of the injection site. (C) The peri-infarct area. Peri-infarct area (gray), infarct core (black). (D) DiI-labeled transplanted SHED (showed by red) migrated from the original injection site to the peri-infarct area in the cortex and striatum. (E–H) High magnification images of DiI-labeled transplanted SHED in D. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Modified Eagle Medium (DMEM; GIBCO, Rockville, MD) containing 20% mesenchymal cell growth supplement (Lonza Inc., Walkersville, MD) and antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin, and 0.25 mg/ml amphotericin B; GIBCO) at 37 °C under 5% CO_2. After primary culture, the cells were subcultured at approximately 1×10^4 cells/cm^2. These cells were used in the experiments from three to five passages.

2.2. Cerebral ischemia model

All animal experiments were approved by the Institutional Animal Care and Use Committee (Nagoya University). Adult male Sprague-Dawley rats (Japan SLC Inc., Shizuoka, Japan) weighing 300–400 g were used. Animals were initially anesthetized with 5% isoflurane (Abbott Laboratories, Chicago) and maintained under anesthesia with 1.5% isoflurane in a mixture of 70% N_2O and 30% O_2. Rectal temperature was maintained at 37 ± 0.5 °C using a heating pad. Focal cerebral ischemia was induced by pMCAO [10]. A 4-0 monofilament nylon suture (Shirakawa, Tokyo, Japan) whose tip was rounded by flame heating and uniformly coated with silicone (KE-200, Shin-Etsu Chemical, Tokyo, Japan) was advanced from the external carotid artery into the internal carotid artery until it blocked the origin of MCA. The regional cerebral blood flow of MCA was measured using a laser-Doppler flowmeter (Omega FLO-N1; Omega Wave Inc., Tokyo, Japan) after occlusion. The response was considered positive and included only if the reduction in regional cerebral blood flow was greater than 70% [6].