Preconditioned protective microglia by oxygen-glucose deprivation promote functional recovery in ischemic rats
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Background

1. Enhancement of angiogenesis might be one strategy for facilitating functional recovery after ischemic stroke. In fact, a previous study using a rodent model demonstrated that enhanced angiogenesis via the intravenous administration of vascular endothelial growth factor (VEGF) at 2 days after cerebral ischemia promoted functional recovery. However, systemic injection of VEGF causes adverse effects. A therapeutic strategy that enhances angiogenesis without these adverse effects is desirable.

2. Cell therapies that invoke pleiotropic mechanisms may facilitate functional recovery in stroke patients. Microglia are the main source of the growth factors in the CNS. Although several studies have demonstrated that microglia might expand cerebral infarct volume in the acute phase, microglia after cerebral ischemia in the subacute and chronic phases are known to play protective roles via tissue and vascular remodeling. These ischemia-induced protective microglia are called M2-like microglia, and their protective effect is considered to be due to the secretion of remodeling factors, such as VEGF and transforming growth factor-beta (TGF-β) after ischemia, that may facilitate anti-inflammation, angiogenesis, and axonal outgrowth after cerebral ischemia (Hu et al. 2012). In addition, transplanted microglia can cross the BBB, particularly in the ischemic condition.

Therefore, we speculated that a cell-based therapy using microglia preconditioned by optimal ischemic condition might be an ideal and convenient therapeutic strategy for ischemic stroke.

Objectives

We hypothesized that intraarterially administered microglia preconditioned by oxygen-glucose deprivation (OGD) can secrete remodeling factors in the injured brain parenchyma, and exert pleiotropic therapeutic effects via promotion of angiogenesis and axonal outgrowth against focal cerebral ischemia even in the subacute phase.

Materials and Methods

This study was conducted in accordance with the Niigata University guidelines for the care and use of animals in research. All in vivo studies were performed according to ARRIVE guidelines (Kilkenny et al., 2010).

1. Focal cerebral ischemia. Eight-week-old male Sprague-Dawley rats weighing 280–320 g were used. Under anesthesia, transient focal cerebral ischemia was induced, using the suture technique for 90 min (Kanazawa et al. 2011).

2. This model provides an area of the ischemic core and penumbra determined by the presence of MAP2. The definitions of the ischemic core is the MAP2-negative lesion (Zhao et al. 2005).

3. Primary murine microglia, and astrocytes were prepared from the neocortices of mice (Milner et al., 2008, Kanazawa et al., 2016).

4. We examined the therapeutic benefits of intraarterially administered microglia preconditioned by OGD (OGD microglia) at 7 days after focal cerebral ischemia.

5. Sensorimotor assessments were performed at 0 to 28 days after transplantation) after cerebral ischemia using the corner test.

Result 1

Angiogenesis on the border of the ischemic core in the subacute phase of focal cerebral ischemia

(A) Cluster of differentiation 31 (CD31)/microtubule-associated protein 2 (MAP2)/DAPI triple labelling of cerebral cortices in the non-ischemic (sham-operated) and ischemic core and penumbra at 1, 3, 7, and 14 days after cerebral ischemia examined by confocal microscopy. Scale bars, 15 μm.

(B) The immunoreactivity of CD31-positive volume (μm^3) per unit volume (μm^3) at 1 (D1), 3 (D3), 7 (D7), and 14 (D14) days after cerebral ischemia (N = 21). *P < 0.05, **P < 0.01 versus sham-operated rats.

(C) MAP2 (green)/DAPI (red)/DAPI (blue) triple labelling of cerebral cortices in the ischemic core and penumbra at 7 days after cerebral ischemia examined by confocal microscopy. Scale bars, 20 μm.

Discussion

The schema of angiogenesis and axonal outgrowth by OGD-preconditioned microglial transplantation (blue cells) after cerebral ischemia. OGD-preconditioned microglial transplantation markedly activated angiogenesis (red lines) at the angiogenesis-positive core (ischemic border area). In addition, the decrease in the expression of CGRP (gray area), which is known to be an axonal outgrowth inhibitor, was observed in the ischemic penumbra after cerebral ischemia. An axonal outgrowth of neuronal cells (green) by OGD-preconditioned microglial transplantation was observed in the ischemic penumbra.

In conclusion, intravascular administration of protective microglia preconditioned by OGD might be a novel therapeutic strategy against ischemic stroke.