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Short communication

Participation of bone marrow-derived cells in hippocampal vascularization after status epilepticus

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ABSTRACT

Purpose: Diseases such as temporal lobe epilepsy, brain trauma and stroke can induce endothelial cell proliferation and angiogenesis in specific brain areas. During *status epilepticus* (SE), bone marrow-derived cells are able to infiltrate and proliferate, dramatically increasing at the site of injury. However, it is still unclear whether these cells directly participate in vascular changes induced by SE.
Method: To investigate the possible role of bone marrow-derived cells in angiogenesis after seizures, we induced SE by pilocarpine injection in previously prepared chimeric mice. Mice were euthanized at 8 h, 7 d or 15 d after SE onset.
Results: Our results indicated that SE modified hippocampal vascularization and induced angiogenesis. Further, bone marrow-derived GFP$^+$ cells penetrated through the parenchyma and participated in the formation of new vessels after SE. We detected bone marrow-derived cells closely associated with vessels in the hippocampus, increasing the density of blood vessels that had decreased immediately after pilocarpine-induced SE.
Conclusion: We conclude that epileptic seizures directly affect vascularization in the hippocampus mediated by bone marrow-derived cells in a time-dependent manner.

1. Introduction

In neurological disorders such as hypoxia/ischemia, epilepsy and brain tumors, the proliferation of endothelial cells induces the growth of new blood vessels in the brain.[1,2] Recent studies have shown that vascular alterations occur after epileptic seizures in both humans and animals.[3–6] Blood–brain barrier permeability and increased blood flow into the brain are associated with the increased vascular density and angiogenesis that occur in the epileptic brain.[3] In particular, hippocampal vascularization is severely compromised during epileptogenesis in animal models of temporal lobe epilepsy.[4,6] In addition, after brain damage, inflammatory and endothelial progenitor cells within the bone marrow cell population infiltrate into the brain and proliferate, increasing dramatically at the site of injury.[7–10] These circulating bone marrow endothelial cells have been suggested to participate in neovascularization after brain injury.[11]

According to these observations, bone marrow-derived cells can participate in *status epilepticus* (SE)-induced angiogenesis and recruit circulating endothelial progenitor cells from bone marrow to the brain after SE. To test this possibility, we proposed to investigate the role of bone marrow-derived cells in the hippocampal vasculature at various time-points after pilocarpine-induced SE animals. Chimeric mice engrafted with bone marrow cells expressing enhanced green fluorescent protein (eGFP) were used to visualize and easily track bone marrow-derived cells incorporated into the blood vessels.

2. Materials and methods

All animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council). All protocols were approved by the Animal Care and Ethics Committee of the University (no. 0334/08).

2.1. Chimera preparation

We prepared the chimeric mice ($n = 29$) by transplanting bone marrow from the C57BL/6 eGFP transgenic mice into lethally irradiated (600 rad, ^{137}Cesium source irradiator) adult male C57BL/

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6 mice. Bone marrow-derived cells were obtained from adult eGFP-donor mice (20–25 g) by flushing the femurs and tibiae with sterile medium. The cells were washed in Dulbecco's modified Eagle's medium (DMEM, Gibco, San Diego, CA, USA), counted, and resuspended in sterile saline. Approximately 3×10^7 cells were administered into each irradiated recipient animal *via* intravenous injection. The chimeric mice were allowed to recover for one month prior to SE induction.

2.2. SE induction

Chimeric mice were injected with pilocarpine (280 mg/kg, intraperitoneal, i.p., Merck, Brazil) to induce SE. Fifteen minutes after the pilocarpine administration, the animals began showing stereotypic behaviors and acute seizures, as described previously.[12] SE was characterized by continuous epileptic seizure, rearing and falling, straub tail, and repeated head twitches. Because of the high mortality rate of the chimeric mice during SE, we administered thionembutal (25 mg/kg, i.p.) 30 min after the SE onset.[9] Thus, the total SE duration varied from 30 to 50 min, with Racine stage 3–5 in the first 30 min going down to stage 2–3 after thionembutal administration. At the following time-points post-SE, the animals were deeply anesthetized by overdose of thionembutal (200 mg/kg, i.p.): 8 h ($n = 5$), 7 d ($n = 7$) or 15 d ($n = 7$) and control no-SE chimeric animals ($n = 10$).

2.3. Immunofluorescence

Mice were deeply anesthetized and perfused through the heart with 50 mL of phosphate-buffered saline (PBS) followed by

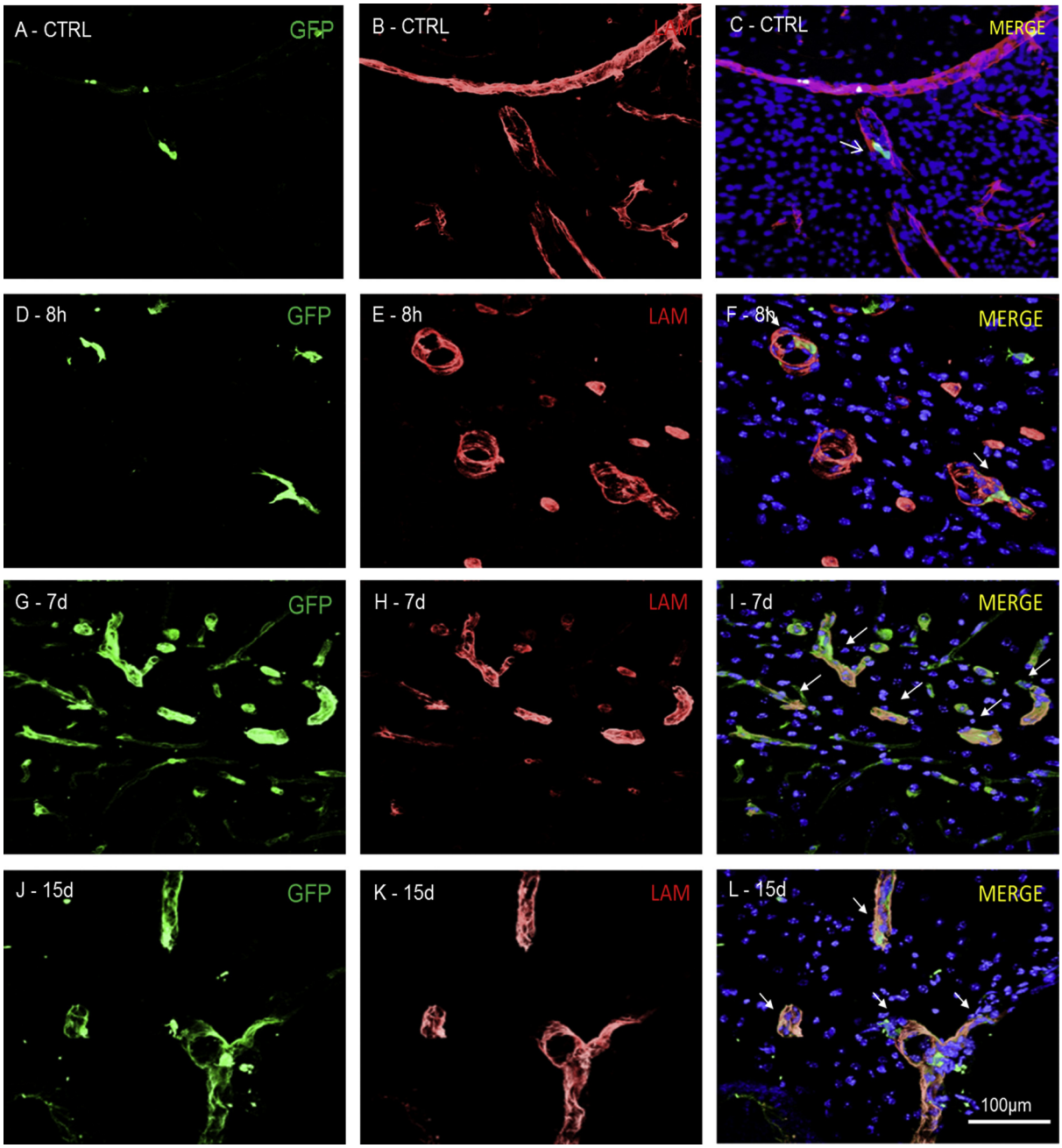

Fig. 1. Photomicrographs showing immunofluorescence representative sections of co-expression GFP and laminin in the CA1 of chimeric SE-induced mice of the four groups, (A–C) control group, (D–F) 8 h after SE, (G–I) 7 days after SE and (J–L) 15 days after SE. Note the low number of GFP^+ cells co-localized with vessels in merged figures at 8 h (F) compared with 7 and 15 days after SE (I and L). Scale bars represent 100 μm.