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Chemokines and their receptors in the brain: Pathophysiological roles in ischemic brain injury

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Abstract

Chemokines constitute a large family of structurally-related small cytokines originally identified as factors regulating the migration of leukocytes in inflammatory and immune responses. Production of chemokines and their receptors in the brain has been reported under various pathological conditions. We revealed that mRNA expression for monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α), members of the CC chemokines, was induced in the rat brain after focal cerebral ischemia, and that intracerebroventricular injection of viral macrophage inflammatory protein-II (vMIP-II), a broad-spectrum chemokine receptor antagonist, reduced infarct volume in a dose-dependent manner. These findings suggest that brain chemokines are involved in ischemic injury, and that chemokine receptors are potential targets for therapeutic intervention in stroke.

Another potential target to suppress the harmful effect of chemokines is the signal transmission system(s) regulating the chemokine production. However, very little is known about how the production of chemokines is regulated in the ischemic brain. We examined the induction of MCP-1 production by excitotoxic injury via activation of NMDA receptors in the cortico-striatal slice cultures, and found that excitotoxic injury induced MCP-1 production in the slice culture. Almost all of the MCP-1 immunoreactivity was located on astrocytes. On the other hand, NMDA-treatment failed to increase the MCP-1 production in the enriched astrocyte cultures, indicating that NMDA dose not directly act on astrocytes. Some signal(s) is likely sent from the injured neurons to astrocytes to induce the MCP-1 production. These results showed that organotypic slice cultures are useful to investigate the molecular mechanism regulating the chemokine production in the injured brain.

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Introduction

Chemokines constitute a large family of structurally related cytokines that includes more than forty members, and the family has been divided into four subfamilies; i.e. CXC, CC, C and CX_3C (Rollins, 1997). Chemokines were originally implicated in the inflammatory and allergic responses in peripheral organs. However, recently, it has been reported that chemokines are also expressed in the brain under various pathological conditions including mechanical injury (Hausmann et al., 1998), Alzheimer's disease (Horuk et al., 1997) and multiple sclerosis (McManus et al., 1998). In this study, we examined the expression of chemokine mRNAs in the brain after focal cerebral ischemia and assessed the effect of chemokine receptor antagonist on the ischemic brain injury. The results suggest that brain chemokines and their receptors play a crucial role in ischemic injury, and that chemokine receptors as well as the signal transmission system(s) regulating the chemokine production are potential targets for therapeutic intervention in stroke.

Induction of chemokine mRNAs in the ischemic brain

The expression of the mRNAs for MCP-1 and MIP-1α was investigated in Male Wistar rats (10 weeks old) after transient focal cerebral ischemia. Focal cerebral ischemia was imposed to the animals under anesthesia by occluding the left middle cerebral artery (MCA) with a silicone rubber coated nylon filament (Nagasawa and Kogure, 1989). The nylon filament was pulled out under anesthesia at 2 h after the onset of occlusion to reperfuse the ischemic area. Animals were killed by decapitation at 0, 2, 4, 12 and 24 h after the onset of reperfusion. The brains were removed rapidly and frozen in powdered dry ice. Coronal sections were prepared in a cryostat, thaw-mounted onto gelatin-coated slides. The expression of the mRNAs for MCP-1 and MIP-1α were examined by in situ hybridization using ^{35}S-labeled antisense RNA probes.

Film-autoradiography revealed the expression of MCP-1 and MIP-1α mRNAs after transient MCA occlusion (MCAO) (Fig. 1). In this figure, each pair of brain sections for the expression of MCP-1 and MIP-1α mRNAs at each time point were derived from the same animal. Although both mRNAs were not detected in the sham-operated rat brain, they had been induced by 2 h after the onset of occlusion. The expression of MCP-1 mRNA peaked at 2 h after the onset of reperfusion, and another peak was observed at 24 h. On the other hand, expression of MIP-1α mRNA peaked at 4 h, and gradually decreasing levels of the expression were observed at 12 and 24 h. Although both mRNAs were expressed in the penumbra region rather than ischemic core region, the distributions of these chemokine mRNAs were deferent from each other.

Double in situ hybridization histochemistry was carried out to clarify the cell species expressing these chemokine mRNAs. MCP-1 mRNA was expressed in the GFAP mRNA-positive cells as well as in the Mac-1α mRNA-positive cells, suggesting that both astrocytes and microglia expressed MCP-1 mRNA. On the other hand, a considerable portion of MIP-1α mRNA was expressed in the Mac-1α mRNA-positive cells, but not in the GFAP mRNA-positive cells, indicating that MIP-1α mRNA was expressed in microglia/macrophages rather than in astrocytes.

These results demonstrating the induction of the expression of chemokine mRNA by ischemic insult suggest the involvement of chemokines in the ischemic brain injury. To address this issue, we examined the effect of a chemokine receptor antagonist on the ischemic brain injury.

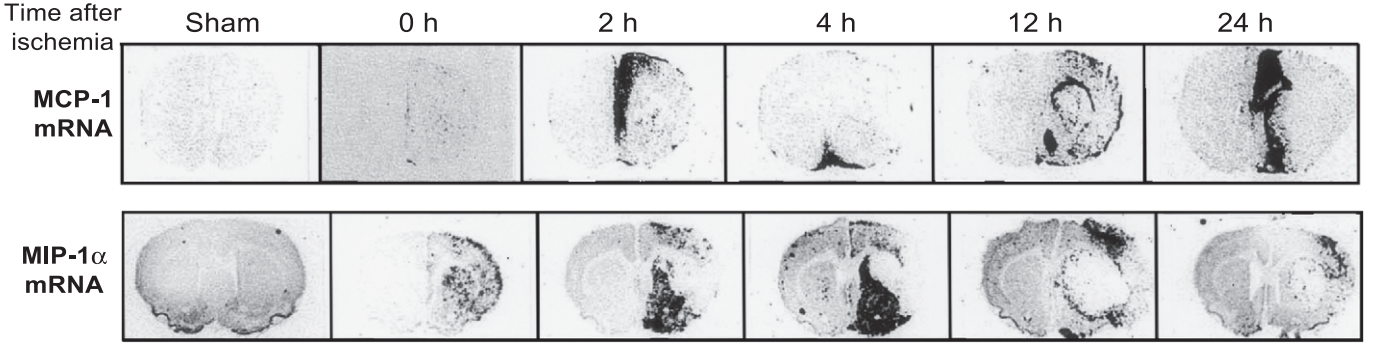

Fig. 1. Film-autoradiograms showing the expression of MCP-1 and MIP-1α mRNAs in the rat brain after transient focal cerebral ischemia induced by MCAO. The autoradiograms at a level of bregmma 1.7 mm are shown.

Protective effect of chemokine receptor antagonist against ischemic brain injury

The effect of a peptidic chemokine antagonist, viral macrophage inflammatory protein-II (vMIP-II) on the ischemic brain injury was examined in a mice stroke model. This peptide was originally identified as a chemokine-like peptide produced by Kaposi's sarcoma-associated herpes virus, and has broad spectrum antagonistic effect against the chemokine receptors (Kledal et al., 1997). Focal cerebral ischemia was imposed to male ddY mice (4 weeks old) under anesthesia by occluding the MCA with a silicone resin coated nylon filament (Takami et al., 2001). After 1 hour of occlusion, the filament was withdrawn to reperfuse the ischemic area. vMIP-II (0.01–1 μg in 5 μL) was administered by intracerebroventricular injection at 1 h before MCA occlusion and 1 h after the onset of reperfusion. Forty-eight hours after reperfusion, the animals under deep anesthesia were killed by transcardial perfusion with 10% formalin in 0.1 mol/L PBS. Coronal sections (50 μm thick) were prepared at an interval of 500 μm throughout the cerebrum (from bregma 3.2 mm to bregma -3.8 mm) (Franklin and Paxinos, 1997), mounted onto gelatin-coated glass slides, and stained with hematoxylin and eosin. The infarct area in each section was measured using an image analysis system, and integrated to give the total infarct volume.

Intracerebroventricular injection of vMIP-II reduced infarct volume in a dose-dependent manner. Total infarct volume of the vehicle-injected group was 43.1 ± 14.1%, but was decreased to 31.6 ± 12.9%, 29.3 ± 12.3 and 26.0 ± 6.1% in the groups injected with 0.01, 0.1 and 1μg of vMIP-II, respectively. To assess the target cell(s) of vMIP-II to produce its effect, we examined the effect of this peptide on the activation of glial cells and on the infiltration of leukocytes by immunohistochemical analyses at 48 h after reperfusion. The brain sections were immunostained using anti-Mac-1α (a marker for activated macrophages/microglia), anti-myeloperoxidase (a marker for neutrophils) and anti-GFAP (a marker for activated astrocytes) antibodies (Fig. 2). There was no significant difference in the numbers

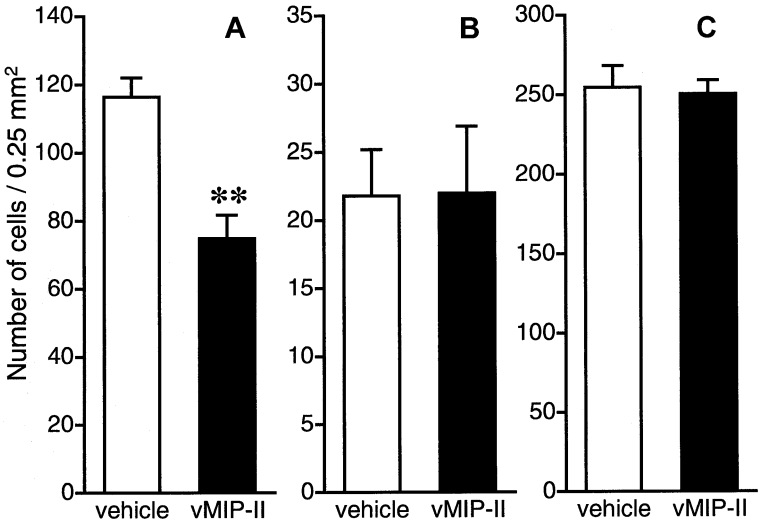

Fig. 2. Number of Mac-1α- (A), myeloperoxidase- (B) or GFAP- (C) positive cells in an area of 500 × 500 μm^2 in the ischemic penumbra (bregma 0.7 mm) at 48 hours after reperfusion. Mice were treated with vehicle or vMIP-II (1μg). Data are expressed as means ± SD. **, $p < 0.01$ compared with the vehicle-injected group (Student's *t*-test, n = 5).

of myeloperoxidase positive cells and GFAP-positive cells between vehicle- and vMIP-II (1μg/mouse)-injected groups. On the other hand, the number of Mca-1α positive cells was significantly decreased by the treatment with vMIP-II.

These findings suggest that brain chemokines play a crucial role in the ischemic injury, at least in part, by enhancing the infiltration of macrophages and/or activation of microglia. Chemokine receptors are likely potential targets for therapeutic intervention in stroke.

Organotypic slice culture as a useful in vitro system to investigate the molecular mechanism regulating chemokine production

Another potential target to suppress the harmful effect of chemokine is the signal transmission system(s) regulating the chemokine production. However, very little is known about how the chemokine production is regulated in the ischemic brain. As the first step to addressing this issue, we investigated the induction of MCP-1 production by excitotoxic injury via activation of NMDA receptors in the cortico-striatal slice cultures.

Organotypic cortico-striatal slice cultures were prepared from postnatal day 2–3 Wistar rats according to the method of Noraberg et al. (1999) with minor modifications. Briefly, coronal slices (300 μm thick) were prepared using a tissue chopper and transferred onto a Millicell-CM insert membrane (Millipore, Bedford, MA) in 6-well plates containing 700 μl/well of culture medium. Four slices were cultivated on each insert membrane. After 10 days of cultivation, the cultures were treated with NMDA. Enriched astrocyte cultures were prepared as previously described (Katayama et al., 2002).

In the non-treated slices, only a few MCP-1-positive cells were observed (Fig. 3A). Treatment with 50 μM NMDA for 4 h induced the production of MCP-1 especially in the cortical area. The production of MCP-1 peaked at 4–8 h after the treatment (Fig. 3B, C). The number of MCP-1-positive cells had decreased to nearly the basal level by 72 h (Fig. 3D). Since the cells producing MCP-1 appeared morphologically to be glial cells (Fig. 3B, C), double fluorescence immunohistochemistry was carried out using an anti-MCP-1 antibody and antibodies against the marker proteins for astrocytes (GFAP and S100β) or microglia (OX42 and ED1). MCP-1 immunoreactivity was colocalized with GFAP and S100β immunoreactivities, but not with OX42 or ED1 immunoreactivity (*see* Katayama et al., 2002), indicating that MCP-1 was produced in astrocytes. ELISA revealed that NMDA-treatment markedly increased the release of MCP-1 in the slice cultures (Fig. 4). The concentrations of MCP-1 in the media were significantly higher in the NMDA-treated cultures than in the vehicle-treated ones at 24 h after the treatment. In contrast, NMDA-treatment did not increase the release of MCP-1 in the enriched astrocyte culture (data not shown).

The results showed that NMDA-evoked excitotoxic injury induced the production of MCP-1 in the cortico-striatal slice cultures. Production of MCP-1 was observed in astrocytes, while NMDA caused severe damage in neurons, but not in astrocytes. Furthermore, the treatment with NMDA failed to increase the release of MCP-1 in the enriched astrocyte cultures, suggesting that NMDA did not directly act on the astrocytes. These findings indicate that some signal(s) is likely sent from the injured neurons to astrocytes to induce the MCP-1 production. This signal transmission molecule(s) or system(s) might be a potential target for the novel drug therapy to protect the brain from ischemic or excitotoxic injury.

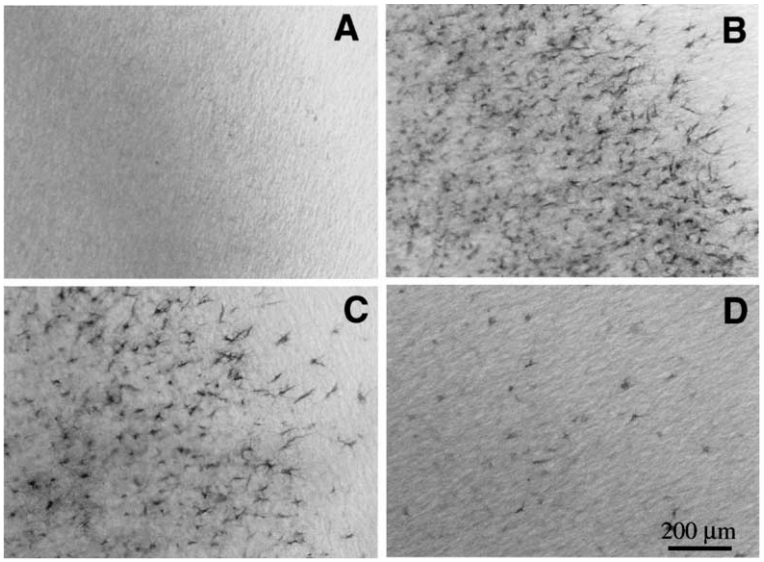

Fig. 3. MCP-1 production in the cortico-striatal slice cultures treated with 50 μM NMDA for 4 h. Slight numbers of MCP-1-positive cells were observed in the non-treated slice (A). The treatment with NMDA induced the production of MCP-1 with the peak at 4–8 h after the treatment (B, C). The number of MCP-1-positive cells had decreased to nearly the basal level by 72 h (D). Bars = 200 μm.

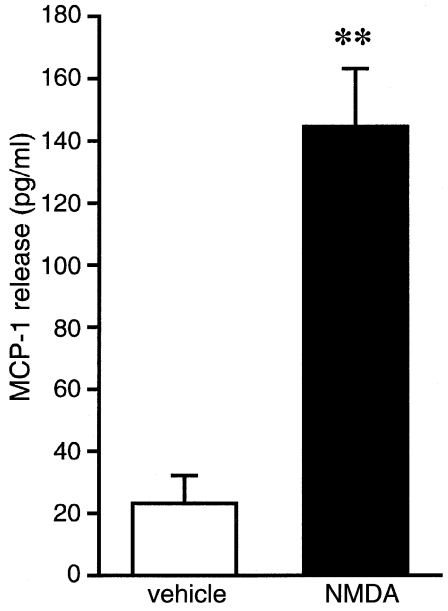

Fig. 4. Release of MCP-1 from the slice cultures treated with 50 μM NMDA (closed column) or vehicle (open column) for 4 h were determined by ELISA 24 h after the NMDA-treatment. Data are presented as means ± SEM. **, $p < 0.01$ compared with the vehicle-treated cultures(Mann-Whitney *U*-test, n = 5).

Conclusion

We showed the expression of chemokine mRNAs in the ischemic brain, and the protective effect of a chemokine receptor antagonist against ischemic brain injury. These findings suggest that brain chemokines and their receptors play a crucial role in ischemic injury, and that chemokine receptors as well as the signal transmission system(s) regulating the chemokine production are potential targets for therapeutic intervention in stroke.

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