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BRAIN RESEARCH

Research Report

Endothelin$_A$ receptor antagonist BSF-208075 causes immune modulation and neuroprotection after stroke in gerbils

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ABSTRACT

Leukocytes contribute to the ischemia-reperfusion injury. Recent studies suggested endothelins could be important mediators for leukocyte activation in stroke. We tested if the endothelin$_A$ receptor antagonist BSF-208075 (ambrisentan) could reduce an ischemic lesion by modulation of leukocyte–endothelium interactions. Twenty-four gerbils underwent either a sham operation ($n=6$) or 15 min of bilateral carotid artery occlusion resulting in global cerebral ischemia. Ischemic animals received normal saline ($n=6$), 5 mg/kg BSF-208075 ($n=6$) or 30 mg/kg ($n=6$) administered intravenously at 10 min of reperfusion. Leukocytes rolling or adhering to endothelium were counted by intravital microscopy in parietal subsurface venules through a closed cranial window. BSF-208075 dose-dependently reduced postischemic leukocytes rolling (7.3±2.3 vs. 3.3±1.4 vs. 0.7±0.7 [n/100 μm/min]; $p<0.05$) and adhering (5.3±1.4 vs. 2.7±1.6 vs. 1.3±0.5 [n/100 μm/min]; $p<0.05$). Cerebral blood flow was not significantly changed by BSF-208075. Cortical neurons [n/mm^2] in an area corresponding to the in vivo microscopy were dose-dependently preserved 7 days after ischemia (2456±687 vs. 3254±245 vs. 3780±168; $p<0.05$). Conclusion: Endothelins mediate leukocyte activation in ischemic stroke. The endothelin$_A$ receptor antagonist BSF-208075 administered during reperfusion reduces the postischemic leukocyte activation and causes neuroprotection.

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1. Introduction

Endothelins (ET) are potent vasoactive peptides originally isolated from porcine endothelial cells (Yanagisawa et al., 1988). The isoforms ET-1 and ET-3 found in the brain (Matsumoto et al., 1989; Shinmi et al., 1989) target two receptor subtypes (ET$_A$ and ET$_B$), which both have been identified on cerebral microvessel endothelium, neurons and glia (Rubanyi et and Polokoff, 1994). The ET$_A$ receptor mainly sensitive to ET-1 influences the development of the cerebral ischemia-reperfusion injury (Barone et al., 1994; Feuerstein et al., 1994; Nikolov et al., 1993; Fuxe et al., 1992). Previous studies have focused on the measurement of cerebral blood flow (CBF) in ischemic brain after blockage of ET receptors. Inhibition of ET$_A$ receptors reduces the postischemic hypoperfusion (Lehmberg et al., 2003; Spatz et al., 1996) and preserves microvascular blood flow (Dawson et al., 1999). In contrast, Patel and McCulloch (1996) report no change of CBF in rats exposed to transient global cerebral ischemia after treatment with a combined ET$_{A/B}$ receptor antagonist. These opposite findings

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Abbreviations: Ca, cornu ammonis; CBF, cerebral blood flow; ET, endothelin(s); LEI, leukocyte–endothelium interactions; MABP, Mean arterial blood pressure; PMNs, polymorphonuclear leukocytes

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may suggest that treatment with ET_A receptor antagonists could prevent neuronal loss in ischemic brain possibly by additional mechanisms other than change of CBF.

Recent studies suggest that inflammatory responses contribute to cerebral ischemia-reperfusion injury, particularly during the postischemic reperfusion (Bramlett and Dietrich, 2004; Takeda et al., 2002; Becker et al., 1997). Stimulated leukocytes mediate the reperfusion injury by production of proteases, active oxygen radicals and lipid-derived mediators (Baggiolini et al., 1994). Further studies show the pro-inflammatory action of endothelins in addition to their vasoactive effects. Chen et al. (2001) demonstrated that ET-1 up-regulates the monocyte chemo-attractant protein-1 (MCP-1), a marker protein of the inflammatory response, via its action on ET_A receptors in the cerebral vascular endothelial cells. McCarron et al. (1993) have shown that the expression of intercellular adhesion molecule-1 (ICAM-1) can be up-regulated by ET. Based on these results, we wanted to test in a gerbil ischemia model if the ET_A receptor antagonist BSF-208075 (ambrisentan) infused intravenously during reperfusion reduces the postischemic inflammatory response in subsurface venules and further causes neuroprotection in the corresponding cerebral cortex. Ambrisentan (LU-208075, BSF-208075) is a new endothelin receptor antagonist currently developed by Myogen under license from Abbott (formerly BASF Pharma) for the potential treatment of postischemic acute renal failure and cardiovascular disease (Billman, 2002). Ambrisentan inhibits predominantly ET-1 effect via ET_A receptors with a selectivity of 77/1 ET_A/ET_B (Motte et al., 2006).

2. Results

2.1. *Systemic parameters and CBF*

Mean arterial blood pressure (MABP; Fig. 1) remained constant

animals. During global ischemia, CBF was reduced to less than 15% of baseline in experimental animals compared to sham ($p<0.001$). A brief phase of hyperperfusion followed at 8 min of reperfusion. CBF then continued to decrease compared to the baseline and sham group ($p<0.05$) to values between 50% and 70% of baseline at 40 min of reperfusion (delayed hypoperfusion). There was no significant difference in CBF among experimental animals.

2.2. *Postischemic leukocyte–endothelium interactions (LEI)*

In none of the groups were rolling or adhering leukocytes observed in postcapillary venules at baseline conditions (Fig. 2). Sham-operated animals did not reveal LEI, with exception at a time corresponding to 120 min of reperfusion in ischemic animals, where we observed 0.5±0.3 rolling and 0.2±0.2 sticking leukocytes [*n*/100 μm/min]. Ischemic control animals had progressive LEI compared to sham animals at 60, 90 and 120 min of reperfusion ($p<0.05$), with 7.3±2.3 rolling leukocytes and 5.3±1.4 sticking leukocytes at 120 min [*n*/100 μm/min]. LEI was reduced dose-dependently in experimental animals treated with the ET_A receptor antagonist BSF-208075 compared to ischemic controls at 60, 90 and 120 min of reperfusion ($p<0.05$). After 120 min of reperfusion, animals treated with 5 mg/kg BSF-208075 had 3.3±1.4 rolling leukocytes and 2.7±1.6 sticking leukocytes [*n*/100 μm/min], while those treated with 30 mg/kg BSF-208075 had only 0.7±0.7 rolling leukocytes and 1.3±0.5 sticking leukocytes [*n*/100 μm/min] ($p<0.05$, compared to control).

2.3. *Histology*

Sham animals had 3686±337 viable nerve cells per mm^2 in the parietal cortex while this number was reduced to 2456±281 cells per mm^2 in ischemic controls ($p<0.05$; Fig. 3). BSF-208075 significantly increased cell survival in the cortex in a dose-dependent manner (3254±245 cells per mm^2 at 5 mg/kg and 3780±69 cells per mm

reperfusion after treatment with ET_A receptor antagonists such as BQ123, Ro 61-1790 or BQ610 (Lehmberg et al., 2003; Dawson et al., 1999; Spatz et al., 1996). In our experiments, however, treatment with BSF-208075 during reperfusion did not change CBF possibly due to lower dosing, different pharmacological properties such as penetration of the blood–brain barrier and different timing of the application. As well, the wide range of ET_A selectivity might account for differences in CBF/anti-inflammatory action with different ET_A antagonists (Motte et al., 2006). For example, Dawson et al. (1999) used a more selective ET_A receptor antagonist Ro 61-1790 (ET_A/ET_B 1000/1) compared to BSF-208075 (77/1) in our study with different effects on CBF.

Increased CBF after treatment would increase shear stress and reduce LEI (Russell et al., 2003). In previous experiments (Lehmberg et al., 2003), the increase of CBF could account for an increase in shear stress resulting in LEI reduction. However, our data show that the observed reduction of LEI in treated