available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

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

Erik F. Hauck[a,b], *Julie F. Hoffmann*[b], *Axel Heimann*[b], *Oliver Kempski*[b,*]

[a]*Division of Neurosurgery, University of Texas Medical Branch, Galveston, USA*
[b]*Institute for Neurosurgical Pathophysiology, Johannes Gutenberg University of Mainz, Langenbeckstr. 1, 55101 Mainz, Germany*

ARTICLE INFO

Article history:
Accepted 19 April 2007
Available online 25 April 2007

Keywords:
Endothelin
Postischemic inflammation
Leukocyte activation
Intravital microscopy

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.

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

* *Corresponding author*. Fax: +49 6131 17 6640.
E-mail address: oliver.kempski@uni-mainz.de (O. Kempski).

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

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.04.047

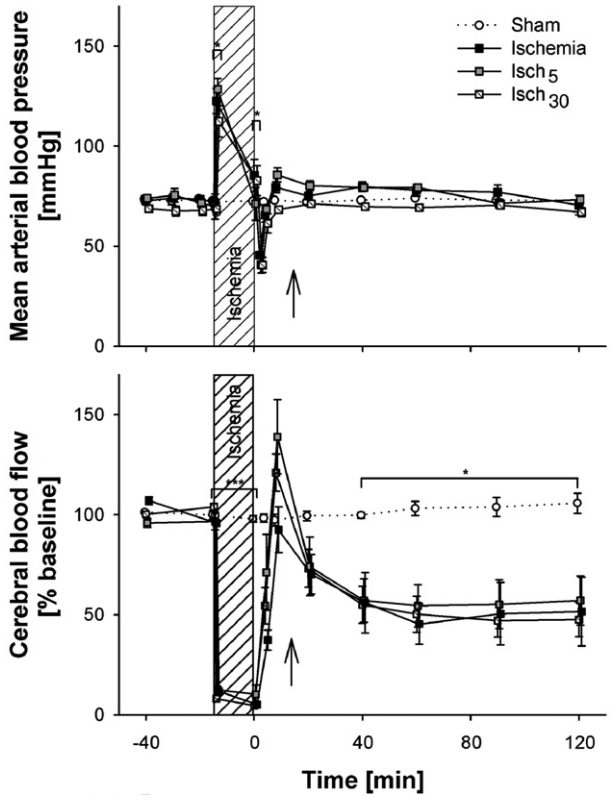

Fig. 1 – Mean arterial blood pressure (MABP) [mmHg] and CBF measured by Laser Doppler [% baseline value] in sham animals (Sham), ischemic controls (Ischemia) and animals treated with 5 mg/kg ($Isch_5$) or 30 mg/kg body weight ($Isch_{30}$) of the $endothelin_A$ receptor antagonist BSF-208075. $^*p<0.05$; $^{***}p<0.001$; ANOVA, Tukey test. CBF and MABP remained stable in sham animals. Note the significant change in blood pressure at the beginning of ischemia and reperfusion in experimental animals compared to sham. CBF is significantly reduced during ischemia and delayed hypoperfusion at 40 min of reperfusion in experimental animals compared to sham. No significant differences in MABP and CBF were observed in experimental animals. Particularly, differences during hyperperfusion were not statistically significant and secondary to larger sample variation. Arrow marks the time of vehicle or BSF-208075 injection.

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 vaso-active 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 in sham animals (72.5±2.0 mmHg; $n=6$). After bilateral carotid artery occlusion in experimental animals, MABP increased from 71.6±5.4 mmHg baseline to 120.7±15.4 mmHg ($p<0.05$; $n=18$). During reperfusion, MABP returned to baseline within the first 5 min without significant difference in ischemic animals regardless of whether they received treatment or not. Similarly, hematocrit, pH, pO_2, pCO_2 and HCO_3 remained constant during the experiment without significant differences among the groups (Table 1). The tympanic membrane temperature in sham animals was constant during the experiment (36.7±0.3 °C). In ischemic animals ($n=18$), however, the tympanic membrane temperature dropped from a baseline of 36.5±0.6 °C to 35.5±0.9 °C at the end of ischemia ($p<0.05$), peaked at hyperperfusion (37.1±0.5 °C; $p<0.05$) and finally reached a plateau during the delayed hypoperfusion at 40 min of reperfusion until the end of the experiment (36.2±0.6 °C; $p<0.05$). There was no statistically significant difference among experimental groups. CBF remained stable in sham

Table 1 – Arterial blood gas analysis in sham animals (Sham), ischemic controls (Ischemia) and treated with 5 mg/kg BSF-208075 ($Isch_5$) or 30 mg/kg body weight ($Isch_{30}$) at baseline and 2 h of reperfusion

	Sham	Ischemia	$Isch_5$	$Isch_{30}$
Baseline				
pH	7.32±0.05	7.36±0.02	7.35±0.01	7.32±0.04
pCO_2	44.5±2.6	43.1±3.0	42.9±3.2	45.6±2.3
pO_2	109.2±12.4	119.4±13.2	135.8±26.5	124±19.1
HCO_3	21.8±2.1	23.7±2.1	23.1±1.5	22.6±1.3
Hct	42.7±2.2	45.2±2.2	45.4±4.0	45.0±1.0
2 h of reperfusion				
pH	7.32±0.05	7.31±0.01	7.28±0.05	7.31±0.01
pCO_2	41.7±1.7	42.9±4.3	42.8±6.9	47.6±0.5
pO_2	124.8±12.0	114.3±38.7	118.6±17.7	121.0±6.3
HCO_3	20.5±2.2	21.0±2.2	19.6±0.6	23.0±0.7
Hct	41.0±1.3	45.4±3.9	45.1±1.1	45.9±3.4

"Hct" represents the hematocrit. Mean±SEM.