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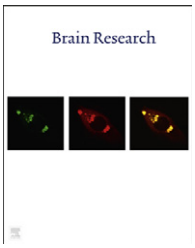

Research Report

Impact of 5-lipoxygenase inhibitors on the spatiotemporal distribution of inflammatory cells and neuronal COX-2 expression following experimental traumatic brain injury in rats

Wolfgang Härtig[a,1], Dominik Michalski[b,*,1], Gudrun Seeger[a], Cornelia Voigt[c], Cornelius K. Donat[d], Julia Dulin[c], Johannes Kacza[e], Jürgen Meixensberger[c], Thomas Arendt[a], Martin U. Schuhmann[c,f]

[a]Paul Flechsig Institute for Brain Research, University of Leipzig, Jahnallee 59, 04109 Leipzig, Germany
[b]Department of Neurology, University of Leipzig, Liebigstr. 20, 04103 Leipzig, Germany
[c]Department of Neurosurgery, University of Leipzig, Liebigstr. 20, 04103 Leipzig, Germany
[d]Department of Neuroradiopharmaceuticals, Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, Leipzig-Site, Permoserstr. 15, 04318 Leipzig, Germany
[e]Institute of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 43, 04103 Leipzig, Germany
[f]Department of Neurosurgery, Eberhard Karls University Tübingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany

ARTICLE INFO

Article history:
Accepted 17 December 2012
Available online 23 December 2012

Keywords:
Traumatic brain injury
Neuronal death
Macrophage
Granulocyte
COX-2

ABSTRACT

The inflammatory response following traumatic brain injury (TBI) contributes to neuronal death with poor outcome. Although anti-inflammatory strategies were beneficial in the experimental TBI, clinical translations mostly failed, probably caused by the complexity of involved cells and mediators. We recently showed in a rat model of controlled cortical impact (CCI) that leukotriene inhibitors (LIs) attenuate contusion growth and improve neuronal survival. This study focuses on spatiotemporal characteristics of macrophages and granulocytes, typically involved in inflammatory processes, and neuronal COX-2 expression. Effects of treatment with LIs (Boscari/MK-886), started prior trauma, were evaluated by quantifying $CD68^+$, $CD43^+$ and $COX\text{-}2^+$ cells 24 h and 72 h post-CCI in the parietal cortex (PC), CA3 region, dentate gyrus (DG) and visual/auditory cortex (v/aC).

Abbreviations: 5-LOX, 5-lipoxygenase; ANOVA, analysis of variance; BP, band pass; CA3, region 3 of the hippocampal cornu ammonis; CCI, controlled cortical impact; COX-2, isoform 2 of cyclooxygenase; CSF, cerebrospinal fluid; Cy, carbocyanine; DG, hippocampal dentate gyrus; GFAP, glial fibrillary acidic protein; h, hour/s; ICAM-1, intercellular adhesion molecule 1; FLAP, 5-LOX activating protein; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; MRI, magnetic resonance imaging; NeuN, Neuronal Nuclei; NDS-TBS-T, TBS containing 5% normal donkey serum and 0.3% Triton X-100; PFA, paraformaldehyde; PMN, polymorphonuclear leucocytes; STL, Solanum tuberosum lectin; TBI, traumatic brain injury; TBS, Tris-buffered saline; TBS-BSA, TBS containing 2% bovine serum albumin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; VCAM-1, vascular cell adhesion molecule-1

*Corresponding author. Fax: +49 341 9724199.
E-mail address: dominik.michalski@medizin.uni-leipzig.de (D. Michalski).
[1]These authors contributed equally.

0006-8993/$ - see front matter
http://dx.doi.org/10.1016/j.brainres.2012.12.022

Correlations were applied to identify intercellular relationships. At 24 h, untreated animals showed granulocyte invasion in all regions, decreasing towards 72 h. Macrophages increased from 24 h to 72 h post-CCI in PC and v/aC. COX-2^{+} neurones showed no temporal changes, except of an increase in the CA3 region at 72 h. Treatment reduced granulocytes at 24 h in the pericontusional zone and hippocampus, and macrophages at 72 h in the PC and v/aC. COX-2 expression remained unaffected by LIs, except of time-specific changes in the DG (increase/decrease at 24/72 h). Interrelations confirmed concomitant cellular reactions beyond the initial trauma site. In conclusion, LIs attenuated the cellular inflammatory response following CCI. Future studies have to clarify region-specific effects and explore the potential of a clinically more relevant therapeutic approach applying LIs after CCI.

1. Introduction

Traumatic brain injury (TBI) still represents a socio-economic relevant disease, although extensive preclinical and clinical research during the last decades yielded increasing insights into pathophysiological processes (Maas et al., 2008). Numerous efforts were made to develop neuroprotective approaches, mainly addressing the typically associated neuronal death with far-reaching consequences, e.g., long-term motor and cognitive impairment or death. However, successful translation of 'neuroprotectants' into clinical application mostly failed (Jain, 2008; Loane and Faden, 2010). As a consequence, an extended perspective of tissue damage gains increasing acceptance which involves not only neurones but also associated cell types – e.g., astro- and microglia – and time-dependent alterations such as delayed secondary injury (Loane and Faden, 2010; Lynch and Dawson, 1994). Despite the fact that post-traumatic inflammatory cascades are considered as complex due to the high number of involved cell types, mediators and interactions, they are still perceived as a promising therapeutic target (Harting et al., 2008; Kadhim et al., 2008; Morganti-Kossmann et al., 2007; Petraglia et al., 2011). Addressing the cellular part, an increase of microglia and macrophages following experimental brain injury was found, starting at the injured region but also occurring in the hippocampus, thalamus and corpus callosum, indicating cell alterations far beyond the initial trauma site (Aihara et al., 1995; Chen et al., 2003). Furthermore, evidence exists that these region-specific findings need to be seen in a temporal context, as indicated for example by a time-specific course of apoptosis in neurones and astrocytes following fluid percussive brain injury (Conti et al., 1998).

Transmigrating cells (e.g., macrophages) were found to produce a range of mediators such as cytokines with opposing effects: Enforcing the inflammatory response at the contusion zone and in remote areas, thought to be deleterious on one hand, and promoting neuroprotection and regeneration on the other hand (Harting et al., 2008; Kadhim et al., 2008; Lenzlinger et al., 2001; Morganti-Kossmann et al., 2007). A group of relevant mediators are leukotrienes and prostaglandins, originating from arachidonic acid, enzymatically produced by 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) (Brain and Williams, 1990; Brock and Peters-Golden, 2007; Funk, 2001). In contrast to prostaglandins, usually synthesised at the endoplasmic reticulum and nuclear membrane of most cell types, leukotrienes are mainly generated at the nuclear membrane of inflammatory active cells, notably macrophages and granulocytes (Funk, 2001). Concerning their pathophysiological role after TBI, leukotrienes are considered to mediate vascular permeability contributing to oedema formation, which is further potentiated by prostaglandins (Brain and Williams, 1990). Beside these vascular effects, prostaglandins and leukotrienes are also known to mediate neurotoxicity via glutamate targeting N-methyl-D-aspartate receptors, whose activity is potentiated by arachidonic acid and its derivatives (Bazan et al., 1995). These cytotoxic effects may help to understand oedema formation following TBI, since a link between blood–brain barrier opening and oedema formation has been refuted (Beaumont et al., 2000). Concerning the location of arachidonic acids in the post-traumatic brain, experimental studies revealed an increased leukotriene concentration in the brain (Dhillon et al., 1996; Farias et al., 2009; Kiwak et al., 1985) and in the cerebrospinal fluid (CSF; Schuhmann et al., 2003), suggesting effects also apart from the contusion site.

Based on the perspective of arachidonic acids and its derivatives as mediators in traumatic brain injury, efforts have been made to interrupt these inflammatory cascades in order to inhibit neuronal cell death and to improve clinical outcome (Jain, 2008). For this purpose, we recently tested in the controlled cortical impact (CCI) model of focal brain injury two leukotriene inhibitors with different modes of action: MK-886 (Gugliucci et al., 2002; Rouzer et al., 1990) and Boscari as a mixture of boswellic acids (Ammon, 2006; Moussaieff et al., 2008). In these experiments, we demonstrated an attenuation of brain contusion size and improved neuronal survival following treatment (Voigt et al., 2012).

As a possible side effect of leukotriene inhibition, an increase of prostaglandins and increased expression/activity of the mediating enzyme COX (particularly the neuronal isoform 2, COX-2) has been discussed, potentially resulting in both excitotoxic and neuroprotective effects (Choi et al., 2009; Serhan and Chiang, 2008). Furthermore, earlier studies revealed COX-2 in the native and also post-traumatic brain and postulated that synaptic activity is critically involved in COX-2 regulation (Dash et al., 2000; Kaufmann et al., 1996, 1997; Kunz et al., 2002; Strauss et al., 2000; Strauss, 2008; Yamagata et al., 1993).