Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Regulation of rotenone-induced microglial activation by 5-lipoxygenase and cysteinyl leukotriene receptor 1

Xiao-Yan Zhang[a], Lu Chen[a], Yi Yang[a], Dong-Min Xu[b], Si-Ran Zhang[b,c], Chen-Tan Li[a], Wei Zheng[a], Shu-Ying Yu[b], Er-Qing Wei[b], Li-Hui Zhang[a,*]

[a]Department of Pharmacology, Hangzhou Key Laboratory of Medical Neurobiology, School of Medicine, Hangzhou Normal University, 16 Xue Lin Road, Hangzhou 310036, PR China
[b]Department of Pharmacology, School of Medicine, Zhejiang University, 388 Yu Hang Tang Road, Hangzhou 310058, PR China
[c]Department of Clinical Medicine, School of Medicine, Zhejiang University, 388 Yu Hang Tang Road, Hangzhou 310058, PR China

ARTICLE INFO

Article history:
Accepted 13 May 2014
Available online 22 May 2014

Keywords:
Rotenone
Neuroinflammation
Microglial activation
5-lipoxygenase
Cysteinyl leukotriene receptor

ABSTRACT

The 5-lipoxygenase (5-LOX) products cysteinyl leukotrienes (CysLTs) are potent pro-inflammatory mediators. CysLTs mediate their biological actions through activating CysLT receptors ($CysLT_1R$ and $CysLT_2R$). We have recently reported that 5-LOX and $CysLT_1R$ mediated PC12 cell injury induced by high concentrations of rotenone (0.3–10 μM), which was reduced by the selective 5-LOX inhibitor zileuton and $CysLT_1R$ antagonist montelukast. The purpose of this study was to examine the regulatory roles of the 5-LOX/$CysLT_1R$ pathway in microglial activation induced by low concentration rotenone. After mouse microglial BV2 cells were stimulated with rotenone (0.3–3 nM), phagocytosis and release of pro-inflammatory cytokine were assayed as indicators of microglial activation. We found that rotenone (1 and 3 nM) increased BV2 microglial phagocytosis and the release of the pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Zileuton and montelukast prevented rotenone (3 nM)-induced phagocytosis and cytokine release. Furthermore, rotenone significantly up-regulated 5-LOX expression, induced 5-LOX translocation to the nuclear envelope, and increased the production of CysLTs. These responses were inhibited by zileuton. Rotenone also increased $CysLT_1R$ expression and induced nuclear translocation of $CysLT_1R$. In primary rat microglia, rotenone (10 nM) increased release of IL-1β and TNF-α, whereas zileuton (0.1 μM) and montelukast (0.01 μM)

Abbreviations: 5-LOX, 5-lipoxygenase; CNS, central nervous system; COX, cyclooxygenase; $CysLT_1R$, cysteinyl leukotriene receptor 1; CysLTs, cysteinyl leukotrienes; DA, dopaminergic; DAPI, 4′, 6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; IL-1β, interleukin-1β; MTT, 3-(4, 5-dimehythiazol-2-yl)-2, 5-diphenyltetrazolium bromide; OGD, oxygen-glucose deprivation; PD, Parkinson's disease; TNF-α, tumor necrosis factor-α.

*Corresponding author.
E-mail addresses: zxy19870910@126.com (X.-Y. Zhang), chenlu19891128@163.com (L. Chen), yyang@hznu.edu.cn (Y. Yang), 286993845@qq.com (D.-M. Xu), 3100102379@zju.edu.cn (S.-R. Zhang), lct@hznu.edu.cn (C.-T. Li), Wei.zheng@hznu.edu.cn (W. Zheng), shuying.101@163.com (S.-Y. Yu), weieq2006@zju.edu.cn (E.-Q. Wei), lhzhang@hznu.edu.cn (L.-H. Zhang).

http://dx.doi.org/10.1016/j.brainres.2014.05.026

significantly inhibited this response. These results indicated that 5-LOX and $CysLT_1R$ might be key regulators of microglial activation induced by low concentration of rotenone. Interference of 5-LOX/$CysLT_1R$ pathway may be an effective therapeutic strategy for microglial inflammation.

1. Introduction

Microglia are the resident innate immune cells in the central nervous system (CNS) and play key roles in the progression of neurodegenerative diseases, such as Parkinson's disease (PD) (Blandini, 2013; Tansey and Goldberg, 2010; Tufekci et al., 2012). Accumulating evidence indicates that exposure to the environmental toxicant rotenone can activate microglia in animals (Sherer et al., 2003; Zhou et al., 2007, 2008) and primary mesencephalic neuron-glial cultures (Gao et al., 2002, 2003, 2011; Zhou et al., 2007, 2008), which contributes to the dopaminergic (DA) neuron death. The inhibition of microglial activation and the resultant neurotoxicity have been considered as a new neuroprotective strategy (Qian et al., 2010; Tansey and Goldberg, 2010; Tufekci et al., 2012).

Microglial activation is regulated by various factors, such as arachidonic acid-derived pro-inflammatory mediators (Ballerini et al., 2005; Klegeris and McGeer, 2003). 5-lipoxygenase (5-LOX) is the key enzyme metabolizing arachidonic acid to produce potent pro-inflammatory mediators, cysteinyl leukotrienes (CysLTs), namely leukotriene (LT) C_4, LTD_4 and LTE_4. CysLTs induce inflammatory responses by activating their receptors, $CysLT_1R$ and $CysLT_2R$ (Kanaoka and Boyce, 2004; Rovati and Capra, 2007). 5-LOX and $CysLT_1R$ are involved in microglial activation and neuroinflammation following cerebral ischemia. 5-LOX expression increased in the neurons and microglia (Chu et al., 2010; Tu et al., 2009; Zhou et al., 2006a), and the 5-LOX inhibitors BW-B 70C, zileuton and caffeic acid inhibited 5-LOX expression and attenuated post-ischemic inflammation and brain injury after focal cerebral ischemia (Jatana et al., 2006; Tu et al., 2010; Zhou et al., 2006b). Inhibition of 5-LOX activity by MK886 attenuated microglial activation, motor dysfunction and axonal damage in mice with multiple sclerosis (Yoshikawa et al., 2011). On the other hand, $CysLT_1R$ was up-regulated in microglia 14 days after focal cerebral ischemia, and the $CysLT_1R$ antagonist pranlukast protected against post-ischemic inflammation and brain damage (Fang et al., 2006). In rat primary microglial cultures, CysLTs/CysLTR participated in microglial inflammatory response (Ballerini et al., 2005; Zhang et al., 2013). Recently, we reported that rotenone increased $CysLT_1R$ expression in BV2 cells (Luo et al., 2011). However, the roles of 5-LOX and $CysLT_1R$ in microglial activation induced by rotenone have not yet been reported.

The mouse microglial BV2 cells (Blasi et al., 1990) possess many characteristics of microglial cells, and have been extensively used for investigating microglial activation *in vitro* (Henn et al., 2009; Jin et al., 2012; Kwon et al., 2008; Liu et al., 2006; Song et al., 2012; Svensson et al., 2010). It has been reported that low concentrations of rotenone (1–10 nM) induced activation of primary microglia (Gao et al., 2002, 2003, 2011; Zhou et al., 2007, 2008) and BV2 cells (Liu et al., 2006). In the present study, we determined the roles of 5-LOX and $CysLT_1R$ in microglial activation induced by low concentration of rotenone in BV2 cells as well as rat primary microglia, and clarified the effects of the selective 5-LOX inhibitor zileuton and the $CysLT_1R$ antagonist montelukast.

2. Results

2.1. *Rotenone-induced microglial activation in BV2 cells*

At first, we confirmed the effects of various concentrations of rotenone on microglial activation. Low concentrations of rotenone (<10 nM) were nontoxic to BV2 cells and mildly increased cell viability as determined by MTT reduction assay (Fig. 1). However, high concentrations of rotenone (≥10 nM) significantly reduced cell viability (Fig. 1). Therefore, the effect of rotenone on microglial activation was assessed at the concentrations of 3 nM or less in this study. Flow cytometric analysis showed that rotenone concentration-dependently enhanced phagocytic activity of BV2 cells, and the effective concentrations were 1 and 3 nM (Fig. 2). Moreover, rotenone (1 and 3 nM) significantly increased release of the pro-inflammatory cytokines interleukin-1β (IL-1β) (Fig. 3A) and tumor necrosis factor-α (TNF-α) (Fig. 3B) from BV2 cells. Thus, the non-toxic low concentrations (1 and 3 nM) of rotenone could efficiently activate microglia.

2.2. *Effect of the 5-LOX inhibitor on rotenone-induced microglial activation in BV2 cells*

Then, we determined whether 5-LOX is involved in rotenone-induced microglial activation. The 5-LOX selective inhibitor

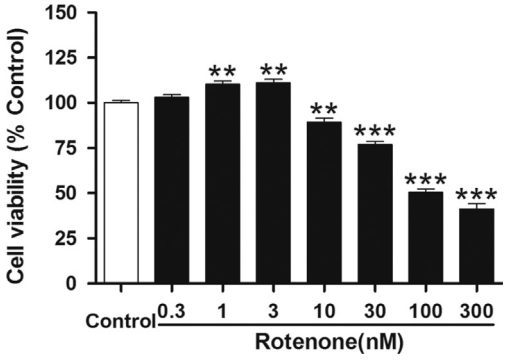

Fig. 1 – Viability of BV2 cells after exposure to rotenone. Cell viability was determined by MTT reduction assay 24 h after rotenone exposure. $n=8$; $^{**}P<0.01$ and $^{***}P<0.001$ compared with control.