Journal of Neuroimmunology 264 (2013) 54–64

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Potential role of A_{2A} adenosine receptor in traumatic optic neuropathy

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ARTICLE INFO

Article history:
Received 30 June 2013
Received in revised form 12 September 2013
Accepted 14 September 2013

Keywords:
Traumatic optic neuropathy
Adenosine A_{2A} receptor
Microglia
Inflammatory cytokines
MAPKinase
Oxidative stress

ABSTRACT

In traumatic optic neuropathy (TON), apoptosis of retinal ganglion cells is closely related to the local production of reactive oxygen species and inflammatory mediators from activated microglial cells. Adenosine receptor A_{2A} (A_{2A}AR) has been shown to possess anti-inflammatory properties that have not been studied in TON. In the present study, we examined the role of A_{2A}AR in retinal complications associated with TON. Initial studies in wild-type mice revealed that treatment with the A_{2A}AR agonist resulted in marked decreases in the TON-induced microglial activation, retinal cell death and releases of reactive oxygen species and pro-inflammatory cytokines TNF-α and IL-6. To further assess the role of A_{2A}AR in TON, we studied the effects of A_{2A}AR ablation on the TON-induced retinal abnormalities. A_{2A}AR −/− mice with TON showed a significantly higher mRNA level of TNF-α, Iba1-1 in retinal tissue, and ICAM-1 expression in retinal sections compared with wild-type mice with TON. To explore a potential mechanism by which A_{2A}AR-signaling regulates inflammation in TON, we performed additional studies using hypoxia- or LPS-treated microglial cells as an in vitro model for TON. Activation of A_{2A}AR attenuates hypoxia or LPS-induced TNF-α release and significantly repressed the inflammatory signaling, ERK in the activated microglia. Collectively, this work provides pharmacological and genetic evidence for A_{2A}AR signaling as a control point of cell death in TON and suggests that the retinal protective effect of A_{2A}AR is mediated by attenuating the inflammatory response that occurs in microglia via interaction with MAPKinase pathway.

1. Introduction

Traumatic optic nerve injury is commonly seen in motor vehicle accidents, assaults, war and in the natural disaster. Traumatic optic nerve injury is usually the consequence of a severe blunt head trauma, often a frontal blow severe enough to cause loss of consciousness. Unfortunately, there are currently no proven treatments that can prevent the damage associated with an acute traumatic optic neuropathy (TON). Prognosis for the recovery of vision in TON is still poor, nevertheless, animal models for TON are often used, mostly because they are easy to perform and can be well standardized (Levkovitch-Verbin, 2004). Retinal ganglion cell (RGC) death is known to be a fundamental pathological process in traumatic optic injury including TON. Several common mechanisms have been hypothesized to underlie apoptotic processes, including interruption of trophic support, oxidative stress, and increased extracellular glutamate levels that result in excitotoxicity. These stimuli associated with the injured RGCs often activate retinal microglia, which release pro-inflammatory cytokines and cytotoxic molecules to further exacerbate the degenerative process (Kreutzberg, 1996). These findings suggest that pharmacological interventions that reduce inflammation may be effective neuroprotectants for TON.

Under stress and ischemic conditions, the local tissue concentration of extracellular adenosine is increased due to its synthesis from the released ATP. This nucleoside has been proposed to modulate a variety of physiological responses by stimulating specific adenosine receptors (AR), which are classified as A1, A2A, A2B, and A3 subtypes (Collis and Hourani, 1993). These receptors can be distinguished based on their affinities for adenosine agonists and antagonists. In addition, these receptors are classified based on their mechanism of signal

Abbreviations: TON, traumatic optic neuropathy; TNF-α, tumor necrosis factor-α; ELISA, Enzyme-linked immunosorbent assay; ROS, reactive oxygen species; MAP kinase, Mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; AR, adenosine receptor; CGS21680, 2-p-[2-Carboxyethyl]phenethylamino-5′-N-ethylcarboxamidoadenosine; LPS, lipopolysaccharides; NECA, 5′-*N*-Ethylcarboxamidoadenosine; ZM241385, 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol.

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http://dx.doi.org/10.1016/j.jneuroim.2013.09.015

Table 1
The primer sets used for the detection of mouse genes by quantitative real-time PCR analysis.

Gene	Primer sequence (5′–3′)	Accession number
TNF-α	CCCTCACACTCAGATCATCTTCT GTCACGACGTGGGCTACAG	NM_013693.2
ICAM-1	CGCTGTGCTTTGAGAACTGTG ATACACGGTGATGGTAGCGGA	NM_010493
Iba-1	GTCCTTGAAGCGAATGCTGG CATTCTCAAGATGGCAGATC	NM_019467
A_2aAR	TCCACTCCGGTACAATGGCTTGGT AGCATGGGGGTCAGGCCGAT	NM_009630.2
Mice IL-6	TAGTCCTTCCTACCCCAATTTCC TTGGTCCTTAGCCACTCCTTC	NM_031168.1
GAPDH	CAT GGC CTC CAA GGA GTAAGA GAG GGA GAT GCT CAG TGT TGG	M32599
18S	AGT GCG GGT CAT AAG CTT GC GGG CCT CAC TAA ACC ATC CA	NR_003278

transduction. A1 and A3 receptors interact with pertussis toxin-sensitive G proteins of the Gi and Go family to inhibit adenylate cyclase. The A2A receptor stimulates adenylate cyclase through Gs coupling (Fredholm et al., 1994). A2B receptor stimulates phospholipase C activity through Gq (Feoktistov et al., 1999). The increased adenosine at inflamed sites exhibits anti-inflammatory effects to protect against cellular damage through A_{2A}AR (Bong et al., 1996; Ralevic and Burnstock, 1998; Ohta and Sitkovsky, 2001). A_{2A}AR agonist treatment blocks the inflammation, and functional and histological changes associated with diabetic nephropathy in wild-type diabetic mice but not in the A_{2A}AR −/− diabetic mice (Awad et al., 2006). We found that treatment with the A_{2A}AR agonist resulted in marked decreases in diabetes-induced retinal cell death and TNF-α release (Ibrahim et al., 2011a). We also found that activation of A_{2A}AR in the stressed retinal microglial cells was the most efficient in mediating TNF-α inhibition (Liou et al., 2008). Furthermore, our work showed that diabetic A_{2A}AR −/− mice had significantly more TUNEL-positive cells, TNF-α release, and ICAM-1 expression compared with diabetic wild-type mice (Ibrahim et al., 2011a). The proposed mechanism of chronic retinal injury in diabetic retinopathy is RGC death associated with activation of an inflammatory pathway and an anti-inflammatory pathway involving A_{2A}AR signaling. We propose that A_{2A}AR signaling may also play a similar role in the acute treatment of TON.

Recent efforts to understand how neurotoxic inflammatory cytokines are produced have shown that MAPKinase signaling pathway is one of the attractive targets for intervention in human inflammatory-associated diseases such as diabetes. However, this pathway does not operate alone, but rather interacts with other signaling systems, such as Gs-coupled receptor transducing pathway. Activation of this pathway results in accumulation of cAMP that interacts with the MAPKinase signaling pathway to regulate cell functions (Gerits et al., 2008).

Previously we demonstrated the anti-inflammatory effect of A_{2A}AR in acute (Liou et al., 2008) and chronic (Ibrahim et al., 2011a) retinal

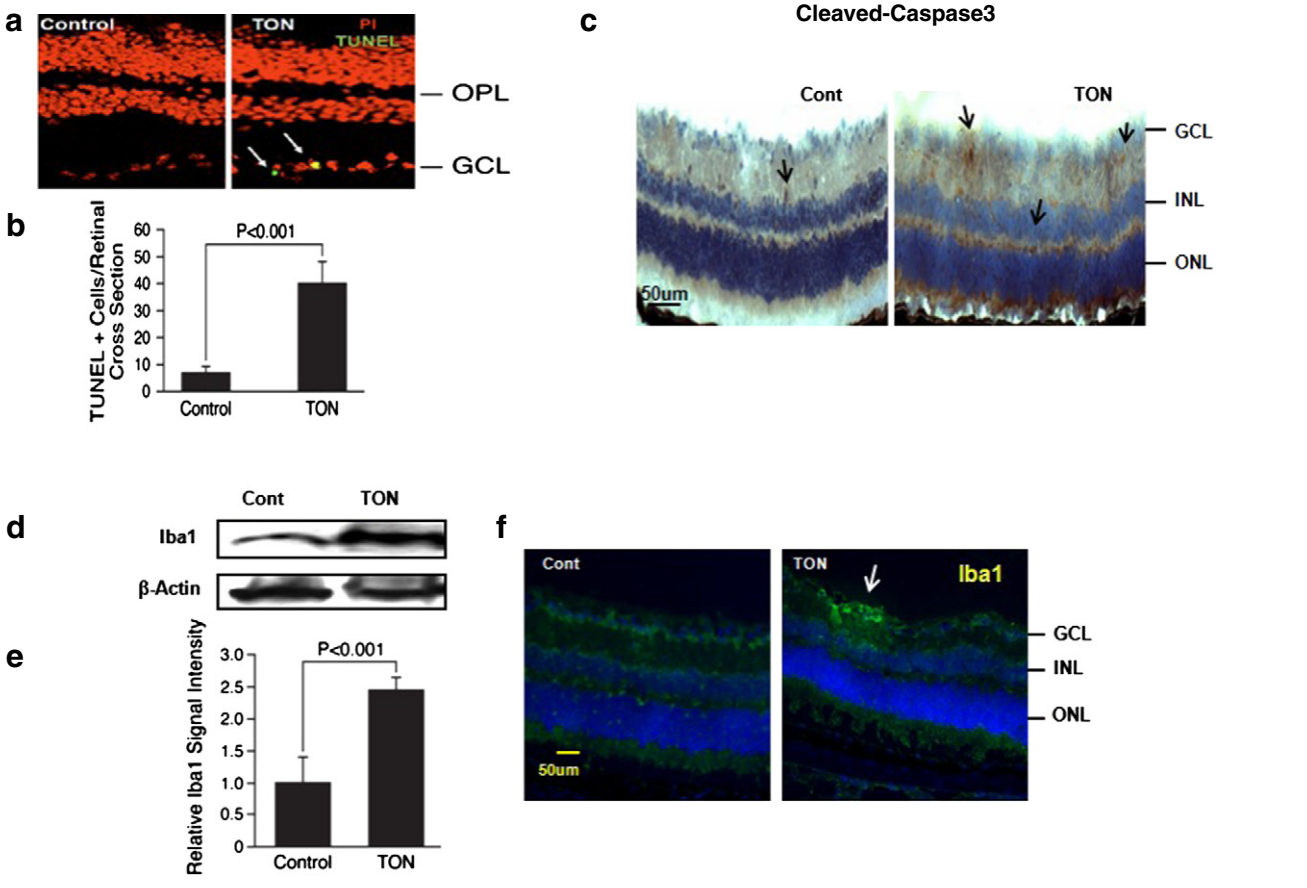

Fig. 1. TUNEL assay, cleaved-caspase3 activation and Iba1 expression on the retina of the mouse model of TON. a–b) Retinal distribution of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive cells identified in TON and control. Sections were counterstained with propidium iodide (PI). c) Quantitative analysis of TUNEL-positive cells in the retinal cross section of TON and control. TUNEL+ cells were counted in 10 adjacent locations along the vertical meridian within 4 mm of the optic disk (10 fields/retina sections). c) Immunohistochemical analysis of activated caspase3 in retinal section of TON vs. control. DAB (3, 3′-diaminobenzidine) produces a dark brown reaction product of cleaved caspase3. d) Immunoblotting analysis of microglial activation marker Iba1 expression in TON vs. control in the retina. e) Densitometry analysis of Iba1 and actin ratio by Image J software, NIH. f) Immunolabeling of Iba1 (green) with nuclear marker, DAPI (blue) in retinal section of TON vs. control. Data shown are the mean ± SD (n = 4). *P < 0.01, **P < 0.001, ***P < 0.0001.