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Research Report

Idazoxan attenuates spinal cord injury by enhanced astrocytic activation and reduced microglial activation in rat experimental autoimmune encephalomyelitis

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ABSTRACT

Idazoxan, an imidazoline 2 receptor (I_2R) ligand, has been shown to protect against brain injury in several animal models of neurological disorders. In the present study we investigated the effect of idazoxan on experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. EAE was induced by immunizing Wistar rats with guinea pig spinal cord homogenates emulsified in CFA, followed by daily treatment of idazoxan (0, 0.5 mg/kg, 1.5 mg/kg, 4.5 mg/kg, i.p, bid) for 10 days. The results showed that the treatment of idazoxan (1.5 mg/kg and 4.5 mg/kg) significantly decreased the incidence and alleviated inflammatory cell infiltration and demyelination in spinal cords and cerebral cortex. Furthermore, the protective effect of idazoxan on EAE was associated with the enhanced astrocytic activation and attenuated microglial activation and with the subsequent down-regulated expression of proinflammatory cytokines IL-12p40 and IFN-γ and up-regulated expression of anti-inflammatory cytokines IL-10 and TGF-β_1. Thus, the daily treatment of the I_2R ligand idazoxan for 10 days attenuates EAE pathology by differential modulation of astrocytic and microglial activations, raising a possibility that the I_2R ligand may be a novel strategy for treating EAE.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is the most thoroughly studied animal model of multiple sclerosis (MS) based on their shared characteristics of focal inflammation and demyelination in the central nervous system (CNS) and consequent paralytic presentation. The pathogenesis of EAE is believed to be mediated by autoimmune $CD4^+$ T cells, i.e. T helper (T_H) cells, which attack self components of myelin, such as myelin basic protein, myelin oligodendrocyte glucoprotein and proteolipid protein. Inflammatory cytokines are closely involved in control of immune responses of EAE. T_H1 cytokines such as IFN-γ and IL-12 promote inflammatory demyelinating disease (Vass et al., 1992; Kim and Voskuhl, 1999; Smith et al., 1997) while cytokines secreted by T_H2 and Treg cells such as TGF-β1 and IL-10 are associated with remission of the disease (Zhang et al., 2004; Khoury et al., 1992). $CD4^+$ T cells and microglia/macrophages are the

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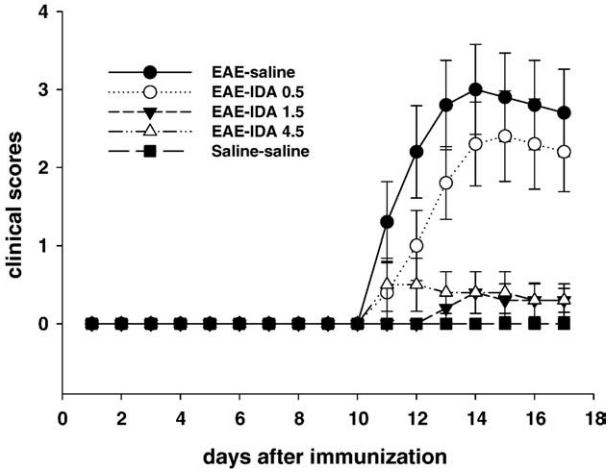

Fig. 1 – The effect of the treatment with idazoxan on the incident, clinical score in EAE rats induced by GPSCH. Immediately after immunization with GPSCH-CFA, Wistar rats were daily treated with IDA (0.5, 1.5 or 4.5 mg/kg) or water/saline for 10 days as described in the Experimental procedures. The onset of clinical signs of EAE rats began at 11 dpi and the mean clinical signs reached the peak around 13 dpi, lasted for additional 4–6 days and followed by gradual recovery. The daily treatment of IDA (at 1.5 and 4.5 mg/kg) for 10 days markedly reduced the mortality and mean maximal clinical scores as compared to the EAE-saline group.

ultimate effector cells in the progression of demyelinating inflammation (Bauer et al., 1995) while astrocytic activation is thought to be related to the remission of EAE (Matsumoto et al., 1992; Liedtke et al., 1998). Neurons and glial (microglial and astrocytic) cells and their inflammatory cytokines act in concert to affect the pathogenesis of EAE.

The concept of imidazoline receptors was first suggested by Bousquet in 1984. According to the binding affinity to different ligands and the discrepancy of biological characters and body distribution, the imidazoline receptors were divided into three subunits: those with high affinity to [3H]-para-amino clonidine was named as imidazoline 1 receptors (I_1R) and those with high affinity to [3H]-idazoxan as imidazoline 2 receptors (I_2R), and recently, a non-I_1 non-I_2 imidazoline receptor was found to be related to the secretion of insulin and designated as imidazoline 3 receptors (I_3R). I_2R was Initially labeled by [3H] idazoxan and more recently by more specific ligands such as 2-BFI (2-(2-benzofuranyl)-2-imidazoline) and BU224 (2-[4,5-dihydroimidaz-2-yl] -quinoline). The widely accepted endogenous ligand of I_2R was agmatine, a decarboxylation product of the amino acid arginine and an intermediate in polyamine biosynthesis. Radioautographic study with [3H]2-BFI revealed that I_2R was distributed throughout the mouse brain, especially nucleus raphes dorsalis, thalamic paraventricular nucleus and nucleus accumbens (MacInnes and Handley, 2005). The I_2R binding was mainly localized in the outer membrane of mitochondria of astrocytes (García-Sevilla et al., 1999). Interactions of I_2R and its ligands including agmatine, was associated with various brain disorders such as feeding behavior (Polidori et al., 2000), depression (Finn et al., 2003), morphine analgesia (Sanchez-Blazquez et al., 2000), Huntington's disease (Reynoldsa et al., 1996), Parkinson's disease (MacInnes and Duty, 2004), heroine addiction (Sastre et al., 1996) and glial tumors (Callado et al., 2004). Especially, activation of I_2R appears to be protective against motoneuron death caused by neurectomy of facial motor nerve (Casanovas et al., 2000) and focal ischemic infarction (Maiese et al., 1992; Reis et al., 1994).

Though the relevance of I_2R and demyelinating neuroinflammation, such as MS, has not been reported, it seemed that activation of I_2R may also have a protective effect on demyelinating neuroinflammation since the cellular and molecular events resident in the neuroprotective effect of I_2R ligands were also correlated with the remission of EAE. First, the neuroprotective effect of I_2R is associated with increased expression of GFAP in astrocyte (Olmos et al., 1994; Garcia-Sevilla et al., 1995), which correlates to the relief of EAE since GFAP knockout mice displayed more severe EAE than wild-type littermates (Liedtke et al., 1998). Second, ligands of I_2R, such as idazoxan (IDA), suppress production of nitric oxide synthase (NOS) (Feinstein et al., 1998, Feng et al., 2002) and interestingly, inhibition of NOS relieves the clinical and histological severity of EAE and up-regulates the expression of regulatory cytokines such as IL-10 and TGF-β1 while down-regulates proinflammatory cytokines such as TNF-α (Brenner et al., 1997). Moreover, activation of I_2R blocks voltage-gated calcium channel and thereby reduces intracellular calcium overload (Weng et al., 2003), which also significantly ameliorated EAE (Brand-Schieber and Werner, 2004).

So, based on the documented I_2R's neuroprotective effects against motoneuron and ischemic injury and I_2R's ability to influence GFAP, NOS and calcium channels which were also influential factors of EAE as described above, we proposed that activation of I_2R may have protective effect on brain and spinal cord injury in EAE. In support of this working hypothesis, we have previously found that the density of I_2R is up-regulated in EAE rats (Yin et al., 2006). Furthermore, levamisole (LMS), a synthetic imidazothiazole derivative which contains imidazolyl and competitively binds to I_2R (Yin et al., 2007), aggravated spinal cord extracts-induced EAE in rats (Xing et al., 2001, 2002) and even induced EAE by itself (Li et al., 2003).

In the present study, we investigated the effect of the I_2R ligand IDA on EAE. Furthermore, we explored the mechanisms associated with IDA-induced potential neuroprotection by determining the effect of IDA on the activation of astrocyte (labeled by GFAP) and microglia/macrophages (labeled by Iba-

Table 1 – Effects of idazoxan on clinical signs of EAE rats

Group	Clinical morbidity	Latency (days, $\overline{X}\pm$SE)	Score (*n*)					
			0	1	2	3	4	5
Saline-saline	0/10	-	0	0	0	0	0	0*
EAE-saline	8/10	11.37±0.26	2	0	0	2	4	2
EAE-IDA 0.5	8/10	12.14±0.69	2	0	2	2	4	0
EAE-IDA 1.5	2/10*	13.50±0.50*	0	0	2	0	0	0**
EAE-IDA 4.5	3/10*	12.67±1.67*	0	1	1	1	0	0**

* $p<0.05$, compared with EAE-saline group.
** $p<0.01$, compared with EAE-saline group.