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BRAIN RESEARCH

Research Report

Thirteen-month inhibition of aldose reductase by zenarestat prevents morphological abnormalities in the dorsal root ganglia of streptozotocin-induced diabetic rats

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ABSTRACT

The dorsal root ganglia (DRG) have been identified as the target tissue in diabetic somatosensory neuropathy. It has been reported that, in the chronically diabetic state, DRG sensory neurons may undergo morphological changes. In this study, we examined the effect of zenarestat, an aldose reductase inhibitor, on the morphological derangement of the DRG and the sural nerve of streptozotocin-induced diabetic rats (STZ rats) over a 13-month period. The cell area of the DRG in STZ rats was smaller than that in normal rats. A decrease in fiber size was apparent in the sural nerve of the STZ rats, and the fiber density was greater. These morphological changes were reversed in zenarestat-treated STZ rats. The data suggest that, in peripheral sensory diabetic neuropathy, hyperactivation of the polyol pathway induces abnormalities not only in peripheral nerve fiber, but also in the DRG, which is an aggregate of primary sensory afferent cell bodies.

1. Introduction

Peripheral sensory neuropathy is a common complication suffered by diabetic patients (Vinik et al., 1992). Although the pathogenesis of this condition has not been fully elucidated, some abnormalities have been reported in the early stages of experimental diabetic neuropathy (Jakobsen and Lundbæk, 1976; Sharma and Thomas, 1987). Recently, disorders have been reported in both the peripheral nerve and the dorsal root ganglia (DRG) of longstanding streptozotocin (STZ)-induced diabetic rats (Sasaki et al., 1997; Zochodne et al., 2001; Kishi et al., 2002). In these reports, a reduction in blood flow and cell size as well as the existence of oxidative injury were observed in the DRG, accompanied by dysfunctional peripheral nerve blood flow and the slowing of peripheral nerve conduction velocity, in chronic (12 months or more) diabetic rats. The reports also mention that both the neuronal soma of peripheral sensory nerves and the peripheral nerve tissue are important neuropathic target tissues.

Aldose reductase (AR) is a rate-limiting enzyme that converts glucose to sorbitol in the polyol pathway. Although there are controversies about whether AR is the key molecule in the development of diabetic neuropathy, the hyperactivation of the polyol pathway is considered to be a

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Table 1 – Body weight, plasma glucose and HbA1c 2-week and 13-month after STZ treatment

Group	Body weight (g)	Plasma glucose (mg/dL)	HbA1c (%)
Normal control			
2-week	347.8±2.2	122.3±2.3	2.8±0.1[a]
13-month	868.3±27.7	123.1±5.4	3.0±0.1
STZ control			
2-week	272.1±8.2[**]	593.8±22.9[**]	6.7±0.1[**]
13-month	319.1±16.3[**]	602.5±35.3[**]	8.9±0.9[**]
STZ zenarestat			
2-week	275.1±4.2	559.4±32.0	6.6±0.2
13-month	318.9±15.9	798.6±90.1	9.3±0.4

Mean±S.E.M., $n=8$ expect for a.
[a] $n=7$, HbA1c of a rat was under the detection limit (<2.5%).
[**] $p<0.01$ vs Normal control, any parameter of STZ zenarestat is not significant compared with those of STZ control.

cause (Greene et al., 1993; Yagihashi, 1995; Oates, 2008). Hyperglycemia induces the hyperactivation of the polyol pathway and the excessive nerve accumulation of sorbitol and fructose. These abnormalities under diabetes lead to non-enzymatic glycation, oxidative injury, hypoxia and so on (Sima, 2006; Pop-Busui et al., 2006). Over the past several decades, a number of newly discovered AR inhibitors (ARIs) have been shown to improve clinical and experimental diabetic neuropathy (Tomlinson et al., 1984; Kamijo et al., 1994; Nakamura et al., 1997; Mizisin et al., 1997; Raccah et al., 1998; Mizuno et al., 1999; Hotta et al., 2001, 2006; Bril et al., 2006). Long-term ARI treatment reportedly improved the abnormalities in the peripheral sural nerve seen in chronically diabetic rats (Kato et al., 2000). Although this report suggests that AR plays an important role in the pathogenesis of chronic experimental diabetic neuropathy in the peripheral nerve, the role of AR in the DRG has not yet been studied.

Zenarestat is one of the specific ARIs (Ao et al., 1991; Takakura et al., 2001). It has already been reported that zenarestat improves diabetic neuropathy in rats during the early stages of diabetes. This therapeutic effect is accompanied by the inhibition of sorbitol accumulation in both the DRG and the peripheral nerve (Ao et al., 1991; Shimoshige et al., 2000; Takakura et al., 2001; Yamamoto et al., 2001). In order to elucidate the role of AR in chronic experimental diabetic neuropathy in STZ rats, we examined the effects of a 13-month administration of zenarestat on morphological disorders of the DRG as well as those of the sural nerve.

2. Results

2.1. *Animal number analyzed for the morphometry of nerve tissue*

The number of rats assigned to the normal control, STZ control, and STZ zenarestat group, were 16, 28, and 28, respectively. During the study, 2, 8, and 11 rats in the normal control, STZ control, and STZ zenarestat groups respectively had died. The plasma glucose levels of 8 rats from STZ control group and 2 rats in the STZ zenarestat group were lower than 400 mg/dL at the time of harvest. Of the remaining rats, 6 were chosen for sural nerve morphometry, and 8 for DRG morphometry, based on the body weights in each group.

2.2. *Body weight, plasma glucose, and HbA1c*

The body weight, plasma glucose, and hemoglobin A1c (HbA1c) values are shown in Table 1. The body weight of the STZ control rats was significantly less than that of normal control rats through the study. The plasma glucose and HbA1c levels in the STZ control rats were significantly higher than those of normal control rats before and after the drug treatment. These parameters were not changed by zenarestat treatment.

2.3. *Morphological analysis of the sural nerve*

The effects of zenarestat on the morphological changes in the sural nerve are shown in Table 2. Because one sural nerve in the STZ zenarestat group was not adequately fixed, and some artifacts were observed, the total fascicular area (TFA) of the affected rat was excluded. The number of fibers analyzed in normal control, STZ control, and STZ zenarestat rats were 175.2±10.7, 223.8±29.8, and 175.8±24.5, respectively (mean±SD). Compared with those of normal control rats, the TFA and fiber size of STZ control rats decreased, while fiber density and the G-ratio (axon diameter/fiber diameter) increased significantly. The fiber density and fiber size of STZ zenarestat rats was significantly improved, compared with those of STZ control rats.

2.4. *Morphological analysis of the DRG*

Typical light microscopic images of the DRG are shown in Fig. 1. In the DRG of both normal and STZ rats, the cell nuclei are clearly observable, and some cells have vacuoles in the cytoplasm. The areas of the DRG cells are shown in Fig. 2.

Table 2 – Morphological analysis in sural nerve after the 13-month zenarestat treatment

Group	TFA (mm^2)	Fiber density ($/mm^2$)	Fiber size (μm^2)	Axon size (μm^2)	G-ratio
Normal control	0.074±0.006	9616±704	47.6±3.7	17.4±1.4	0.61±0.01
STZ control	0.045±0.006[**]	14353±558[**]	33.3±1.0[*]	13.8±0.4	0.64±0.01[**]
STZ zenarestat	0.068±0.010	11282±542[***]	42.3±2.0[***]	15.2±1.3	0.61±0.02

Mean±S.E.M., $n=6$ except for TFA of STZ zenarestat ($n=5$).
[*] $p<0.05$.
[**] $p<0.01$ vs Normal control.
[***] $p<0.01$ vs STZ control, TFA; total fascicular area, G-ratio; the ratio of axon diameter/fiber diameter.