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Research Report

Increased susceptibility to ischemia and macrophage activation in STZ-diabetic rat nerve

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ABSTRACT

Ischemic vulnerability in diabetic nerve plays a paramount role in the development of diabetic neuropathy, yet little is known of the underlying mechanism. Diabetes enhances the inflammatory response to ischemia and reperfusion. We investigated pathological characteristics of nerve fibers and endoneurial macrophages along the length of sciatic–tibial nerves before and after ischemia (60 to 90 min) and reperfusion (6 h to 7 days) in 8 weeks of STZ-induced diabetic rats. Without ischemia, diabetic nerves revealed significantly increased the density of Iba-1-positive endoneurial macrophages when compared with controls. Most of macrophages appeared slim and triangular in shape, but in diabetic nerves, some were rounded with bromodeoxyuridine (BrdU) incorporation, suggesting proliferating macrophages. Seventy-five minutes of ischemia is the minimal ischemic time to cause pathological changes in diabetic nerves. Following 90 min of ischemia and 6 h of reperfusion in diabetic rats, the number of Iba-1-positive endoneurial macrophages was increased significantly at the thigh level of sciatic nerve when compared with those before ischemia. Endoneurial macrophages in diabetic nerves increased in number further significantly after 24 and 48 h of reperfusion and underwent morphological alterations; swollen and rounded including phagocytosis. After 90 min of ischemia and 7 days of reperfusion, severe pathological alterations, e.g., demyelination and endoneurial edema at proximal nerves and axonal degeneration distally, were observed in diabetic nerves, while control nerves showed normal morphology. We conclude that macrophage proliferation occurs in STZ-diabetic nerves. The acute inflammatory response after ischemia and reperfusion was intensified in diabetic nerves. Activation of resident macrophages and infiltration by recruited macrophages could be casually linked to ischemic susceptibility in diabetic nerve.

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Abbreviations: BrdU, bromodeoxyuridine; GFP, green fluorescent protein; Iba-1, ionized calcium-binding adaptor molecule 1; STZ, streptozotocin

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1. Introduction

Diabetes enhances ischemic/reperfusion injury in various tissues. We first demonstrated that peripheral nerves in STZ-diabetic rat are susceptible to acute ischemia by either arterial ligation or microsphere embolization (Nukada, 1986, 1992, 1993). A brief or mild ischemia, insufficient to cause nerve fiber damage in normal nerve, results in endoneurial edema, demyelination, axonal degeneration, and necrosis in STZ-diabetic sciatic and tibial nerves. Zochodne and his colleagues demonstrated this property by applying endothelin-1, the most potent vasoconstrictor, in the epineurium of sciatic nerve in STZ-diabetic rats (Zochodne et al., 1996; Zochodne and Cheng, 1999).

We also found aggravated reperfusion injury electrophysiologically and morphologically in STZ-diabetic nerve; delayed recovery of compound muscle action potential, greater endoneurial edema, and prominent axonal degeneration when compared with those in controls (Baba et al., 2006; Nukada et al., 2002). Low and his colleagues confirmed reperfusion exaggerated morphological pathology in STZ-diabetic nerve (Wang et al., 2004). They also showed enhanced inflammatory response nuclear factor-kappa B (NF-κB) activation after reperfusion in STZ-diabetic nerve (Wang et al., 2006). Similar vulnerability to ischemia and reperfusion has been reported in various tissues of diabetes, e.g., brain, heart, and kidney (Anzawa et al., 2006; Di Filippo et al., 2005; Ding et al., 2004; Hearse et al., 1978; Melin et al., 2003; Panagia et al., 2005; Thakker et al., 2008; Yue et al., 2005), and both acute and chronic hyperglycemia aggravate ischemic brain damage (Capes et al., 2001; Gisselsson et al., 1999; Martin et al., 2006; Muranyi et al., 2003; Nedergaard, 1987).

The macrophage has been emerged as an important player in the pathogenesis of both diabetes and diabetic complications. Macrophage accumulation is a feature of diabetes and is associated with development of vascular complications, including both macro- and microangiopathy (Boyle, 2007; Fernandez-Real and Pickup, 2008; Kolb and Mandrup-Poulsen, 2005; Odegaard and Chawla, 2008; Schenk et al., 2008; Tesch, 2007; Toso et al., 2008; Wellen and Hotamisligil, 2005). Macrophages mediate diabetic injury through a variety of mechanisms, including production of reactive oxygen species and cytokines. Reperfusion nerve injury is also a state where oxidative stress has been implicated (Anderson et al., 1997; Frangogiannis et al., 1998; He et al., 1999, 2003; Wang et al., 2005, 2008). Diabetes exaggerates inflammatory responses after ischemia and reperfusion: increased leukocyte–endothelial cell adhesion, albumin extravasation, and oxidant production by endothelial cells in post-capillary venules (Panes et al., 1996; Salas et al., 1998, 1999).

In the current study, we assessed pathological characteristics along the length of sciatic and tibial nerves in STZ-diabetic rats before and after ischemia and reperfusion. We also addressed the hypothesis that macrophage activation and proliferation could be enhanced in reperfused diabetic nerve.

2. Results

Mean body weights of diabetic and control rats at onset were 231±8 (n=46) and 231±8 (n=46) g, respectively (p>0.05). STZ treatment was associated with a significant attenuation of weight gain at the time of experiment; 343±7 g in diabetic rats and 491±7 g in controls (p<0.0001). STZ-treated rats displayed significant elevation in blood glucose; 29.9±0.5 mmol/l in diabetic rats and 5.5±0.1 mmol/l in controls (p<0.0001). When glucose level was above the scale (>33.3 mmol/l), it was calculated as 33.3 mmol. Motor nerve conduction velocity in sciatic–tibial nerves was reduced in diabetic nerve, being 43.3±0.8 and 53.6±1.0 m/s in diabetic rats and controls respectively (p<0.0001). Results of nerve conduction studies during and after reperfusion in STZ-diabetic rats have been detailed previously (Baba et al., 2006).

2.1. *Nerve pathology*

After 8 weeks of STZ-induced diabetes, there was no morphological change in sciatic–tibial nerves. Reperfusion after 60 min of ischemia did not cause any pathological abnormalities in both diabetic and control nerves. However, following 75 min of ischemia and 7 days of reperfusion, diabetic rats revealed focal or multifocal lesions consisting of axonal degeneration, which is the hallmark of an acute ischemic injury (Nukada and Dyck, 1984), at lower thigh and upper calf levels of sciatic and tibial nerves in 4 out of 6 nerves (Fig. 1), whereas nerve morphology was normal in controls.

After 90 min of ischemia and 7 days of reperfusion, diabetic rats exhibited invariably severe pathological abnormalities in sciatic and tibial nerves. At the upper thigh level of sciatic nerve in diabetic rats, the most proximal level evaluated, isolated demyelinated, or thinly myelinated nerve fibers and macrophages with myelin debris were found (Fig. 2A, B). Minimal endoneurial edema was also observed at this level (Fig. 2C, D). At the lower thigh level of diabetic sciatic and tibial nerves, endoneurial edema was more obvious and demyelinated fibers and macrophages were still observed (Fig. 2E, F).

At the upper calf level of tibial nerves after 90 min of ischemia and 7 days of reperfusion in diabetic rats, clusters of demyelinated fibers, macrophages with myelin debris, and severe endoneurial edema with intra-myelinic edema were found (Fig. 3). Demyelinated fibers were often located either near the vessel or macrophages. Control nerves did not reveal demyelinated nerve fibers. Reperfused diabetic nerves also exhibited frequent axonal degeneration (Fig. 3C, D). The density (/mm^2) of nerve fibers with axonal degeneration was significantly increased at the upper calf level in diabetic nerve than in controls; 24.0±6.5 and 1.2±2.8, p<0.0001, respectively.

At the lower calf and ankle levels of diabetic tibial nerve, axonal degeneration was prominent. Nerve fibers with axonal degeneration were co-existed with normal myelinated fibers, and focal lesions of axonal degeneration were found though not prominent as at proximal levels (Fig. 4A, B). Diffuse axonal degeneration was also seen in some diabetic rats (Fig. 4C). In contrast, there was normal morphology in control nerves after 90 min of ischemia and 7 day of reperfusion (Fig. 4D). Because of consistent severe pathology, we used 90 min of ischemia for the study of acute inflammatory response after ischemia and reperfusion.

2.2. *Endoneurial macrophages*

Immunohistochemical expression of Iba-1 was observed at thigh, knee, and calf levels of sciatic and tibial nerves before