Impaired neurovascular repair in subjects with diabetes following experimental intracutaneous axotomy

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Diabetic complications and vascular disease are closely intertwined. Diabetes mellitus is a well-established risk factor for both large and small vessel vascular changes, and conversely other vascular risk factors confer increased risk for diabetic complications such as peripheral neuropathy, nephropathy and retinopathy. Furthermore, axons and blood vessels share molecular signals for purposes of navigation, regeneration and terminal arborizations. We examined blood vessel, Schwann cell and axonal regeneration using validated axotomy models to study and compare patterns and the relationship of regeneration among these different structures. Ten subjects with diabetes mellitus complicated by neuropathy and 10 healthy controls underwent 3 mm distal thigh punch skin biopsies to create an intracutaneous excision axotomy followed by a concentric 4-mm overlapping biopsy at different time points. Serial sections were immunostained against a pan-axonal marker (PGP9.5), an axonal regenerative marker (GAP43), Schwann cells (p75) and blood vessels (CD31) to visualize regenerating structures in the dermis and epidermis. The regenerative and collateral axonal sprouting rates, blood vessel growth rate and Schwann cell density were quantified using established stereology techniques. Subjects also underwent a chemical ‘axotomy’ through the topical application of capsaicin, and regenerative sprouting was assessed by the return of intraepidermal nerve fibre density through regenerative regrowth. In the healed 3 mm biopsy sites, collateral and dermal regenerative axonal sprouts grew into the central denervated area in a stereotypic pattern with collateral sprouts growing along the dermal–epidermal junction while regenerative dermal axons, blood vessels and Schwann cells grew from their transected proximal stumps into the deep dermis. Vessel growth preceded axon and Schwann cell migration into the denervated region, perhaps acting as scaffolding for axon and Schwann cell growth. In control subjects, Schwann cell growth was more robust and extended into the superficial dermis, while among subjects with diabetes mellitus, Schwann tubes appeared atrophic and were limited to the mid-dermis. Rates of collateral (P = 0.0001), dermal axonal regenerative sprouting (P = 0.02), Schwann cell migration (P < 0.05) and blood vessel growth (P = 0.002) were slower among subjects with diabetes mellitus compared with control subjects. Regenerative deficits are a common theme in diabetes mellitus and may underlie the development of neuropathy. We observed that blood vessel growth recapitulated the pattern seen in ontogeny and preceded regenerating nerve fibres, suggesting that enhancement of blood vessel growth might facilitate axonal regeneration. These models are useful tools for the efficient investigation of neurotrophic and regenerative drugs, and also to explore factors that may differentially affect axonal regeneration.

Keywords: diabetic neuropathy; Schwann cells; blood vessels; peripheral nerve regeneration; stereology
Abbreviations: GAP = growth-associated protein

Received November 2, 2010. Revised March 2, 2011. Accepted March 14, 2011
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Introduction

Epidemiological studies indicate that 25–60% of diabetic patients develop a progressive peripheral neuropathy that leads to significant morbidity (Ziegler et al., 1992; Dyck et al., 1993). Axonal atrophy and degeneration are the pathological hallmarks for the development of severe sensory loss and have been shown to occur both in nerve and skin biopsies of diabetic patients (Dyck et al., 1986; Lehtinen et al., 1989; Malik et al., 2001; Polydefkis et al., 2001; Singleton et al., 2005; Quattrini et al., 2008).

Many mechanisms including direct metabolic effects due to hyperglycaemia, oxidative stress, mitochondrial dysfunction and disruption of axonal transport by advanced glycation end products have been implicated in the pathogenesis of diabetic polyneuropathy (Jakobsen and Sidenius, 1979; Jakobsen et al., 1981; Katz et al., 1989; Jaap et al., 1996; Brownlee, 2001; Gibran et al., 2002; Yasuda et al., 2003; Kennedy and Zochodne, 2005). In addition to peripheral nerve abnormalities, vascular abnormalities are also present in diabetes mellitus, and human and experimental studies have suggested that diabetes impairs ischaemia-driven neovascularization (Yasuda and Dyck, 1987; Yarom et al., 1992; Rivard et al., 1999).

In addition to the loss of sensory fibres resulting in neurological deficits, regenerative failure has been observed in experimental nerve lesions of animals (Kennedy and Zochodne, 2000). We have also observed that regenerative axonal growth is impaired in human subjects with diabetes even in the setting of no evidence of peripheral neuropathy (Polydefkis et al., 2004). Previous studies have shown that molecular signals are shared by nerve and blood vessels, Schwann cells and axonal regeneration in healthy control subjects and subjects with diabetes. This approach allows us to dissect and study the different components of axonal and blood vessel growth in the skin of human subjects.

Materials and methods

Study subjects

Healthy control subjects were determined to be neuropathy-free by neurological examination, symptom score, and were screened by laboratory testing for peripheral neuropathy risk factors (HIV serology, vitamin B12, fasting blood glucose, glycated haemoglobin, thyroid-stimulating hormone, complete blood count and metabolic panel). The median age of controls was 47 years, range 23–60 (Table 1).

Subjects with diabetes had a median age of 51 years, range 37–64 (*P* = 0.24) (Table 1). Subjects with diabetes were examined by a neurologist trained in neuromuscular disease and determined to have peripheral neuropathy attributed to diabetes. All subjects had Michigan Diabetic Neuropathy Score 18 > (MDNS) > 6. Diabetes was confirmed by review of medical records. Diabetes type and absence of retinopathy were determined by subject report and proteinuria was assessed through a urine dipstick. No subject had a history of retinopathy while four subjects had proteinuria (Table 2). Electrophysiology was performed on the subjects with diabetes using a uniform protocol in order to further objectively quantify the neuropathy (Table 3). All studies were approved by the Johns Hopkins University Institutional Review Board.

Capsaicin model

All subjects in the study underwent both capsaicin denervation as well as excision biopsies. For the capsaicin ‘chemical axotomy’, a standardized area was demarcated on the distal lateral thigh. An occlusive bandage measuring 35 × 50 mm, and containing 1.8 g of 0.1% capsaicin cream (Chattem Inc), was applied to the area for two consecutive 24 h periods as previously described (Polydefkis et al., 2004).

Table 1 Demographics of study subjects

<table>
<thead>
<tr>
<th>n</th>
<th>Age* (years)</th>
<th>Gender* (M/F)</th>
<th>Height (cm)</th>
<th>Race (% Caucasian)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>10</td>
<td>37–64</td>
<td>5/5</td>
<td>170 (150–191)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>23–60</td>
<td>6/5</td>
<td>166 (149–179)</td>
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*Median (range); DM = diabetes mellitus.*

Table 2 Glycaemic control and diabetes complications among subjects with diabetes mellitus

<table>
<thead>
<tr>
<th>n</th>
<th>Age (years)</th>
<th>A1C (%)</th>
<th>Weight (kg)</th>
<th>Neuropathy duration (years)</th>
<th>Diabetes duration (years)</th>
<th>Proteinuria (1 + or trace)</th>
</tr>
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<tbody>
<tr>
<td>DM</td>
<td>10</td>
<td>35–64</td>
<td>7.4 (5.9–10.1)</td>
<td>103 (68–148)</td>
<td>7 (1–10)</td>
<td>13 (0.5–34)</td>
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<tr>
<td>Type I</td>
<td>3</td>
<td>35–47</td>
<td>7.0 (6.4–9.9)</td>
<td>76 (68–82)</td>
<td>5 (1–10)</td>
<td>28 (5–34)</td>
</tr>
<tr>
<td>Type II</td>
<td>7</td>
<td>44–64</td>
<td>7.7 (5.9–10.1)</td>
<td>116 (88–148)</td>
<td>8 (3–10)</td>
<td>10 (0.5–17)</td>
</tr>
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*All values represent medians (range).*
Table 1  Immunohistochemistry of diabetic patients

<table>
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<tbody>
<tr>
<td>Median, range</td>
<td>30.5, 25.2-41.4</td>
<td>6.25, 3.8-12.3</td>
<td>52.8, 40.8-57.9</td>
<td>5.3, 6.1</td>
<td>1.8-38.2</td>
<td>31.9-54.3</td>
<td>2.15, 0.1-4.7</td>
<td>43, 35.4-45.1</td>
<td>2.15, 0-9</td>
<td>38.4, 31.1-53.8</td>
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</table>

F-wave latency in ms, motor amplitudes in mV. c.v. = conduction velocity in m/s. Sensory amplitudes are reported in microvolts. Normal values: >10 mV for age <60 years; >5 mV for age ≥60 years. CV >49 m/s in the upper extremity; >39 m/s in the lower extremity.

**Excision model**

All subjects also underwent a 3 mm distal thigh punch skin biopsy (Acupunch™, Acuderm) to create an intracutaneous excision axotomy. This was followed by a 4 mm overlapping biopsy after 2–3 months (Fig. 1) (Rajan et al., 2003; Ebenezer et al., 2009). The first biopsy produces a denervated and avascular zone that subsequently fills in; the subsequent biopsy allows assessment of nerve and blood vessel regrowth and expansion (Rajan et al., 2003; Hahn et al., 2006; Ebenezer et al., 2009; Griffin et al., 2010).

**Immunohistochemistry**

Biopsies were fixed in paraformaldehyde/lysine/periodate, cryoprotected and sectioned at 50 μm intervals. Four sections from each biopsy were selected, bleached in 0.25% potassium permanganate to remove melanin pigment, rinsed in Tris-buffered saline and washed with 5% oxalic acid for 2 min. Blocking was performed in Tris-buffered saline 3% normal serum with 0.4% Triton X-100. The sections were incubated overnight at 4°C with the primary antibodies suspended in 0.1% Triton X-100 and 3% normal serum in Tris-buffered saline.

Primary antibodies used were PGP 9.5 (Chemicon, dilution 1:2000 in triton buffer), mouse anti-growth associated protein (GAP) -43/B-50 (Millipore, dilution 1:500 in triton buffer), mouse anti-p75 nerve growth factor receptor (Chemicon, dilution 1:500 in triton buffer) and mouse anti-CD31, PECAM (BD Pharmingen, dilution 1:100 in Triton buffer).

Non-specific binding of secondary antibodies was blocked with 4% normal goat serum, 1.0% Triton X-100, 0.5% non-fat powdered milk in Tris-buffered saline, pH 7.4. Sections were incubated in secondary antibody (goat anti-rabbit IgG and goat anti-mouse IgG) diluted 1:200 in Tris-buffered saline 0.1% Triton X-100, 3% normal serum for 1 h at room temperature. Endogenous peroxidase was blocked with 0.5% H2O2 in methanol and sections were then incubated with Avidin-Biotin Complex solution (Vector Labs) for 1 h followed by substract incubation for 5–6 min (Vector SG substrate and diaminobenzidine). Eosin (1%) and Mayer’s haematoxylin were used as counterstains.

**Immunofluorescence**

Sections were rinsed in Tris-buffered saline, pH 7.4, and incubated with secondary antibodies prepared in 2% normal goat serum, 0.5% Triton X-100, 0.5% non-fat powdered milk for 2 h at room temperature on a shaker table. The secondary antibodies Cy3 goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc) were used at 1:150 dilution and Alexa-Fluor goat anti-mouse IgG (Molecular Probes) at 1:500 dilutions. The fluorescence-labelled sections were mounted in Mowiol 4-88 (Calbiochem) to prevent fluorescent quenching.

DRAQ5 (Biostaus Ltd) was used for nuclear staining. Fluorescent samples were viewed and scanned using a Zeiss LSM510 confocal imaging system.

Intraepidermal nerve fibre density calculations were determined using established counting rules and the rate of regeneration among the capsaicin series biopsies was assessed by the return of intraepidermal nerve fibre density following confirmed denervation. The regeneration rate was determined from the slope of the regression line of the Day 2, 30, 60 and Day 90 biopsies, as previously described (Polydefkis, 2004).

**Morphometric studies**

During healing of the 3-mm biopsy, a collagen plug (scar) forms in the subepidermal dermis and the 3-mm excision site is easily identified histologically by the homogenous densely stained collagen in the dermis and the corresponding thinned overlying epidermis.

**Measurement of blood vessels and axonal regeneration in dermis**

The area of interest was defined as the dermal region extending from the epidermal/dermal junction to a dermal depth of 2000 μm. The length of blood vessels and dermal nerves growing into the excision site were measured using a design-based unbiased stereology methodology. With ×2.5/0.075 Plan-Neofluor objective of a Zeiss light microscope, the area of interest was marked and under ×63/1.40, oil Plan-Neofluor objective using hemisphere probe with a radius of 15 μm of the Stereo Investigator space ball probe (Fig. 2B) (MBF Bioscience) (Mowton et al., 2002; Tang et al., 2009), the growth rate was expressed as length of nerve or vessels divided by the number of days separating the 3 and 4 mm biopsies (mm/day). The Gundersen coefficient of error for the parameters described was 0.07 for blood vessels and 0.12 for dermal axons.

**Measurement collateral axonal sprouts**

The collateral sprouting distance was defined as the farthest centripetal distance from the 3-mm biopsy incision and was measured along the basement membrane of the epidermis with Bioquant software (R&M Biometrics). The collateral sprouting distance divided by the number of days between the 3 and 4 mm biopsies was calculated to yield the growth rate expressed in mm/day (Hahn et al., 2006).

**Statistics**

Comparisons between groups were made using the non-parametric Wilcoxon rank-sum test. To assess the effect of age or gender on
growth rates, linear regression was performed using the growth rate as the dependent variable and the presence/absence of diabetes, age and gender as independent variables.

**Histomorphometry of Schwann cells**

Schwann cell bands within the dermis were identified in the skin sections by the presence of brown coloured cytoplasmic staining by p75 in the setting of elongated blue-staining nuclei (counter stained with Mayer’s haematoxylin) (Fig. 2A).

Schwann cell nuclei were quantified using a similar approach as was done for the blood vessel and dermal axon measurements. A contour was made at the area of interest under a ×2.5/0.075 Plan-Neofluor objective of a Zeiss light microscope and the cells were counted using ×63/1.40, oil Plan-Neofluor objective. A sampling grid 200 μm × 150 μm, with a dissector height of 15 μm and guard zones of 2 μm (Fig. 2A) was used and the absolute numbers of Schwann cells were obtained using the ‘.DAT’ files of optical fractionator probe of Stereo Investigator (MBF Bioscience) (West et al., 1991; Peterson and Jones, 1993). The sampling design achieved a Gundersen coefficient of error 0.07. The density of Schwann cells in the dermis (cells/mm³) was determined and the rate of Schwann cell growth/day calculated.

All morphometric measurements were performed blinded of all clinical or identifying data.

**Microscopic observation and photography**

Preparations were viewed with Olympus BH2 and Axioscop microscopes and photographed with digital cameras. Using Adobe Photoshop CS v. 8.0 Software (Adobe Systems), the photomicrographs were adjusted evenly across the entire field for contrast and brightness levels, for optimal quality and positioned into the montages.
Results

Capsaicin model

There was complete epidermal denervation in both control and diabetes mellitus subject skin biopsies taken 48 h after capsaicin application (Fig. 3A and D). GAP43 expression was present in deep dermal nerve fibres (Fig. 3A and B) and by Day 60, attained the mean normative density (controls: 10 fibres/mm, diabetes mellitus: 7 fibres/mm) (McArthur et al., 1998). The controls showed a steady growth but the regenerative capacity lagged behind at all time points in subjects with diabetes mellitus (Fig. 3E) and findings has been consistent with our previous studies (Polydefkis et al., 2004) The median rate of post-capsaicin reinnervation among the subjects with diabetes was 0.092 (range 0.01–0.19) fibres/mm/day and was reduced compared with control subjects [median rate 0.19 fibres/mm/day, range (0.05–0.347)] consistent with our previous studies. This difference in regeneration rate persisted if baseline differences in epidermal nerve fibre density, age or gender were adjusted for.

Excision axotomy model

The skin sections showed a thin layer of epithelialization and basement membrane formation in the central denervated zone and the excision edges showed epithelial hyperplasia. The central dermal core area was easily observed with identifiable fibrin, fibroblasts and interlacing bundles of collagen (Fig. 1). Blood vessel growth into the healed excision site was robust in both groups of subjects and preceded Schwann cell and axonal sprouting into denervated regions. The proliferating blood vessels were aligned perpendicularly, towards the epidermis and extended into the papillary dermis (Fig. 4A). Blood vessel growth was significantly delayed (P = 0.004) among subjects with diabetes compared with controls (Fig. 4B).

The earliest axonal growth into the excision site was the collateral sprouts from epidermal nerve fibres just peripheral to the excision margin; these sprouts grew in a stereotypic pattern along the dermal–epidermal junction close to the basement membrane on the side of the basal keratinocytes (Fig. 5A). Few axons extended vertically towards the skin surface to produce intraepidermal axons into the stratum spinosum and when this occurred these were seen only at the edge of the axotomy site. These axons were ensheathed by Schwann cells only for a short distance along the dermal–epidermal junction (P = 0.003) among subjects with diabetes (median 0.01 mm/day, range 0.007–0.021) in comparison to controls (median 0.02 mm/day, range 0.016–0.044) (Fig. 5C).

In the central denervated zone, axons from the cut edge of the dermal nerves at the base of the excision grew through the dermal collagen in small bundles. These nerve bundles were oriented in a predominantly horizontal direction parallel to the epidermal surface (Figs 1B and 5B) and relatively few were directed perpendicularly towards the skin surface, suggesting attempted but incomplete regenerative sprouting by the injured fibres. In both control and diabetes subjects, the regenerative sprouts never reached the papillary dermis and the growth rate was significantly lower among subjects with diabetes compared with controls, P = 0.01 (Fig. 5D).

Schwann cell growth and migration was more robust at the base of the healed excisional biopsy site with cells appearing as
elongated bands. These became thinner and more clumped as they approached the superficial dermis (Fig. 6A). Schwann cell growth extended into the papillary dermis in control subjects. Among subjects with diabetes mellitus, growth was predominantly limited to the mid-dermis and relatively few Schwann cell bands in the superficial dermis contained axonal fragments and co-localized with PGP 9.5 (Fig. 6B). Among the subjects with diabetes mellitus, the Schwann cell migration rate was slowed ($P < 0.05$) compared with control subjects [median 143 cells/day (range 100–213) versus median 96 cells/day (range 42–185)] (Fig. 6C).

Because our groups differed slightly with respect to gender and age (Table 1), we investigated the effect of both these variables.

Figure 3 Double stained confocal images of PGP9.5 (red) with GAP43 (green) on the cutaneous regenerative fibres after chemical axotomy. (A) Forty-eight hours post capsaicin biopsy showing loss of epidermal fibres and few PGP9.5 expressed fibres (red arrow) remaining in the dermis. Note the absence of GAP43-positive fibres. (B) A large dermal nerve bundle reveals PGP 9.5-positive fibres (red arrow) co-localizing with GAP43 fibres (green arrow). (C) At Day 60, the PGP9.5 positive fibres regenerating (red arrow) into the epidermis and the Gap43-positive fibres remain at the level of the papillary dermis (yellow arrow). (D) The deep dermal larger nerve bundles revealing dense expression of GAP43 co-localizing with PGP9.5 fibres. Scale bar = 20 μm. (E) The regenerative rate after chemical axotomy was consistently lower in subjects with diabetes at all post capsaicin time points.
In multivariable regression models, gender was not associated with the growth rates of any epidermal or dermal structure. Age did reach significance in several instances, though the magnitude of the effect was small compared with the association with diabetes, and diabetes remained a significant variable even after adjusting for age (Supplementary Tables 1 and 2).

**Discussion**

In this study, we efficiently measured different forms of axonal, Schwann cell and vascular growth using relatively non-invasive 3 mm and overlapping 4 mm skin punches in the same subjects. These tools will allow us to dissect different mechanisms and
components of neurovascular outgrowth. There are several notable findings from this study. First, we observed that ‘pure’ axonal regenerative sprouting after capsaicin chemical axotomy occurred at a constant rate and was more complete than collateral sprouting, neovascularization or regenerative sprouting of the dermal Schwann cell/axon complex. Second, we observed that blood vessel growth into the excision site preceded regenerating dermal nerve fibres in both subject groups, perhaps providing scaffolding for subsequent axon and Schwann cell growth. Third, collateral sprouting from uninjured nerve fibres was more robust than regenerative sprouting of transected dermal nerve fibres. Finally, growth of blood vessels, dermal nerve fibres and Schwann cells into the axotomy site were significantly reduced among subjects with diabetes compared with healthy controls and appears to be a common theme in diabetes mellitus.

We assessed axonal sprouts through immunohistochemical staining against ubiquitin hydrolase, which is a cytoplasmic marker. It is possible that expression of this protein is altered during regeneration and that other markers would be preferable to assess nascent sprouts. In the past, we observed co-localization between PGP 9.5 and a membrane-bound axonal marker, Gα0, suggesting that this was not the case. In the present study, we also evaluated staining against GAP43, a known regenerative marker. Dermal axons were strongly GAP43/PGP9.5 positive while epidermal nerve fibres were visualized primarily with PGP9.5, which is consistent with PGP9.5 being an appropriate marker for regeneration studies.

Studies have shown that diabetes contributes to impaired axon sprouting (Bisby, 1980) and elongation (Ekstrom and Tomlinson, 1989) in experimental rat models. Schwann cells are essential for guiding regenerating axons to the denervated targets and proliferating Schwann cells within the basal lamina of axons and are regulated by nerve growth factors (Anton et al., 1994; Terenghi, 1999; Bentley and Lee, 2000; Hoke et al., 2002; Griffin, 2006; Song et al., 2006; Taniguchi et al., 2007).

In rodent models of diabetes, there are deficits in nerve growth factor expression (Tomlinson et al., 1996, 1997) and under experimental conditions, high glucose levels are implicated in neuritic outgrowth deficits (Tomlinson et al., 1996, 1997; Tosaki et al., 2008). The reduced regenerative and collateral sprouting that we observed in diabetes mellitus may be attributable to deficits in nerve growth factor production. Alternatively, or in
addition, diabetes mellitus may lead to a cascade of events that result in an inhospitable environment for axonal outgrowth to occur in. Advanced glycation end product residue content of endoneurial extracellular matrix proteins increases markedly in STZ-induced diabetes, and glycation of extracellular matrix proteins causes loss of charge and structural distortion of extracellular matrix (Duran-Jimenez et al., 2009). Glycation of laminin and fibronectin causes a reduction in neurotrophin-stimulated neurite outgrowth (Charonis and Tislbary, 1992; Thornalley, 2002). Studies also suggest that hypoxia causes injury of Schwann cells resulting in a significant decrease in Schwann cell survival (Zhu et al., 2010). The slow proliferation and migration of Schwann cells, and subsequently axons, through the dermal collagen in our study suggests that the surrounding extracellular matrix was relatively hostile and retarded axonal regrowth.

Vascular regrowth after injury is critical for re-establishment of metabolic support as well as production and delivery of growth factors. Vascular growth factors have been implicated in neuronal patterning, migration and neural repair (Hobson et al., 2000; Bates et al., 2003; Schwarz et al., 2004; Yu et al., 2008) and therefore may be well suited as a treatment target. We observed, as have others, that blood vessel and nerve growth recapitulated the pattern seen in ontogeny (Tesfaye et al., 1993; Carmeliet and Tessier-Lavigne, 2005; Eichmann et al., 2005). Subjects with diabetes mellitus exhibited slow proliferation and migration of endothelial cells in the dermal skin possibly attributable to extracellular matrix alterations, which supports previous observations that in diabetes, the endothelial progenitor cells are functionally deficient in their ability to respond to stresses (Tepper et al., 2002; Sigaudo-Roussel et al., 2004; Capla et al., 2007).

Our study indicates that regenerative deficits are a common theme in subjects with diabetes, extend beyond deficits in axonal regrowth and may underlie the development of neuropathy. The local environment in diabetic subjects appears to be deleterious to the growth, maturation, migration of both neuronal (axons and Schwann cells) and vascular structures. Vascular risk factors such as hypertension, elevated cholesterol and smoking have all been associated with the development of peripheral neuropathy in diabetes (Tesfaye and Selvarajah, 2005). Furthermore, axonal and vascular growth ligands, the semaphorins and vascular endothelial growth factors, are known to share a common receptor in neuropilin-1 (Gu et al., 2003). It is possible that diabetes mellitus leads to a cascade of events whereby advance glycation end product formation results in altered neuropilin-1 expression (Bondeva et al., 2009), which in turn mitigates semaphorin and vascular endothelial growth factor activity resulting in slowed axonal and vascular outgrowth.

Vascular endothelial growth factor-A is a well-known angiogenic factor, now considered to have neurotrophic, neuroprotective and neurogenesis functions (Sondell et al., 1999; Sondell et al., 2000; Jin et al., 2002; Gu et al., 2003; Schwarz et al., 2004; Gora-Kuplas and Josko, 2005; Brockington et al., 2006). Preliminary studies have shown that plasmid-mediated delivery of vascular endothelial growth factor-A can reverse neuropathy in diabetic animals and has been shown to improve diabetic neuropathy symptoms in a clinical trial (Schatzberger et al., 2001; Ropper et al., 2009). Our results suggest that such a strategy may be attractive as it may address the initial abnormality in neurovascular repair that appears to be central to diabetic polyneuropathy. Our observations might also have implications for the design of cancer chemotherapy regimens since bevacizumab (AvastinTM) is increasingly used for treatment of subjects with metastatic cancer of the colon or rectum, vestibular schwannomas and glioblastoma multiforme. To our knowledge, no studies have been conducted to evaluate peripheral nerve function in bevacizumab recipients.

This study demonstrates slowed neuronal and vascular growth in subjects with diabetes compared with age-matched controls following standardized injury models. This finding is consistent with alterations in the extracellular matrix and/or alterations in the shared molecular signals responsible for axonal and vascular growth playing a central role in the development of diabetic polyneuropathy. We suggest that the methods developed here are well suited to study vascular and axonal growth in regenerative treatment trials and to dissect mechanisms influencing vascular and nerve growth.

Acknowledgements

The authors thank Erin Burke Voelkel and Scott Sultzberger for expert technical assistance and Dr Jack Griffin for helpful discussions.

Funding

Juvenile Diabetes Research Foundation (to M.P., G.J.E., R.O.); 1P30MH075673 (to J.C.M.); UL1RR025005 (CRC to J.H.U.).

Supplementary material

Supplementary material is available at Brain online.

References


