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The Involvement Of Effector Cells In Diabetic Neuropathy In Drug-Induced Diabetic Rats

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ABSTRACT

Changes in neuronal morphology have been shown to occur in many pathological conditions involving the nervous system. The effector cells of the monocyte/macrophage system have been involved in myelin removal during nerve degeneration. The present study was aimed at investigating the involvement of the effector cells in diabetic neuropathy of peripheral nerves in drug-induced diabetic rats. Albino (Wistar) rats used in the study was administered with diabetogenic dose of alloxan and were separated into 3 groups (A, B and C). Group A was the control while groups B and C were used as the test groups. Parts of the peripheral nerves were transacted and prepared for microscopy. The result from the test groups revealed morphological changes; myelin sheaths were collapsed, axonal structures degenerated and numerous infiltrated cells of the monocyte/macrophage system. The number of axons and myelin density were significantly decreased ($p<0.05$) while the number of Schwann cell nuclei and infiltrated macrophages were significantly increased ($p<0.05$). the observation suggests that the axonal breakdown play important role in the recruitment of effector cells of the macrophage system in the degenerating nerves in diabetic neuropathy of rats.

Keywords: Effector cells, Neuropathy, Diabetic rats.

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia (Danbauchi, 2000). Diabetes can be responsible for disorders of the nervous system because of treatment with insulin leading to hypoglycemia, sometimes because of vascular changes and sometimes because of metabolic disturbance in the peripheral and autonomic nervous system. (Bloom and Ireland, 1980; Kumar and Clark, 1992).

It has been shown that segmental demyelination is the fundamental histological changes in diabetic neuropathy (Edwards, et al, 1995). In the early stages of neuropathy, the axons remain interact, allowing demyelination, and recovery while, in more advanced cases, the axons fragment and remyelination ceases (Bloom and Ireland, 1980; Williams et al; 1995). The combination of neuropathy; ischemia and infection leads to chronic indolent sepsis involving the kin, the tissues and the bones (Bloom and Ireland 1980; Edwards, et al. 1995). Lesions of the peripheral nerves can give rise to weakness, wasting, pain and sensory ,loss appropriate to the nerves involved (Weetman, 1991; Persson and Hanson, 1996; Nakamura et al, 2001, donnini, et al., 2000).

Relative changes in neuronal morphology have been shown to occur in many pathological conditions involving the nervous systems (Friede and Bruck, 1993; Bruck, et al, 1994). These structural changes are often followed by alterations in the morphometry of the nerves. The present study was aimed at investigating the role played by the effector cells in diabetic neuropathy of peripheral nerves in drug-induced diabetic rats.

MATERIALS AND METHODS

Thirty adult male albino (Wistar) rats were separated into three groups (A,B and C). group A was used as the primary control group while groups B and C was the test groups and as such was administered with diabetogenic dose of Alloxan and was confirmed to be diabetic according to Barnes and Eltherington, (1964); Barbato and Landau; (1977); Bowman and Rand, (1985); Greenspan and Baxter, (1994). The animals in group B were the first test group and were used to study the changes that occur in diabetic neuropathy while the second test Group (C) was used to study the influence of insulin

treatment in the resuscitation of these changes in the nerves.

Nerve samples from part of sciatic and sural nerves, about 3-5mm in length were exercised. Some were fixed in neutral formol saline, embedded in paraffin and sectioned, while some were freshly prepared using Cryostat method. Sections were made between 6-8 and 10-20 microns thick for paraffin and frozen sections respectively. The tissues were stained using Haematoxylin and Eosin (H & E); Periodic Acid Schiff (PAS) and Luxol Fast Blue (LFB) methods. The number ofaxons, myelin density, macrophages and Schwann cell nuclei per square millimeter of nerve .tissue was determined according to the methods of Friede and Bruck, (1993) and Bruck, et al; (1995). Macrophages were recognized as phagocytic cells with pseudopodia containing myelin debris and lipid vacuoles intheir cytoplasm while Schwann cells in the nerves were identified by their encompassing basement membrane.

Morphometry And Statistical Analysis

Morphometric analysis was done using a Leitz Dialux microscope at the magnification of 450 with the help of both stage and eyepiece micrometers. The myelin density, the number of axons, macrophages and Schwann cell nuclei were determined as pooled measurements for each group and expressed as mean and standard deviation (SD). The students' t-test was used for testing the level of difference. A, P-value less than 0.05 was considered significant. One-way Analysis of Variance (ANOVA) was used for comparing the means while the Multiple Range Test was used to find the Least Significant Difference (LSP) between the groups.

RESULTS

The result showed morphological changes resembling that seen in Wallerian degeneration. The peripheral nerves in drug-induced diabetic rats showed decreased number of axons in the experimental groups with much decreased in group B as shown in Fig. 1. The rate of decrease was statistically significant ($p<0.05$) between the groups. the myelin density as determined by point sampling showed a statistically significant decrease ($p<0.05$) between the control and the test groups as shown in fig. 2. there was a significant increase in the number of macrophages in the nerves of the test groups when compared with the control as shown in Fig. 3 ($p<0.05$). The number of Schwann cell nuclei per square millimeter was increased indicating proliferation of these cells as shown in fig. 4. The increase was statistically significant between the control group and the test groups ($p<0.05$).

DISCUSSION

Structural changes in peripheral nerves have often been shown to be followed by changes in their morphometry. The changes observed in the peripheral nerves in drug-induced diabetic rats resemble those seen in many pathological conditions involving the nervous system. Examples of which are Wallerian degeneration and Multiple Sclerosis (Bruck, et al; 1996).

The axons were decreased in the experimental groups, which may be due to the axonal disintegration and degeneration of microfilaments. Bloom and Ireland (1989) have shown that in early stages of diabetes mellitus, the axons remain intact and this is due to the remyeliantion and recovery, while in more advanced stages, axons fragment and rate of demyelination increased and remyeliantion ceases (Yki-Jarvinen, 1996).

This may explain the significant decrease in myelin density in the experimental groups with that of group B animals more pronounced than group C animals. This is because, chronic hyperglycemia alters metabolism in peripheral nerves due to the accumulation of sorbital and the diversion of glucose in the sorbital fructose pathway (Bloom and Ireland, 1980; Edwards, et al; 1995). It has been shown that, apart from the metabolic factors vascular anomalies also play a part (Donnini 2000). This is because thrombotic lesions have been demonstrated in the small intraneural and perineural blood vessels and changes in platelet behaviour (Bloom and Ireland, 1980; Farkas, et al 2000).

The results suggest that axonal breakdown plays an important role in the

initiation of macrophage recruitment in degenerating peripheral nerves and insulin possesses therapeutic value for the treatment of diabetic neuropathy.

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