

The comparative effects of metformin, pioglitazone and rosiglitazone on the sciatic nerve of alloxan-induced diabetic male rats



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ABSTRACT

Diabetes mellitus is the most common metabolic disease with neuropathy as its most common complication. In the present study, the effects of oral hypoglycaemic drugs (metformin, pioglitazone and rosiglitazone) on the morphology of the sciatic nerve were investigated. Forty male Wistar rats (140 g) divided into 5 groups control, diabetic, and 3 experimental groups (n=8) were used for the study. The 3 experimental groups were rendered diabetic by intraperitoneal injection of alloxan (150 mg/kg body weight) and subsequently treated with metformin (150 mg/kg/d), pioglitazone (3 mg/kg/d) and rosiglitazone (10 mg/kg/d) respectively. At 28 days of treatment, sciatic nerve morphology was studied by the Bielschowsky's Silver Nitrate (BSN) and Luxol Fast Blue (LFB) techniques. Blood glucose levels were monitored and recorded throughout the experiment. In the diabetic rats with oral hypoglycaemic interventions, blood glucose was not significantly different ($P>0.05$) from the control at 28 days of treatment. The body weight of Rosiglitazone-treated rats showed significant increase when compared with the control and other oral hypoglycaemic drug-treated rats. The axon and myelin fibers showed relatively strong affinity for BSN and LFB in the control and oral hypoglycaemic drug-treated diabetic rats contrary to the weak affinity for the stains in the untreated diabetic rats. These results suggest that oral hypoglycaemic drugs exerted positive effects on the treatment and improvement of sciatic nerve morphology of alloxan-induced diabetic rats.

Introduction

One of the several complications of diabetes mellitus is neuropathy, which could involve both the peripheral

and central nerve tissues [1]. Diabetic neuropathy is microvascular complication which leads to distressing neurological complaints and ultimately diabetic foot [2].

Recent studies are unraveling the structural changes characteristic of the peripheral nerve in chronic diabetic patients and experimental animals. Most characteristic findings of the peripheral nervous system in diabetic patients

are distal and sensory predominant nerve fibre degeneration, axonal loss and endoneurial microangiopathy [3, 4].

However, it has been shown that with optimal glycaemic control, diabetic nerve lesions could be prevented or ameliorated. In the study by Kazuhiro treatment of streptozotocin-induced (STZ-induced) diabetic rats with low dose insulin ameliorated peripheral nerve dysfunction in rats. In the present work, we studied the effect of induced diabetes on the morphology of the sciatic nerve. We also tested the hypothesis that the treatment of rats with hypoglycaemic drugs is protective against peripheral nerve lesions.

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KEYWORDS

- Metformin
- Pioglitazone
- Rosiglitazone
- Sciatic nerve
- Diabetes

Materials and Methods**■ Animals**

Forty male Wistar rats (140 g) were bred and maintained on rodent chow from Bendel feed (Ewu, Nigeria) in the animal house of the Department of Anatomy, University of Ilorin. All animals were maintained in an environment of 12 hour-light and 12 hour dark cycle, at a room temperature between 23°C and 25°C. Special care was provided to minimize animal suffering. Animals were acclimatized to laboratory conditions before the tests for a period of two weeks. The animals were divided into five groups of eight rats each.

■ Induction of experimental diabetes

Diabetes was induced in each fasted rat by administering alloxan monohydrate (150 mg/kg body weight; intraperitoneal) in normal saline. Blood glucose levels of the rats were determined using a glucometer (Roche Diagnostic, Germany) and glucometer strips. Blood samples were obtained from cut-tail tip of conscious rats. Only rats with established hyperglycaemia (blood glucose >300 mg/dL) were treated subsequently.

■ Experimental procedure

The control group was administered normal saline intraperitoneally throughout the experiment, while untreated diabetic group received only distilled water for 28 days. Three oral hypoglycaemic drugs (metformin, rosiglitazone and pioglitazone) were used in the present study. Each drug was administered orally to a group of hyperglycaemic rats (n=8) at 7:00–9:00 each day for 28 days. Metformin (Merck, Germany) was administered at 150 mg/kg body weight/day [5]; rosiglitazone (GlaxosmithKline, USA) at 3 mg/kg body weight/day [6], and pioglitazone (Sun, India) at 10 mg/kg body weight/day [7]. All animals had their body weights and blood glucose measured, and recorded using weighing balance (Scout Pro, Ohaus Corp, USA) and a one touch glucometer. At 28 days of treatment with oral hypoglycaemic drugs, all rats were anaesthetized with ether (Sigma, MO), 24 hours after the last dose of the drugs.

■ Histological processing

The rats were sacrificed and the sciatic nerves were taken and fixed in formaldehyde solution.

The fixed tissues were dehydrated and embedded in paraffin wax. Eight micrometer-thick sections were cut on a rotary microtome. Sections were either stained by Bielschowsky's Silver Nitrate (BSN) or Luxol Fast Blue (LFB) technique [8]. The resulting sections were examined by light microscopy.

■ Statistical analysis

Data collected on blood glucose were analyzed and presented as mean \pm standard error of mean (mean \pm SEM). Means were compared by analysis of variance; followed by the Bonferroni post-hoc test. $P < 0.05$ was accepted as significant.

Results**■ Histologic findings**

In the non-treated diabetic rats, the sciatic nerve fibres showed poorly stained axons, as demonstrated by the BSN technique (FIGURE 1). However, in the non-diabetic control and hypoglycaemic drug-treated rats, the sciatic nerve sections showed well stained myelinated axons. Similarly, LFB technique showed poorly stained myelin fibres in the sciatic nerve of the non-treated diabetic rats (FIGURE 2). However, myelination was preserved in the non-diabetic control and hypoglycaemic drug-treated diabetic rats (FIGURE 2).

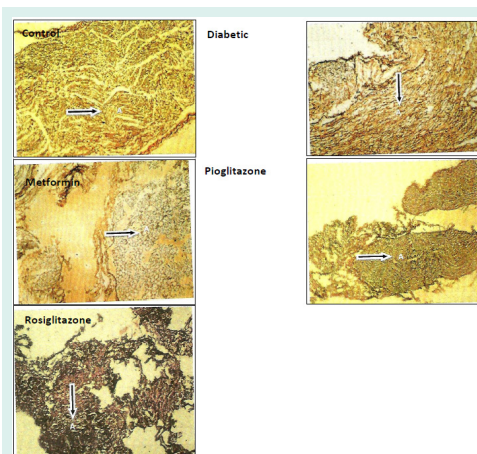


FIGURE 1. Photomicrographs of sections in the sciatic nerve of the control, diabetic, metformin-treated, pioglitazone-treated and rosiglitazone-treated rats. Axonal loss is observable in the diabetic group (arrow), in contrast to the control and oral hypoglycaemic-treated groups. Bielschowsky's Silver Nitrate (Magnification, x200).

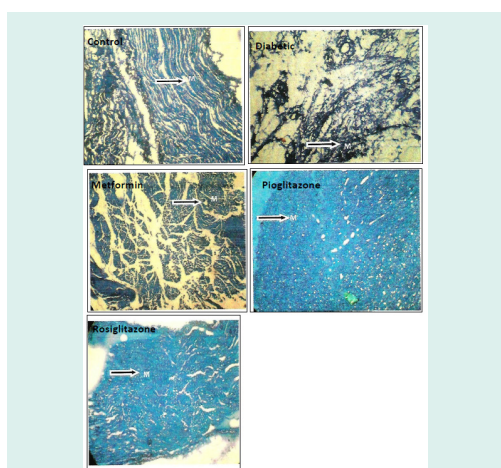


FIGURE 2. Photomicrographs of sections in the sciatic nerve of the control, diabetic, metformin-treated, pioglitazone-treated and rosiglitazone-treated rats. Demyelination is observable in the diabetic group (arrow), in contrast to the control and oral hypoglycaemic-treated groups. Luxol Fast Blue (Magnification, x200).

Metabolic parameters

The glycaemic responses to oral hypoglycaemic drugs in alloxan-induced diabetic rats are shown in the Table. After 4 weeks of treatment, no statistically significant rise in blood glucose levels occurred in all the groups compared to the control rats ($P > 0.05$), except in the non-treated diabetic rats, where significant rise in blood glucose levels occurred ($P < 0.05$). All non-treated diabetic rats showed weight gain disorder in the 4th week after Alloxan injection. As it has been shown, weight of all non-treated diabetic animals had significantly decreased in the 4th week compared to the control group. Treatment of diabetic neuropathy with rosiglitazone significantly affected weight gain of diabetic rats in comparison with diabetic and vehicle groups at 28 days of treatment (TABLE 1).

TABLE 1: Effect of oral hypoglycaemic drugs on blood glucose levels of alloxan-induced diabetic rats (mg/dl).

	n	Before Treatment		After Treatment	
		Blood glucose	Body weight	Blood glucose	Body weight
Control	8	110 ± 5	131 ± 2	125 ± 2	160 ± 1
Non-treated diabetic	8	480 ± 20a	168 ± 1	410 ± 26a	152 ± 2
Diabetic + metformin	8	380 ± 16a	160 ± 10	150 ± 10b	150 ± 2
Diabetic + pioglitazone	8	400 ± 16a	187 ± 3	180 ± 4	187 ± 5
Diabetic + rosiglitazone	8	410 ± 17a	168 ± 2	135 ± 6b	229 ± 2

Note: Mean ± SEM (mg/dl); a = $P < 0.05$ compared with control; b = $P < 0.05$ compared with non-treated diabetic.

Discussion

The study demonstrated that experimental diabetes resulted in nerve fibre degeneration and axonal loss. Hyperglycaemia and Hypoglycaemia are both risk factors of neurostructural impairment of diabetes mellitus. Diabetes produces a wide variety of pathological changes in peripheral nerves in human diabetic neuropathy [9].

Previous study on mature rats confirms the relative lack of morphological changes in experimental diabetic neuropathy [9].

In the present work, we studied the sciatic nerve morphology in alloxan-induced diabetic rats with and without treatment with oral hypoglycaemic drugs. Histologic study of the sciatic nerve of the non-treated diabetic rats showed structural impairment characterized by the loss of axons and myelin fibres. Therefore, the present

morphologic findings from animal studies are experimental evidence of the structural changes of the sciatic nerve in diabetic conditions. Although, neurobehavioral and motor tests were not performed in the present animal study, the observed morphologic impairment could provoke functional deficits of the sciatic nerve in the non-treated diabetic rats. The presence of abnormal fibres in sciatic nerve with axonal degeneration and myelin breakdown is one of the symptoms of streptozotocin-induced diabetic rats [10]. The latter agrees with our findings in the non-treated diabetic rats. It is possible that with a longer duration, the morphological changes in this animal model may lead to the more prominent changes that are observed in established cases of human diabetic neuropathy.

The treatment of the alloxan-induced diabetic rats with the oral hypoglycaemic drugs (metformin, pioglitazone and rosiglitazone)

protected the animals from nerve abnormalities observed in the non-treated diabetic rats. This neuroprotective effect of oral hypoglycaemic drugs was possibly the result of optimum glycaemic control [1]. Diabetes is associated with weight loss due to poor utilization of consumed food substances [11]. On the contrary, oral hypoglycaemic drug-treated animals appreciate in body weight. The results obtained in this research work showed no significant difference in the blood glucose levels of the non-diabetic control and oral hypoglycaemic drug-treated rats at 28 days. However, the blood glucose levels of the hypoglycaemic drug-treated rats were lower than the non-treated diabetic group. This suggests that optimum glycaemic control protects against nerve abnormalities in diabetic

rats. Moreover, in human studies, meticulous glycaemic control could delay the onset or slow the progression of diabetic neuropathy in diabetic subjects [12,13]. This agrees with our histologic findings in the present animal study, and underscores the importance of optimum glycaemic control to nerve morphology and functions in the hyperglycaemic condition.

Conclusion

Data from the present animal study shows that nerve abnormalities are induced by untreated diabetes. Metformin, pioglitazone and rosiglitazone are capable of slowing down the deleterious effect of diabetes on body weight and the sciatic nerve.

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