Neuroprotective Effect of Early Treatment with Pioglitazone and Pyridoxine Hydrochloride in Alloxan Induced Diabetes in Rats

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Summary

Neuropathic pain in diabetics is characterized by both hyperalgesia and allodynia. This is attributed to both uncontrolled glycemia and the further complications which it leads to. We evaluated the effect of concurrent administration of Pioglitazone along with pyridoxine hydrochloride in diabetic rats. The parameters used were hyperalgesia produced by tail immersion (hot water), hot plate method and allodynia produced by tail immersion in cold water. A gradual reduction in body weight and locomotor activity along with a lowered pain latency was observed in alloxan treated animals. No change in pain latency was observed in pioglitazone and pyridoxine treated animals compared to control group of animals indicating that the early treatment with pioglitazone and pyridoxine could protect the animal from early hyperalgesia and allodynia produced by alloxan induced diabetes in rats.

Keywords: Pioglitazone, pyridoxine, Alloxan induced diabetic neuropathy, rats.

Shortened Tittle: Pioglitasone and pyridoxine in alloxan induced diabetic neuropathy….

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Introduction

Diabetes mellitus is associated with several long term complications, prominent among them is peripheral neuropathy. Spontaneous pain, allodynia and hyperalgesia are frequently encountered in diabetic patients **[1,2,3,4,5]**. A review of published work on painful diabetic neuropathy suggests that a significant degree of neuropathic pain is more likely to occur in patients with uncontrolled diabetes and has been proposed that acute biochemical alterations in neural tissues might result from prolonged hyperglycemia and could contribute to the development of diabetic neuropathy **[1,6]**. Four main molecular mechanisms, namely increased polyol pathway flux, increased advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC) isoforms and increased hexosamine pathway flux have been implicated in glucose-mediated vascular damage and all seems to reflect a single hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain **[7]**. Clinical and experimental studies have revealed that reactive oxidant species (ROS) play a significant role in pathophysiology of neuropathic pain in diabetes **[2]**. Adequate metabolic control may reduce the symptoms of painful diabetic neuropathy **[6]**. Pioglitazone, a member of thiazolidinedione (TZD) family sensitizes peripheral tissues to insulin thereby ameliorating hyperglycemia and hyperinsulinaemia **[8]**. It has been reported that diets deficient in Pyridoxine (Vitamin B6) could cause peripheral neuropathy **[9]**. Vitamin B6 supplementation is recommended for patients suffering from Carpel tunnel syndrome **[10]**. Also in a study it was shown that Vitamin B6 (33mg/kg and 100 mg/kg) was shown to reduce thermal hyperalgesia in a model of mononeuropathy caused by loose ligation of sciatic nerve **[11]**. The objective of this study was to evaluate the effect of early treatment with the coadministration of pioglitazone with pyridoxine hydrochloride in prevention of neuropathic complications in alloxan induced diabetic rats.

Materials and Methods

Animals: Adult male Wistar rats (200-250g) were obtained from National Toxicology Centre, Pune, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 hr light : dark cycle was followed. All animals had free access to water and standard pellet laboratory animal diet. Animals were acclimatized to laboratory conditions before the experiments which were carried out between 0900 and 1700 hr. All the experimental protocols were reviewed and carried out in accordance with that described by Zimmerman et al **[12]** and approved by Institutional Animal Ethics Committee for Poona College of Pharmacy, Pune, India.

Preparation of the drug solution

Pyridoxine hydrochloride:- Solution was prepared by dissolving pyridoxine hydrochloride (Himedia) in distilled water. The drug solution was stored in air tight bottles at room temperature. The solutions were freshly prepared for daily administration.

Pioglitasone: Solution was prepared by dissolving pioglitasone (Alembic) in distilled water. The drug was prepared daily.

Alloxan treatment: Following a 48 hr fast, the animals received intraperitoneal (i.p) injections of alloxan monohydrate (120mg/kg) dissolved in 0.9% sodium chloride. Control animals received i.p injections of 0.9% sodium chloride. Within 48 hrs following alloxan administration, blood glucose concentrations were estimated by enzymatic GOD-POD (glucose oxidase-peroxidase) diagnostic kit method (Accurex) **[13, 14]**. The rats having serum glucose levels more than 250mg/dl were selected and used for the present study. After the induction of diabetes in the animals diabetic rats were randomly selected into three groups of 8 animals each. The groups were labeled as control (Group 1) wherein the animals were non-diabetic, diabetic untreated (DU, Group 2). The DU animals were administered with distilled water daily and diabetic animals treated with

coadministration of pioglitazone (10mg/kg,O.D) with pyridoxine hydrochloride (100mg/kg, O.D) (DPP, Group 3) treated. Treatment begain from the day of BSL detection after the alloxan treatment. Body weight was cheaked daily and serum glucose level were measured on 48 hr post alloxan treatment, 15th and 30th day of study.

Assessment of thermal hyperalgesia and cold allodynia [2]

Tail immersion (warm water) test: Tail of rat was immersed in warm water (47±1°C) bath until tail withdrawal (flicking response) or signs of struggle were observed (cut-off time 15 sec).

Tail-immersion (cold water) test: The procedure was same as warm water test but the temperature of water was set at 10±0.5°C, a temperature that is normally innocuous. The cut-off time was 15 sec.

Hot plate test: In this test, animals were individually placed on a hot plate (Ugo Basile hot plate, Versace Italy) with the temperature adjusted to 55±1°C. The latency to the first sign of paw licking or jump response to avoid heat pain was taken as an index of pain threshold. The cut-off time was kept 10 sec so as to avoid damage to the paw. Both hyperalgesia and allodynia were assessed weekly till the end of the study.

Statistical Analysis

Data was expressed as mean ± SEM of animals in each group. To determine the statistical significance, ANOVA followed by Tukey-Kramer test (Instat/ Graphpad) was used. Differences between means were considered statistically significant if $p < 0.001$.

Results

Effect of DPP on blood sugar level (BSL) in alloxan induced diabetes in rats.

Blood sugar levels of control group of animals did not alter throughout the experiment. In DU group blood glucose levels increased steadily on day 15th (346.19±6.86 mg/dl) and day 30th (384.81±4.91 mg/dl) after alloxan treatment indicating the incidence of diabetes in the animals throughout the experiment. There was no significant difference in BSL of DPP treated animals (278.72±6.11 mg/dl) 48 hrs post alloxan treatment as compared to DU group of animals (269.4±8.16 mg/dl) indicating the incidence of diabetes. But a gradual reduction in the BSL of DPP group was observed on day 15th (121.16$^{\#}$±5.66 mg/dl) and day 30th (113.64$^{\#}$±5.16 mg/dl) indicating glycemic controls by drug treatment.

Table 1. Effect of DPP on blood sugar levels (BSL) in alloxan induced diabetes in rats.

	Blood sugar level in mg/dl			
	Pre-Alloxan treated	***48 hr post alloxan treated***	***Day 15***	***Day 30***
Control	97.62 ±5.35	106.64±6.16	102.59±4.8	104.23±3.84
DU	94.51±6.21	269.4±8.16	346.19±6.86	384.81±4.91
DPP	99.2±3.68	278.72±6.11	121.16$^{\#}$±5.66	113.64$^{\#}$±5.16

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test.. p <0.001 = *. Data of DPP was compared with that of DU. n=8.

Effect of DPP on hyperalgesia produced by tail immersion (hot water) in alloxan induced diabetes in rats.

There was no change in tail flick latency (sec) observed in control group of animals throughout the experiment. A gradual decline in the latency was observed in DU group of animals from day 7th (8.1±0.26 s) onwards which was observed minimum on day 28th (4.1±0.76 s), indicating the presence of neuropathic pain due to diabetes. DPP group of animlas recoeded a reduction in latency on day 7th (10.6±0.34 s) which was followed by increase in pain threshold time on subsequent days, indicating absence of algesia produced by tail immersion in hot water.

Table 2. Effect of DPP on hyperalgesia produced by tail immersion (hot water) in alloxan induced diabetes in rats.

	Tail Flick latency in seconds				
	Pre-Alloxan treated	***Day 7***	***Day 14***	***Day 21***	***Day 28***
Normal	11.6 ±0.7	12.4 ±0.61	10.81±0.42	12.0 ±0.91	11.21±0.41
DU	10.9±0.51	8.1±0.26	6.1±0.82	5.3± 0.81	4.1±0.76
DPP	11.2±0.45	10.6±0.34	11.5$^{\#}$±0.94	11.4$^{\#}$±0.64	11.9$^{\#}$±0.31

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test.. p <0.001 = *. Data of DPP was compared with that of DU. n=8.

Effect of DPP on allodynia produced by tail immersion (cold water) in alloxan induced diabetes in rats.

No significant change in latency was observed in control group of animals throughout the study. For DU group of animals there was a gradual reduction in latency (sec) observed from day 7^{th} (10.2±0.49 s) till day 28^{th} (4.1±0.8 s) indicating the presence of allodynia. In the DPP group of animals, no significant lowering in pain latency was exhibited which implies the protective action of drug treatment on allodynia produced by cold water.

Table 3. Effect of DPP on allodynia produced by tail immersion (cold water) in alloxan induced diabetes in rats.

	Tail Flick latency in seconds				
	Pre-Alloxan treated	***Day 7***	***Day 14***	***Day 21***	***Day 28***
Normal	13.6 ±0.41	14.1±0.21	13.2±0.6	13.9±0.36	13.0±0.7
DU	14.0±0.6	10.2±0.49	7.4±0.51	5.5± 0.94	4.1±0.8
DPP	13.4±0.48	12.6±0.26	$13.9^{\#}$±0.19	$12.9^{\#}$±0.31	$13.7^{\#}$±0.16

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test.. p <0.001 = *. Data of DPP was compared with that of DU. n=8.

Effect of DPP on thermal hyperalgesia (hot plate) in alloxan induced diabetes in rats.

No significant change in latency was observed in control group of animals throughout the study. For DU group of animals there was a gradual reduction in latency (sec) observed from day 7th (8.0±0.44 s) till day 28th (3.8±0.19 s) where the pain was observed to be maximum, indicating the presence of algesia by heat. In the drug treated group of animals, no significant lowering in pain latency was exhibited which implies the protective action of DPP on hyperalgesia produced in diabetic animals.

Table 4. Effect of DPP on thermal hyperalgesia (hot plate) in alloxan induced diabetes in rats.

	latency in seconds				
	Pre-Alloxan treated	***Day 7***	***Day 14***	***Day 21***	***Day 28***
Normal	9.8 ±0.4	9.4 ±0.16	9.7±0.34	9.7 ±0.1	9.1±0.84
DU	9.4±0.64	8.0±0.44	6.4±0.52	5.0± 0.32	3.8±0.19
DPP	9.2±0.81	8.4±0.6	9.0$^{\#}$±0.28	9.7$^{\#}$±0.14	9.5$^{\#}$±0.51

Data expressed in Mean ± S.E.M. ANOVA followed by Tukey test.. p <0.001 = *. Data of DPP was compared with that of DU. n=8.

Discussion

A strong relationship exixts between glycemia and diabetic microvascular complications in both type 1 and type 2 diabetes **[7]**. Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy **[2]**. Early in the course of diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability.

Quantative and qualitative abnormalities of extracellular matrix contribute to an irreversible increase in vascular permeability. With time microvascular cell loss occurs in part as a result of programmed cell death. Hyperglycemia may also decrease production of trophic factors for endothelial and neuronal cells. Together, these changes lead to odema, ischaemia and hypoxia induced neovascularization in the retina, proteinurea, messengial matrix expansion, glomerulosclerosis in the kidney and multifocal axonal degeneration in peripheral nerves **[7]**. Impaired blood flow also seems to contribute to noxious stimulus hypersensitivity. Oxidative stress related reduction in perfusion is thought to play a part in cardiac autonomic dysfunction and also in small fiber sensory neuropathy **[2]**.

Alloxan and the products of its reduction, dialuric acid, establish a redox cycle with formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxidant species (ROS) with a simultaneous massive increase in cytosolic calcium concentration cause rapid Beta cell destruction **[15]**. Early pharmaceutical intervention against the long-term consequences of hyperglycemia-induced cross-linking prevents the development of severe late complications of diabetes. Pioglitasone, a PPAR gamma receptor agonist has been approved by FDA for lowering blood sugar in type 2 diabetes. Also studies indicate that pioglitasone is a powerful inhibitor of glycation, AGE formation and cross-linking **[8]**. Pyridoxine has established therapeutic uses in the treatment of neuropathy produced by antituberculosis drugs, alcohol induced neuropathy and pellagra syndromes. Studies have shown that diets totally deficient with pyridoxine would result in peripheral neuropathy **[9]** but if overdose of pyridoxine is given might result in neuropathy induced by pyridoxine toxicity **[10]**. Wang et al (2005) **[11]** reported that Pyridoxine (33mg/kg and 100mg/kg) inhibits thermal hyperalgesia in another model of mononeuropathy caused by chronic constriction injury(CCI).

It was observed that alloxan treated rats (DU) exhibited a marked increase in glycemia **[Table 1]** and water intake and a clear cut reduction in the progressive gain in body weight as compared to control and drug treated group (DPP). In addition, polydipsia, polyphagia, polyurea and reduced motility was also observed in DU group of animals.

These results were in accordance with Aubel et al (2004). Both hyperalgesia and allodynia were established after 14 days of alloxan treatment which was observed behaviorally **[Table 2,3,4]**. The hyperalgesic response in tail-withdrawal test is generally attributed to central mechanisms whereas the hyperalgesic response on hot plate is attributed to the combination of both central and peripheral mechanisms **[2]**

Our results indicate that in untreated group of animals (DU), there was no glycemic control, which led to hyperalgesia and allodynia in the models of tail immersion (hot and cold) and hot plate mothod. Whereas by a proper check on blood glucose levels by Pioglitazone 10 mg/kg, O.D alongwith the supplementation of a vitamin, pyridoxine hydrochloride 100 mg/kg, O.D protected the diabetic animals from algesia produced by noxious and non-noxious stimuli.

We conclude that the onset of neuropathic complications could be prevented by early glycemic controls by pioglitazone and concurrent administration of Pyridoxine in diabetic rats.

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