PROTECTIVE EFFECT OF NATEGLINIDE ALONE AND ITS COMBINATION WITH PIOGLITAZONE ON LIVER FUNCTIONS IN NONDIABETIC AND DIABETIC RATS

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Summary

In the present study effect of Nateglinide (NAT) alone and its combination with Pioglitazone (PIO) was investigated in streptozotocin- nicotinamide induced diabetic and associated hepatic dysfunctioning in rats. Nateglinide (30 mg/kg/day, p.o) alone and its combination with Pioglitazone (10 mg/kg/day, p.o) was administered for 28 days in rats injected with single dose of streptozotocin (65 mg/kg, i.p, STZ) and nicotinamide (110 mg/kg, i.p, NIC). STZ–NIC induced animals showed a significant ($p<0.001$) increased in the level of serum glucose, glycosylated heamoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (γGTP). Treatment with Nateglinide (30 mg/kg/day, p.o) alone and in combination with Pioglitazone (10 mg/kg/day, p.o) showed a significant alteration in all the serum markers towards normal. This study indicates that NAT alone may be better than NAT and PIO with combination in protecting hepatic functions in diabetic conditions.

Keywords: Pioglitazone, Nateglinide, Streptozotocin, Nicotinamide

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Introduction

Recent epidemiological studies suggested that patients with diabetes are twice as likely to suffer hepatic failure compared to patients who do not have diabetes. Increased incidences of hepatotoxicity have been observed in patients with diabetes receiving drug therapies. Neither the mechanisms nor the predisposing factors underlying hepatotoxicity in patients with diabetes are clearly understood (1). Type 2 diabetes (T2D) is a progressive disorder with a consistent and steady increase in glycosylated hemoglobin (HbA_1c) over time associated with enhanced risk of micro-complication (retinopathy, nephropathy and neuropathy) and macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease) and a substantial reduction in life expectancy. Liver disease complication is one of the most common causes of morbidity and mortality in diabetic patients. Liver disease may cause or contribute to, be coincident with, or occur as a result of diabetes mellitus (2).

Nateglinide is a novel, highly physiologic, mealtime glucose regulator recently approved for the treatment of type II diabetes mellitus. Nateglinide has a rapid onset and short duration of insulinotropic action that results in reduction of mealtime glucose rise and lowers the postabsorptive potential for hypoglycemia in humans and experimental animals (3-5). In contrast to sulfonylureas, Nateglinide increases pancreatic -cell sensitivity to ambient glucose without increasing basal insulin secretion (6).

PIO is a type of antidiabetic medicine known as a thiazolidinedione or glitazone. It helps to control blood sugar levels by increasing the sensitivity of liver, fat and muscle cells to insulin. This enables these cells to remove sugar from the blood more effectively. PIO also reduces the amount of glucose produced by the liver, and preserves the functioning of the cells in the pancreas (beta cells) that produce insulin. NAT and PIO are used for people with type 2 diabetes who do not use daily insulin injections. PIO hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. PIO showed dose dependant beneficial effects in many of the pathological conditions including reduction in blood glucose lowers blood pressure and restores endothelial function in animals (7). Troglitazone one of the drug from the PIO class, which was withdrawn from the U. S. market in 2000 because of his high incidence of hepatotoxicity (8).

Literature survey showed that, there was no report regarding the effect of Nateglinide (NAT) alone and its combination with Pioglitazone (PIO) on the hepatic function diabetic rats. Therefore the above study was designed to evaluate the effect of NAT alone and along with PIO on hepatic functions in STZ-NIC induced diabetic model in rats.

Materials and Method

Drugs And Chemicals

Pioglitazone hydrochloride and Nateglinide was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained form

SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of The M.S. University, Baroda. Sprague–Dawley rats (210±15 g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitium*.

Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg STZ, 15 min after the i.p administration of 110 mg/kg of NIC (9). After 7 days following STZ and NIC administration, blood was collected from tail vein and serum samples were analyzed for blood glucose. Animals showing fasting blood glucose higher than 300 mg/dl were considered as diabetic and were used for the study.

Experimental Protocol

Animals were divided in to following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks.

Group 1: Nondiabetic control, received CMC as vehicle (1 ml/kg/day, p.o, ND-CON).

Group 2: Nondiabetic group treated with NAT (30 mg/kg/day, p.o, ND-NAT).

Group 3: Nondiabetic group treated with PIO (10 mg/kg/day, p.o) and NAT (300 mg/kg/day, p.o, ND-NAT+PIO).

Group 4: Diabetic control, single injection of STZ (65 mg/kg, i.p) and NIC (110 mg/kg, i.p, DB-CON).

Group 5: Diabetic rats treated with NAT (30 mg/kg/day, DB-NAT).

Group 6: Diabetic rats treated with PIO (10 mg/kg/day, p.o) and NAT (30 mg/kg/day, p.o, DB- NAT+ PIO).

Biochemical Estimations

Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring no fasting serum glucose (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic (DB) state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Estimation of Serum Markers

On 4th weeks blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2300 rpm for 20 minutes to separate serum. Glucose, HbA1c, AST, ALT, ALP and γGTP were estimated from serum sample using standard Diagnostic Kit. In vitro quantitative determination of the activity of AST and ALT (SPAN Diagnostics Pvt., India) ALP, γGTP (Crest Biosystems, India) were done using enzymatic kit in serum.

Statistical Analysis

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $p < 0.05$.

Results

Characterization of Type 2 Diabetes.

As shown in table1, treatment with NAT (30 mg/kg) and combination with PIO (10 mg/kg, p.o) showed a significant (P<0.01) increase in body weight as compared to control non-diabetic (ND) rats and DB-CON rats. The levels of glucose and HbA1c was significant (P<0.001) decreased after treatment with NAT (30 mg/kg) alone and combination with PIO (10 mg/kg, p.o) as compared to DB-CON rats.

Table 1. Effect of Nateglinide (30 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, p.o) on changes in Body weight, serum glucose and HbA1c level in nondiabetic and diabetic rats.

Group	Body weight (gm)	Glucose (mg/dl)	Glycosylated heamoglobin (%, HbA1c)
ND-CON	248.3± 11.86	99.32±7.34	5.48±0.37
ND-NAT	224.0± 7.23	70.21±5.6	5.04±0.30
ND-NAT+PIO	310.2± 9.5$^{\$\$}$	65.42±8.68	3.10±0.55
D-CON	208.3± 8.92	400.9±8.52$^{\$\$\$}$	11.07±0.55$^{\$\$\$}$
D-NAT	219.8±10.27	170.7±13.31***	6.38±0.99***
D- NAT+PIO	261.03±7.64**	88.8±12.63***	4.98±0.33***

Values are expressed as mean ± SEM for six animals in the group. $^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, considered statistically significant as compared to ND-CON group. *P<0.05, ** P<0.001; ***P<0.001 considered statistically significant as compared to D-CON group.

Effect of PIO on serum marker enzymes

Table 2 showed a significant (P<0.001) increase in serum AST and ALT levels in STZ-NIC treated rats (DB-CON) as compared to ND-CON animals. Treatment with NAT (30 mg/kg) for 4 weeks, showed further decrease in serum AST and ALT level (P<0.01) as compared to DB-CON group alone. Whereas treatment with NAT (30 mg/kg) combination with PIO (10 mg/kg) for 4 weeks showed no significant changes in the serum levels AST and ALT level as compared to DB-CON group alone.

Administration of STZ-NIC alone significantly increases ALP (P<0.001) and γGTP (P<0.01) levels as compared to control rats. As shown in table 2, treatment with NAT (30 mg/kg, p.o) showed a significant (P<0.05) decrease in ALP and γGTP as compared to DB control rats. Whereas treatment with NAT (30 mg/kg) combination with PIO (10 mg/kg) for 4 weeks showed no significant changes in the serum levels ALP and γGTP level as compared to DB-CON group alone.

Table 2. Effect of Nateglinide (30 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day p.o) on changes in ALT, AST, ALP and γGTP level in nondiabetic and diabetic rats.

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	γGTP (IU/L)
ND-CON	35.41±3.27	104.5±5.65	134.9±7.08	74.91±4.15
ND-NAT	27.67±11.33	90.67±5.38	110.48±11.6	77.55±5.64
ND-PIO+NAT	55.83±7.94	110.0±8.64	150.3±8.11	114.80±8.21$^{\$}$
DB-CON	73.76±7.52$^{\$\$\$}$	160.3±6.12$^{\$\$\$}$	194.3±9.11$^{\$\$\$}$	107.7±4.05$^{\$\$}$
DB-NAT	50.09±5.13**	116.59±7.53**	149.9±6.87*	110.4±7.03*
DB-PIO+NAT	81.02±8.49	170.4±10.33	210.6±8.69	129.44±11.99

Values are expressed as mean ± SEM for six animals in the group. $^{\$}$P<0.05, $^{\$\$}$ <0.01, $^{\$\$\$}$P<0.001 considered statistically significant as compared to ND-CON group; * P<0.05, ** P<0.001, ***P<0.001 considered statistically significant as compared to D-CON group.

Discussion

The present study was undertaken with the objective of exploring the hepatic function of NAT (30 mg/kg) alone and its combination with PIO (10 mg/kg) in STZ-NIC induced diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Peroxisome proliferator-activated receptors are nuclear transcription factors that play a role in insulin sensitivity (10).

In STZ-NIC induced diabetes, the characteristic loss of body weight caused by an increase in muscle wasting (11). In the present study treatment with NAT (30 mg/kg) with PIO (10 mg/kg) showed significant increase in body weight which may be because of formation of oedema in the tissue. In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction

of diabetes mellitus. Significant decrease was observed in the glucose and HbA1c level in diabetic rats after treatment with NAT (30 mg/kg) alone and alone with PIO (10 mg/kg) when compared with DB-CON rats at the end of experimental period. STZ causes diabetes by the rapid depletion of β-cells and thereby brings about an eduction in insulin release. HbA1c level has been reported to be increased in patients with diabetes mellitus (12). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (13).The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes. Elevated levels of HbA1c observed in our study reveal that diabetes animals had prior high blood glucose level.

In STZ induced animals a change in the serum enzymes is directly related to changes in the metabolic functions of AST, ALT, ALP and γ- GTP (14-16). It has been reported that the increased levels of transaminases under insulin deficiency were responsible for the increased gluconeogenesis and ketogenesis during diabetes. The increased levels of serum AST, ALT ALP have already been reported to be associated to liver dysfunction and leakage of these enzymes to the liver cytosol in to the blood stream in diabetes (17). Decreased in the activity of AST, ALT, ALP and γGTP in NAT (30 mg/kg) with PIO (10 mg/kg) treated diabetic rats indicate the protective role of the NAT combination with PIO against STZ–NIC induced hepatocellular necrotic changes.

This study concluded that NAT alone and combination with PIO may show some protection in STZ-NIC induced diabetic rats whereas with doses and chronic treatment it showed further liver protection but NAT alone may be better than NAT combination with PIO in protecting hepatic functions in diabetic conditions.

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