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Effect of rosiglitazone on the nicotinamide-streptozotocin induced type-2 diabetes mellitus mediated defects in sperm abnormalities and oxidative defense system in male Wistar rats

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Abstract

The nuclear damages due to the generation of reactive oxygen species is the leading cause for the male infertility in diabetic patients. Rosiglitazone, a PPAR-γ ligand was tested against the nicotinamide (230 mg/kg) and streptozotocin (65 mg/kg) induced reproductive defects in experimental type-2 diabetes mellitus. Rosiglitazone was tested in two doses (1 and 10 mg/kg, p.o daily for 4 weeks) to evaluate its role on the type-2 diabetes induced changes in the sperm shape and sperm count in rats, besides, estimating the serum antioxidant enzyme levels. The results indicate that administration of rosiglitazone produced a dose-dependent inhibition against the alterations induced by the diabetic condition. At 10 mg/kg, rosiglitazone significantly ($P<0.001$) prevented the abnormalities in sperm shape and increased the sperm count, while the lower dose increased ($P<0.01$) only the sperm count in the diabetic animals. The antioxidant study indicated that administration of rosiglitazone to diabetic animals improves the antioxidant status. These observations suggest that the antioxidant property of rosiglitazone might have contributed for its ability to decrease the type-2 diabetes mediated sperm abnormalities.

Key words: Rosiglitazone, sperm shape abnormality, sperm count, antioxidant.

Introduction

Type-2 diabetes mellitus (T2DM) is a chronic metabolic disease that has a significant impact on the health, quality of life, and life expectancy of patients as well as on the health care system. T2DM associated DNA damage may occur due to the disease induced oxidative stress (Rehman et al. 2001). The human spermatozoon is reported to be highly susceptible to the oxidative stress. The peroxidative damage in the sperm plasma membrane causes DNA fragmentation in both the nuclear and mitochondrial genomes. Many reports have associated reactive oxygen species with defective sperm function in diabetes resulting in impaired sperm motility, abnormal morphology and decreased sperm–egg penetration (Rehman et al. 2001, Amaral et al. 2006). However, at lower levels of oxidative damage, spermatozoa may retain the capacity for fertilization while carrying significant levels of oxidative damage in their DNA (Amaral et al. 2006).

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Epidemiological evidence suggests that subsequent aberrant repair of such damage in the zygote may result in the creation of mutations associated with pre-term pregnancy loss and a variety of pathologies in the off spring, including childhood cancer (Olsen et al. 2005).

Peroxosome proliferators-activated receptors (PPARs) are nucleus lipid-activated transcription factors that regulate the expression of genes involved in the control of lipid and lipoprotein metabolism, glucose homeostasis and inflammatory processes (Guo and Tabrizchi 2006). Their wide range of potential therapeutic actions make them attractive targets for the development of oral agents targeting risk factors associated with the metabolic syndrome, type-2 diabetes, cardiovascular diseases etc (Rubenstruck et al. 2007). Thiazolidinediones (TZDs) are the PPAR-γ ligands, known to ameliorate hyperglycemia by sensitizing the insulin action in the peripheral tissues. Presently, two derivatives of TZDs, pioglitazone and rosiglitazone are therapeutically used to overcome the insulin resistance in the T2DM (El-Batron et al. 2006). Apart from hypoglycemia, the administration of TZDs are reported to produce beneficial effects in hyperlipidemia, inflammation, lipodystrophies, non alcoholic fatty liver, poly cystic ovary syndrome and cancer (Rubenstruck et al. 2007, El-Batron et al. 2006, Wise et al. 2007 and Wang et al. 2006). Some of the earlier findings revealed that TZDs possess a significant antioxidant potential against the oxidative stress (Collino et al. 2006). Our recent study indicated that administration of rosiglitazone (RSG) and pioglitazone for acute and chronic durations did not cause any significant damage to the germinal cells in the normal male Wistar rats (Rabbani et al. 2008). Also, we found that administration of RSG to the experimental type-2 diabetic rats reduced the frequency of nuclear damage using bone marrow micronucleus test (Rabbani et al. 2008).

This study was planned to evaluate the effect of rosiglitazone on the sperm shape abnormality and sperm count in diabetic male Wistar rats using nicotinamide-streptozotocin induced experimental T2DM.

Materials and Methods

Chemical

A gift sample of Rosiglitazone (RSG) was obtained from Biocon (India) Ltd, Bangalore. The stains and other reagents/chemicals used in this study were of analytical grade and procured from the regular suppliers.

Animals

Eight week-old healthy, laboratory bred, male Wistar rats weighing 180±10 gm were maintained under standard laboratory conditions such as temperature 22-25 °C, 12 h light / dark cycle and provided water and pellet food *ad libitum*. The experiments were conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals, Chennai, India) approved animal house after obtaining the prior approval from the Institutional Animal Ethics Committee.

Induction of diabetes (Masiello et al. 1998)

Experimental NIDDM was developed in adult rats by administering streptozotocin (STZ) and nicotinamide (NA). The animals received intraperitoneal administration of NA - 230 mg/kg (SD Fine-Chem Ltd, Mumbai, India) dissolved in saline 15 min before an administration of STZ – 65 mg/kg, ip (Sigma Aldrich, USA) dissolved in 0.1 M citrated buffer (pH 4.5) immediately before use. Blood glucose was estimated after 2 days and the animals with glucose level ≈ 180 ± 8 mg/dl are only selected for the study.

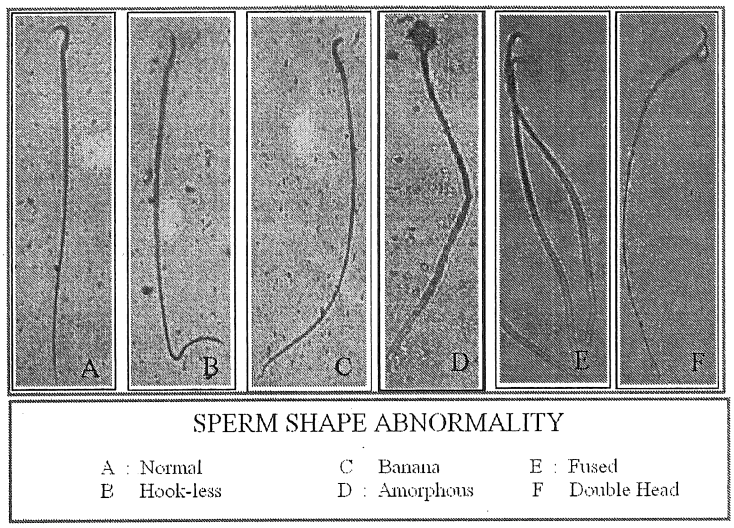
Dosage, treatment and sampling

The animals were divided mainly in to three groups ie., control, diabetic and treatment, consisting of eight animals per group. The treatment group received two doses of RSG (1 and 10 mg/kg) (Villegas et al. 2004, Cuzzocrea et al.

2004) orally per day as gavage for 4 weeks after the induction of diabetes. The control and diabetic animals were administered saline (0.5 ml/kg) daily through out the treatment period. In this study, α-tocopherol (20 mg/kg, p.o) (Ihara et al. 2000) and insulin (5 IU/kg) (Klein et al. 2004) were used as standard antioxidant and hypoglycemic agents respectively. Before the administration, α-tocopherol and rosiglitazone were suspended in 1% carboxy methyl cellulose (CMC) while insulin was reconstituted in water for injection to obtain the required dose.

Sperm morphology and sperm count assay

The procedure described by Wyrobek and Bruce (1975) was followed to study the sperm shape abnormality in cauda epididymis of the rats. One thousand sperms per animals were screened to find the different types of abnormality in one of the cauda epididymis. Five types of abnormalities such as amorphous, hookless, banana shape, fused and double headed (Photo-1) were evaluated and finally represented as percentage total abnormality (Narayana et al. 2002).



The caudal sperm count test was performed according to D'Souza (2004). The spermatozoa count was obtained by counting the number of sperm cells in the four WBC chambers using a neubauer's slide.

Estimation of serum antioxidant enzyme levels

Blood samples after the respective treatment were collected from the retro-orbital plexus under light anesthesia. The serum was separated by centrifugation (1000 rpm) and immediately analyzed to determine the antioxidant status.

Serum lipid peroxidation

The procedure described by Okhawa et al. (1979) was followed to estimate the lipid peroxidation. The principle depends on the reaction between thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation at pH 4. A reddish pink color developed was estimated at 532 nm which indicates the extent of peroxidation. The extent of lipid peroxidation was expressed as η mol/ mg protein.

Catalase (CAT)

The estimation of catalase activity was done by determining the decomposition of H_2O_2 at 240 nm in an assay mixture containing the phosphate buffer (0.25 M, pH 7). One international unit of catalase utilized is that amount which catalyzes the decomposition of 1 mM H_2O_2 per min at 37 °C and expressed in terms of unit / mg protein (Sinha 1972).

Superoxide dismutase (SOD)

The principle for measuring the SOD depends on the detecting the $O2^{-}$ generated during auto-oxidation of hydroxylamine. During the oxidation, nitro blue tetrazolium (NBT) is reduced and nitrite is produced in the presence of EDTA which can be detected colorimetrically at 560 nm. The concentration of SOD is expressed as units / mg protein (Kono 1978).

Glutatione reduced (GSH)

The reduced glutathione (GSH) was estimated using the procedure described by Beutler et al (1963). The sulfhydril groups present in the glutathione forms a colored complex with DTNB {5, 5' dithiobis – (2-nitrobenzoic acid)}, which was measured colorimetrically at 412 nm. GSH levels were expressed as µg / mg protein (Beutler et al. 1963).

Glutathione peroxidase (GPx)

GPx activity was assayed based on the modified method of Paglia and Valentine described by Heath and Tappel (1976). A 100 µl of the serum sample is incubated for 5 min at 37° with stock solution (0.25 mM GSH, 0.12 mM NADPH and 1 unit of glutathione reductase prepared in the tris buffer) in a final volume of 1.65 ml. 50 µl of cumene hydroperoxide (1 mg/ml) are added to start the reaction, and the absorbance at 340 nm is monitored for the rate of disappearance of NADPH and the GPx value was represented as µg of glutathione consumed/min/mg protein.

Statistics

The statistical analyses for the sperm abnormality results was done by One-way Anova followed by multiple comparisons by Mann- Whitney U test (Rai and Vijayalaxmi 2001) while the antioxidant data was analyzed by student 't' test followed by Anova. $P<0.05$ was considered to indicate the significance.

Results

A. Effect of RSG on the sperm shape abnormality and sperm count in the NA-STZ induced type-2 diabetes

Administration of NA with STZ significantly ($P<0.001$) increased the sperm shape abnormality and reduced the sperm count compared to the normal animals. The treatment of RSG has shown dose dependent protection against the sperm shape abnormality and sperm count in the NA-STZ induced T2DM. RSG at lower dose (1 mg/kg) produced significant ($P<0.01$) increase in the sperm count but did not affect the total sperm shape abnormality compared to the diabetic condition. The percentage increase in the sperm count was found to be 10.19 % compared to the diabetic group. Further, RSG at 10 mg/kg) significantly ($P<0.001$) prevented the abnormalities in the sperm shape and enhanced the spermatozoa number. The percentage inhibition was 18.52 % for sperm abnormality and 11.27 % for sperm count compared to the diabetic animals. Similarly, when α-tocopherol (20 mg/kg) was administered to the T2DM animals, a reversal ($P<0.001$) was observed in the sperm shape morphology and caudal sperm count compared to the diabetic group. However, administration of insulin (5 IU/kg) did not produce significant change in the sperm shape abnormality and sperm count in the diabetic condition (Table 1).

B. Effect of RSG on the serum antioxidant status in NA-STZ induced type-2 diabetes

The diabetic condition after the administration of NA and STZ significantly ($P<0.001$) increased the lipid peroxidation (LPO) and reduced the serum catalase (CAT), superoxide dismutase (SOD) glutathione (GSH) and glutathione peroxidase (GPx) levels compared to the control. The antioxidant study indicated that administration of RSG enhanced the antioxidant enzymes in experimental T2DM. Administration of RSG at higher dose (10 mg/kg) produced significant ($P<0.05$) reduction in the LPO and enhanced ($p<0.01$) the serum levels of CAT, SOD, GSH and GPx in the diabetic animals. However, the lower dose of RSG (1 mg/kg) did not change the antioxidant profile in the diabetic animals. The administration of α-

tocopherol (20 mg/kg) produced a significant (P<0.001) suppression of LPO and enhanced the CAT, SOD, GSH and GPx levels in the diabetic animals. Further, insulin did not alter the oxidative stress mediated by the NA-STZ diabetes (Table 2).

Table 1. Effect of Rosiglitazone on sperm shape abnormality and sperm count in NA-STZ induced type-2 diabetes

Treatment and Dose (mg/kg)	Sperm morphology test						Sperm count (10^6)
	Amorphous	Hook less	Banana Shape	Double headed	Fused	Total % Abnormality	
Control (Saline-0.5 ml/kg)	1.82±0.21	0.83±0.31	1.02±0.09	0.16±0.17	2.67±0.42	0.73±0.05	33.18±0.36
NA (230 mg/kg)+STZ (65 mg/kg)	2.72±0.18	2.28±0.29	3.57±0.30	1.71±0.29	4.28±0.18	1.62±0.06^{c}	7.77±1.31^{c}
NA-STZ+RSG (1 mg/kg)	2.33±0.21	2.33±0.21	3.5±0.22	3.66±0.33	2.5±0.43	1.56±0.09^{c}	30.60±1.67**^{c}
NA-STZ+RSG (10 mg/kg)	2.8±0.45	2.4±0.90	2.6±1.82	1.20±0.09	3.4±0.15	1.32±0.07***^{c}	30.90±0.44***^{c}
NA-STZ+α-Tocopherol (20 mg/kg)	1.0±0.00	1.16±0.17	2.17±0.31	1.0±0.26	2.83±0.17	0.91±0.17***^{a}	32.46±0.59***^{a}
NA-STZ+Insulin (5 IU7kg)	2.67±0.09	2.33±0.82	3.50±0.05	1.83±0.09	3.50±0.11	1.52±0.25c	27.33±1.99c

Values are expressed as Mean ± SD, NA – Nicotinamide, STZ – Streptozotocin, RSG – Rosiglitazone, n=8, *Statistics: One way Anova followed by Mann-Whitney U test,* ^{a}p<0.05, ^{c}p<0.001 *compared with the control*, **p<0.01, ***p<0.001 *compared with the Diabetic group*

Table 2. Effect of rosiglitazone on the serum anti-oxidant status in NA- STZ induced type-2 diabetes.

Treatment and Dose (mg/kg)	Sperm antioxidant status				
	Lipid peroxidation (η mol/mg protein)	SOD (Units/mg protein)	Catalase (Unit/mg protein)	Glutathione (GSH) (µg/mg protein)	Glutathione Peroxidase (GPx) (µg of glutathione consumed/mg protein)
Control (Saline-0.5 ml/kg)	2.32±0.24	0.47±0.06	6.26±0.36	3.36±0.31	1.60±0.051
NA (230 mg/kg)+STZ (65 mg/kg)	3.35±0.22c	0.22±0.06c	3.12±0.38c	1.75±0.27c	1.16±0.19c
NA-STZ+RSG (1 mg/kg)	3.07±0.42b	0.26±0.01c	3.38±0.24c	1.59±0.18c	1.34±0.26a
NA-STZ+RSG (10 mg/kg)	2.80±0.55*	0.33±0.01**c	4.38±0.84**c	1.98±0.02*c	1.45±0.12**a
NA-STZ+α-Tocopherol (20 mg/kg)	2.40±0.04***	0.44±0.02***	5.21±0.16***	3.04±0.152***a	1.41±0.09*b
NA-STZ+Insulin (5 IU7kg)	3.30±0.11c	3.15±0.24c	0.28±0.06c	1.76±0.02c	1.16±0.23b

Values are expressed as Mean ± SD, NA – Nicotinamide, STZ – Streptozotocin, RSG – Rosiglitazone, n=8 *Statistics: Student 't' test followed by Anova* ^{a} p<0.05, ^{b}p<0.01, ^{c}p<0.001 *compared with the Control* ,*P<0.05, **P<0.01, ***p<0.001 *compared with the Diabetic group*

Discussion

Male reproductive health has received considerable attention in recent years. In addition to declining sperm quality, fertility problems and increased incidence of testicular cancer, there is accumulating evidence that genetic damage, in the form of unrepaired DNA lesions or mutation, may be transmitted through sperm to the offspring (Olsen et al. 2005) Such genetic damage may arise from environmental exposure or via endogenously formed reactive oxygen species (ROS) during spermatogenesis. Free radical production and lipid peroxidation are known to be important mediators in testis physiology (Amaral et al. 2006). Although testis is endowed with antioxidant enzymes like SOD, catalase, GPx etc. however, their concentrations are known to be relatively lower compared to hepatic (Olsen et al. 2005, Aitken and Baker 2006).

Among the battery of tests available, analyzing the sperm shape abnormalities and sperm count are considered to be a useful assay in determining the cytogenetic changes induced by a drug/disease on the germinal cells (Wyrobek and Bruce 1975, Kumar et al. 2002). In our study, NA-STZ induced T2DM enhanced the abnormalities in the morphology of sperms and reduced the sperm count indicating diabetes

mediated germinal cell damages (Table 1). The experimental T2DM has also reduced the serum levels of catalase, SOD, GSH and GPx and increased the LPO (Table 2). As reported, SOD is an enzyme that catalyzes the dismutation of superoxide ion in to oxygen and hydrogen peroxide, thus protecting the cell from the superoxide toxicity (Kono 1978). CAT present in many plants, animals and aerobic bacteria, efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen (Kumar et al. 2002). The function of GPx is to remove the H_2O_2 generated by metabolic action or oxidative stress. The activity of GPx is highly dependent on GSH concentration. GSH scavenges peroxynitrite and $HO^{\cdot}$ as well as convert H_2O_2 to water with the help of GPx (Aitken et al. 1995). The result of our studies indicates that the NA-STZ induced hyperglycemia could have increased the oxidative stress which might have either exhausted the antioxidant enzymes or inhibited their proliferation.

LPO occurs when ROS attacks the polyunsaturated fatty acid residues of phospholipids of cell membrane which is extremely sensitive to the oxidation. Spermatozoa are highly susceptible to damage by excess concentrations of ROS due to high content of polyunsaturated fatty acid with in their plasma membrane (Olsen et al. 2005, Kumar et al. 2002 and Aitken et al. 1995). Increased LPO and altered membrane can affect the sperm function through impaired metabolism, motility, acrosome reaction as well as oxidative damage to sperm DNA (D'Souza 2004, Aitken et al. 1995). The present study indicated that the administration α-tocopherol and RSG prevented the T2DM changes in sperm shape and sperm count besides exhibiting antioxidant activity. In the earlier study, α-tocopherol, a known antioxidant, has suppressed the oxidative stress mediated changes in rat epididymal sperms (Krishnamoorthy et al. 2007). An increase in the levels of catalase, SOD, GSH and GPx and decrease in LPO followed by the α-tocopherol treatment supports its potential to maintain the redox state and to cope with the oxidative stress (Taylor 2001). These data indicate that the antioxidant activity of an agent play a beneficial role in preventing the ROS mediated damages on male spermatozoa cells. Further, the non-significant alteration in the sperm shape abnormality, sperm count and oxidative stress after the administration of insulin suggests that hypoglycemia alone might not reduce the fertility related complications caused by the oxidative stress in diabetes. The antioxidant potential of RSG has already been reported in the literature (Collino 2006). Besides, a recent study has indicated the protective effect of RSG against the ischemia/reperfusion induced oxidative damage in the cerebrum of rats (Shen et al. 2003). Administration of PPAR-γ agonist has been reported to affect NF-kB activation by interfereing with the MAPK signaling cascade which is responsible for the generation of ROS (Collino 2006). Based on the parallelism between the antioxidant activity and reduced sperm abnormalities by RSG in the present study, we can suggest that RSG might help in preventing the male reproductive toxicity associated with diabetes.

Conclusion

The study indicated that administration of nicotinamide with STZ increased the sperm shape abnormality and reduced the sperm count along with the increased oxidative stress. RSG treatment to the diabetic animals decreased the abnormalities in sperm shape and enhanced the number of sperms. Further, the administration of RSG produced a significant elevation in the serum levels of antioxidant enzymes in the diabetic animals. The observations suggest that inhibitory effect of RSG against the NA-STZ mediated changes in the sperm shape and sperm count could be related to the antioxidant property of RSG.

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