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Atorvastatin prevents type 2 diabetes mellitus—An experimental study

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ABSTRACT

Recent reports of increased diabetes risk have raised concerns regarding the use of statins. The present study was therefore planned to clarify whether atorvastatin can prevent diabetes development in a rat model of type 2 diabetes mellitus. Eight week old male Wistar rats were randomized into three groups ($n=12$ each group). Group A was given standard chow diet, while group B and group C were offered high sucrose diet. In addition to high sucrose diet, group C was given atorvastatin (20 mg/kg/day) from beginning of study till 26th week. After 26 weeks, a low dose of streptozotocin (15 mg/kg, i.p.) was given to all 3 groups and further followed for 4 weeks. Oral glucose tolerance tests were done at week 4, 26 and week 30. Development of impaired glucose tolerance at week 26 (16.66% vs 100%, $P=<0.001$) and diabetes at week 30 (16.66% vs 81.81%, $P=0.002$) was significantly lower in rats pretreated with atorvastatin along with high sucrose diet viz group C compared to group B rats who received high sucrose diet only respectively. Also, metabolic indices like body weight, hypertriglyceridemia, glucose area under the curve (Gl-AUC) were significantly lower in group C compared to group B ($P=<0.05$) while insulin resistance (HOMA-IR) was also lower in group C ($P=0.05$). This study clearly demonstrates for the first time in a rat model of type 2 diabetes mellitus that atorvastatin prevents development of type 2 diabetes.

1. Introduction

Atorvastatin, a drug of class statin known to inhibit HMG CoA (3-hydroxy-3-methyl-glutaryl coenzyme A reductase is a well accepted drug for treatment of dyslipidemia in patients with cardiovascular disease and in high risk subjects. There have been recent concerns over the possible role of statins in causation of insulin resistance and type 2 diabetes mellitus (T2DM). However, literature is divided on this issue with studies supporting and refuting such hypotheses. Short term treatment with statins have shown significantly improved insulin sensitivity, glycemic control, reduced risk of T2DM (Huptas et al.,2006; Guclu et al., 2004; Sonmez et al., 2003) and improved adipocyte insulin responsiveness (Horvath et al., 2008). Recent experimental studies in rats and mice have also shown that atorvastatin treatment reduces insulin levels, restores whole body insulin sensitivity and may be used in insulin resistance and associated T2DM (Furuya et al., 2010; Zhang et al., 2010).

Evidence for an adverse role of statins comes from recent clinical trials which demonstrate that statins significantly increase fasting insulin and HbA1c levels and incidence of new onset diabetes in hypercholesterolemia patients (Koh et al., 2010; Ridker et al., 2008; Peter et al., 2003; Collins et al., 2003). Several experimental studies on statins have also reported suppressed insulin secretion (Yada et al., 1999) reduced insulin sensitivity (Kanda et al.,2003) and impaired glucose tolerance (Nakata et al., 2006).

The animal model used in the present study is a new rat model of type 2 diabetes developed in recent years (Wang et al., 2007). In this model, Wistar rats are kept on high calorie diet that leads to the development of metabolic syndrome. After the development of metabolic syndrome, a low dose of streptozotocin is given to induce partial β-cell destruction and consequently type 2 diabetes develops. The diabetes developed in this fashion simulates the pattern of disease as it occurs in humans. The advantage of this type of animal model is that it exhibits all the classical features that are known to occur in the natural history of human type 2 diabetes viz obesity, dyslipidemia, insulin resistance and impaired glucose tolerance over a period of time and a partial destruction of β-cells unmasks type 2 diabetes.

To the best of our knowledge, this is the first study which has prospectively examined the effect of statin therapy on incident

Abbreviations: CHL, cholesterol; Gl-AUC, glucose area under the curve; ns, not significant; OGTT, oral glucose tolerance test; STZ, streptozotocin; T2DM, Type 2 diabetes mellitus; TG, triglyceride; wk, week

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diabetes. The present study was therefore planned to investigate the effect of long term atorvastatin pretreatment on diabetes risk in a rat model of type 2 diabetes mellitus.

2. Materials and methods

2.1. Animals

All animal experiments were performed according to the guidelines of local institutional ethics committee. Thirty six selectively bred male Wistar rats of 6 week age were procured from central animal house UCMS (Delhi, India). Animals were grouped and housed under controlled environment (temp 20–25 °C, humidity 50 ± 20%) and diurnal cycle (12-h light/dark). After 2 weeks of acclimatization (day 1), animals were randomized into stratified groups to ensure equal body weight means.

2.2. Study design

At day 1 animals were randomly divided into three groups ($n=12$ in each group). Group A was given standard chow diet (control diet), while group B and group C were offered high sucrose diet. In addition to their respective diets, group C was given atorvastatin orally (20 mg/kg body weight/day, Zydus Cadila) whereas group A and B were given vehicle (0.5% carboxymethyl cellulose) only till 26th week. After 26 weeks of follow up from day 1, mild dose of streptozotocin, 15 mg/kg, intraperitonial (sigma chemicals, USA) was given in all the 3 groups and were followed for further 4 weeks. Body weight was recorded, fasting blood samples were collected for estimation of lipids and insulin followed by oral glucose tolerance tests (OGTTs) at week 4 and 26. OGTT was repeated at week 30 i.e., 4 weeks after streptozotocin treatment and outcome of incident diabetes was observed. Animals with fasting plasma glucose value of 126 mg/dl (7 mmol/l) and or postprandial plasma glucose value of 200 mg/dl (11.1 mmol/l) were considered as having diabetes.

2.3. Diets and feeding protocol

After acclimatization i.e., day 1 of study, control group A was continued on standard chow diet (carbohydrate 60–70%, fat 5%, protein 25%), group B and group C were given high sucrose diet used by Kamgang et al. (2005) with slight modification (carbohydrate 60–70% predominantly sucrose, fat 20–25%, protein 15–20%) keeping in view the long term follow up period of 30 weeks.

2.4. Oral glucose tolerance test

After overnight fasting, rats were administered 2 g/kg of glucose and blood samples were collected from tail vein and blood glucose was measured by Glucometer (LifeScan, OneTouch) in fasting and after 30, 60, 90 and 120 min of glucose loading.

2.5. Lipids and insulin

Serum triglyceride, cholestrol were measured by commercially available kits (Labkit, Spain) and HDLc was measured by third generation direct homogeneous assay (Auto pure, Accurex, India). Serum insulin levels were measured by commercially available radioimmuno assay kit (Millipore Corp, USA). LDL and VLDL values were calculated as following (Friedwald equation) LDL=[Total CHL]−[HDL]−([TG]/5), VLDL=[TG]/5.

HOMA-IR was calculated as applicable to rats (Cacho et al., 2008) using fasting glucose and insulin values as follows: Fasting insulin (μU/ml) × Fasting glucose (mg/dl)/2430

2.6. Statistical analysis

Repeated measure ANOVA followed by tukey's test and fischer's exact test were applied for statistical analysis of data and SPSS 20.0 software was used for this purpose. Data was analyzed for $n=11$ in group A and group B at week 26 and 30 as one rat from each group died during the follow up.

3. Results

3.1. Body weight

Body weight was found to be similar in all the three groups till week 4. However, it was observed to be significantly lower in atorvastatin treated group C at week 26 and week 30 compared to high sucrose diet group B. Also, Body weight in atorvastatin treated rats was found to be similar to controls at both these time points (Table 1).

3.2. Blood glucose

Highest fasting blood glucose levels were observed in rats receiving high sucrose diet compared to atorvastatin pretreated and control rats at week 26 and 30 though this difference was statistically significant at 30th week only. Also, fasting blood glucose levels were comparable in atorvastatin pretreated and control rats at week 26 and 30. Similar trend was observed in 2 h postprandial blood glucose levels following oral glucose tolerance test that were found to be comparable in controls and atorvastatin pretreated rats and were significantly lower when compared with rats receiving high sucrose diet only at both of these time points (Table 2).

3.3. Fasting serum insulin

Fasting serum insulin levels were found to be significantly higher in high sucrose diet group compared to controls at week 26. Also, serum insulin levels were observed to be significantly higher in rats receiving high sucrose diet only compared to atorvastatin pretreated and control rats at 30th week (Table 2).

Table 1
Body weight at different time points.

Parameter	Time points	Group A Mean ± S.D.	Group B Mean ± S.D.	Group C Mean ± S.D.	Significance
Body weight (g)	Wk 0	184.80 ± 14.26	179.90 ± 19.54	179.40 ± 13.08	*a*=ns, *b*=ns, *c*=ns
	Wk 4	252.45 ± 24.58	252.27 ± 20.70	239.45 ± 24.94	*a*=ns, *b*=ns, *c*=ns
	Wk 26	351.09 ± 26.14	354.70 ± 38.06	329.90 ± 32.15	*a*=ns, *b*=ns, *c*=0.03
	Wk 30	345.63 ± 30.53	355.30 ± 35.98	334.40 ± 29.84	*a*=ns, *b*=ns, *c*=0.04

a=Group A vs. Group B, *b*=Group A vs. Group C, *c*=Group B vs. Group C.