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Metabolic effects of various antidiabetic and hypolipidaemic agents on a high-fat diet and multiple low-dose streptozocin (MLDS) mouse model of diabetes

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Abstract

Insulin resistance and subsequent insulin secretory defect are two main features of type 2 diabetes and associated metabolic disorders. The animal models of type 2 diabetes are very complex and are as heterogeneous as the disease. We have evaluated the effect of various antidiabetic and lipid lowering agents (fenofibrate, rosiglitazone, glimepiride, metformin and simvastatin) on the metabolic abnormalities induced by combining a high-fat diet and multiple low-dose streptozocin (MLDS) in mice. Male Swiss albino mice were orally treated with the above agents and fed with a diet containing high fat for 28 days. On day 15 the animals were injected intraperitoneally with low-dose streptozocin (40 mg kg^{-1}), which was administered for five consecutive days. At the end of the 28-day treatment plasma metabolic parameters (glucose, triglyceride and immunoreactive insulin) were estimated. The antidiabetic and hypolipidaemic agents exhibited differential effects on these metabolic parameters. With the exception of fenofibrate all these agents reduced the plasma glucose levels, and the effects of metformin and rosiglitazone on glucose were found to be statistically significant. Although the effect of the test drugs on cholesterol was modest, a significant decrease in triglyceride levels was observed with sub-chronic treatment with these agents. Interestingly, glimepiride mildly elevated the insulin levels while the other antidiabetics and hypolipidaemics reduced the insulin levels, with metformin and rosiglitazone exhibiting statistically significant effects on insulin. To our knowledge this is the first report on the effect of various peroxisome proliferator-activated receptor modulators and newer antidiabetics on the metabolic effects induced by the combined high-fat diet and MLDS model of type 2 diabetes in Swiss albino mice. The results suggested the complexity of the hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia induced by the high-fat diet and MLDS mouse model, and their correction by various antidiabetics and antihyperlipidaemics may have involved diverse mechanisms.

Introduction

Type 2 diabetes is a heterogeneous disease with a complicated pathogenesis which is related to genetic susceptibility and life style, especially the dietetic style (Roith & Zick 2001). Type 2 diabetes is a multifactor disease characterized by insulin resistance with a relative impairment in insulin secretion and is often associated with hypertension and hyperlipidaemia. Indeed, studies in type 2 diabetes patients suggest that, although poor metabolic control is the most important determinant of the development of nephropathy, hypertension and hyperlipidaemia are also considered to be involved (Miranda et al 2005). Most of the individuals diagnosed with type 2 diabetes are found to be obese. The two metabolic defects characterizing type 2 diabetes are derangement of insulin secretion that is delayed or is insufficient relative to glucose load and inability of peripheral tissues to respond to insulin—called insulin resistance. Studies of the natural history of type 2 diabetes have shown that the prediabetic state is characterized by resistance to insulin-mediated glucose disposal and compensatory hyperinsulinaemia. The transition from pre-diabetes to type 2 diabetes occurs when the secretory

capacity of the pancreatic β cell is no longer able to compensate for the insulin resistance (Simmons 2005; Miranda et al 2005; Arulmozhi & Portha 2006).

There are two major underlying causes for this metabolic syndrome: obesity and insulin resistance. These two parameters are closely and reciprocally interrelated. It is well established that obesity can cause insulin resistance, but underlying genetic forms of insulin resistance seem to increase susceptibility for the syndrome by recapitulating the metabolic abnormalities of abdominal obesity (Grundy 2006). The core risk factors of the metabolic syndrome are atherogenic dyslipidaemia, elevated blood pressure, and elevated plasma glucose. In addition to hyperglycaemia, systemic or local elevations in insulin may contribute to aberrant lipid metabolism and vascular wall function (Kunjathoor et al 1996).

In spite of the availability of many animal models for type 2 diabetes mellitus, including both genetic and chemically induced models, none of them simulate human type 2 diabetes mellitus, as spontaneous animal models of type 2 diabetes mellitus are highly heterogeneous. At one end of the spectrum there is a mild hyperglycaemia associated with obesity and hyperinsulinaemia. At the other extreme, animal models of type 2 diabetes mellitus can develop a severe form of diabetes with extensive β-cell degeneration, occasionally resulting in ketosis and requirement of exogenous insulin to sustain life (Arulmozhi et al 2004). High doses of β-cell toxins such as streptozocin and alloxan induce insulin deficiency and type 1 diabetes with ketosis. However, doses calculated to cause a partial destruction of β-cell mass can be used to produce a mild insulin deficient state of type 2 diabetes mellitus, without tendency to cause ketosis. The development of hyperglycaemia in animals following streptozocin injection is primarily due to the direct pancreatic β-cell destruction, and resulting in insulin deficiency rather than the consequence of insulin resistance. An ideal model should simulate the metabolic characteristics of patients with type 2 diabetes and should be cost effective (Arulmozhi et al 2004).

The combination of a high-fat diet and multiple low-dose streptozocin (MLDS) injections to animals is reported to have the characteristic features of human type 2 diabetes. Recently various investigators have reported the use of MLDS animals as a model of metabolic abnormalities as the clinical presentations of MLDS animals are multifaceted. Through evidence available on the autoimmune destruction of pancreatic β cells by multiple low-dose streptozocin (McEvoy et al 1984; Ogawa et al 1999), high-fat feeding of MLDS animals provides a new animal model for type 2 diabetes, importantly with lipid abnormalities (Kunjathoor et al 1996; Luo et al 1998; Takamura et al 1999; Reed et al 2000; Srinivasan et al 2005). Sugano et al (2006) reported that a high-fat diet and MLDS animals would serve as a model for diabetic nephropathy.

The high-fat MLDS animal is an emerging animal model for the metabolic abnormalities seen with type 2 diabetes. Therefore, we have investigated the oral subchronic administration of various classes of antidiabetic and antihyperlipidaemic agents (glimepiride, fenofibrate, metformin, rosiglitazone and simvastatin) on various metabolic parameters in MLDS-high fat-induced metabolic abnormalities in male Swiss albino mice. We interpreted the intervention of these treatments in MLDS-induced hyperglycaemia and aimed to shed some light on the mechanisms underlying the effects of these agents.

Materials and Methods

Animals

Adult male Swiss albino mice (20–30 g) were obtained from the Research Animal Facility of Poona College of Pharmacy (PCP), Pune, India. On arrival, the animals were placed at random and allocated to treatment groups (6–10 animals per treatment) in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C on a 12-h light–dark cycle with free access to water and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of PCP, Pune, India, and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India. Rules of CPCSEA are laid down as per the guidelines of the Institute of Laboratory Animal Resources, US.

Drugs and chemicals

Fenofibrate and streptozocin were obtained from Sigma Chemical Co. (St Louis, MO). Rosiglitazone, glimepiride, metformin and simvastatin were gifts from Lupin Ltd, Pune, India. All other chemicals and reagents were of pure analytical grade and obtained from local suppliers.

Drug treatments and MLDS-high-fat diet-induced metabolic abnormalities

After acclimation in the laboratory for one week, the mice were divided into six groups to receive the following treatments: group 1, 0.3% Tween 80 10 mL kg^{-1}; group 2, fenofibrate 100 mg kg^{-1}; group 3, metformin 10 mg kg^{-1}; group 4, glimepiride 10 mg kg^{-1}; group 5, rosiglitazone 10 mg kg^{-1}; and group 6, simvastatin 100 mg kg^{-1}. The animals were fed with a high-fat diet comprising 40% fat, 42% carbohydrates and 18% protein (Nutrilabs, Bangalore, India). The drugs were solubilized in 0.3% Tween 80 and administered by oral gavage between 10.00–11.00 h from day 7 to day 28. The doses of the medications were chosen from preliminary studies and previous reports (Chaput et al 2000; El-Swefy et al 2000; Ramadan et al 2006).

On day 15, the animals were injected inraperitoneally with a low dose of streptozocin (40 mg kg^{-1}) dissolved in citrate buffer (pH 4.5) for five consecutive days. The mice were kept on the above treatments and fed with the high-fat diet until day 28.

A separate group of animals were fed a normal chow diet and did not receive streptozocin injections (non-diabetic control).

On day 28, blood samples were collected from mice (under mild anaesthesia) via the retro-orbital sinus 1 h after

the administration of the last dose. No significant effect of ether anaesthesia was observed on the plasma parameters measured.

Determination of plasma metabolic parameters

Plasma obtained from the mice was used to estimate the metabolic parameters. Glucose, triglyceride and total cholesterol levels were measured spectrophotometrically using commercially available kits (Bayer Diagnostics, India). Plasma insulin was assayed using an ELISA kit (Crystal Chemicals, Chicago, IL).

Statistical analysis

Results were expressed as mean ± s.e.m. Comparisons between groups were made by Student's *t*-test or analysis of variance and Dunnett's post-test as per suitability. A P value of < 0.05 was considered as significant.

Results

Effect of various antidiabetics and antihyperlipidaemics on glucose levels in MLDS-high-fat-fed mice

Though the MLDS-high-fat-fed mice showed a significant increase in body weight when compared with mice fed a normal chow diet (non-diabetic control), no significant difference in body weight of the mice in the various treatment groups was observed during or after the study (data not shown). After the high-fat diet and MLDS the mice exhibited obvious hyperglycaemia, as evidenced by elevated plasma glucose levels (Figure 1). With the exception of fenofibrate, the drugs reduced the elevated glucose level with the order of potency being rosiglitazone > metformin > glimepiride > simvastatin. Rosiglitazone and metformin significantly ($P < 0.05$) reduced the glucose levels ($216.25 \pm 16.5\,mg\,dL^{-1}$ (vehicle) vs 137.96 ± 19.80 and $145.93 \pm 11.28\,mg\,dL^{-1}$, respectively).

Effect of various antidiabetics and antihyperlipidaemics on triglyceride levels in MLDS-high-fat-fed mice

A high-fat diet and MLDS treatment significantly increased triglyceride levels in the mice but the test drugs significantly ($P < 0.01$) reduced these elevated levels. Surprisingly, rosiglitazone, fenofibrate, metformin and glimepiride exhibited a similar reduction (~50–60%) in triglyceride levels (Figure 2).

Effect of various antidiabetics and antihyperlipidaemics on total cholesterol levels in MLDS-high-fat-fed mice

A moderate and significant ($P < 0.05$) elevation of total cholesterol levels was observed after the high-fat diet and MLDS treatment in mice. The test drugs did not significantly reduce the elevated total cholesterol levels, but a trend to decrease was seen (Figure 3).

Effect of various antidiabetics and antihyperlipidaemics on insulin levels in MLDS-high-fat-fed mice

Rosiglitazone and metformin significantly ($P < 0.01$ and 0.05, respectively) reduced the insulin levels when compared with the vehicle-treated animals ($1.08 \pm 0.15\,ng\,mL^{-1}$ (vehicle) vs $0.44 \pm 0.10\,ng\,mL^{-1}$ (rosiglitazone) and $0.67 \pm 0.06\,ng\,mL^{-1}$

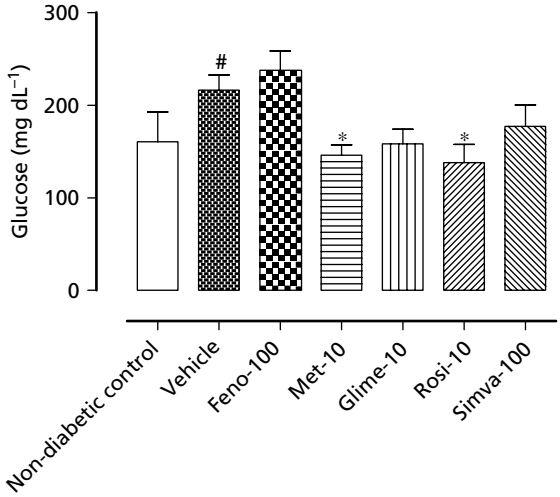

Figure 1 Effect of fenofibrate (Feno; $100\,mg\,kg^{-1}$, p.o.), metformin (Met; $10\,mg\,kg^{-1}$, p.o.), glimepiride (Glime; $10\,mg\,kg^{-1}$, p.o.), rosiglitazone (Rosi; $10\,mg\,kg^{-1}$, p.o.) and simvastatin (Simva; $100\,mg\,kg^{-1}$, p.o.) on plasma glucose levels in high-fat-diet-MLDS male Swiss albino mice after 28-days treatment. Bars represent means ± s.e.m. from n = 6–10. $^{*}P < 0.05$ compared with vehicle treatment; $^{\#}P < 0.05$ compared with non-diabetic control animals.

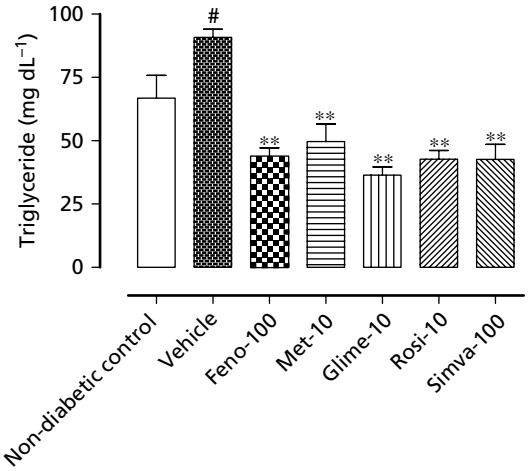

Figure 2 Effect of fenofibrate (Feno; $100\,mg\,kg^{-1}$, p.o.), metformin (Met; $10\,mg\,kg^{-1}$, p.o.), glimepiride (Glime; $10\,mg\,kg^{-1}$, p.o.), rosiglitazone (Rosi; $10\,mg\,kg^{-1}$, p.o.) and simvastatin (Simva; $100\,mg\,kg^{-1}$, p.o.) on plasma triglyceride levels in high-fat-diet-MLDS male Swiss albino mice after 28-days treatment. Bars represent means ± s.e.m. from n = 6–10. $^{**}P < 0.01$ compared with vehicle treatment; $^{\#}P < 0.05$ compared with non-diabetic control animals.

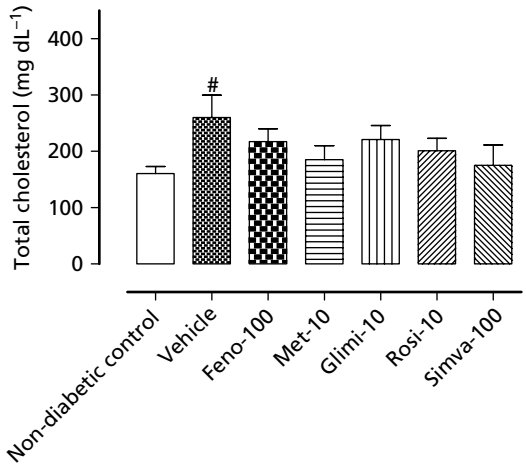

Figure 3 Effect of fenofibrate (Feno; 100 mg kg^{-1}, p.o.), metformin (Met; 10 mg kg^{-1}, p.o.), glimepiride (Glime; 10 mg kg^{-1}, p.o.), rosiglitazone (Rosi; 10 mg kg^{-1}, p.o.) and simvastatin (Simva; 100 mg kg^{-1}, p.o.) on plasma total cholesterol levels in high-fat-diet-MLDS male Swiss albino mice after 28-days treatment. Bars represent means ± s.e.m. from n = 6–10. $^{\#}P < 0.05$ compared with non-diabetic control animals.

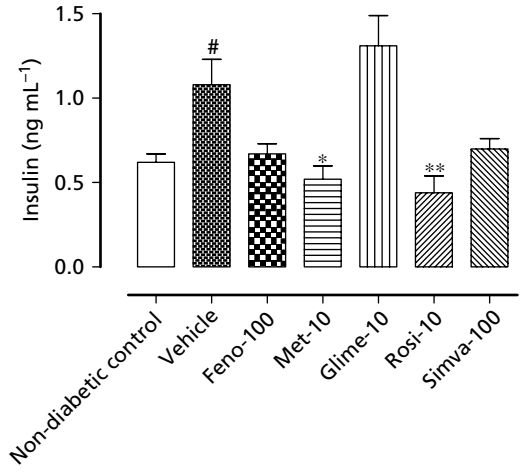

Figure 4 Effect of fenofibrate (Feno; 100 mg kg^{-1}, p.o.), metformin (Met; 10 mg kg^{-1}, p.o.), glimepiride (Glime; 10 mg kg^{-1}, p.o.), rosiglitazone (Rosi; 10 mg kg^{-1}, p.o.) and simvastatin (Simva; 100 mg kg^{-1}, p.o.) on plasma insulin levels in high-fat-diet-MLDS male Swiss albino mice after 28-days treatment. Bars represent means ± s.e.m. from n = 6–10. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle treatment; $^{\#}P < 0.05$ compared with non-diabetic control animals.

(metformin)). Interestingly, glimepiride elevated (21.3%) the insulin levels from the vehicle-treated animals. Fenofibrate and simvastatin reduced insulin levels from the vehicle-treated animals but insignificantly (Figure 4).

Discussion

The primary aim of this study was to develop a rodent model of type 2 diabetes and to investigate various classes of antidiabetic and hypolipidaemic agents to understand the effectiveness of these agents in this model. Insulin resistance and compensatory hyperinsulinaemia have been shown to predict the development of type 2 diabetes. To the best of our knowledge this has been the first report on the effect of novel peroxisome proliferator-activated receptor (PPAR) modulators and the newer lipid lowering agents on the metabolic abnormalities induced by combining a high-fat diet with MLDS in Swiss albino mice.

Type 2 diabetes often co-exists with other metabolic risk factors, including dyslipidaemia, hypertension and obesity, in the clustering known as insulin resistance syndrome (or metabolic syndrome). The limitation of the current treatments for type 2 diabetes highlights the inadequacy of addressing elements of insulin resistance syndrome in isolation (Plutzky et al 2002).

This is the first study to report the effect of the newer thiazolidinedione rosiglitazone in the MLDS and high-fat diet mouse model of diabetes. Rosiglitazone significantly reduced the three parameters glucose, triglycerides and insulin. Thiazolidinedione antidiabetic agents that act to directly improve insulin sensitivity, increasing glucose uptake by adipose tissue and skeletal muscle (Young et al 1995), are agonists for PPARγ, a member of the PPAR family of nuclear receptors that also includes PPARα and PPARδ (also known as PPARβ). PPARγ, highly expressed in adipose tissue, plays an important role in the regulation of adipogenesis lipid metabolism and glucose homeostasis (Auboeuf et al 1997; Lehrke & Lazar et al 2005). Increasing evidence suggests roles for PPARγ in cellular processes outside adipose tissue, including for example in skeletal muscle (Loviscach et al 2000). Activation of PPARγ in fat and possibly skeletal muscle is believed to contribute to the antihyperglycaemic activity of the thiazolidinediones, possibly through mechanisms including up-regulation of transport proteins for glucose, including GLUT1 and GLUT4 (Young et al 1995; Shimaya et al 1998), and fatty acids (Frohnert et al 1999).

In addition, in mouse models of insulin resistance, retinoid X receptor (RXR) agonists also ameliorate the typical symptoms of insulin resistance (Keller et al 1993). RXR has been shown to act synergistically with PPARγ, and RXR agonists are capable of increasing the activity of both types of receptors (Mukherjee et al 1997). In addition to combating hyperglycaemia, thiazolidinediones are reported to mitigate cardiovascular complications associated with type 2 diabetes (Lehrke &Lazar 2005).

PPARα is a nuclear receptor that regulates liver and skeletal muscle lipid metabolism as well as glucose homeostasis. Acting as a molecular sensor of endogenous fatty acids and their derivatives, PPARα regulates the expression of genes encoding enzymes and transport proteins controlling lipid homeostasis, thereby stimulating fatty acid oxidation and improving lipoprotein metabolism. PPARα also exerts pleiotropic anti-inflammatory and antiproliferative effects and prevents the proatherogenic effects of cholesterol accumulation in macrophages by stimulating efflux (Lefebvre et al 2006).

The use of PPARα agonists, fibrates, as hypolipidaemic agents for several decades has demonstrated their safety and efficacy for lipid lowering, an important parameter in the prevention of cardiovascular diseases. Moreover, increasing evidence attributing anti-inflammatory activity to PPARα has emerged, documented largely in in-vitro and animal studies.

Predictable metabolic syndrome patients with athereogenic dyslipidaemia (inflammation, low HDL, high triglycerides and small dense LDL) are highly susceptible to cardiovascular morbidity and respond extremely well to fibrate treatment. Rosiglitazone and fenofibrate are potent PPARγ and PPARα agonists, respectively (Berger et al 2005; Staels & Fruchart 2005).

The effect of rosiglitazone and fenofibrate in this high-fat diet and MLDS model of type 2 diabetes was in accordance with a report by Chaput et al (2000), in which both agents improved the elevated triglyceride and insulin levels in *db/db* mice and fatty (*fa/fa*) Zucker rats.

Glimepiride, a third generation sulfonylurea class of antidiabetic, has a mild effect on insulin secretion and is reported to have additional extra-pancreatic effects including activation of insulin-mediated glycogen synthesis, inhibition of hepatic gluconeogenesis and enhancement of glucose uptake (Müller & Wied 1993). Fukuen et al (2005) reported glimepiride to possess agonist activity for PPARγ and affected adipose gene expression and was considered a partial agonist for PPARγ. In line with its mechanism of action, glimepiride reduced the glucose levels with a mild elevation in insulin. Interestingly, the correction observed in triglyceride levels could provide the in-vivo evidence for the in-vitro studies reported by Fukuen et al (2005) and Inukai et al (2005).

The biguanide metformin was introduced as an antidiabetic drug several decades ago (Schafer 1983). The agent has been found to lower blood glucose levels in patients with type 2 diabetes by facilitating glucose utilization in skeletal muscle and reducing hepatic glucose production (Galuska et al 1991; Hundal et al 1992, 2000; Bailey &Turner 1996). These effects appear to be mediated through inhibition of complex 1 in the mitochondrial respiratory chain (Owen et al 2000; El-Mir et al 2000) and/or stimulation of AMP-activated protein kinase (AMPK) (Zhou et al 2001). AMPK is activated under conditions that deplete cellular ATP and elevate AMP, such as glucose deprivation, heat shock, hypoxia, and ischaemia (Hardie et al 1998; Salt et al 1998). AMPK then phosphorylates and inactivates a number of metabolic enzymes involved in fatty acid and cholesterol synthesis, including acetyl-CoA carboxylase and HMG-CoA reductase (Brown et al 1975; Carling et al 1987). In the liver, AMPK activation by metformin results in reduced gluconeogenesis. Recent studies indicated that metformin may have activated AMPK through AMP-independent pathways (Kishi et al 2000; Hawley et al 2002; Fryer et al 2002; Kefas et al 2004). In agreement with earlier reports on the other genetic models of type 2 diabetes, in this study, metformin alleviated the hyperglycaemia, hypertriglyceridaemia and hyperinsulinaemia in the high-fat diet and MLDS model of diabetes.

Statins are first-line therapy in the treatment of cholesterol-induced atherosclerotic cardiovascular diseases (Grundy et al 2004). They are structural analogues of mevalonate and affect, as 3-hydroxymethyl-3-glutaryl coenzyme A reductase (HMG-CoAR) inhibitors, the rate limiting step in the cholesterol biosynthesis cascade. They inhibit conversion of HMG-CoA to mevalonate by competitive blocking of the responsible enzyme, HMG-CoAR. The endogenous cholesterol synthesis in the liver is remarkably reduced and, consequently, levels of circulating LDL-cholesterol are decreased due to the increasing number of LDL receptors on cell surfaces (Brown & Goldstein 1981). Statins lower all of the apolipoprotein B (apo B)-containing lipoproteins and induce a 30–50% reduction in apo B levels, depending on the dose used. This reduction is accompanied by a 30–50% decrease in risk for major coronary events, most of which seem to be secondary to a decrease in apo-B-containing lipoproteins. Some investigators (Jialal et al 2001; Ridker 2003), but not all (Robinson et al 2005), hold the position that statins are anti-inflammatory beyond their effects on total apo B concentrations. One argument for a direct anti-inflammatory effect of statins is that they significantly reduce C-reactive protein, which is a marker of inflammatory processes (Jialal et al 2001; Ridker 2003). If this is true, then an argument can be made that statins are useful for treating the pro-inflammatory component of the metabolic syndrome. Whether an anti-inflammatory action beyond apo-B-lowering actually exists, however, is of little practical importance, because the benefit of statin therapy in high-risk patients is already well established. Reductions in apo-B-containing lipoproteins in the range 30–40%, which can be achieved by moderate (standard) doses of statins, will reduce risk for atherosclerotic cardiovascular disease events by 30–40%. The benefits of lowering lipoproteins containing apo B extend to patients with both metabolic syndrome and type 2 diabetes. Recent studies have shown that greater reductions of apo B will cause an even greater lowering in atherosclerotic cardiovascular disease risk (LaRosa et al 2005). However, the general principle is emerging that the earlier LDL-lowering is started, the greater will be the long-term risk reduction (Grundy 2006). As people with metabolic syndrome are at higher long-term risk for atherosclerotic cardiovascular disease, they might be good candidates for low doses of LDL-lowering drugs when their LDL cholesterol (LDL-C) levels begin to rise (Grundy 2006).

In this model of type 2 diabetes and hyperlipidaemia, simvastatin significantly reduced the triglyceride levels and exhibited a mild to moderate reduction in glucose and insulin levels.

For a long time there have been reports of the involvement of immune systems in the pathogenesis of diabetes induced by subdiabetogenic doses of streptozocin (McEvoy et al 1984; Mensah-Brown et al 2001). Also the role of various inflammatory mediators including tumour necrosis factor-α, interferon-γ, interleukin-10 (Lau et al 2006), NFκB (Mabley et al 2002) and cyclooxygenase-2 (Tabatabaie et al 2000) have been reported in the diabetogenic effect of low-dose streptozocin in various mouse models. It is noteworthy to mention that most of the antidiabetic hypolipidaemic agents used in this study were reported for their potential anti-inflammatory and immunosuppressive (statins) effects in various animal models, and apart from their modulation through their primary mechanisms of action, these drugs could substantially reduce the risk of diabetes by virtue of their anti-inflammatory effect against streptozocin.

Conclusions

This study has validated the use of the high-fat diet and MLDS model of type 2 diabetes with a variety of antidiabetic and hypolipidaemic agents. The model used Swiss albino mice, a commonly available and economically advantageous laboratory rodent. The study design was limited only to the major

metabolic parameters of glucose, triglyceride and insulin, hence, further detailed pharmacodynamic investigations are required to elucidate the precise mechanisms of action of these agents in this model, as the antidiabetics and antihyperlipidaemics acted on multiple pathways and mechanisms.

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