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The development of overt diabetes in young Zucker Diabetic Fatty (ZDF) rats and the effects of chronic MCC-555 treatment

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1 Young (6-week-old) pre-diabetic Zucker Diabetic Fatty (ZDF) rats displaying impaired glucose tolerance (IGT), moderate hyperglycaemia and hyperinsulinaemia were treated with the novel thiazolidinedione, MCC-555, for 28 days, during which time β-cell failure and progression to overt diabetes occurs.

2 Treated ZDF rats exhibited consistently lower blood glucose levels than vehicle-treated diabetic controls, with a delayed rise and lower plateau levels. MCC-555 maintained plasma insulin levels throughout the treatment period, whereas these fell by 40% in untreated ZDF rats.

3 The rise in body weight was maintained in MCC-555-treated rats, whereas vehicle-treated rats exhibited blunted body weight gain after 8 weeks of age. Daily food intake was higher in diabetic, as compared to non-diabetic rats, but treatment did not modify food intake in diabetic rats. Water intake was lower in treated ZDF rats, concomitant with lowering of blood glucose.

4 The hyperinsulinaemic-euglycaemic clamp technique was applied to all rats after treatment to examine the effects of MCC-555 on insulin sensitivity. The glucose infusion rate to maintain normoglycaemia was lower in diabetic than in non-diabetic rats, demonstrating reduced glucose entry into insulin-sensitive tissues in diabetic rats. Increased glucose infusion rates were required to maintain euglycaemia in treated diabetic rats, demonstrating increased insulin sensitivity in these animals.

5 In conclusion, chronic MCC-555 treatment of young ZDF rats displaying IGT attenuates the development of overt diabetes through improved insulin sensitivity and maintenance of β-cell function. MCC-555 may thus be beneficial in humans with IGT, to prevent or delay the progression of diabetes.

Keywords: ZDF rats; MCC-555; thiazolidinediones; diabetes; insulin; impaired glucose tolerance

Introduction

The thiazolidinediones, such as troglitazone (Nolan *et al*., 1994; Whitcomb & Saltiel, 1995) and MCC-555 ((±)-5-[{6-(2-fluorobenzyl)oxy-2-naphthyl}methyl]-2,4-thiazolidinedione; Ishii *et al*., 1996; Upton *et al*., 1997), represent a novel class of antidiabetic compounds that improve insulin sensitivity in subjects with insulin-resistant states, including non-insulin dependent diabetes mellitus (NIDDM or type 2 diabetes) and impaired glucose tolerance (IGT), but have little or no insulin secretagogue activity (Saltiel & Olefsky, 1996). In the early stages of the development of NIDDM, insulin sensitive tissues, such as adipose tissue and skeletal muscle, become insulin resistant, leading to the development of IGT, which may occur over several years (DeFronzo, 1988). Increased insulin secretion initially compensates for insulin resistance and is generally able to prevent the development of severe hyperglycaemia (DeFronzo, 1988). However, for reasons that are not fully understood, pancreatic β-cells ultimately become 'exhausted', and insulin secretion falls to normal or lower levels, which, in the presence of insulin resistance, allows glucose levels to rise into the range of overt diabetes (Harris, 1996; Polonsky *et al*., 1996). NIDDM is characterized by hyperglycaemia and osmotic symptoms (polydipsia and polyuria) and carries an increased risk of cardiovascular damage, especially when combined with the commonly associated high blood pressure and dyslipidaemia (Williams, 1994). Recent clinical studies have suggested that early intervention with troglitazone therapy in subjects with IGT can delay the onset or attenuate the development of overt diabetes (Nolan *et al*., 1994). We have therefore examined the effect of a novel thiazolidinedione, MCC-555, in an animal model of insulin resistance and NIDDM, namely the Zucker Diabetic Fatty (ZDF) rat. This mutant exhibits IGT at an early age, with moderate hyperglycaemia, insulin resistance and hyperinsulinaemia, and subsequently progresses to overt diabetes through a failure of β-cell function (Clark & Palmer, 1982; Peterson, 1994). The diabetes-prone ZDF strain is derived from the obese (fa^{-1}/fa) Zucker rat; both have the primary genetic defect of the Gln 279 Pro (*fa*) mutation affecting the extracellular domain of the leptin receptor, which results in impaired leptin signalling, and early-onset obesity attributable to both hyperphagia and reduced thermogenesis (Terrettaz & Jeanrenaud, 1983).

Experiments were performed to determine whether MCC-555 treatment of young IGT ZDF rats could prevent the development of diabetes in this genetic model of NIDDM.

Methods

Six-week-old male ZDF pre-diabetic (240 g) and non-diabetes prone ZDF controls (200 g) (Genetic Models Inc., Indianapolis, IN, U.S.A.) were divided into three groups ($n=9$ each). Rats were housed individually and kept at $22\pm2^{\circ}$C on a 12-h light/dark cycle (lights on at 07 00 h) and with free access to standard rodent chow (CRM, Biosure, Cambridge, U.K.) and tap water. One group of pre-diabetic ZDF rats received daily oral doses of MCC-555 [10 mg kg^{-1} (Mitsubishi Chemical

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Corporation, Yokahama, Japan)], suspended in 0.5% sodium carboxymethycellulose vehicle (Sigma Chemical Company, Poole, Dorset, U.K.), for 28 days, whilst the other pre-diabetic rats were given vehicle alone (5 ml kg^{-1} d^{-1}). A group of non-diabetic rats also received daily oral doses of vehicle.

Body weight and food and water intake were measured daily, and every 4 days, tail-vein blood glucose concentrations were measured under the fed state, using an Exactech electrochemical meter (Medisense, Abingdon, Oxon, U.K.). At the end of each week, animals were lightly anaesthetized with i.p. injections of Diazemuls : Hypnorm : water mixture (1 : 1 : 2; Diazemuls: Dumex Ltd., Tring, Herts., U.K.; Hypnorm: Janssen Pharmaceutical Ltd., Oxford, U.K.) and 200 μl of tail-vein blood collected and placed in cooled microfuge tubes. The blood was immediately centrifuged (14,000 r.p.m. for 3 min) and 100 μl of plasma frozen for the later measurement of plasma insulin concentrations.

After 28 days, rats were anaesthetized with pentobarbitone (30 mg kg^{-1}; Sagatal: Harlow, Essex, U.K.) and their insulin sensitivity measured using the hyperinsulinaemic-euglycaemic clamp technique, as previously described (Terretaz & Jeanrenaud, 1983; Upton *et al*., 1997). Human soluble insulin (Humulin-S, Eli Lilly and Company, Basingstoke, Hants, U.K.) in isotonic saline containing 1% (w/v) bovine serum albumin (Sigma Chemical Co.) was infused at a constant flow rate of 14 mU min^{-1} into the right jugular vein to produce hyperinsulinaemia. A 5% glucose solution in isotonic saline was co-infused with the insulin at variable rates to maintain euglycaemia. All rats were also infused with 6-[^{3}H]glucose (0.2 mCi min^{-1}; Amersham International, Little Chalfont, Bucks., U.K.) prior to the clamping procedure, as described previously (Upton *et al*., 1997), in order to measure whole-body glucose uptake and hepatic glucose production. At the end of the clamp, rats were sacrificed by cardiac exsanguination and the perirenal and epididymal (gonadal) fat pads dissected free and weighed. Final plasma glucose concentrations were measured using a standard kit (Boehringer Mannheim, Milton Keynes, Bucks., U.K.), and plasma insulin concentrations were measured using a radioimmunoassay kit (Pharmacia/Upjohn Diagnostics U.K., Lewes, Sussex, U.K.).

Statistical analyses

Differences in body weight, daily food and water intake, plasma insulin, blood glucose and glucose infusion rates during clamping between MCC-555-treated diabetic vehicle-treated diabetic and vehicle-treated non-diabetic rats were analysed using 2-way analysis of variance followed by Bonferroni modified *t*-tests for multiple comparisons.

Results

At the start of the experiment, pre-diabetic ZDF rats had significantly greater body weight, daily food intake and plasma insulin concentrations than non-diabetic controls (all $P<0.0001$; Figures 1 and 2). Pre-diabetic ZDF rats also displayed moderate hyperglycaemia, as compared to non-diabetic rats ($P<0.05$; Figure 2). At the end of the second week of the study, plasma insulin concentrations in the vehicle-treated pre-diabetic rats began to fall, as compared to their levels at the start of the study, becoming significantly lower by 32% and 52% at the end of weeks 3 and 4, respectively ($P<0.01$; Figure 2). These animals also became severely hyperglycaemic and polydipsic by day 10, as compared to vehicle-treated non-diabetic rats ($P<0.0001$ and $P<0.01$, respectively; Figure 2). As a result of the development of overt diabetic symptoms, the body weight gain in vehicle-treated diabetic rats was blunted by day 14, although the rats remained significantly heavier than non-diabetic control rats throughout the study ($P<0.0001$; Figure 1).

In contrast to vehicle-treated pre-diabetic rats, MCC-555-treated pre-diabetic rats maintained initial plasma insulin concentrations throughout the study ($P<0.01$). Concomitantly, the rise in hyperglycaemia and polydipsia in MCC-555-treated rats was attenuated, as compared with vehicle-treated diabetic rats (both $P<0.01$; Figure 2). MCC-555-treated diabetic rats continued to gain weight throughout the study, as did vehicle-treated non-diabetic rats, but MCC-555 did not alter daily food intake in diabetic rats, as compared with vehicle-treated diabetic rats ($P>0.05$; Figure 1). Gonadal fat pad mass was significantly greater in MCC-555-treated diabetic rats, as compared with vehicle-treated diabetic rats at the end of the study ($P<0.01$), whilst both gonadal and perirenal fat pad masses were significantly greater in both diabetic rat groups than in the non-diabetic rats ($P<0.01$; Figure 3).

Steady-state final glucose infusion rates during the hyperinsulinaemic-euglycaemic clamp were significantly greater in

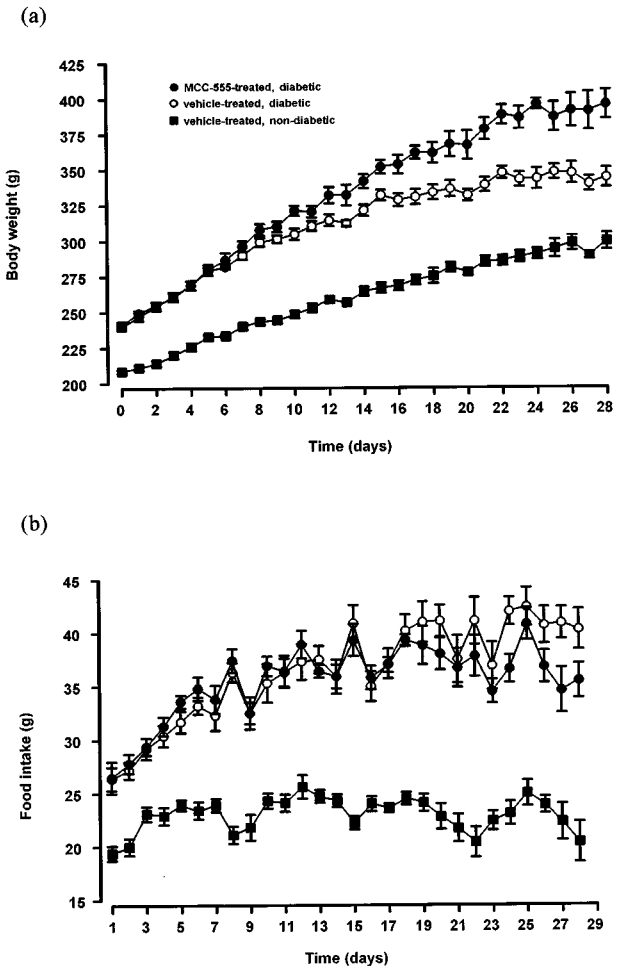

Figure 1 (a) Body weight (g) and (b) daily food consumption (g) in vehicle-treated IGT ZDF rats, MCC-555-treated IGT rats and vehicle-treated non-IGT rats over the 28-day experimental period. Data are shown as means ± s.e.mean for groups of nine rats.

the MCC-555-treated than in the vehicle-treated diabetic rats (diabetic = 0.06 ± 0.03 mg min^{-1}; MCC-555-treated = 0.72 ± 0.22 mg min^{-1}; $P<0.01$), demonstrating significant improvements in glucose sensitivity under hyperinsulinaemic conditions (+10.6%; $P<0.05$).

Basal hepatic glucose production (HGP) and whole-body glucose uptake (WBU) rates were significantly greater in diabetic ZDF rats, as compared to lean rats ($P<0.0001$). During the clamp, there was a significant suppression of HGP in both vehicle-treated lean and MCC-555-treated diabetic rats (both $P<0.01$). Under clamp conditions, HGP and WBU in vehicle-treated diabetic rats did not alter significantly from basal levels ($P>0.05$; Figure 4).

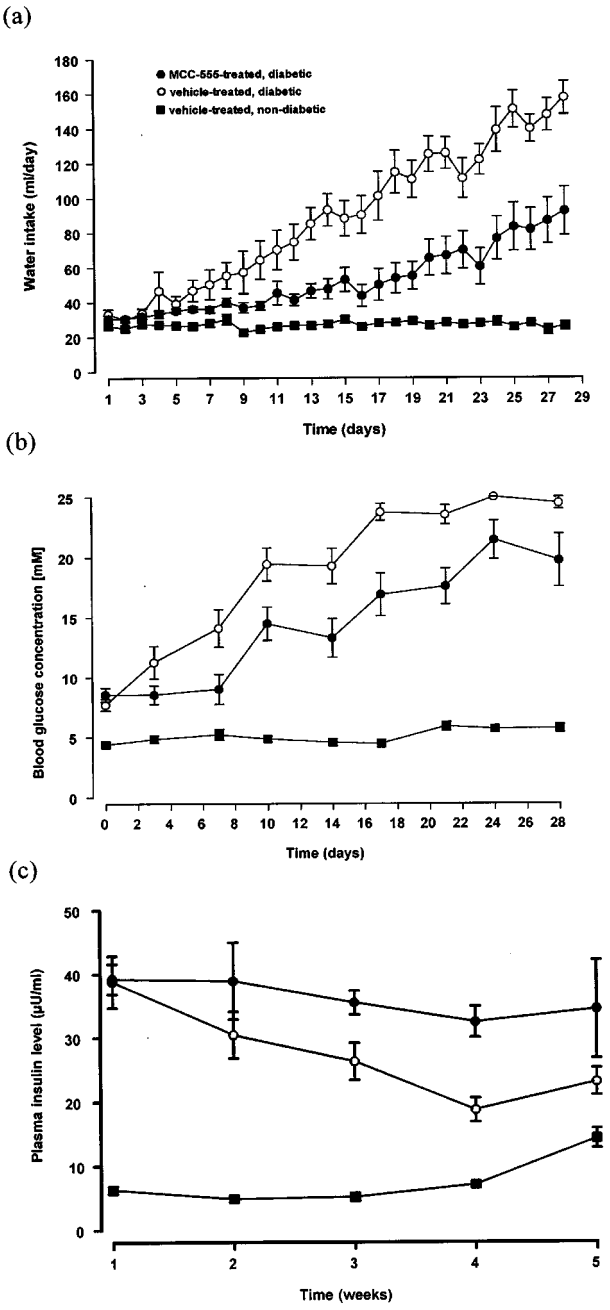

Figure 2 (a) Daily water intake (ml), (b) blood glucose (mmol l^{-1}) and (c) plasma insulin (μU ml^{-1}) concentrations in vehicle-treated IGT ZDF rats, MCC-555-treated IGT rats and vehicle-treated non-IGT rats over the 28-day experimental period. Data are shown as means ± s.e.mean for groups of nine rats.

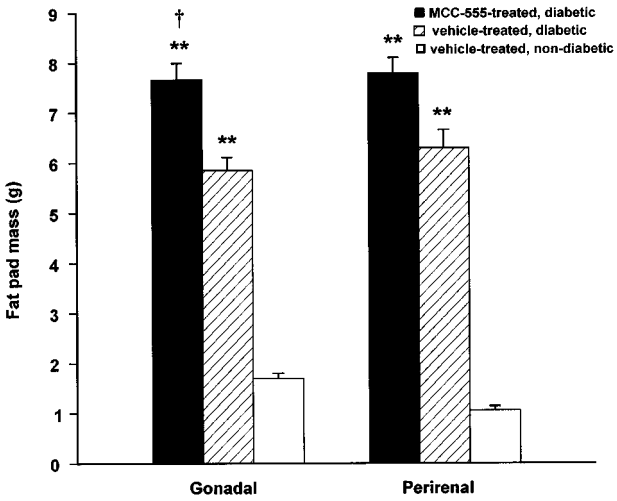

Figure 3 Gonadal and perirenal fat pad mass in MCC-555-treated diabetic rats, vehicle-treated diabetic rats and non-diabetic rats. Data shown as means ± s.e.mean for $n=9$. $^{**}P<0.01$ as compared to non-diabetic rats; $\dagger P<0.05$ as compared to vehicle-treated diabetic rats.

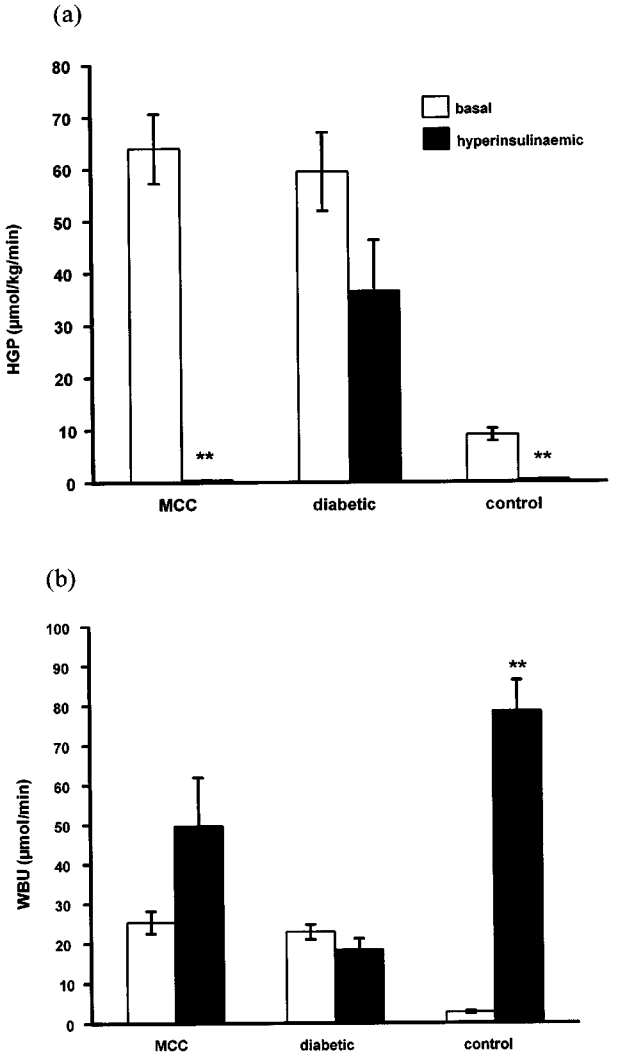

Figure 4 (a) Hepatic glucose production (HGP) and (b) whole body glucose uptake (WBU) in vehicle-treated IGT ZDF rats, MCC-555-treated IGT rats and vehicle-treated non-IGT rats under basal and hyperinsulinaemic conditions after 28 days of treatment. Data are shown as means ± s.e.mean for groups of nine rats. $^{**}P<0.01$ as compared to basal HGP or WBU.

Discussion

These data demonstrate that, as described previously (Clark & Palmer, 1982; Peterson, 1994), pre-diabetic ZDF rats displaying hyperinsulinaemia and moderate hyperglycaemia progress to β-cell failure and overt diabetes between 6 and 10 weeks of age. Overt diabetes is characterized by the development of severe hyperglycaemia (>20 mmol l^{-1}), polydipsia and attenuated body weight gain. We have also demonstrated that these diabetic ZDF rats have a reduced insulin sensitivity, as compared to their non-diabetic counterparts, as described previously in older diabetic ZDF rats (Upton *et al*., 1997).

MCC-555 treatment of young pre-diabetic rats, which display IGT, significantly improved insulin sensitivity, as shown by the hyperinsulinaemic-euglycaemic clamp technique. Improved metabolic status was evident in the return of insulin-sensitive inhibition of hepatic gluconeogenesis observed under hyperinsulinaemic-euglycaemic clamp conditions in the drug-treated group. This has also been shown previously in older diabetic ZDF rats (Upton *et al*., 1997). As a result of the sustained hyperinsulinaemia in the pre-diabetic MCC-555-treated rats, coupled with a partial restoration in insulin sensitivity, the development of overt diabetes was significantly attenuated in these rats, as demonstrated by the lower blood glucose levels, reduced polydipsia and continued increase in body weight gain and maintenance of body fat levels. The significant increase in gonadal fat pad mass in MCC-555-treated ZDF rats, as compared to vehicle-treated diabetic ZDF rats, is probably a consequence of three processes. Firstly, the partial restoration in insulin sensitivity results in increased glucose entry into adipose tissue with subsequent conversion to triglycerides. Secondly, there is attenuation of the lipolysis resulting from catabolic metabolic processes associated with overt diabetes. Thirdly, thiazolidinedione compounds are selective agonists for peroxisome proliferator-activated receptor gamma (PPARγ; Forman *et al*., 1995; Lehmann *et al*., 1995), through which they induce adipogenesis by regulating expression of adipocyte-specific genes (Harris & Kletzien, 1994). MCC-555-mediated attenuation of the overt diabetic symptoms, including the development of severe hyperglycaemia in young ZDF rats, has also been reported following treatment with two other thiazolidinediones, troglitazone (Sreenan *et al*., 1996) and BRL 49653 (rosiglitazone; Smith *et al*., 1997).

In conclusion, we have demonstrated that chronic treatment of young ZDF rats displaying IGT with the thiazolidinedione MCC-555 can attenuate the symptoms of overt diabetes through a combination of partial restoration of insulin sensitivity and maintenance of β-cell function. The probable mechanism for the maintenance of insulin levels is the alleviation of glucose toxicity (see Yki-Järvinen, 1997); i.e., lowering glucose will non-specifically improve β-cell function (which is impaired by high blood glucose). We suggest that MCC-555 may be of significant potential therapeutic value in humans with IGT in preventing or attenuating the development of type 2 diabetes, as has been demonstrated for troglitazone (Nolan *et al*., 1994).

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