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Troglitazone prevents insulin dependent diabetes in the non-obese diabetic mouse

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Abstract

Troglitazone has recently been introduced in the treatment of Type 2 diabetes. In addition to its anti-diabetic effects it acts as a perixosome proliferator activated receptor-gamma (PPAR-γ) agonist and has anti-inflammatory properties by inhibiting macrophage tumour necrosis factor-alpha (TNF-α) secretion. It also inhibits the production of endothelial selectin (e-selectin). Troglitazone also reduces interleukin-1α induced nitric oxide production in pancreatic beta-cells, which may be relevant in preventing nitric oxide mediated damage to these cells in the Type 1 diabetes process. We tested troglitazone in the spontaneous model of autoimmune diabetes, the non-obese diabetic (NOD) mouse, to determine its effect on the disease process. When administered by gavage from weaning at a dose of 400 mg/kg body weight ($n = 32$), troglitazone reduced the incidence of diabetes by 16 weeks compared to controls ($n = 32$) in a pattern that was maintained up to the conclusion of the experiment at 31 weeks of age ($p < 0.05$). Insulitis was unaltered (index $= 1.05 \pm 0.71$ vs. 1.13 ± 0.82, treated vs. controls, $p = 0.78$). The study was repeated using troglitazone in the diet of NOD mice ($n = 24$) to give a dose of approximately 200 mg/kg body weight in order to provide a more consistent level of troglitazone during the time course of the experiment. There was a reduction of diabetes incidence in this group but it did not reach significance. Insulin levels were reduced in gavage treated mice although such reduction did not reach significance ($p < 0.07$). We conclude that, in view of its effect on this model of autoimmune diabetes and because of its known function as an insulin sensitiser, troglitazone might be considered for potential use in those patients with Type 1 masquerading as Type 2 diabetes.

Keywords: NOD (non-obese diabetic) mouse; Troglitazone; Diabetes, type 1

1. Introduction

The non-obese diabetic (NOD) mouse is a spontaneous animal model of insulin dependent (Type 1) diabetes (IDDM) which develops features including hyperglycemia, polydipsia, polyuria and weight loss as well as histological findings in the pancreatic islets, resembling those found in humans with the disease (Makino et al., 1980; Tochino, 1986; Pozzilli et al., 1993). In both humans and NOD mice, hyperglycemia occurs when the majority of beta-cells have been destroyed, usually at 15–22 weeks of age in the mouse. Insulitis, the infiltration of the islets of Langerhans by mononuclear cells, is well-documented and is characterised by the presence of monocytes and lymphocytes which migrate from small blood vessels to surround and infiltrate the islets (Signore et al., 1989; Anderson et al., 1993; Signore et al., 1994). Because of the similarities in the pathogenesis of the disease in mouse and humans, the NOD mouse can be usefully taken as a model for testing preventive therapies for Type 1 diabetes (Bowman et al., 1994).

Perixosome proliferator activated receptor-gamma (PPAR-γ) agonists have recently been introduced in the therapy of Type 2 diabetes and deserve particular attention in the field of diabetes prevention because of their effect in reducing insulin resistance (Saltiel and Olefsky, 1996). The interest in such compounds, and in particular, troglitazone, is due to their effect in protecting islets from interleukin-1α cytotoxicity by inducing lymphopenia (Shima-

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bukuro et al., 1997). Troglitazone also inhibits tumour necrosis factor-alpha (TNF-α) which is a regulator of insulin resistance and is also involved in cytotoxicity to beta-cells (Hotamisligil and Speigelman, 1994). Moreover, an analogue of troglitazone has been shown to inhibit both TNF-α production in vivo (Hofmann et al., 1994) and TNF-α activity on adipocyte de-differentiation in vitro (Ohsumi et al., 1994). Therefore, a compound such as troglitazone may be of interest in the prevention of Type 1 diabetes due to its capacity to interfere with some of the potential mechanisms associated with the disease, namely islet inflammation and insulin resistance.

In this study, we examined the capacity of troglitazone to modify the natural course of the disease in NOD mice and studied its possible mechanism of action.

2. Materials and methods

The mice used in this study came from the NOD/Ba colony established in 1987 at St. Bartholomew's Hospital Medical College, London, UK and which was originally derived from Dr. E. Leiter's laboratory (Bar Harbor, ME, USA). There is a stable cumulative incidence of diabetes of approximately 60% in females and 15% in males at 30 weeks of age (Pozzilli et al., 1993). The colony is housed in a purpose-built area (Mansfield et al., 1992) and maintained strictly according to international (NIH, 1985) and UK (HMSO, 1986) guidelines for animal care.

2.1. Troglitazone by gavage

2.1.1. Diabetes incidence

Thirty-two female mice were given troglitazone at a final dose of 400 mg/kg body weight. Methyl cellulose (0.5%) was used as a carrier and the drug administered five times a week by gavage, from weaning at 3 weeks of age until the end of the study at 31 weeks of age. A further 32 age-, sex- and litter-matched mice were used as controls and received carrier alone.

2.1.2. Insulitis studies

Twenty-four female NOD mice were treated with troglitazone at a dose of 400 mg/kg body weight. Methyl cellulose (0.5%) was used as a carrier and the drug administered five times a week by gavage, from weaning at 3 weeks of age until 12 weeks of age, in order to determine the effect on the early stages of insulitis. A further 24 age-, sex- and litter-matched mice received carrier alone as controls.

A standard maintenance diet (RM1[E]-Special Diet Services, Witham, Essex, UK) was provided ad libitum, and the food and water intakes, as well as body weights of all animals, were measured weekly.

2.2. Troglitazone in diet

Twenty-four female NOD mice were given troglitazone in diet at a concentration of approximately 200 mg/kg body weight. Modified diet was given from weaning at

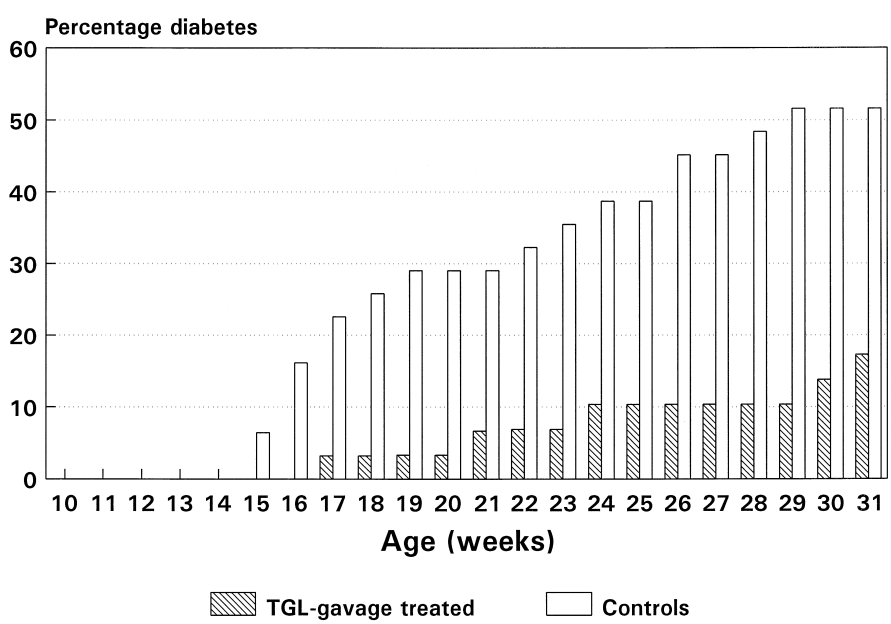

Fig. 1. Diabetes incidence in NOD mice given troglitazone by gavage. Thirty-two female NOD mice were given 400 mg/kg body weight troglitazone by gavage five times per week in 0.5% methyl cellulose carrier from 3 weeks of age onwards. Thirty-two age-, sex- and litter-matched animals received carrier alone as controls. From 16 weeks of age onwards, there were statistically fewer diabetics in the treated group compared with the controls ($p < 0.03$).

3–4 weeks of age until the conclusion of the experiment at 31 weeks of age. Twenty-four age-, sex- and litter-matched mice were used as controls, receiving unmodified diet.

In all experiments, mice were screened weekly for diabetes from 10 weeks of age by urinary glucose testing (Diabur-Test 5000, Boehringer Mannheim, Germany). The occurrence of diabetes was diagnosed on the detection of a repeated glucosuria equal to, or greater than, 56 mmol/l, diabetic animals were removed from the study. Diabetes was confirmed by a non-fasting blood-glucose level greater than 12 mmol/l at culling.

2.3. Histological studies

Mice treated by gavage from 3–12 weeks of age were culled and their pancreata removed, snap-frozen in liquid nitrogen and stored at −70°C until processed. Cryostat sections were prepared as follows: a 5 μm section was cut and put on a microscope slide. Approximately 300 μm were then cut and discarded, after which another section was cut and put on the slide. This was repeated until 10 sections per pancreas were obtained. This process allowed the identification of 10–30 islets per pancreas with the separation between sections resulting in each section containing different islets. For morphological analysis, sections were stained with haematoxylin, counterstained with eosin and examined in a 'blind' controlled manner at ×250 magnification. Islet infiltration was scored as: no infiltration (grade 0); peri-insulitis (grade I), where about 10% of the islet area was infiltrated by a peripheral ring of lymphocytes; medium (grade II) 10–50% or severe (grade III) >50% of islet area is infiltrated, respectively. The number of islets was noted for each pancreas and an index calculated by multiplying the number of islets in each category by the grade of infiltration (I–III) and adding these together. This was then divided by the total number of islets observed in all categories (0–III).

At culling all mice had a serum sample taken for insulin measurement by radioimmunoassay and for measurement of circulating troglitazone levels (Glaxo Research, Bio-Met Division, Herts, UK).

2.4. Troglitazone measurement

A sample of 50 μl and 50 μl of acetonitrile were mixed and the samples analyzed using High Performance Liquid Chromatography (HPLC) with a detection wavelength of 260 nm, all samples were analyzed in blind.

2.5. Insulin measurement

Insulin in serum was measured using a commercially available equilibrium assay (Incstar, Stillwater, MN, USA).

3. Results

3.1. Diabetes outcome

There was a significant reduction in diabetes incidence in gavage treated mice from 16 weeks of age onwards in the treated vs. the control group ($p < 0.05$—Chi-squared)

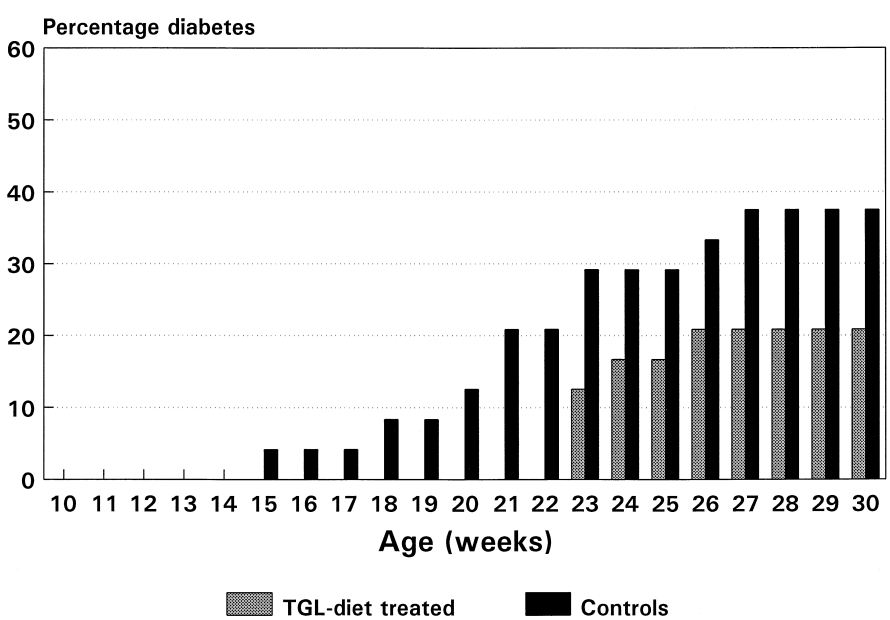

Fig. 2. Diabetes incidence in NOD mice given troglitazone in diet. Twenty-four female NOD mice were given troglitazone in diet at a dose of approximately 200 mg/kg body weight, from 3–4 weeks of age onwards. Twenty-four age-, sex- and litter-matched mice were used as controls, receiving unmodified diet. Although there was a reduction in diabetes incidence in the treated group compared with the controls, this did not reach significance ($p = 0.07$).

(Fig. 1). There was a tendency for a reduction in diabetes incidence in animals treated with dietary troglitazone but this did not reach significance (21 vs. 39% by 31 weeks, troglitazone vs. controls, p = not significant) (Fig. 2).

3.2. Insulitis

The index of insulitis of troglitazone treated animals was not statistically different from that of controls (1.05 ± 0.71 vs. 1.13 ± 0.82; p = not significant).

3.3. Troglitazone measurement

Detectable circulating levels of troglitazone were found. Levels of troglitazone in the serum of gavage and diet treated mice were 0.81 ± 1.5 and 3.7 ± 2.1 $\mu g/ml$, respectively.

3.4. Insulin

Levels of circulating insulin were not significantly reduced in either of the troglitazone treated groups with respect to their controls although in the troglitazone gavage treated animals insulin levels appeared lower. (Diet dosed animals 28.9 ± 8.5 vs. 27.0 ± 9.2 $\mu U/ml$, vs. controls, p = not significant; gavage dosed animals 29.6 ± 8.8 vs. 33.6 ± 16.2 $\mu U/ml$, vs. controls, $p = 0.07$).

4. Discussion

We have shown that troglitazone is able to prevent diabetes in NOD mice when given by gavage but not when administered in the diet. It is interesting to note that the levels of troglitazone in the serum of diet treated mice were much higher than in gavage treated mice, although the former received half the daily dose of the gavage group. As culling normally took place approximately 3 h after administration of the final bolus, this meant that the gavaged dose probably had time to be removed from the circulation to some extent and must have declined from the time of administration. The different effect of troglitazone on diabetes outcome could, therefore, be due to a difference in availability of the drug by the two delivery methods with the constant nibble-feeding pattern of mice allowing a higher dose to be continuously maintained, although paradoxically this did not reduce diabetes incidence. We suggest that a high peak dose of troglitazone might be more important than a constant level of the compound.

The pathogenesis of IDDM involves several processes where the immune system plays a major role as shown by the mononuclear cells infiltrating the islets, leading to the characteristic pattern of insulitis. A possible hypothesis to explain the effect of troglitazone is its binding and activation of the nuclear receptor PPAR-γ (Ibrahgimi et al., 1994). PPAR-γ receptors are expressed in a variety of haematopoietic cells, including neutrophils, peripheral blood lymphocytes and some leukemia cells (Greene et al., 1995). It has also been reported that prostaglandins of the J_2 sub-family and their metabolites, in particular 15-deoxy-delta^{12,14}-prostaglandin J_2, binds to, and activates, PPAR-γ (Kliewer et al., 1995). These observations are consistent with the suggestion of Yu et al. (1995), that the PPAR-γ sub-family of receptors is activated by eicosanoid-like molecules and may thus play a role in inflammatory processes.

The regulation of the immune system appears to be mediated mainly by two sub-sets of CD4 + lymphocytes, Th1 and Th2, and a dysregulation of this balance has been suggested to be important in the pathogenesis of Type 1 diabetes (Rabinovitch, 1995). It has been shown that a Th2 response, involving the production of cytokines such as interleukin-4, is characteristic of a non-destructive pattern of infiltration, whereas a Th1-like response, involving release of cytokines such as interferon-γ, is clearly involved in the progression to overt diabetes by destroying the insulin-producing cells. The drug troglitazone has a pattern of action that seems to stimulate the production of cytokines such as interleukin-4 or granulocyte-macrophage colony stimulating factor (GM-CSF) and also reduce interferon-γ production (Giorgini et al., 1997). Troglitazone, therefore, could act by altering a Th1 response to a Th2 type.

Preliminary studies with TNF-α, which involved the stimulation of endothelial cells, indicated that troglitazone can also down-regulate expression of e-selectin and other adhesion molecules such as Vascular Cell Adhesion Molecules (VCAM) and Endothelial Cell Adhesion Molecules (ECAM) (Sironi et al., 1986). E-selectin which is expressed on most mononuclear cells (Lasky, 1992; Lorant et al., 1993; Pozzilli et al., 1994) is also known to be involved in lymphocyte trafficking to some extranodal sites of inflammation, including the thymus, skin, and pancreas (Larson and Springer, 1990) and this could be a possible mechanism for troglitazone's interference with the inflammatory process leading to Type 1 diabetes. However, the fact that insulitis is similar in troglitazone treated and control groups suggests that the products of lymphocyte activation (e.g., released cytokines in the islets) may be different.

The recent withdrawal from use of troglitazone due to hepatotoxicity is unfortunate. However, it is likely that similar compounds will be introduced with the same mode of action but without harmful side effects.

In conclusion, although the precise role of troglitazone as an anti-inflammatory agent is unclear at present, there is an intriguing network of immunological connections which require further investigation. Nevertheless, troglitazone and other compounds of this type which may become available, do appear to be an interesting family of compounds to consider as agents to test for the prevention of Type 1 diabetes and this may prove to be especially of interest in

those patients with 'type 1 masquerading as type 2 diabetes' (Leslie and Pozzilli, 1994) as it also reduces blood-glucose levels.

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