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Dissociation of antihyperglycaemic and adverse effects of partial perioxisome proliferator-activated receptor (PPAR-γ) agonist balaglitazone

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ABSTRACT

Balaglitazone is a novel thiazolidinedione in clinical development for the treatment of type 2 diabetes. Common side effects associated with PPARγ receptor agonists are weight gain, oedema and adipogenesis. Balaglitazone is a selective partial PPARγ agonist and it has been speculated that such compounds have a more favourable safety margin than full agonists. We have compared impact of equi-efficacious antihyperglycaemic doses of balaglitazone with full PPARγ agonist rosiglitazone on body fluid accumulation, cardiac enlargement, and adipogenesis. Equi-efficacious antihyperglycaemic doses (ED_{90}) of balaglitazone (3 mg/kg/day) and rosiglitazone (6 mg/kg/day) were determined in male diabetic *db/db* mice. In adult male rats treated for up to 42 days, feeding, drinking, anthropometry, and plasma volumes were measured. Total plasma volume was measured with dye dilution technique. Compared to vehicle, rosiglitazone consistently increased food intake throughout the 42 day treatment period. In contrast, balaglitazone increased food intake in the last week of the experiment. However, both rosiglitazone and balaglitazone increased water intake. After 42 days, rosiglitazone treated rats displayed significantly elevated adiposity. Rosiglitazone increased total blood and plasma volumes throughout the treatment. Twenty-one days of balaglitazone treatment had no significant impact on blood or plasma volumes, whilst 42 days of balaglitazone increased plasma volume but to a significantly lesser extent than seen for rosiglitazone (vehicle: 46.1 ± 1.5; balaglitazone: 50.8 ± 1.21; rosiglitazone: 54.6 ± 1.6 ml/kg). Heart weight was significantly elevated only in rosiglitazone treated animals. At doses inducing comparable antihyperglycaemic control, the full PPARγ agonist, rosiglitazone, induces more pronounced body fluid retention and heart enlargement than seen for the partial PPARγ agonist, balaglitazone. Thus, partial agonists may pose safer alternative to current anti-diabetic therapy with full PPARγ agonist.

1. Introduction

Small molecule agonists acting at perioxisome proliferator-activated receptors (PPARs) have beneficial effects not only on peripheral insulin sensitivity but also on the cardiovascular system via mechanisms largely independent of their ability to lower blood glucose (Willson et al., 2001). Thus, PPARγ agonists lower blood pressure in both humans and in animal models of arterial hypertension (Diep et al., 2002; Ghazzi et al., 1997; Kaufman et al., 1995; Yoshioka et al., 1993). PPARγ activators also exert anti-inflammatory actions in the intimal lining of arteries (Duval et al., 2002), and together with anti-proliferative and anti-migratory influences on vascular smooth muscle cells PPARγ agonists are likely to reduce neointimal tissue proliferation after coronary intervention (Kintscher et al., 2000; Law et al., 1996). The therapeutic applicability of this knowledge was recently demonstrated in a small prospective, randomised, case-controlled study of the preventive effect of rosiglitazone upon restenosis after coronary stent implantation (Choi et al., 2004). Results from long-term cardiovascular outcome studies designed to study prevention or even reversal of atherosclerosis by PPARγ agonism have so far been ambiguous for pioglitazone (Charbonnel et al., 2004; Dormandy et al., 2005) and we are still awaiting the outcome of another long-term prospective study (RECORD) to assess potential preventive effects of rosiglitazone on cardiovascular events (Home et al., 2005).

However, despite these beneficial effects of PPARγ activators on the cardiovascular system, both clinically approved PPAR compounds and several of the new chemical entities currently being developed in

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the class have adverse effects influencing the cardiovascular system. Based on a meta-analysis of 42 clinical studies, it was recently suggested that short term use of rosiglitazone is associated with increased risk of cardiovascular events particularly in high risk populations (Nissen and Wolski, 2007). Both rosiglitazone and pioglitazone induce fluid retention, which can aggravate pre-existing heart failure (Gillies and Dunn, 2000). Based on summarised data from several clinical studies, it appears that rosiglitazone causes higher degree of fluid retention than pioglitazone as evidenced by lesser degree of haemodilution and oedema (Berlie et al., 2007). Not all patients display fluid retensive response to PPARγ treatment, but the serious perspective of aggravating chronic heart failure contra-indicates prescription of rosiglitazone and pioglitazone to type 2 diabetes patients with CHF classified as NYHA-II or worse.

The underlying mechanism determining the fluid retention is less well understood but recent studies in man and rat have led to the conclusion that renal sodium and water resorption occurring in response to lowered arterial pressure are likely to be significant contributors (Song et al., 2004; Zanchi et al., 2004). With the recent observation that PPARγ receptors in collecting ducts of the kidney are responsible for mediating PPARγ agonist induced expression of the epithelial Na^+ channel (ENaCγ) (Guan et al., 2005), a molecular target for PPARγ induced fluid retention has been identified. However, additional modes of action such as increased endothelial permeability and enhanced hydrostatic pressure in capacitance vessels may promote fluid retention and oedema. Because PPARγ agonists are notoriously known for their adipogenic effects, body weight gain is a poor parameter for drug induced fluid retention. Therefore, development of a reliable animal model of PPARγ induced fluid retention is required to substantiate the aforementioned hypotheses.

Agonists of the nuclear PPARγ receptor display differential physical interaction with the receptor which ultimately leads to variable levels of efficacy. Based on maximal efficacy in cell based transactivation assays, PPARγ agonists are classified as either full or partial agonists (Berger et al., 2003). From *in vivo* pharmacological experience it is has become evident that PPARγ induced end points are differentially sensitive to full and partial agonists. Thus, maximal *in vivo* insulin sensitising efficacy is obtained with PPARγ agonists inducing more than 30% of maximal efficacy, whereas the upper dynamic range of PPARγ agonists on adipogenesis and fluid retention is much closer to maximal *in vitro* transactivation efficacy (Berger et al., 2005). Therefore, it is has been proposed that partial PPARγ agonists possess improved safety margins compared to full PPARγ agonists and consequently much effort has been put in promoting partial PPARγ agonists for clinical development.

The aim of the current study was to compare side effects of equi-efficacious antihyperglycaemic doses of the partial PPARγ agonist balaglitazone with the full PPARγ agonist rosiglitazone. Monitored side effects were fluid retention, haemodilution, cardiac enlargement, and adipogenesis. To elucidate the underlying mechanisms of PPARγ weight gain we have developed a method to assess PPARγ induced fluid retention in rats (Wulff et al., 2004).

2. Methods

2.1. Animals

To assess equi-efficacious antihyperglycaemic doses of rosiglitazone and balaglitazone, glycaemic control was studied in male *db/db* mice (C57BL/KsBom-*db/db*, Taconic M&B A/S, Denmark). The *db/db* mice were 12–14 weeks of age with a mean body weight 47.6±2.3 g at the start of the experiment. Mice were housed at 23–24 °C, humidity at 60–70% and a normal 12 h/12 h light/dark cycle (lights on at 6:00 AM). All animal procedures were conducted according to Rheoscience A/S Animal Care approved protocols, and the experiments were done in compliance with internal animal welfare and national guidelines. The animals were allowed to adapt to the laboratory conditions for 1 week prior to the start of the study. Normal chow (Altromin 1324) and tap water were freely available in the home cages throughout the study.

To assess impact of PPARγ agonist therapy on body fluid and fat compartments, adult male Sprague Dawley rats weighing 290–320 g were used. Rats were housed under a normal light cycle (light from 0600–1800 h) at controlled temperature conditions with *ad libitum* access to chow (Altromin 1324) and water. Measurements of 24 h food and water intake were carried out bi-weekly on days −1, 6, 20, and 35 (final measurement of 24 h food intake on the numbered day). All animal experiments were conducted in accordance with Rheoscience bioethical guidelines, which are fully compliant to internationally accepted principles for the care and use of laboratory animals. The described rat experiments were covered by personal licenses to PJL issued by the Danish Committee for Animal Research.

2.2. Drugs

Balaglitazone (DRF2593) and rosiglitazone (Avandia) were synthesised in compliance with GMP principles at Novo Nordisk A/S (Bagsværd, Denmark).

2.3. Transactivation assay

The PPARγ transactivation profile of the compounds were assessed *in vitro* using a cell based assay containing human PPARγ subtype (Sauerberg et al., 2002). Briefly, the ligand binding domains of the human PPARγ receptor subtype as well as that of rat PPARγ (amino acids 167–469 (end)) were fused to the DNA binding domain (amino acids 1–147) of the yeast transcription factor Gal4. HEK293 cells were transiently transfected with an expression vector for the respective PPAR chimera along with a reporter construct containing five copies of the Gal4 DNA binding site driving expression of a luciferase reporter gene. All compounds were dissolved in DMSO and diluted 1:1000 upon addition to the cells. Compounds were tested in five concentrations ranging from 0.01 to 30 μM. Cells were treated with compound for 24 h followed by luciferase assay. EC_{50} values were calculated via nonlinear regression using GraphPad PRISM 3.02 (GraphPad Software, San Diego, CA).

2.4. Antihyperglycaemic effects

Antihyperglycaemic effects of balaglitazone and rosiglitazone were assessed in adult male diabetic *db/db* mice. At 14 weeks of age, animals were randomised according to fasting blood glucose into 11 groups (n=6). Mice were dosed orally once daily for 9 days with vehicle (0.2% carboxymethyl cellulose (CMC)+0.4% Tween-80 in saline) or increasing doses of either balaglitazone (0.1; 0.3; 1.0; 3.0; 10.0 mg/kg/day) or rosiglitazone (0.2; 0.6; 2.0; 6.0 mg/kg/day). After 7 days of treatment, plasma samples obtained in the morning (between 8:00 and 10:00 AM) were analysed for glucose and insulin. After 9 days of treatment, animals were exposed to an oral glucose tolerance test (OGTT; 3.0 g/kg). The resulting area under the curve was calculated for each of the doses.

2.5. Volume expansion

A preliminary experiment was conducted to assess the temporal profile of normalisation of fluid retention after cessation of treatment with rosiglitazone (3 mg/kg BID) for 21 days. In this experiment it was seen that plasma volume increased approximately 7% in rosiglitazone treated animals, and that volume expansion was partially reversed after 3 days and fully reversed after 7 days of discontinuation of rosiglitazone administration (data not shown).

Guided by these preliminary data, the following study design was implemented: Ninety rats were stratified by weight into 9 groups