Rapid Communication

Rosiglitazone and pulmonary oedema: an acute dose-dependent effect on human endothelial cell permeability

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Abstract

Aims/hypothesis. Peripheral and pulmonary oedema has emerged as the most common drug-related side effect of rosiglitazone in clinical practice, but the underlying mechanisms are not clear. Fluid retention and changes in vascular tone could contribute to oedema formation, but the interpretation of clinical and in vivo studies is particularly difficult and the direct effects of thiazolidinediones on endothelial barrier function have not been previously reported.

Methods. Human pulmonary artery endothelial cells were seeded and grown on 0.4 µm collagen-coated filters to form a tight monolayer (transendothelial electrical resistance 9–11 ohms·cm⁻² after 2–3 days). Transendothelial albumin flux (expressed as the percentage clearance of albumin relative to control) was measured using Evans blue-labelled albumin after exposure to rosiglitazone 1–100 µmol/l for 1 h to 48 h, and after removal of drug from the monolayer.

Results. Incubation of pulmonary artery endothelial cells with rosiglitazone for 4 h produced immediate

concentration-dependent increases in transendothelial albumin flux: e.g., relative to control (100%), 113%±13% (1 µmol/l), 215%±37% (10 µmol/l, $p=0.01$) and 461%±96% (100 µmol/l, $p=0.002$) ($n=12$). There was no effect after 1 h. The acute hyperpermeability response to rosiglitazone, maximal after 4 h, was fully reversible after washing the monolayer. After incubation for 24 to 48 h the effect of rosiglitazone on pulmonary endothelial permeability tended to subside: e.g., 210%±59% (24 h) for rosiglitazone 100 µmol/l ($p=0.06$).

Conclusion/interpretation. Exposure to high-therapeutic concentrations of rosiglitazone causes a reversible fourfold increase in pulmonary endothelial permeability which could be clinically relevant especially at higher doses and at times of peak plasma drug concentration. [Diabetologia (2003) 46:288–290]

Keywords Rosiglitazone, endothelial function, permeability, oedema, pulmonary, heart failure, thiazolidinedione.

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Abbreviations: EGM, endothelial growth media; HPAECs, human pulmonary artery endothelial cells; PPAR-γ, peroxisome proliferator activator receptor-gamma; TEAF, transendothelial albumin flux; TEER, transendothelial electrical resistance; VEGF, vascular endothelial growth factor (also known as vascular permeability factor).

Although hepato-toxicity was the major concern with first-generation thiazolidinediones, peripheral and pulmonary oedema has emerged as the most common serious adverse drug reaction with rosiglitazone and pioglitazone in current practice. For example, rapid weight gain and peripheral oedema have been attributed to fluid retention [1], and clinical features of heart failure, including pulmonary oedema and pleural effusions, are aggravated by thiazolidinediones [2, 3], although there seems to be no evidence of a direct negative effect on myocardial function. Surprisingly little is known about the aetiology, risk factors and treatment or avoidance of oedema associated with thiazoli-

dinediones but worldwide awareness and concern about this side effect has increased as a result of reports on spontaneous adverse drug reactions.

The mechanisms of peripheral and pulmonary oedema formation in patients treated with glitazones (especially those on insulin), are likely to be complex and multifactorial, but clinical and *in vivo* studies are not able to dissect the relative contributions of systemic changes in fluid balance and vascular tone from possible direct effects on regional endothelial barrier function. Clinical studies cannot easily dissociate the adverse effects attributable to the thiazolidinedione from numerous potentially confounding factors such as diabetes, insulin and blood pressure, which also affect oedema formation [4]. Thus, the purpose of this study was to investigate the direct effects of rosiglitazone on human pulmonary artery endothelial cell permeability to macromolecules using an established *in vitro* model.

Materials and methods

The method of Hippenstiel et al. [5], as reported previously by our group [6], was used to measure albumin flux across an endothelial monolayer. In brief, human pulmonary artery endothelial cells (HPAECs, Clonetics, Buckingham, UK) were trypsinised from tissue culture flasks, seeded onto 0.4 µm filter inserts pre-coated with collagen in 12-well plates (3×10^5 cells/insert) and grown in modified endothelial growth media (EGM-2, Biowhittaker, UK) until a tight monolayer was achieved (approximately 2–3 days). The integrity of the HPAEC monolayer was assessed by direct visualisation and measurement of transendothelial electrical resistance (TEER) using an Endohm chamber (World Precision Instruments, Stevenage, UK) [5, 6]. TEER of 10 ohms-cm² is consistent with tight intercellular adherence of the monolayer [5].

Rosiglitazone was applied to confluent HPAEC monolayers in varying concentrations (1 to 100 µmol/l) and incubated for 1 h, 4 h, 24 h and 48 h prior to measurements of transendothelial albumin flux (TEAF). To assess the reversibility of the rosiglitazone-induced hyperpermeability response, monolayers were thoroughly washed and reincubated with fresh media for a further 2 h prior to repeating the permeability assay.

Measurement of transendothelial albumin flux. The growth media (and test substance) bathing the monolayer was removed at the end of the incubation period, before the permeability assay started. The monolayer was then covered with HBSS containing an Evans Blue (0.67 g/l)-BSA (40 g/l) complex and incubated for 30 min. Thereafter, the fluid in the lower wells was agitated and sampled to measure the light absorbance at 610 nm [5, 6].

Measurements of TEAF are expressed as the mean percentage albumin flux across the endothelial monolayer (relative to control) $\pm$ SEM and data were analysed using a student's *t*-test with unequal variance. Statistical significance was at a *p* value of less than 0.05.

Results

Incubation of the HPAEC monolayers with rosiglitazone (1–100 µmol/l) for 4 h produced a concentration-dependent increase in TEAF; e.g. relative to control (100%), 113% $\pm$ 13% (rosiglitazone 1 µmol/l), 215% $\pm$ 37% (rosiglitazone 10 µmol/l, *p*=0.01) and 461% $\pm$ 96% (rosiglitazone 100 µmol/l, *p*=0.002) (Fig. 1). This acute hyperpermeability response to rosiglitazone exposure was fully reversible by washing the monolayer and incubating in fresh media (Fig. 1), indicating that irreversible toxicity or endothelial cell lysis was not the underlying mechanism.

Time-course studies were undertaken (Fig. 2). There was no effect of rosiglitazone on permeability after 1 h, and the effect of rosiglitazone on transendothelial albumin flux tended to subside after 24 h and 48 h of exposure: e.g. relative to control (100%), 210% $\pm$ 59% for rosiglitazone 100 µmol/l after 24 h (*p*=0.06) (Fig. 2).

Fig. 1. Concentration-dependent increase in transendothelial albumin flux after incubation for 4 h with rosiglitazone 1 µmol/l, 10 µmol/l and 100 µmol/l, relative to control (media without rosiglitazone). TEAF returned towards control values after washing rosiglitazone 100 µmol/l from the monolayers and incubating with fresh (glitazone-free) media for 2 h

Fig. 2. Time-dependent effect of rosiglitazone 100 µmol/l on pulmonary endothelial permeability. The hyperpermeability response was maximal after 4 h and was decreasing after 24–48 h in contact with the monolayer

Discussion

Considerable attention has been focused on the hepatic safety of thiazolidinediones, but increasing numbers of reports on spontaneous adverse reactions suggest that with rosiglitazone and pioglitazone the side-effects of peripheral and pulmonary oedema, especially in patients with known heart failure, have been underestimated. Very little is known about the underlying mechanisms. Fluid retention, haemodilution and weak vasodilator activity are associated with PPAR- γ agonists but there is no evidence of a direct negative inotropic effect on the heart and modest changes in fluid balance and vascular tone cannot fully explain the pattern of clinical observations. PPAR- γ receptors are heavily expressed on endothelial cells and seem to regulate endothelial-dependent vasodilator mechanisms [7] but the extent to which thiazolidinediones directly affect endothelial barrier function has not been clear.

Our study shows that direct exposure to rosiglitazone is associated with a marked concentration-dependent increase in human pulmonary endothelial cell permeability, an effect that was maximal after 4 h and was decreasing after 24 to 48 h. There is evidence showing that PPAR- γ agonists can cause endothelial cell apoptosis [8], but in this study the hyperpermeability effect of rosiglitazone was reversible by washing the drug off of the monolayer. Thus, cell lysis or nonspecific toxicity cannot explain these experimental observations. The underlying mechanism is unclear, and three possible pathways merit further investigation. Firstly, PPAR- γ stimulation increases gene expression and peptide production of vascular endothelial growth factor (VEGF, also known as vascular permeability factor) [9], which is a powerful permeability-inducing agent [6]. Secondly, it is possible that PPAR- γ -mediated effects on intracellular contractile proteins induce endothelial cell shape-change, intercellular gaps and increased leakage of macromolecules. Thirdly, rosiglitazone-induced changes in endothelial cell nitric oxide (NO) production or availability could increase endothelial permeability [10] as well as promoting vasorelaxation. The time-course of rosiglitazone's effect in these experiments, i.e. increased permeability at 4 h but not after 1 h, would be consistent with a PPAR γ -mediated mechanism, perhaps on VEGF production.

These results are potentially of clinical relevance. For example, the hyperpermeability response was most pronounced (a two- to fourfold increase) at concentrations of rosiglitazone (10–100 $\mu\text{mol/l}$) that correspond to the upper range of therapeutic plasma drug concentrations in routine clinical practice. Thus, a direct undesirable effect of rosiglitazone on endothelial barrier function could be clinically relevant at higher doses, or transiently over several hours when the pulmonary vasculature is exposed to peak plasma drug concentrations. In addition, the concentration-dependent and time-dependent effects of rosiglitazone on

endothelial permeability in the present study suggest that pulmonary oedema could be more common with dosing regimens that produce pharmacokinetic profiles with higher peak plasma drug concentrations (C_{max}); e.g. C_{max} is higher during steady-state treatment with 8 mg o.d. compared with 4 mg bid, but pharmacoepidemiology studies have yet to explore the relationship between dosing frequency and the adverse reports of pulmonary oedema.

Because heart failure has been reported more often among patients receiving combined insulin and glitazone treatment, it has been suggested that peripheral and pulmonary oedema are more likely to be caused by insulin. This study, however, clearly shows a direct effect of rosiglitazone on pulmonary endothelial permeability, independent of other *in vivo* variables such as glucose, insulin and blood pressure. Further studies are obviously required to extend these preliminary observations, e.g. to identify the mechanism of glitazone-induced pulmonary endothelial hyperpermeability and the extent to which this adverse effect might be positively or negatively influenced by insulin, other PPAR agonists and/or concomitant medications (e.g. ACE inhibitors) which might stabilise endothelial barrier function.

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