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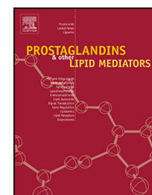

Original research article

In silico modelling of prostacyclin and other lipid mediators to nuclear receptors reveal novel thyroid hormone receptor antagonist properties

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Abbreviations:
MLS, MLS000389544
NSAID, non-steroidal anti-inflammatory drug
PPARβ/δ, peroxisome proliferator activated receptor
PGI_2, prostacyclin
PAH, pulmonary artery hypertension
T3, triiodothyronine
TRα, thyroid hormone α receptor
TRβ, thyroid hormone β receptor

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ABSTRACT

Prostacyclin (PGI_2) is a key mediator involved in cardiovascular homeostasis, acting predominantly on two receptor types; cell surface IP receptor and cytosolic peroxisome proliferator activated receptor (PPAR) β/δ. Having a very short half-life, direct methods to determine its long term effects on cells is difficult, and little is known of its interactions with nuclear receptors. Here we used computational chemistry methods to investigate the potential for PGI_2, beraprost (IP receptor agonist), and GW0742 (PPARβ/δ agonist), to bind to nuclear receptors, confirmed with pharmacological methods.

In silico screening predicted that PGI_2, beraprost, and GW0742 have the potential to bind to different nuclear receptors, in particular thyroid hormone β receptor (TRβ) and thyroid hormone α receptor (TRα). Docking analysis predicts a binding profile to residues thought to have allosteric control on the TR ligand binding site. Luciferase reporter assays confirmed that beraprost and GW0742 display TRβ and TRα antagonistic properties; beraprost IC_{50} 6.3×10^{-5} mol/L and GW0742 IC_{50} 4.9×10^{-6} mol/L. Changes to triiodothyronine (T3) induced vasodilation of rat mesenteric arteries measured on the wire myograph were measured in the presence of the TR antagonist MLS000389544 (10^{-5} mol/L), beraprost (10^{-5} mol/L) and GW0742 (10^{-5} mol/L); all significantly inhibited T3 induced vasodilation compared to controls.

We have shown that both beraprost and GW0742 exhibit TRβ and TRα antagonist behaviour, and suggests that PGI_2 has the ability to affect the long term function of cells through binding to and inactivating thyroid hormone receptors.

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1. Introduction

Prostacyclin (PGI_2) is a prostaglandin that was first described by Sir John Vane in 1976 as a potent vasodilator and anti-thrombotic agent of the vasculature [1]. The long term effects of PGI_2 is known to be important in different organ systems, for example PGI_2 is known to suppress airway remodelling in experimental asthma models [2], and has important anti-inflammatory effects in pulmonary artery and bronchi. PGI_2 has a very short half-life of 42 s, which makes direct pharmacological analysis difficult, although there are a large number of PGI_2 mimetics available such as iloprost, beraprost and treprostinil, which have half-lives of 30 min, 40 min and 4 h respectively. Prostanoid signalling is of key importance in the lung, as evidenced by the use of PGI_2 mimetics in pulmonary artery hypertension and non-steroidal anti-inflammatory drug (NSAID) induced asthma [3], and involvement in the development of asthma and COPD [4]. Lung diseases such as pulmonary artery hypertension (PAH) are treated with PGI_2 mimetics, which greatly improve the symptoms but fail to reverse or cure the vascular remodelling. The use of PGI_2 mimetics in the clinic has so far only attempted to replace the acute IP receptor mediated changes related to PGI_2, and have not yet attempted to mimic the genomic changes affected by PGI_2 binding to nuclear receptors. Before this

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can be attempted we need to address our understanding of how PGI_2 and mimetics signal via nuclear receptors.

PGI_2 has been described as the putative endogenous ligand for peroxisome proliferator activated receptor β/δ (PPARβ/δ) nuclear receptors [5]. Signalling via PPARβ/δ has a number of effects on cell function and has been shown to be involved in fatty acid metabolism, lipid transport as well as proliferation and differentiation of cells. There are a large number of PPARβ/δ agonists, both endogenously produced (PGI_2, fatty acids including the 'omega 3' fatty acids) and synthetic compounds (GW0742, GW501516), however the use of PPARβ/δ agonists in different cellular models and at widely differing concentrations has led to a controversy in regards to the role of PPARβ/δ in the cell [6]. The PPARβ/δ agonist GW501516 completed proof-of-concept clinical trials successfully for dyslipidaemia [7] and hypercholesteraemia [8], although further clinical trials were halted due to toxicology issues [9]. Since this point, much interest has gathered as to the use of PPARβ/δ agonists in various disease settings, as the nuclear receptor has multiple regulatory roles in cell function and differentiation from controlling smooth muscle tone to remodelling of the pulmonary circulation [9–11].

There are fundamental questions as to the long term direct actions of PGI_2 and related compounds on the cell and the wide variety of receptors which remain to be addressed. The aim of this study was to identify novel binding targets of PGI_2, and other lipid mediators using computational chemistry methods, validated on gene reporter assays and alterations to vascular function measured by myography.

2. Materials and methods

2.1. In silico methods

OpenVirtualToxLab .5,21 [12] was used to predict toxic potential by predicting binding affinities to 10 nuclear receptors. The default values of the software for the predictions of toxic potentials for prostacyclin, beraprost and GW0742 were used as described previously [12].

The Pharmmapper, freely available web server (http://59.78.96.61/pharmmapper), was used to predict potential target candidates for all drugs. The mol2 files for all molecules were submitted to the Pharmmapper server by using default settings and limiting the target set to human targets [13].

The ability of drugs to bind into protein active sites was investigated using Glide (Small-molecule Drug Discovery Suite 2014-3: Glide, Version 6.4 Schrödinger, LLC, New York, NY (2014)) with Maestro as a graphical user interface. The protein preparation wizard was utilized to adjust charges and protonation states of proteins using relevant data bank entries, 1Q4X for TRβ, 1NAV and 4LNX for TRα, and 3GZ9 for, PPARβδ. Prepared protein structures were used to build energy grids with enclosing boxes of default sizes centred on co-crystalized ligands. The molecules PGI_2, beraprost and GW0742 were docked flexibly using XP docking protocol; ligands were minimized onto OLSA-2005 non-bonded interaction grid, with all other parameters set to their default values.

2.2. Reporter assays

Human TRα, TRβ and glucocorticoid reporter (GR) assay systems were purchased from INDIGO Bioscience (State College, PA). Assays were performed according to the manufacturer's instructions for both agonist and antagonist activity. Briefly, reporter cells were dispensed into the wells of the assay plate and immediately dosed with L-triiodothyronine (T3), beraprost and GW0742. Following 24 h incubation at 37 °C, treatment media was discarded and the Luciferase Detection Reagent added. Light emission from each sample well was quantified using a plate reading luminometer.

In order to assess TRα and TRβ antagonistic activities the protocol was adjusted; Reporter Cells were exposed to a sub-maximal concentration of a suitable agonist: dexamethasone for the GR assay and T3 for TRα and TRβ assays, followed by increasing concentrations of GW0742 and beraprost.

2.3. Myography

Male Wistar rats (350–450 g) were housed in pairs, and killed by CO_2 asphyxiation. The care and use of the rats were carried out in accordance with UK Home Office regulations, UK Animals (Scientific Procedures) Act of 1986 under PPL70/6788.

First and second order mesenteric arteries were removed and prepared as described previously [14]. Briefly, artery segments were dissected in Kreb's buffer (pH 7.4, 1.18×10^{-3} mol/L NaCl, 4.7×10^{-3} mol/L KCl, 1.2×10^{-3} mol/L $MgSO_4$, 1.2×10^{-3} mol/L KH_2PO_4, 2.5×10^{-3} mol/L $CaCl_2$, 2.5×10^{-2} mol/L $NaHCO_3$ and 1.1×10^{-2} mol/L glucose), and loaded onto isometric wire myographs. The bath solution was continuously bubbled with 95% O_2 and 5% CO_2. All vessels were allowed to equilibrate for 30 min prior to being set at a 'normalised' internal circumference $0.9.L_{100}$; this is estimated to be 0.9 times the circumference they would maintain if relaxed and exposed to 100 mm Hg transmural pressure. This was calculated for each individual vessel on the basis of passive length-tension characteristics of the artery and the Laplace relationship [15]. The average diameter of the mesenteric arteries were 239.4 ± 10.9 μm.

Arteries were incubated with 10^{-5} mol/L MLS, 10^{-5} mol/L beraprost or 10^{-7} mol/L GW0742 for 30 min prior to addition of increasing concentrations of U46619 (10^{-9} mol/L to 10^{-6} mol/L). Arteries were washed four times with Krebs buffer, and once tone had returned to basal levels, arteries were incubated with MLS, beraprost and GW0742 for a further 15 min. Arteries were then pre-contracted with 3×10^{-7} mol/L U46619; once plataeu was acheived, vasodilation in response to increasing concentrations of L-triiodothyronine (T3; 10^{-10} to 3×10^{-7} mol/L) was measured.

2.4. Materials

All chemicals and reagents were obtained from Sigma Aldrich unless otherwise stated. Drugs were dissolved in DMSO to a stock of 10^{-2} mol/L, and then further dilutions made in water for further dilutions.

2.5. Data analysis

Reporter assay data are expressed as a mean ± SEM percent induction, with 100% induction defined as the activity achieved with 10^{-7} mol/L of T3.

Contractile data are expressed in changes in tension (mN) minus baseline tension. Vasodilation is expressed as percentage of the U46619 mediated pre-contraction obtained prior to the addition of T3. Data are presented as mean ± SEM; n refers to the number of rats used. Statistical analysis was performed by two way analysis of variance using GraphPad Prism 5.0, and differences were considered to be significant when P was less than 0.05.

3. Results

3.1. Virtualtox screening and docking programmes

Using the Virtualtox screening programme, structures for PGI_2, beraprost and GW0742 were assessed for the potential binding to a series of target protein known to be correlated with the side effects,

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