PGN 1091, A NOVEL 5-HT$_{2B}$ RECEPTOR ANTAGONIST FOR THE TREATMENT OF IRRITABLE BOWEL SYNDROME

R. Borman, A. Oxford, S. Denny, A. Sykes, S. Samra, A. Woodrooffe, M. Eagle, R. Coleman & K. Clark,
Pharmagene Laboratories Ltd, Orchard Road, Royston, SG8 5HD, UK

Introduction

Serotonin (5-HT) has been proposed to play a key role in the aetiology of irritable bowel syndrome (IBS). In a previous study, we have shown that 5-HT$_{2B}$ receptors are highly expressed in human colon, with predominant localisation on longitudinal smooth muscle and the myenteric nerves that control its motility (Figure 1). Stimulation of these 5-HT$_{2B}$ receptors results in a significant potentiation of neuronally mediated contractions (Figure 2), leading to smooth muscle hyper-excitability, and it has been proposed that this effect is key to the aetiology of IBS. In the present study we aimed to identify selective antagonists of this receptor, in the search for a novel treatment for the disease.

Methods

All samples of colon were obtained through medically qualified intermediaries with the informed consent of the donor, and with approval of the local ethics committee. Longitudinal muscle strips of human colon were mounted in organ baths, where they were electrically stimulated to induce neurally-mediated contractions. The ability of 5-HT to potentiate these contractions was then determined in the absence and presence of test compounds, in order to detect compounds that could inhibit the excitatory effects of 5-HT in human colon. The compounds that blocked the effects of 5-HT in this assay then underwent selectivity and ADMET profiling, in order to identify drug-like compounds that were suitable for development for the treatment of IBS.

Briefly, compounds were incubated in the presence of microsomes or hepatocytes from rat and human liver, to determine metabolic stability, intrinsic clearance and metabolic profile. Samples were analysed by LC-MS. In addition, the ability of compounds to induce or inhibit human CYPs was investigated. In toxicity studies using human hepatocytes, the effect of compounds on LDH leakage, cellular ATP content and MTT metabolism was determined, together with the effect on actin mRNA. In parallel, the bi-directional transport of compounds was assessed across Caco-2 cell monolayers, used as a surrogate marker for intestinal absorption.

In rat PK studies, the half life and oral bioavailability of compounds was determined after dosing by iv (1mg/kg) or oral (10mg/kg) routes. These studies were performed at BioDynamics Research Ltd, UK.

Results

Based on data generated from all compounds tested, PGN 1091 was identified as having a suitable candidate profile. The data from PGN 1091 are presented.

Pharmacology

At the human 5-HT$_{2B}$ receptor stably expressed in CHO-K1 cells (Euroscreen), PGN 1091 bound reversibly with high affinity resulting in a Ki of 3nM. The compound was assessed in two functional assays in order to demonstrate antagonism. PGN 1091 inhibited both total phosphate production and calcium mobilisation induced by 5-HT, with a pK_B of 8.6 in the total phosphate assay and pIC_{50} of 8.4 for inhibition of calcium responses.

Human Colon

In human colon, application of 5-HT has been shown to induce excitatory effects, resulting in potentiation of neurally-mediated contractions. PGN 1091 was shown to block these excitatory effects of 5-HT (Figure 3). The compound was a competitive antagonist with a pK_B of 8.0 (10nM).

Selectivity

PGN 1091 shows greater than 800-fold selectivity for the 5-HT$_{2B}$ receptor over both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, with Ki values in excess of 2500nM at both of these receptor sub-types. In addition, PGN 1091 shows little or no affinity (less than 50% inhibition of binding at up to 10µM) for a range of 84 receptors, ion channels and enzymes.

Metabolism

PGN 1091 showed good stability when incubated with human liver microsomes. In addition, in human hepatocytes, PGN 1091 showed low intrinsic clearance indicating good metabolic stability in human.

Absorption

PGN 1091 shows high permeability across Caco-2 cell monolayers, (with A:B > B:A transport) indicating good absorption. This was confirmed in rat *in vivo*, with acceptable bioavailability (30%) following oral dosing.

CYP induction/inhibition

PGN 1091 did not induce CYP gene expression or activity at concentrations up to 100µM. PGN 1091 did not inhibit human CYPs, and showed a Ki of greater than 20µM against CYP3A4 activity.

Toxicity

At concentrations up to 100µM, PGN 1091 had no effect on LDH leakage, cellular ATP content, MTT metabolism or cellular actin mRNA levels.

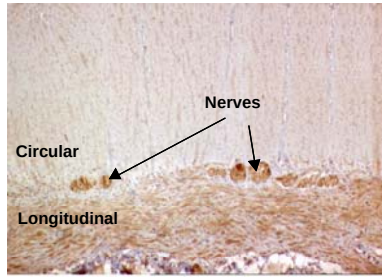

Figure 1. 5-HT$_{2B}$ receptor antibody staining in human colon. Figure shows intense staining in colon longitudinal smooth muscle and myenteric nerves.

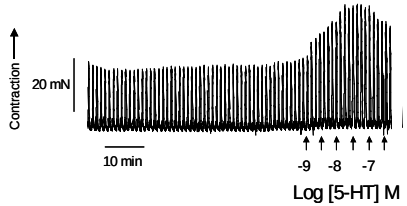

Figure 2. Effect of 5-HT on electrically-induced contractions in human colon smooth muscle. 5-HT causes potentiation of neurally-mediated contractions.

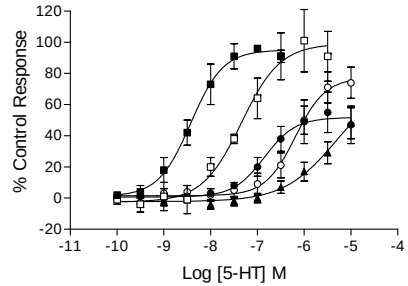

Figure 3. Effect of PGN 1091 on the excitatory effect of 5-HT in human colon smooth muscle. Increasing concentrations of PGN 1091 (0.1-3.0µM) cause a rightward shift of the concentration-effect curve to 5-HT

Summary

It has previously been proposed that antagonists at 5-HT$_{2B}$ receptors may be beneficial in the treatment of IBS. We have now identified a compound that is a potent and selective antagonist at this receptor in human colon, with a suitable *in vitro* ADMET profile, and propose that PGN 1091 is suitable for further pre-clinical development towards the treatment of IBS.

PGN 1091 Profile

- Competitive antagonist of the 5-HT$_{2B}$ receptor in human colon
  - Concentration-dependent inhibition of the excitatory effects of 5-HT
- Affinity at the 5-HT$_{2B}$ receptor of 3 nM and antagonist pK_B of 10nM in human colon
- Over 800-fold selectivity over 5-HT$_{2A}$ and 5-HT$_{2C}$
- No significant affinity for 84 other receptors, ion channels and enzymes
- Highly permeable across Caco-2 cells, acceptable bioavailability in rat *in vivo*
  - likely to be well absorbed orally
- Metabolically stable in human
  - Stable in presence of human liver microsomes
  - Low clearance observed in human hepatocytes
- No significant toxicity up to 100µM
- No significant CYP induction/inhibition
- Novel, “drug-like” compound – low MW (<300)

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