A Phase 2 Randomised Controlled Trial of Serelaxin to Lower Portal Pressure in Cirrhosis (STOPP)

Fiona J Gifford
Royal Infirmary of Edinburgh

Philip DJ Dunne
Royal Infirmary of Edinburgh

Graeme Weir
Royal Infirmary of Edinburgh

Hamish Ireland
Royal Infirmary of Edinburgh

Catriona Graham
The University of Edinburgh

Sharon Tuck
The University of Edinburgh

Peter C Hayes
Royal Infirmary of Edinburgh

Jonathan Fallowfield
The University of Edinburgh

Research

Keywords: Cirrhosis, portal hypertension, serelaxin

Abstract

Background: In preclinical models recombinant human relaxin-2 (serelaxin) had anti-fibrotic effects and ameliorated portal hypertension (PH). A small exploratory study in patients with cirrhosis also suggested that serelaxin could reduce portal pressure.

Methods: In a phase 2 double-blind randomised controlled study conducted in a single centre (Royal Infirmary of Edinburgh, UK), male and female adult participants with cirrhosis and clinically-significant PH (CSPH; hepatic venous pressure gradient (HVPG) >10 mmHg) were enrolled. Participants were allocated to serelaxin or placebo in a 3:1 ratio. The placebo control group was used to maintain the blind. The primary endpoint was the change from baseline in fasting HVPG after 2 h peripheral i.v. serelaxin infusion (80 µg/kg/day for 60 min followed by 30 µg/kg/day for at least 60 min). Secondary endpoints included the change from baseline in hepatic blood flow and systemic haemodynamics (cardiac index, systemic vascular resistance index and aortic pulse wave velocity). Short-term safety and tolerability of serelaxin was also assessed.

Results: A total of 17 participants were screened, 15 were randomised and 11 completed the study (n=9 serelaxin, n=2 placebo). Reasons for withdrawal were baseline HVPG <10 mmHg (n=2) and technical failure (n=2). Median age was 56 (range 43-69) and 73% of participants were male. Cirrhosis aetiologies were alcohol (n=10), non-alcoholic fatty liver disease (n=2), hepatitis C (n=2) and hepatitis B (n=1). Participants were Child-Pugh class A (60%) and B (40%) with a median Model for End-Stage Liver Disease score of 10 (range 6-14). Mean baseline HVPG was 16.3 mmHg (range 10.3-21.7). Individual responses were variable, but overall there was no statistically significant change in HVPG after 2 h i.v. serelaxin (mean±SD 0.4±3.5 mmHg; p=0.76). There were also no substantial changes from baseline in hepatic blood flow or systemic haemodynamic measures following serelaxin. We recorded 12 adverse events in 7 participants treated with serelaxin; none were significant, and most were unrelated to the investigational medicinal product. There were no serious adverse events.

Conclusion: In a small randomised phase 2 proof-of-concept study in patients with cirrhosis and CSPH, serelaxin infusion was safe and well-tolerated but had a neutral effect on HVPG.

Background

Standardised mortality rates for liver disease in the UK have increased 400% since 1970, and in people younger than 65 years have increased by almost 500%.[1] In patients with cirrhosis of the liver, portal hypertension (PH) is the main cause of death and of liver transplantation. In Europe alone it is estimated that 29 million patients suffer from chronic liver disease, and that 170,000 die each year from complications of cirrhosis, a number exceeding the mortality due to breast cancer.[2] Patients with an hepatic venous pressure gradient (HVPG) ≥10 mmHg (clinically-significant PH, CSPH) are at increased risk of hepatic decompensation[3] and of hepatocellular carcinoma.[4] Variceal bleeding occurs when the HVPG is >12 mmHg. A reduction in HVPG to <12 mmHg or by >20% from baseline are reported to improve

clinical outcomes and represent targets for haemodynamic response in interventional studies.[5] Despite a significant improvement in outcomes over the past 30 years, the average 6-week mortality of the first episode of variceal bleeding in most studies is reported to be up to 20%.[6]

Terlipressin, a synthetic analogue of vasopressin, has an immediate systemic vasoconstrictor action followed by portal haemodynamic effects due to slow conversion to vasopressin. It is the only pharmacological agent used in acute variceal bleeding that has been shown to reduce mortality in placebo controlled trials.[6] Terlipressin decreases failure of initial haemostasis by 34%, decreases mortality by 34%, and is considered a first-line treatment for bleeding oesophageal varices, when available. However, off-target effects include peripheral and coronary ischaemia, and adverse events (AEs) occur in 10–20% of patients.[7] Terlipressin is not licensed in the USA, where octreotide (a somatostatin analogue) is most commonly used. Octreotide is also thought to act as a mesenteric arterial vasoconstrictor, but in an acute haemodynamic study, octreotide was found to only transiently reduce HVPG and portal venous flow.[8] Nevertheless, octreotide has recently been shown to be as effective as terlipressin in the control of acute variceal bleeding.[9]

We have previously shown that serelaxin, a recombinant form of the human peptide hormone relaxin–2, had anti-fibrotic and portal hypotensive effects in cirrhotic rats.[10] Moreover, serelaxin reduced portal pressure by decreasing intrahepatic vascular resistance (IHVR) through augmentation of nitric oxide (NO) bioavailability and signalling, thus maintaining or enhancing hepatic blood flow. In a recent small exploratory open-label phase 2 study,[11] Part B showed that serelaxin induced a rapid and potentially clinically significant reduction in portal pressure in patients with cirrhosis, PH and a transjugular intrahepatic portosystemic shunt (TIPSS). Following at least 120 min of serelaxin infusion there was a 31.3% (95% CI −66.5, 71.6) reduction in the portal pressure gradient (PPG) compared to baseline. During the infusion there was a progressive reduction in the portal vein pressure (PVP) reaching a decrease of 25.2% (95% CI −12.7, 50.3) from baseline at the 120-min time point. The reduction in PVP started at 30 min and continued through to the 135-min time point. With serelaxin infusion, there were no newly occurring liver enzyme abnormalities, no clinically significant changes in blood pressure, and no discontinuations due to AEs. Indeed, in a separate study the pharmacokinetic and safety profiles of serelaxin were not affected in patients with mild, moderate or severe hepatic impairment.[12]

The objective of this double-blind, randomised placebo-controlled study was to evaluate the safety and efficacy of serelaxin in reducing portal pressure, as determined by HVPG in patients with cirrhosis and CSPH.

Methods

Study participants

We enrolled male or female adult participants over the age of 18 with cirrhosis and PH. Inclusion criteria included: ability to provide written informed consent and to understand and willingness to comply with

the requirements of the study; clinical/imaging-diagnosed or biopsy-proven liver cirrhosis of any aetiology; evidence of PH either on imaging or previous endoscopy (patients with large/grade 3 varices as identified by endoscopy within 6 months of screening had to be in an endoscopic band ligation program at the time of study entry); suspected HVPG ≥10 mmHg at baseline (if the baseline HVPG was subsequently found to be <10mmHg on direct measurement, the participant was withdrawn from the study). Exclusion criteria included: pregnancy or breast-feeding; women of child-bearing potential not using highly effective methods of contraception; severe liver failure defined by one of the following: prothrombin activity < 40%, bilirubin > 5 mg/dL (85 μmol/L), hepatic encephalopathy > grade I; presence of any non-controlled and clinically significant disease that could affect the study outcome or that would place the patient at undue risk; history of variceal bleed within 1 month prior to visit 1; hepatocellular carcinoma or history of malignancy of any organ system (other than localised basal cell carcinoma of the skin) treated or untreated; portal vein thrombosis; previous surgical shunt or TIPSS; current use of beta-blockers or nitrates, or any other drug therapy known to have an influence on portal pressure (diuretics were permitted provided patients had been on a stable dose for at least 30 days); history of drug or alcohol abuse within 1 month of enrolment; sitting systolic blood pressure <110 mmHg at screening visit or within 10 min prior to starting study drug infusion; use of other investigational drugs within 5 half-lives of enrolment, or within 30 days/until the expected pharmacodynamic effect had returned to baseline, whichever was longer; significant arrhythmias, which included any of the following: sustained ventricular tachycardia, bradycardia with sustained ventricular rate < 45 beats per min or atrial fibrillation/flutter with sustained ventricular response of > 90 beats per min at rest, or long QT syndrome or QTc > 450 msec (QT correction performed using the Fredericia correction method: QTcF = QT/RR0.33) for males and > 460 msec for females at screening visit 1; documented hypersensitivity to i.v. contrast agents and/or iodine; severe renal impairment (eGFR < 30 mL/min/1.73 m^2); significant left ventricular outflow tract obstructions (e.g., severe valvular aortic stenosis, obstructive cardiomyopathy), severe mitral stenosis, restrictive amyloid cardiomyopathy, acute myocarditis; severe aortic or mitral regurgitation for which surgical or percutaneous intervention was indicated; major neurologic event including cerebrovascular events, within 30 days prior to screening; clinical evidence of acute coronary syndrome currently or within 30 days prior to enrolment; history of hypersensitivity to study drug serelaxin or study drug ingredients; inability to follow instructions or comply with follow-up procedures; pacemaker, cardiac resynchronisation device or implantable cardioverter-defibrillator *in situ.*

Data collection

This was a single-site study, undertaken at the Royal Infirmary of Edinburgh (RIE), Edinburgh, UK between October 2017 and August 2018.

Participants attended the RIE Clinical Research Facility (RIE-CRF) for screening (visit 1) consisting of physical examination, blood tests (full blood count, coagulation and biochemistry), electrocardiogram (ECG), blood pressure measurement, and written informed consent. Randomisation was performed once it was known that the participant had passed screening, prior to the study visit.

On the study day (visit 2; ≤7 days after the screening visit), eligible participants attended the RIE-CRF and had baseline haemodynamic measurements performed, following an overnight fast and the avoidance of caffeine for >8 h. After baseline evaluation and confirmation of HVPG ≥10 mmHg, participants received (in a double-blind fashion) either serelaxin or placebo. The haemodynamic measurements were repeated at specified time points. A peripheral blood sample was taken at baseline and after 2 h, processed, and stored for potential future analysis. After the post-treatment assessments, participants were observed for a recovery period of 4 h which included repeat physical examination, blood pressure, ECG measurement and routine laboratory blood tests.

Study design and randomisation

This study was a phase 2, double-blind, randomised controlled trial to investigate the effects of serelaxin on PH in patients with cirrhosis. The control group was used to maintain the blind and provide information to aid the design of future studies and no statistical comparison between treatment and control groups was planned.

Randomisation was carried out after it was confirmed that the participant had passed screening, prior to the study visit (visit 2). The randomisation service was carried out by the Edinburgh Clinical Trials Unit (ECTU), allowing researchers and participants to remain blinded to treatment allocation. Random sequences of block sizes were generated by computer to achieve a 3:1 allocation ratio between serelaxin and placebo; there was no stratification to this allocation. Pharmacy prepared the appropriate treatment after randomisation.

Sample size

The primary efficacy endpoint was the decrease in fasting HVPG between baseline and 2 h post serelaxin treatment, targeting for a 20% reduction. The sample size calculation was based on a previous study in Edinburgh evaluating carvedilol[13] and the data from the previous Novartis-sponsored serelaxin phase 2 study (ClinicalTrials.gov Identifier: NCT01640964). Assuming a mean baseline HVPG of 16.37 (SD = 2.14) mmHg and post-baseline HVPG of 13.1 (SD = 3.91) mmHg (20% decrease), the change from baseline in HVPG was estimated to be 3.3 (SD = 4) mmHg. A sample size of 14 participants in the serelaxin group would provide 80% power to detect at least a 20% decrease from baseline in HVPG using a two-sided paired *t*-test with alpha level 0.05. A small number of placebo-treated patients were included in order to preserve double-blindness, not as a comparison group. Therefore, it was proposed that a total of 20 patients (15 serelaxin and 5 placebo) would be randomised in a 3:1 ratio.

Intervention

Recombinant human relaxin−2 (serelaxin (Novartis Pharmaceuticals, UK)) or placebo (20 mM sodium acetate buffer solution at pH 5.0 (Novartis Pharmaceuticals, UK)) were administered via peripheral i.v. infusion at two different infusion rates: 80 µg/kg/day for 60 min followed by 30 µg/kg/day for at least 60 min (until completion of the final HVPG/ICG measurements). This was achieved by a single infusion bag

with a change in the administration rate. The placebo had an appearance identical to serelaxin to achieve blinding.

Study Endpoints

The primary endpoint was the change from baseline in fasting HVPG after 2 h serelaxin infusion. HVPG was measured as previously described.[13] The procedure was performed after overnight fast and at the same time of day due to circadian variation in HVPG measurements. Prior to catheter insertion, participants were offered low dose (≤ 0.02mg/kg) midazolam to reduce any anxiety. A 7F venous introducer was inserted into the right femoral vein using the Seldinger technique under ultrasound guidance. A balloon-tipped catheter was then advanced into a hepatic vein using fluoroscopy. The free hepatic venous pressure (FHVP) was measured with the balloon deflated and floating freely in the hepatic vein close to its junction with the inferior vena cava (IVC). The wedged hepatic venous pressure (WHVP) was measured with the balloon inflated until the branch of hepatic vein was completely occluded. HVPG was obtained by subtracting the FHVP from the WHVP. All measurements were performed in triplicate and permanent tracings were printed, stored, and read blindly at the end of the study prior to the opening of the randomisation codes. HVPG was measured at baseline, then repeated after 60 min and 120 min of the IMP (either serelaxin or placebo). Baseline HVPG ≥ 10mmHg confirmed the presence of CSPH. If an HVPG < 10mmHg was obtained the study participant was withdrawn. The IVC pressure (IVCP) was measured at baseline and after the final HVPG measurement.

Secondary endpoints included: the change from baseline in fasting HVPG after 1 h serelaxin infusion; the change from baseline in fasting hepatic blood flow (HBF) after 2 h serelaxin infusion (measured from the concentration of indocyanine green (ICG) in the hepatic venous blood vs peripheral venous blood using the Fick Principle); the change from baseline in IVCP after 2 h serelaxin infusion; the change from baseline in cardiac index (CI) after 2 h serelaxin infusion; the change from baseline in systemic vascular resistance index (SVRI) after 2 h serelaxin infusion; the change from baseline in aortic pulse wave velocity after 2 h serelaxin infusion; safety and tolerability of serelaxin infusion (as assessed throughout the study by monitoring AEs, clinical laboratory blood tests, heart rate, blood pressure and ECG); change from baseline in exploratory blood biomarker measurements after 2 h serelaxin infusion (if a demonstrable effect on HVPG was observed).

The total HBF was calculated using the ICG constant infusion method[14,15] and derived from measurements of ICG clearance and extraction. Baseline serum samples were taken prior to each ICG infusion. Thereafter, 10 mg of ICG (10 mL) was given as a slow i.v. bolus via a peripheral cannula, followed by an infusion of 0.2 mg/min (0.2 mL/min or 12 mL/h) by accurate infusion pump (Alaris Asena, Becton Dickinson, USA). After an equilibrium period of at least 40 min, samples were taken simultaneously from the right hepatic vein (via the catheter tip) and the femoral vein (via the side port of the introducer). Paired samples (hepatic and femoral) were taken in triplicate, 2 min apart in order to confirm equilibrium. The HBF was measured in this way both at baseline and after 120 min of IMP infusion.

Cardioscreen 1000 (Medis, Germany) was used for non-invasive measurement of cardiac output (CO = heart rate x stroke volume/1000, L/min), CI (L/min/m^2) and systemic vascular resistance index (SVRI, dyne s/cm^5/m^2) by the bio-impedence technique before and after 120 min of IMP infusion. Similarly, arterial function was measured using an Arteriograph device (TensioMed™, Hungary). Arterial stiffness and central haemodynamics were assessed by the application of an inflatable cuff to the upper arm. Aortic pulse wave velocity (APWV, m/s) was calculated as the distance the pulse wave travels in the aorta (as measured from the suprasternal notch to pubic bone) divided by the measured transit time.

Participants were monitored for 4 h after IMP discontinuation and removal of the catheter and introducer from the femoral vein. Vital signs were recorded every 30 min throughout the infusion and recovery periods, with regular ECG monitoring. Adverse events (AEs) were collected during visit 2 and by follow-up telephone calls at 24 h and 4 weeks. The severity, expectedness and causality of AEs in relation to the study medication were noted by the study team.

Statistical analysis

Summary statistics (n, mean, SD, median, min, max, Q1 and Q3) were generated over time for the baseline, post-baseline and change from baseline measurements for the primary endpoint in the serelaxin and placebo group. The geometric mean was calculated for the baseline value, post-baseline values, and for the ratio to the baseline values. Confidence intervals were calculated for both the arithmetic and geometric means. Paired *t*-tests were used to test the mean change from baseline measurements. The secondary endpoints were subjected to the same analysis as the HVPG.

The placebo control group was used to maintain the blind. We present the baseline to 2 h change in the same way as the primary outcome although as this has not been powered for, no direct statistical comparison was made between serelaxin and placebo.

All participants were analysed in the group to which they were originally assigned irrespective of the treatment received with the exception of AEs which are presented according to allocated treatment and also treatment received. For all analysis unless otherwise specified statistical significance was taken to be p<0.05.

Any missing data as a consequence of the participant not having post-baseline measurement was not imputed and participants with missing post-baseline data were excluded from the analysis at that time point. The number of participants who withdrew during the course of the study is presented broken down by treatment allocation and presented with reasons for withdrawal where available.

Results

Participant flow

Participant disposition is shown in the Consort diagram (Fig. 1). Between 19th October 2017 and 31st August 2018 a total of 17 participants were screened. Of these, 2 had a screening failure and did not proceed to randomisation. Fifteen patients were randomised and 11 completed the trial (n = 9 serelaxin, n = 2 placebo). Reasons for withdrawal were baseline HVPG <10 mmHg (n = 2) and HVPG technical failure (n = 2). The 4 participants who withdrew following randomisation did not receive any study drug and are not included in any primary or secondary analysis. This study was terminated before the recruitment target was met due to a global drug supply issue.

Baseline participant data

Participant characteristics are summarised in Table 1. The median age of participants was 56 (range 43–69) and 73% were male. Cirrhosis aetiologies were alcohol-related liver disease (n = 10), non-alcoholic fatty liver disease (n = 2), chronic hepatitis C (n = 2) and chronic hepatitis B (n = 1). Participants were Child-Pugh class A (60%) and B (40%) with a median MELD score of 10 (range 6–14). The mean baseline HVPG was 16.3 mmHg (range 10.3–21.7).

Primary outcome

The primary outcome was to examine if the baseline to 2 h change in fasting HVPG was clinically significant. In those allocated to the serelaxin arm (n = 11), 2 participants were withdrawn. The (geometric) mean ±SD HVPG at baseline was 15.6 ±3.3 mmHg compared to 15.1 ±4.3 mmHg after 2 h serelaxin infusion. Although individual responses were variable (Fig. 2), there was no evidence of a significant change between baseline and 2 h change in fasting HPVG (p = 0.68). In the 2 placebo-treated patients HVPG decreased over the 2 h observation period, but no further analysis or conclusions can be made in such a small sample (Table 2).

Secondary Outcomes

There was no evidence of a significant change in fasting HVPG between baseline (15.6 ±3.3 mmHg) and 1 h of serelaxin infusion (15.8 ±2.1 mmHg; p = 0.63). However, 5 participants did not have HVPG measurements taken at the 1 h timepoint. This was due to a decision by the study team to focus efforts on maintaining the catheter position for the critical 2 h HVPG measurement (primary outcome).

For all of the other secondary outcomes, that were measured after 2 h serelaxin infusion (including HBF, IVCP, CI, SVRI and APWV), no substantial changes were observed (data summarised in Table 2).

Safety and tolerability of serelaxin

Treatment with serelaxin was well-tolerated. Overall, 12 AEs were reported in 7 participants treated with serelaxin (Table 3). None were serious or considered to be related to the IMP. There were no serious adverse events (SAEs) in this study. No pregnancies were reported. There were no striking changes in laboratory blood tests monitored during the course of the study (Supplementary Fig. 1); in particular, there were no newly occurring liver enzyme abnormalities observed. According to the product label,

hypotension is a potential side effect of serelaxin. Patients with cirrhosis and PH often have lower baseline blood pressure levels, predominantly due to severe splanchnic vasodilatation. Following serelaxin, we observed a statistically significant increase in heart rate (baseline 65 ±8 bpm vs 2 h post serelaxin 72 ±8 bpm, p = 0.02) and decrease in mean arterial pressure (baseline 93 ±7 mmHg vs 2 h post serelaxin 88 ±5, p = 0.02) due to a reduction in diastolic rather than systolic blood pressure (Supplementary Table 1). However, changes were not clinically significant and there were no discontinuations due to tachycardia or hypotension.

Discussion

The aim of the STOPP study was to investigate the safety and efficacy of the vasoactive peptide molecule serelaxin (recombinant human−2 relaxin) in reducing portal pressure, as determined by the HVPG in patients with compensated cirrhosis and PHCSPH. It is important to note that the trial was terminated before the recruitment target was met; consequently, although serelaxin had a neutral effect on HVPG in the treated sample, low statistical power increases the probability of a type II error.

PH is the strongest predictor of decompensation and death in patients with compensated cirrhosis[16] and the major driver for serious complications such as variceal bleeding, ascites and hepatic encephalopathy. At present, non-selective beta-blockers, vasopressin analogues and somatostatin analogues are the mainstay of drug treatment for PH, but these strategies are suboptimal and only target splanchnic hyperaemia. New therapeutic options, particularly drugs that reduce increased intrahepatic vascular resistance in cirrhosis are needed. In preclinical models, serelaxin decreased portal pressure through an increase in intrahepatic nitric oxide (NO) signalling and a reduction in hepatic stellate cell contractility.[10] In an initial small exploratory open-label phase 2 study, serelaxin induced a rapid and potentially clinically-significant reduction in portal pressure in patients with cirrhosis, PH and a TIPSS.[11]

A consistent finding in this (and previous) studies is the good safety profile of serelaxin in patients with cirrhosis and PH. With 2 h of serelaxin infusion, there were no newly occurring liver enzyme abnormalities, no clinically-significant changes in blood pressure, and no discontinuations due to AEs. Additionally, in a separate study the pharmacokinetic and safety profiles of serelaxin were not affected in patients with mild, moderate or severe hepatic impairment.[12] In contrast, terlipressin is associated with a high risk of serious (particularly ischaemic) complications.[17]

Pathophysiological mechanisms of PH differ in patients with mild PH (HVPG >5 but <10 mmHg) compared to those with CSPH (HVPG > 10 mmHg).[18] In mild PH the main mechanism driving raised portal pressure is increased intrahepatic vascular resistance, while in those with CSPH/varices increased portal blood flow plays a substantial role in perpetuating and exacerbating the portal hypertensive state. These pathophysiological differences can influence drug efficacy, depending on the stage of disease and the predominant mechanism of action. For example, patients with mild PH have a significantly lower response to non-selective beta-blockers, which reduce portal inflow, compared to those with CSPH/varices who exhibit a hyperdynamic systemic circulation.[19]

In this study, serelaxin had a neutral effect on HVPG and a range of secondary haemodynamic endpoints in a population of patients with HVPG > 10mmHg. It is possible, given the proposed mechanism of action of serelaxin in cirrhosis (decreased intrahepatic vascular resistance secondary to increased NO bioavailability), that it may have a more pronounced effect on portal pressure in patients with mild PH. We recruited patients with HVPG > 10 mmHg because these individuals are at most risk of decompensation and a decrease in portal pressure in this population would potentially lead to a reduction in clinically-meaningful endpoints (e.g. development of varices, variceal bleeding and ascites).

The acute haemodynamic effects of vasoactive drugs (e.g. propranolol, terlipressin, octreotide) on portal pressure have generally been demonstrated within 20 min after i.v. administration.[8,20] Here, serelaxin was administered over a relatively short time-frame (2 h), at least in part because rapid changes in visceral blood flow had been observed in a previous exploratory study in a similar patient population (ClinicalTrials.gov Identifier: NCT01640964). However, for drugs acting on intrahepatic vascular resistance, previous studies have been much longer (e.g. simvastatin significantly decreased HVPG after 28 days of oral administration).[21] So, it is conceivable that potential changes in HVPG due to a reduction in intrahepatic vascular resistance and/or anti-fibrotic/anti-inflammatory mechanisms were not captured after only a short serelaxin infusion. Whether any portal pressure reducing effect of serelaxin might be demonstrated after prolonged administration would need to be verified in a longer, adequately designed study, if formulation or half-life issues can be resolved to enable chronic exposure to serelaxin (or other RXFP–1 agonist).

Limitations of the study

The main limitation is that the study was terminated before the recruitment target was met due to slow enrollment and ultimately a global drug supply issue (Novartis had stopped manufacturing serelaxin and there was none available with a shelf-life beyond 31st August 2018). Therefore, based on the sample size calculation, the study is underpowered to detect the primary endpoint. The study was double-blind, placebo-controlled which would have addressed potential sources of bias. A formal dose-ranging study of serelaxin in cirrhosis patients has not yet been undertaken. We used the same infusion regimen that had previously shown encouraging haemodynamic effects[22] and had achieved similar steady-state serum concentrations to that observed in our 72 h rat cirrhosis models[10] and in human heart failure following 48 h i.v. serelaxin infusion.[23] However, the biological effects of relaxin are known to follow a U-shaped dose-response curve[24] and we do not know if serelaxin might have induced more pronounced effects on HVPG or secondary haemodynamic endpoints at higher (or lower) doses. Future work should address dose-response relationships.

Conclusions

In summary, this exploratory randomised study showed that an i.v. infusion of serelaxin for 2 h was safe but had a neutral effect on portal pressure in patients with cirrhosis and CSPH (HVPG > 10 mmHg).

Future studies might evaluate the acute effect of serelaxin on mild PH (HVPG 5–10 mmHg) and/or the effect of chronic administration of serelaxin on hepatic fibrosis and portal pressure.

List Of Abbreviations

AE, Adverse Event

APWV, Aortic Pulse Wave velocity

BP, Blood Pressure

bpm, beats per minute

CI, Cardiac Index

(e)CRF, (Electronic) Case Report Form

CSPH, Clinically-Significant Portal Hypertension

ECG, Electrocardiogram

FHVP, Free Hepatic Venous Pressure

GCP, Good Clinical Practice

HR, Heart Rate

HVPG, Hepatic Venous Pressure Gradient

ICG, Indocyanine Green

IMP, Investigational Medicinal Product

IVCP, Inferior Vena Cava Pressure

PH, Portal Hypertension

PV, Portal Vein

SAE, Serious Adverse Event

SVRI, Systemic Vascular Resistance Index

WHVP, Wedged Hepatic Venous Pressure

Declarations

Ethics approval and consent to participate

The clinical trial was conducted according to the ethical principles of the Declaration of Helsinki 2013 and following approval from the West of Scotland Research Ethics Committee 1 (Ref: 16/WS/0070), NHS Lothian Research and Development department and the Medicine and Health Care Regulatory Agency (MHRA-UK, CTA number: 01384/0250/001–0001). The trial was registered in the European Clinical Trial Database (EudraCT Ref: 2015–000963–15). All participants gave written informed consent.

Consent for publication

All participants gave written permission for publication of de-identified study data.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article (and its additional files).

Competing interests

JAF reports personal fees from Ferring Pharmaceuticals, Novartis, Galecto Biotech, Caldan Therapeutics, Gilde Healthcare, Arix Bioscience, NorthSea Therapeutics and grants from GlaxoSmithKline and Intercept Pharmaceuticals, outside the submitted work. PCH is an advisor for AbbVie, BMS, Eisai Ltd, Falk, Ferring, Gilead, Gore, Janssen, Lundbeck, MSD, Norgine, Novartis, ONO Pharmaceuticals, Pfizer and Roche, outside the submitted work. The remaining authors declare that they have no competing interests.

Funding

This was an academic-led study, funded by Novartis (UK CPO - Investigator Initiated Trial; Funder Reference Number: CRLX030C2202T), and sponsored by ACCORD (Academic and Clinical Central Office for Research and Development for NHS Lothian/University of Edinburgh). The funders reviewed, suggested revisions pertaining to, and accepted the study Protocol. The funders had no role in the conduct of the research or the subsequent analysis.

Author contributions

Conceptualisation and design of the work were carried out by JAF, PCH, FJG, CG; the acquisition, analysis, and interpretation of data were performed by FJG, JAF, PCH, PDJD, GW, HI, ST, CG; trial delivery and administration were carried out by FJG and JAF; the original draft of the manuscript was written by FJG and JAF; the draft was reviewed and edited by all the authors.

Acknowledgements

We thank Tony Wackett (Edinburgh Clinical Trials Unit) for building and maintaining the eCRF, Ruaridh Buchan (Advanced Pharmacist/ Clinical Trials, Royal Infirmary of Edinburgh) for pharmacy support,

David Lyall and Mark Chapman (Investigational Supplies Group, University of Edinburgh) for IMP blind labelling/ QP release, radiographers at the Royal Infirmary of Edinburgh (Department of Radiology) for fluoroscopy screening, and staff in the Royal Infirmary of Edinburgh Clinical Research Facility for their assistance in all aspects of the study.

References

1.Williams R, Aspinall R, Bellis M, et al. Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *Lancet* 2014; 384(9958): 1953–97.

2.Blachier M, Leleu H, Peck-Radosavljevic M, et al. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; 58(3): 593–608.

3.Garcia-Tsao G, Groszmann RJ, Fisher RL, et al. Portal pressure, presence of gastroesophageal varices and variceal bleeding. *Hepatology* 1985; 5(3): 419–24.

4.Ripoll C, Groszmann RJ, Garcia-Tsao G, et al. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009; 50(5): 923–8.

5.Garcia-Tsao G, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. *N Engl J Med* 2010; 362(9): 823–32.

6.Tripathi D, Stanley AJ, Hayes PC, et al. U. K. guidelines on the management of variceal haemorrhage in cirrhotic patients. *Gut* 2015; 64(11): 1680–704.

7.Krag A, Borup T, Moller S, et al. Efficacy and safety of terlipressin in cirrhotic patients with variceal bleeding or hepatorenal syndrome. *Advances in Therapy* 2008; 25(11): 1105–40.

8.Baik SK, Jeong PH, Ji SW, et al. Acute hemodynamic effects of octreotide and terlipressin in patients with cirrhosis: a randomized comparison. *Am J Gastroenterol* 2005; 100(3): 631–5.

9.Seo YS, Park SY, Kim MY, et al. Lack of difference among terlipressin, somatostatin, and octreotide in the control of acute gastroesophageal variceal hemorrhage. *Hepatology* 2014; 60(3): 954–63.

10.Fallowfield JA, Hayden AL, Snowdon VK, et al. Relaxin modulates human and rat hepatic myofibroblast function and ameliorates portal hypertension in vivo. *Hepatology* 2014; 59(4): 1492–504.

11.Lachlan NJ, Masson N, Ireland H, et al. Serelaxin reduced portal pressure gradient and portal vein pressure in patients with cirrhosis and portal hypertension. *Hepatology* 2015; 62: (Suppl):585a.

12.Kobalava Z, Villevalde S, Kotovskaya Y, et al. Pharmacokinetics of serelaxin in patients with hepatic impairment: a single-dose, open-label, parallel group study. *Br J Clin Pharmacol* 2015; 79(6): 937–45.

13.Tripathi D, Therapondos G, Lui HF, et al. Haemodynamic effects of acute and chronic administration of low-dose carvedilol, a vasodilating beta-blocker, in patients with cirrhosis and portal hypertension. *Aliment Pharmacol Ther* 2002; 16(3): 373–80.

14.Winkler K, Larsen JA, Munkner T, et al. Determination of the hepatic blood flow in man by simultaneous use of five test substances measured in two parts of the liver. *Scand J Clin Lab Invest* 1965; 17(5): 423–32.

15.Cherrick GR, Stein SW, Leevy CM, et al. Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. *J Clin Invest* 1960; 39: 592–600.

16.Ripoll C, Groszmann R, Garcia-Tsao G, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; 133(2): 481–8.

17.Gifford FJ, Morling JR, Fallowfield JA. Systematic review with meta-analysis: vasoactive drugs for the treatment of hepatorenal syndrome type 1. *Aliment Pharmacol Ther* 2017; 45(5): 593–603.

18.Bosch J, Groszmann RJ, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol* 2015; 62(1 Suppl): S121–30.

19.Villanueva C, Albillos A, Genesca J, et al. Development of hyperdynamic circulation and response to beta-blockers in compensated cirrhosis with portal hypertension. *Hepatology* 2016; 63(1): 197–206.

20.Villanueva C, Aracil C, Colomo A, et al. Acute hemodynamic response to beta-blockers and prediction of long-term outcome in primary prophylaxis of variceal bleeding. *Gastroenterology* 2009; 137(1): 119–28.

21.Abraldes JG, Albillos A, Banares R, et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. *Gastroenterology* 2009; 136(5): 1651–8.

22.Snowdon VK, Lachlan NJ, Hoy AM, et al. Serelaxin as a potential treatment for renal dysfunction in cirrhosis: Preclinical evaluation and results of a randomized phase 2 trial. *PLoS Med* 2017; 14(2): e1002248.

23.Teerlink JR, Cotter G, Davison BA, et al. Serelaxin, recombinant human relaxin–2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *Lancet* 2013; 381(9860): 29–39.

24.Danielson LA, Conrad KP. Time course and dose response of relaxin-mediated renal vasodilation, hyperfiltration, and changes in plasma osmolality in conscious rats. *J Appl Physiol (1985)* 2003; 95(4): 1509–14.

Tables

Table 1. Summary of participant characteristics

	All Participants (n=15)	Serelaxin (n=11)	Placebo (n=4)
Age (years)	58 (43-70)	57 (43-70)	60 (54-64)
Gender (% male)	11 (73%)	8 (73%)	3 (75%)
Ethnicity (% Caucasian)	15 (100%)	11 (100%)	4 (100%)
Aetiology of cirrhosis			
Alcohol alone	10 (67%)	8 (73%)	2 (50%)
NAFLD	2 (13%)	1 (9%)	1 (25%)
HCV alone	1 (7%)	1 (9%)	0
HCV + HBV	1 (7%)	0	1 (25%)
Cryptogenic	1 (7%)	1 (9%)	0
Child-Pugh Class A	9 (60%)	6 (54%)	3 (75%)
Child-Pugh Class B	6 (40%)	5 (45%)	1 (25%)
Child-Pugh Class C	0	0	0
Current/previous liver related complications			
Ascites	8 (53%)	6 (55%)	2 (50%)
Spontaneous Bacterial Peritonitis	0	0	0
Hepatic Encephalopathy	4 (27%)	3 (27%)	1 (25%)
Variceal Bleeding	5 (33%)	5 (45%)	0
BMI (kg/m^2)	27.6 (19.8-36.6)	28.0 (24.1-36.6)	26.5 (19.8-31.8)
Systolic BP (mmHg)	145 (112-173)	155 (126-173)	134 (112-149)
Heart rate (bpm)	71 (46-97)	73 (46-97)	69 (66-71)
MELD score	10 (6-14)	11 (8-14)	7.5 (6-11)
Total bilirubin (μmol/L)	24 (6-44)	28 (7-44)	12 (6-17)
INR	1.2 (1.0-1.6)	1.3 (1.1-1.6)	1.2 (1-1.5)
Albumin (g/dL)	34 (23-40)	34 (23-39)	36 (30-40)
Platelet count (x10^9/L)	71 (26-331)	71 (26-182)	104 (55-331)
AST (U/L)	43 (22-122)	46 (25-122)	28 (22-32)
ALT (U/L)	37 (10-123)	37 (12-123)	26 (10-39)
Ongoing alcohol use			
Yes	4 (27%)	3 (27%)	1 (25%)

NAFLD: non-alcoholic fatty liver disease, HCV: hepatitis C virus, HBV: hepatitis B virus, BMI: body mass index,

BP: blood pressure, bpm: beats per minute, MELD: model for end-stage liver disease, INR: international

normalised ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

Table 2. Secondary Outcomes in participants receiving serelaxin

	HVPG[†] (mmHg)	HBF* (mL/min)	IVCP* (mmHg)	CI* L/min/m^2	SVRI* dynes · sec/cm^5/m^2	APWV* (m/sec)
Mean ±SD Pre serelaxin	15.9 ±3.3	1.5 ±0.8	8.2 ± 3.4	3.8 ±0.5	1716 ±398	8.1 ±1.4
Mean ±SD Post serelaxin	15.9 ±2.1	1.2 ±0.8	9.0 ± 2.4	4.1 ±0.8	1605 ±474	8.0 ±2.1
Arithmetic mean of difference ±SD	-0.4 ±1.9 p=0.69	-0.3 ±0.3 p=0.15	0.4 ±1.7 p=0.58	-0.3 ±0.7 p=0.28	111 ±394 p=0.42	0.2 ±0.7 p=0.49
Geometric mean of difference, CV	1.0, 0.1 p=0.63	0.8, 0.2 p=0.15	1.0, 0.1 p=0.27	1.0, 0.1 p=0.44	1.0, 0.0 p=0.32	1.0, 0.0 p=0.19

[†]Baseline to 1 hour change in HVPG. *Baseline to 2 hour change in all other secondary outcomes. Results of paired *t*-tests using both the arithmetic and geometric mean. HVPG: hepatic venous pressure gradient, HBF: hepatic blood flow, IVCP: inferior vena cava pressure, CI: cardiac index, SVRI: systemic venous resistance index, APWV: aortic pulse wave velocity, SD: standard deviation, CV: coefficient of variation.

Table 3. Adverse Events

SN	IMP	Adverse Event	SAE/SAR	Severity	Relatedness to IMP	Expectedness
002	Placebo	Diarrhoea	No	Mild	N/A	N/A
003	Serelaxin	Syncope on inserting venflon	No	Mild	Unrelated	Unexpected
003	Serelaxin	Syncope on inserting hepatic venous catheter	No	Mild	Unrelated	Unexpected
003	Serelaxin	Syncope on removing hepatic venous catheter	No	Mild	Unrelated	Unexpected
003	Serelaxin	Right upper quadrant ache reported at 24 hour follow up	No	Mild	Unrelated	Unexpected
005	Serelaxin	Mean diastolic BP< 60mmHg (58.3mmHg at IMP+30 mins)	No	Mild	Possibly related	Expected
006	Serelaxin	Prolonged QTc on ECG after 2 hours of serelaxin infusion	No	Mild	Possibly related	Unexpected
008	Serelaxin	Bilirubin rise	No	Mild	Unrelated	Unexpected
012	Serelaxin	Prolonged QTc on ECG during recovery period (normal throughout infusion)	No	Mild	Possibly related	Unexpected
014	Serelaxin	Syncope on inserting venflon	No	Mild	Unrelated	Unexpected
014	Serelaxin	Syncope on inserting hepatic venous catheter	No	Mild	Unrelated	Unexpected
014	Serelaxin	Dental abscess	No	Mild	Unrelated	Unexpected
016	Serelaxin	Femoral artery puncture	No	Mild	Unrelated	Unexpected

SN: subject number, IMP: investigational medicinal product, SAE: serious adverse event, SAR: serious adverse reaction.

Figures
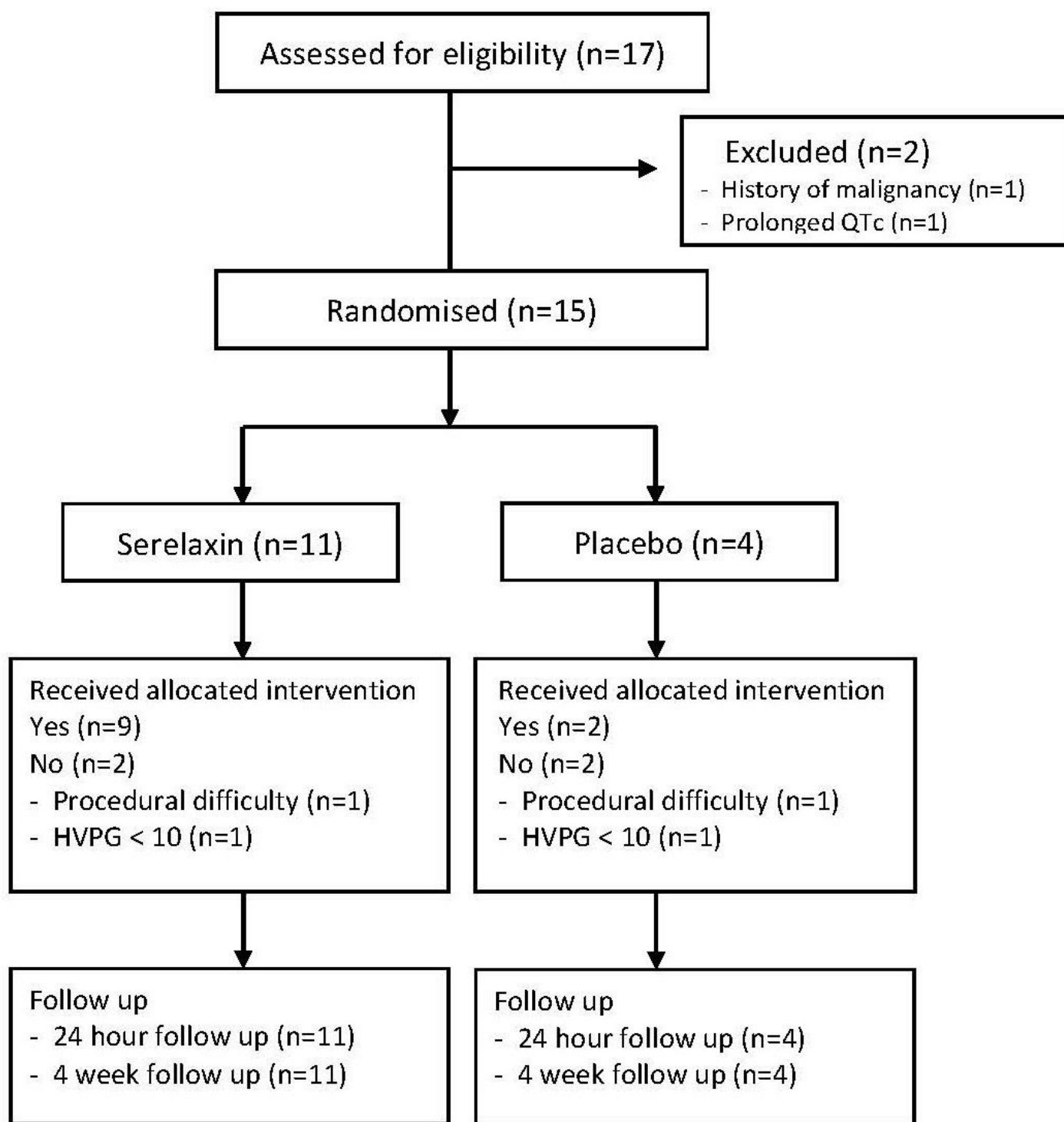
Figure 1

CONSORT diagram. HVPG: hepatic venous pressure gradient, QTc: corrected QT interval.
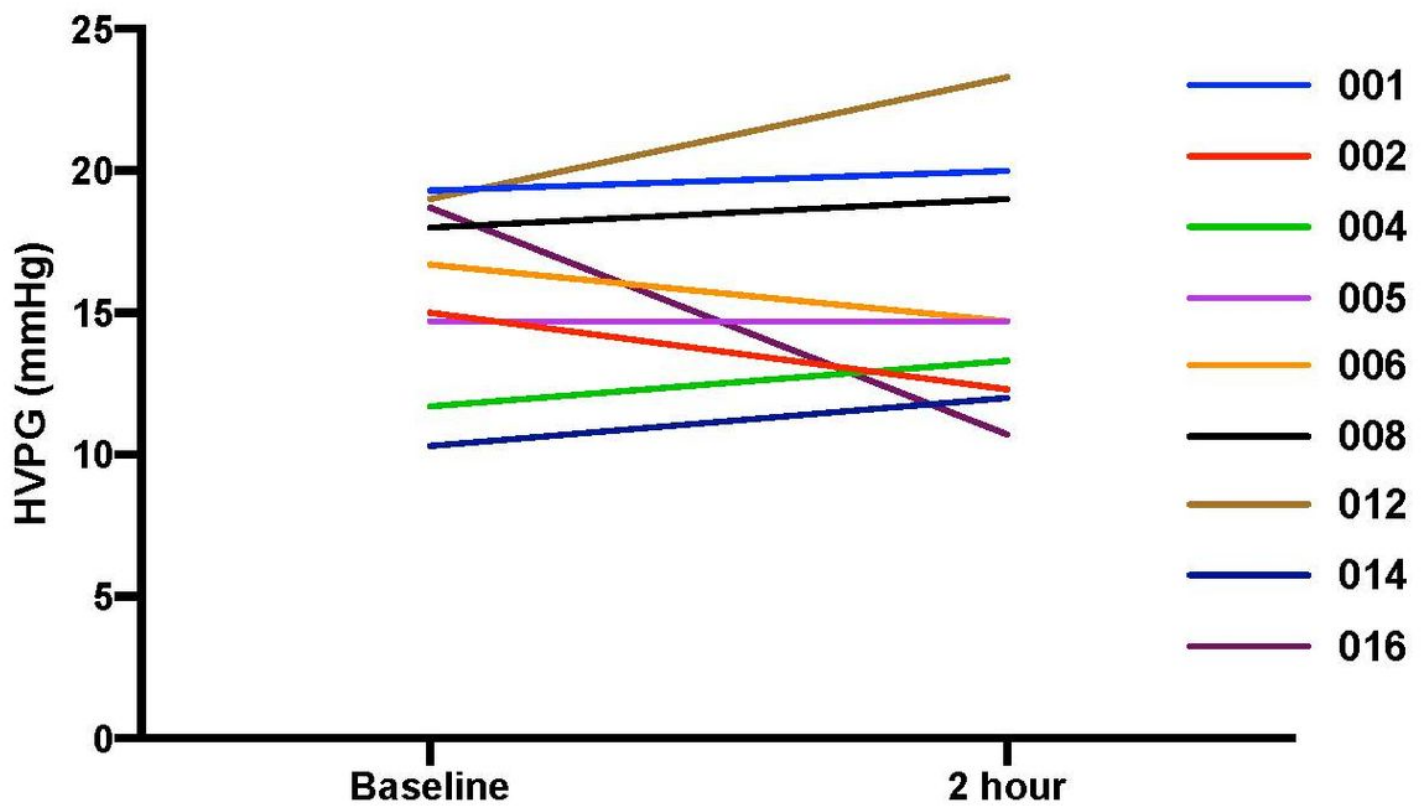
Figure 2

Fasting HVPG response to serelaxin. Lines represent individual participant changes in HVPG following 2 hour infusion of serelaxin (n=9).