Received: 3 June 2020 | Accepted: 29 June 2020

DOI: 10.1111/liv.14586

ORIGINAL ARTICLE

Liver INTERNATIONAL WILEY

Combination of phosphodiesterase-5-inhibitors and beta blockers improves experimental portal hypertension and erectile dysfunction

Frank E. Uschner[1] | Kathleen Glückert[2] | Rafael Paternostro[3,4] | Thorsten Gnad[5] | Robert Schierwagen[1] | Mattias Mandorfer[3,4] | Fernando Magdaleno[2] | Cristina Ortiz[1] | Katharina Schwarzkopf[1] | Patrick S. Kamath[6] | Carlo Alessandria[7] | Christoph Boesecke[2] | Alexander Pfeifer[5] | Thomas Reiberger[3,4] | Wolfgang Kreisel[8] | Tilman Sauerbruch[1] | Arnulf Ferlitsch[3,4] | Jonel Trebicka[1,9] | Sabine Klein[1]

[1]Department of Internal Medicine I, Hospital of the Goethe University, Frankfurt, Germany

[2]Department of Internal Medicine I, University Hospital Bonn, Bonn, Germany

[3]Hepatic Hemodynamic Lab, Medical University Vienna, Vienna, Austria

[4]Division of Gastroenterology & Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

[5]Institute of Pharmacology and Toxicology, University Hospital, University of Bonn, Bonn, Germany

[6]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA

[7]Division of Gastroenterology and Hepatology, Città della Salute e della Scienza Hospital, Turin, Italy

[8]Department of Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Diseases, Faculty of Medicine, Medical Center - University of Freiburg, Freiburg, Germany

[9]European Foundation for the Study of Chronic Liver Failure, Barcelona, Spain

Correspondence
Jonel Trebicka, Department of Internal Medicine I, University Hospital, Goethe

Abstract

Background & Aims: Phosphodiesterase-5 inhibitors (PDE-5-I) are used for treatment of erectile dysfunction (ED), which is common in patients with cirrhosis. They may improve portal hypertension (PH), but contradictory data on efficacy and side-effects have been reported. Non-selective beta blockers (NSBB) reduce portal pressure, but might aggravate ED. Thus, we evaluated the combination of PDE-5-I with NSBB and its impact on PH and ED in experimental cirrhosis.

Methods: ED was assessed in cirrhotic patients (n = 86) using standardized questionnaire. Experimental cirrhosis was induced by bile-duct-ligation or carbon-tetrachloride intoxication in rats. Corpus cavernosum pressure – a surrogate of ED -, as well as systemic and portal haemodynamics, were measured in vivo and in situ after acute administration of udenafil alone or in combination with propranolol. mRNA and protein levels of PDE-5 signalling were analysed using PCR and western Blot.

Results: ED in humans was related to severity of liver disease and to NSBB treatment. PDE-5 was mainly expressed in hepatic stellate cells and upregulated in human and experimental cirrhosis. Propranolol reduced corpus cavernosum pressure in cirrhotic rats and it was restored by udenafil. Even though udenafil treatment improved PH, it led to a reduction of mean arterial pressure. The combination of udenafil and propranolol reduced portal pressure and hepatic resistance without systemic side-effects.

Abbreviations: *Adrb1*, beta-1-adrenoceptor; *Adrb2*, beta-2-adrenoceptor; BDL, bile duct ligation; BW, body weight; CCl_4, carbon tetrachloride; CCP, corpus cavernosum pressure; CCP_{max}, maximum of corpus cavernosum pressure; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; GAPDH, glyceraldehyde- 3-phosphate dehydrogenase; HSC, hepatic stellate cell; IIEF-5, International Index of Erectile Function-5; L-NAME, N_{ω}-nitro-L-arginine methyl ester hydrochloride; LSEC, liver sinusoidal endothelial cells; MELD, Model of End Stage Liver Disease; *Nos2*, inducible nitric oxide synthase; *Nos3*, endothelial nitric oxide synthase; NSBB, non-selective beta-blocker; PCR, polymerase chain reaction; PDE-5/*Pde-5*, phosphodiesterase-5; peNOS, phosphorylated endothelial nitric oxide synthase; SDS-PAGE, sodium dodecyl sulfate - polyacrylamide gel electrophoresis; SEM, standard error of the mean; WT, wild type.

Frank Erhard Uschner and Kathleen Glückert shared first authorship.

University, Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany.
Email: jonel.trebicka@kgu.de

Funding information
The authors received direct funding from Dr Falk Pharma GmbH (Freiburg, Germany; 20.000€). J. Trebicka is supported by grants from Deutsche Forschungsgemeinschaft (Grant SFB TRR57 and CRC 1382), European Union Horizon 2020 Research and Innovation Program GALAXY (Grant 668031), MICROB-PREDICT (Grant 825694) and LIVERHOPE (Grant 731875) as well as Cellex Foundation.

Handling editor: Virginia Hernandez-Gea

Conclusions: ED is common with advanced cirrhosis and concomitant NSBB treatment. The combination of PDE-5-I and NSBB improves ED and PH in experimental cirrhosis.

KEYWORDS
erectile dysfunction, non-selective beta-blocker, PDE-5-inhibitor, portal hypertension

1 | INTRODUCTION

Phosphodiesterase-5 (PDE-5) inhibitors are used for treatment of erectile dysfunction as they prolong sufficient corpus cavernosum pressure. This is because of an inhibition of the conversion of cyclic guanosine monophosphate (cGMP) to biologically inactive 5′-GMP, thereby enhancing nitric oxide bioavailability and causing vasodilation.[1] In cirrhotic livers, nitric oxide production is reduced and its vasodilatory response is further impaired by increased expression of PDE-5.[2-5] Thus, PDE-5 inhibitors have already been proposed as a therapy for cirrhosis with portal hypertension in the past. Nevertheless, previous studies reported inconsistent results with regard to efficacy and potential side-effects in experimental and human cirrhosis. In fact, cirrhotic animal models deliver evidence that PDE-5 inhibitors sufficiently reduce portal pressure and hepatic resistance, but might impact systemic circulation and decrease mean arterial pressure.[6-9] By contrast, conflicting results were reported in patients treated with PDE-5 inhibitors; either no effect on portal pressure with deleterious systemic side-effects was reported or a relevant reduction in portal pressure with minor systemic effects was observed.[10-13]

Furthermore, little is known about the cell specific biological role of PDE-5 in cirrhosis. It is unclear which cells express PDE-5. Moreover the effect of PDE-5 inhibitors on extrahepatic vascular beds, which contribute to portal hypertension, are poorly understood in patients with cirrhosis.

Non-selective beta-blockers (NSBB) are a cornerstone to prevent bleeding in patients with PH. They decrease the portal-venous inflow by reducing cardiac output and by causing splanchnic vasoconstriction.[14-16] Adequate haemodynamic response to NSBB also hampers decompensation of cirrhosis and may improve overall survival.[17,18] NSBB may lead to erectile dysfunction, which impairs life-quality and may influence drug adherence, but this has not been investigated in patients with cirrhosis to date.[19]

Thus, the aim of our study was (a) to assess the effects of NSBB on erectile dysfunction in patients with different severity of cirrhosis, (b) to investigate the effects of PDE-5 inhibition on portal hypertension and on erectile dysfunction in experimental cirrhosis and (c) to test the combination of PDE-5 inhibitors with NSBB on portal hypertension and erectile dysfunction.

Lay Summary

Patients with cirrhosis frequently report erectile dysfunction, which is worsened by beta blocker therapy. The combination of beta blockers and phosphodiesterase-5-inhibitors improves erectile function and portal pressure, in experimental cirrhosis, without major side-effects.

2 | METHODS

2.1 | Patients and data collection

Eighty-six cirrhotic patients were included in this study. The patients were prospectively enrolled between December 2010 and December 2012 at the Division of Gastroenterology and Hepatology, Department for Internal Medicine III, Medical University of Vienna, Austria and between July 2017 and July 2018 at the Department for Internal Medicine I, University Hospital Bonn, Germany. Inclusion criteria were male sex and proven cirrhosis (either by radiologic/clinical parameters or by histology). Exclusion criteria were missing information on NSBB treatment or indication for NSBB treatment other than bleeding prophylaxis (treatment other than propranolol and carvedilol was excluded), age over 80 years, current overt hepatic encephalopathy, previous liver transplantation, extrahepatic malignancies, previous urologic surgery, diabetes, depression and severe cardiac disease. Concomitant medication and concomitant diseases, Model of End Stage Liver Disease (MELD) and Child-Pugh score were recorded.

2.2 | International Index of Erectile Function-5 (IIEF-5)

The 5-item version of the International Index of Erectile Function (IIEF-5) was used to determine the presence and the severity of erectile dysfunction.[20] The score ranges from 5 to 25 points and classifies the severity of erectile dysfunction in five categories: no erectile dysfunction (22-25 points), mild erectile dysfunction (17-21

points), mild to moderate erectile dysfunction (12-16 points), moderate erectile dysfunction (8-11 points) and severe erectile dysfunction (5-7 points).

Ethics

The human studies were approved by the ethics committee of the University Hospital Bonn (Study Number 279/14) and Medical University of Vienna (Study Number 450/2010) and performed in accordance to the current version of the Declaration of Helsinki. All patients signed an informed consent prior to study inclusion. The use of human samples was approved by the ethics committee of the University of Bonn (029/13). Liver and hepatic artery samples were obtained at liver transplantation between 1999 and 2005 and non-cirrhotic donor samples served as controls.

2.2.1 | Reagents

Udenafil (Batch No. AFGH000722) was kindly provided by Dr Falk Pharma GmbH (Freiburg, Germany). Propranolol was purchased from Sigma-Aldrich (Batch-No. P8688, Darmstadt, Germany).

2.2.2 | Animals

In total, 136 male wild type (WT) Sprague Dawley rats were used. The experiments were performed according to the guidelines and regulations approved by LANUV, the responsible committee for animal studies in North Rhine-Westphalia/Germany (permission number 84-02.04.2014.A030). All rats were placed in a controlled environment (12 hours light/dark, temperature between 22°C and 24°C), and received water and standard rat feed (Ssniff, Soest, Germany) ad libitum.

2.3 | Induction of cirrhosis

2.3.1 | Cholestatic model of fibrosis

Bile duct ligation (BDL) was performed in 79 WT rats with an initial body weight (BW) of 180-200 g as described previously.[21-23] After four weeks, when ascites as a definite sign of portal hypertension was present, experiments were performed.

2.3.2 | Toxic model of fibrosis

Forty-two rats with an initial BW of 80-100 g were intoxicated twice weekly by inhalation of 1 l/min carbon tetrachloride (CCl_4) for 12-14 weeks. Experiments were performed when ascites was present as described previously.[21]

2.3.3 | Invasive erectile function measurement

Corpus cavernosum pressure (CCP) was measured invasively in five sham-operated and six BDL rats. This method is described in detail in the Data S1.

2.3.4 | In vivo haemodynamic experiments

In vivo systemic and liver haemodynamic studies were performed in five control, five sham-operated, 43 BDL and 22 CCl_4 intoxicated rats as described previously.[21,24]

To evaluate the effects of udenafil and propranolol, invasive measurements of mean arterial pressure and portal pressure were performed continuously (for a total of 60 minutes after udenafil and 90 minutes after propranolol/udenafil) after acute intravenous drug administration.

2.3.5 | Microsphere technique

To investigate portal and systemic haemodynamics, the colored microsphere technique was carried out before and 60 minutes after acute administration of udenafil and 90 minutes after propranolol/udenafil as described previously.[24-26] 300.000 systemic (red/before; yellow/after udenafil or udenafil/propranol) microspheres (15 μm diameter, Triton-Technologies, San Diego, USA) were injected in the left ventricle. In parallel, peripheral blood was removed from the femoral artery and cardiac output was calculated as microspheres per pre-defined amount of blood (0.65 mL/min femoral artery blood). Mesenteric portal-systemic shunt volume was estimated by injection of 150.000 microspheres (white/before; blue/after) in the ileocecal vein and calculated as microspheres liver/microspheres lung ratio.[21,24-26]

2.3.6 | In situ isolated liver perfusion

In situ isolated liver perfusion was performed in 30 BDL and 20 CCl_4 intoxicated rats. Experiments were performed in a recirculating system as previously described.[21,27] The criteria for liver viability were gross appearance of the liver, stable perfusion, bile production >0.4 μL/min x g in CCl_4 rats and stable buffer pH (7.4 ± 0.1) during the initial stabilization period. Livers were incubated with methoxamine (Batch-No. M6524, Sigma-Aldrich, Darmstadt, Germany) for initial pre-contraction and N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME Batch-No. 5751, Sigma-Aldrich, Darmstadt, Germany) for inhibition of nitric oxide-dependent signalling.

2.3.7 | Quantitative real-time polymerase chain reaction

RNA from human and rodent liver tissue and RNA from samples of rodent hepatic stellate cells (HSC), liver sinusoidal endothelial

TABLE 1 General characteristics of patients

	All	no ED	ED	
Variables	**86 (100%)**	**28 (33%)**	**58 (67%)**	***P***
Etiology (alcoholic/viral/ others)	43/22/21	11/10/7	32/12/14	
Age	54.5 (19-76)	49 (19-68)	57.5 (39-76)	<.001
Child-Pugh class (A/B/C)	29/40/17	15/11/2	14/29/15	
Child-Pugh score	8 (5-13)	6 (5-11)	8 (5-13)	.0069
MELD score	12 (6-22)	11 (6-18)	14 (7-22)	.0103
NSBB (yes/no)	49/37	11 (39%)/ 17 (61%)	38 (65%)/ 20 (35%)	.0212
Grade IIEF-5 (mild/mild-moderate/moderate/ severe)			26/18/7/7	

Note: General characteristics of the patient cohort.

Abbreviations: ED, erectile dysfunction; IIEF, international index of erectile function; MELD, model for end-stage liver disease; NSBB, non-selective beta-blocker; *P*, *P*-value.

cells (LSEC), aorta and penis were isolated. Reverse transcription and detection by real-time polymerase chain reaction (PCR) were performed as described previously.[22,23] Assays were provided by Applied Biosystems (Foster City, USA). 18S rRNA served as endogenous control. Results were expressed as $2^{-\Delta\Delta CT}$, which corresponds to the x-fold increase of gene expression of the reference group.

2.3.8 | Hepatic cell isolation

The subsets of liver cells (hepatic stellate cells, liver sinusoidal endothelial cells) were isolated from healthy and BDL rats, as described previously.[25,27-30] Briefly, primary liver cells were isolated in a two-step pronase-collagenase perfusion fractionated by density gradient centrifugation. Afterwards, the purified cells were subjected to PCR analysis.

2.3.9 | Western blotting

Liver samples were processed using sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE gels and nitrocellulose membranes were used as described previously.[23,25] Ponceau staining and glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) as endogenous control confirmed equal protein loading. Membranes were incubated with the respective primary antibodies: eNOS (BD Bioscience, San Jose, CA, USA; Cat.610296, Lot41425), peNOS (Cell Signalling, Boston, MA, USA; #9571, Lot 14), PDE5 (sigma HPA004729, Lot A91778) or GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-25778, Lot # K1511). A corresponding secondary peroxidase-coupled antibody was added (Santa-Cruz-Biotechnology, Santa Cruz, USA). After enhanced chemiluminescence (ECL, Amersham, UK) digital detection was evaluated using Chemi-Smart (PeqLab Biotechnologies, Erlangen, Germany).

2.3.10 | cGMP determination

cGMP levels were measured by EIA (Cayman Chemical) following the manufacturer's instructions.

2.4 | Statistical analysis

Results are presented as mean and standard error of the mean (SEM) unless otherwise indicated. Statistical analysis of two groups was either performed with Mann-Whitney-*U* test or Wilcoxon-signed-rank test for animal experiments. Only groups with more than three animals were tested statistically. Correlations were analysed by calculating Spearman's Rank Correlation Coefficient. A binary multivariate logistic regression model was fitted using MELD score (MELD was chosen over Child-Score to avoid multicollinearity) and NSBB intake as covariates and statistical significance for the subsequent steps. Statistical analyses and graphing were performed using GraphPad Prism 5.0 (Graph-Pad, San Diego, USA) or SPSS 22 (SPSS Inc Chicago, IL, USA). $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Erectile dysfunction in patients with cirrhosis

Eighty-six patients were prospectively included for erectile dysfunction using standard erectile function questionnaire (International Index of Erectile Function-5/ IIEF-5). Fifty percent of the patients suffered from alcoholic cirrhosis, 26% from chronic viral hepatitis and 24% from other aetiologies. The median age was 54.5 years, the median Child-Pugh Score was eight points and the median MELD score was twelve (Table S1).

Overall, 58 out of 86 patients (67%) reported erectile dysfunction with an IIEF-5 score < 22 (out of a maximum of 25 points). Out of

these, 28% reported mild, 31% mild to moderate, 12% moderate and 12% severe erectile dysfunction (Table 1). Patients with erectile dysfunction were significantly older (48 ± 10.7 years vs 57 ± 8.5 years; $P < .001$) and had a significant higher Child-Pugh score (7 ± 2 vs 8 ± 2 $P = .007$), as well as MELD score (11 ± 4 vs 14 ± 4; $P = .01$) compared to patients without erectile dysfunction.

Age (r_s −.482, $P < .001$), Child-Pugh score (r_s −.357, $P = .001$), as well as MELD score (r_s −.301, $P = .01$) significantly correlated with the IIEF-5 score. Thereby, IIEF-5 score decreased with the severity of disease, assessed by Child-Pugh score (Figure 1A).

Overall, patients with NSBB treatment had significantly lower IIEF-5 scores than patients without NSBB treatment (Figure 1B). Importantly, this association was only present in Child A and B, but not in Child C patients (Figure 1C).

Of note, 65% of patients reporting erectile dysfunction were under NSBB treatment, while only 39% of patients without erectile dysfunction received NSBB treatment ($P = .02$). Logistic regression analysis, using MELD score and NSBB intake as covariates, showed that NSBB intake increases by the factor 3.54 with reported erectile dysfunction (Odds Ratio 3.54, $P < .05$; 1.07-11.69 95% confidence interval) (Figure 1D).

Because of the significant association of erectile dysfunction and NSBB treatment, the effects of NSBB and PDE-5 inhibitors on erectile function were analysed in experimental cirrhosis.

3.2 | Erectile dysfunction in experimental cirrhosis

Erectile dysfunction was evaluated in vivo in rats after cavernous nerve stimulation by measuring invasive corpus cavernosum pressure (CCP) and the effects of propranolol (1 mg/kg BW, single dose, CCP measured after 30 minutes) and udenafil (1 mg/kg BW, single dose, CCP measured after 30 minutes) alone or as add-on to propranolol were analysed (Figure 2A). Erection dependent CCP

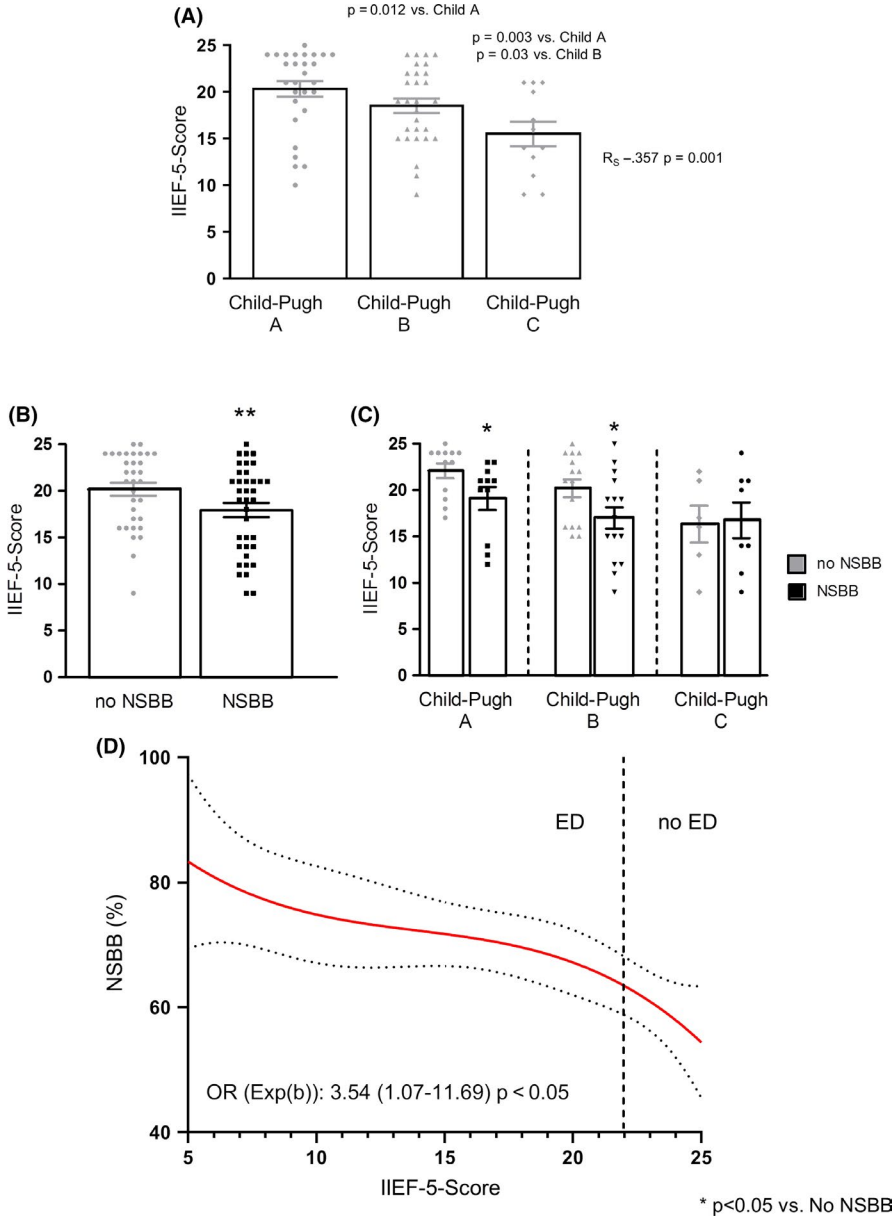


FIGURE 1 Erectile dysfunction in patients with cirrhosis. (A) Differences in IIEF-5 score between Child-Pugh A, B and C patients with a statistically significant negative correlation between categorical Child-Pugh score and IIEF-5 score; R_s −0.357, $P = .001$). (B) Differences in IIEF-5 score according to NSBB treatment in all patients and (C) stratified by Child-Pugh A, B and C. (D) Logistic regression analysis (Exp(B)/OR NSBB: 3.54, $P < .05$; 1.07-11.69 95% CI) on the probability for NSBB treatment in patients with and without ED. Abbreviations: 95%CI, 95% confidence interval; ED, erectile dysfunction; IIEF-5, International Index of Erectile Function 5; NSBB, non-selective beta-blocker; OR, odds ratio

increase was significantly lower in cirrhotic BDL rats compared to sham-operated animals (−46%, $P = .03$). Furthermore, the maximum CCP (CCP_{max}) during erection was lower in BDL than in sham-operated rats (31 vs 56 mmHg, Figure 2B).

Propranolol reduced CCP_{max} in BDL and sham-operated rats and udenafil improved erectile function both in sham and BDL animals either pretreated or not pretreated with propranolol (Figure 2C).

In the corpus cavernosum of BDL rats, treatment with propranolol and udenafil was associated with an increase of endothelial nitric oxide synthase (*Nos3)* ($P < .005$ vs untreated) and inducible nitric oxide synthase (*Nos2)* mRNA, as well as decreased levels of *Pde-5* compared to vehicle-treated animals ($P < .001$ vs untreated, Figure 2D). Combined administration of propranolol and udenafil significantly increased mRNA expression of beta-1- and beta-2-adrenoceptor (*Adrb1* and *Adrb2)* in corpus cavernosum of BDL rats compared to vehicle-treated animals ($P < .001$ vs untreated, Figure 2E).

Since udenafil showed beneficial effects on erectile dysfunction in experimental cirrhosis, we also studied the effects of PDE-5 inhibition on portal and systemic haemodynamics in vivo under the hypothesis that udenafil might have further beneficial effects on portal hypertension.

3.3 | Effect of PDE-5 inhibitors and NSBB on portal hypertension in experimental cirrhosis

Haemodynamic changes were investigated in BDL and CCl_4 rats after acute administration of different doses of udenafil (1 mg/kg BW and 5 mg/kg BW for 60 minutes). Treatment with 1 mg/kg and 5 mg/kg BW udenafil significantly decreased portal pressure in BDL rats (−30% 1 mg/kg; Figure 3A; −23% 5 mg/kg udenafil; Table 2).

As expected, portal pressure was unchanged after vehicle administration (Table 2). Similarly, application of 1 mg/kg udenafil led to a significantly decreased portal pressure in CCl_4 rats (−30%; Figure 3A). No change in portal pressure was observed after administration of 5 mg/kg udenafil, probably because of a decrease in mean arterial pressure (Tables 2 and 3).

A dose of 1 mg/kg but not a dose of 5 mg/kg udenafil significantly reduced hepatic vascular resistance in BDL and CCl_4 rats (Tables 2 and 3). Moreover mesenteric shunt flow was reduced in all treated animals (Tables 2 and 3). Nevertheless, acute administration of udenafil significantly decreased mean arterial pressure (Figure 3B) and splanchnic vascular resistance (Tables 2 and 3) in both models of experimental cirrhosis. But cardiac output was

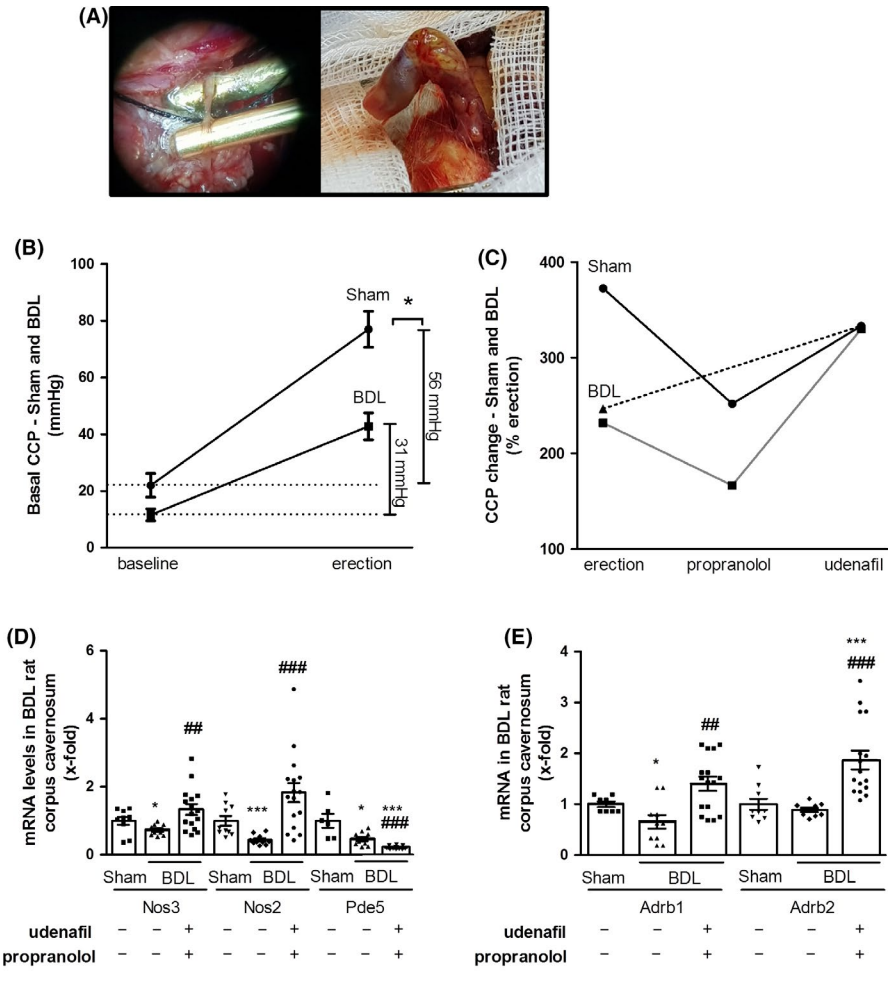


FIGURE 2 Erectile dysfunction in experimental cirrhosis. (A) Experimental setup, preparation and stimulation of cavernous nerve induces erection in BDL rats. (B) CCP in BDL and sham-operated rats at baseline and during erection. (C) Changes in CCP during erection after acute add-on treatment with propranolol and udenafil in BDL and sham-operated rats. (D) mRNA expression levels of *Nos-3*, *Nos-2* and *Pde-5* as well as (E) *Adrb1* and *Adrb2* in corpus cavernosum of BDL and sham-operated rats after treatment with udenafil and propranolol compared to vehicle treatment. Abbreviations: *Adrb1*, beta-1-adrenoceptor; *Adrb2*, beta-2-adrenoceptor; BDL, bile duct ligation; CCP, corpus cavernosum pressure; mRNA, messenger ribonucleic acid; *Nos-2*, inducible nitric oxide synthase; *Nos-3*, endothelial nitric oxide synthase; *Pde-5*, Phosphodiesterase-5

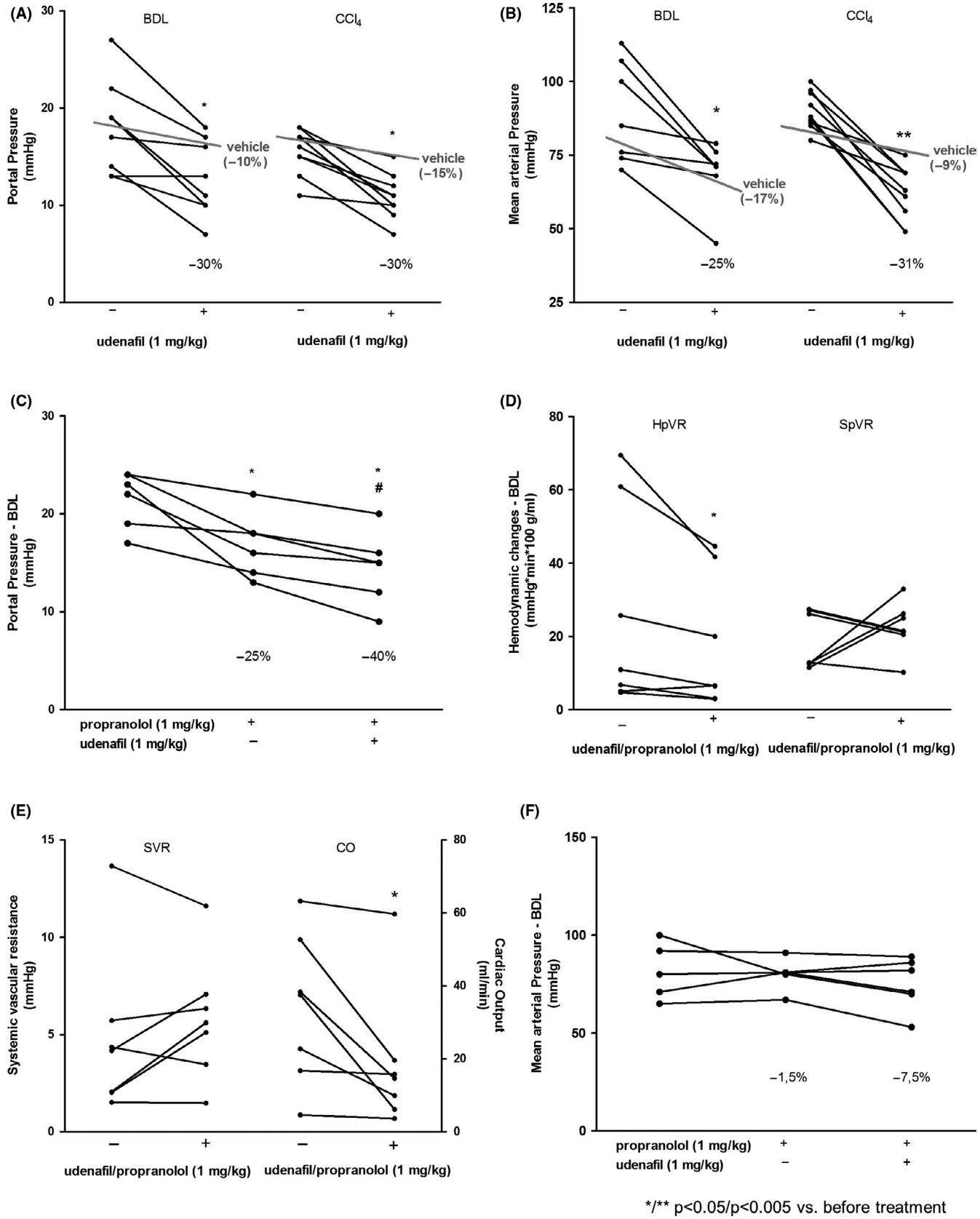


FIGURE 3 Effect of PDE-5 inhibitors and NSBB on portal hypertension in experimental cirrhosis. (A) PP and (B) MAP in BDL and CCl_4 rats before and after monotherapy with udenafil. (C) PP, (D) HpVR and SpVR in BDL rats after combined treatment with propranolol and udenafil. (E) Changes in SVR, CO and (F) MAP after treatment with propranolol and udenafil in BDL rats. Abbreviations: BDL, bile duct ligation; CCl_4, carbon tetrachloride; CO, cardiac output; HpVR, hepatic portal vascular resistance; MAP, mean arterial pressure; NSBB, non-selective beta-blocker; PDE-5, phosphodiesterase-5; PP, portal pressure; SpVR, splanchnic vascular resistance; SVR, systemic vascular resistance

TABLE 2 Haemodynamic changes after udenafil treatment in BDL rats

	Vehicle (n = 5)			1 mg/kg udenafil (n = 6)			5 mg/kg udenafil (n = 5)		
	Before mean ± SEM	After mean ± SEM	*P*-value	Before mean ± SEM	After mean ± SEM	*P*-value	Before mean ± SEM	After mean ± SEM	*P*-value
HpVR (mmHg*min*100 g/mL)	6.8 ± 1.0	7.8 ± 1.6	.8125	6.9 ± 1.1	4.3 ± 0.7	**.0313***	7.6 ±2.0	4.3 ± 1.0	.0938
SpVR (mmHg*min*100 g/mL)	16.3 ± 2.6	14.8 ± 6.3	.6250	17.3 ± 3.3	12.4 ± 2.2	.2188	15.3 ± 1.5	10.3 ± 1.2	**.0313***
SF (%)	15.9 ± 4.8	20.5 ± 6.1	.1250	15.3 ± 3.7	7.1 ± 3.2	.0625	17.8 ± 13.8	8.9 ± 9.3	.0625
CO (mL/min)	31.050 ± 11.580	30.570 ± 7.234	1.0000	44.600 ± 5.883	38.922 ± 8.324	1.0000	39.1 ± 10.7	31.1 ± 8.4	.6250
PP (mmHg)	22.0 ± 2.8	209 ± 8.7	.2785	18.0 ± 1.7	12.8 ± 1.4	**.0223***	20.7 ± 2.4	16.1 ± 2.4	**.0350***
MAP (mmHg)	88.6 ± 6.7	70.4 ± 5.3	.0625	84.1 ± 6.1	67.1 ± 3.5	**.0090****	84.0 ± 3.2	72.1 ± 4.4	**.0247***

Note: Haemodynamic changes after acute treatment with either vehicle, 1 mg/kg BW or 5 mg/kg BW udenafil in BDL rats.

Abbreviations: BDL, bile duct ligation; BW, body weight; CO, cardiac output; HpVR, hepatic portal vascular resistance; MAP, mean arterial pressure; PP, portal pressure; SEM, Standard error of the mean; SF, mesenteric shunt flow; SpVR, splanchnic vascular resistance. Bold is statistically significant, *P < .05 and **P > .01.

TABLE 3 Haemodynamic changes after udenafil treatment in CCl_4 rats

	Vehicle (n = 5)			1 mg/kg udenafil (n = 6)			5 mg/kg udenafil (n = 5)		
	Before mean ± SEM	After mean ± SEM	*P*-value	Before mean ± SEM	After Mean ± SEM	*P*-value	Before mean ± SEM	After mean ± SEM	*P*-value
HpVR (mmHg*min*100 g/mL)	6.3 ± 0.5	8.0 ± 3.3	.6250	6.8 ± 1.2	3.8 ± 1.0	**00355***	6.5 ± 2.7	2.9 ± 0.6	.2188
SpVR (mmHg*min*100 g/mL)	22.4 ± 8.1	26.4 ± 6.4	.3125	25.5 ± 6.3	14.0 ± 5.7	.0625	26.2 ± 2.7	15.4 ± 6.7	.1250
SF (%)	7.4 ±4.0	6.4 ± 2.4	.3125	7.4 ± 2.6	5.1 ± 2.9	**.0313***	7.6 ± 2.2	2.6 ± 1.2	**.0313***
CO (mL/min)	22.0 ± 2.9	26.7 ± 6.3	.6250	21.5 ± 7.8	21.0 ± 9.8	1.000	26.0 ± 3.5	28.2 ± 6.7	.8125
PP (mmHg)	16.8 ± 2.5	15.3 ± 3.0	.4142	17.5 ± 2.1	12.5 ± 1.8	**.0058****	14.0 ± 1.0	14.7 ± 0.9	1.000
MAP (mmHg)	86.5 ± 6.2	72.5 ± 5.2	.0938	86.6 ± 4.0	60.0 ± 3.6	**.0059****	93.5 ± 7.7	62.0 ± 4.4	.0579

Note: Haemodynamic changes after acute treatment with either vehicle, 1 mg/kg BW or 5 mg/kg BW udenafil in CCl_4 intoxicated rats.

Abbreviations: CCl4, carbon tetrachloride; CO, cardiac output; HpVR, hepatic portal vascular resistance; MAP, mean arterial pressure; PP, portal pressure; SEM, Standard error of the mean; SF, mesenteric shunt flow; SpVR, splanchnic vascular resistance. Bold is statistically significant, *P < .05 and **P > .01.

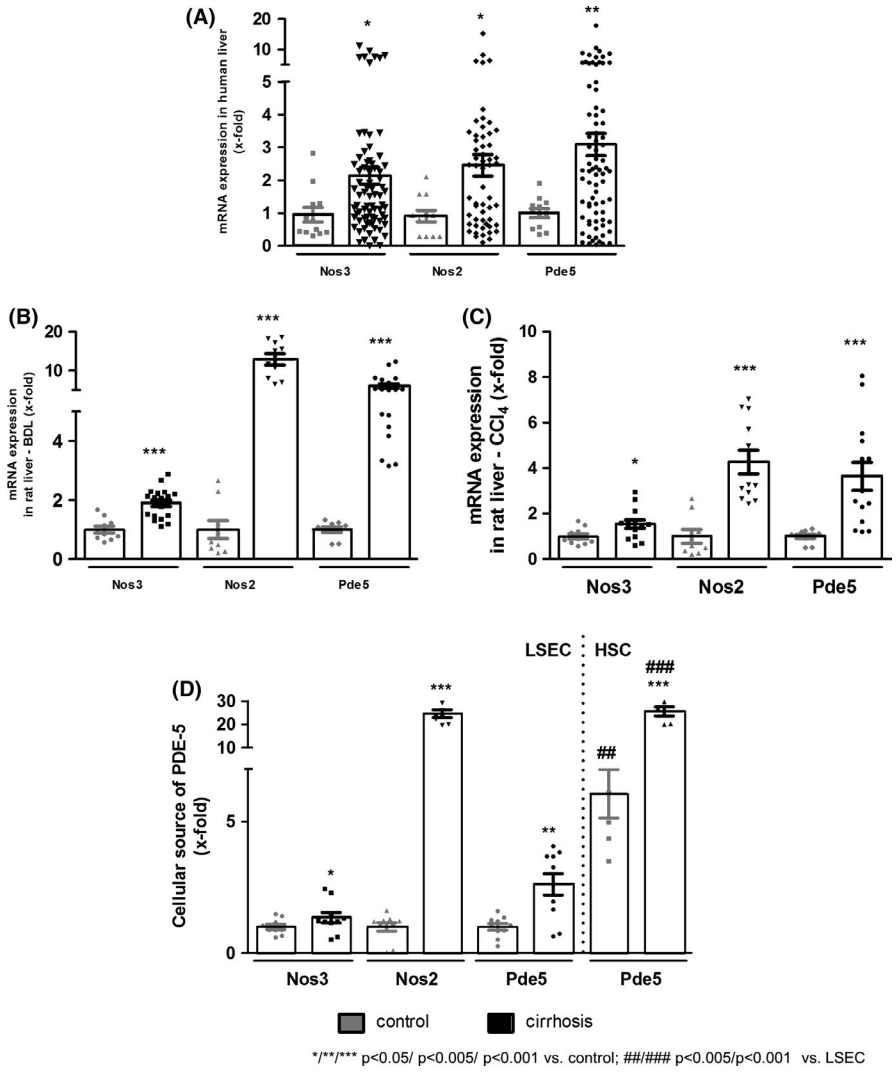


FIGURE 4 Role of PDE-5 in human and experimental cirrhosis. (A) mRNA expression levels of *Nos-3*, *Nos-2* and *Pde-5* in liver tissue of humans and (B-C) of BDL and CCl_4 intoxicated rats compared to healthy controls. (D) *Nos-3* and *Nos-2* mRNA levels in primary isolated LSEC from BDL rats compared to healthy controls and *Pde-5* mRNA expression in LSEC and HSC from healthy and cirrhotic BDL rats. Abbreviations: BDL, bile duct ligation; CCl_4, carbon tetrachloride; HSC, hepatic stellate cells. LSEC, liver sinusoidal endothelial cells; mRNA, messenger ribonucleic acid; *Nos-2*, inducible nitric oxide synthase; *Nos-3*, endothelial nitric oxide synthase; *Pde-5*, Phosphodiesterase-5

not influenced either by the administration of udenafil or vehicle (Tables 2 and 3).

Interestingly, the combination of the NSBB propranolol and udenafil further reduced portal pressure compared to udenafil or propranolol alone (−25% propranolol; 60 minutes after administration vs −30% udenafil; 60 minutes after administration vs −40% combined treatment; after 30 minutes of propranolol and additional 60 minutes of udenafil) (Figure 3C). Additionally, hepatic vascular resistance as well as cardiac output were decreased after combined treatment compared to placebo (Figure 3D-E). Importantly, when propranolol was combined with udenafil, no systemic side-effects were observed, as shown by unchanged mean arterial pressure, splanchnic and systemic vascular resistance. That is in contrast to Figure 3B, where injection of udenafil alone significantly reduced mean arterial pressure (Figure 3E-F).

While PDE-5 inhibition reduced portal hypertension and was associated with systemic side-effects, combination with NSBB improved portal hypertension without negative impact on splanchnic and systemic circulation. Thus, we followed two questions: what is the role of PDE-5 in-, respectively outside the liver and how is it influenced by PDE-5 inhibitor and NSBB treatment.

3.4 | Role of PDE-5 in human and experimental cirrhosis

Liver and vessel samples from healthy liver transplant donors and cirrhotic recipients were used to analyse changes in nitric oxide signalling in human cirrhosis.

Nos3, *Nos2* and *Pde-5* were significantly upregulated in liver samples from liver transplant recipients compared to samples from transplant donors ($P < .05$ vs healthy control. Figure 4A). By contrast, *Pde-5* mRNA levels were reduced and *Nos3* and *Nos2* mRNA levels were increased in human hepatic artery samples from liver transplant recipients compared to transplant donors ($P < .05$ vs healthy control, Figure S1A).

In experimental cirrhosis, induced either by four weeks of BDL or 14 weeks of CCl_4 intoxication, hepatic levels of *Pde-5*, *Nos3* and *Nos2* mRNA were significantly upregulated compared to healthy controls ($P < .001$ vs control, Figure 4B-C). In line with human data, *Nos2* mRNA levels were upregulated ($P < .05$) and *Pde-5* mRNA levels were reduced ($P < .001$) in aorta samples from cirrhotic rats, while *Nos3* mRNA levels remained unchanged (Figure S1B-C).

To determine the source of disturbed nitric oxide signalling in cirrhotic livers, liver sinusoidal endothelial cells (LSEC) and hepatic

FIGURE 5 Molecular mechanisms of PDE-5 effect with and without NSBB in experimental cirrhosis. (A-B) Changes in perfusion pressure in response to udenafil and L-NAME in BDL and CCl_4 rats. (C) Hepatic protein expression of eNOS, p-eNOS, PDE5 and GAPDH in BDL rats after udenafil treatment compared to vehicle control. (D-E) Changes in hepatic and aorta cGMP levels after treatment with udenafil and propranolol. (F) Aorta mRNA expression of *Nos-3*, *Nos-2* and *Pde-5* in BDL rats after udenafil monotherapy or in combination with propranolol. Abbreviations: BDL, bile duct ligation; cGMP, cyclic guanosine monophosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; L-NAME, N-Nitroarginine methyl ester; mRNA, messenger ribonucleic acid; NO, nitric oxide; *Nos-2*/iNOS, inducible nitric oxide synthase; *Nos-3*/eNOS, endothelial nitric oxide synthase; NSBB, non-selective beta-blocker; PDE-5, phosphodiesterase-5; p-eNOS phosphorylated eNOS

stellate cells (HSC) were isolated from healthy and BDL rats and further analysed in vitro.

In LSEC isolated from BDL rats, mRNA levels of *Nos3* and *Nos2* were significantly increased compared to healthy controls ($P < .001$ vs control, Figure 4D). Moreover *Pde-5* mRNA levels were significantly upregulated in LSEC ($P < .005$ vs control) as well as primary isolated rat HSCs compared to their respective controls ($P < .001$ vs control). This effect was even more pronounced in HSC compared to LSEC (Figure 4D).

3.5 | Molecular mechanisms of PDE-5 effect with and without NSBB in experimental cirrhosis

In situ isolated liver perfusion was performed in BDL and CCl_4 rats after initial pre-contraction with methoxamine. To determine NO-dependent effects of the PDE-5 inhibitor udenafil, a subset of livers was perfused with the NO-synthase inhibitor L-NAME. Administration of increasing doses of udenafil (0.1 mg/mL, 0.5 mg/mL, 1.0 mg/mL perfusate) significantly reduced perfusion pressure in BDL rats compared to vehicle ($P < .05$, Figure 5A). This effect was completely blunted by L-NAME in BDL rats (Figure 5A). Similarly, perfusion pressure in isolated liver of CCl_4 rats was reduced after udenafil treatment in a dose-dependent manner, while it remained unchanged after incubation with L-NAME ($P < .05$, Figure 5B).

Udenafil administration led to slightly increased protein expression of endothelial nitric oxide synthase (eNOS) and significantly promoted its phosphorylation (peNOS) in BDL liver samples ($P < .05$ vs untreated, Figure 5C). Consequently, udenafil treatment increased cGMP levels in liver and aorta samples from cirrhotic animals (Figure 5D). By contrast, the combination of propranolol and udenafil particularly blunted cGMP in aorta samples, while it was increased in cirrhotic liver samples (Figure 5D-E). Furthermore, combined acute administration of propranolol and udenafil reduced *Nos3*, *Nos2* and *Pde-5* mRNA expression in aorta samples ($P < .05$ vs untreated, Figure 5F).

These results suggest that PDE-5 inhibition enhances hepatic cGMP levels and induces intrahepatic vasodilation, which is further increased by the combined NSBB treatment in experimental cirrhosis.

4 | DISCUSSION

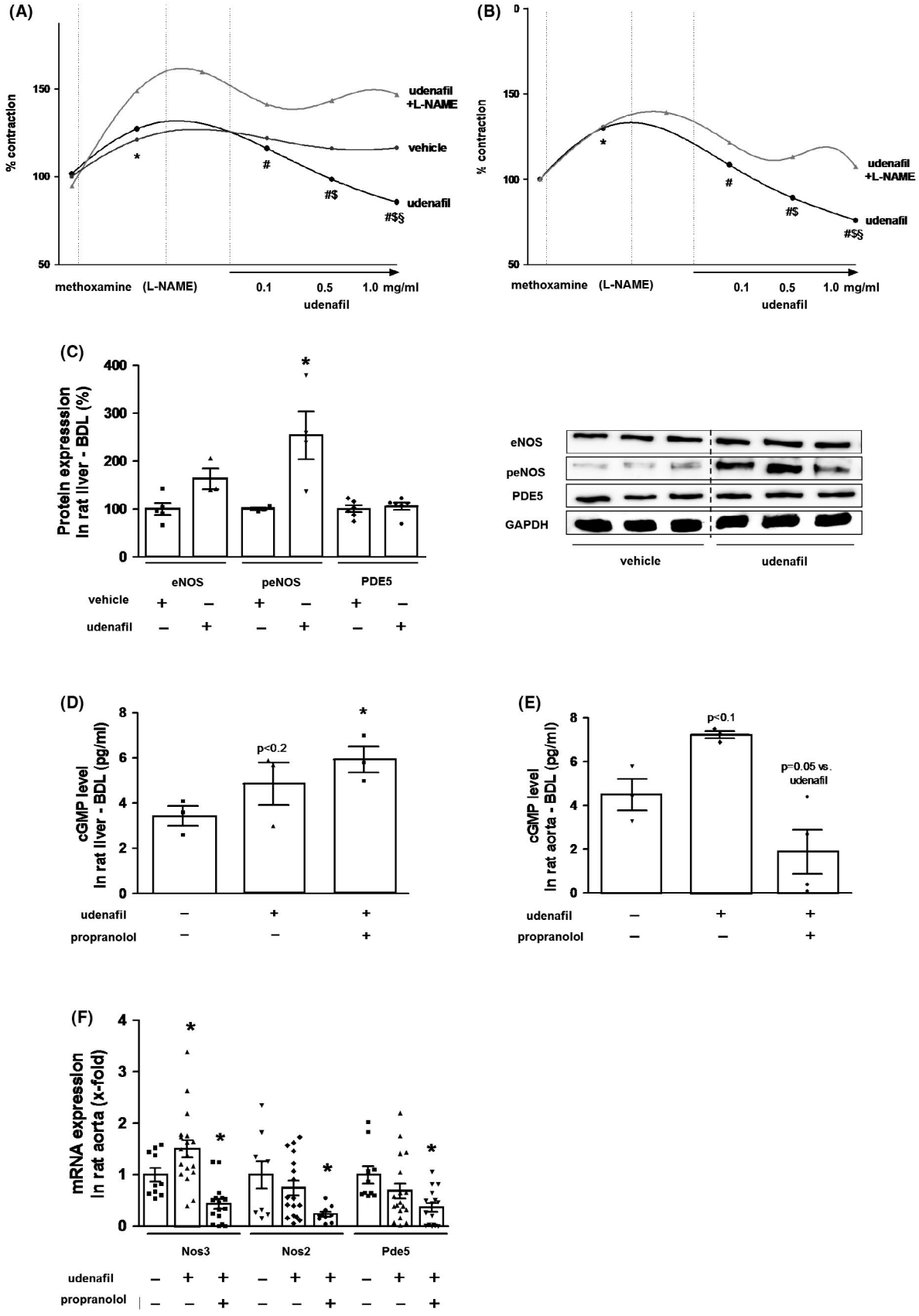
PDE-5 inhibitors restore erectile function in experimental cirrhosis and prevent NSBB-induced erectile dysfunction. Moreover the combination of PDE-5 inhibitors with NSBB decreases portal pressure without extrahepatic systemic effects.

Erectile dysfunction is a common complication in cirrhotic patients and its presence increases with the severity of liver disease.[31-34] NSBB are used as standard medical therapy for the prevention of portal hypertension-related bleeding and erectile dysfunction is known as a side-effect of NSBB in general population.[35,36] Nevertheless, recent studies could not identify a clear association of NSBB with the presence of erectile dysfunction in cirrhosis.[31,34]

This is the first study delivering evidence that NSBB treatment is indeed associated with erectile dysfunction in cirrhotic patients, especially in less-severe (Child-A and –B) disease-stages. The major difference to previous studies is the exclusion of patients with extrahepatic diseases that might interfere with erectile function and exclusion of patients with indication for NSBB other than bleeding prophylaxis (eg arterial hypertension). This potentially leads to a more refined population with regard to the relation of NSBB and erectile dysfunction in cirrhosis. Nevertheless, we could further confirm that NSBB induce erectile dysfunction in a rodent model of cirrhosis. This was the case despite the downregulation of PDE-5, which is known to be an important enzyme in the regulation of erectile function.

PDE-5 inhibitors, originally developed as a therapy for angina pectoris and arterial hypertension, are licensed for the treatment of erectile dysfunction.[1,32,37] Our data show that PDE-5 inhibition restores erectile function, especially after treatment with NSBB, in experimental cirrhosis.

At molecular level, PDE-5 inhibitors block the degradation of cGMP, thereby enhancing nitric oxide and inducing vasodilation.[38] In cirrhosis, an intrahepatic lack of nitric oxide effects aggravates vasoconstriction, while splanchnic overproduction of nitric oxide contributes to extrahepatic vasodilation.[39,40] Thereby, the reduced intrahepatic nitric oxide availability is caused by a disturbed balance of production and degradation through PDE-5.[5,41] In the liver, nitric oxide mainly derives from LSEC, which thereby regulate HSC contraction and vascular tone.[38,40,42,43] Yet, our study delivers evidence that decreased nitric oxide effects are at least partially because of PDE-5 upregulation in cirrhosis and that the main hepatic source of PDE-5 are HSC's, and not due to decreased expression of NOS which are clearly uprgulated in liver cirrhosis as demonstrated in this study and confirming previous studies. This confirms recent reports describing hepatic upregulation of PDE-5 in experimental cirrhosis shown by immunohistochemistry.[5,6] Our



* p<0.05 vs. before treatment; # p<0.05 vs. Methoxamine/propanolol; $ p<0.05 vs. 0.1mg/ml udenafil; § p<0.05 vs. 0.5 mg/ml udenafil

results attribute this effect to HSCs and suggest a specific role of PDE-5 and nitric oxide in the interaction between LSEC and HSC. In several lines of evidence, we could demonstrate in vitro, in situ and in vivo that PDE-5 inhibition decreases intrahepatic resistance and portal pressure.

The potential role of PDE-5 in cirrhosis has been investigated before.[10,12,44-46] While some studies report improved portal haemodynamics, reduced hepatic resistance and reduced fibrosis after PDE-5 inhibitor treatment in rodents and human cirrhosis, others could not confirm these effects in human disease, especially because of severe systemic side-effects.[10-13,47] In our hands, acute PDE-5 inhibition with udenafil decreased portal pressure in different models of cirrhosis. We could demonstrate that the PDE-5 inhibition was nitric oxide-dependent, as shown by our in situ experiments and underlined by our expression data of NOS and PDE-5 in total liver tissue and isolated LSEC and HSC from cirrhotic and healthy animals. Importantly, we demonstrated that PDE-5 inhibition promotes these effects by regulating hepatic cGMP levels in HSC.

Similar to sildenafil,[13,47] udenafil also had significant systemic side-effect because of its lack of liver selectivity. These effects might be especially deleterious in human portal hypertension since *Nos3* and *Nos2* are upregulated in vessel samples from cirrhotic patients, despite the decreased PDE-5 expression. There, further PDE-5 inhibition would aggravate extrahepatic vasodilation. This was clearly demonstrated in our in vivo studies showing a dose-dependent effect of udenafil on systemic circulation and increased cGMP levels in the analysed human hepatic artery samples.

Notably, previous clinical trials, exploring the efficacy of PDE-5 inhibitors in cirrhosis, excluded all patients with previous NSBB treatment or stopped it for at least a few days before intervention.

Thus, our study provides a possible explanation for the observed limited effects of PDE-5 inhibitors. An ideal therapy for PH should induce intrahepatic vasodilation and systemic vasoconstriction. NSBB exert its effects through unselective blocking of beta-1 and beta-2 adrenoceptors, leading to reduced cardiac output and increased systemic vasoconstriction.[14,15,48] Importantly, conventional NSBB have little to no influence on hepatic vascular resistance which is the major cause of portal hypertension.[16]

We demonstrate that combination of NSBB with udenafil has several benefits in cirrhotic portal hypertension. First, it acts synergistic on decreasing portal pressure, since udenafil reduces hepatic vascular resistance and NSBB decrease portal venous inflow by ameliorating splanchnic vasodilation. Second, the systemic effect of PDE-5 inhibition is abolished under NSBB therapy, what means that the mean arterial pressure is maintained. Third, udenafil co-administration with NSBB improves erectile dysfunction and thus may improve patient quality of life, which may eventually have an effect on the adherence to NSBB therapy.

A limitation of this work is that the combination of PDE-5 inhibitors and NSBB was not tested in patients with erectile dysfunction. However, our rodent data provides clear evidence for beneficial effects of combination therapy, and thus, future studies should be performed to investigate its effect on hepatic and systemic haemodynamics, as well as on erectile dysfunction in men.

In conclusion, this study shows for the first time that combining NSBB and PDE-5 inhibitors might be a good strategy to treat portal hypertension without aggravating arterial hypotension or erectile dysfunction.

ACKNOWLEDGEMENTS

The authors thank G. Hack and S. Bellinghausen for excellent technical assistance and S. Dentler for critical reading and correction of the manuscript. Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

AUTHOR'S CONTRIBUTION

JT initiated the work. JT and SK coordinated the work. FEU, KG, RP, FM, TG, CO and SK collected data/performed experiments. FEU, KG, RP, RS, SK, FM, MM, CO, KS and JT performed statistics. AF, TR, AP, WK, CB, PD, PK, CA, TR and TS provided essential materials. FEU, KG, RP, SK, AF and JT drafted the manuscript. All authors critically discussed, corrected and reviewed the manuscript.

ETHICS APPROVAL

The human studies were approved by the ethics committee of the University Hospital Bonn (Study Number 279/14) and Medical University of Vienna (Study Number 450/2010) and performed in accordance to the current version of the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

All patients signed an informed consent prior to study inclusion.

ORCID

Frank E. Uschner https://orcid.org/0000-0002-3760-2887
Rafael Paternostro https://orcid.org/0000-0002-1813-5769
Thorsten Gnad https://orcid.org/0000-0003-0169-3067
Mattias Mandorfer https://orcid.org/0000-0003-2330-0017
Katharina Schwarzkopf https://orcid.org/0000-0002-2147-5051
Alexander Pfeifer https://orcid.org/0000-0001-8805-6831
Thomas Reiberger https://orcid.org/0000-0002-4590-3583
Wolfgang Kreisel https://orcid.org/0000-0001-6884-0135
Jonel Trebicka https://orcid.org/0000-0002-7028-3881

REFERENCES

1. Mónica FZ, Bian K, Murad F. The endothelium-dependent nitric oxide-cGMP pathway. *Adv Pharmacol*. 2016;77:1-27.
2. Rockey DC, Chung JJ. Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. *Gastroenterology*. 1998;114:344-351.
3. Gupta TK, Toruner M, Chung MK, Groszmann RJ. Endothelial dysfunction and decreased production of nitric oxide in the intrahepatic microcirculation of cirrhotic rats. *Hepatology*. 1998;28:926-931.

4. Biecker E, Trebicka J, Kang A, Hennenberg M, Sauerbruch T, Heller J. Treatment of bile duct-ligated rats with the nitric oxide synthase transcription enhancer AVE 9488 ameliorates portal hypertension. *Liver Int*. 2008;28:331-338.
5. Loureiro-Silva MR, Iwakiri Y, Abraldes JG, Haq O, Groszmann RJ. Increased phosphodiesterase-5 expression is involved in the decreased vasodilator response to nitric oxide in cirrhotic rat livers. *J Hepatol*. 2006;44:886-893.
6. Schaffner D, Lazaro A, Deibert P, et al. Analysis of the nitric oxide-cyclic guanosine monophosphate pathway in experimental liver cirrhosis suggests phosphodiesterase-5 as potential target to treat portal hypertension. *World J Gastroenterol*. 2018; 24:4356-4368.
7. Halverscheid L, Deibert P, Schmidt R, et al. Phosphodiesterase-5 inhibitors have distinct effects on the hemodynamics of the liver. *BMC Gastroenterol*. 2009;9:69.
8. Lee K-C, Yang Y-Y, Huang Y-T, et al. Administration of a low dose of sildenafil for 1 week decreases intrahepatic resistance in rats with biliary cirrhosis: the role of NO bioavailability. *Clin Sci*. 2010;119:45-55.
9. Colle I, De Vriese AS, Van Vlierberghe H, Lameire NH, DeVos M. Systemic and splanchnic haemodynamic effects of sildenafil in an in vivo animal model of cirrhosis support for a risk in cirrhotic patients. *Liver Int*. 2004;24:63-68.
10. Kreisel W, Deibert P, Kupcinskas L, et al. The phosphodiesterase-5-inhibitor udenafil lowers portal pressure in compensated preascitic liver cirrhosis. A dose-finding phase-II-study. *Dig Liver Dis*. 2015;47:144-150.
11. Deibert P, Schumacher Y-O, Ruecker G, et al. Effect of vardenafil, an inhibitor of phosphodiesterase-5, on portal haemodynamics in normal and cirrhotic liver – results of a pilot study. *Aliment Pharmacol Ther*. 2006;23:121-128.
12. Lee K-C, Yang Y-Y, Wang Y-W, et al. Acute administration of sildenafil enhances hepatic cyclic guanosine monophosphate production and reduces hepatic sinusoid resistance in cirrhotic patients. *Hepatol Res*. 2008;38:1186-1193.
13. Clemmesen J-O, Giraldi A, Ott P, Dalhoff K, Hansen B-A, Larsen F-S. Sildenafil does not influence hepatic venous pressure gradient in patients with cirrhosis. *World J Gastroenterol*. 2008;14:6208-6212.
14. Turon F, Casu S, Hernández-Gea V, Garcia-Pagán JC. Variceal and other portal hypertension related bleeding. *Best Pract Res Clin Gastroenterol*. 2013;27:649-664.
15. Giannelli V, Lattanzi B, Thalheimer U, Merli M. Beta-blockers in liver cirrhosis. *Ann Gastroenterol*. 2014;27:20-26.
16. Groszmann RJ, Garcia-Tsao G, Bosch J, et al. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med*. 2005;353:2254-2261.
17. Reiberger T, Ulbrich G, Ferlitsch A, et al. Carvedilol for primary prophylaxis of variceal bleeding in cirrhotic patients with haemodynamic non-response to propranolol. *Gut*. 2013;62:1634-1641.
18. Villanueva C, Albillos A, Genescà J, et al. β blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2019;393:1597-1608.
19. Manolis A, Doumas M. Antihypertensive treatment and sexual dysfunction. *Curr Hypertens Rep*. 2012;14:285-292.
20. Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Peña BM. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. *Int J Impot Res*. 1999;11:319.
21. Klein S, Schierwagen R, Uschner FE, Trebicka J. Mouse and rat models of induction of hepatic fibrosis and assessment of portal hypertension. *Methods Mol Biol*. 2017;1627:91-116.
22. Trebicka J, Hennenberg M, Schulze Pröbsting A, et al. Role of β3-adrenoceptors for intrahepatic resistance and portal hypertension in liver cirrhosis. *Hepatology*. 2009;50:1924-1935.
23. Trebicka J, Hennenberg M, Laleman W, et al. Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. *Hepatology*. 2007;46:242-253.
24. Grace JA, Klein S, Herath CB, et al. Activation of the MAS receptor by angiotensin-(1–7) in the renin-angiotensin system mediates mesenteric vasodilatation in cirrhosis. *Gastroenterology*. 2013;145:874–884.e5.
25. Klein S, Klösel J, Schierwagen R, et al. Atorvastatin inhibits proliferation and apoptosis, but induces senescence in hepatic myofibroblasts and thereby attenuates hepatic fibrosis in rats. *Lab Invest*. 2012;92:1440-1450.
26. Uschner FE, Ranabhat G, Choi SS, et al. Statins activate the canonical hedgehog-signaling and aggravate non-cirrhotic portal hypertension, but inhibit the non-canonical hedgehog signaling and cirrhotic portal hypertension. *Sci Rep*. 2015;5:14573.
27. Klein S, Rick J, Lehmann J, et al. Janus-kinase-2 relates directly to portal hypertension and to complications in rodent and human cirrhosis. *Gut*. 2017;66:145-155.
28. Hennenberg M, Trebicka J, Kohistani Z, et al. Hepatic and HSC-specific sorafenib effects in rats with established secondary biliary cirrhosis. *Lab Invest*. 2011;91:241-251.
29. Granzow M, Schierwagen R, Klein S, et al. Angiotensin-II type 1 receptor-mediated Janus kinase 2 activation induces liver fibrosis. *Hepatology*. 2014;60:334-348.
30. Fernández-Iglesias A, Ortega-Ribera M, Guixé-Muntet S, Gracia-Sancho J. 4 in 1: antibody-free protocol for isolating the main hepatic cells from healthy and cirrhotic single rat livers. *J Cell Mol Med*. 2019;23:877-886.
31. Paternostro R, Heinisch BB, Reiberger T, et al. Erectile dysfunction in cirrhosis is impacted by liver dysfunction, portal hypertension, diabetes and arterial hypertension. *Liver Int*. 2018;38:1427-1436.
32. Andersson K-E. PDE5 inhibitors - pharmacology and clinical applications 20 years after sildenafil discovery. *Br J Pharmacol*. 2018;175:2554-2565.
33. Durazzo M, Premoli A, Di Bisceglie C, Bo S, Ghigo E, Manieri C. Male sexual disturbances in liver diseases: what do we know? *J Endocrinol Invest*. 2010;33:501-505.
34. Maimone S, Saffioti F, Oliva G, et al. Erectile dysfunction in compensated liver cirrhosis. *Dig Liver Dis*. 2019;51:843-849.
35. Shiri R, Koskimäki J, Häkkinen J, Auvinen A, Tammela TLJ, Hakama M. Cardiovascular drug use and the incidence of erectile dysfunction. *Int J Impot Res*. 2007;19:208-212.
36. Keene LC, Davies PH. Drug-related erectile dysfunction. *Adverse Drug React Toxicol Rev*. 1999;18:5-24.
37. Unegbu C, Noje C, Coulson JD, Segal JB, Romer L. Pulmonary hypertension therapy and a systematic review of efficacy and safety of PDE-5 inhibitors. *Pediatrics*. 2017;139:e20161450.
38. Kim NN. Phosphodiesterase type 5 inhibitors: a biochemical and clinical correlation survey. *Int J Impot Res*. 2003;15(Suppl 5):S13-S19.
39. Hu LS, George J, Wang JH. Current concepts on the role of nitric oxide in portal hypertension. *World J Gastroenterol*. 2013; 19:1707-1717.
40. DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. *Hepatology*. 2015;61:1740-1746.
41. Loureiro-Silva MR, Cadelina GW, Groszmann RJ. Deficit in nitric oxide production in cirrhotic rat livers is located in the sinusoidal and postsinusoidal areas. *Am J Physiol Gastrointest Liver Physiol*. 2003;284:G567-G574.
42. Marrone G, Shah VH, Gracia-Sancho J. Sinusoidal communication in liver fibrosis and regeneration. *J Hepatol*. 2016;65:608-617.

43. Natarajan V, Harris EN, Kidambi S. SECs (Sinusoidal Endothelial Cells), liver microenvironment, and fibrosis. *Biomed Res Int*. 2017; 2017:4097205.
44. Deibert P, Lazaro A, Stankovic Z, Schaffner D, Rössle M, Kreisel W. Beneficial long term effect of a phosphodiesterase-5-inhibitor in cirrhotic portal hypertension: a case report with 8 years follow-up. *World J Gastroenterol*. 2018;24:438-444.
45. Tahseldar-Roumieh R, Ghali-Ghoul R, Lugnier C, Sabra R. Effect of phosphodiesterase 5 inhibitor on alteration in vascular smooth muscle sensitivity and renal function in rats with liver cirrhosis. *Am J Physiol Heart Circ Physiol*. 2006;290:H481-H488.
46. Choi S-M, Shin J-H, Kim J-M, et al. Effect of udenafil on portal venous pressure and hepatic fibrosis in rats. A novel therapeutic option for portal hypertension. *Arzneimittelforschung*. 2009;59:641-646.
47. Tandon P, Inayat I, Tal M, et al. Sildenafil has no effect on portal pressure but lowers arterial pressure in patients with compensated cirrhosis. *Clin Gastroenterol Hepatol*. 2010;8:546-549.
48. Mandorfer M, Reiberger T. Beta blockers and cirrhosis, 2016. *Dig Liver Dis*. 2017;49:3-10.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Uschner FE, Glückert K, Paternostro R, et al. Combination of phosphodiesterase-5-inhibitors and beta blockers improves experimental portal hypertension and erectile dysfunction. *Liver Int*. 2020;40:2228–2241. https://doi.org/10.1111/liv.14586