

Pharmacological Modulation of Liver and Spleen Stiffness in a Cirrhotic Rat Model

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Purpose: Liver stiffness (LS) assesses liver fibrosis, while spleen stiffness (SS) is a promising marker for portal hypertension (PH), reflecting blood flow and vascular resistance. However, the response of LS and SS to vasoactive drugs is unclear. This study evaluates the effects of various PH-lowering drugs on LS and SS in a rat cirrhosis model.

Patients and Methods: In this study, cirrhosis was induced in 43 male Wistar rats (8 weeks old) via intraperitoneal thioacetamide (TAA) injections (200 mg/kg, twice weekly for six weeks). Rats were divided into six groups: control (sodium chloride), metoprolol, udenafil, enalapril, terlipressin, and carvedilol. LS and SS were measured using μ Fibroscan. Mean arterial pressure (MAP), heart rate (HR), and portal vein pressure (PVP) were continuously monitored. Drugs were administered systemically, with data collected at 0, 15, and 30 minutes.

Results: TAA-treated rats exhibited significantly higher LS and SS compared to controls (22.1 vs 4.2 kPa and 53.7 vs 27.7 kPa; $P < 0.001$). Changes in LS and SS correlated with PVP ($r = 0.670$ for LS and $r = 0.867$ for SS; $P < 0.01$). Metoprolol, udenafil, enalapril, and carvedilol significantly reduced PVP (22–34%, $P < 0.05$), accompanied by decreases in LS and SS (13–37%, $P < 0.05$). Terlipressin did not reduce LS or SS, likely due to opposing effects of increased MAP and reduced PVP.

Conclusion: In conclusion, combined LS and SS measurements may provide valuable non-invasive insights into patient responses and adherence to PH-lowering therapies.

Keywords: spleen stiffness, liver stiffness, cirrhosis, portal pressure, terlipressin

Introduction

The development of clinically significant portal hypertension (CSPH), as defined by a hepatic venous pressure gradient (HVPG) of ≥ 10 mmHg, represents a critical turning point in the natural history of patients with advanced chronic liver diseases (ACLD). After this point, the risk of hepatic decompensation^{1,2} and liver-related mortality is considerably increased.^{3,4} Portal hypertension (PH) has distinct structural and dynamic components⁵ and can originate from prehepatic, intrahepatic, and posthepatic causes. Hepatic PH is further classified into presinusoidal and sinusoidal PH, with some ACLD etiologies, such as cholestatic liver diseases, exhibiting a pronounced presinusoidal PH component.⁶ While HVPG remains the gold standard for assessing sinusoidal PH, it cannot accurately quantify prehepatic and presinusoidal PH.^{7,8}

Traditionally, the increase in portal pressure has been attributed to elevated vascular resistance within the cirrhotic liver.⁹ However, recent insights underscore the importance of increased portal flow resulting from splanchnic vasodilation. This dynamic interplay between portal blood flow and vascular resistance forms the foundation of portal pressure. Through pharmacological interventions, vascular resistance can be reduced by approximately 20–30%, marking a significant advance in managing portal hypertension.¹⁰ The pharmacotherapy landscape for this condition is now well-established, offering hope for prolonging survival in patients with advanced cirrhosis, particularly those facing severe

complications such as variceal bleeding.^{11,12} Key medications in this therapeutic arsenal include non-selective beta blockers, vasopressin, and somatostatin analogues, which effectively mitigate the hyperdynamic circulation, reduce splanchnic hyperperfusion, and enhance effective blood volume.^{10,13}

Historically, portal hypertension has been quantified through the invasive measurement of the hepatic venous pressure gradient (HVPG), which, despite its accuracy, is not widely available due to its invasive nature and high cost.³ Through the Baveno consensus conferences, non-invasive methods such as liver elastography have been identified as important parameters to assess portal hypertension, enhancing treatment monitoring and compliance.^{14–16} These non-invasive approaches also include routine laboratory tests such as the platelet-to-spleen ratio and assessments of liver and spleen stiffness, with transient elastography being particularly noted for its efficacy in predicting portal hypertension and esophageal varices.^{17–19}

Liver stiffness (LS) as measured by various elastographic methods was originally established to screen for fibrosis and cirrhosis in chronic liver disease.¹⁷ LS values <6 kPa are considered normal, with a high negative predictive value for ruling out fibrosis, whereas LS values exceeding 12.5 kPa are generally indicative of cirrhosis.¹⁷ However, beyond this static component of LS (ie, the fibrosis stage), various other conditions have been identified that modulate LS independently of fibrosis. These include hepatic necroinflammation,^{20,21} congestion,²² cholestasis,²³ alcohol,²⁴ and food intake,²⁵ as well as arterial pressure²⁶ and short-term modulations of portal flow, such as variceal ligation or the implantation of a transjugular intrahepatic portosystemic shunt (TIPS).²⁷ Based on these observations, an inflow/outflow model explaining LS based on the resulting intrahepatic sinusoidal pressure has been introduced recently.²⁸

The deployment of μ Fibroscan technology has recently enabled real-time monitoring of these pressure-modulating effects on liver stiffness due to catecholamines and portal pressure-modulating drugs such as Losartan, glycerol nitrate, or propranolol.^{26,29} Yet, despite its utility, liver stiffness cannot serve as a perfect surrogate for HVPG. Beyond certain thresholds, liver stiffness and HVPG do not correlate strictly, and changes in liver stiffness in patients treated with non-selective beta-blockers do not necessarily reflect changes in HVPG.¹⁸ However, spleen stiffness has shown higher correlations with HVPG and improved predictions of portal hypertension, warranting further exploration, particularly concerning the effects of vasoactive drugs on spleen stiffness.^{30–36}

Elastographic methods not only hold great promise in identifying patients with CSPH, but also may also be important for monitoring PH-lowering treatment strategies and fine-tuning the therapy of PH-lowering drugs.³⁷ Therefore, this study aimed to investigate liver and spleen stiffness in a rat model of cirrhosis, their response to various vasoactive drugs, and their association with invasively measured portal²⁶ and arterial pressure parameters.

Material and Methods

Animals

Forty-three male Wistar rats, 8 weeks old, were housed under standardized conditions at the Interfaculty Biomedical Research Facility, University of Heidelberg. The animals were accommodated in Type IV cages under a controlled 12-hour light-dark cycle. Food and water were provided ad libitum until the initiation of the experiment. All procedures were conducted in accordance with the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA) and approved by the Regional Council Karlsruhe (approval number 35–9185.81/G-93/17).

Induction of Cirrhosis

Cirrhosis was induced by administering intraperitoneal injections of thioacetamide (TAA) at a concentration of 50 mg/mL. The injections were given twice weekly for six weeks, with each rat receiving a dose of 200 mg/kg body weight, calculated based on pre-injection weights. 9 rats received 0.9% sodium chloride injections and served as controls.

Vasoactive Drug Administration

The study design, illustrated in [Figure 1](#), included the administration of various vasoactive drugs to five groups: Metoprolol, Udenafil, Enalapril, Terlipressin, and Carvedilol. The mode of action of these drugs is summarized in [Supplementary Table 1](#). The five selected vasoactive agents represent a diverse range of pharmacodynamic mechanisms

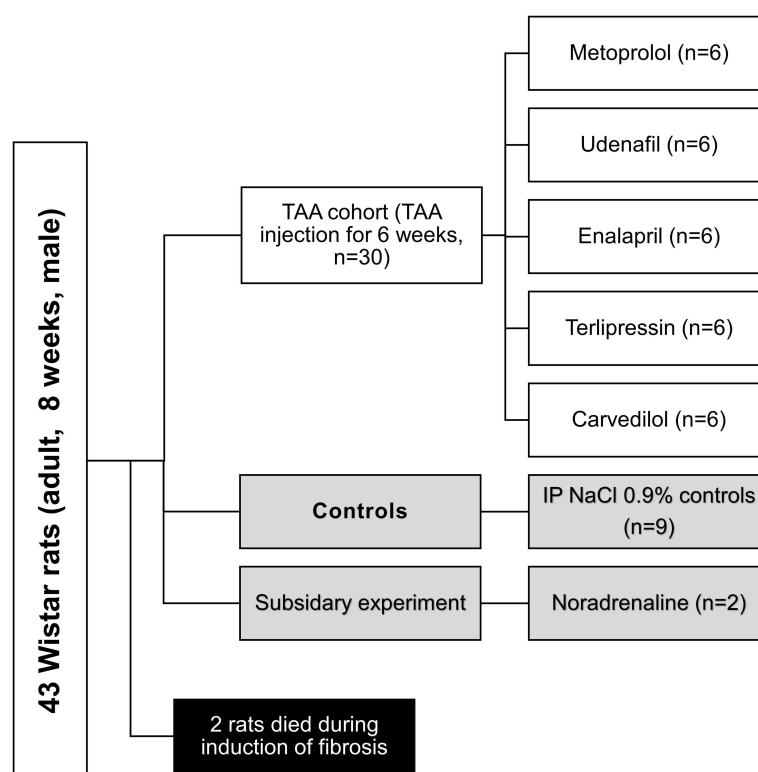


Figure 1 Study design schematic. The diagram outlines the experimental timeline, including cirrhosis induction, drug administration, and subsequent measurements of hemodynamic and stiffness parameters.

with varying clinical and experimental relevance to portal hypertension. Carvedilol, Metoprolol, and Terlipressin are widely used in clinical practice to reduce portal pressure in patients with cirrhosis, with carvedilol being established in variceal bleeding prophylaxis and terlipressin routinely applied in acute variceal bleeding and hepatorenal syndrome. Metoprolol, although β 1-selective, was included to assess the specific effects of adrenergic modulation. Enalapril, a classical systemic vasodilator, was selected to evaluate the impact of arterial pressure changes. Udenafil, a PDE-5 inhibitor, has shown promise in experimental studies as a portal pressure-lowering agent, despite its current approval being limited to erectile dysfunction. This selection allows for a mechanistic comparison of drug-induced hemodynamic effects on liver and spleen stiffness, independent of their current clinical indications.

Drug dosages were calculated by scaling the standard human dose to rat size using the formula: Animal dose = human dose $\times$ 0.1644.³⁸ The drugs were administered by intravenous injections. For human doses not specified in mg/kg, a standard body weight of 70 kg was assumed, as detailed in [Supplementary Table 2](#). Baseline parameters for each animal group are shown in [Table 1](#).

Table 1 Baseline Parameters of the Individual Treatment Groups (Mean $\pm$ Standard Deviation)

	Body Weight (g)	Liver Stiffness (kPa)	Spleen Stiffness (kPa)	SS/LS	PVP (mmHg)	MAP (mmHg)	Heart Rate (bpm)
Metoprolol	426.8 $\pm$ 24.5	20.5 $\pm$ 17.1	58.3 $\pm$ 14.7	4.3 $\pm$ 2.4	15.7 $\pm$ 1.1	124.2 $\pm$ 15.4	324.0 $\pm$ 25.3
Udenafil	456.2 $\pm$ 51.6	21.9 $\pm$ 15.8	58.4 $\pm$ 5.2	3.8 $\pm$ 1.8	14.9 $\pm$ 0.6	105.8 $\pm$ 14.8	300.5 $\pm$ 11.4
Enalapril	486.7 $\pm$ 32.2	19.7 $\pm$ 14.3	47.8 $\pm$ 11.3	3.3 $\pm$ 1.9	17.3 $\pm$ 4.5	120.0 $\pm$ 10.8	308.7 $\pm$ 25.8
Terlipressin	472.5 $\pm$ 23.6	23.8 $\pm$ 15.6	53 $\pm$ 5.3	3.2 $\pm$ 1.9	18.4 $\pm$ 4.0	120.0 $\pm$ 16.4	278.8 $\pm$ 36.0
Carvedilol	425.0 $\pm$ 93.2	24.8 $\pm$ 16.2	50.8 $\pm$ 6.7	2.9 $\pm$ 1.7	16.7 $\pm$ 2.8	132.3 $\pm$ 11.1	301.5 $\pm$ 17.6

Invasive Hemodynamic Measurements

Invasive blood pressure measurements were performed using a PowerLab system (AD Instruments, Dunedin, New Zealand) following the protocol described by Piecha et al (2016).²⁶ Pressure transducers (MLT0699 BP transducers, AD Instruments) were connected to catheters inserted into the abdominal aorta and portal vein. Mean arterial pressure (MAP) and heart rate data were recorded and analyzed using LabChart software (AD Instruments, Dunedin, New Zealand). To minimize respiratory artifacts, mean pressure values corresponding to each liver stiffness measurement were averaged over one-second intervals. Concurrent evaluations of drug effects on liver stiffness (LS) and spleen stiffness (SS) were conducted, with changes in MAP, portal vein pressure (PVP), and heart rate carefully documented.

Liver and Spleen Stiffness Measurements

Liver stiffness (LS) and spleen stiffness (SS) were quantified using the μ Fibroscan device (Echosens, Paris, France) in accordance with protocols described previously.^{26,39,40} For spleen measurements, the organ was positioned in the epigastric area, with the stomach and intestines gently displaced to ensure optimal access. The probe was aligned perpendicularly at a midpoint between the spleen's upper and lower borders, and between the hilum and anterior pole. This specific site was identified through preliminary tests. Measurements taken at this location demonstrated high reproducibility and negligible interobserver variability. LS and SS values were recorded at baseline and at 15 and 30 minutes after drug administration, as shown in Figure 2.

Statistical Analysis

Data analysis was conducted using SPSS Statistics version 25.0 (IBM, USA) and GraphPad Prism 6 (GraphPad Software, USA). Group comparisons were performed using independent samples t-tests, while paired samples t-tests were applied for pre- and post-treatment comparisons. Pearson's correlation coefficient (r , p) was used to evaluate relationships between changes in hemodynamic parameters, heart rate, and organ stiffness metrics. The delta (Δ) values, representing the differences between pre- and post-intervention measurements, were calculated for each parameter and aggregated across intervention groups to investigate correlations between absolute changes. Statistical significance was defined as $P < 0.05$, with highly significant results defined as $P < 0.01$. All reported P-values are two-tailed and uncorrected.

Results

Increased Liver and Spleen Stiffness and Weight in Cirrhotic Rats

Control ($n = 9$) and cirrhotic rats ($n = 30$) without treatment with vasodilating drugs were compared, as illustrated in Figure 3. Cirrhotic rats exhibited significantly higher liver stiffness (22.1 kPa vs 4.2 kPa, $P < 0.001$) and spleen stiffness (53.7 kPa vs 27.7 kPa, $P < 0.001$) compared to controls. Additionally, these animals had markedly larger and heavier

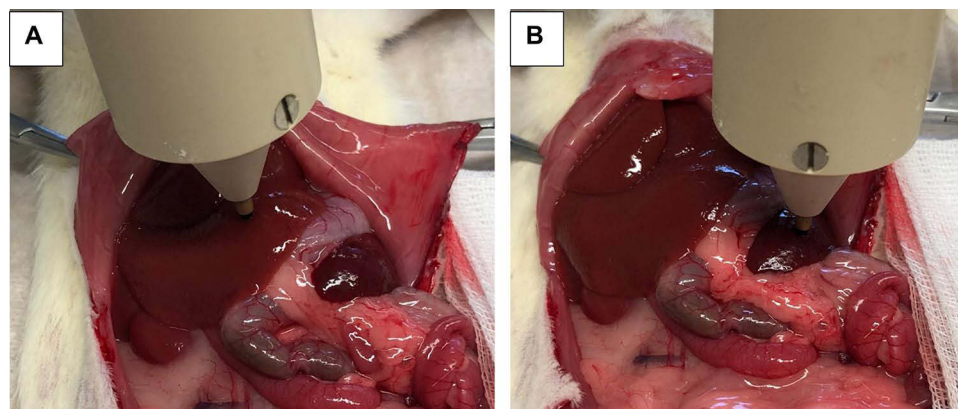


Figure 2 Liver and spleen stiffness measurements using the μ Fibroscan device. (A) Liver stiffness (LS) and (B) spleen stiffness (SS) values obtained with μ Fibroscan. Representative results illustrate stiffness acquisition and variability between treatment conditions.

spleens (5.9 cm vs 4.0 cm, 2.2 g vs 1.1 g, $P < 0.001$) and elevated portal vein pressures (PVP; 16.6 mmHg vs 10.4 mmHg, $P < 0.001$). No significant differences were observed in heart rate or mean arterial pressure between the groups. These findings confirm that an 8-week regimen of TAA administration successfully induces cirrhosis, as evidenced by increased liver stiffness, spleen enlargement, and elevated PVP.

Effects of Vasoactive Drugs on Hemodynamics and Organ Stiffness

The time course of hemodynamic parameters and organ stiffness within 15 minutes after drug administration is presented in [Figure 4](#). All tested vasoactive drugs significantly reduced portal vein pressure (PVP) within the first 15 minutes, with reductions ranging from 21% to 36% ($P < 0.01$; [Figure 4A](#)). Carvedilol demonstrated the most pronounced effect, achieving a 36% reduction in PVP ($P < 0.01$). Except for terlipressin, all drugs significantly decreased liver and spleen stiffness within the same period ($P < 0.01$; [Figure 4B](#) and [C](#)). However, no significant changes were seen for the SS/LS ratio (see [Supplementary Figure S1](#)). Notably, carvedilol yielded the greatest reductions in liver stiffness (37%) and spleen stiffness (31%; $P < 0.01$).

Correlation analyses revealed a stronger relationship between changes in spleen stiffness (ΔSS) and PVP (ΔPVP) across all drug treatments ([Table 2](#)). Detailed individual parameter changes 15 and 30 minutes post-terlipressin administration are shown in [Figure 5](#).

Cardiovascular Responses to Vasoactive Drug Administration

Vasoactive drug administration significantly affected mean arterial pressure (MAP) and heart rate. All drugs except terlipressin significantly decreased MAP within 15 minutes ([Figure 4D](#)). Carvedilol produced the largest MAP reduction (37%; $P < 0.01$), while metoprolol, udenafil, and enalapril caused reductions ranging from 13% to 21% ($P < 0.01$). In contrast, terlipressin uniquely increased MAP by 44% ($P < 0.01$). Heart rate responses varied among treatments. Carvedilol, metoprolol, and terlipressin significantly reduced heart rate by 40%, 23%, and 27%, respectively ($P < 0.01$; [Figure 4E](#)). Udenafil slightly increased heart rate by 8%, while enalapril showed no significant effect.

Correlation of Spleen Stiffness with Portal Pressure

Correlations between parameter changes at 0 to 15 minutes and 15 to 30 minutes for each drug group are summarized in [Table 3](#). The relationship between ΔSS and ΔPVP was consistently stronger than that observed between liver stiffness (ΔLS) and ΔPVP . This trend was particularly pronounced in the carvedilol-treated group, which exhibited the highest correlation ($r=0.946$ vs $r=0.820$, $P<0.001$).

Terlipressin demonstrated a distinct pharmacodynamic profile, with an inverse relationship between MAP and SS, along with increases in liver stiffness, spleen stiffness, and PVP at 30 minutes post-administration. This unique response underscores the complex mechanism of terlipressin compared to other drugs, highlighting the need for further studies to elucidate its hemodynamic effects.

Discussion

This study provides important insights into the dynamic effects of vasoactive drugs on liver stiffness (LS) and spleen stiffness (SS) in a cirrhotic rat model, advancing our understanding of the pathophysiology and treatment monitoring of portal hypertension (PH). Clinically significant portal hypertension (CSPH), defined as a hepatic venous pressure gradient (HVPG) ≥ 10 mmHg, represents a critical turning point in patients with advanced chronic liver disease (ACLD), heralding increased risks of hepatic decompensation and liver-related mortality.¹⁶ Our findings align with these clinical observations by demonstrating that cirrhotic rats exhibit significantly elevated LS, SS, portal vein pressure (PVP), and spleen weight compared to controls, establishing the reliability of the TAA model for studying PH.

The structural and dynamic components of PH are critical for understanding its pathogenesis and treatment. While HVPG is the gold standard for assessing sinusoidal PH, its invasiveness and limited availability necessitate the development of alternative approaches.^{41–43} Emerging technologies, such as elastography, have facilitated non-invasive assessments of LS and SS, which have shown promise in reflecting PH severity.^{30,32,44–47} However, the physiological significance and responsiveness of LS and SS to acute hemodynamic changes induced by pharmacological interventions

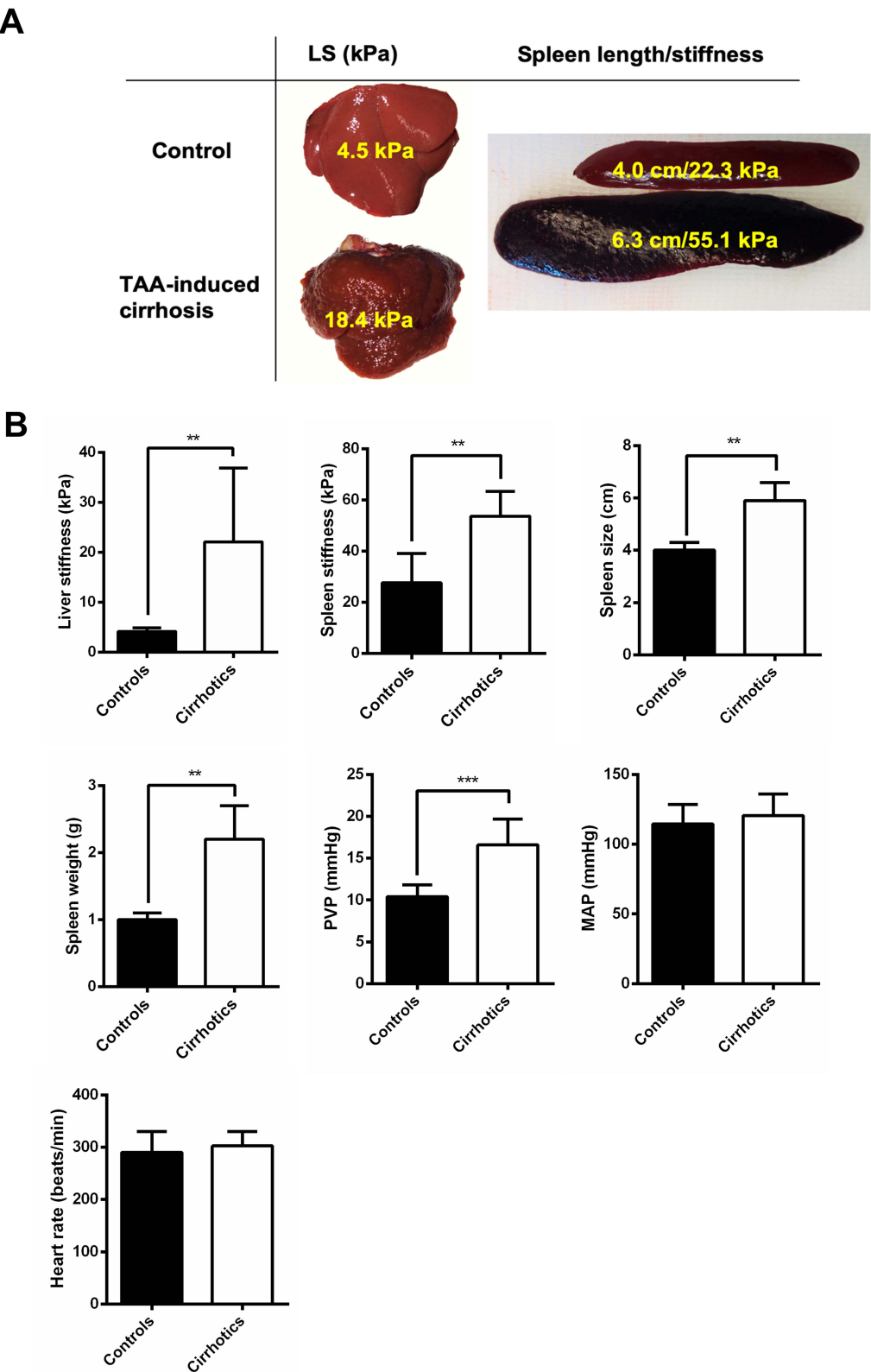


Figure 3 Increased liver stiffness, spleen stiffness, and spleen size in TAA-induced cirrhotic rats compared to controls. **(A)** Representative images of livers and spleens from control (n = 9) and cirrhotic rats (n = 30), highlighting organ enlargement in cirrhotic animals. **(B)** Quantitative analysis of liver stiffness, spleen stiffness, spleen size, spleen weight, heart rate, mean arterial pressure (MAP), and portal vein pressure (PVP). Data are presented as mean ± standard deviation. **P<0.01, ***P<0.001.

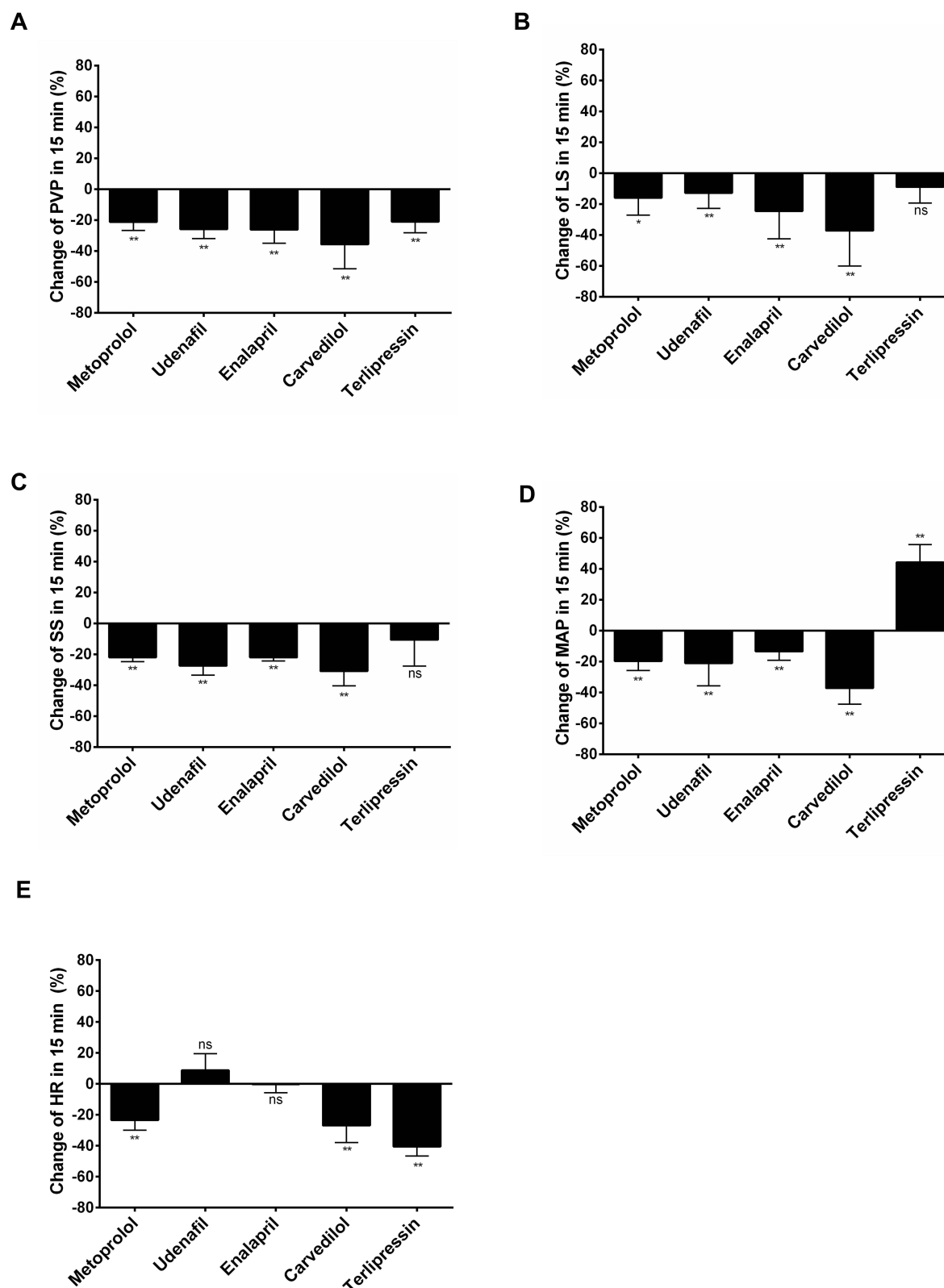


Figure 4 Acute effects of vasoactive drugs on hemodynamic parameters and organ stiffness within the first 15 minutes post-administration. **(A)** Percent change in portal vein pressure (PVP). **(B)** Percent change in liver stiffness (LS). **(C)** Percent change in spleen stiffness (SS). **(D)** Percent change in mean arterial pressure (MAP). **(E)** Percent change in heart rate (HR). Bars represent mean relative changes with standard errors. Significant differences from baseline (0 min) are indicated (* $P < 0.05$, ** $P < 0.01$, ns=not significant).

Table 2 Pearson Correlation Coefficients Across All Drug Groups for Changes in Hemodynamic Parameters and Organ Stiffness Between 0–15 minutes and 15–30 minutes Post-Administration. Correlations Highlight the Relationships Between Relative Changes in Portal Vein Pressure (PVP), Liver Stiffness (LS), Spleen Stiffness (SS), Mean Arterial Pressure (MAP), and Heart Rate (HR) Over Time

Parameter	Δ LS	Δ SS
Δ LS		0.638***
Δ SS	0.638***	
Δ PVP	0.670***	0.867***
Δ MAP	0.324*	0.375**
Δ HR	0.337**	0.360**

Notes: *P<0.05, **P<0.01, ***P<0.001.

remain underexplored.^{29,48} This study addresses this gap by evaluating the differential effects of commonly used vasoactive drugs, providing a comprehensive view of their impact on LS, SS, PP, and systemic hemodynamics.

Our results indicate that all tested drugs significantly reduced PVP, yet their effects on LS and SS varied. Carvedilol and Metoprolol demonstrated pronounced reductions in LS and SS, consistent with their ability to mitigate splanchnic hyperperfusion and reduce vascular resistance. These findings support the established use of non-selective beta-blockers (NSBB) in managing portal hypertension and highlight the potential of SS as a sensitive marker for monitoring therapeutic efficacy.^{12,49–51} In contrast, terlipressin, despite reducing PVP initially, failed to produce sustained decreases in LS and SS. Interestingly, it uniquely increased MAP and heart rate, suggesting a complex interplay between systemic vasoconstriction and hepatic hemodynamics. The hepatic arterial buffer response (HABR) may explain these findings, as it modulates hepatic arterial flow to compensate for changes in portal flow, potentially offsetting reductions in LS and SS.^{28,52–54} This compensatory mechanism underscores the limitations of LS and SS as sole markers for assessing terlipressin’s effects. Our findings align with previous studies that have reported a rebound increase in portal pressure following bolus administration of terlipressin.^{55–57} These results highlight the clinical rationale for utilizing continuous infusion, which offers a more consistent and stable reduction in portal pressure compared to bolus dosing.^{58,59}

As illustrated in Figure 6, terlipressin-induced splanchnic vasoconstriction reduces portal venous inflow, which would be expected to decrease LS and SS. However, this reduction in portal flow activates the hepatic arterial buffer response (HABR), a compensatory mechanism that increases hepatic arterial perfusion to maintain sinusoidal blood supply.⁶⁰ The HABR is thought to be mediated by adenosine and functions unidirectionally, ie, it compensates for reduced portal flow but not vice versa.⁶⁰ In the cirrhotic liver, this buffer mechanism becomes increasingly impaired or overwhelmed due to progressive arterialization of the hepatic circulation.^{61,62} Consequently, increases in arterial pressure—such as those induced by terlipressin—are more directly transmitted to the sinusoidal bed, raising intrahepatic pressure and tissue strain despite lowered portal pressure.^{26,63} These pathophysiological conditions are consistent with the sinusoidal pressure hypothesis (SPH) and may explain the paradoxical elevation of LS and SS observed in our terlipressin-treated animals.

SS exhibited a stronger correlation with PVP than LS, corroborating previous studies that highlight its direct interdependence with portal pressure, especially in the presence of elevated HVPG. SS also demonstrated drug-dependent correlations with systemic parameters such as MAP and heart rate. For example, Carvedilol and Metoprolol

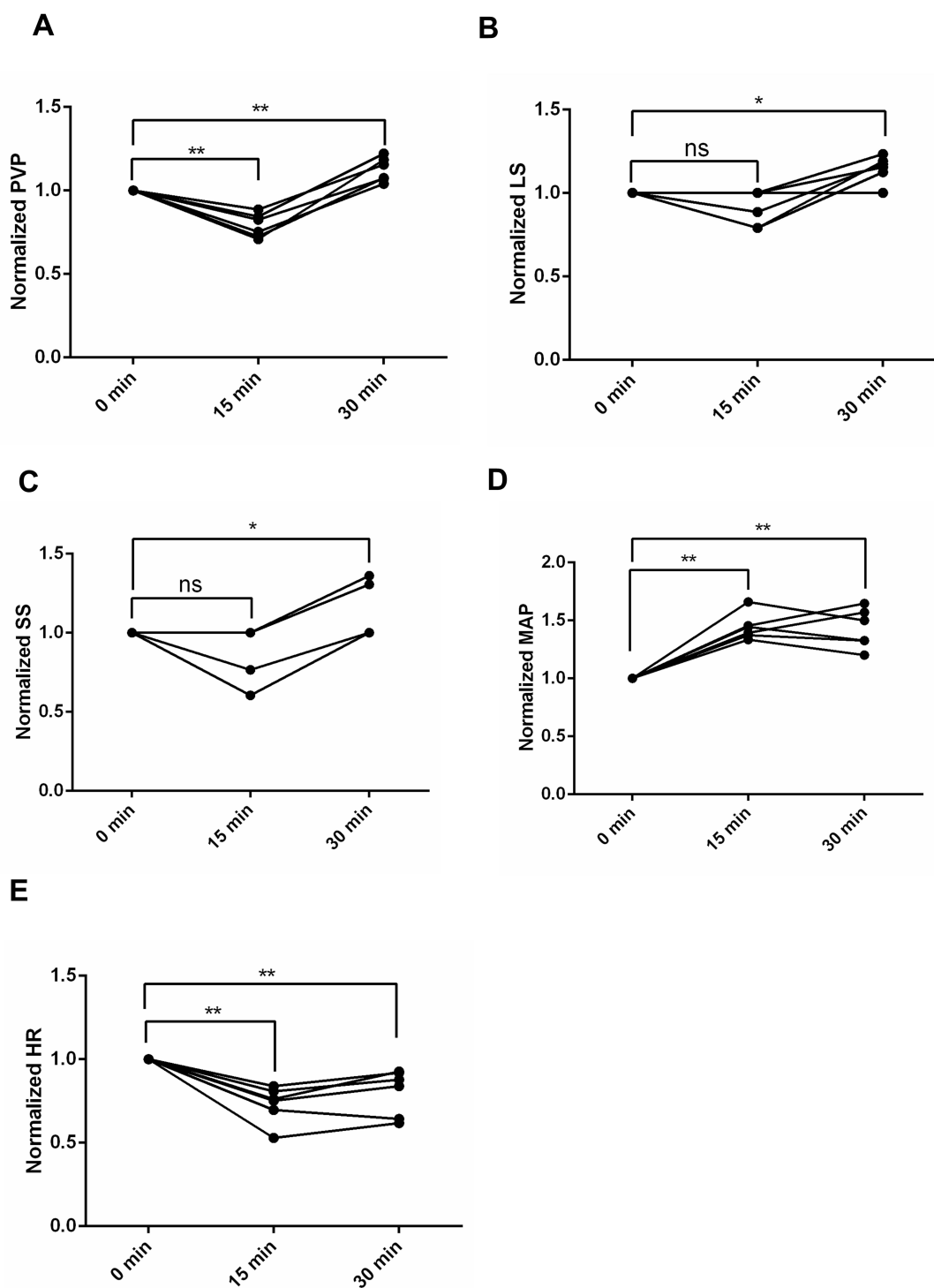


Figure 5 Time course of normalized hemodynamic and stiffness parameters following terlipressin administration. **(A)** Portal vein pressure (PVP). **(B)** Liver stiffness (LS). **(C)** Spleen stiffness (SS). **(D)** Mean arterial pressure (MAP). **(E)** Heart rate (HR). Data are normalized to baseline values and presented at 15 and 30 minutes post-injection, illustrating the temporal effects of terlipressin. * $P < 0.05$, ** $P < 0.01$, ns=not significant.

showed comprehensive associations between SS, MAP, heart rate, and PVP, reflecting their systemic and portal hemodynamic effects. In contrast, terlipressin demonstrated an inverse relationship between SS and MAP, with no significant correlations observed for Enalapril or Udenafil. These findings suggest that SS is a dynamic marker influenced by both portal and systemic hemodynamics, offering a more nuanced understanding of drug effects on PH.

Table 3 Pearson Correlation Coefficients for Combined Relative Changes in Hemodynamic Parameters and Organ Stiffness Across 0–15 minutes and 15–30 minutes, Stratified by Individual Drug Groups. These Correlations Illustrate the Drug-Specific Interdependence of PVP, LS, SS, MAP, and HR During the Observation Period

	Parameter	Δ LS	Δ SS
Metoprolol	Δ LS		0.740**
	Δ SS	0.740**	
	Δ PVP	0.606*	0.860***
	Δ MAP	0.383	0.693*
	Δ HR	0.392	0.739**
Udenafil	Δ LS		0.754**
	Δ SS	0.754**	
	Δ PVP	0.480	0.729**
	Δ MAP	0.281	0.468
	Δ HR	−0.498	−0.183
Enalapril	Δ LS		0.561
	Δ SS	0.561	
	Δ PVP	0.330	0.719**
	Δ MAP	−0.215	0.436
	Δ HR	−0.301	−0.428
Carvedilol	Δ LS		0.766**
	Δ SS	0.766**	
	Δ PVP	0.820**	0.946***
	Δ MAP	0.597*	0.868***
	Δ HR	0.697*	0.950***
Terlipressin	Δ LS		0.788**
	Δ SS	0.788**	
	Δ PVP	0.750**	0.874***
	Δ MAP	−0.750**	−0.757**
	Δ HR	0.789**	0.693*

Notes: *P<0.05, **P<0.01, ***P<0.001.

The clinical relevance of these findings is underscored by parallels in human studies. Patients with hepatitis C virus (HCV) infection have been shown to exhibit higher SS and PVP compared to those with alcoholic liver disease (ALD) at equivalent LS levels.⁶⁴ The higher SS/LS ratio observed in HCV patients is associated with increased risk of complications and earlier mortality due to PH-related events.^{65,66} The SS/LS ratio remained unchanged following portal pressure

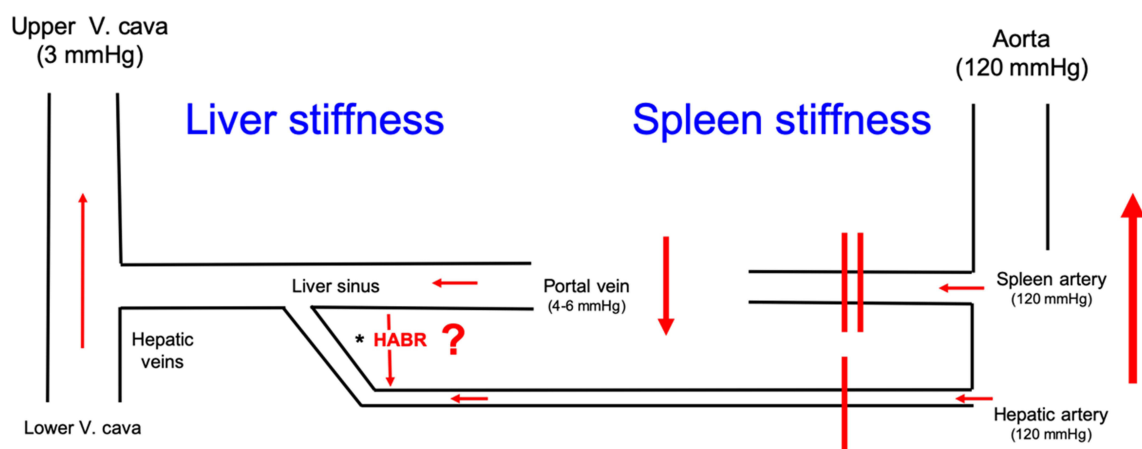


Figure 6 Mechanistic model of terlipressin's vasoconstrictive effects on splanchnic circulation and their impact on liver and spleen stiffness. Terlipressin reduces portal blood flow through splanchnic vasoconstriction while increasing systemic MAP. The hepatic arterial buffer response (HABR) compensates for reduced portal flow by enhancing hepatic arterial blood supply, thereby stabilizing LS and SS. Figure adapted and modified from Mueller S. Does pressure cause liver cirrhosis? The sinusoidal pressure hypothesis. *World J Gastroenterol.* 2016;22(48):10,482–10501. Creative Commons.²⁸ * = hepatic perfusion, ? = uncertain compensatory mechanism, arrows = direction of blood flow changes.

modulation across different drug groups, suggesting its disease-specific nature and its correlation with portal pressure ([Supplementary Figure S1](#)). These observations suggest that SS may serve as a more reliable marker for assessing portal hypertension severity and predicting clinical outcomes.^{30,31,33,46,47,67–70} Furthermore, the differential effects of terlipressin and beta-blockers on SS observed in our study may help refine drug selection and monitoring strategies in clinical practice.

Direct effects of vasoactive agents on the liver sinusoidal bed also warrant consideration. Alpha-adrenergic stimulation may influence sinusoidal vascular resistance, contributing to the complex hemodynamic responses observed with drugs like terlipressin.⁷¹ Similar delayed or paradoxical responses have been reported with vasoconstrictors such as noradrenaline and epinephrine, emphasizing the need to account for both systemic and local factors when interpreting changes in LS and SS.^{26,72} This complexity highlights the importance of integrating multiple metrics, including arterial pressure, pulse, LS, and SS, to provide a comprehensive assessment of portal pressure-lowering therapies.

This study has several limitations. First, the sample size of six animals per treatment group, while consistent with previous preclinical studies, may limit the detection of subtle pharmacodynamic effects and reduces statistical power. Second, although fibrosis induction was confirmed by elastographic and hemodynamic markers, histological validation was not performed in this study to minimize animal use and comply with 3R principles. Third, all measurements reflect acute drug responses; chronic dosing regimens, longer observation times, and clinical correlation were beyond the scope of this study but warrant future investigation. Finally, although spleen and liver stiffness correlated with portal pressure changes, these parameters are influenced by multiple systemic and local hemodynamic factors, which may confound interpretation in more heterogeneous clinical settings.

The findings of this study have important implications for clinical practice and research. In clinical practice, a considerable number of patients experience intolerance or adverse effects to NSBB, particularly at higher doses.^{37,73} The utilization of LS and SS measurements could provide a non-invasive method to monitor the effect of carvedilol on portal pressure. This approach enables clinicians to tailor and optimize the treatment dose more effectively, balancing efficacy and tolerability.^{74,75} To enhance the management of portal hypertension, non-invasive monitoring of PH-lowering drugs should incorporate both LS and SS alongside systemic hemodynamic parameters to capture the multifaceted effects of these interventions. SS, in particular, may provide a more accurate reflection of portal pressure changes, especially in patients with advanced PH.^{30,46,68,69} Additionally, our study underscores the need for further exploration of the mechanisms underlying the differential effects of vasoactive drugs, particularly the role of HABR and systemic vascular responses.

Conclusion

In conclusion, this study demonstrates the potential of SS as a superior biomarker for monitoring portal pressure changes compared to LS. The differential effects of vasoactive drugs on LS, SS, and systemic hemodynamics highlight the complexity of their interactions, emphasizing the need for comprehensive and individualized monitoring strategies. Future studies should aim to validate these findings in clinical settings, explore long-term effects, and investigate novel therapeutic approaches to optimize the management of portal hypertension.

Abbreviations

LS, liver stiffness; MAP, mean arterial pressure; ROC, receiver operating characteristics; TE, transient elastography; US, ultrasound; SL, spleen length; SS, spleen stiffness; PVP, portal vein pressure; TAA, thioacetamide, HR, heart rate; HABR, hepatic arterial buffer response; HVPG, hepatic venous pressure gradient; CVP, central venous pressure.

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Disclosure

The authors report no conflicts of interest in this work.

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