Research Article

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Evaluation of regional hepatic perfusion (RHP) by contrast-enhanced ultrasound in patients with cirrhosis

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Background & Aims: Ultrasonographic contrast agents allow the assessment of myocardial and renal perfusion through the analysis of refill kinetics after microbubbles rupture. This study evaluated the feasibility of contrast-enhanced ultrasonographic (CEUS) estimations of regional hepatic perfusion in patients with cirrhosis, and its correlation with clinical and hemodynamic parameters.

Methods: Fifty-five patients with cirrhosis undergoing hepatic vein catheterization were included. Hepatic perfusion was studied by CEUS (using Contrast Coherent Imaging) during a continuous i.v. infusion of microbubbles (SonoVue®); after their rupture (high insonation power), tissue refill was digitally recorded and time-intensity curves were electronically calculated on a region of interest of the right hepatic lobe. Regional hepatic perfusion (RHP) was calculated as microbubbles velocity × microbubble concentration. During hepatic vein catheterization, we measured hepatic blood flow by indocyanine green (ICG) infusion, hepatic venous pressure gradient (HVPG), and cardiac output (Swan-Ganz catheter).

Results: RHP was higher in patients than in healthy controls (5.1 ± 3.7 *vs.* 3.4 ± 0.7, $p = 0.003$), and correlated with MELD ($R = 0.403$, $p = 0.002$), Child-Pugh score ($R = 0.348$, $p = 0.009$), and HVPG ($R = 0.279$, $p = 0.041$). RHP inversely correlated with ICG extraction ($R = -0.346$, $p = 0.039$), ICG intrinsic clearance ($R = -0.327$, $p = 0.050$), and ICG clearance ($R = 0.517$, $p = 0.001$), and directly correlated with hyperdynamic syndrome markers (cardiac index $R = 0.422$, $p = 0.003$; mean arterial pressure $R = -0.405$, $p = 0.004$; systemic vascular resistance $R = -0.496$, $p = 0.001$).

Conclusions: RHP increases in patients with cirrhosis and correlates with the degree of liver failure and hyperdynamic syndrome. RHP increases along with liver functional reserve decrease, suggesting that RHP increase occurs mainly through anatomical/functional shunts. RHP by CEUS is a feasible novel, objective, quantitative, non-invasive tool, potentially useful for the estimation of hepatic perfusion in patients with cirrhosis.

Introduction

Liver cirrhosis is characterized by anatomical changes within the liver parenchyma including fibrosis and nodular regeneration [1,2]. A direct consequence of these alterations is a disturbance of the hepatic microcirculation, which accounts for the onset of portal hypertension. The study of the hepatic microcirculation could provide accurate information on the degree of structural progression of liver disease, but it is not currently feasible in patients with liver diseases due to the lack of an appropriate non-invasive method. Dynamic contrast-enhanced CT (DCE-CT) [3–5] and DCE-MRI [6,7] have been recently proposed, but their cost still limits the clinical applicability of both techniques.

In recent years, the discovery of some physical properties of ultrasonographic microbubble contrast agents, such as their capability of generating nonlinear signals when insonified [8], led to the possibility of using contrast-enhanced ultrasound (CEUS) to image functional aspects of macro-and microvasculature in biological tissues in real-time [9].

Gas-filled microbubbles used in CEUS remain entirely within the intravascular space, similar to more conventional tracers. It has been shown that during a continuous infusion, up to the achievement of a steady-state, the relation between microbubbles concentration and video-intensity is linear [10]; it is possible to destroy the microbubbles in the insonation area by ultrasound using high emission power (high mechanical index) and to measure the replenishment curve of a region of interest (ROI) using specifically designed computer software. The slope of the replenishment curve represents the microbubbles velocity, and corresponds to the blood flow velocity, and the maximum amplitude of the curve in ROI, estimated in the grey-scale, reflects the microbubbles concentration within the vessel, in other words the tissue blood volume fraction [9]. The product of microbubbles velocity and tissue blood volume fraction reflects regional perfusion [11–13].

Keywords: Portal hypertension; Hepatic blood flow; Indocyanine-green; HVPG.
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Abbreviations: RHP, regional hepatic perfusion; HVPG, hepatic venous pressure gradient; CEUS, contrast enhanced ultrasound.

This technique has the advantage of providing non-invasive, quantitative, and observer-independent data on tissue perfusion *in vivo* and in real time, and has been already successfully used in humans to quantify brain perfusion during stroke [14–16], to assess myocardial flow reserve [11,17–19] and to estimate renal perfusion [13,20].

We previously reported a preliminary study on hepatic perfusion in healthy subjects demonstrating that this method has a good reproducibility, and can reflect the changes in hepatic perfusion after physiological stimuli [21].

The aims of the present study were to evaluate:

(1) the feasibility, applicability and reproducibility of the analysis of refill kinetics by CEUS to assess regional hepatic perfusion (RHP) in patients with cirrhosis;
(2) the correlation of RHP with indicators of liver disease severity (estimated by Child-Pugh and MELD score), portal hypertension (evaluated by HVPG), hepatic blood flow (by Indocyanine Green, ICG, and Doppler), and effective hepatic perfusion (assessed by ICG plasmatic clearance and extraction)
(3) changes in RHP after increasing hepatic blood flow by a standard meal and after decreasing portal blood flow in response to propranolol infusion.

Materials and methods

Patients

The study was performed in a prospective group of 55 consecutive patients with histologically proven liver cirrhosis (34 males, 21 females; mean age 56 ± 8 years) referred for HVPG measurement at our institution. The main clinical, laboratory, and hemodynamic characteristics of the studied population are summarized in Table 1.

Ten healthy subjects (6 males, 4 females, mean age 33 years) were used as controls for the non-invasive evaluations.

Measurements were done in baseline fasting conditions in all patients. In addition, 16 patients received a standard liquid meal (400 ml) containing 25 g of proteins, 80.8 g of carbohydrates, and 19.68 g of lipids for a total of 600 kcal (Ensure Plus; Abbott Laboratories B.V., Zwolle, The Netherlands) and both HVPG and CEUS were repeated after 30 min. Ten additional patients were treated with intravenous propranolol (0.15 mg/kg in 10 min), and HVPG and CEUS were repeated after 20 min.

Exclusion criteria were: ongoing treatment with vasoactive drugs, hepatocellular carcinoma, and important co-morbidity, myocardial infarction in the last 6 months, pregnancy, and denial of informed consent.

Patients' history and routine laboratory tests were obtained on inclusion. Severity of liver disease was assessed by Child-Pugh [22] and MELD scores [23].

This study was approved by the Ethics Committee of Hospital Clinic. The nature of the study was explained to the patients, and a written informed consent was obtained in each case, according to the principles of the Declaration of Helsinki (revision of Edinburgh 2000).

Portal blood flow, hepatic artery blood flow, and total hepatic blood flow by Doppler US

Doppler and CEUS studies were performed with a Siemens ACUSON Sequoia™ 512 (Acuson, Mountain View, CA, USA) using a 3.75 multifrequency sector probe provided with pulsed, color, and power device, by two trained operators (C.N. and A.B.), always after a fasting of at least 6 h and before the hemodynamic study, in the Hepatic Hemodynamics Laboratory. Operators were not aware of the histological, endoscopic, and hemodynamic data of the patients.

The examination included a standard B-mode scan for the assessment of the diameter of portal vein (PV) and hepatic artery (HA), and a color, and pulsed Doppler examination for the assessment of PV and HA blood velocity. Portal blood flow (PBF) was calculated as follows:

PBF (ml/min) = (PV diameter (cm))^2/4Π PV mean blood velocity (cm/s) ∗ 60.

Hepatic artery blood flow (HABF) was similarly calculated.

Total hepatic blood flow (THBF) by Doppler was calculated as the algebraic sum of PBF and HABF.

Regional hepatic perfusion (RHP) by Contrast-Enhanced ultrasound (CEUS)

After standard US-Doppler examination, CEUS with the second-generation intravenous US contrast agent sulfur hexafluoride SonoVue® (Bracco S.P.A., Milan, Italy) was performed. We used the same convex array probe, with focus depth beyond the area of interest located at 3–5 cm from the right hepatic lobe surface, using the following settings: microbubble-specific mode (Contrast Coherent Imaging); insonation frequency, 3.75 MHz; acoustic power, −75 to −90 dB. A low, non destructive emission power (<0.20) was selected to avoid the disruption of microbubbles. Gray-scale gain was adjusted for baseline imaging and was not altered after contrast material injection.

SonoVue® was administered intravenously by a 21 G needle placed in a vein of the left upper arm as a bolus of 0.5 ml, followed by 3 ml/min as a continuous infusion during 3 min. This dose was calculated according to SonoVue® distribution and elimination characteristics [24], to reach and maintain a steady state blood concentration of SonoVue®. At 95 s, when the stable concentration of parenchymatous enhancement was attained, microbubbles were destroyed in a chosen sector of the right hepatic lobe (VII segment), avoiding large vessels such as hepatic artery or portal vein branches, by emission of echo bursts obtained at high emission power. Successively, maintaining a continuous low emission power (0.15–0.19), the contrast replenishment in the selected area was observed and a 30 s film was recorded. Motion artifacts due to breathing were avoided by asking the patient to hold his breath during the acquisition of images of the replenishment period (Fig. 1).

Quantification of the contrast acoustic intensity in three regions of interest (ROIs) of the studied segment was performed off-line on the recorded images using dedicated software (CUSQ 1.3, Siemens Acuson, Mountain View, California). Nonlinear curve fitting was performed using the mathematical model $Y = \text{MinValue} + A\ (1 - e^{-\beta t})$ where β reflects microbubbles velocity and A microbubbles concentration automatically derived from intensity of acoustic backscatter in gray scale.

Regional hepatic perfusion (RHP) was calculated as [9]:

$\text{RHP} = A \times \beta$.

HVPG measurement

Immediately after ultrasound examination, the patients underwent hepatic vein catheterization. Under local anesthesia, a 8F venous catheter introducer (Axcess; Maxxim Medical, Athens, TX, USA) was placed in the right internal jugular vein using the Seldinger technique. Thereafter, a 7F balloon-tipped catheter (OB-Medi-Tech, Boston Scientific Cork Ltd., Cork, Ireland) was advanced into the right hepatic vein, to measure wedged and free hepatic venous pressures (WHVP and FHVP, respectively) by the connection to external electro-mechanical transducer and polygraph (Marquette Electronics, NY, USA). Hepatic venous pressure gradient was calculated as the difference between wedge and free hepatic venous pressure, as previously described [25].

Hepatic blood flow and effective liver perfusion by indocyanine green (ICG)

ICG is an anionic dye that remains within the vascular space following intravenous (i.v.) injection, and it is metabolically cleared in its first passage through the liver, when it is extracted into the bile [26]. The rate of excretion depends on hepatic circulation and active uptake and transportation by hepatocytes, so the test reflects hepatocyte function and effective hepatic blood flow [27].

Preceded by a priming dose of 5 mg, a solution of indocyanine green (Pulsion Medical Systems, Munich, Germany) was infused intravenously at a constant rate of 0.2 mg/min. After an equilibration period of at least 40 min, four separate sets of simultaneous samples of peripheral and hepatic venous blood were obtained for the measurement of hepatic blood flow (HBF) according to Fick's method, as previously described [28]. To avoid interferences from differences in plasma turbidity, the Nielsen correction was used [29].