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World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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ORIGINAL ARTICLE

Basic Study

Morphological alterations and redox changes associated with hepatic warm ischemia-reperfusion injury

Rim Jawad, Melroy D'souza, Lisa Arodin Selenius, Marita Wallenberg Lundgren, Olof Danielsson, Greg Nowak, Mikael Björnstedt, Bengt Isaksson

Rim Jawad, Lisa Arodin Selenius, Marita Wallenberg Lundgren, Olof Danielsson, Mikael Björnstedt, Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm S-141 86, Sweden

Melroy D'souza, Bengt Isaksson, Department of Clinical Science, Intervention, and Technology (CLINTEC), Division of Surgery, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm S-141 86, Sweden

Greg Nowak, Department of Clinical Science, Intervention, and Technology (CLINTEC), Division of Transplantation Surgery, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm S-141 86, Sweden

ORCID number: Rim Jawad (0000-0002-4216-0913); Melroy D'souza (0000-0002-6798-8960); Lisa Arodin Selenius (0000-0002-1148-5897); Marita Wallenberg Lundgren (0000-0003-3256-7063); Olof Danielsson (0000-0002-2558-2401); Greg Nowak (0000-0001-8766-8860); Mikael Björnstedt (0000-0003-2831-3837); Bengt Isaksson (0000-0002-7518-7127).

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Correspondence to: Melroy D'souza, MS, DNB MRCSEd, Department of Clinical Science, Intervention, and Technology (CLINTEC), Division of Surgery, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm S-141-86, Sweden. melroy.dsouza@karolinska.se
Telephone: +46-76-2884290
Fax: +46-85-8582340

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Abstract

AIM

To study the effects of warm ischemia-reperfusion (I/R) injury on hepatic morphology at the ultrastructural level and to analyze the expression of the thioredoxin (TRX)

and glutaredoxin (GRX) systems.

METHODS

Eleven patients undergoing liver resection were subjected to portal triad clamping (PTC). Liver biopsies were collected at three time points; first prior to PTC (baseline), 20 min after PTC (post-ischemia) and 20 min after reperfusion (post-reperfusion). Electron microscopy and morphometry were used to study and quantify ultrastructural changes, respectively. Additionally, gene expression analysis of TRX and GRX isoforms was performed by quantitative PCR. For further validation of redox protein status, immunogold staining was performed for the isoforms GRX1 and TRX1.

RESULTS

Post-ischemia, a significant loss of the liver sinusoidal endothelial cell (LSEC) lining was observed (P = 0.0003) accompanied by a decrease of hepatocyte microvilli in the space of Disse. Hepatocellular morphology was well preserved apart from the appearance of crystalline mitochondrial inclusions in 7 out of 11 patients. Post-reperfusion biopsies had similar features as post-ischemia with the exception of signs of a reactivation of the LSECs. No changes in the expression of redox-regulatory genes could be observed at mRNA level of the isoforms of the TRX family but immunoelectron microscopy indicated a redistribution of TRX1 within the cell.

CONCLUSION

At the ultrastructural level, the major impact of hepatic warm I/R injury after PTC was borne by the LSECs with detachment and reactivation at ischemia and reperfusion, respectively. Hepatocytes morphology were well preserved. Crystalline inclusions in mitochondria were observed in the hepatocyte after ischemia.

Key words: Hepatic ischemia-reperfusion injury; Ischemia reperfusion injury; Warm ischemia-reperfusion injury; Glutaredoxins; Thioredoxins; Electron microscopy; Oxidative stress; Portal triad clamping

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Core tip: The complex mechanisms of warm Ischemia reperfusion (I/R) injury in the liver are diverse and have been widely studied but poorly understood. This study aims to investigate the ultrastructural changes at warm I/R injury induced by portal triad clamping. The effects were mainly borne by the liver sinusoidal endothelial cells (LSEC) which detached from the sinusoidal wall after ischemia. Interestingly we found that the LSECs reattached after reperfusion. Hepatocytes were unaffected except for the appearance of crystalline inclusions in the mitochondria. Investigation of redox related proteins showed no changes within our time frame.

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INTRODUCTION

Ischemia-reperfusion (I/R) injury is a known cause of tissue damage during liver resection and transplantation with direct impact on patients' postoperative morbidity and mortality[1,2]. It is a biphasic phenomenon whereby the initial hypoxic damage is compounded upon restoration of blood supply along with oxygen delivery. The mechanisms of injury are complex and have been widely studied but remain poorly understood. Hepatic I/R injury is classified as warm or cold, where warm ischemia occurs when the blood supply to the liver is interrupted during liver resection, transplantation, trauma, and shock. Cold storage ischemia occurs during organ preservation in cold preservation solutions before transplantation[3]. Although both mechanisms share similarities, there are fundamental differences between warm and cold hepatic I/R injury. Existing knowledge indicates that warm I/R injury inflicts hepatocyte damage, while cold I/R injury is primarily characterized by injury to the sinusoidal endothelial lining[4].

Blood loss is one of the significant determinants of morbidity and tumor recurrence after hepatectomy[5]. Portal triad clamping (PTC), also known as the Pringle maneuver, has been one of the most widely used methods to reduce blood loss during hepatic surgery and involves clamping of the hepatic vascular inflow. PTC causes warm I/R injury in the remnant liver, the consequences of which are determined by the duration of clamping and the underlying health status of the liver parenchyma.

While there is vast literature regarding the biochemical and metabolic alterations associated with hepatic I/R injury, studies investigating the cellular and ultrastructural changes occurring in the liver as a result of I/R injury have mainly involved animal models and data from human studies are limited[6-10].

The ischemic injury occurs as a result of a reduction in blood supply and switching from aerobic to anaerobic metabolism. The initial ischemic insult followed by the sudden oxygen burst upon the reestablishment of vascular flow causes reperfusion injury which to a large extent is ascribed to the production of reactive oxygen species (ROS) and associated cellular injury[11-13]. There are several proteins involved in ROS scavenging and antioxidant defense. Many are regulated at transcriptional level through binding of nuclear factor (erythroid-derived 2)-like 2 (NRF2)[14], to the Antioxidant-Response Element (ARE) localized upstream of the promotor of these genes. By the same mechanism, *Nrf2* regulates glutamate-cysteine ligase (GCLC) and cysteine/glutamate antiporter (xCT), which are essential for glutathione (GSH) synthesis. GSH maintains the cellular redox balance and is considered

as one of the most important cellular antioxidants[15,16]. Thioredoxin (TRX) and glutaredoxin (GRX) are two intricate reduction systems belonging to the thioredoxin superfamily of proteins and are ubiquitously expressed in all cell types[17-19]. There is a lack of information on the involvement of these redox systems in hepatic I/R injury.

The present study aimed at investigating the effects of warm I/R injury induced by PTC in the human liver at the ultrastructural level, determining the degree and character of hepatocyte damage, and the sinusoidal endothelial lining. In addition, the impact of I/R injury on redox proteins was studied, in particular the TRX and GRX systems.

MATERIALS AND METHODS

Patients

Eleven patients (8 men and 3 women), undergoing liver resection for differing indications, but without preoperative clinical or biochemical signs of chronic liver disease, were included in the study. Seven of the patients had colorectal liver metastases and all of them had received preoperative chemotherapy. Two patients had melanoma metastases to the liver and one had metastases from a bowel carcinoid. One patient was operated because of a suspected hepatocellular carcinoma, which on final histopathology turned out to be an inflammatory pseudotumor. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Regional Ethics Committee for human studies, Stockholm, Sweden. All patients were informed orally and in writing and gave written consent.

Study protocol and biopsy acquisition

Laparotomy was performed by a right subcostal incision with an upper midline extension and the falciform ligament then divided. The hepatoduodenal ligament was isolated and a PTC then performed by placing a soft cloth tape around the porta hepatis over which a rubber tubing was then slid. With a hemostat, the rubber tubing was adjusted to constrict the vessels in the porta hepatis. The liver was not manipulated during the experimental time period. One wedge biopsy and two needle biopsies (with a Tru-Cut needle) were taken at three time-points; Baseline (just before the application of PTC), post-ischemia (after 20 min of PTC) and post-reperfusion (after 20 min of reperfusion). The needle biopsies were immediately transferred to the required buffers as detailed below before being stored at 4 ℃ for further analyses. The wedge biopsies were immediately transferred to vials and flash-frozen in liquid nitrogen and stored at -70 ℃ until analysis. The liver resection was then carried out as planned.

Transmission electron microscopy

The needle biopsies were fixed in 2% glutaraldehyde, 1% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4 for 10 min at room temperature and then stored at 4 ℃. The samples were washed with 0.1 mol/L phosphate buffer, pH 7.4 and then postfixed for 2 h in 2% osmium tetroxide, 0.1 mol/L phosphate buffer, pH 7.4 at 4 ℃. Dehydration was performed in ethanol, followed by acetone and the tissue samples were embedded in LX-112. Sections of approximately 70 nm were prepared by an ultramictrotome (Leica EM UC 6). Uranylacetate was added to the sections for contrast followed by lead citrate. A transmission electron microscope (Tecnai 12 Spirit Bio TWIN) was used at 100 kV for examination of the sections and a Veleta® camera used for capturing digital images. Assessment of the electron microscopy (EM) findings included evaluation of the cellular architecture, hepatocyte morphology, sinusoids and bile canaliculi.

Morphometric image analysis

NIS Elements Basic Research software was used for quantification analysis of the sinusoids in the electron micrograph images. Pixel length measurements were applied on the sinusoidal endothelial lining surrounding vessels. The number of pixels was determined on one representative sinusoid for each patient and time point, and the length of the endothelial lining was correlated to the length of the entire sinusoid. The retrieved pixel value was related to actual μm for comparison between different images.

Immunoelectron microscopy

Needle biopsies were fixed in 3% paraformaldehyde in 0.1 mol/L phosphate buffer and rinsed in 0.1 mol/L phosphate buffer with subsequent addition of 2.3 mol/L sucrose and subsequently frozen in liquid nitrogen. An ultramicrotome (Leica EM UC 6) was used for the sectioning on carbon-reinforced formvar coated, 50 mesh Nickel grids. The grids were placed on drops of 0.1 mol/L phosphate buffer, 2% BSA, 2% Fish gelatin. Primary antibodies of TRX1 and GRX1 were applied on the sections (GRX1 1:5, TRX1 1:10, own production as described previously[20], in 0.1 mol/L of phosphate buffer, 0.1% BSA, 0.1% gelatin) overnight in a humidified chamber at room temperature. The sections were rinsed with the same buffer and detection of primary antibodies was achieved by protein A with 10 nm gold at a dilution of 1:100. A second wash of the sections was performed before fixation in 2% glutaraldehyde. Contrast was attained by 0.05% uranylacetate and sections were embedded in 1% methylcellulose. A transmission electron microscope (Tecnai G2 Bio TWIN) was used for examination and digital images captured by a Veleta camera®. Quantification of the staining was performed on 5 hepatocytes in close proximity to vessels for each tissue section. The number of gold particles in the cytosol and the nuclei were recorded.

RNA purification, cDNA synthesis and qPCR

The fresh frozen wedge biopsies (approximately 10

Table 1 Patient demographics

Patient (gender/age)	Diagnosis	Resection performed	Preoperative chemotherapy	Peak AST, μkat/L	Peak ALT, μkat/L	Day of enzyme peak (AST, ALT), (d)	Peak bilirubin, μmol/L, (d)	Fibrosis (stage)	Inflammation	Steatosis
P1 (M/75)	mCRC	Atypical resection	Yes	4.39	2.23	1	24 (1)	1	0	1
P2 (F/71)	mCRC	Multiple resections	Yes	23.05	21.93	2	53 (1)	1	0	2
P3 (M/39)	Inflammatory pseudotumor	Extended right hepatectomy	No	4.19	4.81	1	41 (1)	-	-	1
P4 (M/75)	Malignant melanoma metastasis	Multiple atypical resections	Yes	2.32	1.90	1	12 (2)	1	0	1
P5 (M/63)	mCRC	Bisegmentectomy	Yes	6.25	7.03	2	16 (1)	1	0	1
P6 (F/78)	Carcinoid metastasis	Left hepatectomy	No	3.73	2.89	1	20 (1)	1	0	1
P7 (F/61)	mCRC	Right hepatectomy	Yes	7.14	6.11	1	14 (1)	1	0	0
P8 (M/55)	Malignant melanoma metastasis	Right hepatectomy	No	7.90	8.84	2	48 (2)	0	0	2
P9 (M/68)	mCRC	Right hepatectomy + atypical resection	Yes	3.85	1.91	1	82 (8)	3-4	2	1
P10 (M/37)	mCRC	Right hepatectomy	Yes	6.71	3.96	2	33 (2)	1	0	0
P11 (M/58)	mCRC	Right hepatectomy + atypical resection	Yes	11.16	9.28	1	53 (2)	1	0	3

Steatosis was defined as 1: Mild (< 33%); 2: Moderate (33%-66%); 3: Severe (> 66%). Fibrosis was defined as Stage 1: Portal fibrosis; Stage 2: Periportal fibrosis; Stage 3: Septal fibrosis; and Stage 4: Cirrhosis. mCRC: Metastatic colorectal cancer.

mg) were homogenized and lysed using a TissueLyser LT with RLT plus lysis buffer (Qiagen). RNeasy Plus Mini Kit (Qiagen) was used for RNA purification according to the manufacturer's instructions, and RNA concentration determination was performed on a NanoDrop ND 100 Spectrophotometer (Saveen Werner). In order to validate the purity and quality of the RNA, Experion Automated Electrophoresis System with Experion RNA StdSens Analysis Kit (BIO-RAD) was used according to manufacturer's protocol. The mRNA quality was assessed for all samples and 6 out of 11 patients had good quality of samples from all time points.

For cDNA synthesis, 2 μg RNA was subjected to reverse transcription by Omniscript RT kit (Qiagen) according to manufacturer's instructions. Twenty-50 ng cDNA/reaction was used for qPCR in a BIO-RAD iCycler (BIO-RAD). Forward and reverse primers were designed *via* primer BLAST and Ape and purchased from Invitrogen (primer details in Supplementary Table 1). The genes of interest were isoforms of thioredoxins (*TXN*) and glutaredoxins (*GLRX*) and also the gene of *xCT* and *NRF-2*, which are *SLC7A11* and *NFE2L2* respectively. The operation setting for qPCR instrument was the following: 50 ℃ for 2 min, 95 ℃ for 2 min with 40 amplification cycles of denaturation at 95 ℃ for 15 s. Annealing and elongation temperatures of 60 ℃, 66 ℃ or 55 ℃ were used, depending on the gene of interest, during 30 s. The $2^{-\Delta\Delta CT}$ method was used for quantification by normalizing the CT values against the housekeeping gene β-actin and retrieving fold change relative to the chosen control. A cut off value of 32 cycles was chosen and all primers were optimized for an efficiency of 90%-105%.

Statistical analysis

The statistical analysis was performed using GraphPad Prism 6.0 software. The non-parametric Friedman test followed by Dunn's *post-hoc* test was used for the analysis of endothelial lining, gene expression data, and immunogold staining data. A *P* values of less than 0.05 was considered to be significant.

RESULTS

Patients

The median age of the patients was 68 (range 39-78) years. Light microscopy evaluation of tissue blocks collected for clinical routine diagnostics from macroscopically non-tumorous liver parenchyma revealed no major histological differences in the surgical specimens from the majority of patients. However, one patient had severe fibrosis, suspicious for cirrhosis (stage 3-4) combined with inflammation of grade 1-2 as defined by Batts and Ludwig[21]. Two patients had moderate steatosis (defined as 33%-66% of hepatocytes affected). Furthermore, one patient had pronounced steatosis (> 67% of hepatocytes affected). The relevant patient data has been summarized in Table 1.

Ultrastructural examination

At baseline the biopsies exhibited typical hepatic organization with normal hepatocyte and liver sinusoidal endothelial cell (LSEC) morphology. The hepatocytes had a normal appearance, intact plasma membranes, and large numbers of mitochondria without any discernible morphological aberrations at this point. The presence of lipofuscin lipid lysosomes was observed in most liver sections (Table 2). The morphology of the Space of Disse showed hepatocytes with intact microvilli extensions and normal fenestrated LSECs lining the

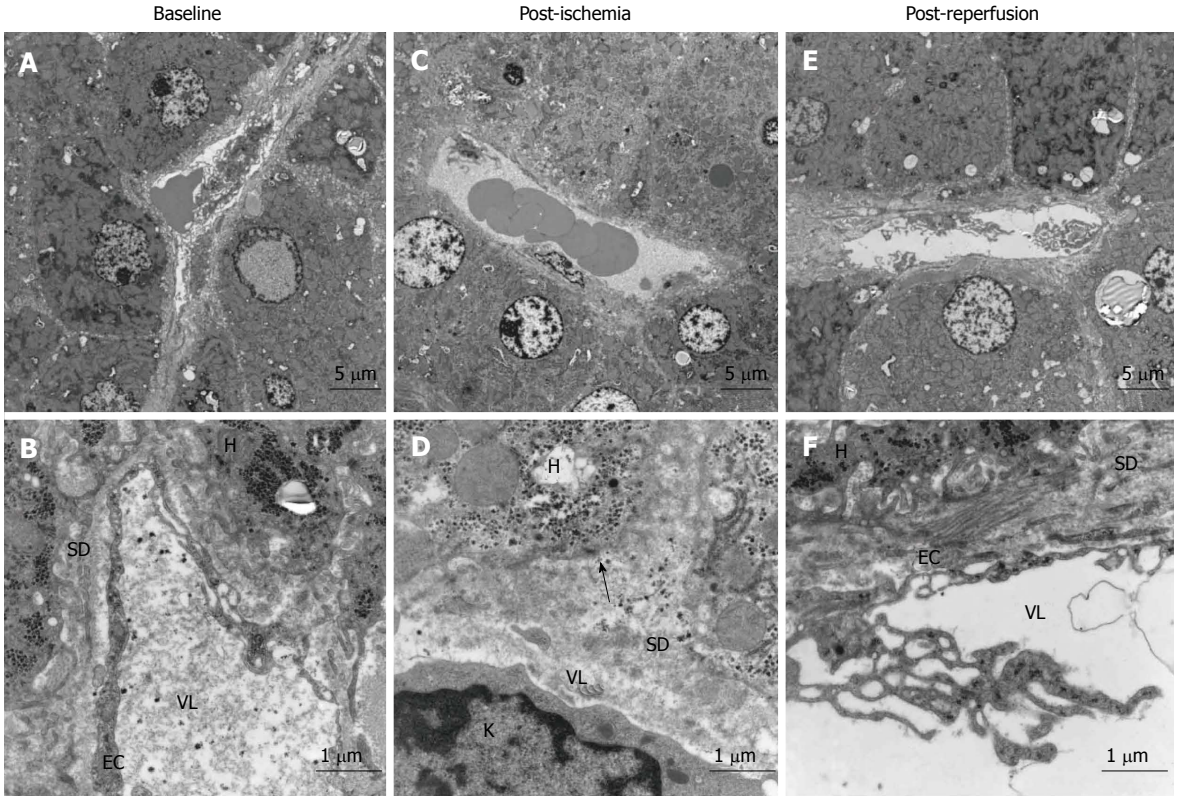

Figure 1 Morphological changes in liver before and after ischemia and reperfusion, transmission electron micrographs of representative images of liver sections from one patient. A: Baseline, before induction of ischemia, shows the normal state of liver morphology at a cellular level; B: Morphology of a sinusoid with neighbouring hepatocytes 20 min after ischemia; C: Representative image of sinusoid and haptocytes 20 min after reperfusion; D: Endothelial lining of a sinusoid with hepatocyte microvilli; E: Morphology of the Space of Disse post-ischemia; F: Morphology of the space of disse post-reperfusion. SD: Space of disse; H: Hepatocyte; EC: Endothelial cells; VL: Vessel lumen; K: Kupffer cell. Arrow shows the absence of hepatocyte microvilli.

Table 2 Quantitative summary of ultrastructural changes studied by endothelial morphology, (*n*) patients out of total (11) patients

	Baseline	Post-ischemia	Post-reperfusion
Disruption of endothelium	2/11	10/11	2/11
Endothelial activation	4/11	3/11	9/11
Mitochondrial inclusions in hepatocytes	1/11	6/11	7/11
Lipids, lipofucsin	10/11	10/11	10/11

sinusoids (Figure 1A and D).

The most noticeable change post-ischemia was a disruption of the LSEC lining (Figure 1B and E) in 10 out of 11 patients (Table 2). Apparent changes were seen in the space of Disse where the hepatocyte microvilli decreased in number and were in some cases undetectable (Figure 1E). There were no signs of hepatocyte plasma membrane rupture in either the ischemic or reperfused states. The hepatocytes exhibited some condensed nuclear chromatin but otherwise preserved hepatocyte morphology (Figure 1). In seven out of eleven patients, the hepatocyte mitochondria exhibited aggregates, so-called crystalline inclusions post-ischemia (Figure 2 and Table 2). These inclusions were accompanied by dilated mitochondria, both round and elongated types.

There was a reactivation of the LSECs with pseudopod-like extensions appearing from the cells' surface (Figure 1F and Table 2) post-reperfusion. The hepatocyte microvilli returned to their normal state within the space of Disse. LSEC apoptosis and phagocytosis by Kupffer cells was noticed in some sections. Hepatocyte morphology remained normal and the mitochondrial crystalline inclusions were persistent.

Morphometric analysis of endothelial cell lining loss

In order to evaluate the apparent loss and re-growth of the LSEC lining, quantitative image analysis was performed. This was expressed as a percentage of intact LSEC lining of the total length of the hepatic sinusoid. There was a quantitatively significant reduction in the lining between baseline and post-ischemia (P = 0.0003) (Figure 3). However, there was no difference between the baseline level and the post-reperfusion level, indicating a recovery of the LSEC lining post-reperfusion.

Gene expression analysis of redox regulating systems

To study the effects on expression of redox proteins during I/R, relative mRNA expression was investigated for genes implicated in the defense against oxidative stress. The gene expression of *NFE2L2* and *SLC7A11*, coding for the redox regulatory proteins NRF-2 and

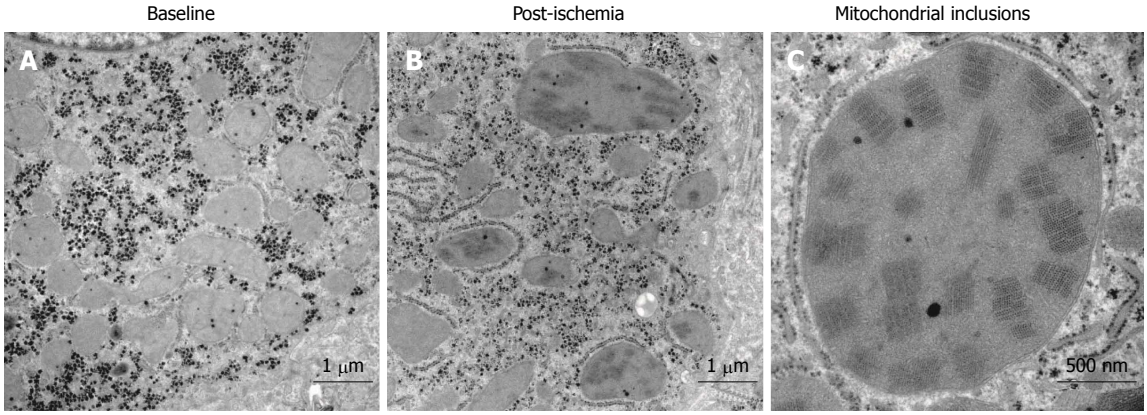

Figure 2 Crystalline mitochondrial inclusions. A: Baseline, before induction of ischemia, shows hepatocyte mitochondria with normal appearance; B: Post-ischemia, showing mitochondria with the crystalline inclusions and a few dilated mitochondria; C: Mitochondrial inclusions, close-up of a single mega-mitochondrion showing the inclusions post-ischemia.

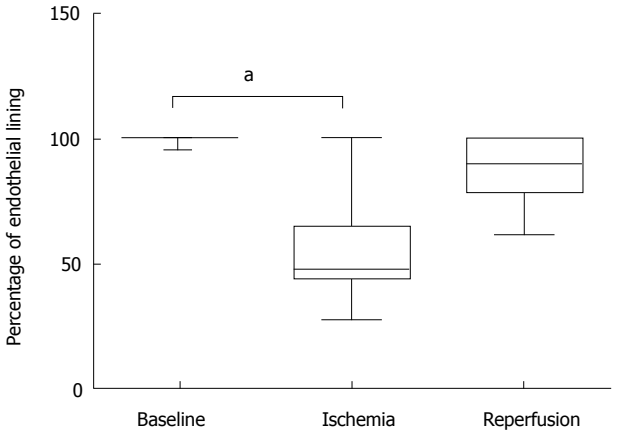

Figure 3 Morphometric analysis of endothelial lining. The percentage of attached endothelial lining along sinusoidal walls was quantified using network information services elements Basic Research Software. Statistical analysis was performed in Graphpad Prism, and differences were determined by the non-parametric Friedman test followed by Dunn's *post-hoc* test ($P < 0.01$). Baseline: Before induction of ischemia; Ischemia: Twenty minutes of ischemia; Reperfusion: Twenty minutes after reperfusion.

xCT, were investigated along with the TRX family of proteins. There were no differences post-ischemia or post-reperfusion compared with baseline (Table 3) of the investigated genes.

Immunoelectron microscopy redox proteins

Immunogold staining for GRX1 and TRX1 was performed in order to study if a translocation of the proteins occurred during I/R. There were no significant changes in the amount of TRX1 either in the nuclei or the cytosol of the hepatocytes during I/R (Figure 4). However, the total amount of TRX differed in the hepatocytes between the time points, and during ischemia the level of TRX decreased in five patients, remained unaffected in three, and increased in two patients (Table 4). The levels of GRX1 did not change in any of the patients during I/R (Figure 4).

DISCUSSION

PTC is an effective method to reduce blood loss during liver resections but is used very selectively in routine clinical practice[22]. In general, the extent of I/R injury depends on the duration and magnitude of the ischemia. This study used PTC to investigate ultrastructural changes associated with warm I/R injury. PTC was carried out for a fixed amount of time in all patients and the liver was not manipulated during the experimental procedure and biopsy acquisition. Thus, controlled experimental conditions were established in order to obtain reliable and comparable data. Since PTC is carried out routinely with a 20 min application time, ethical considerations did not permit a more extensive study. The short ischemia time and defined time points of biopsy acquisition thus limited the experimental scope of this study. The findings, however, provide data from human, eventually facilitating our understanding of the complex pathological alterations associated with warm hepatic I/R injury.

Hepatocytes and the LSECs are the cell types most sensitive to I/R injury. In our study, the most noticeable finding in post-ischemia liver biopsies was the loss of the LSEC lining, which was seen in 10 of 11 patients. This finding was significant as shown by the morphometric analysis. According to the current knowledge, based mainly on animal studies, hepatocytes are more sensitive to warm ischemia and LSECs to cold ischemia[4,23]. Detachment of these specific cells has been previously seen in cold ischemia models in rat[24,25]. On the other hand one animal study reported that LSEC death may precede hepatocyte death in warm I/R injury[26]. Here we show, in the human setting, that the LSECs bore the major impact of warm ischemia, visualized by signs of endothelial cell disruption.

Another striking finding in this study was the formation of pseudopod-like projections from the LSEC surface which has been interpreted as a reactivation or reattachment of the LSECs as a response to reperfusion. This was found in 9 of 11 patients and became evident already after 20 min of reperfusion. This suggests that structural loss of LSEC may be reversible when the duration and magnitude of warm

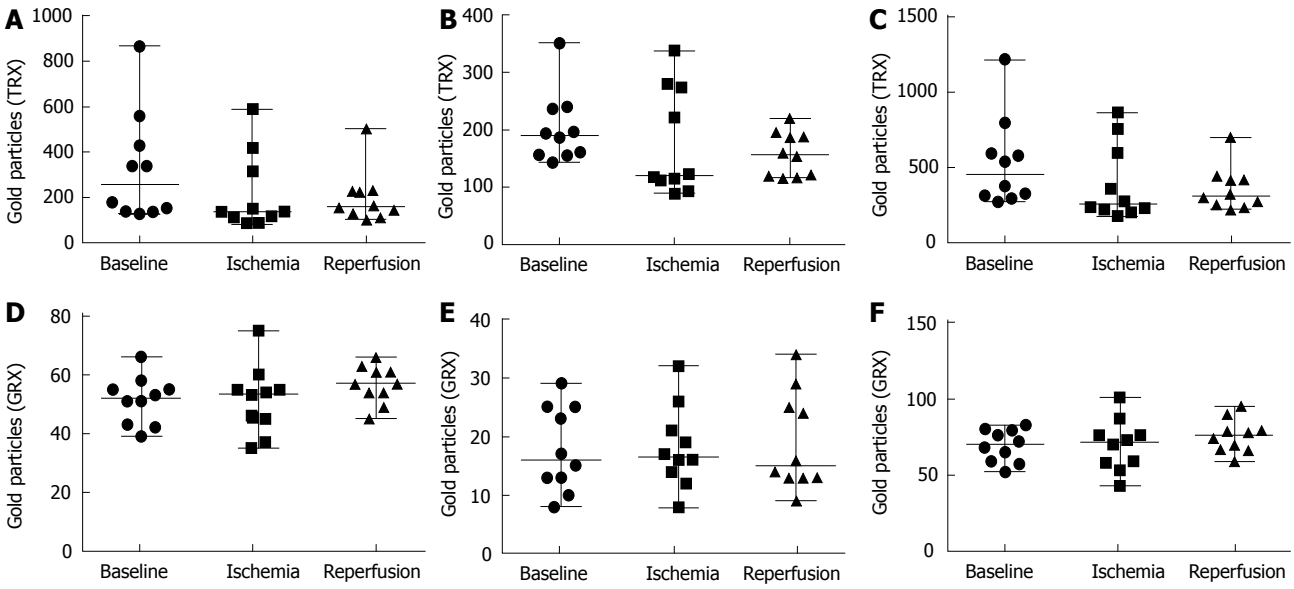

Figure 4 Quantification of immunogold staining. Number of gold particles of 5 hepatocytes for each time point and patient were recorded. A-C: Values from the TRX immunogold staining where A: Quantification of TRX in the cytosol; B: Quantification of TRX in the nuclei; C: Total value from of TRX both in cytosol and nuclei; D-F: Values from the quantification of GRX immunogold staining where D: Quantification of GRX in the cytosol; E: Quantification of GRX in the nuclei; F: Total value of GRX both in cytosol and nuclei. Baseline: Before induction of ischemia; Ischemia: Twenty minute of ischemia; Reperfusion: Twenty minute after reperfusion.

Table 3 Gene expression of redox proteins changes in mRNA expression compared to baseline, calculated using the $2^{-\Delta\Delta CT}$ method

	TNX1	TNX2	TNXR1	TNXR2	GLRX1	GLRX2	GLRX3	GLRX5	NFE2L2	SLC7A11
Ischemia	1.09 ± 0.18	1.14 ± 0.30	1.02 ± 0.28	1.05 ± 0.23	1.19 ± 0.41	1.24 ± 0.46	1.03 ± 0.16	1.20 ± 0.27	0.83 ± 0.15	1.27 ± 1.24
Reperfusion	1.12 ± 0.25	1.31 ± 0.57	0.97 ± 0.33	1.39 ± 0.79	1.12 ± 0.67	1.16 ± 0.43	1.16 ± 0.26	1.18 ± 0.63	1.00 ± 0.44	1.22 ± 0.78

I/R injury is limited. It must be noted, however, that late response to reperfusion was not investigated in this study and secondary phases of injury to the LSECs are therefore unknown, if there are any. In a rat model of liver transplantation cold preservation of donor organs resulted in detachment of LSECs followed by some reattachment after reperfusion[27]. To our knowledge, this phenomenon has never been reported after warm ischemia.

We observed that the hepatocytes lost microvilli in the space of Disse after PTC and some had condensed nuclear chromatin. Furthermore, hepatic mitochondria were dilated and showed the presence of post-ischemic crystalline inclusions which prevailed after reperfusion. Consistent with previous reports, the inclusions almost exclusively appeared in dilated mitochondria[28]. Apart from these findings the overall morphology of hepatocytes was remarkably well preserved. This is in consistency with an earlier report that has shown that the hepatic ultrastructure was unaffected after intermittent PTC, indicating that the tissue might recover from the injury[29].

It is known that early hypoxic changes of the hepatocytes can be detected from the morphological alterations of mitochondria[30]. Mitochondrial crystalline inclusions are commonly seen in early alcohol and non-alcohol related liver diseases and aspirin toxicity[31-33]. These have been previously described as "para"-crystalline inclusions, however, an optical diffraction study showed that they are true crystals. To date, the composition of these inclusions remains unknown[34]. The mitochondria of *E. coli* exhibit crystalline inclusions visually similar to these and may arise from copolymerization of the protein Dps to the bacterial DNA as a protective response against oxidative and nutritional stress[35]. Mitochondrial inclusions could thus be an evolutionarily preserved event and adaptive response to the ischemic state rather than an occurrence secondary to the injury.

Previous studies on oxidative stress in I/R have demonstrated an activation of the transcription factor NRF-2, which regulates a number of redox proteins, including the TRX family of proteins[15,36]. In our study PTC was used as a model of oxidative stress for studying redox proteins from the TRX family and their alterations in I/R injury. However, no changes on the mRNA levels of the redox proteins could be detected during the 20 min of PTC. Immunogold staining for TRX1 and GRX1 showed no changes in the hepatic GRX1 levels, but the levels of TRX1 present in the hepatocytes varied between the time points, suggesting a possible secretion of the protein. Although TRX1 lacks a translocation signal, this protein can be actively secreted through a leaderless

Table 4 Levels of TRX1, evaluated by immunogold staining, total number of gold particles present in five hepatocytes

Patient	Baseline	Post-ischemia	Post-reperfusion
P1	794	357	414
P2	310	202	323
P3	1217	235	219
P4	373	176	298
P5	324	231	232
P6	269	273	443
P7	575	569	252
P8	294	221	699
P9	535	863	417
P10	590	755	272

secretory pathway that is independent of the classical endoplasmic reticulum-Golgi pathway[37]. These findings could support a tentative role for the TRX family of proteins in warm I/R injury, however further studies are needed to elucidate this.

We conclude that in a human experimental model of warm I/R injury the major effect observed at the ultrastructural level was on the non-parenchymal LSECs while hepatocytes morphology remained relatively intact apart from crystalline inclusions in the mitochondria after ischemia. Alterations that arise may be protective adaptations that to some extent seem to be reversible. In situ protein observations were compatible with a tentative role for the thioredoxin family of proteins in I/R injury.

COMMENTS

Background

Portal triad clamping (PTC) is used during liver surgery to reduce blood loss. Limiting the blood supply in a tissue can cause ischemia reperfusion (I/R) injury to the tissue. The ischemic injury occurs initially with a switch from aerobic to anaerobic metabolism. Sudden oxygen burst upon returned vascular flow causes reperfusion injuries related to the production of reactive oxygen species (ROS).

Research frontiers

Primary endpoints of clinical studies concerning the PTC method has involved measurements of liver function test, duration of hospital stay, and post-operative complications. Hepatic tissue injury at the ultrastructural level has been sparsely studied and never has there been a differentiation between the ischemic and reperfusion state due to complexity arising in study design.

Innovations and breakthroughs

This is the first study that investigates ultrastructural changes as a result of warm I/R injury during surgery at each point of tissue insult in the liver.

Applications

Limiting the extent of tissue injury during surgery is important for the post-operative recovery of the patients. Evaluation of morphological changes as a result of PTC can provide insights for clinicians of their preferred methods. The results indicate that the changes on the ultrastructural level upon 20 min of ischemia are mainly localized in the sinusoids with a detachment of liver sinusoidal endothelial cells and a loss of hepatocyte microvilli in the Space of Disse. Upon reperfusion the sinusoids showed a reappearance of some liver sinusoidal endothelial cells. The hepatocytes displayed normal morphology with the exception of crystalline inclusions in mitochondria.

Terminology

Crystalline inclusions are characterized visually by dark lines in the mitochondria in the electron microscope. The true composition of the inclusions remain unknown, however *E.coli* have been recorded to display them as a means of protecting mitochondrial DNA in response to oxidative stress.

Peer-review

The authors present an interesting study on the microstructural alterations of liver in a warm ischemia-reperfusion setting.

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REFERENCES

1 **Zhai Y**, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation--from bench to bedside. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 79-89 [PMID: 23229329 DOI: 10.1038/nrgastro.2012.225]

2 **Nastos C**, Kalimeris K, Papoutsidakis N, Tasoulis MK, Lykoudis PM, Theodoraki K, Nastou D, Smyrniotis V, Arkadopoulos N. Global consequences of liver ischemia/reperfusion injury. *Oxid Med Cell Longev* 2014; **2014**: 906-965 [PMID: 24799983 DOI: 10.1155/2014/906965]

3 **Klune JR**, Tsung A. Molecular biology of liver ischemia/reperfusion injury: established mechanisms and recent advancements. *Surg Clin North Am* 2010; **90**: 665-677 [PMID: 20637940 DOI: 10.1016/j.suc.2010.04.003]

4 **Mendes-Braz M**, Elias-Miró M, Jiménez-Castro MB, Casillas-Ramírez A, Ramalho FS, Peralta C. The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. *J Biomed Biotechnol* 2012; **2012**: 298-657 [PMID: 22649277 DOI: 10.1155/2012/298657]

5 **Kooby DA**, Stockman J, Ben-Porat L, Gonen M, Jarnagin WR, Dematteo RP, Tuorto S, Wuest D, Blumgart LH, Fong Y. Influence of transfusions on perioperative and long-term outcome in patients following hepatic resection for colorectal metastases. *Ann Surg* 2003; **237**: 860-869; discussion 869-870 [PMID: 12796583 DOI: 10.1097/01.SLA.0000072371.95588.DA]

6 **Man K**, Lo CM, Liu CL, Zhang ZW, Lee TK, Ng IO, Fan ST, Wong J. Effects of the intermittent Pringle manoeuvre on hepatic gene expression and ultrastructure in a randomized clinical study. *Br J Surg* 2003; **90**: 183-189 [PMID: 12555294 DOI: 10.1002/bjs.4027]

7 **Nadig SN**, Periyasamy B, Shafizadeh SF, Polito C, Fiorini RN, Rodwell D, Evans Z, Cheng G, Dunkelberger D, Schmidt M, Self SE, Chavin KD. Hepatocellular ultrastructure after ischemia/reperfusion injury in human orthotopic liver transplantation. *J Gastrointest Surg* 2004; **8**: 695-700 [PMID: 15358330 DOI: 10.1016/j.gassur.2004.04.002]

8 **Vollmar B**, Glasz J, Leiderer R, Post S, Menger MD. Hepatic microcirculatory perfusion failure is a determinant of liver dysfunction in warm ischemia-reperfusion. *Am J Pathol* 1994; **145**: 1421-1431 [PMID: 7992845]

9 **Moussa ME**, Sarraf CE, Uemoto S, Sawada H, Habib NA. Effect of total hepatic vascular exclusion during liver resection on hepatic ultrastructure. *Liver Transpl Surg* 1996; **2**: 461-467 [PMID: 9346693 DOI: 10.1002/lt.500020609]

10 **Rodriguez AA**, LaMorte WW, Hanrahan LM, Hopkins SR, O'Keane JC, Cachecho R, Hirsch EF. Liver viability after ischemia-reperfusion. *Arch Surg* 1991; **126**: 767-772 [PMID: 2039366 DOI: 10.1001/archsurg.1991.01410300113018]

11 **Galaris D**, Barbouti A, Korantzopoulos P. Oxidative stress in hepatic ischemia-reperfusion injury: the role of antioxidants and iron chelating compounds. *Curr Pharm Des* 2006; **12**: 2875-2890 [PMID: 16918418 DOI: 10.2174/138161206777947614]

12 **Togashi H**, Shinzawa H, Yong H, Takahashi T, Noda H, Oikawa K, Kamada H. Ascorbic acid radical, superoxide, and hydroxyl radical are detected in reperfusion injury of rat liver using electron spin resonance spectroscopy. *Arch Biochem Biophys* 1994; **308**: 1-7 [PMID: 8311441 DOI: 10.1006/abbi.1994.1001]

13 **Togashi H**, Shinzawa H, Matsuo T, Takeda Y, Takahashi T, Aoyama M, Oikawa K, Kamada H. Analysis of hepatic oxidative stress status by electron spin resonance spectroscopy and imaging. *Free Radic Biol Med* 2000; **28**: 846-853 [PMID: 10802214 DOI: 10.1016/S0891-5849(99)00280-4]

14 **Jaeschke H**, Woolbright BL. Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. *Transplant Rev* (Orlando) 2012; **26**: 103-114 [PMID: 22459037 DOI: 10.1016/j.trre.2011.10.006]

15 **Sasaki H**, Sato H, Kuriyama-Matsumura K, Sato K, Maebara K, Wang H, Tamba M, Itoh K, Yamamoto M, Bannai S. Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. *J Biol Chem* 2002; **277**: 44765-44771 [PMID: 12235164 DOI: 10.1074/jbc.M208704200]

16 **Ishii T**, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, Yamamoto M. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J Biol Chem* 2000; **275**: 16023-16029 [PMID: 10821856 DOI: 10.1074/jbc.275.21.16023]

17 **Lillig CH**, Berndt C, Holmgren A. Glutaredoxin systems. *Biochim Biophys Acta* 2008; **1780**: 1304-1317 [PMID: 18621099 DOI: 10.1016/j.bbagen.2008.06.003]

18 **Lillig CH**, Holmgren A. Thioredoxin and related molecules--from biology to health and disease. *Antioxid Redox Signal* 2007; **9**: 25-47 [PMID: 17115886 DOI: 10.1089/ars.2007.9.25]

19 **Hirota K**, Matsui M, Iwata S, Nishiyama A, Mori K, Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci USA* 1997; **94**: 3633-3638 [PMID: 9108029 DOI: 10.1073/pnas.94.8.3633]

20 **Mollbrink A**, Jawad R, Vlamis-Gardikas A, Edenvik P, Isaksson B, Danielsson O, Stål P, Fernandes AP. Expression of thioredoxins and glutaredoxins in human hepatocellular carcinoma: correlation to cell proliferation, tumor size and metabolic syndrome. *Int J Immunopathol Pharmacol* 2014; **27**: 169-183 [PMID: 25004829 DOI: 10.1177/039463201402700204]

21 **Batts KP**, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* 1995; **19**: 1409-1417 [PMID: 7503362 DOI: 10.1097/00000478-199512000-00007]

22 **Rahbari NN**, Wente MN, Schemmer P, Diener MK, Hoffmann K, Motschall E, Schmidt J, Weitz J, Büchler MW. Systematic review and meta-analysis of the effect of portal triad clamping on outcome after hepatic resection. *Br J Surg* 2008; **95**: 424-432 [PMID: 18314921 DOI: 10.1002/bjs.6141]

23 **Peralta C**, Jiménez-Castro MB, Gracia-Sancho J. Hepatic ischemia and reperfusion injury: effects on the liver sinusoidal milieu. *J Hepatol* 2013; **59**: 1094-1106 [PMID: 23811302 DOI: 10.1016/j.jhep.2013.06.017]

24 **Holloway CM**, Harvey PR, Mullen JB, Strasberg SM. Evidence that cold preservation-induced microcirculatory injury in liver allografts is not mediated by oxygen-free radicals or cell swelling in the rat. *Transplantation* 1989; **48**: 179-188 [PMID: 2667203 DOI: 10.1097/00007890-198908000-00001]

25 **Fratté S**, Gendrault JL, Steffan AM, Kirn A. Comparative ultrastructural study of rat livers preserved in Euro-Collins or University of Wisconsin solution. *Hepatology* 1991; **13**: 1173-1180 [PMID: 2050331 DOI: 10.1002/hep.1840130625]

26 **Kohli V**, Selzner M, Madden JF, Bentley RC, Clavien PA. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. *Transplantation* 1999; **67**: 1099-1105 [PMID: 10232558 DOI: 10.1097/00007890-199904270-00003]

27 **Morgan GR**, Sanabria JR, Clavien PA, Phillips MJ, Edwards C, Harvey PR, Strasberg SM. Correlation of donor nutritional status with sinusoidal lining cell viability and liver function in the rat. *Transplantation* 1991; **51**: 1176-1183 [PMID: 2048194 DOI: 10.1097/00007890-199106000-00007]

28 **Le TH**, Caldwell SH, Redick JA, Sheppard BL, Davis CA, Arseneau KO, Iezzoni JC, Hespenheide EE, Al-Osaimi A, Peterson TC. The zonal distribution of megamitochondria with crystalline inclusions in nonalcoholic steatohepatitis. *Hepatology* 2004; **39**: 1423-1429 [PMID: 15122772 DOI: 10.1002/hep.20202]

29 **Man K**, Liang TB, Lo CM, Liu CL, Ng IO, Yu WC, Fan ST. Hepatic stress gene expression and ultrastructural features under intermittent Pringle manoeuvre. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 249-257 [PMID: 14612278]

30 **Lemasters JJ**. V. Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. *Am J Physiol* 1999; **276**: G1-G6 [PMID: 9886971]

31 **Manov I**, Motanis H, Frumin I, Iancu TC. Hepatotoxicity of anti-inflammatory and analgesic drugs: ultrastructural aspects. *Acta Pharmacol Sin* 2006; **27**: 259-272 [PMID: 16490160 DOI: 10.1111/j.1745-7254.2006.00278.x]

32 **Caldwell SH**, Swerdlow RH, Khan EM, Iezzoni JC, Hespenheide EE, Parks JK, Parker WD Jr. Mitochondrial abnormalities in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 430-434 [PMID: 10488700 DOI: 10.1016/S0168-8278(99)80033-6]

33 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]

34 **Sternlieb I**, Berger JE. Optical diffraction studies of crystalline structures in electron micrographs. II. Crystalline inclusions in mitochondria of human hepatocytes. *J Cell Biol* 1969; **43**: 448-455 [PMID: 5351401 DOI: 10.1083/jcb.43.3.448]

35 **Wolf SG**, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A. DNA protection by stress-induced biocrystallization. *Nature* 1999; **400**: 83-85 [PMID: 10403254 DOI: 10.1038/21918]

36 **Itoh K**, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 1997; **236**: 313-322 [PMID: 9240432 DOI: 10.1006/bbrc.1997.6943]

37 **Rubartelli A**, Bajetto A, Allavena G, Wollman E, Sitia R. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J Biol Chem* 1992; **267**: 24161-24164 [PMID: 1332947]

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ORIGINAL ARTICLE

Observational Study

Liver decompensation predicts ribavirin overexposure in hepatitis C virus patients treated with direct-acting antivirals

Viola Guardigni, Lorenzo Badia, Matteo Conti, Matteo Rinaldi, Rita Mancini, Pierluigi Viale, Gabriella Verucchi

Viola Guardigni, Lorenzo Badia, Matteo Rinaldi, Pierluigi Viale, Gabriella Verucchi, Department of Medical and Surgical Sciences, University of Bologna, S. Orsola-Malpighi Hospital, Bologna 40138, Italy

Viola Guardigni, Lorenzo Badia, Gabriella Verucchi, Research Centre for the Study of Hepatitis, University of Bologna, Bologna 40138, Italy

Matteo Conti, Rita Mancini, Metropolitan Laboratory, Maggiore Hospital, Bologna 40133, Italy

ORCID number: Viola Guardigni (0000-0002-2769-7325); Lorenzo Badia (0000-0001-5694-1158); Matteo Conti (0000-0002-1579-9674); Matteo Rinaldi (0000-0002-3568-5973); Rita Mancini (0000-0002-5491-2449); Pierluigi Viale (0000-0003-1264-0008); Gabriella Verucchi (0000- 0001-6546-0495).

Author contributions: Guardigni V carried out the statistical analysis and drafting the manuscript; Guardigni V and Badia L developed concept and design of the study; all authors were involved in data collection, interpretation of data and editing of the manuscript.

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Correspondence to: Viola Guardigni, MD, Department of Medical and Surgical Sciences, University of Bologna, S. Orsola-Malpighi Hospital, via G. Massarenti 9, Bologna 40138, Italy. viola.guardigni2@unibo.it
Telephone: +39-0512-144301
Fax: +39-0512-144301

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Abstract

AIM

To determine whether ribavirin (RBV) concentrations differ according to cirrhosis stage among cirrhotic patients treated with interferon-free regimens.

METHODS

We included patients with hepatitis C virus and cirrhosis [Child-Pugh (CP) A or B], Glomerular Filtration Rate $\geqslant$ 60 mL/min, who started therapy with DAAs and weight-based RBV between October 2014 and February 2016. RBV plasma levels were assessed during the treatment. We focused our analysis on the first 8 wk of therapy.

RESULTS

We studied 68 patients: 54 with compensated (CP-B) and 14 with decompensated (CP-A) cirrhosis. Patients with

decompensated cirrhosis displayed significantly higher RBV concentrations than those with compensated cirrhosis at week 1, 2, 4 and 8 ($P < 0.035$). RBV levels were positively correlated with Hb loss over the treatment ($P < 0.04$). Majority (71%) of CP-B patients required a RBV dosage reduction during the treatment. After adjustment for confounders, Child-Pugh class remained significantly associated (95%CI: 35, 348, $P = 0.017$) to RBV levels, independently from baseline per-Kg RBV dosage.

CONCLUSION

Liver decompensation might affect RBV clearance leading to an overexposure and increased related toxicities in decompensated cirrhosis. Our findings underscore the importance of an early ribavirin therapeutic drug monitoring and suggest that an initial lower RBV dose, rather than weight-based, might be considered in those with advanced liver disease (CP-B) treated with direct-acting antivirals.

Key words: Hepatitis C; Direct-acting antivirals; Ribavirin; Therapeutic drug monitoring; Decompensated cirrhosis

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Core tip: In this study, patients with decompensated cirrhosis displayed higher plasma ribavirin concentrations in comparison to compensated patients, when treated with Interferon-free regimens for hepatitis C. Higher ribavirin levels were found to lead to greater rates of related toxicities and Child-Pugh class resulted independently associated with ribavirin plasma levels, in our population. Our findings suggest that ribavirin concentrations should be strictly monitored in subjects with advanced liver disease, during direct-acting antivirals-treatment. An early dosage adjustment of ribavirin should be performed when high levels of this antiviral are detected in patients' plasma, in order to avoid toxicities among these frail individuals.

Guardigni V, Badia L, Conti M, Rinaldi M, Mancini R, Viale P, Verucchi G. Liver decompensation predicts ribavirin overexposure in hepatitis C virus patients treated with direct-acting antivirals. *World J Hepatol* 2017; 9(34): 1270-1277 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i34/1270.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i34.1270

INTRODUCTION

Before the advent of direct-acting antivirals (DAAs), the association of Peg-interferon (Peg-IFN) and ribavirin (RBV) represented the standard of care (SOC) for the treatment of chronic C hepatitis, regardless of hepatitis C virus (HCV) genotype and stage of liver disease[1]. Although HCV-induced cirrhosis represents a major cause of liver-related morbidity and mortality and successful therapy of individuals with advanced fibrosis and liver cirrhosis is associated with better outcomes [*e.g.*, decreased incidence of hepatocellular carcinoma (HCC), decompensation][2], patients with cirrhosis were rarely treated because of the high risk of decompensation due to Peg-IFN administration.

New DAAs-regimens have dramatically changed this scenario, leading to the achievement of high rate of HCV eradication, even in individuals with compensated and decompensated cirrhosis. Although the role of RBV in the era of DAAs will probably decrease, it is currently still recommended for difficult-to-treat patients (*i.e.*, experienced and cirrhotic), to optimize sustained virological response (SVR) in many treatment regimens[3].

Monitoring of ribavirin plasma concentration, given its interindividual variability, was used during combination therapy with Peg-IFN, since RBV exposure had been shown to be associated with treatment efficacy (SVR) and side effects (*i.e.*, anaemia)[4,5]. In particular, trough RBV concentration (C_{trough}) at week 4 and 8 of treatment was commonly used[5-7]. Indeed, steady-state concentrations are reached after the first 4 wk of therapy according to scientific literature[8].

Although higher RBV concentration levels have been associated to higher rates of SVR and therapeutic ranges at week 8 have been established also for first-generation DAAs-based HCV therapies (with telaprevir or boceprevir)[9], role of RBV therapeutic drug monitoring (TDM) in the era of second-generation DAAs regimens has not been investigated and elucidated yet.

Thus far, data on ribavirin TDM among cirrhotic patients with advanced liver disease, treated by the combination of RBV and last generation DAAs are lacking.

Multiple factors are involved in RBV pharmacokinetics consequently affecting RBV plasma concentrations, such as creatinine clearance, gender, age, diet, HIV infection, weight[3,10]. Child-Pugh score, a marker of cirrhosis severity, used to estimate the prognosis in these patients, has never been investigated as a determinant of RBV plasma concentrations during HCV treatment.

The aim of our retrospective study was to determine whether ribavirin plasma levels differ according to cirrhosis stage (defined by Child-Pugh class) in a cohort of subjects with liver cirrhosis, treated for HCV with IFN-free DAA-regimens containing RBV and to define effects of plasma RBV levels.

MATERIALS AND METHODS

We retrospectively included in this study all the patients with chronic C hepatitis and cirrhosis (Child-Pugh A or B), without significant renal impairment [Estimated Glomerular Filtration Rate (eGFR) $\geqslant$ 60 mL/min], who started HCV treatment with DAAs in association with ribavirin (weight-based dose $\geqslant$ 11 mg/kg per die) between October 2014 and February 2016 at Infectious Diseases Department of University Hospital of Bologna (Italy). We excluded from the analysis two subjects

who had less than two available plasma ribavirin level measurements throughout the antiviral therapy. Patients who received Peg-IFN in combination with DAAs and RBV were excluded from the study. All the patients were treated for 12 or 24 wk according to the national and international guidelines and were afterwards monitored for at least 12 wk after completing the therapy to assess SVR (SVR12). Patients were categorized into two groups (compensated or decompensated cirrhosis) according to their Child-Pugh score (< 7 or ⩾ 7, respectively) to perform the analysis of interest.

For each subject, we collected the following data at baseline (corresponding with day of treatment start): Demographics (sex, age, race), medical history on previous HCV treatment (naïve or experienced), on chronic C hepatitis (HCV genotype, HCV RNA quantification) and on liver diseases (fibrosis stage, history of previous HCC, Child-Pugh class), HIV and HBV coinfections, creatinine level, eGFR, haemoglobin (Hb), bilirubin, current anti-HCV regimen (administered DAAs and Ribavirin initial dosage).

Data on RBV dose reduction over the course of treatment, therapy duration and response to the HCV treatment (as SVR12) were also recorded. Liver fibrosis was assessed according to the Metavir score either from elastographic measurements or from liver biopsy prior to HCV treatment. HCV RNA levels were detected by using Roche COBAS AmpliPrep/COBAS TaqMan HCV Test v 2.0 (Roche Molecular Systems, Inc., Pleasanton, CA). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.

Reasons for RBV dosage adjustment were the following: Occurrence of anaemia (Hb decline of more than 20 g/L or Hb less than 100 g/L), high RBV trough concentration (> 2500 ng/mL), that was considered at high risk for significant Hb decline[11,12], and other adverse effects (*e.g.*, cutaneous rash, itch).

All the subjects' visits and blood samples were standardized according to treatment schedule and performed at baseline and at week 1, 2, 4, 8, 12, 16, 20, 24 over treatment period. For each patient RBV concentrations were quantified using plasma samples that were collected multiple times during the treatment (average of 6.5 samples per subject), although we mainly focused our analysis on the first 8 wk of treatment because steady concentrations are usually reached between week 4 and 8[8] and because changes in RBV concentrations after week 8 (due to dosage adjustment) were expected to occur in our population.

RBV concentrations were measured in plasma samples, collected before taking the next dose of the drug (C_{trough}), using a novel analytical method, validated according to the ISO 15089 predicaments. In brief, Ribavirin is extracted from plasma by means of a methanol-water (20%-80% mL/L) solution containing $ZnSO_4$ (0.1 mol/L in water). The drug is then resolved chromatographically from endogenous and exogenous isobaric interferences by means of a binary chromatographic gradient. Specific signals for the drug are obtained in multiple reaction monitoring mode, recording the 245.1 > 113.2 and 245.1 > 96.2 m/z mass transitions. Ion extraction chromatograms enable drug determination with great specificity and sensitivity (Limit of Quantification = 1 μg/L).

Statistical analysis

Variables were expressed as mean ± SD or median and range interquartile. Continuous variables were compared by *t*-test or by Mann-Whitney-*U* test, when distribution was normal or not normal, respectively. Categorical variables were compared by chi-square test or Fisher's exact as appropriate. Bivariate association between RBV plasma levels and continuous variables of interest were tested by Spearman rank correlations. Linear regression was first performed to evaluate factors associated with RBV plasma levels in the univariate analysis and afterward to determine independent predictors of RBV plasma levels among variables with significant result at the univariate. All statistical analysis were performed using SPSS software version 21. Tests were considered significant for a *P* value < 0.05.

RESULTS

Characteristics of population

We totally evaluated 68 cirrhotic patients who underwent anti-HCV treatment with DAAs in association with RBV in the study period: 54 had a compensated liver cirrhosis and 14 a decompensated cirrhosis (Child-Pugh A, scored < 7 and B, scored ⩾ 7, respectively). Baseline characteristics of our population are shown in Table 1. No differences in demographics were found between the two groups. Fifty-seven point four percent of the population was composed by HIV co-infected patients who showed a greater, but not significantly, prevalence in decompensated subjects (53.7% *vs* 71.4%). Most of the subjects (63.2%) had been previously treated with Peg-IFN-based regimen, unsuccessfully. Genotype 1a was the most common HCV genotype (33.8%), as well as the association of Sofosbuvir (SOF, 400 mg/die) and Daclatasvir (DCV, 60 mg/die, or 30 mg/die in 13 HIV patients taking atazanavir/rtv-containing antiretroviral regimens) was the most frequently used (42.6%) in the overall population, without any difference according to Child-Pugh class. As expected, significant differences in haemoglobin and total bilirubin levels were detected, before starting antiviral therapy between the two groups: Indeed compensated cirrhotic patients showed higher mean Hb (146 ± 16 g/L *vs* 129 ± 17 g/L, *P* = 0.002) and lower median bilirubin levels [0.47, interquartile range (IQR) 2.8-8.2 mg/L *vs* 7.7 mg/L, IQR 5.3-16, *P* < 0.001] compared to decompensated patients. Creatinine values resulted higher in compensated patients (*P* = 0.011), although eGFR was similar in the two groups. 86.8% of subjects was treated for 24 wk, 13.2% for 12 wk.

Table 1 Subjects' baseline characteristics

Characteristics	Overall (*n* = 68)	Child pugh A (*n* = 54)	Child pugh B (*n* = 14)	*P* value
Age (yr), mean ± SD	55 ± 8.6	55.2 ± 9.5	54.6 ± 5	0.8
Male, *n* (%)	52 (76.5)	43 (79.6)	9 (64.3)	0.2
White race, *n* (%)	66 (97.1)	53 (98.1)	13 (92.9)	0.3
HIV infection, *n* (%)	39 (57.4)	29 (53.7)	10 (71.4)	0.2
HBsAg positive, *n* (%)	1 (1.5)	1 (1.9)	0	-
History of HCC, *n* (%)	6 (8.8)	4 (7.4)	2 (14.3)	0.6
HCV treatment experienced, *n* (%)	43 (63.2)	33 (61.1)	10 (71.4)	0.5
Liver stiffness (kPa), median (IQR)	21.4 (15.9-34.4)	21.2 (15.6-33.1)	31.1 (17.8-54.3)	0.1
HCV genotype, *n* (%)				0.7
1a	23 (33.8)	18 (33.3)	5 (35.7)	
1b	11 (16.2)	9 (16.7)	2 (14.3)	
2	6 (8.8)	6 (11.1)	-	
3	22 (32.4)	17 (31.5)	5 (35.7)	
4	6 (8.8)	4 (7.4)	2 (14.3)	
Weight (kg), mean ± SD	73 ± 14.1	73.2 ± 15.3	72.1 ± 8.5	0.7
Hb baseline (g/L), mean ± SD	143 ± 18	146 ± 16	129 ± 17	0.002
Bilirubin (mg/L), median (IQR)	9.5 (5.6-19)	4.7 (2.8-8.2)	7.7 (5.3-16)	< 0.001
Creatinine (mg/L), mean ± SD	8 ± 1.3	8.2 ± 1.5	7.5 ± 0.8	0.011
eGFR, (mL/min per 1.73 m^2), median (IQR)	101 (90-109)	99 (88-105)	102 (98-107)	0.3
HCV-RNA baseline (IU/mL)	933559 (25667-2128936)	1228876 (410822-2940050)	246455 (126308-712307)	0.007
[1]DAAs regimen, *n* (%)				0.2
SOF, *n* (%)	6 (8.8)	6 (11.1)	-	
SOF + DCV, *n* (%)	29 (42.6)	21 (38.9)	8 (57.1)	
SOF + SMP, *n* (%)	1 (1.5)	-	1 (7.1)	
DCV + SMP, *n* (%)	4 (5.9)	3 (5.6)	1 (7.1)	
SOF + LDV, *n* (%)	12 (17.6)	11 (20.4)	1 (7.1)	
OBV/PTV/r + DSV, *n* (%)	12 (17.6)	9 (16.7)	3 (21.4)	
OBV/PTV/r, *n* (%)	4 (5.9)	4 (7.4)	-	

[1]All regimens are intended in association with ribavirin. HCC: Hepatocellular carcinoma; eGFR: Estimated glomerular filtration rate; DAAs: Direct-acting antivirals; SOF: Sofosbuvir; DCV: Daclatasvir; SMP: Simeprevir; LDV: Ledipasvir; OBV: Ombitasvir; PTV: Paritaprevir; R: Ritonavir; DSV: Dasabuvir.

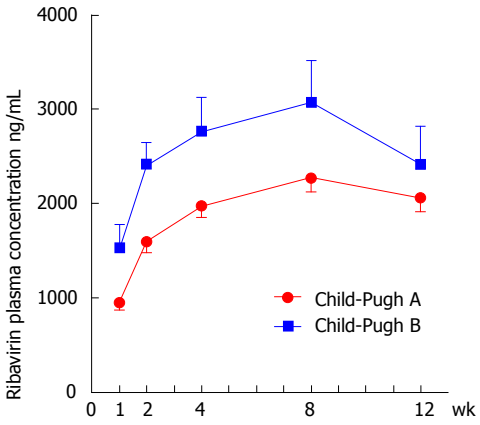

Figure 1 Ribavirin plasma concentrations over the treatment. Error bars represent standard errors. Ribavirin plasma concentrations statistically differ between Child-Pugh A and B patients at week 1, 2, 4 and 8 (all *P* value < 0.025). Legend: Child-Pugh B patients display significantly higher ribavirin concentrations (expressed as C trough) compared to Child-Pugh A patients over the first 8 wk of treatment with direct acting antivirals.

Ribavirin concentrations and dosage

There was no difference in initial mean dose of RBV (mg/kg) between the two groups (*P* = 0.3). Significant differences in RBV plasma concentrations between the two groups were found at each considered time-point (week 1, 2, 4 and 8) with much higher *Ctrough* levels among decompensated (Child-Pugh B) than among compensated (Child-Pugh A) patients. Otherwise, no remarkable difference in overall RBV mean values (measured during all the course of treatment) was revealed (Table 2).

Figure 1 shows the significant different trends of RBV plasma concentration from baseline until week 12 in the two cirrhotic groups.

In almost three-quarters (71.4%) of Child-Pugh B patients a reduction of RBV dosage was required during the treatment period, instead only 50% of Child-Pugh A subjects experienced a dosage adjustment (*P* = 0.15), after an average of 6.8 and 7.1 wk from treatment start among Child-Pugh A and B, respectively. Most common reasons for RBV dosage reduction were onset of anaemia (36.1%) and high RBV concentration revealed by TDM (36.1%), data not shown. Although decompensated patients experienced greater Hb loss (at both week 4 and week 8) than those with compensated cirrhosis, the differences were not significant (Table 2).

Multiple factors potentially associated with RBV concentration were investigated at univariate analysis. The significant predictors of RBV concentrations (as a treatment average between week 1 and 8) were baseline RBV dose per kilogram (β value 205, 95%CI: 77; 332, *P* = 0.002), male gender (β value -539, 95%CI: -975; -103, *P* = 0.016) and Child-Pugh class (β value 232, 95%CI: 66; 399, *P* value= 0.007). After adjusting for all these significant variables, only baseline

Table 2 Ribavirin dosages and plasma levels (C trough) and haemoglobin loss over the course of treatment

Parameters	Overall (*n* = 68)	Child pugh A (*n* = 54)	Child pugh B (*n* = 14)	*P* value
RBV (mg/kg pre die), mean ± SD	14.4 ± 1.7	14.4 ± 1.36	14.9 ± 1.6	0.3
[1]RBV week 1 (ng/L) median (IQR)	9105 (6130-1310)	8750 (5700-11680)	14050 (9010-22280)	0.007
[1]RBV week 2 (ng/L) median (IQR)	15000 (11600-27680)	14150 (11150-20000)	23550 (17200-28930)	0.001
[2]RBV week 4 (ng/L) median (IQR)	18300 (12900-27680)	17850 (12200-26500)	26250 (17700-35130)	0.024
[3]RBV week 8 (ng/L) median (IQR)	23100 (17050-31000)	21500 (16100-27950)	33000 (19800-41380)	0.0034
Mean RBV 1, 2, 4 wk, (ng/L) median (IQR)	14240 (10770-19450)	13280 (10480-18200)	19600 (14670-26870)	0.005
Mean RBV 1, 2, 4, 8 wk, (ng/L) median (IQR)	16490 (12220-21750)	15135 (11850-19830)	21700 (15090-29280)	0.006
Mean RBV, full treatment duration (ng/L), mean ± SD	18079 ± 6609	17528 ± 6020	20327 ± 7700	0.2
RBV dosage reduction, *n* (%)	37 (54.4)	27 (50)	10 (71.4)	0.2
Weeks before reduction, median (IQR)	6.1 (4-9.8)	5 (4-9.7)	7 (4-10)	0.6
Hb loss week 4 (g/L), mean ± SD	19.6 ± 1.8	19 ± 13	21 ± 32	0.5[4]
Hb loss week 8 (g/L), mean ± SD	21 ± 19	20 ± 13	25 ± 35	0.3[4]

[1]Data for 68 subjects; [2]Data for 62 subjects; [3]Data for 95 subjects; [4]Analysis of covariance of Hb at week 4 and haemoglobin (Hb) at week 8, adjusted for baseline Hb levels. RBV: Ribavirin; Hb: Haemoglobin; IQR: Interquartile range.

Table 3 Baseline predictors of average plasmatic Ribavirin levels in the first 8 wk of therapy

	Univariate analysis			Multivariate analysis		
	β value	95%CI	*P* value	β value	95%CI	*P* value
Baseline ribavirin dosage (mg/kg)	205	77; 332	0.002	161	36; 286	0.013
IFN-experienced	97	-303; 497	0.6			
Male gender	-539	-975; -103	0.016	-349	-765; 68	0.099
Age (yr)	13	-9; 34	0.3			
White race	424	-714; 1563	0.5			
Bilirubin (mg/L)	870	-920; 2670	3			
Creatinine (mg/L)	-2890	-16830; 11040	7			
Child-Pugh class	232	66; 399	0.007	192	35; 348	0.017
Liver stiffness (kPa)	-1.6	-14; 11	0.8			
HIV infection	76	-314; 466	0.7			

IFN: Interferon; HIV: Human immunodeficiency virus.

RBV dosage (β value 161.1, 95%CI: 35.6; 286, *P* = 0.013) and Child-Pugh class (β value 191.7, 95%CI: 35; 348, *P* = 0.017) remained significantly associated to RBV levels (Table 3). To investigate whether the association with Child-Pugh class was driven by single items of this composite index, we analysed each of them (*i.e.*, Encephalopathy, Ascites, Bilirubin, INR, Albumin at baseline) in relationship to RBV concentrations, but no significant differences in RBV overexposure were observed. Moreover, clearance of RBV (indirectly assessed by its plasma concentrations) did not seem to be related to portal hypertension signs (*e.g.*, platelet count, esophageal varices), data not shown.

Remarkably, there were no significant differences in RBV concentration (as an average value obtained from the first 8 wk measurements) between subjects who achieved a rapid virological response (RVR, namely HCV undetectability within week 4 of treatment) and those who did not (Figure 2). Furthermore, no correlation between RBV concentrations (at any time-point) and time to reach HCV undetectability was observed in our population (data not shown). The majority of patients included in this study (92.6%) achieved SVR12, without any difference in SVR rate based on Child-Pugh class and RBV levels at week 4 and 8 (data not shown).

Haemoglobin loss

As depicted in Figure 3, Hb loss (g/L) in the first two months of treatment (at the two therapy time-points: Week 4 and 8) was correlated to RBV concentrations measured at the corresponding week (R = + 0.272, *P* value= 0.033 at week 4, R = + 0.279, *P* = 0.025 at week 8), with higher RBV levels associated with greater Hb decline. The relationship between Hb loss at week 8 and RBV levels at that specific week was independent from Child-Pugh class (in a multivariate linear regression, *P* = 0.002).

DISCUSSION

The advent of new IFN-free DAAs regimens has allowed to achieve very high rates of HCV eradication, even in patients with advanced liver disease. Although the role of RBV is now discussed and its relevance will probably decrease over time, this antiviral drug is still recommended in combination with DAAs in several challenging situations: For treatment-experienced but DAAs-naïve patients without cirrhosis or with compensated cirrhosis (Child-Pugh A) to reduce weeks of therapy, for subjects who failed to

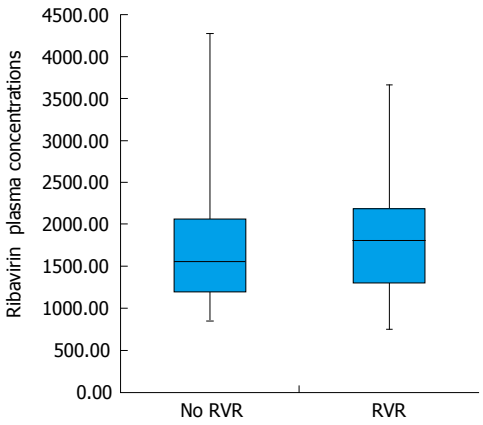

Figure 2 Average of ribavirin plasma concentrations between week 1 and 8 of treatment in association with rapid virological response. Legend: Achievement of Rapid Virological Response (*i.e.*, hepatitis C virus RNA undetectability within week 4 of treatment) was not associated with ribavirin plasma concentrations measured over the first 8 wk of treatment. RVR: Rapid virological response.

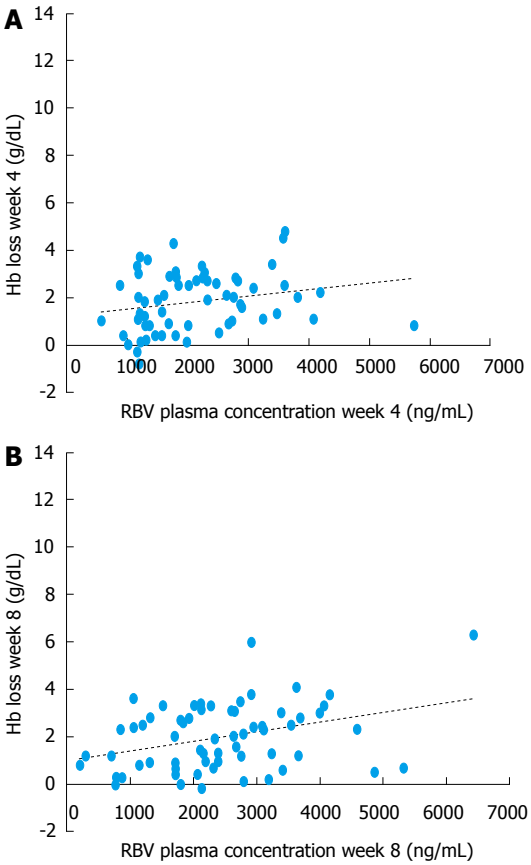

Figure 3 Correlation between ribavirin plasma concentrations and haemoglobin loss at week 4 and 8. Legend: Higher ribavirin plasma concentrations resulted significantly correlated with greater haemoglobin loss at both week 4 (A) and week 8 (B) of therapy in the overall population. RBV: Ribavirin.

achieve SVR on prior antiviral therapy containing DAAs and for those with decompensated cirrhosis (Child-Pugh B or C) to increase SVR rates[13].

To date, specific data regarding ribavirin TDM in cirrhotic patients treated with IFN-free regimens are lacking and daily weight-based ribavirin remains the SOC. In particular, a daily dose of ribavirin higher than 10 mg/kg of body weight had been previously associated to higher rate of SVR among subjects treated with Peg-IFN and RBV[14] and it is still used with DAAs, although the use of RBV according body weight has been occasionally disapproved due to the variability in this antiviral elimination phase[4].

In our study, patients with decompensated cirrhosis (Child-Pugh B) remarkably displayed higher plasma levels of RBV during the first 8 wk of therapy with DAAs compared to those with compensated cirrhosis (Child-Pugh A), although initial weight-dosages were similar. This raises the hypothesis that liver failure might play a meaningful role in RBV clearance, not observed before.

The fact that overall RBV mean plasma concentrations did not differ based on Child-Pugh class in our cohort might be explained by the greater rate of RBV dose reduction (but no discontinuation) and the following RBV exposure decline in those with decompensated cirrhosis, over the course of treatment.

Reduced dosages of ribavirin are currently recommended in patients with moderate or severe renal impairment (Creatinine Clearance $\leqslant$ 60 mL/min), since RBV exposure is predicted to increase up to 17% in this subsets of patients, due to its renal excretion[15]. Also weight, gender, age, fat meals have been shown to clearly affect RBV metabolism[3]. Gastrointestinal tract is another potential site of RBV clearance[16].

To date, no adjustment in ribavirin dosage is required in liver dysfunction, which instead affects metabolism of many drugs[17], since so far liver failure did not seem to impact on ribavirin clearance.

Only Glue[18] assessed the single dose pharmacokinetics of this antiviral in subjects with various degrees of chronic liver disease: In agreement to our results, they observed that C_{max} significantly increased with the severity of liver dysfunction, although there was a remarkable overlap in individual C_{max} values among the groups and they eventually did not propose any dosage reduction in these patients.

Contrarily, we first evaluated RBV pharmacokinetics in patients with liver disease over the course of the treatment in a real-world setting, obtaining then different findings. One could argue that a slower clearance of the drug in our population was due to a cirrhosis-related renal dysfunction (*i.e.*, hepatorenal syndrome), but we included only subjects with eGFR $\geqslant$ 60 mL/min and unexpectedly creatinine was not found to be associated to RBV concentrations over the first 8 wk at univariate analysis in our population.

Conversely, a higher Child-Pugh score determined a RBV overexposure, leading to high rate of dose adjustment in those with Child-Pugh $\geqslant$ 7. To our knowledge, this is the first study exploring ribavirin TDM in decompensated cirrhotic individuals treated with new DAAs regimens and showing an overexposure in this group of patients. Indeed, while few data report RBV plasma levels in therapeutic range in patients with

Child-Pugh A treated with a fixed dose (800 mg daily) of RBV associated with SOF and DCV[19], no information about Child-Pugh B or C patients are currently available. These findings might be useful to clinicians, since an overexposure can be modulated by lowering the initial RBV dosage from the weight-based dosage in certain patients with advanced liver disease, avoiding secondary and detrimental effects.

Furthermore, our data show a positive correlation between RBV concentrations and Hb loss, as already widely reported in literature, even in the context of IFN-free regimens[20]. This finding strengthens the importance of considering a lower RBV dosage in subjects with decompensated cirrhosis, also considering their higher rate of baseline anaemia characterizing this group of patients (confirmed also in our population). Indeed, anaemia occur in about 75% of patients with chronic liver disease and can be linked to different aetiologies (*e.g.*, haemorrhage, splenomegaly)[21], regardless of the use of anti-HCV drugs, such as ribavirin, and represents a condition to be taken into account in the management of cirrhotic patients undergoing anti-HCV treatment.

According to our results, higher RBV concentrations in the first weeks of therapy did not seem to enhance RVR rate in cirrhotic patients treated with IFN-free regimens and no relationship between RBV plasma concentration at week 4 or 8 and rate of SVR was found. This might appear in contrast with prior data regarding Peg-IFN/RBV[22] or, more recently, SOF/RBV treatment (*e.g.*, FISSION, NEUTRINO and FUSION trials)[23,24], in which RBV exposure was one of the factor associated with SVR, with suggested RBV plasma concentration threshold between 2000 and 3000 ng/mL[25,26]. In our cohort, the low rate of patients failing to achieve SVR (7.4%) could explain the lack of relationship between RBV levels and SVR rate: It is in general difficult to identify statistically significant predictive markers with such a high response rate.

This study has some limitation to be considered. The main limitation is the absence of C_{max} and AUC data, due to the observational nature of the study; however C_{trough} can be considered in clinical practice as a surrogate of plasma exposure and its values correlate with drug efficacy and toxicity in other studies[27]. Moreover, this is a retrospective study with a limited population size and this suggests caution prior to extend our results to the all the population with liver cirrhosis treated with RBV and DAAs. The inclusion of patients with mild renal impairment (eGFR 60-89 mL/min) could represent a confounding factor, although baseline creatinine was not found to affect RBV concentrations in our population.

In conclusion, liver failure might affect RBV clearance leading to an overexposure and increased related toxicities (*e.g.*, anaemia) in subjects with decompensated cirrhosis who are now being treated with last-generation DAAs. Our findings underscore the importance of RBV plasma monitoring and early dose adjustments in these patients and suggest that an initial lower dose, rather than weight-based, of RBV might be considered in individuals with advanced liver disease (CP B class) treated with new DAAs in order to reduce toxicities without increasing virological failure rates.

This might improve the knowledge on the tailored use of ribavirin within IFN-free regimens for HCV treatment, in subjects with advanced liver disease. Further studies will be needed to confirm our results in order to determine the optimal dosage of ribavirin in patients with advanced cirrhosis in the era of DAAs-based regimens, since difficult-to-treat patients remain a challenge in HCV treatment and maintaining the benefit of this effective antiviral is still a relevant issue.

COMMENTS

Background

In the era of new direct-acting antivirals (DAAs), ribavirin (RBV) is still recommended for difficult-to-treat hepatitis C virus (HCV) positive patients, but data on RBV therapeutic drug monitoring (TDM) among patients with advanced liver disease treated with RBV and DAAs are lacking.

Research frontiers

TDM represent an innovative technique and is currently widely used approach to personalize and ameliorate anti-infective treatments.

Innovations and breakthroughs

This is the first study showing that patients with decompensated cirrhosis displayed significantly higher RBV concentrations than those with compensated cirrhosis, when treated with new Interferon-free regimens.

Applications

These findings might improve the knowledge on the tailored use of ribavirin within IFN-free regimens for HCV treatment in subjects with advanced liver disease, for whom a lower initial dosage of RBV might be considered, in order to reduce risks related to RBV overexposure.

Terminology

DAAs: Direct-acting antivirals represent the current standard of care for Chronic C Hepatitis; RBV: Ribavirin is an old-fashioned antiviral used in combination with last generation DAAs for difficult-to-treat HCV patients; TDM: Therapeutic drug monitoring by means of blood measurement of a drug concentration; *C trough*: Plasma concentration of a drug measured before taking the next dose of the drug.

Peer-review

This paper offers important approach to the topic and treatment algorithm.

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REFERENCES

1 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]

2 **Van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause

mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]

3 **Pradat P**, Virlogeux V, Gagnieu M-C, Zoulim F, Bailly F. Ribavirin at the Era of Novel Direct Antiviral Agents for the Treatment of Hepatitis C Virus Infection: Relevance of Pharmacological Monitoring. *Adv Hepatol* 2014 [DOI: 10.1155/2014/493087]

4 **Brochot E**, Castelain S, Duverlie G, Capron D, Nguyen-Khac E, François C. Ribavirin monitoring in chronic hepatitis C therapy: Anaemia versus efficacy. *Antivir Ther* 2010; **15**: 687-695 [PMID: 20710050 DOI: 10.3851/IMP1609]

5 **Kuntzen T**, Kuhn S, Kuntzen D, Seifert B, Mullhaupt B, Geier A. Influence of Ribavirin Serum Levels on Outcome of Antiviral Treatment and Anemia in Hepatitis C Virus Infection. *PLoS One* 2016; **11**: e0158512 [PMID: 27388623 DOI: 10.1371/journal.pone.0158512]

6 **Loustaud-Ratti V**, Debette-Gratien M, Jacques J, Alain S, Marquet P, Sautereau D, Rousseau A, Carrier P. Ribavirin: Past, present and future. *World J Hepatol* 2016; **8**: 123-130 [PMID: 26807208 DOI: 10.4254/wjh.v8.i2.123]

7 **Marucco DA**, de Requena DG, Bonora S, Tettoni C, Bonasso M, De Blasi T, D'Avolio A, Sciandra M, Siccardi M, Baietto L, Trentini L, Sinicco A, Cariti G, Di Perri G. The use of trough ribavirin concentration to predict sustained virological response and haematological toxicity in HIV/HCV-co-infected patients treated with ribavirin and pegylated interferon. *J Antimicrob Chemother* 2008; **61**: 919-924 [PMID: 18238889 DOI: 10.1093/jac/dkn013]

8 **Glue P**. The clinical pharmacology of ribavirin. *Semin Liver Dis 19 Suppl* 1999; **1**: 17-24 [PMID: 10349689]

9 **De Kanter CTMM**, Buti M, DeMasi R, Ouwerkerk-Mahadevan S, Dofferhoff ASM, Witek J, Drenth JPH, Zeuzem S, Burger DM. Ribavirin concentration determines treatment success of first-generation DAA-based chronic HCV therapy. *Antivir Ther* 2016; **21**: 153-159 [PMID: 26378941 DOI: 10.3851/IMP2994]

10 **Hatu G**, Bailly F, Pourcelot E, Pradat P1, Miailhes P, Maynard M, Parant F, Chiarello P, Livrozet JM, Zoulim F, Gagnieu MC. Lower ribavirin biodisponibility in patients with HIV-HCV coinfection in comparison with HCV monoinfected patients. *BMC Infect Dis* 2014; **14**: 150 [PMID: 24650094 DOI: 10.1186/1471-2334-14-150]

11 **Bonora S**, Calcagno A, Cometto C, Fontana S, Aguilar D, D'Avolio A, Gonzalez de Requena D, Maiello A, Dal Conte I, Lucchini A, Di Perri G. Short-term additional enfuvirtide therapy is associated with a greater immunological recovery in HIV very late presenters: A controlled pilot study. *Infection* 2012; **40**: 69-75 [PMID: 22135137 DOI: 10.1007/s15010-011-0223-4]

12 **Rendon AL**, Nunez M, Romero M, Barreiro P, Martin-Carbonero L, Garcia-Samaniego J, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Early monitoring of ribavirin plasma concentrations may predict anemia and early virologic response in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2005; **39**: 401-405 [PMID: 16010160 DOI: 10.1097/01.qai.0000170034.90438.68]

13 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C. *J Hepatol* 2017; **66**: 153-194 [PMID: 27667367 DOI: 10.1016/j.jhep.2016.09.001]

14 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]

15 **Polepally AR**, Badri PS, Eckert D, Mensing S, Menon RM. Effects of Mild and Moderate Renal Impairment on Ombitasvir, Paritaprevir, Ritonavir, Dasabuvir, and Ribavirin Pharmacokinetics in Patients with Chronic HCV Infection. *Eur J Drug Metab Pharmacokinetics* 2017; **42**: 1-7 [PMID: 27165046 DOI: 10.1007/s13318-016-0341-6]

16 **Dixit NM**, Perelson AS. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell Mol Life Sci* 2006; **63**: 832-842 [PMID: 16501888 DOI: 10.1007/s00018-005-5455-y]

17 **Tolman KG**. Drugs and the liver. *Med J Aust* 1977; **2**: 655-656 [PMID: 607107]

18 **Glue P**, Schenker S, Gupta S, Clement RP, Zambas D, Salfi M. The single dose pharmacokinetics of ribavirin in subjects with chronic liver disease. *Br J Clin Pharmacol* 2000; **49**: 417-421 [PMID: 10792198 DOI: 10.1046/j.1365-2125.2000.00186.x]

19 **Parisi SG**, Loregian A, Andreis S, Nannetti G, Cavinato S, Basso M, Scaggiante R, Dal Bello F, Messa L, Cattelan AM, Palù G. Daclatasvir plasma level and resistance selection in HIV patients with hepatitis C virus cirrhosis treated with daclatasvir, sofosbuvir, and ribavirin. *Int J Infect Dis* 2016; **49**: 151-153 [PMID: 27378577 DOI: 10.1016/j.ijid.2016.06.020]

20 **Rower JE**, Meissner EG, Jimmerson LC, Osinusi A, Sims Z, Petersen T, Bushman LR, Wolfe P, McHutchison JG, Kottilil S, Kiser JJ. Serum and cellular ribavirin pharmacokinetic and concentration-effect analysis in HCV patients receiving sofosbuvir plus ribavirin. *J Antimicrob Chemother* 2015; **70**: 2322-2329 [PMID: 25971261 DOI: 10.1093/jac/dkv122]

21 **Gonzalez-Casas R**, Jones EA, Moreno-Otero R. Spectrum of anemia associated with chronic liver disease. *World J Gastroenterol* 2009; **15**: 4653-4658 [PMID: 19787828 DOI: 10.3748/wjg.15.4653]

22 **Larrat S**, Stanke-Labesque F, Plages A, Zarski JP, Bessard G, Souvignet C. Ribavirin quantification in combination treatment of chronic hepatitis C. *Antimicrob Agents Chemother* 2003; **47**: 124-129 [PMID: 12499179 DOI: 10.1128/AAC.47.1.124-129.2003]

23 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J of Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]

24 **Jacobson IM**, Gordon SC, Kowdley K V, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR; POSITRON Study; FUSION Study. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J of Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]

25 **Maynard M**, Pradat P, Gagnieu MC, Souvignet C, Trepo C. Prediction of sustained virological response by ribavirin plasma concentration at week 4 of therapy in hepatitis C virus genotype 1 patients. *Antivir Ther* 2008; **13**: 607-611 [PMID: 18672540]

26 **Maeda Y**, Kiribayashi Y, Moriya T, Maruhashi A, Omoda K, Funakoshi S, Murakami T, Takano M. Dosage adjustment of ribavirin based on renal function in Japanese patients with chronic hepatitis C. *Ther Drug Monit* 2004; **26**: 9-15 [PMID: 14749543 DOI: 10.1097/00007691-200402000-00004]

27 **Arase Y**, Ikeda K, Tsubota A, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, Akuta N, Someya T, Hosaka T, Sezaki H, Kobayashi M, Kumada H. Significance of serum ribavirin concentration in combination therapy of interferon and ribavirin for chronic hepatitis C. *Intervirology* 2005; **48**: 138-144 [PMID: 15812187 DOI: 10.1159/000081741]

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