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Reduced serum chemerin in patients with more severe liver cirrhosis

Kristina Eisinger [a], Sabrina Krautbauer [a], Reiner Wiest [b], Thomas S. Weiss [c], Christa Buechler [a,*]

[a] Department of Internal Medicine I, Regensburg University Hospital, Regensburg, Germany
[b] Department of Visceral Surgery and Medicine, University Inselspital, Bern, Switzerland
[c] Department of Pediatrics and Juvenile Medicine, Regensburg University Hospital, Regensburg, Germany

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ABSTRACT

Chemerin is a well-established modulator of immune cell function and its serum levels are induced in inflammatory diseases. Liver cirrhosis is associated with inflammation which is aggravated by portal hypertension. The objective of this study was to evaluate whether chemerin is induced in patients with more severe liver cirrhosis and portal hypertension. Chemerin has been measured by ELISA in the portal venous serum (PVS), systemic venous serum (SVS) and hepatic venous serum (HVS) of 45 patients with liver cirrhosis. Chemerin is higher in HVS compared to PVS in accordance with our recently published finding. SVS, HVS and PVS chemerin decline in patients with more advanced liver injury defined by the CHILD-PUGH score. Hepatic chemerin has been determined in a small cohort and is similarly expressed in normal and cirrhotic liver. MELD score and serum markers of liver and kidney function do not correlate with chemerin. There is a positive correlation of chemerin in all compartments with Quick prothrombin time and of SVS chemerin with systolic blood pressure. PVS chemerin is induced in patients with modest/massive ascites but this does not translate into higher HVS and SVS levels. Chemerin is not associated with variceal size. Reduction of portal pressure by transjugular intrahepatic portosystemic shunt does not affect chemerin levels. These data show that low chemerin in patients with more severe liver cirrhosis is associated with reduced Quick prothrombin time.

1. Introduction

Liver cirrhosis is the end stage of chronic liver injury independent of disease etiology (Pinzani et al., 2011). Viral infections, alcohol abuse and increasing non-alcoholic steatohepatitis (NASH) are the major underlying causes of liver injury (Buechler et al., 2011; Pinzani, 2011; Pinzani et al., 2011). Adipokine imbalance and chronic inflammation modulate pathogenesis of liver disease partly by directly promoting fibrogenesis (Marra et al., 2011; Schaffler et al., 2005). Chemerin is highly expressed in adipocytes and hepatocytes, and is secreted as an inactive pro-protein which can be activated by carboxyl-terminal proteolysis (Krautbauer et al., 2013; Zabel et al., 2014). Chemerin regulates migration of immune cells, angiogenesis and glucose homeostasis (Zabel et al., 2014). Inflammatory cytokines and lipopolysaccharide (LPS) enhance adipocyte chemerin production (Bauer et al., 2011; Zabel et al., 2014) and increased chemerin serum levels in obesity and various diseases are associated with inflammation (Gisondi et al., 2013; Gu et al., 2014; Makrilakis et al., in press; Weigert et al., 2010).

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome (Buechler et al., 2011) and serum chemerin has been described to be increased or unaltered in these patients (Docke et al., 2013; Kukla et al., 2010; Yilmaz et al., 2011). Hepatic chemerin expression is nevertheless induced in human and murine NASH (Docke et al., 2013; Krautbauer et al., 2013). Cytokines like TNF and IL-6, LPS and the adipokines adiponectin and leptin with an established role in NASH (Buechler et al., 2011; Tilg and Moschen, 2010) do not considerably affect human hepatocyte chemerin protein (Docke et al., 2013; Krautbauer et al., 2013). Studies in mice deficient in the chemokine-like receptor 1 (CMKLR1), one of the two so far described chemerin receptors, exclude a role of CMKLR1 in NAFLD. This suggests that higher hepatic chemerin levels are not associated with NAFLD pathology (Gruben et al., 2014).

Liver cirrhosis predisposes to the development of hepatocellular carcinoma (Pinzani et al., 2011). In patients with hepatocellular carcinoma liver chemerin expression is even decreased. Importantly, low levels are linked to worse prognosis indicating that chemerin itself may exert protective functions in liver tumors (Lin et al., 2011). Serum chemerin is, however, not correlated with recurrence-free survival or overall survival. In these patients circulating chemerin is negatively associated with residual liver function defined by the CHILD-PUGH score. Here, serum chemerin correlates with serum alanine aminotransferase, bilirubin and platelet counts (Imai et al., 2014).

Portal hypertension is the major underlying cause of variceal bleeding, ascites and hepatic nephropathy in patients with liver cirrhosis (Iwakiri, 2012; Laleman et al., 2005; Wiest, 2007). Transjugular intrahepatic portosystemic shunt (TIPS) reduces portal pressure

* Corresponding author at: Department of Internal Medicine I, Regensburg University Hospital, 93042 Regensburg, Germany.
E-mail address: christa.buechler@klinik.uniregensburg.de (C. Buechler).

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(Moller et al., 2008) and endotoxemia (Benten et al., 2011; Holland-Fischer et al., 2011).

Chemerin has already been measured in portal and hepatic venous blood obtained from patients with liver cirrhosis before stent insertion (Weigert et al., 2010). In these patients hepatic vein chemerin concentrations are higher than portal vein levels suggesting that chemerin is released from the liver (Weigert et al., 2010).

Here, chemerin concentrations were analyzed in relation to residual liver function, laboratory parameters and complications of liver cirrhosis. Furthermore, it was determined whether TIPS lowers chemerin.

2. Materials and methods

2.1. Transjugular intrahepatic portosystemic shunt (TIPS)

Forty five patients with liver cirrhosis were included in the study and patient demographics and laboratory parameters are summarized in Table 1. Etiology of liver disease was alcoholic in 38 of the patients, hepatitis C infection in 3 and of other reasons in 4 patients. Patients were electively treated by TIPS implantation due to complications of liver cirrhosis such as variceal bleeding (15 patients), hepatorenal syndrome (1 patient), refractory ascites (27 patients) or other reasons (2 patients). The procedure of TIPS implantation has been described earlier and TIPS (Viatorr-Stent, Putzbrunn, Germany) was inserted according to Rossle and Gerbes in the fasted state (Rossle and Gerbes, 2010). During TIPS implantation, samples of one of the hepatic veins (HVS) not being drained by the TIPS-stent, of the portal vein (PVS) and of one peripheral vein such as the superior caval vein (SVS) were obtained simultaneously. Patient samples analyzed herein have been partly used in previous studies (Bauer et al., 2012; Eisinger et al., 2013; Weigert et al., 2010).

Standard laboratory parameters such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured routinely by the Institute for Clinical Chemistry and Laboratory Medicine at the University Hospital of Regensburg. The study complies with the Declaration of Helsinki. All patients gave written informed consent and the study was approved by the ethical committee of the University Hospital of Regensburg.

2.2. Liver tissue samples

Liver tissue was obtained from 6 patients with normal liver function (3 males, 3 females), 4 patients with liver cirrhosis CHILD-PUGH score B (males only) and 5 patients with liver cirrhosis CHILD-PUGH score C (males only). Median age was 67 (22–87) years, 66 (50–70) years and 45 (39–50) years, respectively. Patients with CHILD-PUGH score C were significantly younger than patients with CHILD-PUGH score B ($p = 0.016$). BMI was similar in patients and controls (data not shown). Bilirubin of patients with CHILD-PUGH score B was 4.6 (0.51–6.1) mg/dl and of patients with CHILD-PUGH score C 8.9 (3.9–34.4) mg/dl. Quick prothrombin time of patients with liver cirrhosis CHILD-PUGH score B was 72.5 (38.0–82.0) % and of patients with CHILD-PUGH C 43.0 (19.0–58) % ($p = 0.111$). All patients gave written informed consent and the study was approved by the ethical committee of the University Hospital of Regensburg.

Table 1
Patient demographics and laboratory parameters (alanine aminotransferase, ALT; alkaline phosphatase, AP; aspartate aminotransferase, AST). Median values and range of the values are shown.

	Study cohort
Number	45
Sex (female/male)	9/36
Age (years)	54 (26–81)
Type 2 diabetes yes/no	12/33
CHILD-PUGH stage A/B/C	12/16/17
MELD score	9 (6–21)
Ascites: no/little/modest/massive	6/13/4/22
Variceal size: no/small/large	9/7/29
C-reactive protein (mg/l)	13.4 (1.0–53.5)**
Fibrinogen (mg/dl)	263 (114–520)*
ALT (U/l)	37 (4.0–108.0)
AST (U/l)	28.0 (2.0–84.0)
Albumin (g/l)	31.3 (1.6–47)
Bilirubin (mg/dl)	1.3 (0.3–8.2)
Quick prothrombin time (%)	72 (28–100)
Creatinine (mg/dl)	1.0 (0.5–4.5)
Creatinine clearance (ml/min)	54 (1–204)*

* data of 44 and ** data of 42 patients are given.

2.3. ELISA

Chemerin ELISA was from R&D Systems (Wiesbaden-Nordenstadt, Germany). Serum was diluted 1 to 250 fold.

2.4. SDS-polyacrylamide gel electrophoresis and immunoblotting

GAPDH antibody was from New England Biolabs GmbH (Frankfurt, Germany). Antibody to detect human chemerin by immunoblot was from R&D Systems (Wiesbaden-Nordenstadt, Germany). CMKLR1 antibody was from Abcam (Cambridge, UK). Proteins (10–20 μg) were separated by SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes (Bio-Rad, Munich, Germany). Incubations with antibodies were performed in 1.5% BSA in PBS, 0.1% Tween. Detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany).

2.5. Monitoring of gene expression by real-time RT-PCR

The mRNA expression was investigated by quantitative real-time PCR using a LightCycler FastStart DNA Master SYBR Green I kit from Roche (Mannheim, Germany). Total cellular RNA was isolated with TRIzol reagent from Life Technologies GmbH (Darmstadt, Germany) and 1 μg RNA was reverse transcribed using the Promega Reverse Transcription System (Promega, Madison, WI) in a volume of 40 μl; 2 μl of the cDNA was used for amplification in glass capillaries (LightCycler). Human chemerin was amplified with the primers 5′ GGT CCA CTG CCC CAT AGA G 3′ and 5′ TTA TCA TGG CTG GGG ATA GAA 3′ and 18S rRNA with 5′ GAT TGA TAG CTC TTT CTC GAT TCC 3′ and 5′ CAT CTA AGG GCA TCA CAG ACC 3′ as described (Krautbauer et al., 2013).

2.6. Statistics

Data are shown as median values and range of the values (IBM SPSS Statistics 21.0). Statistical differences were analyzed by two-tailed Mann–Whitney U Test (IBM SPSS Statistics 19.0). Paired data were analyzed by t-test (MS Excel). Spearman correlation was calculated using the IBM SPSS Statistics 21.0 software.

3. Results

3.1. Chemerin in serum of patients with liver cirrhosis

Forty five patients suffering from clinically diagnosed liver cirrhosis were included in the study. In this cohort chemerin concentrations were similar in female and male serum (data not shown). Chemerin did not correlate with the age of the patients (data not shown). Only SVS chemerin correlated with systolic blood pressure ($r = 0.390$, $p = 0.013$) which has been documented for 40 patients. SVS chemerin positively correlated with PVS ($r = 0.798$, $p < 0.001$) and HVS (0.728, $p < 0.001$) levels. Significant positive correlations were also identified for PVS and HVS chemerin ($r = 0.839$, $p < 0.001$). Chemerin was higher in HVS compared to PVS levels which were similar to SVS chemerin (Fig. 1A), in accordance with already published data (Weigert et al., 2010). Twelve of the patients had type 2 diabetes but chemerin was