Increased plasma levels of relaxin-2 in liver cirrhotic patients

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Research article

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Abstract

Background: Relaxin-2 has been found to alleviate fibrosis in liver cirrhosis, and our previous study showed that relaxin-3 inhibits ROS-induced hepatic cell apoptosis. In this study, we investigated the levels of plasma relaxin-2 or relaxin-3 in patients with liver cirrhosis and their relationship to component traits in patients with liver cirrhosis.

Aims: To determine the levels of plasma relaxin-2 or relaxin-3 in patients with liver cirrhosis and their relationship to component traits in patients with liver cirrhosis.

Methods: A total of 75 patients with liver cirrhosis and 41 age-matched healthy control subjects were enrolled in this study. Relaxin-2 and relaxin-3 expression levels in plasma were investigated by radioimmunoassay.

Results: Plasma relaxin-2 levels were significantly higher in patients with liver cirrhosis than in control subjects ($p=0.004$). Specifically, plasma relaxin-2 levels were significantly positively correlated with Child-Pugh classification, white blood cell, activated partial thromboplastin time and indirect bilirubin. Plasma relaxin-2 levels were significantly negatively correlated with albumin. There were no significant differences in plasma relaxin-3 concentrations between the two groups ($p=0.932$). Receiver operating characteristic curve analysis yielded an area under the curve for relaxin-2 of 0.665 in liver cirrhosis.

Conclusions: Plasma relaxin-2 levels were higher in patients with liver cirrhosis than in control subjects, and plasma relaxin-2 levels were significantly positively correlated with Child-Pugh classification, and negatively correlated with albumin.

Introduction

Liver injury results in a repair response, and causes remodel of the extracellular matrix from one composed predominantly of basement membrane-type collagens to one with increased collagens at the injured liver area[1]. However, in the case of persistent injury, the collagen accumulates, resulting in fibrosis characterized by scarring and eventual impairment of liver function. Ultimately, if the fibrotic process is not reversed, the scarring will progress to liver cirrhosis. Currently, few options are available for the treatment of liver fibrosis, therefore, it is very important to clarify the pathogenesis of liver cirrhosis for discovering the new treatment methods. Cumulative evidence has shown that endogenous bioactive peptides play an important role in risk stratification, diagnostic assessment, treatment and monitoring of liver cirrhosis.

Relaxin-2 and relaxin-3 are two members of the relaxin family of peptides[2]. Relaxin-2 has proved effective in the treatment of experimental models of fibrosis in vivo, including pulmonary, renal, dermal and cardiac fibrosis[3–7]. [Bennett RG](#) et al reported that in normal rat liver, relaxin family peptide receptor 1(RXFP1) was expressed at low levels, in cirrhotic liver, expression of RXFP1 significantly increased[8–9]. Relaxin-2 induced a selective and significant reduction in portal pressure in pathologically distinct

portal hypertension in cirrhosis models, through augmentation of intrahepatic NO signaling and a dramatic reduction in contractile filament expression in hepatic stellate cell(HSC)-myofibroblasts[10]. Relaxin-2 reduced collagen deposition and HSC activation in established hepatic fibrosis despite the presence of continued hepatic injury. The results suggest that relaxin-2 may be an effective treatment for established hepatic fibrosis[11]. A single adenoviral delivery of relaxin-2 in the liver attenuated established hepatic fibrosis by suppressing collagen cross-linking and enhancing collagen degradation[12]. Our previous study showed that relaxin-3, the 'ancestral' member of the relaxin peptide family, inhibits ROS-induced hepatic cell apoptosis[13]. However, the relationship between the endogenous peptide hormone relaxin-2 and relaxin-3 levels in liver cirrhosis remains to be elucidated.

The aim of this study was to explore the clinical relevance of relaxin-2 and relaxin-3 in liver cirrhosis patients. Towards that end, we measured the plasma levels of relaxin-2 and relaxin-3 in control subjects and liver cirrhosis patients and analysed its association with clinical parameters.

Methods

2.1 Patients

Our study selected 75 patients with liver cirrhosis and 41 healthy subjects with no clinical diseases. (Selection and exclusion criteria for patients and Ethics of study are the same as Ma et al. [BBRC.2018.505:40e44.](#))

2.2 Measurement of plasma relaxin-2 and relaxin-3

The levels of plasma relaxin-2 and relaxin-3 were measured by Radioimmunoassay (RIA) according to the manufacturer's instructions.

2.3 Measurement of other blood parameters

Blood samples were collected, and blood routine, liver and kidney function, and the level of lipid were assessed by routine biochemical analyses.

2.4 Statistical analysis

Data consistent with normal distribution is expressed in means (standard deviations), and data that does not conform to the normal distribution is represented by medians (interquartile range, IQR). Categorical variables are evaluated by χ^2 tests, and continuous variables are evaluated by Wilcoxon's test. The associations between plasma relaxin-2/relaxin-3 levels and other variables are analyzed by Spearman's correlation co-efficient. Comparison between two groups is analyzed by an unpaired Student's t-test. All data are performed with GraphPad Prism 5.0 software, $P < 0.05$ was considered statistically significant.

Results

3.1 Baseline clinical and demographic characteristics of the study population

The baseline clinical and demographic characteristics of the study population are shown in Table 1. No significant differences in age or gender were found between the control and liver cirrhosis groups. Patients with liver cirrhosis had significantly higher creatinine, aspartate aminotransferase, alanine aminotransferase, TB, DB, IDB and PT levels, and significantly lower ALB, CHE, hemoglobin levels and PLT counts than control subjects. Seventy-five patients were grouped by different Child-Pugh class: sixty-six Child-Pugh class A patients, 7 Child-Pugh class B patients, and 2 Child-Pugh class C patients.

3.2 Relaxin-2 and relaxin-3 levels in human plasma

Plasma relaxin-2 levels were significantly higher in patients with liver cirrhosis than in control subjects. Specifically, the median plasma relaxin-2 concentration in patients with liver cirrhosis was 71.5 pg/mL (range, 63.8–82.0 pg/mL), and the median plasma relaxin-2 concentration in control subjects was 65.7 pg/mL (range, 52.1–74.2 pg/mL) ($p = 0.004$)(Figure 1a). The median plasma relaxin-3 concentration in patients with liver cirrhosis was 179.2 pg/mL (range, 157.1–202.4 pg/mL) and the median plasma relaxin-3 concentration in control subjects was 184.1 pg/mL (range, 168.2–192.9 pg/mL). There were no significant differences in plasma relaxin-3 concentrations between the two groups ($p = 0.932$)(Figure 1b).

3.3 Relation between plasma relaxin-2 and relaxin-3 levels and liver cirrhosis parameters

We analysed the correlations between plasma relaxin-2 and relaxin-3 levels and liver cirrhosis parameters. Plasma relaxin-2 levels were significantly positively correlated with Child-Pugh classification($r_s = 0.244$; $p = 0.035$), WBC($r_s = 0.265$; $p = 0.022$), APTT($r_s = 0.233$; $p = 0.044$) and IDB($r_s = 0.229$; $p = 0.048$). Plasma relaxin-2 levels were significantly negatively correlated with ALB($r_s = -0.240$; $p = 0.038$). There were no significant correlations between plasma relaxin-3 concentrations and cirrhosis patient clinico-pathologic characteristics(Table 2). Additionally, plasma relaxin-3 concentrations were significantly positively correlated with relaxin-2 concentrations in patients with liver cirrhosis ($r_s = 0.322$; $p = 0.005$).

3.4 Validity and predictive values of relaxin-2

We found that the specificity (45%) was observed using a relaxin-2 cut-off value of 60.78 pg/ml, and the sensitivity (85.3%) was obtained using 60.78 pg/ml as a cut-off. Moreover, a cut-off value of 60.78 pg/ml offers the maximal combination of sensitivity and specificity. The area under the ROC curve indicated that the diagnosis value of liver cirrhosis was 0.665(Figure 2).

Discussion

In our study, we found that plasma relaxin-2 levels were significantly higher in patients with liver cirrhosis than in control subjects. Specifically, plasma relaxin-2 levels were significantly positively correlated with Child-Pugh classification, white blood cell, activated partial thromboplastin time and indirect bilirubin.

Plasma relaxin-2 levels were significantly negatively correlated with albumin. There were no significant differences in plasma relaxin-3 concentrations between the two groups.

In 1929, Hisaw identified relaxin-2 as a corpus luteum-derived hormone that could induce relaxation of the pelvic ligament in experimental settings. Recent studies have shown that relaxin-2 can be used to treat liver, kidney and cardiac fibrosis by binding to RXFP1. In this study, we found that plasma relaxin-2 levels were significantly increased in patients with liver cirrhosis compared with control subjects. Moreover, numerous studies have suggested that relaxin-2 ameliorates fibrosis in rat models and inhibits HSC phenotypic transformation in vivo[10–12]. We surmised that the higher endogenous relaxin-2 (an anti-fibrotic peptide) levels noted in patients with liver cirrhosis protected against liver fibrosis development and progression. We also found that plasma relaxin-2 levels were significantly correlated with Child-Pugh classification, white blood cell, activated partial thromboplastin time and indirect bilirubin in patients with liver cirrhosis. Although Child-Pugh classifications are subjective and serve as broad indices of liver function, they can indirectly serve as indices of liver fibrosis and portal hypertension severity. In addition, plasma relaxin-2 levels were significantly negatively correlated with albumin. The lower the albumin level, the worse the liver function, which is consistent with the Child-Pugh classifications.

Relaxin-3, which was first identified in 2002, is the ancestral peptide of the human relaxin subclass of the insulin superfamily[14]. Currently, the mechanisms underlying the functions of relaxin-3 remain to be fully elucidated. Some studies suggested that relaxin-3 was involved in modulating brain function in combination with RXFP3[15], and one study showed that relaxin-3 exerts its effects by binding to RXFP1 (the primary relaxin-2 receptor)[16]. Our previous studies demonstrated that relaxin-3 may attenuate hepatocyte apoptosis and protect against liver injury by inhibiting endoplasmic reticulum stress[13]. We found that plasma relaxin-3 levels were not significantly different between patients with liver cirrhosis and control subjects, however, plasma relaxin-2 concentrations were significantly positively correlated with relaxin-3 concentrations in patients with liver cirrhosis. The small sizes of the samples included in this study may be responsible for the lack of a significant difference in relaxin-3 concentrations between the two groups.

Conclusions

In this study, we demonstrated that plasma relaxin-2 levels are elevated in patients with liver cirrhosis. Relaxin-2 levels are also positively correlated with Child-Pugh classification and relaxin-3 levels in patients with liver cirrhosis, negatively correlated with albumin.

Abbreviations

RXFP1: relaxin family peptide receptor 1; HBV: viral hepatitis type B; RIA: Radioimmunoassay; ALB: albumin; RGT: gamma glutamyl transpeptidase; AP: alkaline phosphatase; CHE: choline esterase;

TB:total bilirubin; DB: direct bilirubin; IDB: indirect bilirubin; APTT:activated partial thromboplastin time; PT:prothrombin time; PLT:platelet; WBC:white blood cell.

Declarations

Ethical approval and consent to participate

This study was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University (reference number KY2016–126) and complied with the Declaration of Helsinki. Written informed consent to participate was obtained from all participants.

Consent for publication

Not Applicable

Conflict of interest statement

On behalf of all authors, the corresponding author states that there are no conflicts of interest regarding the publication of this manuscript.

Role of the funding source: no

Informed consent: Informed consent was obtained from all subjects before the study was initiated.

Authors' contributions:

Xiao Ma, Yu Pan, Xiaohui Zhang designed and wrote the manuscript. Ruiling Xu, Mingna Liu collected the samples. The interpretation of the results was done by Xiao Ma, Yu Pan, Xiaohui Zhang. All authors contributed to and commented on the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due patient information are private, but are available from the corresponding author on reasonable request.

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Reference

1. Friedman SL. Mechanisms of hepatic fibrosis. *Gastroenterology*. 2008 (134):1655-69.
2. Kong RC, Shilling PJ, Lobb DK, Gooley PR, Bathgate RA. Membrane receptors: structure and function of the relaxin family peptide receptors. *Mol Cell Endocrinol* 2010;320(1-2):1-15.
3. Unemori EN, Pickford LB, Salles AL, Piercy CE, Grove BH, Erikson ME. Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts in vitro and inhibits lung fibrosis in a murine model in vivo. *J Clin Invest* 1996;98(12):2739-45.
4. Garber SL, Mirochnik Y, Brecklin CS, Unemori EN, Singh AK, Slobodskoy L. Relaxin decreases renal interstitial fibrosis and slows progression of renal disease. *Kidney Int* 2001;59(3):876-82.
5. Samuel CS, Hewitson TD, Zhang Y, Kelly DJ. Relaxin ameliorates fibrosis in experimental diabetic cardiomyopathy. *Endocrinology* 2008;149(7):3286-93.
6. Unemori EN, Beck LS, Lee WP, Xu Y, Siegel M, Keller G. Human relaxin decreases collagen accumulation in vivo in two rodent models of fibrosis. *J Invest Dermatol*. 1993;101(3):280-5.
7. Zhang J, Qi YF, Geng B, Zhao J, Chen L, Yang J. Effect of relaxin on myocardial ischemia injury induced by isoproterenol. *Peptides* 2005;26(9):1632-9.
8. Bennett RG, Dalton SR, Mahan KJ, Gentry-Nielsen MJ, Hamel FG, Tuma DJ. Relaxin receptors in hepatic stellate cells and cirrhotic liver. *Biochem Pharmacol* 2007;73(7):1033-40.
9. Bennett RG, Mahan KJ, Gentry-Nielsen MJ, Tuma DJ. Relaxin receptor expression in hepatic stellate cells and in cirrhotic rat liver tissue. *Ann N Y Acad Sci* 2005;1041:185-9.
10. Fallowfield JA, Hayden AL, Snowdon VK. Relaxin modulates human and rat hepatic myofibroblast function and ameliorates portal hypertension in vivo. *Hepatology*. 2014;59(4):1492-504.
11. Bennett RG, Heimann DG, Singh S, Simpson RL, Tuma DJ. Relaxin decreases the severity of established hepatic fibrosis in mice. *Liver Int* 2014;34(3):416-26.
12. Kim JK, Lee JI, Paik YH, Yun CO, Chang HY, Lee SY, et al. A single adenovirus-mediated relaxin delivery attenuates established liver fibrosis in rats. *J Gene Med* 2016;18(1-3):16-26.
13. Ma X, Han S, Zhang W, Fan YJ, Liu MN, Liu AY, et al. Protection of cultured human hepatocytes from hydrogen peroxide induced apoptosis by relaxin 3. *Mol Med Rep* 2015;11(2):1228-34.
14. Bathgate RA, Samuel CS, Burazin TC, Layfield S, Claasz AA, Reytomas IG, et al. Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene. Novel members of the relaxin peptide family. *J Biol Chem* 2002;277(2):1148-57.
15. Chan LJ, Hossain MA, Samuel CS, Separovic F, Wade JD. The relaxin peptide family—structure, function and clinical applications. *Protein Pept Lett* 2011;18(3):220-9.
16. Hossain MA, Man BC, Zhao C, Xu Q, Du XJ, Wade JD, et al. H3 relaxin demonstrates antifibrotic properties via the RXFP1 receptor. *Biochemistry* 2011;50(8):1368-75.

Tables

Due to technical limitations, all Table(s) are only available as a download in the supplemental files section.

Figures

Figure 1

plasma levels of relaxin-2 and relaxin-3 in patients with liver cirrhosis. (a) Relaxin-2 level in patients with liver cirrhosis. (b) Relaxin-3 level in patients with liver cirrhosis. *P<0.05 vs. control, **P<0.01 vs. control.

Figure 2

Receiver operating curve of relaxin-2 value for liver cirrhosis diagnosis according to the analysis of 75 liver cirrhosis and 41 healthy controls.

Supplementary Files

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- [Table1.pdf](#)
- [Table2.pdf](#)