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BRIEF ARTICLE

Role of serotonin in development of esophageal and gastric fundal varices

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Abstract

AIM: To determine the effect of free serotonin concentrations in plasma on development of esophageal and gastric fundal varices.

METHODS: This prospective study included 33 patients with liver cirrhosis and 24 healthy controls. Ultrasonography and measurement of serotonin concentration in plasma were carried out in both groups of subjects. The upper fiber panendoscopy was performed only in patients with liver cirrhosis.

RESULTS: The mean plasma free serotonin levels were much higher in liver cirrhosis patients than in healthy controls (219.0 ± 24.2 nmol/L *vs* 65.4 ± 18.7 nmol/L, $P < 0.0001$). There was no significant correlation between serotonin concentration in plasma and the size of the esophageal varices according to Spearman coefficient of correlation ($r_s = -0.217$, $P > 0.05$). However, the correlation of plasma serotonin concentration and gastric fundal varices was highly significant ($r_s = -0.601$, $P < 0.01$).

CONCLUSION: Free serotonin is significant in pathogenesis of portal hypertension especially in development of fundal varices, indicating the clinical value of serotonergic receptor blockers in these patients.

Key words: Serotonin; Portal hypertension; Esophageal varices; Fundal varices; Platelets

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INTRODUCTION

Portal hypertension has been increasingly regarded as a multi-organ disease with complex blood flow changes in the systemic and splanchnic vascular network.

The hepatic stellate cell (HSC) has a significant position in the sinusoid for regulation of portal flow. During liver damage, HSCs are "activated" which leads to HSC transformation into myofibroblast-like cells with a resulting increased collagen production[1,2]. Several mitogens are included in the triggering of HSC proliferation: platelet-derived growth factor (PDGF), insulin-like growth factor 1 and connective-tissue growth factor. During activation,

HSCs acquire the ability to express PDGF receptors on the cell membrane surface[3]. The HSC membrane contains numerous receptors whose expression is increased with the extent of liver damage, to which different vasoconstrictors are bound: catecholamines, endothelin, angiotensin I and Ⅱ, leukotrienes and serotonin [5-hydroxytriptamin (5-HT)][4,5].

Serotonin, at the level of hepatic sinusoids, causes endothelial fenestrae contractions of liver sinusoids through 5-HT_1 receptors mediated by a Ca^{2+}-dependent process. Due to different proinflammatory mediators releasing from the damaged liver, it comes to platelet adherence to sinusoidal endothelium, translocation into Disse's space and serotonin release. Thereafter, serotonin binds to receptors (5-HT_{2A}, 5-HT_{1B}, 5-HT_{1F} and 5-HT_7) which are expressed on HSC and hepatocytes, which additionally interferes with HSC proliferation[6,7].

The aim of our study was to determine to what extent a free serotonin concentration in plasma has an effect on development of esophageal and gastric fundal varices.

MATERIALS AND METHODS

The study included 33 patients with liver cirrhosis who were examined and treated at the Clinic of Gastroenterology, Clinical Center of Serbia, and 24 healthy subjects who made up the control group. The study was prospective and conducted during the period 2008-2009.

Ultrasonography was carried out by Toshiba Core Vision, with 3.5 MHz duplex Doppler convex tube in a standard procedure. Ultrasonography examined the liver size, echo structure of the hepatic parenchyma and possible focal changes with a view to rule out the patients with primary and secondary liver tumors from the study. To determine the spleen size, standard parameters were used, according to which in physiological conditions the spleen diameter measured in the X intercostal space exceeded no more than 12.0 cm and anteroposterior diameter was not over 5.0 cm.

The upper endoscopy was performed by endo-video system Olympus GIF-Q 165. To measure the esophageal varices size, Paquet's classification was used: I degree-lesser snake-like mucosal protrusions, Ⅱ degree-varices were predominating up to a half of the esophageal lumen radius, Ⅲ degree-varices were in contact at some points, and Ⅳ degree-heralds of the imminent rupture (cherry red spots)[8]. Endoscopic examination showed portal hypertensive gastropathy (snake skin mucosa) and varices of the gastric fundus.

Platelet poor plasma (PPP) was obtained from the venous blood which was collected in 3 mL original Vacutainer "BD" tubes, with 75 g/L K_3EDTA 0.072 mL. Blood samples were taken between 8 and 9 a.m. Platelet rich plasma (PRP) was obtained by low speed centrifugation (200 *g*, 10 min) on "Heraeus Digifuga GL". Exactly 1 mL of PRP was centrifuged at 1000 *g*, 10 min. The obtained PPP was separated and stored at -20℃ for no longer than 20 d[9].

The number of PPP serotonin samples was estimated in one series. One hundred μL plasma samples were spiked with 10 μL of original N-methyl serotonin solution (Recipe, Munchen), which was an internal standard. After that, PPP samples were deproteinized with 100 μL original deproteinating reagent (Recipe, München), and centrifuged at 10 000 *g*[10]. The obtained 20 μL supernatants were analyzed on reverse phase HPLC column (Recipe, München), with original mobile phase for serotonin (Recipe, München). Original "Recipe" external standard solution was used for calibration. The HPLC system consisted of "Bio-Rad AS 100" HRLC automatic sampling system with "Rheodine 7125 valve", "Bio-Rad 1350" HPLC pump and "Bio-Rad 1640" electrochemical detection. Chromatographic data were calculated using the "Chrome Line V 4.20" HPLC software. Amperometric detection was done at 0.6 V. The duration of chromatographic separation was 10 min.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS®, version 17.0). Basic descriptive statistics included means, standard deviations, ranges and percentages. Differences between groups were compared with parametric *t*-test because data had a Gaussian distribution. Correlation analysis was processed *via* the Spearman method. Values at the $P \leqslant 0.05$ level were considered statistically significant.

RESULTS

The study included 11 (33.3%) female and 22 (66.7%) male patients, mean age of 52.32 (SD ± 11.55) years. The most common cause of liver cirrhosis was alcohol-in 15 (45.4%) cases. The incidence of posthepatitic cirrhosis was lower; HCV-8 (24.2%), HBV-5 (15.1%), while autoimmune diseases were quite rare-in 5 (15.1%) patients.

Splenomegaly was detected in 28 (84.8%) patients with liver cirrhosis. An average longitudinal splenic diameter was 17.5 ± 3.57 cm, and transversal diameter was 6.8 ± 1.77 cm, which was significantly different in relation to the controls, in whom an average longitudinal diameter was 10.21 ± 1.65 cm and transversal diameter was 3.03 ± 0.87 cm (*t*-test, $P < 0.05$).

There was a highly significant difference between the platelet count in the studied groups of patients ($t = -9.779$, $P < 0.01$).

The mean plasma free serotonin level was much higher in liver cirrhosis patients than in healthy controls (219.0 ± 24.2 nmol/L *vs* 65.4 ± 18.7 nmol/L; *t*-test, $P < 0.0001$).

There was no significant correlation between serotonin concentration in plasma and the platelet count according to Spearman coefficient of correlation ($r_s = 0.158$, $P > 0.05$).

Esophageal varices were not detected in 5 (15.1%) patients, grade Ⅰ-Ⅱ varices were detected in 9 (27.2%), grade Ⅱ-Ⅲ in 15 (45.4%) and the remaining 4 patients (12.1%) were grade Ⅳ. Gastric fundal varices were found in 7 (21.2%) patients, out of whom 2 had Ⅰ-Ⅱ, 4 had grade Ⅱ-Ⅲ esophageal varices, and one patient had Ⅳ degree varices.

Spearman's rank correlation verified a statistically significant correlation between the platelet count and varices size ($r_s = -0.479$, $P < 0.05$).

Spearman's rank correlation verified no significant difference between the serotonin concentration in plasma in relation to the size of esophageal varices (r_s = -0.217, P > 0.05). However, the mean plasma free serotonin level was higher in patients with esophageal varices than in patients without varices (t = -2.301, P < 0.05). Furthermore, the correlation of plasma serotonin concentration and fundal varices was highly significant (r_s = -0.601, P < 0.01). Also, we proved that the mean plasma free serotonin level was much higher in patients who had esophageal and gastric fundal varices than in patients who had only esophageal varices (t = -5.862, P < 0.01).

DISCUSSION

Different factors may affect the concentrations of circulating serotonin in liver cirrhosis, such as: impaired serotonin catabolism due to higher activity of the monoamino oxidases; impaired metabolism of tryptophane as a serotonin precursor; platelet sequestration in the spleen and/or platelet activation[11]. In addition, 5-HT as well as other vasoactive substances synthesized in the gastrointestinal tract *via* porto-systemic collaterals bypass the liver and directly enter the systemic circulation[12].

In the study of Beaudry *et al*[13], in 1994, the whole-blood serotonin levels were significantly lower in 30 patients with cirrhosis than in the age-matched controls, and no correlation was found between these levels and the severity of cirrhosis. However, in the same study the unconjugated plasma serotonin levels, an indication of the active form of serotonin, were significantly higher in patents with cirrhosis than in the controls.

In our previous study, free or unconjugated serotonin levels were investigated. The levels of free serotonin were higher in patients with liver cirrhosis than in healthy subjects[14].

In the study of Vorobioff *et al*[15], in 1989, it was confirmed that the application of ketanserin and ritanserin (serotonergic receptor inhibitors) caused the lowering of portal hypertension in patients with liver cirrhosis. The authors reported that the spleen congestion in liver cirrhosis brought about the platelet breakdown. Free serotonin, released in the sinusoidal spaces of the spleen, induced by S-2 receptor produced an intense vasoconstricting response in portal circulation which led to maintenance and elevation of the portal pressure. Moreover, it was documented that the reaction of the isolated mesenteric vein in rats with portal hypertension to 5-HT was hypersensitive, which was additional evidence of the role of this substance in pathogenesis of portal hypertension.

In our study, the correlation of unconjugated serotonin concentration (active form of serotonin) in plasma and varices of the gastric fundus was highly significant while the plasma unconjugated serotonin concentration did not correlate with the size of the esophageal varices. Moreover, mean longitudinal and transversal diameters of the spleen in patients was significantly higher as compared to controls.

The spleen has a crucial role in pathogenesis and maintenance of portal hypertension[16,17]. In portal hypertension, the anatomic changes of the spleen (pulp hyperplasia, congestion and fibrosis) and specific vascularization affect the hemodynamics of the splenic circulation[18].

Our finding may be accounted for different porto-systemic collateral pathways in esophageal and fundal varices as well as valuable flow changes in the left part of the portal venous system. Perisic *et al*[19] reported, in 2005, that the splenic vein flow in patients with liver cirrhosis was slower in comparison with healthy controls. In addition, in healthy controls, the splenic vein flow was significantly slower than in the portal vein. However, in patients with liver cirrhosis splenic vein flow was significantly faster than in the portal vein, probably because of the splenic venous congestion and compensatory hemodynamic mechanisms of the spleen.

Gastric varices are drained through the short gastric veins into the splenic vein. Serotonin released by platelet sequestration in the enlarged spleen reaches the lienal vein where the blood flow is faster than in the portal vein, and directly, *via* short gastric veins, it enters the fundal gastric veins, leading to vasoconstriction.

Our conclusion is that free serotonin is significant in pathogenesis of portal hypertension especially in development of gastric fundal varices which may have clinical value in use of serotonin receptor blockers in these patients.

COMMENTS

Background

In acute and chronic hepatic insufficiency, the serotonin system changes lead to development of hepatic encephalopathy, portal hypertension and hyperdynamic circulation. Portal hypertension has been increasingly regarded as a multi-organ disease with complex blood flow changes in systemic and splanchnic vascular network. The hepatic stellate cell (HSC) has a significant position in sinusoid for regulation of portal flow and during liver damage serotonin binds to receptors [5-hydroxytriptamin (5-HT)$_{2A}$, 5-HT$_{1B}$, 5-HT$_{1F}$ and 5-HT$_7$] which are expressed on HSC and hepatocytes, which additionally interferes with HSC proliferation.

Research frontiers

Free serotonin, released in the sinusoidal spaces of the spleen, induced by S-2 receptor, produces an intense vasoconstricting response in the portal circulation, which leads to maintenance and elevation of portal pressure. The highlight of our study was to determine to what extent a free serotonin concentration in plasma has an effect on development of esophageal and gastric fundal varices.

Innovations and breakthroughs

In the study of Vorobioff *et al*, in 1989, it was confirmed that the application of ketanserin and ritanserin (serotonergic receptor inhibitors) caused the lowering of portal hypertension in patients with liver cirrhosis. Moreover, it was documented that the reaction of the isolated mesenteric vein in rats with portal hypertension to 5-HT was hypersensitive, which was additional evidence of the role of this substance in pathogenesis of portal hypertension. In this study, the correlation of unconjugated serotonin concentration (active form of serotonin) in plasma and varices of the gastric fundus was highly significant while the plasma unconjugated serotonin concentration did not correlate with the size of the esophageal varices. The authors' finding may be accounted for by different porto-systemic collateral pathways in the esophageal and fundal varices as well as valuable flow changes in the left part of the portal venous system.

Applications

The conclusion is that free serotonin is significant in pathogenesis of portal hypertension especially in development of gastric fundal varices. This may have clinical value in use of serotonin receptor blockers in these patients.

Terminology

Portal hypertension: Portal hypertension (> 10 mmHg) most commonly results from increased resistance to portal blood flow. Cirrhosis is the most common

cause of portal hypertension. One of the major clinical manifestations of portal hypertension includes life-threatening hemorrhage from gastrointestinal varices. Serotonin: Serotonin is a vasoactive substance, synthesized by the intestinal enterochromaffin cells, which is actively incorporated into platelets and stored in platelet dense-storage granules.

Peer review

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