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RAPID COMMUNICATION

Plasma and platelet serotonin levels in patients with liver cirrhosis

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Abstract

AIM: To analyze the relationship between plasma and platelet serotonin levels and the degree of liver insufficiency.

METHODS: The prospective study included 30 patients with liver cirrhosis and 30 healthy controls. The degree of liver failure was assessed according to the Child-Pugh classification. Platelet and platelet poor plasma serotonin levels were determined.

RESULTS: The mean plasma serotonin level was higher in liver cirrhosis patients than in healthy subjects (215.0 ± 26.1 *vs* 63.1 ± 18.1 nmol/L; $P < 0.0001$). The mean platelet serotonin content was not significantly different in patients with liver cirrhosis compared with healthy individuals (4.8 ± 0.6; 4.2 ± 0.3 nmol/platelet; $P > 0.05$). Plasma serotonin levels were significantly higher in Child-Pugh grade A/B than in grade C patients (246.8 ± 35.0 *vs* 132.3 ± 30.7 nmol/L; $P < 0.05$). However, platelet serotonin content was not significantly different between Child-Pugh grade C and grade A/B (4.6 ± 0.7 *vs* 5.2 ± 0.8 nmol/platelet; $P > 0.05$).

CONCLUSION: Plasma serotonin levels are significantly higher in patients with cirrhosis than in the controls and represent the degree of liver insufficiency. In addition, platelet poor plasma serotonin estimation is a better marker for liver insufficiency than platelet serotonin content.

Key words: Serotonin; Plasma; Platelet; Liver cirrhosis

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INTRODUCTION

The serotonergic system plays a critical role in a wide variety of physiological and behavioral processes. In the circulation, serotonin synthesized by the intestinal enterochromaffin cells, is actively incorporated into platelets and stored in platelet dense-storage granules. Nearly all of the serotonin in circulating blood is concentrated in the platelets and minimally in plasma (which is the interactive pool). The integral membrane protein of mucosal epithelial cells is the major protagonist in regulating the extracellular serotonin concentration[1]. Serotonin is mostly metabolized into 5-hydroxyindoleacetic acid by monoamine oxidase in hepatic and lung endothelial cells[2]. The effects of serotonin are most prominent in the cardiovascular system, with additional effects in the respiratory system and the intestines. Vasoconstriction is a classic response to administration of serotonin. However, serotonin induces smooth muscle cell contraction and proliferation and stimulates endothelial cells to release vasodilating substances and acts as "helper agonist" of platelet aggregation in humans[3,4]. Altered concentrations of circulating serotonin have been implicated in several pathologic conditions including hypertension, primary pulmonary hypertension, liver cirrhosis, and psychiatric disorders[5-8].

The acute and chronic hepatic insufficiency gives rise to serotonin system changes, contributing to the development of hepatic encephalopathy, portal hypertension, and hyperdynamic circulation[9]. Hepatic encephalopathy is followed by changes of serotonin neurotransmission, including the catabolic enzymes, receptors, and metabolites[10]. After application of serotonin inhibitors (ketanserin and ritanserin), portal pressure is decreased in patients with liver cirrhosis, confirming the importance of serotonin in the pathogenesis of portal hypertension[11]. The aim of this study was to characterize the relationship between plasma and platelet serotonin levels and the degree of liver insufficiency.

MATERIALS AND METHODS

Subjects

In the period January-April, 2007, the prospective study included 30 patients with liver cirrhosis and 30 healthy controls, examined at the Institute of Digestive

Diseases, Clinical Center of Serbia, Belgrade. Hepatologic examinations were based on medical history, physical examination, laboratory tests, and liver biopsy. Laboratory tests included hepatocyte integrity, cholestasis, synthetic liver function, and specific (etiological) tests. Puncture liver biopsy was performed in 16 (53.3%) patients, using Menghini needle of 1.4 mm. The degree of liver failure was assessed according to the Child-Pugh classification.

Biochemistry

Platelet and platelet poor plasma (PPP) venous blood 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 am. Platelet number was immediately determined on "Coulter A^c T Diff" analyser. Platelet rich plasma (PRP) was obtained by low speed centrifugation (200 g, 10 min) on "Heraeus Digifuga GL". Platelet count was determined again and taken into consideration in final determination of serotonin concentration in platelets. Exactly 1 mL of PRP was centrifuged on 1000 × g for 10 min. The obtained PPP was separated, and together with platelet pellets, stored at -20℃, not longer then 20 d[12]. The whole number of platelet pellets and PPP serotonin samples were estimated in one series.

Platelet pellets were spiked with 10 μL N-methyl serotonin solution (Recipe, Munchen) as an internal standard. All pellets were diluted with 100 μL high performance liquid chromatography (HPLC) ultra pure water (Recipe, Munchen). Platelets were destroyed with 100 μL of 700 g/L perchloric acid (Merck Darmstad) and centrifuged at 10 000 × g for 5 min. 100 μL of PPP samples was deproteinized with 100 μL deproteinating reagent (Recipe, Munchen) and centrifuged in same way, as for platelets[13].

Twenty μL of the supernatants were analyzed by reverse phase HPLC (Recipe, Munchen) with original mobile phase for serotonin (Recipe, Munchen). Original, "Recipe" external standard solution has been 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 has been done on 0.6 V. Duration of chromatographic separation was 10 min.

Statistical analysis

Statistical analyses were performed using SPSS statistical software (SPSS for windows, release 10.0, SPSS, Chicago, Il). Descriptive statistics are presented as mean ± SE. Differences between groups were compared with parametric *t*-test because data had a Gaussian distribution. Because we performed 6 consecutive statistical analyses, we chose a level of significance 0.05/6 = 0.008 (α-adjustment according to the modified Bonferroni procedure).

RESULTS

The most common cause of liver cirrhosis was alcohol in 12 individuals (40.0%). The incidence of cirrhosis due to viral infection was lower with HCV in 6 (20.0%) and HBV in 5 subjects (16.6%); autoimmune diseases were quite rare with 3 cases (10.0%), while etiology was unknown in 4 (13.3%) cases. The patients were classified according to the Child-Pugh system. Child-Pugh class A included 12 (40%) patients, Child-Pugh class B 8 (26.6%), and Child-Pugh class C 10 (33.3%) patients. All patients had clinically evident portal hypertension, none of them had episodes of bacterial peritonitis, and none of them had a beta blocker medication.

The mean plasma free serotonin levels were much higher in liver cirrhosis patients than in healthy individuals. A statistical significant difference was found between serotonin plasma values in patients with liver cirrhosis and healthy subjects (215.0 ± 26.1 *vs* 63.1 ± 18.1 nmol/L; t = 3.868, $P < 0.0001$).

The mean platelet serotonin content was not significantly different in patients with liver cirrhosis compared to healthy individuals. There was no statistically significant difference between platelet serotonin content in patients with liver cirrhosis and healthy subjects (4.8 ± 0.6 *vs* 4.2 ± 0.3 nmol/platelets; t = 0.881, $P > 0.05$).

Plasma free serotonin levels were significantly higher in Child-Pugh grade A/B than in grade C patients (246.8 ± 35.0 *vs* 132.3 ± 30.7 nmol/L; t = 1.938, $P < 0.05$). However, platelet serotonin content was not significantly different between Child-Pugh grade C and grade A/B (4.6 ± 0.7 *vs* 5.2 ± 0.8 nmol/platelets; t = 0.48, $P > 0.05$).

In addition, plasma free serotonin levels were not significantly different between alcohol liver cirrhosis and cirrhosis of other etiology (143.9 ± 29.3 *vs* 224.84 ± 34.8 nmol/L; t = 1.6, $P > 0.05$). Also, platelet serotonin content was not significantly different between alcohol liver cirrhosis and cirrhosis of other etiology (4.2 ± 0.7 *vs* 5.3 ± 0.9 nmol/10^9 platelets; t = 0.91; $P > 0.05$).

DISCUSSION

Marasini *et al*[7] described a significant reduction of serotonin, determined by high-performance liquid chromatography, in platelets of 14 patients with liver cirrhosis, although levels of free circulating plasma serotonin were within the normal range. In the study of Beaudry *et al*[14], the whole-blood serotonin levels were significantly lower in 30 patients with cirrhosis than in age-matched controls, and no correlation was found between these levels and the severity of cirrhosis. This difference might be the result of low platelet count observed in patients with cirrhosis; however, in this series of patients, the significant difference persisted when the whole-blood serotonin levels were expressed by platelet count, but it was less expressed. Thus, in patients with cirrhosis, low whole-blood serotonin levels probably depend on reduction of both uptake, retention of serotonin by platelets, and low platelet number.

However, in the same study of Beaudry *et al*[14], unconjugated plasma serotonin levels, an indication of the active form of serotonin, were significantly higher in patients with cirrhosis than in the controls, and in patients with cirrhosis these levels were higher in Child-Pugh grade A than in grade C patients. In our study, we investigated levels of free or unconjugated serotonin. However, the levels of free serotonin in patients with liver cirrhosis were

also higher than in healthy subjects. Also, the levels of free serotonin in patients with Child-Pugh grade C is less than in grade A/B patients. In addition, platelet serotonin content was not significantly different when patients with liver cirrhosis were compared to healthy subjects.

The discrepancy of our and Beadry's study may be explained by the fact that whole blood is a similar, but not the same kind of biological material, as the platelet pellet is. In addition, we used HPLC as basic technique in our work, which significantly differs from Beadry's study, who also emphasized this difference in his study. The reasons for high levels of plasma serotonin in the liver cirrhosis could be slow uptake and storage of serotonin by the platelets (as could be the sequelae of the kinetic change of serotonin transport mechanisms) or abnormal serotonin release from dense granules of activated platelets.

Laffi *et al*[15] gave the evidence for significant reduction of substances that are deposited in thick (adenosine triphosphate and serotonin) and in alpha granules (B-thromboglobulin and platelet factor 4) in patients with liver cirrhosis in comparison to controls. It is supposed that platelet disorder in deposition of substances mentioned above, in patients with liver cirrhosis, is in relation to platelet activation, a condition defined as "platelet exhaustion".

The causes of platelet activation in liver cirrhosis are complex and not yet fully understood. Hyperdynamic portal circulation and retention in the spleen microcirculation in liver cirrhosis might stimulate platelets. Immunological and inflammatory phenomena in the liver tissue and its influence are other possible reasons for platelet activation. In addition, endotoxemia is often associated with severe liver cirrhosis and it causes platelet activation[16]. In liver cirrhosis, thrombocytopenia is associated with a shorter life span of platelets and is the result of constant platelet activation by cytokines (IL2, IL6, TNFα), it is mediated by subclinical DIC, and intensified elimination by reticuloendothelial system of the spleen and liver[17]. Platelet activation with the increase of both βTG serum concentration and elevation of platelet population (CD62P and CD63 as well as medial intensity of fluorescence CD62P and CD63) becomes higher as liver cirrhosis develops and thrombocytopenia rises. Concurrently with thrombocytopenia in liver cirrhosis, platelet CD63+ population increases, clearly indicating the platelet activation with elevated medial intensity of fluorescence CD62P and CD63[18].

Concentration of circulating serotonin in liver cirrhosis can be influenced by other factors, such as altered serotonin catabolism due to an elevated activity of monoamino oxidase and impaired metabolism of tryptophan, as serotonin precursor[19]. Impaired metabolic function in liver cirrhosis contributes to elevated plasma serotonin. Moreover, vasoactive substances, produced in the splanchnic circulation, bypass the liver in the presence of porto-systemic collaterals and directly enter the systemic circulation. In conclusion, plasma serotonin levels are significantly higher in patients with cirrhosis than in the controls, and represent the degree of liver insufficiency. In addition, PPP serotonin estimation is a better marker of liver insufficiency than platelet serotonin content.

COMMENTS

Background

The acute and chronic hepatic insufficiency gives rise to serotonin system changes, contributing to the development of hepatic encephalopathy, portal hypertension, and hyperdynamic circulation. In patients with liver cirrhosis, low whole-blood serotonin levels depend probably on reduced uptake, retention of serotonin by platelets, and low platelet number. It is supposed that reduced platelet deposition of substances in thick and alfa granules, is in relation to platelet activation, caused by hyperdynamic circulation and endotoxemia. Also, concentration of circulating serotonin in liver cirrhosis can be influenced by other factors, such as altered serotonin catabolism due to elevated activity of monoamino oxidase and impaired metabolism of tryptophan, as a precursor of serotonin.

Research frontiers

The serotonergic system plays a critical role in a wide variety of physiological and behavioral processes. Altered concentrations of circulating serotonin are implicated in several pathologic conditions including hypertension, primary pulmonary hypertension, liver cirrhosis, and psychiatric disorders. The highlight of our research was to characterize the relationship between plasma and platelet serotonin levels and the degree of liver insufficiency.

Innovations and breakthroughs

In Beadry's study, unconjugated plasma serotonin level, an active form of serotonin, was significantly higher in patients with cirrhosis than in the controls, and in cirrhotics this level was higher in Child A than in Child C patients. In our study, the levels of free serotonin in patients with liver cirrhosis were also higher than in healthy subjects. However, the levels of free serotonin in patients with Child C cirrhosis were less compared to Child A/B patients. Furthermore, platelet serotonin content was not significantly different in cirrhotics compared to healthy controls. The discrepancy of our and previous study could be explained by the fact that we used high performance liquid chromatography as basic technique in our work, which significantly differs from the technique used in the previous study.

Applications

The results in the study suggest that plasma serotonin levels may represent the degree of liver insufficiency. Also, platelet poor plasma serotonin estimation is a better marker for liver insufficiency than platelet serotonin content.

Terminology

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. Integral membrane protein of mucosal epithelial cells is the major protagonist in regulating the extracellular serotonin concentration. Serotonin is mostly metabolized into 5-hydroxyindoleacetic acid by monoamine oxidase in hepatic and lung endothelial cells.

Peer review

This is a case controlled study demonstrating that plasma but not platelet serotonin levels are increased in patients with liver cirrhosis and correlate with liver insufficiency. The subject is novel and interesting.

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