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Clinical Study

Melatonin Levels in Serum and Ascitic Fluid of Patients with Hepatic Encephalopathy

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Cirrhotic patients exhibit disturbed melatonin homeostasis, which may lead to sleep disturbances, but an influence on the hepatic encephalopathy has not been elucidated. *Aim*. In the present study, the association of melatonin levels in serum and ascitic fluid and ammonia concentration related to the intensity of hepatic encephalopathy (HE) was investigated. *Material and Methods*. The study included 90 alcoholic patients with hepatic encephalopathy and 30 healthy volunteers (C). Patients were divided in three groups according to 0–4 West-Haven Score: HE_1 ($n = 28$), HE_2 ($n = 30$), and HE_3 ($n = 32$). Melatonin was measured by radioimmune assay. *Results*. In fasting patients with hepatic encephalopathy we noted higher melatonin serum levels [pg/mL] than in healthy subjects groups: C—11.3 ± 3.9, HE_1 – 34.3 ± 12.2 ($P < 0.01$), HE_2—54.8 ± 23.9, and HE_3—119.8 ± 96.4 ($P < 0.001$). No correlation between melatonin and ammonia levels was found. Melatonin was detected in ascetic fluid in 24 patients of group HE_2 and 27 patients of group HE_2 of hepatic encephalopathy. *Conclusions*. Our results suggest that high blood levels of melatonin in cirrhotic liver patients may account for some of the clinical manifestations of hepatic encephalopathy, for example, daytime sleepiness, fatigue.

1. Introduction

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome which is characterized by disturbances in behavioral and consciousness as well as neurological symptoms. Detailed pathophysiology of this disease remains unknown, but some of the pathogenetic factors have been identified. This includes disturbances of hepatocyte function because of toxic substances produced during metabolism. As result, toxic substances are transported via the blood from the portal vein into various organs including brain where they induce metabolic disturbances.

Ammonia has been identified as a factor of hepatic encephalopathy pathogenesis. It disturbs enzymatic processes in brain tissue, inhibits the activity of acetylcholine and dopamine, and increases accumulation of false neurotransmitters [1]. However, high levels of ammonia were not observed in all hepatic encephalopathy patients, indicating that other chemical substances should be also considered as pathogenetic factors. Possible candidates are the excess of methionine and its mercaptan derivatives as well as aromatic amino acids (phenylalanine, tyrosine, tryptophan, and methionine). These agents block the synthesis of physiological neurotransmitters and induce the production of false ones, including octopamine, which may replace normal neurotransmitters, especially noradrenaline and dopamine in synapses. This causes muscle tremors and psychoemotional disturbances [2, 3]. Disturbances in consciousness may also be a consequence of increased concentration of some neurotransmitters, including gamma-aminobutyric acid (GABA) and other biologically active compounds. Melatonin has beneficial effects on a variety of central nervous system (CNS) diseases [4, 5]. However, the influence of melatonin on CNS in individuals with liver insufficiency has not been sufficiently recognized.

Melatonin is released by the pineal gland according to the circadian rhythm, and its peak levels are near the middle of the dark phase [6]. Nocturnal concentrations of melatonin are severalfold higher than in the daytime [7]. Due to its high lipophilicity melatonin released from pinealocytes easily penetrates all cells and enters the body fluids [8, 9]. The gastrointestinal tract (GIT) is also a source of melatonin, from where it is released following various stimuli, including alimentary signals. In this location it seems to be synthesized when L-tryptophan is supplied in the diet [10, 11]. The precise mechanisms of synthesis and regulation of melatonin in the GIT are not known. Melatonin released from enteroendocrine cells (ECs) plays an important enteroprotective role via paracrine mechanisms [10–12]. Melatonin is metabolized in enterocytes by cytochrome CYP1B1 fraction [13]. The majority of melatonin is transported to the liver through hepatic portal vein [14, 15]. About 90% of alimentary tract-released melatonin is inactivated during the first passage through the liver [16]. As in the got, melatonin is metabolized in hepatocytes by the cytochrome P-450 enzymes (CYP1A1 and CYP1A2) to 6-sulfatoxymelatonin and 6-hydroxymelatonin glucuronide, which are removed with the urine [17–19]. A fraction of melatonin may be released into bile in its unchanged form, since the concentration of indoleamine in the bile is extremely high [20, 21]. The melatonin receptors are expressed in human gallbladder epithelia [22]. High concentrations of melatonin in the bile may prevent the epithelium of bile tracts and intestines from injury by biliary acids.

Melatonin release and metabolism seem to be affected by various pathological states, especially those associated with liver disease. Disturbances in circadian rhythm of melatonin release from the pineal gland with its peak in the morning were observed in patients with cirrhosis [23, 24]. It was suggested that this occurred as a result of hepatic insufficiency-related metabolic disturbances [25]. Similar changes were observed in rats after installation of portosystemic shunt [26, 27]. In this situation, administration of neomycin correct at the rhythm to a melatonin release [28]. These findings promoted conclusion that increased concentrations of ammonia in the blood exerted toxic effects on brain structures, including the pineal gland, and changed the rhythm of melatonin release [29–31]. This may indicate that the increase of melatonin concentration in the morning may depend on the ability of liver to metabolize it; this is possible in patients with hepatic insufficiency. A fivefold increase in the concentration of melatonin was observed in the fasting state in cirrhotic patients with portal hypertension as compared with controls. The concentration of melatonin increased almost four-fold after test meal when taken with 10 mg melatonin [32]. Such high concentration of melatonin in hepatic insufficiency might result from its disturbed metabolism and its discharge from portal to systemic circulation.

In the present study we evaluated the melatonin concentration in the blood and ascitic fluid in patients with hepatic encephalopathy.

Table 1: Characteristics of the subjects enrolled in the study (mean values ± SEM).

Feature/parameters	Cirrhotic patients	Healthy subjects
Age (years)	44.1 ± 9.9	43.1 ± 6.8
Sex (F, M)	F-24, M-66	F-11, M-19
Bilirubin (mg/dL)	8.3 ± 7.1	0.7 ± 0.2
Ammonia (μg/dL)	90.2 ± 41.0	30.4 ± 8.9
Albumins (g/dL)	3.1 ± 1.4	5.4 ± 0.6
AST (U/L)	81.6 ± 78.0	21.0 ± 4.8
ALT (U/L)	102.3 ± 121.6	23.1 ± 5.9
GGTP (U/L)	135.6 ± 90.4	26.0 ± 6.1
ALP (U/L)	49.4 ± 30.2	37.4 ± 10.8
GFR (mL/min)	97.0 ± 11.9	108.9 ± 9.6

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGTP: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; GFR: glomerular filtration rate.

2. Material and Methods

2.1. Patients

2.1.1. Including Criteria. Ninety liver cirrhosis patients with postal hypertension and ascites (grade B, C acc. to Child-Pugh Score) [33] and thirty sex- and age-matched healthy volunteers were enrolled in this study. The study was approved by the Local Ethics Committee (RNN272/05/KB), and each patient gave written consent. Table 1 presents general characteristics of the subjects.

The diagnosis of liver cirrhosis was based on the results of social, clinical, imaging (USG, panendoscopy, and CT), and laboratory investigations. Liver biopsy with histopathological analysis was performed in 11 patients. Each patient abused alcohol for 6–21 years. All patients also underwent neurological, psychological, psychiatric and in 58 patients psychometrical examinations, including the number connection tests NCT-A and NCT-B and the line tracing test (LTT) in order to determine the intensity of hepatic encephalopathy [34]. The results of these examinations allowed for categorization of patients into group HE_1 ($n = 28$), HE_2 ($n = 30$), and HE_3 ($n = 32$) according to the West-Haven criteria [35].

2.1.2. Excluding Criteria. Viral hepatitis, hepatic coma, delirium, psychic illness, renal insufficiency, and post-surgical state.

2.2. Analytical Procedures. The following biochemical examinations were performed on the patients: blood cell count, bilirubin, urea, creatinine, ALT, AST, GGTP, ALP, glucose, cholesterol, INR, prothrombin, APTT, albumins, globulins, GFR, HBsAg, and anti-HCV. In particular, ammonia in serum was performed by enzymatic method using glutaminic dehydrogenase.

The concentration of albumins and globulins as well as the number and kind of cells was determined in ascitic fluid. Sixteen patients were additionally examined to exclude

bacterial infection. Blood was taken from basilic vein, and ascitic fluid was obtained by abdominocentesis and collected at the same time, at 09:00 h at which time the patients were in fasting state and in the daylight for 2 hrs. Three days before the examination, the patients were on the same standard diet consisting of 3 × 400 mL (1800 kcal) Nutridrink (Nutricia, Poland) (1800 kcal) and 1500 mL mineral water. Samples of serum and ascitic fluid were centrifuged and kept at −70°C until the measurement of melatonin; samples were never kept longer than 4 months before the assays were performed. The concentration of melatonin was determined with the Melatonin Direct RIA (IBL, Hamburg, Germany) kit with the detection limit 2.5 pg/mL.

2.3. Statistical Analysis. To compare serum melatonin levels among all groups the nonparametric Mann-Whitney test was applied, since the distribution of the analyzed parameters significantly differed from the normal distribution. To compare melatonin levels between groups with 1–3 degree of hepatic encephalopathy the nonparametric Kruskal-Wallis test was used with the subsequent Mann-Whitney test. The Mann-Whitney test was also used to compare melatonin concentrations in blood and ascitic fluid. The correlation between the concentrations of ammonia and melatonin in blood was evaluated by linear regression equation with the Pearson's correlation coefficient (r). All statistical analyses were performed with the use of STATISTICA, v. 9.0 package (Tulsa, OK, USA).

3. Results

Serum melatonin level in healthy subjects was 11.3 ± 3.9 pg/mL, and it was significantly higher in cirrhotic patients and strongly depended on the degree of hepatic insufficiency (Figure 1). In patients classified into group HE_1 the level of melatonin was 34.3 ± 12.2 pg/mL ($P < 0.01$), in group HE_2—54.8 ± 23.9 pg/mL ($P < 0.001$), and in group HE_3—119.8 ± 96.4 pg/mL ($P < 0.001$).

Serum ammonia concentrations (μg/dL) were in groups: C—30.4±8.9, HE_1—52.6±26.0 ($P < 0.05$), HE_2—74.2±30.6 ($P < 0.001$), and HE_3—109.5 ± 38.9 ($P < 0.001$).

We did not find any correlation between melatonin and ammonia levels in all groups of patients with hepatic encephalopathy. Value of correlation coefficient (r) in group HE_1 was $r = -0.157$ (Figure 2), in group HE_2 ($r = 0.024$) (Figure 3), and in group HE_3 ($r = 0.142$) (Figure 4).

Increased melatonin concentration in ascitic fluid was detected in 24 patients out of 30 (80%) in group HE_2 and 27 patients out of 32 (84,3%) in group HE_3, and it was 16.4 ± 14.9 pg/mL and 45.5 ± 48.6 pg/mL, respectively ($P < 0.05$, Figure 5). Melatonin concentrations in both groups were about threefold lower than in serum ($P < 0.001$, Figure 5).

Melatonin levels in ascitic fluid of two patients of group HE_3 exceeded 600 pg/mL (609 and 632 pg/mL) and were similar to their respective serum concentrations (641 and 667 pg/mL); those were excluded from the statistical analyses. The remaining 11 patients displayed no melatonin in ascitic fluid.

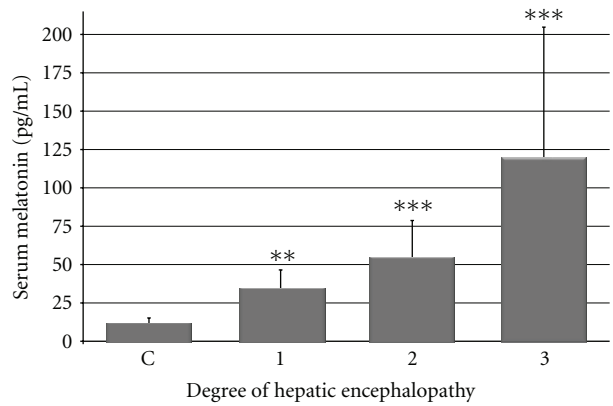

Figure 1: Serum melatonin levels in healthy subject (C) and in cirrhotic patients with different (1, 2, 3) degree of hepatic encephalopathy—according to West-Haven Score. $^{**}P < 0.01$, $^{***}P < 0.001$.

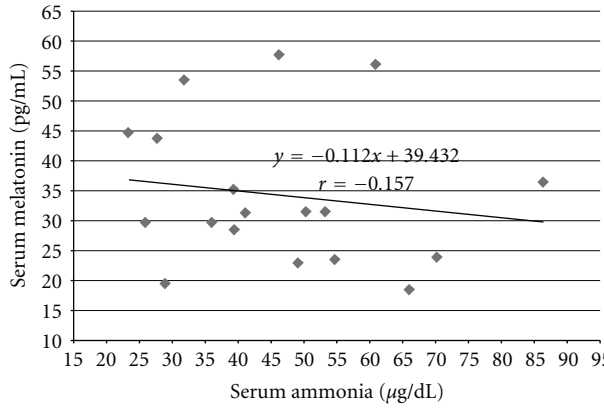

Figure 2: Correlation between ammonia and melatonin serum levels in cirrhotic patients with 1st degree of hepatic encephalopathy (group HE_1) according to West-Haven Score; y: regression equation, r: correlation coefficient, statistically insignificant.

4. Discussion

Results of many studies suggest that melatonin, both produced in the pineal gland and GIT, reaches the liver via blood circulation. Its concentration in biliary fluid is manyfold higher than diurnal concentrations in blood [20, 21]. Melatonin fulfills many important functions before its degradation, especially in reference to antioxidative defense. Mitochondria of hepatocytes are sites of intensive metabolism which lead to detoxification of many substances which are harmful for the organism. However, these detoxification processes are associated with the release of ROS, which can damage hepatocytes. Melatonin protects these cells against detrimental action of ROS because of its multiple antioxidative actions [36–38]. Melatonin protects hepatocytes against derivatives of oxygen metabolism and improves mitochondrial functions [39]. Likewise, melatonin displays preventive properties against harmful consequences of age-related changes in oxygen metabolism in rats [40]. The hepatoprotective action of melatonin was also documented

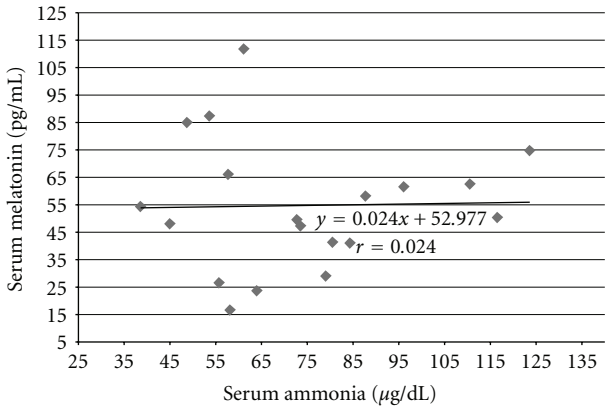

Figure 3: Correlation between ammonia and melatonin serum levels in cirrhotic patients with 2nd degree of hepatic encephalopathy (group HE_2) according to West-Haven Score; y: regression equation, r: correlation coefficient, statistically insignificant.

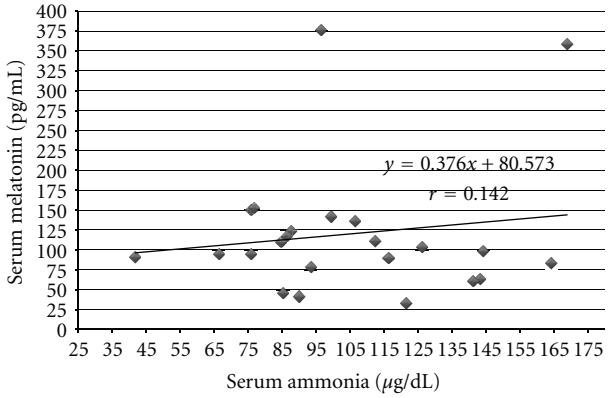

Figure 4: Correlation between ammonia and melatonin serum levels in cirrhotic patients with 3rd degree of hepatic encephalopathy (group HE_3) according to West-Haven Score; y: regression equation, r: correlation coefficient, statistically insignificant.

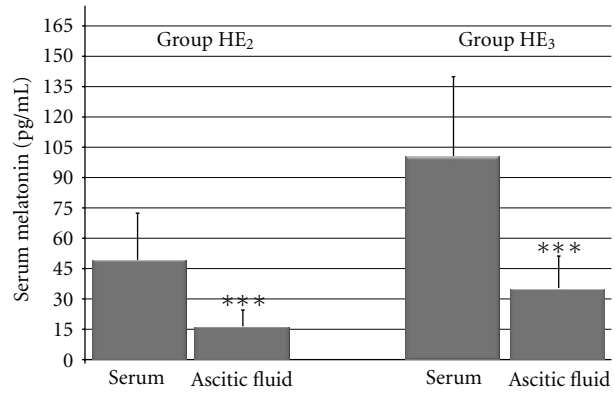

Figure 5: Comparison of melatonin concentration in serum and ascetic fluid in cirrhotic patients with 2nd (group HE_2, $n = 24$) and 3rd (group HE_3, $n = 25$) degrees of hepatic encephalopathy. $^{***}P < 0.001$. There are significant differences between mean values obtained in ascitic fluid in groups HE_2 and HE_3—$P < 0.01$.

against ionizing radiation [41], carbon tetrachloride [42], cold storage and reperfusion [43–46], hepatotoxic compounds [47–51], and bile duct ligation [52–54]. Melatonin also exerts anti-inflammatory actions in bile ducts and the pancreas [55, 56]. These beneficial actions of melatonin are attributed to its antioxidant properties [57, 58] although its receptor-mediated effects may also be beneficial [59]. Melatonin also decreases the production of proinflammatory cytokines, IL-1β and TNF-α and inhibits fibrogenesis in the liver [60, 61]. These results suggest the possibility for testing melatonin for therapeutic purposes. Melatonin protected against fat-rich diet-induced liver cirrhosis in rats [62]. A decrease in the activity of gamma-glutamyl transpeptidase and the level of proinflammatory cytokines was observed in patients with nonalcoholic steatohepatitis (NASH) after 4 weeks of L-tryptophan (the precursor of melatonin) administration [63]. A progressive reduction in the level of aminotransferases ALT and AST has been observed in patients with NASH after daily (2×5 mg) melatonin administration [64]. Melatonin at a dose of 50 mg/kg body weight improved the postoperational state of patients after partial liver resection [65].

It would be expected that the highest concentration of melatonin in cirrhotic patients would occur in the morning following the night time rise. The elevated serum levels are likely due to the decrease in the metabolic clearance rate, probably related to reduced liver blood flow, lowered activity of 6 β-hydroxylase, and competition with bilirubin in the intrahepatic transport system [66]. Results obtained in various laboratories are ambiguous, however, which may result from the differences in the patient-recruiting procedures. It has been observed that morning melatonin concentrations are higher in patients with minimal hepatic insufficiency compared to patients with a higher degree of this disease [67]. The highest concentration of morning melatonin, 102 pg/mL, was observed in the study in which only patients classified into group B according to Child-Pugh criteria were enrolled. We obtained a substantially lower concentration of morning melatonin, that is, 48.7 pg/mL, in the current report performed in a similar population of patients with hepatic insufficiency (group B), but this value was significantly higher in patients with more serious hepatic insufficiency. The difference may be related to the differences between the classifications, since our group C patients displayed extreme hepatic insufficiency. Moreover, we did not observe any correlation between the results of laboratory tests and concentrations of melatonin in blood; in particular no association between the concentrations of melatonin and ammonia was seen. This may result from different degrees of disturbances in the metabolic pathway of urea and melatonin. We did observe a relationship between the degree of liver encephalopathy and melatonin concentrations; however that high daytime melatonin concentration could result in daytime sleepiness and fatigue [68–70]. The involvement of melatonin in the pathogenesis of liver encephalopathy should be considered in patients with a high melatonin concentrations both in the day and at night.

Interestingly, the great majority of patients with portal hypertension had measurable levels of melatonin in their ascitic fluid. The mechanism of its leakage of melatonin from portal veins into peritoneal cavity is not completely clear. Also it is unknown why the concentration of melatonin in ascitic fluid in some cirrhotic patients was very high, while this indoleamine was not detected in this fluid in other patients. It is worth noting that in every case, melatonin concentration in blood was higher than in ascitic fluid. Melatonin in the ascitic fluid may be derived from the blood, from the GIT, or from both. Certainly, in patients with hepatic insufficiency the leakage of melatonin from these two sites could occur. A better understanding of the transfer of GIT and/or blood melatonin into ascitic fluid of patients with severe liver damage should be examined in greater depth.

In summary, our results indicate that increased concentration of melatonin in blood of patients with liver cirrhosis may be the consequence of both hepatic insufficiency and transport of melatonin from gastrointestinal tract to systemic circulation through the portosystemic shunts. High concentrations of melatonin in blood may influence the clinical features of hepatic encephalopathy.

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