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THE EFFECTS OF L-TRYPTOPHAN AND MELATONIN ON SELECTED BIOCHEMICAL PARAMETERS IN PATIENTS WITH STEATOHEPATITIS

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Nonalcoholic fatty liver disease is the most common chronic liver disease and nonalcocholic steatohepatitis (NASH) is its advanced form. Oxidative stress and hepatocyte apoptosis may be involved in pathogenesis of NASH and particularly in progress of NASH to liver fibrosis and cirrhosis, which are initiated by the inflammation and which promote the progress of the disease. The aim of this study was to evaluate the effects of melatonin and L-tryptophan on selected biochemical parameters of blood in patients with NASH. Forty five patients with NASH, confirmed by histopathological examination of liver biopsy samples, were admitted to the study. They were divided into three groups (I, II and III). The first group (group I, n=15) received preparation Essentiale forte 3 times a day and tryptophan 500 mg twice daily for 4 weeks. In the second group (group II, n=15), Essentiale forte three times a day was administered with melatonin 5 mg applied twice a day for 4 weeks. The third group (group III, n=15) received only Essentiale forte with placebo three times a day for 4 weeks. After four-week treatment we found statistically significant reduction in GGTP, triglycerides and proinflammatory cytokine levels in the melatonin-treated (group I) and the L-tryptophan-treated patients (group II). Plasma level of melatonin was significantly elevated in groups treated with tryptophan (group I) and melatonin (group II), but remained unchanged in placebo-treated group (group III). Among patients from the third group (treated with placebo) no statistically significant differences in the measured biochemical parameters were observed. The present study suggests that melatonin and tryptophan have the significant impact on the reduction in plasma levels of proinflammatory cytokines and may be useful in the treatment of patients with NASH.

Key words: *antioxidants, L-tryptophan, melatonin, nonalcocholic steatohepatitis, proinflammatory cytokines*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in industrialized countries. It may occur in 20-30% of adults. Nonalcoholic fatty liver disease includes two forms; simple steatosis and non alcoholic steatohepatitis (NASH) (1, 2). NASH has a potential to progress to liver fibrosis and cirrhosis. Oxidative stress, excessive hepatocyte apoptosis, abdominal obesity, increased volume of visceral adipose tissue and hepatic insulin resistance are considered to be the main factors in the pathogenesis of NASH. Melatonin is not only as a powerful endogenous antioxidant but also is considered an anti-inflammatory and anti-apoptotic agent (3-6). A proven medical treatment for NASH is not available. Therapeutic approach to the patients with NASH at present includes the treatment of components of metabolic syndrome such like obesity, hyperlipidemia, diabetes, arterial hypertension, usually some kind of hepatoprotection is required.

MATERIAL AND METHODS

Forty five patients with NASH were admitted to the study. Among them were 18 women aged from 22 to 46 years and 27 men aged from 26 to 58 years. Diagnosis of NASH was based on the medical history, clinical features and laboratory results. It was confirmed by liver biopsy. No alcohol history was obtained by a face-to-face interview in all participants. The other causes of steatohepatitis (viral, autoimmunological hepatisis) were excluded. At the onset of the study the following: plasma parameters were measured in all participants including; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma gluthamylo transpeptydase (GGTP), bilirubin, the profile of the lipidic parameters called as lipidogramme (total cholesterol, triglycerides, LDL, HDL) and plasma cytokines IL-1, IL-6 and TNF-alfa levels. Plasma levels of ALT, AST, ALP, GGTP, bilirubin and the profile of the lipidic parameters were determined spectrophotometrically with a Advia 1650 analyzer (Siemens, Germany). Serum levels of cytokines were analyzed with immune-enzymatic (ELISA) test (R&D Systems Inc., USA). Plasma levels of melatonin were measured using radioimmunoassay kit from DRG Instruments in Marburg (Germany) as described before (7). The melatonin radioimmunoassay kit utilized a specific sample preparation containing 5-methoxytryptamine-125-Bolton-Hunter-conjugate as a tracer and a specific anti-melatonin antibody. This antiserum did not show cross-reactivity with any product related to melatonin metabolism such as serotonin, 5-metoxytryptophan or N-acetyl-serotonin. The assay reliably detected melatonin

concentrations as low as 1 pg/mL and intra-assay and inter-assay variations were 7 and 8 pg/mL, respectively (8).

Forty five patients were divided into three groups (I, II and III). The first group (group I, n=15) received preparation Essentiale forte 3 times a day and L-tryptophan 500 mg twice a day for 4 weeks. (Ardeydorm, Ardeypharm, Germany). In the second group (group II, n=15) Essentiale forte in the above doses and melatonin 5 mg twice a day for 4 weeks were administered (Melatonina, Lekam, Zakroczyn, Poland). The third group (group C) received only Essentiale forte three times a day for 4 weeks. The Essentiale forte, Rhone-Poulenc Rorer GmBH, contains 300 mg natural "essential" phospholipids, diglyceride esters of cholinephosphoric acid (enriched with unsaturated fatty acids (linolic, linoleic, oleic), vitamin B1- 6 mg, vitamin B2- 6 mg, vitamin B6- 6 mg, vitamin B12- 6 micrograms, nicotinamide- 30 mg, vitamin E- 6 mg.

In all participants biochemical parameters and cytokine tests presented above were measured after 4 weeks of treatment and were compared with the results evaluated at the start of the study.

The study was approved by the Local Ethics Committee of medical University Lublin and all subjects gave written informed consent for examinations.

Statistical analysis

All results were expressed as the mean standard errors. Numeric data were analyzed by Kruskal-Wallis test. Statistical significance between the differences was assumed p- values <0.05. All calculations were done by the means of STATISTICA PL software.

RESULTS

The mean ALT, AST, ALP, GGTP, bilirubin levels and the profile of the lipidic parameters measured at the start of the study and after 4 weeks of the treatment are presented in the *Table 1*. After 4 week treatment with tryptophan or melatonin, we found statistically significant reduction in plasma levels of GGTP and triglycerides in the first (group I) and the second group (group II) of NASH patients. Among patients from third group (placebo group) no statistically significant differences in the evaluated biochemical parameters were observed. The mean levels of GGTP after 4 week administration of Essentiale and tryptophan in patients of group I (treated with tryptophan) decreased from initial value of 196±41 U/L to 139±26 U/L and in patients treated with melatonin in II group, the GGTP values declined from 189±28 to 112±21 U/L, respectively. These decreases in GGTP values after 4 week treatment with Essentiale plus tryptophan or melatonin were statistically significant different from those measured at the start of the study. Also the mean levels of triglycerides after 4 week treatment with Essentiale plus tryptophan or melatonin were statistically significantly reduced in group I and II when compared to the initial values, falling to about 218±39 mg/dL and 224±39 mg/dL, respectively.

Table 2 illustrates the plasma levels of measured cytokines and melatonin. After 4 week treatment with Essentiale plus tryptophan (group I) or melatonin (group II), the mean IL-1, IL-6, TNF-alfa serum levels decreased statistically significantly only in patients of the first and the second group.

Table 1. Biochemical parameters measured at the start of the study and after 4 weeks of treatment in examined groups.

	I group		II group		III group	
Analyzed parameter	at the start of the study	after 4 weeks of the treatment	at the start of the study	after 4 weeks of the treatment	at the start of the study	After 4 weeks of the treatment
ALT (U/L)	84±25	92±27	97±19	95±26	89±23	83±30
AST (U/L)	45±20	38±14	26±11	29±7	44±13	36±12
ALP (U/L)	117±21	109±26	107±11	112±21	94±32	101±36
GGTP (U/L)	196±41	139±26*	189±28	112±21*	202±41	177±21
Bilirubin	0.8±0.02	0.8±0.01	0.7±0.02	0.8±0.01	0.8±0.02	0.6±0.02
Total cholesterol (mg/dL)	228±37	298±63	248±43	218±33	231±63	275±51
Triglycerides (mg/dL)	318±44	218±39*	295±58	224±39*	271±28	276±37
LDL (mg/dL)	212±28	241±39	169±58	172±33	278±37	278±33
HDL (mg/dL)	38±7	42±9	37±7.1	40±6.2	42±6	38±9.1

The values are given as mean standard error. Asterisk indicates statistically significant (P<0.05) decrease in plasma levels of GGTP and triglycerides measured after four weeks of treatment with tryptophan or melatonin compared to the values recorded at the start of the study. The differences between the remained measured parameters were not statistically significant.

Table 2. Level of cytokines and melatonin measured at the start of the study and after 4 weeks of treatment in examined groups.

	I group		II group		III group	
Cytokines & Melatonin	At the start of the study	after 4 weeks of treatment	at the start of the study	After 4 weeks of treatment	at the start of the study	After 4 weeks of treatment
IL-1 (pg/mL)	9.1±2.5	5.2±2.7*	9.67±1.12	6.07±2.61*	10.1±2.64	9.79±2.08
IL-6 (pg/mL)	28.04±9.01	12.1±4.28*	26.11±6.32	17.32±4.9*	24.73±6.5	21.11±5.9
TNF-alfa (pg/mL)	2.14±0.72	1.16±0.66*	2.43±0.68	1.52±0.07*	2.82±0.52	2.01±0.38
Melatonin(pg/mL)	18.7±2.6	64.5±7.2+	21.3±3.1	75.±12.3+	19.8±2.6	22.3±4.1

The values are given as mean standard error. Asterisk indicates statistically significant decrease below the values recorded at the start of the study in treated patients (p<0.05). Cross indicates statistically significant (p<0.05) increase above the values recorded at the start of the treatment.

After 4 week treatment the mean plasma IL-1 and IL-6 decreased significantly in groups I and II patients to 5.2±2.7 pg/mL (group I) and 6.07±2.61 pg/mL (group II). Also the plasma value of TNF-alpha decreased significantly from the initial value of 2.14±0.72 pg/mL to 1.16±0.66 pg/mL in the first group and from 2.43±0.68 to 1.52±0.07 pg/mL in the second group of patients. The patients from the third group did not show any statistically significant differences in the evaluated plasma cytokine levels. Initial plasma levels of melatonin were similar in all three groups tested and averaged 18.7±2.6 pg/mL in group I, 21.3±3.1 pg/mL in group II and 19.8±2.6 pg/mL in group III. In patients treated with Essentiale plus melatonin or tryptophan, plasma levels of melatonin gradually increased and were significantly higher than those recorded in patients treated with Essentiale plus placebo at all periods of testing (*Table 2*). No significant changes in plasma melatonin were observed in patients treated with Essentiale plus placebo.

DISCUSSION

This study carried out on 45 patients with NASH confirmed by liver biopsy shows that the addition of melatonin or its precursor, L-tryptophan, to typical treatment with Essentiale forte, improves the therapy of this liver condition and this might be attributed to the antioxidant action of melatonin documented by the significant reduction in plasma levels of major proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha. These effects were also accompanied by the fall in liver tests such as GGTP and the reduction in plasma levels of triglycerides.

The term NAFLD is used to describe a wide spectrum of fatty liver changes ranging from fatty liver and steatosis on one side to non-alcoholic steatohepatitis (NASH) and cirrhosis on the other. NAFLD is an extremely common liver disease in Western countries affecting about 20% of adult population. Among obese persons, the prevalence raises to about 50% and to about 75% amongst obese diabetics (9, 10). It should be emphasized that simple liver steatosis is defined as a benign form of NAFLD with minimal risk of progression in contrast to NASH, which tends to progress to cirrhosis in up to 20% of patients and can subsequently lead to liver failure or hepatocellular carcinoma (11).

Rationale for investigating different potential of NAFLD and NASH treatment modalities comes from understanding the proposed mechanisms in the pathogenesis of this disease. According to currently accepted theory, the first step in disease genesis if the accumulation of fat caused by changes in lipid metabolism favoring triglyceride accumulation in hepatocytes, as a result of insulin resistance (IR). This may be followed by increased oxidative stress within the hepatocytes with characteristic excessive production of reactive oxygen species by mitochondria. Subsequently, these reactive oxygen species result in lipid peroxidation and excessive proinflammatory cytokine induction. Indeed, most of our patients with NASH show excessive production of proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha.

The mainstays of therapy of patients with metabolic syndrome is simple modifications of lifestyle such as reduction in body weight with or without physical exercise. Some case reports showed improvement in liver biochemical test levels and remission in few patients who achieved weight loss with orlistat, a reversible inhibitor of gastric and pancreatic secretion (12), but therapeutic benefit of weight loss achieved with diet, medical aids, exercise or even surgery has not been examined in randomized, prospective studies with firm histological evaluation of the liver. In fact, rapid weight loss can exacerbate NASH in morbidly obese people, especially after bariatric surgery (13).

As the hepatic steatosis is established, the liver become susceptible for to the oxidative stress promoting the progression of NAFLD to NASH. In this process, free fatty acids (FFA) , originating from peripheral lipolysis, become oxidized in mitochondria and reactive oxygen species (ROS) leak mostly as hydrogen peroxide. ROS trigger steatohepatis by lipid peroxidation and cytokine induction. In the presence of steatosis, mitochondrial ROS oxidase accumulated hepatic fat causing lipid peroxidation that may cause cell death explaining liver cell necrosis. ROS induce also the secretion of cytokines such as TNF-alpha, IL-1 and IL-6 and IL-8. The former is a chemoattractant that gives rise to neutrophil infiltration. Due to anti-oxidant depletion and impaired ROS inactivation, there is rationale for antioxidant supplementation in the treatment of NAFLD and NASH. The treatment with vitamin E, a potent lipid soluble antioxidant, appears to be effective against lipid peroxidation, resuling in suppression of TNF-alpha, IL-1 and IL-8. Lavine (14) in his elegant pivotal studies in children with established diagnosis of NASH and persistently elevated transaminase concentrations showed that 1-3 months therapy resulted in improvement of liver transaminase tests but the lack of liver biopsy and histological liver assessment limit the therapeutic impact of this study (15),

Melatonin is a well-known lipophylic hormone produced at night by pineal gland and after feeding with tryptophan containing protein or tryptophan itself by neuroendocrine cells of the digestive system (16). It acts through high-affinity G-protein-coupled membrane receptors through endocrine, paracrine or neurocrine pathway to protect the mucosa of the upper gastrointestinal tract from various irritants and ulcerogens. Although melatonin is uptaken by the liver and secreted in the bile, little is known about its possible protective action on the liver. Houssein *et al* (17) using white rabbits on regular or high-fat diet for 12 weeks observed increased serum lipids and the fatty changes in the liver, kidneys and blood vessels. Administration of melatonin for four weeks significantly reduced the metabolic pathologies associated with the intake of high-fat diet and this favorable effects of this indole has been attributed to its antioxidant and anti-inflammatory receptor-mediated activities.

In our study based on 15 patients with confirmed NASH by liver biopsy, the treatment with melatonin or its precursor L-tryptophan was found to be highly effective in reducing the proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha, suggesting that such treatment with melatonin should be practically very important in prevention of the progression of liver damage in NAFLD and NASH. Since plasma levels of melatonin in our patients not only treated with melatonin itself but also with L-tryptophan, were significantly increased it is suggestive that the major factor responsible for the favorable effects of this aminoacid could be attributed to its enzymatic transformation in the gastrointestinal tract into melatonin, which in turn reduced the formation of anti-inflammatory cytokines produced by fatty liver disease. The involvement of lipids in the improvement of liver with NASH is supported in our study by the demonstration of significant fall of circulating lipids, particularly plasma triglycerides, but the encouraging results indicate that further evaluation with larger controlled trials treated with melatonin or tryptophan over a longer period is necessary to substantiate the promising initial findings. In conclusion, the present study suggests that L-tryptophan and melatonin have significant impact by reducing the level of proinflammatory cytokines in patients with NASH. Consequently, they may be considered as potential anti-oxidant and anti-inflammatory factors in treatment of NASH.

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