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BRIEF ARTICLE

Secretion of melatonin and 6-sulfatoxymelatonin urinary excretion in functional dyspepsia

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Abstract

AIM: To evaluate blood concentration of melatonin and urinary excretion of its metabolite, 6-sulfatoxymelatonin (6-OHMS), in functional dyspepsia (FD).

METHODS: Ninety individuals were enrolled in the study: 30 in each study group: patients with postprandial distress syndrome (PDS), epigastric pain syndrome (EPS), and controls. Blood samples were drawn at 02:00 and 09:00 h and 24-h urine collection was performed. Serum melatonin and urinary 6-OHMS concentrations were measured by enzyme-linked immunosorbent assay.

RESULTS: Serum melatonin concentration at night and in the morning was significantly ($P < 0.001$) higher in PDS patients [at 02:00 h-93.3 pg/mL, quartile range (QR): 79.8-116.2; at 09.00 h-14.3 pg/mL, QR: 7.06-19.0] than in EPS (57.2 pg/mL, QR: 42.6-73.1; 8.1 pg/mL, QR: 4.1-9.3) and control patients (57.7 pg/mL, QR: 51.2-62.5; 8.1 pg/mL, QR: 5.4-10.3). A similar relationship was observed for urinary 6-OHMS excretion. Patients with severe PDS symptoms had a higher melatonin concentration than these with moderate syndromes, whereas patients with severe EPS had a lower urinary 6-OHMS excretion than patients with moderate symptoms.

CONCLUSION: Evaluation of melatonin serum concentrations and 24-h urinary 6-OHMS excretion are useful methods for differential diagnosis of various clinical forms of FD.

Key words: Functional dyspepsia; Postprandial distress syndrome; Epigastric pain syndrome; Melatonin; 6-sulfatoxymelatonin

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INTRODUCTION

Melatonin is synthesized by pinealocytes and in the gastrointestinal (GI) tract. Enterochromaffin (EC) cells are widely distributed in the GI tract mucosa, and are a rich source of this hormone, with the total amount of melatonin greatly exceeding that in the pineal gland[1,2]. Melatonin

displays endocrine, paracrine and autocrine properties, which may account in part for its neuroprotective action, and melatonin and its metabolites are powerful antioxidants[3-5]. The hormone also plays a role in the modulation of prostaglandin secretion and nitric oxide generation, as well as stimulation of bicarbonate secretion in the duodenum and pancreas[6-8]. Melatonin exerts an inhibitory effect on gastric acid secretion and myorelaxation effects on the smooth muscles of the GI tract[9,10]. Melatonin anti-inflammatory and immunomodulatory properties may also play a role in its general protective action in the GI tract[11,12].

An obvious question is whether the protective actions of MEL are exerted only in the case of a threat, or whether they are indispensible under physiological conditions. A growing body of evidence suggests the latter possibility and it has become clear that melatonin deficiency plays an important role in the pathogenesis of certain GI diseases[13,14]. Moreover, melatonin may protect gastric mucosa from stress-mediated lesions at a level comparable to or better than ranitidine and omeprazole[15,16].

Patients with duodenal ulcer disease have lower melatonin concentrations in the blood than healthy individuals have. The difference is most pronounced in the autumn and at night, but they it does not depend on the clinical phase (exacerbation or remission) of peptic ulcer disease[17-19]. It has been suggested that fasting and night abdominal pain in ulcer-like dyspepsia could be associated with lower than normal melatonin secretion, but there are some contradictory data[20,21]. Thus, an unequivocal link between melatonin deficiency and occurrence of disease symptoms in the GI tract has not been established. In our previous study, we recommended that patients with GI pain syndromes took 5 mg/d melatonin for 12 wk. In most patients (56.6%), the symptoms resolved after melatonin treatment; in 30% there was some amelioration of symptoms; while only 13.6% reported no clinical effect[22]. These results prompted us to carry out the present study.

The clinical picture of functional dyspepsia (FD) is rather complex. According to the Rome Ⅲ criteria, there are two major forms of this disease: epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS). In patients with EPS, abdominal pain in the epigastrium dominates, but fasting and nocturnal pain also occur. On the other hand, PDS patients rarely suffer from epigastric pain, but they complain of discomfort and distension in the epigastrium after meals, and they often have morning satiety and nausea.

In the present work, we determined the level of melatonin in serum and measured urinary excretion of the main and immediate metabolite of melatonin, 6-sulfatoxymelatonin (6-OHMS), in patients with EPS or PDS.

MATERIALS AND METHODS

Patients

Ninety subjects were enrolled in this study, 58 women and 32 men, aged 19-45 years (mean, 30.9 years). Clinical characteristics of the patients are presented in Table 1. The subjects were divided into three groups: healthy persons with no signs of GI dysfunction; patients with EPS; and patients with PDS without symptoms of irritable bowel syndrome (IBS).

Table 1 Clinical characteristics of the study subjects: controls and patients with postprandial distress syndrome and epigastric pain syndrome

	Controls	PDS	EPS
Number	30	30	30
Age (yr), mean ± SD	28.6 ± 9.4	31.8 ± 12.4	32.3 ± 14.1
Sex (M/F)	12/18	14/16	13/17
Normal gastric mucosa	18/30	17/30	15/30
Superficial gastritis	12/30	13/30	15/30

PDS: Postprandial distress syndrome; EPS: Epigastric pain syndrome.

Diagnosis of FD was based on the Rome Ⅲ Criteria. To exclude other diseases, all the patients underwent upper GI endoscopy with histopathological evaluation of gastric mucosa biopsy specimens, abdominal ultrasound, and laboratory tests including blood cell morphology, C-reactive protein, glucose, electrolytes, bilirubin, urea, creatinine, cholesterol, triglycerides, thyrotropin, and activity of aspartate transaminase, alanine transaminase, γ-glutamyl transpeptidase, alkaline phosphatase, amylase and lipase. To exclude Helicobacter pylori infection, the urea breath test (UBT; FANCI-2 System, Fisher Instruments, Germany) was performed. Patients on any long-term treatment as well as cigarette smokers were not enrolled. Furthermore, Beck Depression Inventory and Hamilton Depression Scale were used to assess mental status and to exclude patients with psychiatric disease.

Methods

Seven days prior to the start of the study, patients were recommended to stop taking any medication and remain on a similar diet, which contained the same amount of tryptophan-rich products. Symptoms reported were graded according to a 10-point scale and the subjects were grouped into categories with moderate (1-5 points, 14 individuals with EPS, 16 with PDS) or exacerbated (6-10 points, 15/15) symptoms. On the day of the study, the patients were in the room with only red light exposure between 21:00 h to 07:00 h, and on the same liquid diet (Nutri Drinks; Nutricia, Warsaw, Poland) that consisted of 3 × 400 mL, which contained 18.9 g carbohydrate, 6.0 g protein and 5.8 g lipid per 100 mL, with a total caloric value of 1800 kcal. They also consumed 1.5 L isotonic gas-free water. Blood samples were drawn from the antecubital vein at 02:00 h and 09:00 h and serum was frozen at -70℃. At the same time, 24-h urine samples were collected and stored at 4℃. At the end of 24-h urine collection, the volume of urine was measured and the samples were frozen at -70℃. Serum melatonin and urinary 6-OHMS were measured by enzyme-linked immunosorbent assay using IBL antibodies (RE-54021 and RE-54031; Nordic Immunological Laboratories, Tilburg, Holland) and Expert 99 MicroWin 2000 Reader (BMG Labtech, Offenburg, Germany).

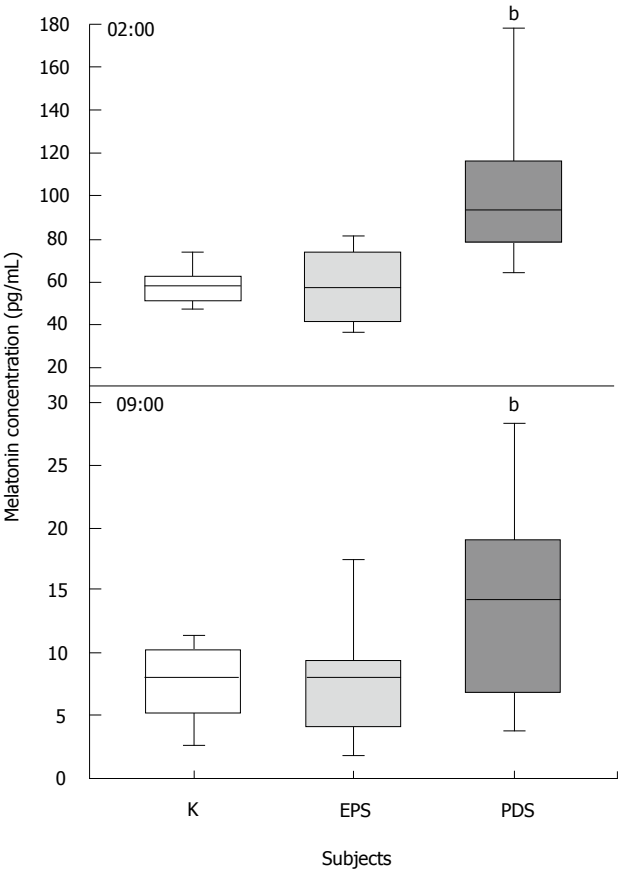

Figure 1 Serum melatonin concentrations at 02:00 h and 09:00 h in healthy subjects (K, clear bar, *n* = 30) and epigastric pain syndrome (grey bar, *n* = 30) and postprandial distress syndrome (dark grey bar, *n* = 30). Box represents median with 25th and 75th percentiles (lower and upper quartiles, respectively). The ends of the error bars represent the smallest and largest measurements in the study groups. $^{b}P < 0.001$. PDS: Postprandial distress syndrome; EPS: Epigastric pain syndrome.

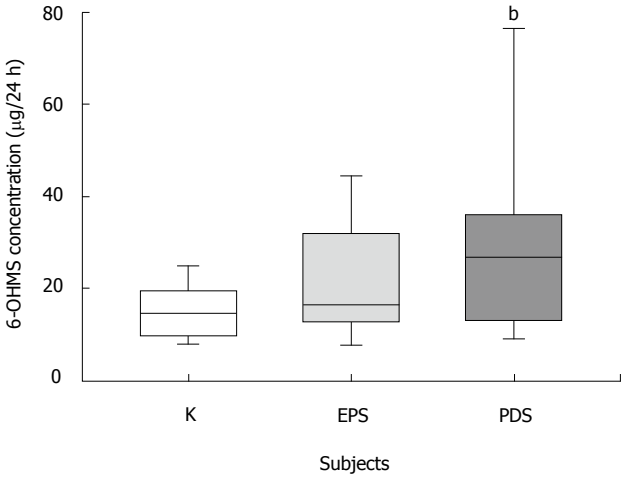

Figure 2 Twenty-four-hour urinary excretion of 6-OHMS in healthy subjects (K, clear bar, *n* = 30) and in patients with epigastric pain syndrome (grey bar, *n* = 30) and postprandial distress syndrome (dark grey, *n* = 30). Box represents median with 25th and 75th percentiles (lower and upper quartiles, respectively). The ends of the error bars represent the smallest and largest measurements in the study groups. $^{b}P < 0.01$. PDS: Postprandial distress syndrome; EPS: Epigastric pain syndrome.

Ethics

The study was conducted in accordance with the Declaration of Helsinki and with the principles of good clinical practice. These studies were approved by the Bioethics Committee of the Medical University of Lodz, Poland (permission no. RNN/26/04/KB). Each patient was acquainted with the aim of the study and gave written informed consent.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine whether data fitted a normal distribution. Differences between groups were evaluated using the Mann-Whitney rank sum test, with $P < 0.05$ regarded as statistically significant.

RESULTS

The median serum melatonin concentration at 02:00 h in patients with PDS [93.3 pg/mL, quartile range (QR): 79.8-116.2] was about two times higher than in the control subjects (57.7 pg/mL, QR: 51.2-62.5, $P < 0.001$) and patients with EPS (57.2 pg/mL, QR: 42.6-73.1, $P < 0.001$) (Figure 1). We observed a similar relationship at 09:00 h when melatonin concentration in the PDS group (14.3 pg/mL, QR: 7.060-19.0) was significantly ($P < 0.001$) higher than in the controls (8.1 pg/mL, QR: 5.4-10.3) and in EPS subjects (8.1 pg/mL, QR: 4.1-9.3) (Figure 1). We also observed higher 24-h urinary 6-OHMS levels in patients with PDS (26.8 μg, QR: 13.4-35.6) as compared with controls (14.6 μg, QR: 10.6-19.4) and patients with EPS (16.4 μg, QR: 13.4-30.7) (Figure 2). The PDS patients with severe symptoms displayed a higher melatonin concentration at 02:00 h (100.0 pg/mL, QR: 91.0-115.0) and 09:00 h (16.1 pg/mL, QR: 12.9-23.4) as compared with patients with moderate symptoms (84.0 pg/mL, QR: 66.1-101.7, $P < 0.05$ and 11.9 pg/mL, QR: 5.2-14.4, $P < 0.001$) (Figure 3). The 24-h urinary excretion of 6-OHMS was also higher in these patients (30.3 μg, QR: 22.9-45.7) in comparison with moderate symptom patients (84.0 pg/mL, QR: 66.1-101.7; 11.9 *vs* 17.5 μg, QR: 9.7-28.9, $P < 0.01$) (Figure 4). We also observed that patients with EPS with severe symptoms had a reduced 24-h 6-OHMS urinary excretion as compared with patients with moderate symptoms (13.2 μg, QR: 10.3-16.6 *vs* 22.8 μg, QR: 16.5-43.3; $P < 0.01$) (Figure 4).

DISCUSSION

Clinical studies of melatonin secretion are usually based on its serum concentrations. These values are relatively low during the daytime and increase significantly at night; especially in the absence of white light. The circadian rhythm of melatonin is a result of secretion by the pineal gland. In pinealectomized animals, no night-time rise in blood melatonin levels is observed, and its concentration throughout a 24-h period remains at a low and relatively constant level[23]. The low residual amounts of melatonin are from sources other than pineal gland, for example, mainly from the GI tract. These sources of melatonin are regulated by mechanisms that do not involve the pi-

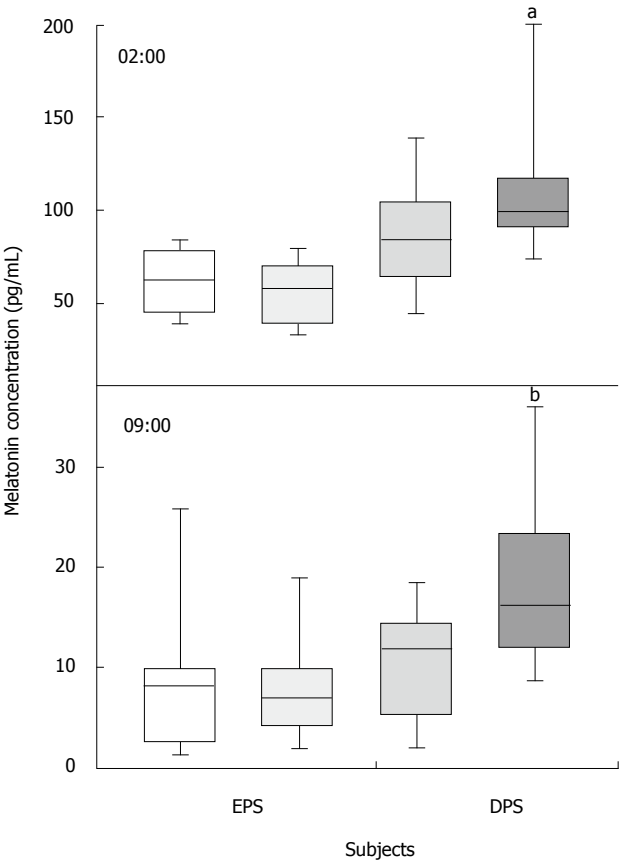

Figure 3 Serum melatonin concentrations at 02:00 h and at 9.00 a.m. in patients with epigastric pain syndrome (two shades of light grey, *n* = 30) and postprandial distress syndrome (two shades of dark grey, *n* = 30). Darker bars represent patients with severe symptoms [*n* = 16 for epigastric pain syndrome (EPS) and *n* = 15 for postprandial distress syndrome (PDS)] and lighter bars, patients with moderate symptoms (*n* = 14 for EPS and *n* = 15 for PDS). Box represents median with 25th and 75th percentiles (lower and upper quartiles, respectively). The ends of the error bars represent the smallest and largest measurements in the study groups. $^{a}P < 0.05$, $^{b}P < 0.01$.

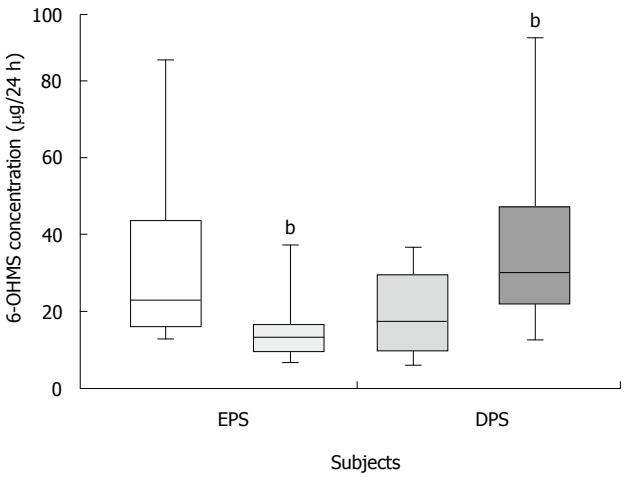

Figure 4 Twenty-four-hour urinary 6-OHMS excretion in patients with epigastric pain syndrome (two shades of light grey, *n* = 30) and postprandial distress syndrome (two shades of dark grey, *n* = 30). Darker bars represent patients with severe symptoms (*n* = 16 for epigastric pain syndrome (EPS) and *n* = 15 for postprandial distress syndrome (PDS)) and lighter bars, patients with moderate symptoms (*n* = 14 for EPS and *n* = 15 for PDS). Box represents median with 25th and 75th percentiles (lower and upper quartiles, respectively). The ends of the error bars represent the smallest and largest measurements in studied groups. $^{b}P < 0.01$.

neal gland. Melatonin secretion from EC cells of the gut increases as a consequence of the activity of muscarinic M3 and β-adrenergic receptors, but also after the intake of food[24]. Large meals rich in L-tryptophan cause satiety and sleepiness in humans and they stimulate the release of melatonin from EC cells. Postprandial melatonin peripheral serum concentrations do not rise markedly because melatonin is absorbed into the portal circulation and transported to liver, where 90% of it is metabolized, mainly to 6-OHMS, which is then excreted by kidneys into the urine[25]. It is accepted that evaluation of urinary 6-OHMS excretion is a useful index of the secretory activity of all cells that produce melatonin[26]. Collecting blood every 1-2 h for the purpose of measuring melatonin concentration is troublesome for patients, disturbs their normal circadian rhythms, and increases emotional stress, which may significantly influence their melatonin levels. As a result, it is widely accepted to perform the measurements twice daily: usually at 02:00 h (in darkness) and at 09:00 h (in daylight)[27].

Melatonin, due to its interaction with receptors[28] and because of its antioxidative properties[29,30], plays an important role in the function of the GI tract and this indoleamine deficiency is likely involved in the pathogenesis of some GI diseases, including gastric and duodenal peptic ulcers[14]. However, the role of melatonin in the etiology of FD has not been established. The diagnosis of FD is still based on patient complaints. Objective indices of this disease are generally lacking. In the current study, the subjects with EPS had melatonin serum concentrations similar to those in healthy controls, but higher urinary 6-OHMS excretion. This suggests enhanced melatonin secretion from the GI tract in these patients. This enhancement might be sufficient to prevent formation of peptic lesions, but not sufficient to prevent the occurrence of dyspeptic symptoms. In this context, increased melatonin release from the GI tract may be a consequence of pathogenic factors such as chronic stress, increased vegetative system tension, and other processes.

We observed a higher concentration of melatonin in PDS patients than in the control subjects; both during the day and at night, but whether this increase was the reason or a consequence of FD is unresolved. A relative melatonin deficiency may have pathogenic implications for the GI tract, as it has in sleep disturbances, depression and in some organic diseases, including cancer. However, elevated melatonin concentrations have been observed in some diseases of the endocrine and reproductive systems[31], liver cirrhosis[32], and anorexia nervosa[33]. The results of the current study show that FD could be one of these diseases. Different melatonin levels in different forms of FD may have diagnostic significance. Melatonin, or its precursor L-tryptophan, could also be administered in cases of their deficiency, or in patients who are receiving gastro toxic drugs. Rapaport *et al*[34] have recommended that melatonin should be taken by patients with duodenal ulcer

disease, after they observed its beneficial clinical effect and ability to heal inflammatory lesions of the gastric antral mucosa. In our previous studies, the therapeutic efficacy of melatonin in patients with ulcer-like dyspepsia has been shown. Moreover, Konturek *et al*[35] have reported a clear gastroprotective effect of L-tryptophan in patients who are taking acetylsalicylic acid. To date, however, melatonin and its analogs have been used mainly in the treatment of sleep disorders and depression[36,37]. Functional diseases of the GI tract are often associated with psycho-emotional disorders. Dyspeptic symptoms are often the main or even the only manifestation of depression, because it is not associated with bad mood. In several cases of sleep disorders, GI complaints and the fear of severe, chronic non-curable disease are markers of depression.

Disturbance of melatonin level is also observed in patients with IBS, which is more likely to share a common background with FD. To date, the role of melatonin in the etiology of IBS has not been established. Melatonin is known to be a regulator of intestinal motility, therefore, it could act by relaxing bowel muscles. It has been observed that melatonin reduces the tone, but not the amplitude or frequency of contractions[38]. This effect is probably due to stimulation of melatonin receptors and regulation of Ca^{2+}/K^{+} channels, and indirectly by the nervous system[39,40]. Other results suggest that melatonin has a peripheral anti-serotonin-like effect[41]. Serotonin (5-hydroxytryptamine; 5-HT) is thought to be a likely contender in the induction and maintenance of visceral hypersensitivity associated with IBS. 5-HT acts mostly at 5-HT3 or 5-HT3-like receptors, and enhances the sensitivity of visceral neurons that project between the gut and central nervous system.

In conclusion, melatonin levels in the peripheral blood are significantly higher in patients with severe symptoms of PDS than in those with EPS. Thus, these levels may be useful in the differential diagnosis of these diseases. Evaluation of 24-h urinary 6-OHMS excretion is a useful method for the estimation of melatonin secretion and may be important in the differentiation of various clinical forms of FD.

COMMENTS

Background

Melatonin is synthesized by pinealocytes and in the gastrointestinal (GI) tract. Melatonin displays endocrine, paracrine and autocrine properties, which may account in part for its neuroprotective action. Moreover, melatonin and its metabolites are powerful antioxidants.

Research frontiers

An obvious question is whether the protective actions of melatonin are exerted only in the case of a threat or whether they are indispensible under physiological conditions. A growing body of evidence suggests the latter possibility, and it has become clear that melatonin deficiency plays an important role in the pathogenesis of certain GI diseases.

Innovations and breakthroughs

This is believed to be the first study to report an association between functional dyspepsia (FD) and melatonin serum level and 6-sulfatoxymelatonin (6-OHMS) urinary excretion.

Applications

By connecting melatonin levels in the peripheral blood with FD, this study may help to establish the new differential diagnosis of various clinical forms of FD. The authors also proved that evaluation of 24-h urinary excretion of 6-OHMS is a useful method for the estimation of melatonin secretion, and together with melatonin levels in the peripheral blood, may be important for the differentiation of various clinical forms of FD.

Terminology

Melatonin is a naturally occurring compound in animals, plants and microbes. Many biological effects of melatonin are produced through the activation of its receptors, while others are due to its role as a pervasive and powerful antioxidant. FD is a disease without organic evidence that is likely to explain the symptoms. These symptoms include: upper abdominal pain, belching, nausea, abdominal bloating and early satiety. FD is estimated to affect about 15% of the general population in western countries.

Peer review

This was a well-designed study that examined the association between FD and melatonin serum level and 6-OHMS urinary excretion.

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