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

Cezary Chojnacki, Tomasz Poplawski, Grażyna Klupinska, Janusz Blasiak, Jan Chojnacki, Russel J Reiter

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.

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. Melatonin

© 2011 Baishideng. All rights reserved.

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

Peer reviewer: Menachem Moshkowitz, Department of Gastroenterology, Ichilov Hospital, Tel-Aviv Sourasky Medical Center, 6, Weizman St, Tel-Aviv, 64239, Israel

Chojnacki C, Poplawski T, Klupinska G, Blasiak J, Chojnacki J, Reiter RJ. Secretion of melatonin and 6-sulfatoxymelatonin urinary excretion in functional dyspepsia. World J Gastroenterol 2011; 17(21): 2646-2651 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i21/2646.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i21.2646
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. Melatonin exerts an inhibitory effect on gastric acid secretion and myorelaxation effects on the smooth muscles of the GI tract. Melatonin anti-inflammatory and immunomodulatory properties may also play a role in its general protective action in the GI tract.

An obvious question is whether the protective actions of MEL are exerted only in the case of a threat, or whether they are indispensable 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. Moreover, melatonin may protect gastric mucosa from stress-mediated lesions at a level comparable to or better than ranitidine and omeprazole.

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 do not depend on the clinical phase (exacerbation or remission) of peptic ulcer disease. 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. 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. 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-sulfoaxon melatonin (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).

**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 (Nuti 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°C. At the same time, 24-h urine samples were collected and stored at 4°C. At the end of 24-h urine collection, the volume of urine was measured and the samples were frozen at -70°C. 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).

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

| Diagnosis              | 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.
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).
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. 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. It is accepted that evaluation of urinary 6-OHMS excretion is a useful index of the secretory activity of all cells that produce melatonin. 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).

Melatonin, due to its interaction with receptors and because of its antioxidative properties, 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. 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, 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, liver cirrhosis, and anorexia nervosa. 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. have recommended that melatonin should be taken by patients with duodenal ulcer
Melatonin secretion in functional dyspepsia

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 indispensable 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.

REFERENCES
1. Bubhen G, Localization, physiological significance and possible clinical implication of gastrointestinal melatonin. Biol Signals Recept 2001; 10: 350-366
2. Messner M, Huether G, Lorf T, Ramadori G, Schwörer H. Presence of melatonin in the human hepatobiliary-gastrointestinal tract. Life Sci 2001; 69: 543-551
3. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim Pol 2003; 50: 1129-1146
4. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Bandypadhyay D. Neuromediators and independently beneficial actions of melatonin in the gastrointestinal tract. J Physiol Pharmacol 2003; 54 Suppl 4: 113-125
5. Hardeland R, Tan DX, Reiter RJ. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. J Pineal Res 2009; 47: 109-126
6. Cabeza J, Alarcón-de-la-Lastra C, Jiménez D, Martín MJ, Motilva V. Melatonin modulates the effects of gastric injury in rats: role of prostaglandins and nitric oxide. Neurosignals 2003; 12: 71-77
7. Sjöblom M, Flemström G. Melatonin in the duodenal lumen is a potent stimulant of mucosal bicarbonate secretion. J Pineal Res 2003; 34: 288-293
8. Jaworek J, Brzozowski T, Konturek SJ. Melatonin as an organoprotector in the stomach and the pancreas. J Pineal Res 2005; 38: 73-83
9. Kato K, Murai I, Asai S, Takahashi Y, Matsuho Y, Komuro S, Kurosaka H, Iwasaki A, Ishikawa K, Arakawa Y. Central nervous system action of melatonin on gastric acid and pepsin secretion in pylorus-ligated rats. Neuroreport 1998; 9: 3989-3992
10. Kasimay O, Cabir B, Dsveren E, Yegen BC. Exogenous melatonin delays gastric emptying rate in rats: role of CCK2 and 5-HT3 receptors. J Pineal Res 2005; 40: 316-320
11. Carrillo-Vico A, Guerrero J, Lardone P, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine 2005; 27: 189-200
12. Lahiri S, Singh P, Singh S, Rasheed N, Palit G, Pant KK. Melatonin protects against experimental reflux esophagitis. J Pineal Res 2009; 46: 207-213
13. Konturek PC, Konturek SJ, Brzozowski T, Dembinski A, Zembala M, Mytar B, Hahn EG. Gastroprotective activity of melatonin and its precursor, L-tryptophan, against stress-induced and ischaemia-induced lesions is mediated by scavenging of oxygen radicals. Scand J Gastroenterol 1997; 32: 433-438
14. Konturek SJ, Brzozowski T, Konturek PC, Zwierska-Korczał K, Reiter RJ. Day/night differences in stress-induced gastric lesions in rats with an intact pineal gland or after...
15 Sener-Muratoglu G, Paskaloglu K, Arbak S, Hurdag C, Ayanoğlu-Dünger G. Protective effect of famotidine, omeprazole, and melatonin against acetylsalicylic acid-induced gastric damage in rats. Dig Dis Sci 2001; 46: 318-330

16 Bandypadhyay D, Bandypadhyay A, Das PK, Reiter RJ. Melatonin protects against gastric ulceration and increases the efficacy of ranitidine and omeprazole in reducing gastric damage. J Pineal Res 2002; 33: 1-7

17 Malinovskaya N, Komarov FI, Rapoport SI, Voznesenskaya LA, Wetterberg L. Melatonin production in patients with duodenal ulcer. Neuro Endocrinol Lett 2001; 22: 109-117

18 Komarov FI, Rapoport SI, Malinovskaya NK, Voznesenskaya LA, Sharov AA, Wetterberg L. [Melatonin production in patients with duodenal ulcer at different stages of disease]. Klin Med (Mosk) 1998; 76: 15-18

19 Komarov FI, Rapoport SI, Malinovskaya NK, Voznesenskaya LA, Wetterberg L. [Melatonin: ulcer disease and seasons of the year]. Klin Med (Mosk) 2003; 81: 17-21

20 Klupińska G, Wiśniewska-Jarosińska M, Harasiuk A, Chojnacki C, Stoc-Michalska K, Blasiak J, Reiter RJ, Chojnacki J. Nocturnal secretion of melatonin in patients with upper digestive tract disorders. J Physiol Pharmacol 2006; 57 Suppl 5: 41-50

21 Klupińska G, Harasiuk A, Poplawski T, Chojnacki C, Blasiak J, Chojnacki J. Nocturnal secretion of melatonin in patients with functional dyspepsia. Gastroenterol Pol 2007; 14: 103-106

22 Klupińska G, Poplawski T, Drzewoski J, Harasiuk A, Reiter RJ, Blasiak J, Chojnacki J. Therapeutic effect of melatonin in patients with functional dyspepsia. J Clin Gastroenterol 2007; 41: 270-4

23 Bubenik GA, Brown GM. Pineallectomy reduces melatonin levels in the serum but not in the gastrointestinal tract of rats. Biol Signals 1997; 6: 40-44

24 Konturek SJ, Konturek PC, Brzozowska I, Pawlik M, Sliwowski Z, Czesniewicz-Guzik M, Kwiecien S, Brzozowski T, Bubenik GA, Pawlik WW. Localization and biological activities of melatonin in intact and diseased gastrointestinal tract (GIT). J Physiol Pharmacol 2007; 58: 381-405

25 Baskett JJ, Cockrem JF, Antunovich TA. Sulphatoxymelatonin excretion in older people: relationship to plasma melatonin and renal function. J Pineal Res 1998; 26: 58-61

26 Arendt J, Bojkowski C, Franey C, Wright J, Marks V. Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. J Clin Endocrinol Metab 1985; 60: 1166-1173

27 Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia M, Ferrer J, de la Rosa A, Vargas M, Reiter RJ. Light/dark patterns of interleukin-6 in relation to the pineal hormone melatonin in patients with acute myocardial infarction. Cytokine 2004; 26: 89-93

28 Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005; 27: 101-110

29 Peyrot F, Ducrocq C. Potential role of tryptophan derivatives in stress responses characterized by the generation of reactive oxygen and nitrogen species. J Pineal Res 2008; 45: 235-246

30 Reiter RJ, Paredes SD, Manchester LC, Tan DX. Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem Mol Biol 2009; 44: 175-200

31 Luboshitzky R, Herer P, Shen-Orr Z. Urinary 6-sulfatoxymelatonin excretion in hyperandrogenic women: the effect of cyproterone acetate-ethinyl estradiol treatment. Exp Clin Endocrinol Diabetes 2004; 112: 102-107

32 Steindl PE, Finn B, Bendok B, Rothke S, Zee PC, Blei AT. Disruption of the diurnal rhythm of plasma melatonin in cirrhosis. Ann Intern Med 1995; 123: 274-277

33 Tortosa F, Puig-Domingo M, Peinado MA, Oriola J, Webb SM, de Leiva A. Enhanced circadian rhythm of melatonin in anorexia nervosa. Acta Endocrinol (Copenh) 1989; 120: 574-578

34 Rapoport SI, Raitkhlin NT, Malinovskaya NK, Lakshin AA. [Ultrastructural changes in cells of the antral gastric mucosa in patients with duodenal ulcers treated with melatonin]. Ter Arkh 2003; 75: 10-14

35 Konturek PC, Celinski K, Slomka M, Cichoz-Lach H, Burnat G, Naegel A, Bielanski W, Konturek JW, Konturek SJ. Melatonin and its precursor L-tryptophan prevent acute gastric mucosal damage induced by aspirin in humans. J Physiol Pharmacol 2008; 59 Suppl 2: 67-75

36 Wasdell MB, Jan JE, Bompen MM, Freeman RD, Rietveld WJ, Tai J, Hamilton D, Weiss MD. A randomized, placebo-controlled trial of controlled release melatonin treatment of delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. J Pineal Res 2008; 44: 57-64

37 Maldonado MD, Reiter RJ, PÁarez-San-Gregorio MA. Melatonin as a potential therapeutic agent in psychiatric illness. Hum Psychopharmacol 2009; 24: 391-400

38 Bubenik GA. The effect of serotonin, N-acetylserotonin, and melatonin on spontaneous contractions of isolated rat intestine. J Pineal Res 1986; 3: 41-54

39 Reyes-Vázquez C, Naranjo-Rodríguez EB, García-Segovia-NO JA, Trujillo-Santana JT, Prieto-Gómez B. Aparin blocks the direct relaxant effect of melatonin on rat ileal smooth muscle. J Pineal Res 1997; 22: 1-8

40 Forster ER, Green T, Elliot M, Bremner A, Dockray GJ. Gastro-empting in rats: role of afferent neurons and cholecystokinin. Am J Physiol 1990; 258: G552-G556

41 Lu WZ, Gwee KA, Mochhalla S, Ho KY. Melatonin improves bowel symptoms in female patients with irritable bowel syndrome: a double-blind placebo-controlled study. Aliment Pharmacol Ther 2005; 22: 927-934

S-Editor Tian L  L-Editor Kerr C  E-Editor Ma WH