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EVALUATION OF MELATONIN EFFECTIVENESS IN THE ADIUVANT TREATMENT OF ULCERATIVE COLITIS

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Ulcerative colitis (UC) is a chronic disease characterized by the variable clinical picture with the inflammatory changes which can involve the whole colon or its distal part. The current treatments for UC are mostly nonspecific, not always effective, and often accompanied by serious side effects. Therefore, there is a considerable interest in finding alternative and more tolerable treatments for this serious disease. Several lines of experimental studies have shown that melatonin (MEL) regulates the extensive gut immune system and exerts antiinflammatory and immunomodulatory effects suggesting its beneficial action in UC by reducing and controlling inflammation. The study aimed at evaluating the effect of MEL on the activity of inflammatory process and sustaining the remission in patients with UC. It comprised 60 patients with left-sided UC, divided in two equal groups of 30 patients each (38 women and 22 men, aged 26-49 years), similar in both groups, who were in clinical remission for the last 12 months. Patients, during a next period of 12 months, were given mesalazine in daily doses 2 x 1.0 g and melatonin 5 mg daily at bedtime (group I) or placebo (group II). All the patients on MEL adjuvant treatment remained in remission during 12 months of observation with The Mayo Clinic Disease Activity Index (MCDAI) values 1.50±0.51 at the beginning and 2.75±1.86 points after 12 months. In the placebo group significantly higher MCDAI values were observed than in patients on MEL after 6, 9 and 12 months. At the inclusion MCDAI was 1.61±0.68 points and at the end of observation it reached the value of 5.10±2.22 points. In MEL group CRP level remained within the normal range during the course of the study (from 3.49±1.40 to 4.17±2.10 mg/dl). Whereas in the placebo group from the end of the third month the steady rise in CRP blood concentration was noted from 3.85±1.29 to 13.13±6.08 mg/dl. Parallelly to CRP rise a significant decrease in hemoglobin concentration in blood from 12.05±0.69 to 10.93±0.81 g/dl was observed in patients receiving placebo and the values significantly differed between the groups after 3 ($p<0.05$), 6, 9 and 12 months ($p<0.01$). The level of anxiety and the intensity of depression in patients on adjuvant MEL decreased during the study but there were no statistical differences noted between the groups. The results of the study allowed drawing the conclusion that adjuvant melatonin therapy may help in sustaining remission in patients with UC.

Key words: *inflammatory bowel disease, c-reactive protein, adjuvant treatment, melatonin, ulcerative colitis, anxiety*

INTRODUCTION

Ulcerative colitis (UC) is a chronic disease characterized by the variable clinical picture. The inflammatory changes begin in the rectum and they can involve the whole colon or its distal part. The course of the disease may be very severe leading to cachexia or toxemia, what sometimes demands even surgical treatment, on the other hand in some cases the symptoms are mild and they do not largely influence patient quality of life. The pharmacotherapy mainly aims at achieving a long-term remission and preventing of the disease recurrence, no matter of the clinical form of UC. In severe cases it is a rather difficult task, demanding administration of immunosuppressive drugs and monoclonal antibodies. Whereas in mild cases usually low doses of 5ASA and azathioprine, rarely with glicocorticosteroids, are enough to maintain remission. In patients with severe UC glicocorticosteroid-dependent or glicocorticosteroid-resistent are not rare. Azathioprine intolerance is a separate therapeutic problem, which can occur in several percent of patients. Moreover a lot of patients are aware of untoward drug effects as well as the consequences of a long pharmacological treatment. These fears most often concern young women planning maternity and in elderly patients with concomitant diseases (1-3). On the other hand a question is put forward whether 5ASA compounds, as the only treatment, can protect against the disease relapses. This explains the need for research on other drugs, which could be an effective adjuvant therapy in this chronic and serious disease. For the same reason the antioxidant vitamins were used in the treatment of IBD (4). In our present study melatonin (MEL), which is one of the most potent antioxidants (5), was chosen as an adjuvant drug. Moreover MEL possesses anti-inflammatory and immunomodulative properties (6). Melatonin and its analogies improve sleeping (7)

and acts as an antidepressant (8), which is particularly important in patients with UC, who due to the chronic and recurrent nature of their disease often present psychoemotional disturbances.

In experimental studies in rodents have shown that MEL administration reduced the severity of colitis (9-11). These beneficial effects were attributed to a variety of mechanisms including: inhibition of NO production and COX-2 expression (12, 13), inhibition of NF-kappa activation (14), reduction of colon immunological injury through regulation of macrophage activity (15, 16), reduction of bacterial translocation (17, 18), reduction of matrix metalloproteinase-2 and-9 activity (19) and modulation of signal transduction pathways and apoptosis (20, 21).

In addition to the basic science data and experimental studies, results from human studies provide several lines of evidence that suggest that melatonin supplementation could have an ameliorative effect on the course of UC. Studies in humans have indicated that MEL supplementation can help combat inflammation and oxidative stress, two of the major pathophysiological factors involved in the pathogenesis of inflammatory bowel disease (IBD). For example Malhotra *et al*. (22) observed some improvement in patient with severe, refractory colitis by MEL administration. Jan *et al*. (23) described that MEL used to treat children with sleep disorders significantly improved gastrointestinal (GI) conditions, including UC. Shernhamer *et al*. (24) in a large follow-up observational study in Germany found increased prevalence of UC and total IBD among men and women who engaged in extended or irregular shift work, which itself has been associated with lower endogenous melatonin levels. The same authors also observed the link between the shift work and decreased colorectal cancer risk (25). Boznanska *et al*. (26) in the study performed at our department showed that a major melatonin metabolite, 6-hydroxymelatonin sulphate in urine was decreased in patients with UC compared to healthy controls.

This study aimed at evaluating the effect of MEL on the activity of inflammatory process and sustaining the remission in patients with UC.

MATERIAL AND METHODS

Subjects

Sixty-four patients with left-sided ulcerative colitis (39 female and 25 male, aged 26-49 years mean age 36.1±12.5 years) were enrolled in the study. Patients were randomly selected from 380 subjects those attending outpatients clinics at the University Hospitals in Lodz. The recruitment of patients was conducted from October 2004 to December 2007. The patients from group I and group II were under medical supervision, aimed at achieving clinical and endoscopic remission of ulcerative colitis all the time. Using mesalazine, corticosteroids and azathioprine remission was achieved within 28 to 42 months. During remission only mesalazine treatment was continued. Patients who signed the written consent were enrolled to participate in this prospective study.

Inclusion criteria Demographic data and relevant medical history were obtained from each patient and physical examination was done. The characteristic of the studied patients are presented in *Table 1.* Clinical and endoscopic remission was according to the Mayo Clinic Score System, and defined as less than 2 points.

Exclusion criteria. Patients with concomitant diseases, pregnant and lactating women, with history of surgical procedure, mechanical obstruction or perforation of gut and with using another drug were excluded of study.

Protocol

Patients who met all inclusion and exclusion criteria were assigned randomization numbers and treated according with protocol study. Subject were enrolled in a randomized, double-blind, placebo-controlled, crossover trial consisting of screening visit (performed within 14-21 days from the start of treatment), followed by twelve months, during each patient received mesalazine at the dose of 2.0 g daily and melatonin (LEK-AM, Poland) 5 mg at bedtime (group I, n=32) or placebo (group II, n=32). Placebo (saccharine) in identical form as melatonin free of charge from producer (LEK-AM) were received.

The Mayo Clinic Score System was applied to evaluate UC activity with the total Mayo Clinic Disease Activity Index (MCDAI) ranging from 0 to12 points. Four parameters (scored 0-3 each) were taken into account to calculate MCDAI: stool frequency, rectal bleeding, endoscopic findings, physician's global assessment. Disease activity was evaluated after 3, 6, 9 and 12 months of treatment. According to the Mayo Clinic Score System a clinical remission was defined as: MCDAI 0-2 points with no individual subscore >1, an endoscopic remission was defined as endoscopic findings scored 0 or 1 and a clinical exacerbation was defined as: MCDAI 5 points together with an increase of endoscopic score of at least 1 point (27).

At the same points of time in each patient the level of anxiety and intensity of depression was assessed. The level of anxiety was measured according to the 14-parameter (scored from 0 to 4 points each) Hamilton anxiety scale (HAMA). The levels of anxiety were divided into three categories depending on the number of points scored (28):
18-24 points - mild anxiety
25-30 points - moderate anxiety
>30 points - severe anxiety

The intensity of depression was evaluated according to the 21-question (each scored from 0 to 3 points) Beck depression inventory (BDI). The severity of depression, based on the German reference values, was divided into four categories depending on the number of points scored (29, 30):
0-11 points - no depression
12-19 points - mild depression
20-25 points - moderate depression
>26 points - severe depression

Compliance and safety

At each visit patients returned their medication containers and tablets were counted. Global tolerability of used medication was good. During the treatment two patients from group I, and two from group II were excluded for a reason of pregnancy (one woman) and at his own request (three men). No serious adverse events were reported. Some patients recurrent headaches felt, but probably not related to melatonin or placebo. At baseline and

Table 1. The patient characteristics.

	Group I (MEL)	**Group II (placebo)**
Number of patients	30	30
Age	35.6±11.4 years	33.9±11.7 years
Gender (F/M)	20/10	18/12
UC duration	9.2±6.4 years	10.2±5.3 years
Employed	26	24
Smokers/Non-smokers	2/28	4/26

after 3, 6, 9 and 12 months of treatment a wide array of laboratory tests was performed including blood cell count, hemoglobin concentration, CRP, glucose, electrolytes, bilirubin, urea, creatinine, cholesterol, triglyceride, TSH and activity of AST, ALT, GGTP, FA, amylase, lipase.

Statistical evaluation

Comparison between groups were performed with nonparametric Mann-Whitney rank - sum test and Kruskal-Wallis test. All calculations were made using StatSoft software. A p - value < 0.05 was considered statistically significant. The study was approved by the Bioethics Committee of Medical University of Lodz (number RNN/242/06/KB) and all performed in accordance with Declaration of Helsinki.

RESULTS

All the patients on MEL adjuvant treatment remained in remission during 12 months of observation. At the beginning of the study MCDAI value in this group was 1.50±0.51 points and after 12 months it was 2.75±1.86 points (*Fig. 1*). Only two of the patients on MEL treatment reported increased number of stools during the seventh and the eight month but the observed symptoms did not require an increase of 5ASA daily dose. During the treatment three persons from group I (received melatonin) and four persons from group II (received placebo) recurrent headaches felt, but the other factors may be their cause and no seasonal differences were observed. No real changes and differences between both group in laboratory tests were observed.

In the placebo group MCDAI at the inclusion was 1.61±0.68 points and it gradually increased to the value of

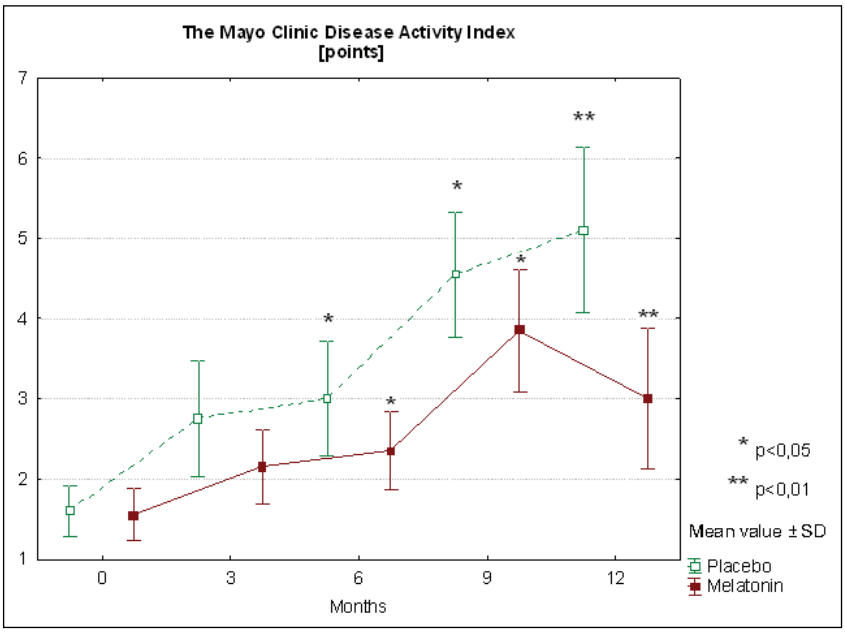

Fig. 1. Ulcerative colitis activity according to the Mayo Clinic Scoring System.

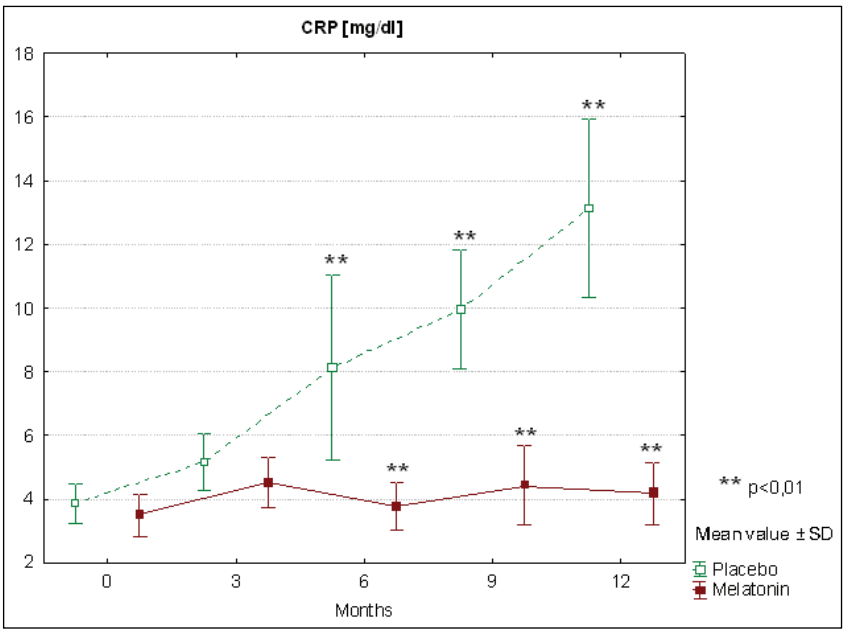

Fig. 2. CRP concentration in patients on adiuvant melatonin (Group I) and placebo (Group II).

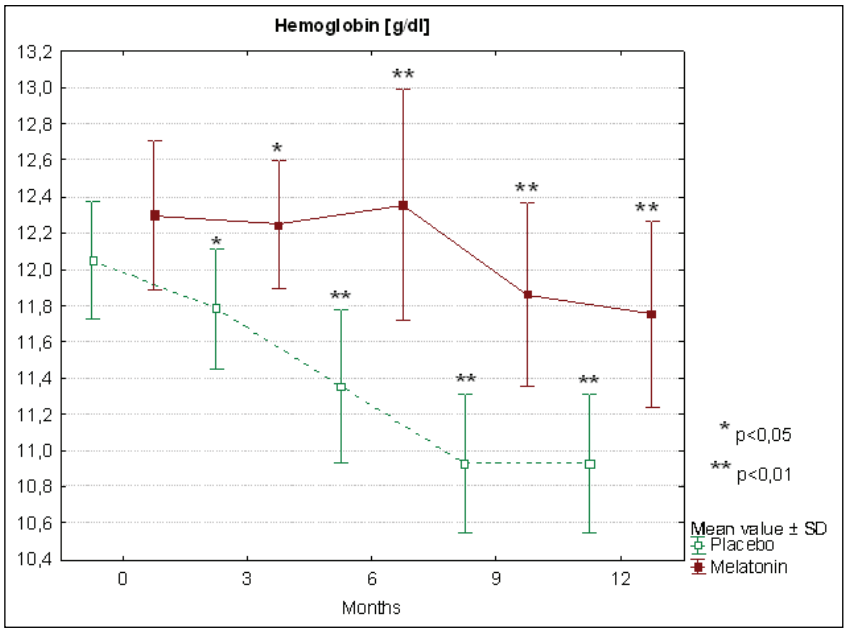

Fig. 3. Hemoglobin concentration in patients on adiuvant melatonin (Group I) and placebo (Group II).

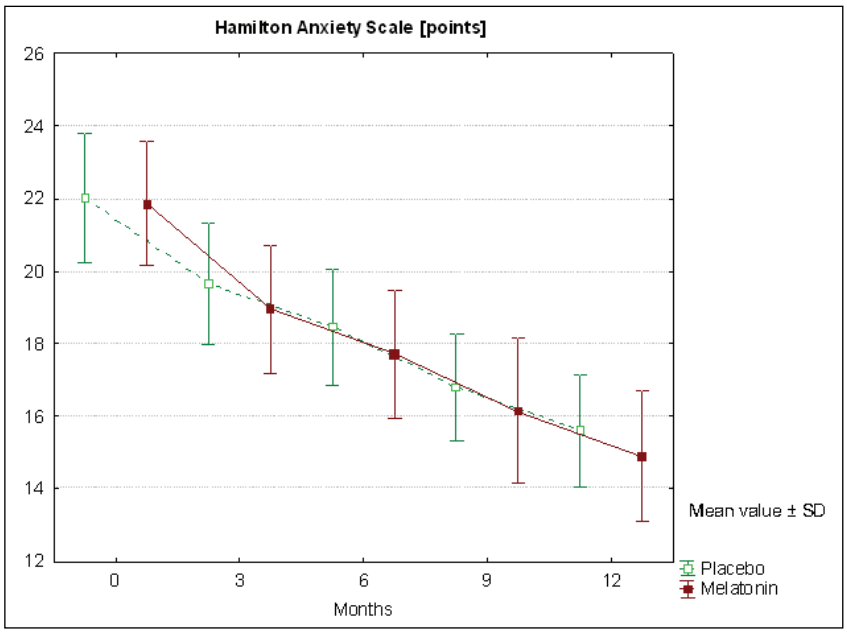

Fig. 4. The level of anxiety in patients on adiuvant melatonin (Group I) and placebo (Group II) according to Hamilton anxiety scale.

5.10±2.22 points at the end of the study. In the placebo group significantly higher MCDAI values were observed than in patients on MEL treatment after 9 and 12 months ($p<0.05$, $p<0.01$ respectively) (*Fig. 1*).

Similar differences were noted in CRP concentration in blood. In MEL group CRP levels were within the normal range during the course of the study (from 3.49±1.40 to 4.1±72.10 mg/dl). Whereas in the placebo group from the end of the third month the steady rise in CRP blood concentration was noted from 3.85±1.29 to 13.13±6.08 mg/dl (*Fig. 2*). Paralelly to CRP rise a significant decrease in hemoglobin concentration in blood from 12.05±0.69 to 10.93±0.81 g/dl was observed in patients receiving placebo. In MEL group there was only a slight decrease in hemoglobin concentration in blood from 12.29±0.87 to 11.76±1.09 g/dl. The observed values of hemoglobin concentrations in blood differed significantly between the studied groups after 3, 6, 9 and 12 months (*Fig. 3*).

The level of anxiety in patients on adjuvant MEL decreased during the study and it remained at the same level as in the placebo group according to Hamilton anxiety scale (*Fig. 4*). Similarly the intensity of depression in patients on both MEL and placebo decreased during the course of the study (*Fig. 5*). There were no statistical differences in the level of anxiety and the intensity of depression noted between the groups (*Fig. 4* and *5*).

DISCUSSION

The pineal gland is not the only rich source of MEL in vertebrate species. MEL is also derived in vast amounts from the alimentary system, where it is produced in enterochromaffin cells (ECs). ECs are found throughout the GI tract with greater density in the duodenum and rectum. Calculating MEL concentration in 1 gram of tissue and taking into account a large

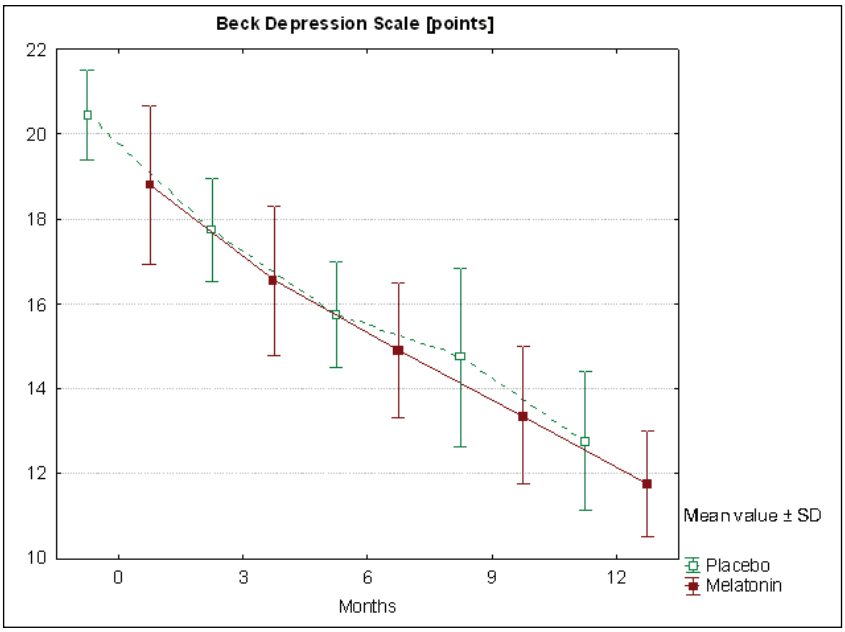

Fig. 5. The intensity of depression in patients on adiuvant melatonin (Group I) and placebo (Group II) according to Beck Depression Scale.

surface of the gut it has been shown that MEL concentrations in the GI system surpasses blood levels by 10-100 times and there is at least 400 times more of MEL in the gut that in the pineal gland (5, 31, 32). MEL was found in all parts of the gut wall with the highest concentration in the mucosa (33). Messner *et al*. (33) described the highest concentration of this hormone in the small and large intestine. MEL secretion from ECs exhibit cicardian rhythm, but also depends on the food components, particularly L-tryptophan, MEL precursor (31-35). MEL may serve as an endocrine, paracrine or autocrine hormone (5, 36). Its action in the GI tract concerns motor and secretory function and indirectly MEL is also involved in the process of digestion. In experimental studies in animals it has been shown that melatonin stimulates bicarbonate secretion from gastric and duodenal mucosal glands and pancreas (37), exerts an effect on NO production and COX-2 expression (12, 38), presents anti-inflammatory (10-15, 17), immunomodulative properties (7, 16) and anti-oxidant properties (39-42). All the mentioned above MEL properties determine its enteroprotective action, which was mostly proved in the upper part of the alimentary system. Konturek *et al*. (43) described that MEL administration in rats decreased an injury of the oesophageal mucosa caused both by hydrochloric acid and bile. In multiple experimental models it has been shown that MEL protects gastric mucosa from many harmful factors, such as stress, alcohol and non-steroidal antiinflammatory drugs (NSAIDs) (44-50).

In the clinical studies Klupinska *et al*. (51) observed relatively lower MEL concentration in blood in patients with erosive form of GERD, duodenal ulcer disease and ulcer-like functional dyspepsia. Similar observations in duodenal peptic ulcer disease have been done by other authors (52). It can be speculated that a relative deficiency of MEL can lead to a reduction in anti-oxidative activity, decrease of mucous and bicarbonate secretion and disturbances of blood distribution in the mucosal microcirculation. This can result in imbalance between aggressive and protective factors and further lead to destructive changes in the mucosa of the upper GI tract.

There are far less studies on MEL beneficial effect on colitis. It has been well recognised that MEL exerts regulatory effect on gut motor function, among others also by antagonizing serotonin gastrointestinal actions. Most of experimental data have shown MEL inhibitory action on motor function due to smooth muscle relaxation. This effect can be exerted directly by stimulating specific receptors, regulation of Ca^{2+}/K^{+} ion channels of the cell membrane activity as well as indirectly *via* the enteral nervous system (ENS). This hormone blocks the nicotinic acetylocholine receptors on the nerve endings of the submucosal plexi and activates afferent vagal fibres *via* an increased release of CCK and CCK1/CCK2 receptors activation (53-56). MEL also exerts its effect on visceral sensation, which can be of a particular importance in patients with irritable bowel syndrome (IBS). It has been shown that there are differences in the number of ECs in patients with different forms of IBS. Spiller *et al*. (57) described increased in the number of ECs in constipation predominant IBS (IBS-C). Inversely, El Sahy *et al*. (58) observed a decreased number of ECs. Indirectly the changes in the number of ECs lead to disturbances of MEL secretion and metabolism. Higher MEL secretion was observed in IBS-C and decreased excretion of MEL main metabolite, 6-hydroksymelatonin sulphate (6-OHMS), in urine in both forms of IBS (59).

Most of data on ameliorative effect of MEL on colitis come from *in vitro* studies in animals, so far there has been only a limited number of studies performed in humans in organic gut diseases and among them also IBD. Pentney and Bubenik (60) in the experimental study in mice with dextran-induced colitis ten times higher MLT concentration in blood than in healthy animals. MLT exerts enteroprotective action in the gut inhibiting tissue injury *via* receptor signalling and metabolic paths. Pentney (60), Cuzzocrea (61) and Marquez (62) observed a graduate amelioration of the bloody diarrhoea and an improvement of the clinical state after intraperitoneal administration of MEL. Cuzzocrea *et al*. (61) also described a decrease of myeloperoxidase, COX-2 and NO synthetase, oxygen peroxidation products, ICAM-1 expression and P-selectin in colon mucosa. Dong *et al*. (63) also observed decreased COX-2 and NO synthetase activity as well as PGE2 and NO concentrations. Necefli *et al*. (64) showed significantly lower colonic activities of myeloperoxidase (MPO) and caspase-3 and decreased levels of oxygen peroxidative products, such as, malondialdehyde (MDA), together with an increase in glutathione (GSH) levels. Mei *et al*. (65) described reduced colon immunological injury in rats given melatonin. It was attributed to regulating activity of macrophages by MEL. In the animals, which were on MEL, the authors observed decreased

myeloperoxidase activity, as well as Il-1, TNF-α and NO concentrations. Morover, Mazzon (66) proved that MEL modulates signal transduction pathways and apoptosis in experimental colitis. The authors showed that treatment with MEL was associated with a remarkable amelioration of colonic disrupted architecture as well as a significant reduction of TNF-α. Melatonin also reduced the NF-kappaB activation, phosphorylation of c-Jun, Fas ligand and Bax expression, prevented and the loss of Bcl-2 proteins as well as the presence of apoptotic cells in the model of colitis caused by dinitrobenzene sulfonic acid (DNBS).

In the study performed in the patients with UC by Boznanska *et al*. (26) the changes in MEL metabolism correlated with the severity of the disease were demonstrated. Daily excretion of the main MEL metabolite -6-hydroxymelatonin (6-HMS) sulphate in urine was significantly lower in patients with UC compared to healthy controls, and its levels in UC patients were inversely correlated with the disease severity. This inverse correlation between 6-HMS excretion in urine and disease activity was confirmed in all the criteria of UC activity evaluation: clinical assessment, endoscopic and pathomorphological changes. The authors suggest that MEL secretion in severe UC is decreased as well as its metabolism is disturbed. The metabolism of MEL to 6-HMS takes place mainly in the liver, but to some extend it also occurs in the colonic wall with the engagement of an isoform CYP1B1 of cytochrome P450. It has been postulated that in IBD overproduction of proinflammatory cytokines can lead to a decrease in an expression of enzymes of MEL metabolic pathway in hepatocytes. On the other hand, the inflammatory process of the colonic wall causes dysfunction of the metabolic processes in the gut mucosa as well as disruption of the epithelium, what further results in a decreased expression of CYP1B1. Lower urinary excretion of 6-HMS in severe UC, as a consequence may lead to overproduction in the cells of the body immunological system of other MEL metabolites. MEL metabolites can also be produced as a result of the reactions with free radicals in the places of inflammation.

There are experimental have provided the evidence on enteroprotective action of MEL encourage to its therapeutic use in the treatment of the diseases of the alimentary tract. So far, the beneficial effect of MEL administration has been shown in GERD (66-68), duodenal ulcer disease (69) and functional dyspepsia (70).

Some of data in literature confirms the beneficial therapeutic effect of MEL in patients with IBS. Saha *et al*. (71) observed significant improvement of the quality in life in patients with IBS treated with MEL in a daily dose of 3 mg for 8 weeks. Whereas, Song *et al*. (72) described the beneficial therapeutic effect of MEL administered in the same daily dose for two weeks on abdominal pain and rectal pain sensitivity. Therapeutic effectiveness of MEL in women with IBS treated for 8 weeks was also proved by the study performed by Lu *et al*. (73), in which the authors observed amelioration in abdominal pain, bloating and urge for defecation.

There is a lack of data in literature from the randomized studies on the therapeutic effectiveness of MEL in IBD. Nevertheless, considering the antiinflammatory action of this enterohormone, some researchers, among them Terry and colleagues (74) suggest the potential therapeutic usage of MEL in the treatment of IBD. The results of our study also support this opinion.

IBD is a chronic disease with the periods of remissions and relapses. Because of such a course of this disease and particularly UC we decided to treat the patients with MEL for 12 months observing its effect on the disease activity in remission. Good tolerance of MEL and its beneficial effects seen in both somatic and psychoemotional sphere encourage its implementation in the adjuvant treatment of UC. The dose of MEL, which should be used in order to obtain a therapeutic effect, is still discussed. Usually melatonin is applied in daily dose of 3 mg. In our study we chose the dose of 5 mg MEL daily. We took into the consideration that even in a higher dose most of exogenic MEL is absorbed from the gut and *via* the portal circulation it reaches the liver, where 60-80% is metabolized. Melatonin can act both locally and systematically, this second effect being attributed to the fraction, which reaches the systemic circulation. This systemic action is seen in the beneficial effect of MEL on sleep disturbances as well as its antidepressant action. Certainly, it is worth noting that a main metabolite of MEL-6-HMS possesses also antioxidative action. Preferentially MEL is given at the bedtime in order not to disturb its circadian rhythm of secretion. MEL is most often given in the evening because the relation between the pineal and the gut fractions of MEL secreted by ECs of the alimentary system is still not fully explained. (75).

In our study we did not observe any significant differences between the level of anxiety and intensity of depression between the patients treated with MEL and these receiving placebo, although there was an obvious amelioration of the psychoemotional staus of both the studied groups. Similarly to our results Lu *et al*. (73) described that MEL treatment (3 mg at bedtime) improved bowel symptoms in IBS, but the mean sleep, anxiety and depression scores were similar after melatonin and placebo administration. The authors concluded that its beneficial effect in IBS must be independent of its action on sleep disturbances and psychological profile (73).

The results of our study suggest that adjuvant melatonin therapy may help in sustaining remission in patients with UC.

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Conflict of interests: None declared.

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