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-resistant 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 psychomotional 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 patients 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. Shernhammer 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 to 12 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. |
|-------------------------------|----------------|----------------|
| 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           |
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 adjuvant melatonin (Group I) and placebo (Group II).
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
It has been well recognised that MEL exerts regulatory effect on MEL inhibitory action on motor function due to smooth muscle. Most of experimental data have shown gut motor function, among others also by antagonizing serotonin destructive changes in the mucosa of the upper GI tract. Between aggressive and protective factors and further lead to the mucosal microcirculation. This can result in imbalance bicarbonate secretion and disturbances of blood distribution in reduction in anti-oxidative activity, decrease of mucous and speculated that a relative deficiency of MEL can lead to ulcer disease have been done by other authors (52). It can be functional dyspepsia. Similar observations in duodenal peptic erosive form of GERD, duodenal ulcer disease and ulcer-like relatively lower MEL concentration in blood in patients with harmful factors, such as stress, alcohol and non-steroidal 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++/K+ ion channels of the cell membrane activity as well as indirectly via the enteral nervous system (ENS). This hormone blocks the nicotinic acetylcholine 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-hydroxymelatonin 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. Necelli 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
disrupted architecture as well as a significant reduction of TNF-
experimental colitis. The authors showed that treatment with
modulates signal transduction pathways and apoptosis in
concentrations. Moreover, Mazzon (66) proved that MEL
in patients with IBS. Saha
dyspepsia (70).
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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 defection.

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 psychosomatic
status 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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