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ORIGINAL ARTICLE

Melatonin production in patients with duodenal ulcer

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Abstract

OBJECTIVES. This work was designed to study melatonin (M) production levels in duodenal ulcer (DU) patients in the exacerbation and remission stages and in DU patients with a different course of DU in the exacerbation and remission stages.

METHODS. DU patients (15 men aged 20 to 45, mean age 34,2 ± 1,1 years) were studied. The control group included 11 healthy volunteers (men aged 20–45, mean age 32,5 ± 1,4 years). M was measured by RIA - method using H^3–labeled M in daily urine collected at 3-hour intervals (8 portions per day). Mathematical processing was carried out using ANOVA, Student's t-test, and cosinor analysis. Differences at $p \leq 0,05$ were considered to be significant.

RESULTS. A strong disturbance of M secretion in DU patients both in the exacerbation and remission stages and a direct correlation between the degree of M production disturbance and severity of clinical course of DU were revealed.

CONCLUSION. The obtained results suggest that circadian rhythms of M production are altered in DU patients. M was supposed to be involved in the pathogenesis of DU.

Introduction

The role of M in the human body is difficult to overestimate. This is connected with a wide spectrum of its effects and their importance for survival. Since M was discovered [1] and virtually until recently, considerable attention has been given to its following functions: 1) rhythm-regulating, 2) antioxidant, 3) immunomodulating, 4) involvement in sex development, and 5) sleep regulation. Though the presence of M was confirmed immunohistochemically as early as 1975 [2] and the high density of M receptors and the enzymes required for its synthesis from tryptophan were revealed in the gastrointestinal tract (GIT) tissues in 1991 [3] and 1976 [4], respectively, it is only in recent years that a few studies of the role of M in the regulation of GIT functions were undertaken [5, 6, 7, 8]. However, even these studies lead us to consider that M plays an essential role in the physiology of GIT and disruption of its secretion may result in a different pathology of GIT organs.

At present there is no doubt that M regulates GIT motility. A series of recent research works have given conclusive evidence of the existence of a balanced system regulating gastrointestinal motility, the so-called "serotonin-melatonin system" which is regulated by its constituent parts on the feedback principle [9, 5, 7]. Undoubtedly, research into gastrin -M interaction [10] arouses considerable interest. Based on the fact that the chemical structure of M and the gastrin receptor antagonist benzotript are close to each other and that M and gastrin act differently on GIT motility, cell proliferation, and the intracellular cAMP content, the authors reason that M might mediate its effects on GIT both by activating its own receptors and blocking gastrin ones.

The experimental studies on animals showed the efficacy of M in preventing gastric ulceration in different ulcer models: ethanol and ischemia-reperfusion model [11], shift light -dark rhythm model [12], as well as in the gastric ulcer model using nonsteroid antiinflammatory drugs (NSAD) [13]. The authors showed that the antiulcerous effects of M were due to its antioxidant effect, stimulation of PgE_2 synthesis by the gastric mucous membrane, and improvement in microcirculation.

Considering the recently obtained data on the genetic nature of human biorhythms [14, 15] and the role of M in the human body as well as on the essence of the dysadaptation phenomenon, which is the result of a mismatch between genetically determined endogenous body rhythms and exogenous rhythms (environmental rhythms) [16], we can state that scientists are close to a new insight into both the pathogenesis of duodenal ulcer proper and the nature of its seasonal exacerbation and the daily rhythmic pattern of its clinical manifestations.

Based on the spectrum of M effects [17, 18], it is natural to suggest that disruption of its production, likely to be genetically determined, might play a leading role in the pathogenetic mechanisms of DU. DU is as such the original cause of all the pathogenetic links to follow, which are the changes in rhythm and secretory function of the stomach, motility and evacuation disorders, activation of free radical processes, impairment of local and general immunity, reduction of cytoprotection, and unbalance of the gastrointestinal hormones.

The aims of our research were to study M secretion in DU patients in the exacerbation and remission stages and in DU patients with a different course of DU in the exacerbation and remission stages.

Materials and methods

DU patients (15 men aged 20 to 45, mean age 34.2 ± 1.1 years) were studied. Depending on the character of the clinical course, all the patients were assigned to two groups. Group I included 5 patients with rare exacerbation of DU (one episode over 2–3 years), group II included 10 patients with frequent exacerbation of this disease (once or twice a year). All the patients were subjected to clinical evaluation twice, namely, in the exacerbation and remission (exacerbation-free period = 1 month) stages. The disease stage was verified endoscopically. The control group included 11 healthy volunteers (men aged 20–45, mean age 32.5 ± 1.4 years). The functional status of the liver and kidneys of all the patients and controls was assessed by the biochemical blood tests, complete blood count, and urinalysis. Included in the study were only individuals with normal functional values of the liver and kidneys. M was determined in daily (24-h) urine at 3-hour intervals (8 portions daily). Diuresis was measured, and urine samples of 4 ml each were frozen at –20°C. M was determined by RIA-method using H^3-labelled M [19] in the laboratory of St. Goran's Hospital, Karolinska Institute, Stockholm, Sweden. In the exacerbation stage, urine samples were examined before the institution of antiulcer therapy. Nocturnal urine was collected at twilight. Alcohol consumption and medication were ruled out at least three days before sampling. The material was processed mathematically using ANOVA, Student's t-test, and cosinor analysis. The difference was considered to be significant at $p \leq 0.05$.

Table 1. Urinary melatonin concentration (nmol/L) in DU patients in exacerbation and remission stages and in controls.

	Melatonin concentration in urine (M ± m)		
Time (hours)	Exacerbation	Remission	Control
6–9	0.25 ± 0.024	0.241 ± 0.017	0.263 ± 0.029
9–12	0.238 ± 0.028	0.201 ± 0.022	0.189 ± 0.017
12–15	0.238 ± 0.033	0.16 ± 0.018	0.12 ± 0.011
15–18	0.143 ± 0.013	0.15 ± 0.011	0.09 ± 0.006
18–21	0.128 ± 0.008	0.146 ± 0.01	0.10 ± 0.008
21–24	0.181 ± 0.021	0.182 ± 0.015	0.131 ± 0.011
24–3	0.186 ± 0.034	0.227 ± 0.022	0.219 ± 0.019
3–6	0.275 ± 0.026	0.253 ± 0.019	0.238 ± 0.021
Day	0.186 ± 0.013	0.165 ± 0.008	0.123 ± 0.006
Night	0.221 ± 0.014	0.225 ± 0.009	0.211 ± 0.011
24 h	0.203 ± 0.01	0.193 ± 0.006	0.166 ± 0.007

Table 2. Twenty-four-hour rhythm in urinary concentration of melatonin (nmol/L) in DU patients in exacerbation and remission stages and in controls.

	Exacerbation	Remission	Control
Amplitude (concentration)	0.074 ± 0.004	0.054 ± 0.005	0.087 ± 0.01
Acrophase (clock time)	0430 ± 90 min	0430 ± 90 min	0730 ± 90 min
Mesor (concentration)	0.203 ± 0.01	0.193 ± 0.006	0.166 ± 0.007

Fig. 1.

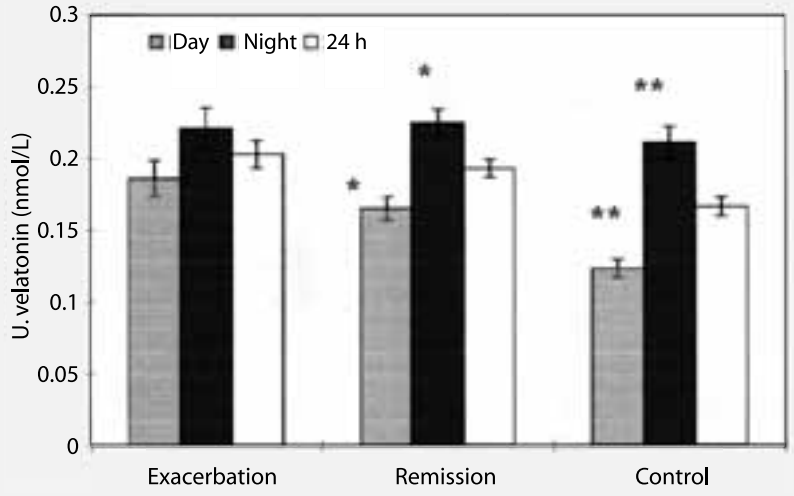

Fig. 1. Daily rhythm of urinary melatonin concentrations in DU patients in exacerbation and remission as well as in controls.
*** - *** - significant differences between melatonin concentrations in diurnal and nocturnal urine samples of remission-stage patients.
**** - **** - significant differences between melatonin concentrations in diurnal and nocturnal urine samples of controls.

Results

Table 1 shows urinary M concentrations in DU patients in the exacerbation and remission stages at a whole as well as in controls at different time intervals.

Daily urine output in DU patients in the exacerbation and remission stages was 920 ± 80 and 870 ± 70 ml, respectively, while that in the control group was 910 ± 60 ml.

The study revealed (Fig. 1, Table 2) the absence of the daily M production rhythm in DU patients in the exacerbation stage and a dramatic decrease in the daily M production amplitude in the exacerbation and remission stages, as compared with the control group. The absence of significant differences between diurnal, nocturnal, and daily M secretion levels in DU patients as compared with those in different stages was established. Despite the apparent absence of differences in M production as judged by the day- and nighttime means and the interstage average daily level, the daily M secretion curve (Fig. 2) in exacerbation-stage patients differs dramatically from that in the remission stage in the absence of rhythm and the presence of maximum production in the daylight. As compared with the controls, patients in the exacerbation stage demonstrated an increased diurnal and daily (due to the daylight fraction) M production level. The same trends are noted in the remission stage of duodenal ulcer as compared with the control group; however, the daily production curve, though gently sloped due to the high diurnal secretion, is of normal sinusoidal character (Fig. 2).

Table 3 shows urinary concentrations of M in patients with different clinical forms of DU course in the exacerbation and remission stage and in the control group at different time intervals. Figure 3 shows diurnal, nocturnal, and daily (24-h) M production rhythmic pattern in patients with rare and frequent exacerbation of DU in the exacerbation and remission stage as well as in the controls. As seen from Fig. 3, DU patients with rare exacerbation have no daily M production rhythm in the exac-

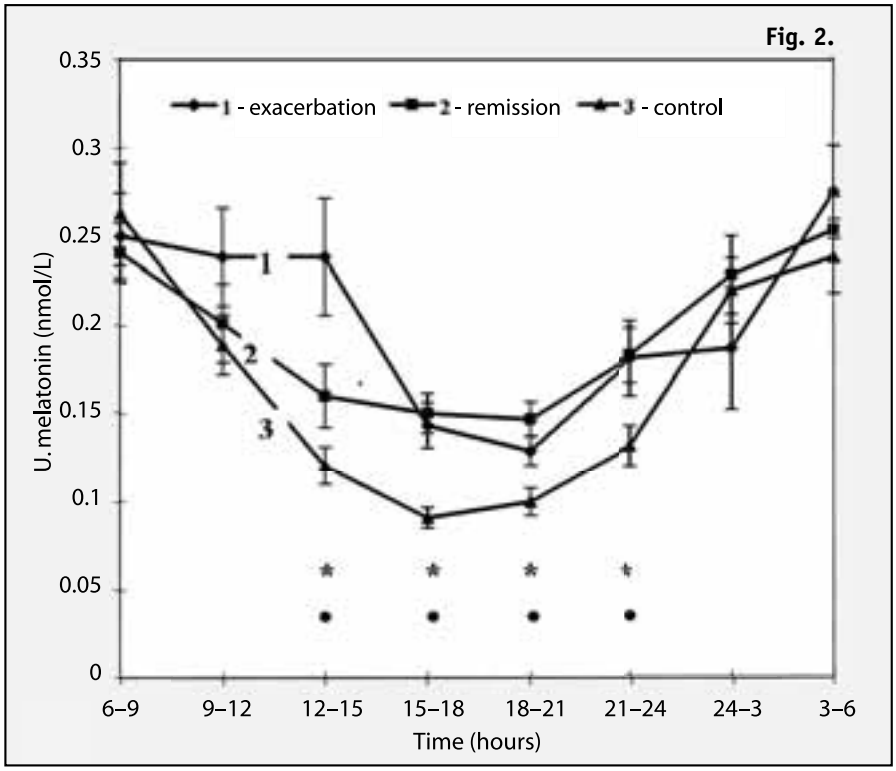

Fig. 2. Daily urinary melatonin concentration curve in DU patients in exacerbation and remission and in controls.
*** - * -** significant differences between the levels of urinary melatonin concentrations at each time interval in exacerbation-stage patients and controls.
• - • - significant differences between the levels of urinary melatonin concentrations at each time interval in remission-stage patients and controls.

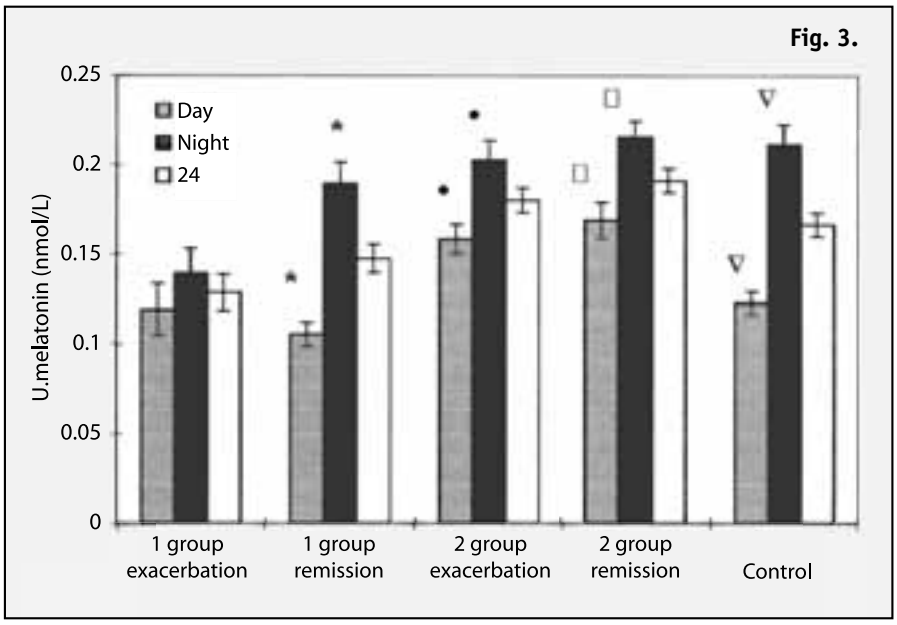

Fig. 3. Daily rhythm of urinary melatonin concentrations in DU patients with different course of disease in exacerbation and remission as well as in controls.
*** - *** - significant differences between melatonin concentrations in diurnal and nocturnal urine samples in DU patients with rare exacerbations in remission stage.
• - • - significant differences between melatonin concentrations in diurnal and nocturnal urine samples in DU patients with frequent exacerbations in exacerbation stage.
☐ - ☐ - significant differences between melatonin concentrations in diurnal and nocturnal urine samples in DU patients with frequent exacerbations in remission stage.
∇ - ∇ - significant differences between melatonin concentrations in diurnal and nocturnal urine samples of controls.

erbation stage. The level of diurnal M production in the exacerbation stage in these patients is significantly higher than in the controls, whereas the level of nocturnal and daily M secretion is lower than in the controls. In the remission stage, M secretion rhythm is restored, and the level and rhythm of M secretion in these patients in the remission stage does not virtually differ from those of daily M secretion in the controls, which is clearly seen in Fig. 3. That is, in remission-stage patients with rare exacerbation of DU, the level and rhythm of M secretion are normalized due to both a decrease in diurnal and an increase in nocturnal M production, which is evidenced by normalization of the amplitude of the daily M production rhythm in this stage of DU (Table 4). In patients with frequent exacerbation of DU, both the exacerbation and remission stages are characterized by the presence of a daily rhythmic pattern of M secretion with significant differences between the levels of diurnal and nocturnal secretion (Fig. 3). However, the rhythm amplitude is sharply decreased as compared to that of the control group (Table 4). The level and rhythm of M secretion in these patients in the exacerbation stage does not virtually differ from those in the remission stage, and the diurnal M production level in these patients both in the exacerbation and remission stages is higher than in the controls (Fig. 3). In other words, despite the presence of differences between the

Table 3. Urinary melatonin concentration (nmol/L) in DU patients with different course of disease in exacerbation and remission stages and in controls (M ± m).

Time(hours)	(I group)		(II group)		Control
	Exacerbation	Remission	Exacerbation	Remission	
6–9	0.132 ± 0.016	0.22 ± 0.028	0.24 ± 0.025	0.25 ± 0.023	0.263 ± 0.029
9–12	0.133 ± 0.028	0.133 ± 0.017	0.197 ± 0.018	0.20 ± 0.021	0.189 ± 0.017
12–15	0.125 ± 0.028	0.10 ± 0.01	0.194 ± 0.021	0.192 ± 0.03	0.12 ± 0.011
15–18	0.108 ± 0.02	0.083 ± 0.013	0.129 ± 0.01	0.14 ± 0.01	0.09 ± 0.006
18–21	0.11 ± 0.035	0.107 ± 0.011	0.114 ± 0.007	0.143 ± 0.012	0.099 ± 0.008
21–24	0.123 ± 0.028	0.144 ± 0.02	0.149 ± 0.015	0.163 ± 0.013	0.131 ± 0.011
24–3	0.128 ± 0.007	0.207 ± 0.022	0.175 ± 0.02	0.213 ± 0.016	0.219 ± 0.019
3–6	0.178 ± 0.043	0.185 ± 0.024	0.25 ± 0.021	0.235 ± 0.014	0.238 ± 0.021
Day	0.119 ± 0.014	0.106 ± 0.007	0.158 ± 0.008	0.169 ± 0.01	0.123 ± 0.006
Night	0.139 ± 0.014	0.189 ± 0.012	0.203 ± 0.011	0.215 ± 0.009	0.211 ± 0.011
24 h	0.129 ± 0.01	0.147 ± 0.008	0.18 ± 0.007	0.191 ± 0.007	0.166 ± 0.007

Table 4. Twenty-four-hour rhythm in urinary concentration of melatonin (nmol/L) in DU patients with different course of disease in exacerbation and remission stages and in controls.

	(I group)		(II group)		Control
	exacerbation	remission	exacerbation	remission	
Amplitude (concentration)	0.035 ± 0.002	0.069 ± 0.004	0.068 ± 0.002	0.056 ± 0.005	0.087 ± 0.01
Acrophase (clock time)	0430 ± 90	0730 ± 90 min	0430 ± 90	0730 ± 90 min	0730 ± 90 min
Mesor(concentration)	0.129 ± 0.01	0.147 ± 0.008	0.18 ± 0.007	0.191 ± 0.007	0.166 ± 0.007

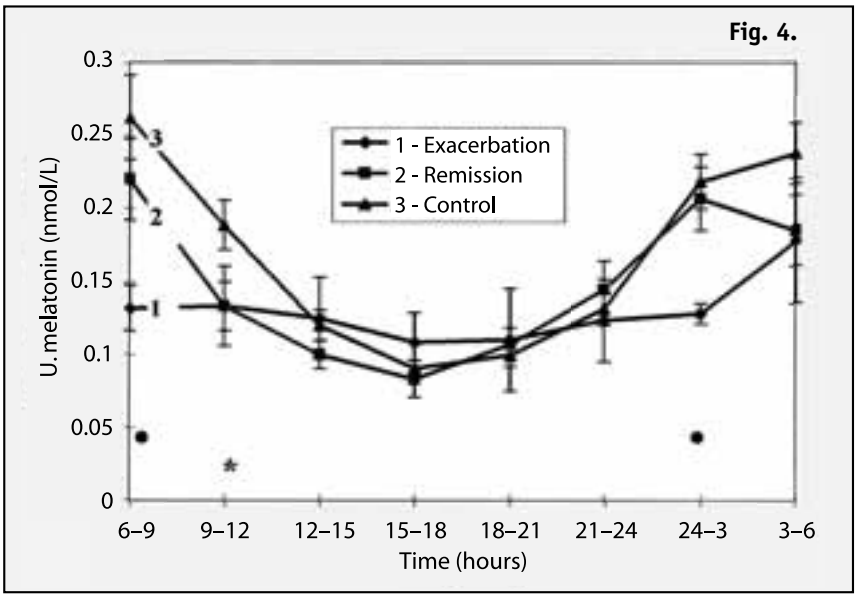

Fig. 4. Daily urinary melatonin concentration curve in DU patients with rare exacerbations in exacerbation and remission stages and in controls.
● - significant differences between the levels of urinary melatonin concentrations at each time interval in DU patients with rare exacerbations in exacerbation - stage and controls.
* - significant differences between the levels of urinary melatonin concentrations at each time interval in DU patients with rare exacerbations in remission-stage and controls.

diurnal and nocturnal M production in patients with frequent exacerbation of DU, the rhythmic pattern of M secretion in these patients is disrupted both in the exacerbation and remission stages.

Figure 4 shows the character of the daily M production curve in patients with rare exacerbation in both stages as well as in the controls. It can clearly be seen that in the exacerbation stage the daily M production rhythm is virtually absent, but there is a slight rise from 3 to 6 a.m. In the remission stage, the character of the M production curve closely resembles that of the control group with a significant difference only from 9 to 12 a.m.

Figure 5 shows the character of the daily M production curve in patients with frequent exacerbation of DU in the exacerbation and remission stages and in the control group. As seen from Fig. 5, the daily M production curves in patients with frequent exacerbation of DU in the exacerbation and remission stages are virtually similar and do not differ significantly

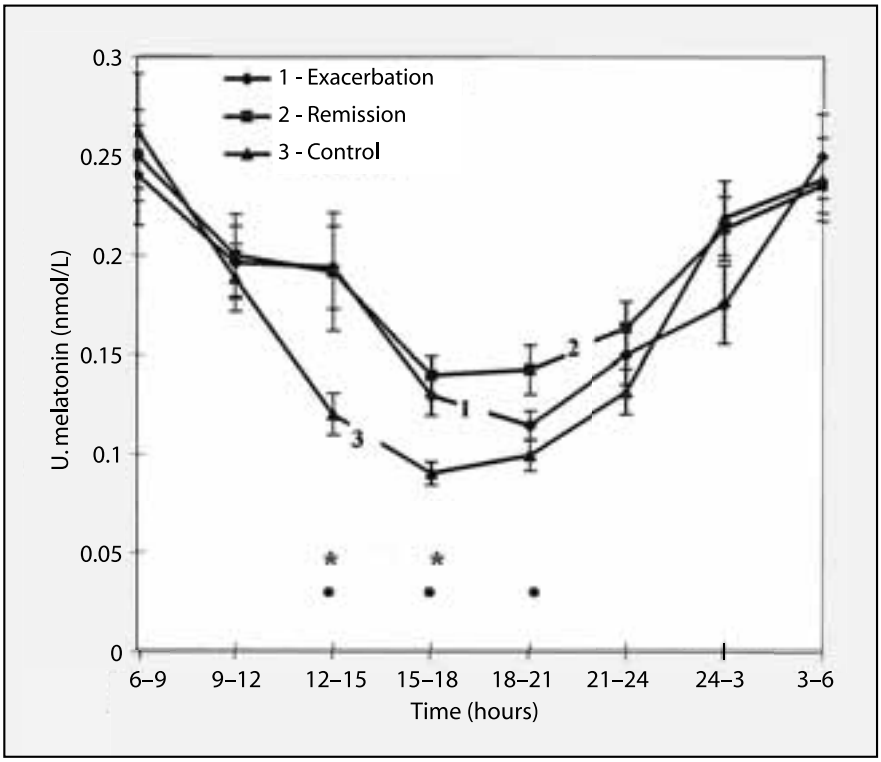

Fig. 5. Daily urinary melatonin concentration curve in DU patients with frequent exacerbations in exacerbation and remission stages and in controls.
* - significant differences between the levels of urinary melatonin concentrations at each time interval in DU patients with frequent exacerbations in exacerbation - stage and controls.
● - significant differences between the levels of urinary melatonin concentrations at each time interval in DU patients with frequent exacerbations in remission-stage and controls.

at any of the time intervals studied. This is also evidenced by virtually single-type differences between them and the daily M production curve in the controls.

Discussion

The secretion of M in humans is subject to a well-defined daily rhythm with maximum production at night and minimum production during the daylight hours. The peak of M production occurs from 2 to 4 o'clock a.m. [20]. The suprachiasmatic nuclei of the hypothalamus are endogenous regulators of M production [21], and the photoperiod is an external modulator of endogenous melatonin rhythm [20, 21]. The character of the daily M production curve changing subsequent to the photoperiod provides information about the environmental changes and is a crucial factor for the daily sleep- wakefulness pattern and circannual rhythms including seasonal changes in the human body [20]. The effects of M depend not only on its blood level but also on the amplitude of its daily production rhythm and duration of a nocturnal rise in M secretion [20, 21]. Hence, to assess the level of M secretion and to derive information on the presence or absence of changes in its production, it is not enough to study its basal level at one time point, as is customary for most of the hormones. For some reasons, preference has been given to noninvasive methods of investigation in recent years. Due to the existence of a direct correlation between the blood and urinary concentrations of M when the functional status of the liver and kidneys is normal [22, 23], as well as owing to the fact that they correlate with the level of its secretion by the epiphysis, because pinealocytes do not accumulate M [21], we assessed the level of M secretion on the basis of its urinary concentrations.

The fundamental studies in the field of clinical biorhythmology allow a contention that any disease is accompanied by a mismatch of endogenous biological rhythms of the organism [24, 16]. The mismatch of biorhythms may precede the pathology of the internal organs or may occur due to the disease itself [24, 16].

At present it is legitimate to state that the discovery of M [1] appeared to be a link that allowed the knowledge of biological rhythms of the body to be integrated into an elegant system. The studies performed *in vitro,* on animals and volunteers allow us to consider M to be a unique substance possessing the whole gamut of vitally important effects, part of which is inherent to M only. The wide spectrum of M effects appears to be due to the fact that it existed as long ago as the early steps of evolution, which is evidenced by its presence and the circadian rhythm of its production in unicellular organisms and plants [25, 26]. The study of the biorhythmological functions of M allowed us to determine that M is not only a messenger of the main endogenous rhythm generated by the suprachiasmatic nuclei of the hypothalamus and synchronizing all the other biological rhythms of the body but also a modulator of this endogenous rhythm relative to the environmental rhythms [21]. Hence,

any change in its production beyond the range of normal physiological fluctuations can cause a mismatch of the biological rhythms of the body (internal rhythm disturbance) as well as a mismatch of the bodily rhythms with those of the environment (external rhythm disturbance). Both internal and external rhythm disturbances may be the cause of different pathological conditions, a dramatic example of which is rhythm disturbance in shift work and flights through several time zones [27], and accompany diseases of the internal organs. In recent years it was revealed that the only cause of sudden infant death syndrome [28], idiopathic infant colic [29], seasonal affective disorders [30], and different sleep disorders [31] is a disturbed rhythm of M production.

Numerous studies led us to establish that M is one of the most powerful endogenous antioxidants [32]. Moreover, as distinct from most of the other intracellular antioxidants localized predominantly in certain cell structures (vitamin E in cell membranes, vitamin C in cytosol), the presence of M and, hence, its antioxidant activity, was revealed in all cell structures, including the nucleus [32, 33]. This fact testifies to the universality of the antioxidant effect of M, which is confirmed by the experimental studies demonstrating the protective properties of M against the free radical damage to DNA, proteins, and lipids [32, 33]. Since its antioxidant effects are not mediated through its membrane receptors, M can affect free radical processes in any human cell rather than in the cells having M receptors.

A large number of studies were devoted to immunomodulating functions of M. Summarizing the literature data, we may conclude that M is involved in the regulation of the immune functions in humans, which is evidenced by the presence of M receptors on the peripheral immune human cells and M-potentiated production of cytokines by the latter as well as by its immunostimulating effect in animal experiments when simulating conditions. The physiological responses to the above are identical in the human and animal body [34, 35]. A close relationship between M and the immune system is corroborated by γ-interferon-stimulated M production by the epiphysis, a fact testifying to the immune system regulating M secretion [36].

Today the role of M in the regulation of gastrointestinal motility [7, 9, 37], microcirculation [11], proliferative activity of the mucosa [38, 39], and stimulation of the synthesis of prostaglandins by the mucosa [13] was proven.

All the above-mentioned leads us to believe that changes in M production relating to its production level or daily rhythmic pattern can play a considerable role in the pathogenetic mechanisms of DU.

The data obtained give evidence of a dramatic disturbance of M secretion in DU patients both in the exacerbation and remission stages of the disease. In the exacerbation stage, the rhythmic pattern of daily M production is virtually absent, which is well demonstrated in Figs. 1 and 2. A sharp increase in the diurnal M production is noted, whereas nocturnal M production is similar to that in the control group. The daily M secretion curve (Fig. 2, Table 2) indicates a delay (6 hours) in the morning fall in M production as compared to the controls. In the remission stage (Fig. 2), the daily M production curve assumes the form of a sinusoid typical of a normal production curve; however, the amplitude of M production is sharply decreased (Table 2) due to an increased secretion of M in the daylight. In the remission stage, one can see a significant difference between diurnal and nocturnal M secretion levels (Fig. 1) as compared to M secretion in the exacerbation stage. However, compared with the control group, one can see a similar regularity both in the remission and exacerbation stage of DU.

The results obtained testify to a disturbed production of M in DU patients both in the exacerbation and remission stages. Based on the effects of M, the well-known fact of rigorous genetic determination of the level and character of M secretion [40, 41] and the phenomenon of conservation of changes in M production in the remission stage of DU revealed by us in the process of work, we cannot but suggest that M is likely to be involved in the pathogenesis of DU, in its exacerbation in particular. In connection with the fact of the daily rhythmic pattern of M production in remission-stage patients being disturbed, we feel it right to consider that not only does a change in M production appear to be the body's response to DU exacerbation but is also a probable factor of the occurrence of the exacerbation itself.

The data obtained allow us to state that the changes in M secretion revealed by us in patients with different clinical forms of DU show that, irrespective of the disease stage, patients with frequent exacerbation of DU (Fig. 3, Fig. 5, Table 4) have significant M production and rhythmic pattern disorders, which may be one of the causes of more frequent exacerbation of DU in this group of patients. In patients with rare exacerbation of DU (Fig. 3, Fig. 4, Table 4), dramatic changes in M production in the exacerbation stage are virtually leveled off in the remission stage as compared to the control group, and the daily M production curve (Fig. 4) and M production amplitude (Table 4) as one o f the main characteristics of the circadian rhythm become close to those in the control group. The fact that the daily M production rhythm and amplitude are almost normalized in the remission stage in these patients

appears to be very important, because it may explain rare exacerbation of DU in this group of patients.

Thus, the data obtained in the process of work show the presence of a direct correlation between the severity of M production disorders and that of the clinical course of DU, which counts once again in favor of M being involved in the pathogenetic mechanisms of DU.

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