Melatonin (MT) and its precursor L-tryptophan (Trp) are implicated in the protection of gastric mucosa against noxious agents. However, the role of MT and Trp on the gastric mucosal injury induced by aspirin (ASA) in humans has not been investigated. Studies in animals showed that both MT and Trp given intragastrically prevents the formation of gastric mucosal lesions induced by ASA. The aim of the present study was to determine the influence of MT and Trp given orally to healthy humans on gastric mucosal lesions induced by ASA. The present study included 21 healthy, Hp-negative male volunteers with intact gastro-duodenal mucosa aging 20-50 yr. They were divided in 3 groups; group 1: 7 volunteers receiving daily 2 x 1g ASA (Polfa, Rzeszow) during 11 days; group 2: 7 healthy volunteers receiving 2x1g ASA and MT (Lekam, Zakroczyn) (5 mg 30 min prior to ASA) during 11 days and group 3: 7 healthy volunteers receiving 2x1g ASA and Trp (Ardeytropin, Germany) (0.5 g 30 min prior to ASA) during 11 days. Mucosal damage was evaluated at 3rd, 7th and 11th days of ASA administration by endoscopy using Lanza score. Plasma melatonin was measured using RIA and gastric mucosal generation of PGE2 was assessed also by RIA. ASA caused marked mucosal injury at all days of its administration except day 11th when only moderate lesions were evident. Pretreatment with MT or Trp alone was accompanied by a significant decrease in gastric mucosal lesion score. Gastric mucosal generation of PGE2 was suppressed by about 90% in subjects treated with ASA without or with MT or Trp. We concluded that: MT and its precursor Trp significantly attenuate gastric mucosal lesions induced by aspirin. The action of Trp may be be mediated by MT produced in gastrointestinal tract from Trp. The gastroprotective action of MT and Trp is independent on gastric mucosal PGE2 generation.

**Key words:** aspirin, melatonin, L-tryptophan, humans, gastric mucosal damage
INTRODUCTION

Melatonin (MT), the principal secretory product of the pineal gland, is known to influence a variety of biological processes including circadian rhythms, neuroendocrine, cardiovascular or immune functions (1). MT is an indole originating from L-tryptophan (L-Trp) which was discovered by Lerner at al in 1958 in pineal gland (2). Initially pineal gland was considered as the sole source of circulating hormone, but more recently MT was found to be an ubiquitous molecule produced in various tissues, particularly in the gastrointestinal tract (GIT) and liver (3). Studies on the distribution of MT in the digestive system showed that this indole is generated in the entero-endocrine (EE) cells of GIT mucosa in significantly larger amounts than in pineal gland. MT acts partly in paracrine fashion in GIT mucosa and partly released to portal circulation to be accumulated in the liver for metabolism and excretion with the bile to small bowel and to undergo in entero-hepatic circulation (4).

MT is a derivative of an essential amino acid L-Trp and it is synthesised in a four step pathway. First, L-trp is converted to 5-hydroxytryptophan by tryptophan 5-monooxygenase. Aromatic-1-amino acid decarboxylase then catalyses the conversion of 5-hydroxytryptophan to serotonin (5-hydroxytryptamine). Arylalkylamine N-acetyltrasferase (AA-NAT) acetylates serotonin to N-acetylserotonin, the immediate precursor of melatonin. The last step in this biosynthetic pathway is catalysed by hydroxyindol-O-methyltransferase (HIOMT) which leads to the formation of melatonin (5).

MT acts via specific receptors. To date, three mammalian melatonin receptors have been identified; MT₁, MT₂ and MT₃. First two receptors are G-protein-coupled receptors and their activation modulate a wide range of intracellular messengers, e.g. cAMP, cGMP or [Ca²⁺]. The MT₃-binding site has been identified as quinine reductase protein and its physiological significance remains to be clarified (6).

Nonsteroidal anti-inflammatory drugs (NSAID), such as aspirin (ASA), are known to induce gastric mucosal damage and to interfere with healing of acute or chronic gastric ulcerations. Despite these side effects these drugs are among the most widely used medications in the world because of their demonstrated efficacy in reducing pain, inflammation, and protection against stroke and myocardial infarction (7). Patients at risk for development of serious gastrointestinal side effects are considered for prevention with stable PGE₂ analogs such as misoprostol, proton pump inhibitors or COX-2 (coxibs) selective inhibitor therapy. In addition to prostanoids, two important gaseous mediators, nitric oxide (NO) and hydrogen sulphide (H₂S) exert protective effects against ASA-induced damage of gastric mucosa. This last observation led to the development of NO- and H₂S releasing NSAID derivatives (8, 9).

The gastric mucosal injury induced by NSAID is associated with the detrimental effect of these drugs on prostaglandin synthesis and increased
oxidative stress due to neutrophil activation (PMN) (10). Due to high free radical scavenging activity, MT and its precursor L-Trp, are important candidates for protection of gastric mucosa against NSAIDs (11).

The purpose of the present study was: 1) to investigate the effect of MT and L-Trp co-treatment on the gastric mucosal injury induce by aspirin in healthy, Hp-negative men; 2) to analyze the effect of therapy with ASA alone or in combination with MT or Trp on the serum level of immunoreactive MT.

**MATERIAL AND METHODS**

**Subjects and study design**

21 *H. pylori* negative healthy male volunteers (age 20-50yr) entered the study. The study was approved by the appropriate Institutional Review Committee at University Lublin (Poland) and all subjects gave written informed consent to participate prior to inclusion. All subjects underwent routine endoscopy at day 0 during which the gastric mucosa was evaluated and gastric biopsies were taken for *H. pylori*. On the day following endoscopy, patients were randomized to following treatment groups: 1) 2x1g of ASA per day during 11 days; 2) 2x1 g ASA and Trp in the dose 0.5 g 30 min prior to ASA application during 11 days; 3) 2x1 g ASA and MT in the dose 5 mg 30 min prior to ASA application during 11 days. Each group contained 7 volunteers. For evaluation of gastric mucosal damage, the gastroscopy with GIFQ160 was performed on days 3, 7 and 11 after start of treatment. The standard endoscopy was performed by one investigator using Olympus GIF Q160 endoscope and recorded on video tape that was evaluated for mucosal damage using the Lanza score system by the second investigator, being unaware of the treatment given as described before (12).

**Determination of plasma melatonin and generation of gastric mucosal prostaglandin E₂ (PGE₂)**

At day 0, 3, 7 and 11 after start of the treatment, the blood samples were collected for determination of plasma melatonin levels. The plasma samples were stored at -20°C. The measurement of plasma melatonin level was performed using RIA kit from DRG Instruments GmbH in Marburg (Germany) as described before (13). The MT RIA-kit utilized a specific sample preparation containing 5-methoxytryptamine-125-Bolton-Hunter-conjugate as a tracer and a specific anti-MT antibody. This antisera did not show cross-reactivity with any product related to MT metabolism such as serotonin, 5-methoxytryptophan or N-acetyl-serotonin. The assay reliably detected MT concentrations as low as 1 pg/mL and the intra-assay and interassay variations were 7 and 8 pg/mL, respectively.

The biopsy samples obtained from gastric mucosa were measured for ex vivo PGE2 measurement as previously described. Briefly, the mucosal sample was minced with scissors for 1 min in microfuge plastic tube containing 1 mL of phosphate buffer (pH=7.4) and centrifuged in a fixates-speed bench centrifuge at 15.000 r.p.m. for 30 s. After the supernatant was discarded, the tissue was suspended in 1 mL of phosphate buffer and mixed by vortex for 1 min at room temperature. Then, 10μL indomethacin was added to each sample to inhibit further formation and release of prostaglandins (PG). The samples were centrifuged for 1 min and the supernatant stored at -20°C until assay was performed. The measurement of PGE₂ generation was performed using RIA kit (PGE₂ ¹²⁵I-PGE₂ kit, NEN Life Science Products Inc., Boston, MA, USA). The assay medium was
0.0255 mL/L phosphate-buffered medium azide (0.05%), pH 6.8. Generative capacity was expressed as nanograms of PGE₂ per g wet tissue weight (ng/g). Sensitivity of the assay was 1 pg/ml.

**Statistical analysis**

Results are expressed as means ± SEM. This significance of the difference between means was evaluated using analysis of variance followed by Duncan’s test or, when appropriate, by Wilcoxon’s rank sum test with a level of confidence at p<0.05.

**RESULTS**

All subjects completed the study and no major side effects were recorded. Minor side effects include dyspepsia observed in some subjects. In the group treated with ASA alone, a significant increase in gastric mucosal damage according to Lanza score was observed at day 3 after start of treatment (Fig. 1). At day 7 and 11 a gradual decrease in the gastric mucosal lesion score was found (Figs 2 and 3). Pre-treatment with MT or Trp prevented almost completely the

**Table 1** Lanza score - Modified erosive mucosal changes injury scoring system

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal stomach or proximal duodenum</td>
</tr>
<tr>
<td>1</td>
<td>Mucosal haemorrhages only</td>
</tr>
<tr>
<td>2</td>
<td>One or two erosions</td>
</tr>
<tr>
<td>3</td>
<td>1 to 10 erosions</td>
</tr>
<tr>
<td>4</td>
<td>Large number of erosions (&gt;10) or an ulcer</td>
</tr>
</tbody>
</table>

*Fig. 1* Endoscopic picture of bleeding multiple erosions in antral portion of the stomach observed in humans ingesting ASA (2g/day) for 3 days.
higher lesion scores at all days of ASA administration (Fig. 4). At day 11, the endoscopic lesion score in patients treated with ASA with MT or Trp was still significantly lower than in patients treated with ASA alone. Plasma levels of melatonin was similar in all subjects tested but after the administration of ASA it tended to increase throughout the administration of ASA.

Gastric mucosal generation of PGE$_2$ was suppressed by about 90% in all subjects treated with ASA alone or with MT or Trp (Fig 5).

**DISCUSSION**

This study demonstrates for the first time in humans the protective effects of MT and its precursor Trp against gastric mucosal damage induced by ASA. This
observation is in keeping with the previous observations of MT-induced gastroprotection shown in different animal models of gastric mucosal injury induced by NSAIDs. According to study by Alarcon de la Lastra et al (14), the protective activity afforded by MT was mainly due to the radical scavenging activity of this indol. Similar results were obtained by Bandyopadhyay et al (15) who demonstrated a significant increase in the generation of free radicals after treatment with piroxicam in gastric mucosa and attenuation of this phenomenon by MT treatment. However, our results indicate that MT and its precursor show similar gastroprotective effects in humans treated with aspirin shedding the new light on the pathophysiological role of MT in human GIT.

The mechanism of gastroprotective effect of melatonin was not revealed in the present study. Importantly, MT and Trp protected gastric mucosa despite almost complete inhibition of endogenous prostaglandin synthesis. This observation indicates that MT and Trp protects gastric mucosa independently on gastric mucosal generation of PGE$_2$ playing a central role in gastric mucosal protection (16). Our results document a highly protective efficacy of MT and its
precursor Trp against ASA-induced gastric mucosal damage, suggesting that Trp/MT system is more effective in maintaining mucosal integrity COX-PG system because it maintains mucosal integrity under conditions when endogenous generation of PG is almost completely suppressed.

The fact that Trp given orally protects gastric mucosa similarly to MT indicates that Trp given p.o. is converted to MT in the gastrointestinal mucosa possibly due to activity of NAT and HIOMT two, important enzymes involved in the biosynthesis of MT in EE cells (17). This is supported by an increase in plasma MT level in subjects taking ASA. This observation suggests that the acute gastric mucosal damage of gastric mucosa is accompanied by increased MT synthesis in gastric mucosa. This is keeping with our results obtained in animal model for acute gastric injury in which the damage of gastric mucosa was associated with increased release of MT into the circulation (18).

It is of interest that at day 11, we observed a significant decrease in mucosal lesion score in patients taking daily large dose of ASA (2 g/day). This may be due to the phenomenon of gastric mucosal adaptation. Further studies should clarify whether MT may be involved in the mechanism of already described in humans gastric mucosal adaptation to ASA (19) and whether regular pretreatment with MT or Trp could be useful in protecting the gastric mucosa from the damage in patients treated regularly for long period of time with ASA, e.g. in prevention of cardiac or cerebral vascular diseases.

Fig. 5. PGE2 generation in gastric mucosa in subjects treated with aspirin alone, or treatment (day 0). Asterisk indicates a significant change as compared to value obtained at day 0
In summary, MT and its precursor Trp significantly attenuate gastric mucosal lesions induced by ASA in humans. The action of Trp appears to be mediated by MT produced from Trp in gastrointestinal mucosa. The gastroprotective action of MT and Trp appears to be independent on gastric PGE₂ generation.

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Conflict of interest statement: None declared.

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