Changes of endogenous melatonin and protective effect of diet containing pleuran and extract of black elder in colonic inflammation in rats

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The possible involvement of endogenous melatonin in the effects of pleuran (β-glucan isolated from Pleurotus ostreatus), and of black elder (Sambucus nigra) was studied in a model of acute colitis in rats. Pleuran as 0.44% hydrogel drink and black elder as a 4% extract added to food were administered for over 4 weeks. Colitis was then induced by intraluminal instillation of 4% acetic acid and after 48 hours the extent of colonic damage was examined. Melatonin concentrations were measured in the pineal gland, plasma, duodenum and colon. Both pleuran in drinking fluid and black elder extract food supplementation significantly decreased the disposition to colitis. The macroscopic colonic damage score was reduced by 67.4% and 46.5% by pleuran and black elder, respectively. A significant increase of melatonin in the pineal gland was observed in rats given pleuran hydrogel, which paralleled the maximum protective effect of pleuran against colonic inflammation. There were no significant differences in the concentration of melatonin in plasma or in the intestinal wall between control and experimental groups and between vehicle and acetic acid treated rats. Results are discussed in the context of a possible involvement of endogenous melatonin in the protective effect of pleuran, but not of black elder, against colonic inflammation.

Key words: melatonin, pleuran, black elder, colon, inflammation, antioxidants.

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Introduction

Ulcerative colitis belongs to chronically recurrent nonspecific inflammatory bowel diseases (IBD) with yet unknown etiology and pathogenesis (Fiocchi, 1998). Of the various etiopathogenic mechanisms, immunological processes and reactive oxygen metabolites (ROM) have been proposed to contribute considerably to the development of tissue injury (Keshavarzian et al., 1996; Grisham, 1994). The main sources of colonic ROM are activated phagocytic leukocytes present in large numbers in the inflamed mucosa, capable of producing superoxide and a cascade of various ROM leading to hydroxyl radical and peroxide. The xanthine oxidase pathway in colonocytes also produces superoxide by conversion of xanthine/hypoxanthine to uric acid. A third possible source is the oxidation of arachidonic acid producing prostaglandins and leukotrienes (Yamada & Grisham, 1991). Since the colonic mucosa contains a relatively small amount of antioxidant enzymes, the balance between prooxidant and antioxidant mechanisms may be easily disturbed, resulting in oxidative stress with subsequent tissue injury (Loguercio et al., 1996).

In the studies of the metabolic effects of oyster mushroom, pleuran (β-1,3-D-glucan, isolated as the main fibre from this fungus) added to the diet was found to reduce lipoperoxidation and increase the activity of antioxidant enzymes (Bobek & Galabov, 2001). Glucans, a structurally diverse group of glucose polymers from microbial or fungal sources (Augustín, 1998), may act as effective free radical scavengers (Patchen et al., 1987). In addition, glucans possess "biological response modifier" properties (Bohn & Bemiller, 1995). Their immunomodulating effects include the capacity to render hosts more resistant to infections, radiation and neoplasms (Chihara et al., 1982; Deluzio & Williams, 1985).

Melatonin is a neurohormone synthesised predominantly in the pineal gland and involved in the control of circadian and photoperiodic systems in mammals (Reiter, 1991). Synthesis and secretion is strictly rhythmic, high concentrations are found during the dark time and low during the light time. In addition to the pineal gland, melatonin production was confirmed in other tissues, like the retina and Harderian glands (Reiter, 1995). High melatonin concentrations were found in the gastrointestinal tract (GIT) of all species studied (Bubenik et al., 1998) and enterochromaffine cells of GIT were suggested to have a capacity to synthesize melatonin (Quay & Ma, 1976). The high melatonin content in GIT might be of physiological significance since melatonin modulates gastrointestinal functions, such as food transit time (Bubenik & Dhavanantari, 1989) and gut motility (Harlow & Weekly, 1986).

Recent data showed melatonin to be one of the most potent antioxidants (for a review, see Reiter et al., 1997). Besides its direct radical scavenger activity, melatonin stimulates several antioxidant enzymes, e.g. superoxide dismutase (Antolin et al., 1997) glutathione peroxidase (Severynek et al., 1996) and glutathione reductase (Barlow-Walden et al., 1995). Owing to the high content of gastrointestinal melatonin and its antioxidant property, possible protective effects of this compound in the GIT are of special importance. Several studies indicate that pharmacological doses of melatonin can protect against gastric injury induced by different pathological attacks (De La Lastra et al., 1997; Konturek et al., 1997). Moreover, melatonin was found to eliminate most signs of experimental colitis induced by dextran sodium sulphate (DSS) in mice and serum melatonin concentrations were increased in mice with induced colitis (Pentney & Bubenik, 1995).

Based on these findings, it was of interest to study the possibility of endogenous melatonin involvement in the nutritional antioxidant treatment of colitis induced by intraluminal administration of acetic acid in rats.

Material and methods

General procedure

Male Wistar rats with initial body weight of 170–185 g were housed in wire-mesh cages and given standard laboratory rodent diet and tap water ad libitum. Lighting regimen 12 hours light and 12 hours dark was used and samples were taken during the light time. The rats were acclimatised for one week before experiment and then randomly assigned to three groups (15 animals in each group): the control group was fed standard laboratory rodent diet (control diet), the second group was given the control diet with pleuran hydrogel prepared by 1:5 dilution of 2.2% pleuran suspension in water as the only source of drinking, and the third group received the chow supplemented by 4% extract of black elder (Sambucus nigra). After four-week feeding of the above-mentioned diets, colitis was induced by intracolonial instillation of acetic acid (Fedorak et al., 1992; Nosálová & Bauer, 1996).

Induction of colitis

The animals were weighed and anaesthetised by thiopental (50 mg/kg i.p.). After laparatomy, 4% acetic acid (10 animals in each group) or saline (5 animals in each group) was administered in a volume of 2 mL.
After 50 sec exposure, the excess fluid was withdrawn and the abdomen was sutured. The rats were allowed to recover with food and water and the resulting injury was assessed after 48 hours.

Assessment of colonic damage
The rats were weighed, inspected for the presence of diarrhea and sacrificed by decapitation in light diethyl ether anesthesia. The colon was excised and opened longitudinally, rinsed with cold saline, and observed under a dissecting microscope. The colonic damage was scored using the criteria of Wallace et al. (1985): 0) no damage, 1) hyperaemia, 2) hemorrhage, 3) one ulcer, 4) two or more ulcers, 5) ulcer extending > 1 cm along the length of the colon, 6–10) if damage covered > 2 cm along the length of the colon, the score was increased by 1 for each additional cm. The scoring of damage was performed by an observer unaware of the treatment.

Melatonin assay
The pineal gland and samples of duodenal and colonic tissue were taken for the determination of melatonin concentrations immediately after colonic damage scoring and were kept along with plasma at −20°C until analysis. Melatonin was measured by radioimmunoassay (Fraser et al., 1983), directly in plasma and after methanol and chloroform extraction in pineal glands and gut tissue, respectively. Details of extraction are given elsewhere (Vician et al., 1999). Sheep anti-melatonin serum (G/S 704-8486 Stockgrand Ltd., Guildford, UK), and 3H-labelled melatonin with a specific activity of 1.3 TBq/mmol (NEN Life Science Products, Bad Homburg, Germany) were used. The parallelism of regression between the standard curve and successive dilutions (n = 5) of blood and an extracted homogenate of tissue showed an absence of interference in the assay. The sensitivity of the method was 2.5–3.0 pg/tube.

Chemicals
Pleuran was kindly provided by Ing. Kuniar, the co-author of the isolation procedure and the author of the relevant patent (1992). Pleuran was isolated from fresh Pleurotus ostreatus fruiting bodies by extraction with 0.15 M NaOH, the product was re-extracted by acetone and dried at 60°C. Extract of black elder is marketed under the commercial name “Coloring concentrate from black elder pomace” and its preparation is to be found in PUV 32/98. The pomace was extracted by 70% ethanol and the extract was vacuum-concentrated to the 50–60% content of dry matter. The product is supposed to be used for coloring or additional coloring of drinks, fruit, confectionery or bakery products.

Statistical analysis
The results were statistically evaluated by one-way analysis of variance with Tukey-Kramer multiple comparison test or by the Mann-Whitney U test.

Results
Intracolonic administration of acetic acid caused diffuse hyperaemia and bleeding with focal erosions and ulcerations. Pleuran supplementation in drinking fluid as well as black elder food supplementation significantly decreased the disposition to colitis. The macroscopic colonic damage score of 4.1±0.1 in the vehicle treated (control) rats exposed to acetic acid was significantly reduced to 1.4±0.3 after pleuran hydrogel (67.4% reduction) and to 2.3±0.6 (46.5% reduction) after black elder diet (Fig. 1).

Melatonin concentrations in the pineal glands and plasma were low as expected for samples collected during the light time (Fig. 2). Concentrations of melatonin in the duodenum (Fig. 3) were 10-times and in the colon (Fig. 4) 7-times higher than those in plasma.

Induction of colitis by acetic acid administration did not induce any changes in melatonin concentrations in comparison with vehicle treated rats (Tab. 1). A significant increase of melatonin concentration in the pineal gland (Fig. 2) was observed in the group given pleuran hydrogel (2.6 times higher than in the control group). Melatonin levels in rats treated with black elder in the diet were in the same range as those without any food supplementation. There were no significant differences in concentrations of melatonin in plasma or in the gut tissue.

Fig. 1. Colonic damage score (mean ± SEM) after acetic acid induced colitis in rats. Rats were without supplementation (C; n = 5) or supplemented by 0.44% pleuran (PL) in drinking water (n=10) or 4% extract of black elder (BE) in chow diet (n = 10). *P < 0.05 vs control.
in the intestinal wall between colitic treated and untreated rats (Figs. 2,3). The pattern of melatonin concentrations in plasma, duodenum and colon does not suggest that a potential melatonin synthesis would contribute to levels found in the general circulation under our experimental conditions.

**Discussion**

Several studies have suggested that melatonin is effective in the prevention or treatment of gastric (Konturek et al., 1997) or gut injury (Pentney & Bubenik, 1995) in rats. The mechanism of protective action of melatonin has not yet been elucidated but the diminution of free radical production and modulation of gut blood flow have to be considered. An antagonistic effect of melatonin against serotonin is also possible since overproduction of serotonin is connected with several pathological situations in the gut (Bubenik et al., 1998). In all studies demonstrating protective effects of melatonin in the gut, high pharmacological concentrations were used. It is however not clear whether endogenous melatonin present in the gut may exhibit a protective action under physiological conditions. Our data demonstrated that melatonin concentrations in gut tissue were substan-
Table 1. Melatonin concentrations (mean ± SEM) in rats supplemented with pleuran in drinking water or black elder in the food and treated with acetic acid to induce colitis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Pineal gland (pg/gland)</th>
<th>Plasma (pg/mL)</th>
<th>Duodenum pg/g of wet tissue</th>
<th>Colon pg/g of wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>10</td>
<td>259 ± 44</td>
<td>38 ± 11</td>
<td>281 ± 58</td>
<td>172 ± 63</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>218 ± 16</td>
<td>15 ± 4</td>
<td>245 ± 88</td>
<td>220 ± 79</td>
</tr>
<tr>
<td>Pleuran 0.44% hydrogel</td>
<td>10</td>
<td>163 ± 26</td>
<td>33 ± 9</td>
<td>198 ± 55</td>
<td>176 ± 34</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>157 ± 38</td>
<td>47 ± 22</td>
<td>303 ± 103</td>
<td>243 ± 58</td>
</tr>
<tr>
<td>Black elder 4% in diet</td>
<td>10</td>
<td>333 ± 52</td>
<td>24 ± 9</td>
<td>216 ± 78</td>
<td>214 ± 83</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>303 ± 135</td>
<td>37 ± 18</td>
<td>322 ± 116</td>
<td>200 ± 40</td>
</tr>
</tbody>
</table>

Partialy higher than those in the circulation. Moreover, significantly higher melatonin concentrations were found in pineal glands of rats treated by pleuran in comparison with control and black elder treated rats, and the same trend was seen also in plasma and duodenum. The highest melatonin levels paralleled the highest protective effect of pleuran and it is possible that endogenous melatonin is involved in mediating the protective effect of pleuran hydrogel, but not of black elder, against inflammation.

Owing to low concentrations of endogenous melatonin, its direct scavenging activity against ROS may have been less important for the affected animals. A more substantial role of melatonin involves stimulation of antioxidant enzymes, as well as immunological effects (Bubenik et al., 1998). Moreover, melatonin may influence GIT activity through interactions with the enteric nervous system, which controls complex contraction patterns of the gut, transport across the mucosal lining and intramura blood flow (Wood, 1999). It was demonstrated (Merle et al., 2000) that endogenous melatonin was physiologically involved in the control of pre- and postprandial motility of the gut at night, and this effect may be manifested through interaction with the neurohorome cholecystokinin (Benouali-Pellissier, 1994).

We observed no significant changes in melatonin content in plasma, pineal glands, and GIT after induction of colitis by acetic acid administration. This finding does not corroborate data of Pentney & Bubenik (1995) who found 10-times higher serum melatonin concentrations in rats with colitis induced by DSS. The different model of colitis may explain the discrepancy and further studies are needed to elucidate this problem.

Our results showed that nutritional supplementation both in drinking fluid and food significantly increased resistance to acetic acid induced colitis. The effect of the diet used was confirmed by an about one-half reduction of the colonic macroscopic damage score. In our previous experiments (Bobek et al., 2001), the antioxidant effect of orally administered pleuran and of black elder was documented by significantly lower levels of the primary products of lipoperoxidation, conjugated dienes, in the colon, liver, and erythrocytes. The changes of antioxidant enzyme activity were not significant, except the increased activities of SOD and GST. The activities of the antioxidant enzymes studied were substantially lower in the colon than in erythrocytes or liver, corresponding with the finding of Blau et al. (1999) who observed a difference in the reducing power along the rat gastrointestinal tract with the lowest antioxidant capacity in the colon. This may facilitate ROM-induced injury in the colon and lead to inflammation disease such as ulcerative colitis.

In the colonic mucosa of patients with ulcerative colitis and Crohn’s disease, an increased content of ROM positively correlated with the intensity of the disease (Simmonds et al., 1999).

A diet containing black elder extract may also increase the antioxidant defense of the organism. The crucial antioxidant agents of the black elder extract are flavonoids, phytochemicals that cannot be synthesised by humans. The six classes of flavonoids vary in their structural characteristics. Some of them have been shown to attenuate colonic damage: e.g. the orally administered phycoerythrin extract was found to reduce myeloperoxidase activity and colonic damage in colitis induced by acetic acid (Gonzales et al., 1999). The synthetic flavonoid DA-6034 decreased the lesion score more effectively than did prednisolon or sulfasalazine, used as standard therapy in ulcerative colitis (Kim et al., 1999). Similarly, the orally administered flavonoid morin decreased the colonic damage induced by TNBS in rats (Ocete et al., 1998). Endogenous melatonin does not appear to
have contributed to the protective effect of black elder feeding since its concentration remained unchanged.

In conclusion, these results indicate that oral pleuran and black elder diet supplementation may enhance the intestinal integrity and antioxidant defense of the colon. Such drugs of readily available source appear to be a promising supplement of elemental diets. In addition to their direct antioxidant action, pleuran hydrogel may affect melatonin concentrations in the gut, which can partially contribute to protection of mucosal cells against injury.

Acknowledgements

The authors are grateful to the Slovak Academy of Science for partially supporting this work by VEGA grant No. 1/8208/01 and GAV No. 6024. The authors would like to thank Mrs. H. Bobeková, D. Čepcová and A. Sochorová for technical assistance.

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Received June 25, 2001
Accepted September 14, 2001