Indian J Physiol Pharmacol 2002; 46 (2): 229–234

EFFECT OF MELATONIN AND BETA-CAROTENE ON INDOMETHACIN INDUCED GASTRIC MUCOSAL INJURY

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(Received on October 25, 2001)

Abstract : The study was conducted to examine the role of free radicals in Indomethacin induced gastric mucosal injury and to evaluate the gastroprotective effects of melatonin and beta–carotene. Gastric mucosal injury was produced in rats by administering indomethacin 30 mg/kg subcutaneously. Melatonin was administered in three different doses of 5, 10 and 20 mg/kg, 30 minutes prior to the administration of indomethacin. Beta–carotene was administered as a single dose of 100 mg/kg. Following parameters were calculated: ulcer index, lipid peroxidation and antioxidant defense enzymes i.e. superoxide dismutase, glutathione peroxidase and catalase. Indomethacin caused gastric mucosal injury in the form of haemorrhages, increased the lipid peroxidation and decreased the levels of the antioxidant defense enzymes. Melatonin (20 mg/kg) and beta–carotene decreased the ulcer index and lipid peroxidation, and reduced the decrease in antioxidant enzyme levels. These findings suggest the melatonin and beta–carotene show protective effect against indomethacin induced gastric injury and this effect is mediated by scavenging of oxygen derived free radicals.

Key words : indomethacin, gastric injury, free radical, melatonin, beta-carotene, rats

INTRODUCTION

It has been recently proposed that agents, like melatonin (1), beta-carotene (2), allopurinol (3) and superoxide dismutase (4). protect the gastrointestinal mucosa against NSAID and ethanol-induced injury. Beta-carotene, an antioxidant, has been shown to prevent the gastric mucosal injury caused by ethanol induced oxidative stress (2). Similarly, melatonin has been shown to reduce the gastroduodenal injury caused by ethanol in rats (1). However, some studies have shown that lipid peroxidation might not be an important process in the development of mucosal lesions due to NSAIDs (5). Some other studies have failed to show the protective effect of beta-carotene (6), allopurinol (7) and superoxide dismutase (7) against gastric mucosal injury caused by ethanol in rats. These findings are thus, not unequivocally accepted.

Till date, the therapeutic potential of antioxidants for gastric mucosal injury caused by NSAIDs has not been firmly

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established, so the present study was aimed to confirm the role of antioxidants: beta-carotene and melatonin in gastric mucosal injury induced by a NSAID (indomethacin).

METHODS

Subjects

Male wistar rats (150–200 g) housed under standard laboratory conditions with 12 hour day/night cycle with food and water ad libitum, were used. The experiments were initiated only after the approval of the Institutes Ethics committee. The animals were kept fasting for 48 hours prior to the experiment. The rats were divided into seven treatment groups of 12 animals each. Out of each group, 6 animals were used to calculate ulcer index and 6 to assess lipid peroxidation alongwith free radical enzyme activity.

Administration of Drugs

Different groups of animals were administered melatonin (5, 10 and 20 mg/kg; intragastrically) and B-carotene (100 mg/kg; intragastrically). Melatonin was dissolved in 0.25% carboxymethyl cellulose (CMC) and B-carotene in vegetable oil. Drugs were given 30 minutes prior to administration of indomethacin. For each treatment, respective vehicle controls were used.

(i) Melatonin dissolved in 0.25% carboxymethyl cellulose (CMC) was administered in doses of 5, 10 and 20 mg/kg.

(ii) Beta-carotene dissolved in vegetable oil as vehicle was administered in single dose of 100 mg/kg in the other group, 30 minutes prior to indomethacin administration.

Indomethacin was administered subcutaneously in a dose of 30 mg/kg dissolved in normal saline with 100 µl of tween-80. The animals were sacrificed 6 hours after indomethacin administration. Saline control for this model was used by administration of 0.2 ml of normal saline subcutaneously.

Macroscopic examination

The stomach was cut open along the greater curvature and washed in normal saline. The it was laid flat and ulcer area calculated under a dissecting microscope (x10) with a square grid. Gastric mucosal lesions were seen in the form of haemorrhages or linear breaks.

Ulcer index was calculated using the following method (8):

Ulcer index = 10/X

Where X = Total mucosal area/Total area of mucosal lesions.

Preparation of Gastric Mucosal Homogenate

The stomach was removed and kept in ice-cold phosphate buffer (pH 7.2). It was cut along greater curvature and washed with ice-cold phosphate buffer to remove the debris. The gastric mucosa was scraped with a plastic scraper, weighed and homogenized by a hand homogeniser in phosphate buffer to prepare 4.5 ml homogenate. The homogenate was used for the assay of lipid peroxidation and protein concentration.

The homogenate was sonicated for two minutes on ice and ultracentrifuged at 20,000 g for 20 minutes. The supernatant (cytosolic fraction) was used for the estimation of the antioxidant enzymes and protein concentrations.

Estimations

Measurement of proteins and lipid peroxidation :

Protein was estimated according to the method of Lowry et al (9) using bovine serum albumin as the standard. The extent of lipid peroxidation was measured by assaying malondialdehyde (MDA) formation according to the method of Utley et al (10).

Antioxidant defense enzymes :

Superoxide dismutase (SOD) activity was estimated by the method of Kono (11).

Glutathione peroxidase (GPx) activity was determined by following the procedure of Paglia and Valentine (12) as modified by Lawerence and Burk (13) with H_2O_2 as a substrate.

Catalase was assayed according to the method of Veers and Sizers (14).

Statistical analysis

All the results were expressed as mean ± SD. One-way analysis of variance with post-hoc scheffe's test was used to analyse ulcer index, lipid peroxidation and enzyme assays. 'P' value of <0.05 was considered significant.

RESULTS

Ulcer Index

Melatonin administered in doses of 5, 10 and 20 mg/kg orally, caused a significant and dose–dependent decrease in ulcer index as compared to vehicle control (0.15 ± 0.02, 0.11 ± 0.03 and 0.06 ± 0.02, respectively Vs 0.21 ± 0.02; $P<0.05$) (Table I). Similarly,

TABLE I: Effect of melatonin in indomethacin–induced gastric mucosal injury, values are in mean ± SD. (n=6)

Groups	*Ulcer Index*	*Lipid peroxidation nmol MDA/mg*	*SOD U/mg protein*	*GPx nmol/mg protein*	*Catalase U/ml protein*
Saline Control	0	1.7±0.5	5.7±1.3	170.8±23.6	20.3±1.7
Vehicle control	0.21±0.02*	3.6±0.8	3.6±1.2*	59.3±11.4	12.4±3.1*
Indomethacin (30 MG/KG)+ Melatonin (5mg/kg)	0.15±0.01*	3.4±0.3	3.8±0.7	69.4±8.6	14.6±1.7
Indomethanin (30 mg/kg)+ Melatonin(10mg/kg)	0.11±0.03*	2.5±0.4	4.0±0.9	90.6±15.6*	14.6±1.8
Indomethacin (30 mg/kg)+ Melatonin (20 mg/kg)	0.06±0.02*	2.3±0.3*	4.8±1.0	147.4±15.9*	18.2±2.1*

* = P<0.05 vs saline control
* = P<0.05 vs vehicle control

TABLE II: Effect of betacarotene on indomethacin–induced gastric mucosal injury, values are presented as mena ± SD. (n=6).

Groups	*Ulcer Index*	*Lipid peroxidation (nmol MDA/mg)*	*SOD (U/mg protein)*	*GPx (nmol/mg protein)*	*Catalase (U/mg protein)*
Saline control	0	1.7±0.5	5.7±1.3	170.8±23.6	20.3±1.7
Vehicle control	0.18±0.03*	3.7±0.7	3.1±0.6*	58.5±8.6*	12.1±2.9*
Indomethacin (30 mg/kg)* Betacarotene (100 mg/kg)	0.07±0.02#	2.3±0.4#	4.7±1.0	149.5±15.3#	18.3±1.8#

* = P<0.05 vs saline control
# = P<0.05 vs vehicle control

Beta-carotene administered in a single dose of 100 mg/kg caused a significant decrease in ulcer index as compared to the control (0.07 ± 0.02 Vs 0.18 ± 0.03; P<0.05) (Table II).

Biochemical parameters

i) *Lipid peroxidation :* Melatonin reduced indomethacin induced increase in lipid peroxidation as compared to the vehicle control (Table 1). However, this reduction was statistically significant only at 20 mg/kg dose of melatonin. (2.3 ± 0.3 Vs 3.6 ± 0.8 nmol MDA/mg protein; P<0.05). Beta-carotene administered in a single dose of 100 mg/kg caused a significant decrease in lipid peroxidation as compared to the control (2.3 ± 0.4 Vs 3.7 ± 0.7; P<0.05) (Table II).

ii) *Antioxidant defence enzymes* (SOD, GPx, Catalase) : Melatonin prevented the reduction in the level of SOD enzyme but this was not significant at any of the doses used in comparison to the vehicle control. The 20 mg/kg dose of melatonin significantly prevented the reduction of GPx levels by indomethacin (147 ± 15.9 Vs 59.3 ± 11.4 nmol/mg protein; P<0.05) and also prevented the reduction of catalase (18.2 ± 2.1 Vs 12.4 ± 3.1; P<0.01). Beta-carotene caused an increase in concentration of SOD but this was not significant as compared to vehicle control. It caused a significant increase in GPx levels (149.5 ± 15.3 Vs 58.8 ± 8.6; P<0.05) and catalase levels (18.3 ± 1.8 Vs 12.1 ± 2.9; P<0.05) when compared to vehicle.

DISCUSSION

Indomethacin a non-steroidal anti-inflammatory drug is known to induce erosions and ulcers in the gastrointestinal tract. Although it has been proposed that a deficiency of endogenous prostaglandins due to inhibition of cyclo-oxygenase enzyme by indomethacin is involved in these effects, the exact pathogenic mechanism remains to be elucidated. Much recent attention has been focused on the role of reactive oxygen species in mediating indomethacin-induced gastric mucosal injury (15).

The results of the present study show that indomethacin caused a significant

increase in lipid peroxidation along with gastric mucosal injury. The levels of antioxidant defense enzymes also decreased following administration of indomethacin. These results are in accordance with a previous report of Yoshikawa et al (16) and Tanaka et al (17) who showed that the area of gastric erosions and the amount of thiobarbituric acid reactive substances in gastric mucosal were significantly increased after indomethacin administration.

In a recent study, Alarcon et al demonstrated the protective effect of melatonin on indomethacin induced gastric mucosal injury (18). It was shown that pretreatment with melatonin resulted in a significant increase of glutathione peroxidase levels which was decreased by indomethacin. This was accompanied with a decrease in ulceration. Also, the increased thiobarbituric acid reactants after indomethacin administration were inhibited by melatonin. Our findings confirm that of the mechanisms by which indomethacin causes gastric mucosal injury is by generation of free radicals and increased lipid peroxidation and melatonin may have gastroprotective effect due to its free radical scavenging properties.

Like melatonin, single administration of beta-carotene (100 mg/kg) significantly reduced the area of gastric mucosal injury and reduced the increase in lipid peroxidation. In addition to this, beta-carotene also significantly prevented the reduction of antioxidant defense enzymes namely superoxide dismutase, glutathione peroxidase and catalase, induced by indomethacin.

The above results lead us to conclude that indomethacin-induced damage of the gastric mucosa can be counteracted by melatonin or beta-carotene and the antioxidants might play an adjuvant role in the pharmacotherapy of gastric ulcers.

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