Pharmacological Research 64 (2011) 249–257

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Melatonin independent protective role of L-tryptophan in experimental reflux esophagitis in rats☆

Pratibha Singh[a], Neetu Singh[a], Ausaf Ahmad[a,b], Omprakash Singh[a], Gautam Palit[a,*]

[a] Division of Pharmacology, Central Drug Research Institute, CSIR, Lucknow 226001, Uttar Pradesh, India
[b] Amity Institute of Biotechnology, Lucknow campus, Amity University, Lucknow 226016, India

ARTICLE INFO

Article history:
Received 1 November 2010
Received in revised form 6 March 2011
Accepted 11 April 2011

Keywords:
L-Tryptophan
Melatonin
Gastroesophageal reflux disorder
PGE_2
Arylalkylamine-N-acetyltransferase

ABSTRACT

Melatonin is implicated in sustaining the esophageal integrity in gastro-esophageal reflux disease. However, the role of its synthetic precursor L-tryptophan is not clear in this pathology. The present study was designed to explore the effects of L-tryptophan on esophageal damage following reflux esophagitis (RE)-establishment and concurrent alterations in factors possibly influencing esophageal integrity such as esophageal melatonin level, luminal acidity, H^+K^+-ATPase activity, mucin and gastric PGE_2 levels. RE was established in rats by simultaneous ligation of pylorus region and fore-stomach. RE significantly decreased the esophageal-melatonin level and the expression of its synthesizing enzymes: arylalkylamine-N-acetyltransferase (AA-NAT) and hydroxyindole-O-methyltransferase (HIOMT). Administration of L-tryptophan significantly decreased the RE-induced esophageal mucosal damage, without altering the levels of melatonin. L-Tryptophan pretreatment also normalized the esophageal mucosal damage caused by melatonin receptor antagonist-luzindole. Simultaneously, L-tryptophan significantly increased the RE-decreased expression of AA-NAT with insignificant effect on HIOMT gene expression. In contrast, L-tryptophan *per se* caused a significant elevation in the esophageal melatonin level, with no significant effect on the expression of AA-NAT and HIOMT enzymes. Further, L-tryptophan significantly normalized the RE-induced changes in the gastric juice volume, acidity and pH. However, it did not significantly inhibit the H^+K^+-ATPase activity in vitro. Also, L-tryptophan significantly increased the RE-reduced mucin level, COX-2 activity and thereby PGE_2 levels. Interestingly, indomethacin (PGE_2 synthesis blocker), aggravated the RE-induced tissue injury with simultaneous changes in the gastric volume, acidity, pH and mucin content, which L-tryptophan failed to reverse, suggesting that the attenuating effect of L-tryptophan on gastric secretions could be PGE_2 driven. Thus the current study provide evidences that protective functions of L-tryptophan against RE is independent of its conversion into melatonin, and possibly involve mobilization of factors such as COX-2 derived PGE_2 and mucin that counterbalance the detrimental effect of gastric acid on esophageal mucosa, signifying the therapeutic efficacy of L-tryptophan against the esophageal pathologies.

1. Introduction

Gastroesophageal reflux disorder (GERD) is classified as a functional gastrointestinal (GI) disorder, in which gastric acid refluxes into the esophagus, causing irritations and inflammation of its mucosa. The prevalence of GERD is ranging from less than 5% in Asia to 10–20% in the Western world [1]. It is also reported that chronic heart burn-symptoms may lead to the development of oesophageal adenocarcinomas [2].

The present mainstay therapy for GERD is based on gastric acid-suppression, and healing of injured oesophageal mucosa to prevent further complications [3]. Although these therapies are effective in producing acute symptom relief and mucosal healing, there is still a need of alternative treatment strategies because of their limitations such as daily administration, failure to provide complete symptom relief and frequent relapses following drug withdrawal [3].

The major breakthrough in the search for alternative approaches for GERD treatment is the discovery of the protective influences of melatonin in GERD patients [4,5]. Supplementation of melatonin, alone or in combination with acid suppressant is found to bring rapid regression of GERD associated symptoms [6]. Moreover, the esophageal erosions in GERD are associated with declined levels

Abbreviations: RE, reflux esophagitis; AA-NAT, arylalkylamine-N-acetyltransferase; HIOMT, hydroxyindole-O-methyltransferase; PGE_2, prostaglandinE_2; GERD, gastroesophageal reflux disorder; GI, gastrointestinal; GIT, gastrointestinal tract.

☆ CDRI communication no. 8050.

* Corresponding author. Tel.: +91 522 2612411 418x4303; fax: +91 522 2623405/2623938.
E-mail addresses: pratibha02in@gmail.com (P. Singh), gpalitcdri@gmail.com (G. Palit).

1043-6618/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phrs.2011.04.004

of circulating melatonin, signifying its role in the protection of esophageal integrity [7].

Melatonin has been isolated primarily from the pineal gland [8], but following studies have shown its widespread occurrence in other organs also such as GIT, suprarenal gland, thyroid gland, thymus, placenta, etc. [9,10]. Besides the presence of melatonin in enterochromaffin (EC) cells [11], its receptors and biosynthetic enzymes arylalkylamine-N-acetyltransferase (AA-NAT; EC 2.3.1.37) and hydroxyindole-O-methyltransferase (HIOMT; EC 2.1.1.4) were also detected in GIT [12,13]. The clinical applicability of melatonin in the treatment of GERD patient and the evidences of its possible role in the maintenance of esophageal integrity intrigued us to evaluate the role of its precursor, L-tryptophan in the esophageal pathology. Also in GERD subjects, a supplementary formulation containing L-tryptophan were administered and found extremely effective in reducing the GERD associated symptoms [4]. The author's rationale of using it was to alleviate the pain by enhancing the availability or efficacy of neuro-inhibitor noradrenalin and serotonin. Several studies indicate that GIT loading of L-tryptophan, increases the levels of circulating melatonin in the day time [14,15]. It was also hypothesized that the protective influences of L-tryptophan may depend on its conversion to melatonin [16,17]. Thus, it is essential to understand the intricate relationship between L-tryptophan and melatonin system in the protection of esophageal integrity and to elucidate their mode of function by deciphering their synergism or independent action. In the current study we also focused an important issue that the effect of L-tryptophan on the melatonin levels in the esophagus is subjected to change under pathological conditions such as RE when the esophagus mucosal tissue become injured.

In this regard, our finding address following two issues. Our first concern was to evaluate the effect of oral administration of L-tryptophan on the esophageal melatonin and expression levels of AA-NAT and HIOMT. It is well perceived that RE is not a simple consequence of acid accumulation, and a number of factors possibly governing through L-tryptophan are involved. Thus, our second issue was to evaluate that whether the changes in L-tryptophan and/or melatonin system would lead to the protection against RE associated mucosal injury, and if so, these systems work synergistically or independently. Hence, parallel perturbations in factors influencing esophageal integrity such as luminal acidity, H^+K^+-ATPase activity, mucin and gastric mucosal PGE_2 level were examined in all the groups.

2. Materials and methods

2.1. Materials

All the chemicals used in the study were obtained from M/s. Sigma Chemicals, St Louis, MO, USA unless otherwise mentioned.

2.2. Animals

Experimental protocols were approved by the Institutional Ethical and Usage Committee of Central Drug Research Institute (CDRI), Lucknow, following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Adult Sprague Dawley rats, weighing 180–220 g procured from National Laboratory Animal Centre, CDRI, were used in the study. Rats were housed three to four per cage, in a room with temperature regulated at $22 \pm 2\,^{\circ}C$, with a 12 h/12 h light/dark cycle (lights on 07:00 h, lights off 19:00 h). Standard chow pellets and water were given *ad libitum*, except during the period when food deprivation was applied.

2.3. Induction of reflux esophagitis

Animals were deprived of food for 18 h before induction of esophagitis but water was provided *ad libitum*. RE was induced in rats according to the method described earlier [18] with minor modifications. Briefly, under pentobarbital anesthesia (30 mg/kg, i.p.) the abdomen was incised along the midline and then both the pyloric end of the stomach and limiting ridge (transitional region between the fore stomach and corpus) were simultaneously ligated tightly, resulting in the reflux of the gastric juice into the esophagus. After 5 h of ligation animals were killed, esophagus was removed and incised lengthwise. Haemorrhagic lesions were observed under Trinocular zoom microscope (SZ-CTV Olympus) and area of lesions (sq.mm) developed was measured using Biovis image analyzer software (Expert Vision Lab Private Ltd., Mumbai, India).

2.4. Experimental procedure

After 1 week of acclimatization, the rats were randomly divided into various groups, each consisting of 6 animals.

Normal control: Rats underwent sham operation and were not given any treatment.

Reflux esophagitis control: RE was induced in rats as described above (RE) and given same vehicle solutions as used for the dissolution of respective drugs and used for the purpose of comparison accordingly.

L-tryptophan per se group: Normal rats were pretreated with L-tryptophan (100–400 mg/kg) alone to evaluate drug *per se* effect (L-100–400 *per se*).

Treatment groups: Rats were treated with graded doses of L-tryptophan (100–400 mg/kg; L-100–400 + RE), omeprazole (10 mg/kg; Omz + RE) and indomethacin (5 mg/kg; IND + RE), 45 min prior to the induction of RE. The dose of omeprazole and indomethacin was selected on the basis of our pilot studies and available literature [19,20].

In a separate experimental group, rats were pretreated with indomethacin (5 mg/kg) followed by L-tryptophan (200 mg/kg) 45 min prior to the induction of RE (IND + L-200 + RE).

Additionally, rats were divided into two more groups: in one group rats were treated with melatonin receptor antagonist-luzindole (2 mg/kg) 45 min prior to the induction of (LUZ + RE). In another group rats were pretreated with luzindole followed by graded doses of L-tryptophan (100–400 mg/kg) 45 min prior to RE-induction (LUZ + L-100–400 + RE).

L-Tryptophan was dissolved in normal saline with a drop of 0.1 HCl. Omeprazole was suspended in aqueous solution containing 0.5% carboxymethyl cellulose (CMC). L-Tryptophan and omeprazole were administered orally using a ball ended feeding needle. Both L-tryptophan and omeprazole were administered to animals at a volume of 1 ml/200 g body weight. Indomethacin was dissolved in the minimal amount of DMSO and diluted in water. Indomethacin was administered subcutaneously. Luzindole was dissolved in DMSO, diluted in water and given intraperitoneally (i.p.).

2.5. Estimation of melatonin and L-tryptophan level in esophageal mucosa

Immediately after 5 h of RE-induction, rats were killed by inhalation of anesthetic ether, and the esophageal tissues were immediately removed. The esophageal tissues were first weighed and 200 ng/ml of internal standard HPA (4-hydroxyphenylacetic acid) was added to each sample. Homogenization was performed in 0.1 M perchloric acid with an Ultra-Turrax homogenizer (Model T25, IKA-Laborthechnik, Germany). Homogenates were then centrifuged at $13\,200 \times g$ (Sigma centrifuge, model 3K30, USA) at $4\,^{\circ}C$,