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Research Article

ADMINISTRATION OF THREONINE PREVENTS STRESS-INDUCED ULCERS INVOLVING INTRASPECIES EMOTIONAL COMMUNICATION IN A MURINE ULCEROGENIC MODEL

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ABSTRACT

Psychological stress was produced by intraspecies emotional communication in a jumping box. Mice were randomly divided into two groups, one was used as the 'sender' group that received electrical foot shocks (10 s duration with 50 s intervals; starting at 0.2 mA and gradually increased to 25 mA; for 3h/day till 3 days) and showed emotional responses such as abnormal squealing and jumping. The other group called the 'responder' group were able to observe the 'sender' group mice but were not subjected to the shocks & they were sacrificed and were observed for the presence of gastric leisons. Separate groups of mice were treated half an hour before with Threonine (25, 50, and 100 mg/kg, i.p.) The results revealed marked ulcers in the responder's mice with mean ulcer index of 1.1 ± 0.75. Acute administration of Threonine (50 and 100 mg/kg) marked prevented the induction of stress ulcers with mean ulcer indiex of 0.125 ± 0.25. However, the lower dose was found to be ineffective. Histopathology corroborated the results. The anti-stress activity of hydrogen sulfide precursor Threonine in mice.

Key words: Conditioned emotional stimuli; Gastric mucosal lesions, ulcer index, hydrogen sulphide, H & E staining.

INTRODUCTION

Increasing demands and overwhelming environmental stimuli in our modern society continuously heighten the stress level of humans and escalate the pathogenesis of stress-associated illness. When demands imposed by events exceed a person's ability to cope, a psychological stress response composed of negative cognitive and emotional states is elicited [1].Hydrogen sulfide (H_2S) is generally known as a toxic (in high concentration) and colourless gas with a strong odour produce from a variety of environments, but it is also found in mammalian tissues, where it is generated during cysteine metabolism [2]. H_2S is formed in

mammalian cells by the activity of two pyridoxal phosphate –dependent enzymes: cystathionine – γ lyase (CSE) and cystathionine β-synthetase (CBS) [3]. Hydrogen sulfide is a gaseous neurotransmitter and amongst pleotropic effects it is proposed to be anti-stress, anti-inflammatory and mucosal blood flow regulator. In addition, controversy exists over its role in the process of ulcerogenesis. It is reported that administration of H_2S modulators reduces the amount of cortisol secretion from adrenal gland after subjection to stress [4]. Similar to other gaseous mediators (NO and CO), H_2S seems to present paradoxical effect in the inflammatory process;there is evidence that it is involved both as an agent preventing tissue damage ,leucocyte migration,and edema [5].

Gastric mucosa expressed both CSE and CBS, which have the ability to mediate H_2S synthesis[6]. In the gastrointestinal tract (GI), recent studies suggest that H_2S may contribute to mucosal defence against injury caused by nonsteroidal anti-inflammatory drugs, and it is also seems to play a significant role in regulating gastric mucosal blood flow. The mechanism through which H_2S exerts these anti-inflammatory properties are not fully understood. However , the involvent of ATP-sensitive potassium K_{ATP} channel activation [7] . In contrast, Wallace demonstrated that H_2S promotes healing of gastric ulcers in rats by a mechanism that is not associated with K_{ATP} channel, because neither glibenclamide(K_{ATP} channel antagonist) nor pinacidil(K_{ATP} channel activator)affected ulcer healing.

The maintenance of GI mucosal integrity depends on protective mechanisms in the face of pending injury. Capsaicin sensitive sensory afferent fibers subserve this goal through different mechanisms. The protective responses triggered by sensory neurons comprise alterations in GI blood flow, secretion and motility as well as modifications of the immune function [8]. However, there are only a few correlative reports between H_2S and sensitive sensory afferent neurons and the involvement of the former upon GI functions. Considering the limited information on the pathophysiological role of H_2S on gastric damage, we evaluated the protective effect of H2S on psychological stress induced gastric lesions in mice.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade. They were products of Loba Chemie Pvt. Ltd. Mumbai.

Animals.

Male Swiss mice (20-25g) were used in this experiment. 20-25 animals were housed in cage (32×28×20) in groups of 6 each and allowed free access to food and water. They were maintained in a breeding room at a temperature of 20˚-24˚C and relative humidity of about 60%.

Communication Box.

A Communication box apparatus was used to expose the mice to conditioned emotional stimuli (CES).This apparatus consisted of (25×25×25)cm. The floor of the communication box is equipped with grids for electrical shock. The inside is divided into small compartments (12.5×12.5cm), consisting of foot- shock compartments with a grid floor and non-foot-shock compartments with a grid floor covered by transparent plastic boards. The foot-shock compartments are arranged such as to surround the non-foot shock compartments.

Procedure

The experimental groups consist of the following 2 groups: sender group, responder group. Sender animals received a foot shock of 10 sec duration at intervals of 50 sec for 3 hour. The electric current for the shock is increased step-wise from 1.6 mA to 2.0 mA at a rate of 0.2mA per hour .Responders are exposed daily to the emotional responses of sender animals, 3h per day for 3 day sender animals are changed daily to naive mice to prevent a reduced emotional response to foot shock based on adaptation or learned helplessness due to repeated exposure. Both sender and responder animals are placed individually in each compartment of the communication box 15 min before beginning of the shock period. On day-1, responder animals are returned to their home cages after the 3 hr foot shock period. An day-2, after the completing of foot-shock period, they are transferred to metal cages and are housed in the cages with 4 animals per cages under food – deprivation condition. Food yoked control animals are maintained to the metal cage during the foot-shock period under the aggregated housing condition (5-animals each) and they are returned to the home cages after the foot-shock period. From beginning of the day-2 experiment, they are maintained in the metal cages under aggregating housing. On day-3, just after completing the foot-shock period, the responders are sacrificed by chloroform, and their stomach was removed. The stomach are visually inspected for lesions. Drug (Threonine) are administered orally with low (25mg/kg), medium (50mg/kg) and high (100mg/kg) dose on day-3,30min before the shock period.

Immediately after the end of CES, animals were decapitated under light either anaesthesia and laparotomized to isolate the stomach. The stomach was immersed in 10% formaldehyde for about 10 sec, incised along the greater curvature, and the existence or absence of gastric mucosal lesion was determined macroscopically.

RESULTS

Psychological stress induced ulcer model set up

The results revealed that exposure of responder mice to sender mice, which received 3 h of footshocks daily were given showed pronounced ulcer at the end of the protocol. The mean ulcer index was found to be 1.125 ± 0.75. It was associated with increased gastric volume secretion. Thus, the set up of the model was established successfully.

Effect of Threonine administration on the mean ulcer index of mice

The effect of Threonine administration on the mean ulcer index of mice is shown in fig. 1C. One-way ANOVA revealed a significant influence of Threonine treatment on the UI of mice [F (3, 15) = 4.633; P = 0.0255]. Further, analysis revealed that Threonine reduced the gastric erosions significantly at 50 and 100 mg/kg ($P < 0.05$), whereas it was ineffective at 25 mg/kg.

Effect of Threonine administration on the gastric pH of mice

The effect of Threonine administration on the gastric pH of mice is shown in fig. 1B. One-way ANOVA revealed a significant influence of Threonine treatment on the gastric pH of mice [F (3, 15) = 36.05; $P < 0.001$]. Further, analysis revealed that Threonine increased the gastric pH significantly at all doses ($P < 0.05$), with maximum increase observed at 100 mg/kg dose.

Effect of Threonine administration on the gastric volume of mice

The effect of Threonine administration on the gastric volume of mice is shown in fig. 1A. One-way

ANOVA revealed a significant influence of Threonine treatment on the gastric pH of mice [F (3, 15) = 4.622; P = 0.0227]. Further, analysis revealed that Threonine reduced the gastric volume significantly at 50 and 100 mg/kg ($P < 0.05$), whereas it was ineffective at 25 mg/kg.

Effect of Threonine administration on the histopathology

The results of haematoxylin and eosin staining are shown in Fig.2. The control mice exhibited marked mucosal damage. The mucosal lining appears to be completely eroded in the lesion area. Threonine administration appears to have preserved the intestinal preserved.

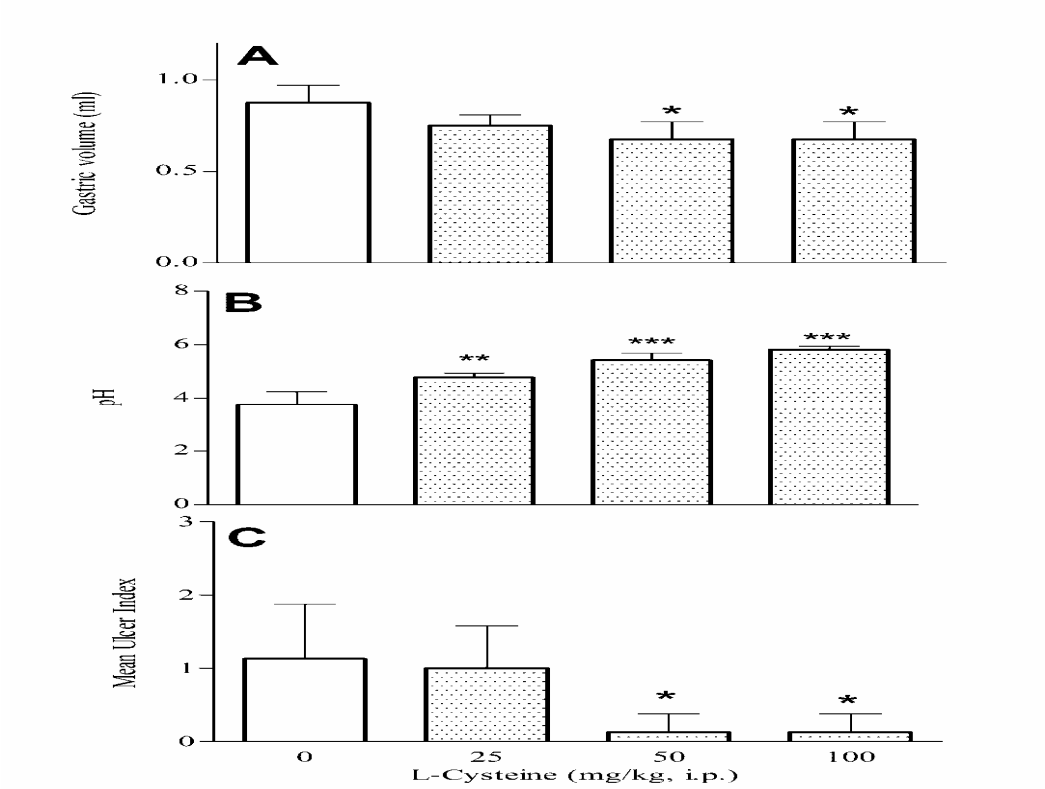

Fig.1. Influence of Threonine administration on the gastric volume (A), pH (B), and mean ulcer index (C) in psychological stress induced mice.

Different groups of mice were subjected to psychological stress in a communication box for 3 days and were treated with saline or Threonine (25, 50, and 100 mg/kg). At the end of 3 days each mice was sacrificed and evaluated gastric contents, gastric pH and gastric lesions. The gastric lesions were scored and mean ulcer index was calculated.

Each bar represents mean ± S.D. of five observations

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. Control group (One-way ANOVA followed by Dunnett's test)

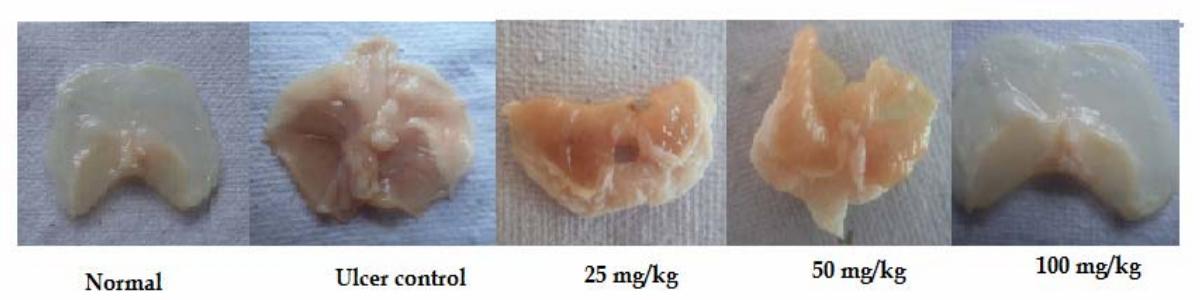

Fig. 2. Photographs of mice stomach exposed to psychological stress
Different groups of mice were subjected to psychological stress in a communication box for 3 days and were treated with saline or Threonine (25, 50, and 100 mg/kg). At the end of 3 days each mice was sacrificed, the stomach was cut along the greater curvature and the lesions were observed.

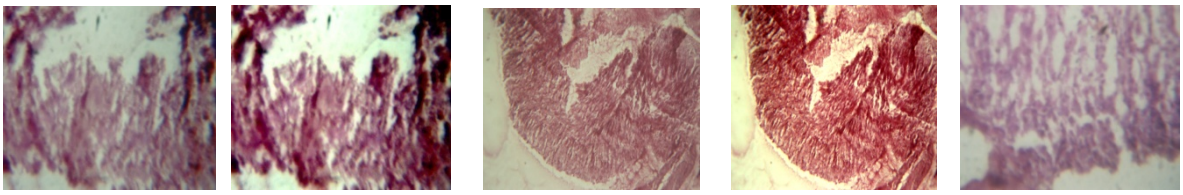

Fig. 3. Photomicrographs of mice exposed to psychological stress
Different groups of mice were subjected to psychological stress in a communication box for 3 days and were treated with saline or Threonine (25, 50, and 100 mg/kg). At the end of 3 days each mice was sacrificed, the stomach was cut along the greater curvature and the lesions were observed. The stomach were treated with series of alcohol and benzene and then fixed in paraffin. Later 10 µ sections were taken on a microtome. Fixed on slide and stained with haematoxylin and eosin and evaluated at 10 x and 40 x magnification.

DISCUSSION

Numerous reports have indicated the role of hydrogen sulphide and its precursors in the prevention of gastric ulcers induced by variety of chemicals. It is also reported to exhibit antistress effect in numerous animal models. Hence, this study evaluated the influence of Threonine administration, which is a substrate for hydrogen sulphide on psychological induced stress ulcers.

The psychological induced stress ulcer is a novel paradigm to screen the effect of centrally acting agents on ulcerogenesis. In this paradigm, intraspecies emotional communication is used to induce ulcers. A group of mice called the senders are exposed to foot shocks (0.2 mA – 25 mA) for 10 s duration every min for 3 h. During this period another group of mice are kept in the communication box, besides the sender mice and are called as responder mice. The foot shocks precipitate vocalization, urination, defecation and increased activity in the senders. Most important in the

conditioned stimulus i.e. the buzzer after which the shock ensues. After some time the responder mice become restless and stress is precipitated in them after the buzzer. Such trial continues for 3 days at the end of which the receiver group animals are sacrificed and evaluated for the presence of gastric ulcers. Different sender animals are used each day. This model appears excellent for the use of evaluation of antistress drugs on ulcerogenesis and hence was used in the present investigation.

The results revealed a dose dependent effect of Threonine administration on the prevention of psychological stress induced ulcers. Threonine administration increased the pH of the gastric fluid with a concomittent reduction in its content. It appears that Threonine administration has reduced the central stress response, which in turn resulted in the reduction of acid secretion which was accompanied by reduced gastric volume. The histological finding also support the above results. The incidence of gastric mucosal lesions of the responder was significantly suppressed by the administration of the conventional anti ulcer drug Threonine at 25 mg/kg, 50 mg/kg and 100 mg/kg. However dose at 50 mg/kg showed slight increase in gastric Ph but not suppress gastric mucosal lesion, but at dose 100 mg/kg gastric mucosal lesion suppress significantly. Thus the suppressive effect of anti-ulcer drug Threonine was more in dose at 100 mg/kg. When gastric mucosal lesion were made to responder showed stronger suppressive effect at dose 100 mg/kg. These results suggest the possibility that the central and peripheral actions of anti-ulcer drugs might be distinguished by the communication box method, and that there might be a difference in the mechanism of development of gastric mucosal lesion in responders. These points remain to be further studied with the use of other drugs. The communication box method, which produces psychological stimuli without using physical stimulus (like foot shock), is useful for the evaluationt of the effect of anti-ulcer agents.

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