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Gastroprotective potential of risperidone, an atypical antipsychotic, against stress and pyloric ligation induced gastric lesions

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ABSTRACT

Risperidone has been used in some stress disorders and may be potentially protective against stress-induced gastric lesions. Thus, the aim of the present study is to investigate, whether risperidone, a D_2 receptor and 5-HT_{2A} receptor antagonist, would be able to result in gastroprotective effect in stress-induced lesions and also explore the possible mechanism of action behind its gastroprotective activity. Gastroprotective activity of risperidone was evaluated both by single treatment and 21 days repeated (0.03, 0.1, 0.3 and 1 mg/kg, *p.o.*) treatment in the cold restraint stress (CRS) model and 21 days repeated treatment in the pyloric ligation (PL) model and compared with that of sulpiride (D_2 receptor antagonist) and ketanserin (5-HT_2 receptor antagonist) as standard. Histopathological assessment was done to evaluate the gastroprotective activity of risperidone in CRS model. The roles of nitric oxide (NO), sulfhydryl (SH) group, ATP-sensitive K^+ channels (K_{ATP} channels) and prostaglandins (PGs) in the gastroprotective effect of risperidone against CRS were also investigated. PGE_2, hexosamine as a marker of mucus barrier and microvascular permeability were also estimated. Results show that repeated treatment of risperidone, sulpiride and ketanserin exhibited a gastroprotective effect against CRS-induced lesions while single administration of risperidone was found to be ineffective. Moreover, repeated treatment of risperidone and ketanserin was found to be ineffective in case of PL in contrast to sulpiride. Risperidone pretreatment reverses the stress induced alteration in hexosamine, PGE_2 and microvascular permeability. Pretreatment with L-NAME, NEM, glibenclamide and indomethacin reversed the gastroprotective effect of risperidone. The results suggest that risperidone has significant gastroprotective effects in CRS-induced gastric lesions models, which appears to be mediated by endogenous NO, SH, PGs and K_{ATP} channel opening.

1. Introduction

Gastric ulcer, a multi-etiologic disease, is one of the most common chronic illnesses among adults. Despite great research, its etiology has not been completely elucidated. Various factors, such as imbalance between aggressive (increased acid and pepsin secretions) and protective factors (mucous and bicarbonates), stress, trauma, sepsis, hemorrhagic shock, burns, pulmonary and liver diseases, helicobacter pylori, use of cigarettes and alcohol, steroidal and non-steroidal drugs, have been shown to play a role in gastric ulcerogenesis [1,2].

Stress is one of the important factors for gastric lesions. Stress-related mucosal disease (SRMD) remains significant concern in critically ill patients and places them at a high risk of death. Clinical studies indicate that 75–100% of patients in the intensive care unit (ICU) suffer from SRMD [3]. SRMD not only increases the mortality due to excessive GI bleeding and multiple organ failure [4] but also increases total cost of treatment by increasing the stay of patient at hospital [3]. Thus, due to the above facts stress ulcer prophylaxis has become an established routine practice in ICU [3].

Apart from this, depression with psychotic and somatic symptoms has been seen in patients with gastrointestinal tract (GIT) diseases [5]. Further, one report shows that stress and psychosis shares common neuro-physiological pathways [6]. Both preclinical and clinical evidences suggest that atypical antipsychotics may modulate the stress response [7]. Glavin and Hall [8] reported clozapine, an atypical antipsychotic is beneficial in stress-induced gastric lesions. Another known atypical antipsychotic drug is risperidone, known for antagonistic activities toward both dopamine (D_2) and 5-hydroxytryptamine (5-HT_{2A}) receptors [9]. However, as of now risperidone has not been explored for its gastroprotective activity against stress-induced gastric lesions.

Risperidone has been shown to be effective in the treatment of post-traumatic stress disorder (PTSD) [10]. As stress is common denominator between stress related mucosal disorder and post-traumatic stress disorder, risperidone may be beneficial for the treatment of stress-induced gastric lesions. Further, it has been reported that risperidone has anxiolytic activity [11] and certain

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anxiolytic drugs [12] have shown gastroprotective activity against stress-induced gastric lesion in experimental models. Thus, risperidone may be potentially effective against stress-induced gastric lesions.

In several preclinical as well as clinical conditions, ischemia is one of the important factors for pathogenesis of stress-induced gastric lesions [13]. Reperfusion of ischemic tissues further aggravates the injury process which results in inflammation [14,15]. It is also known that several endogenous factors such as nitric oxide (NO) and sulfhydryl (SH) compounds are related to the pathophysiology of gastric ulcer [16]. In the stomach mucosa, the prostaglandins (PGs) and opening of ATP-sensitive K^+ (K_{ATP}) channels seem to stimulate the secretion of bicarbonate and mucus, maintain the mucosal blood flow, inhibit the acid secretion, as well as regulate the mucosal cell turnover and repair [17,18]. Among all prostaglandins, PGE_2 is mainly responsible for gastroprotective activity. Based on the above facts, our present investigation evaluates the gastroprotective effect of risperidone against the cold restraint stress (CRS) induced gastric lesions as well as pyloric ligation (PL) models. CRS models are commonly used for evaluating drugs having gastroprotective activity against stress-induced gastric lesions [19] while pyloric ligation model is used for the evaluating drugs which are effective by decreasing acid and pepsin secretion [20]. Risperidone is known for antagonistic activities toward both D_2 and 5-HT_{2A} receptors [9]. Therefore for better understanding of the receptor involved in the gastroprotective activity of risperidone, the reduction in ulcer index afforded by risperidone in both cold restraint stress as well as pyloric ligation model was compared with that of sulpiride and ketnaserin. Apart from studying the ulcer index and histopathological examination, the present investigation also includes estimation of gastric PGE_2, mucus secretion in terms of hexosamine and microvascular permeability. The involvement of NO, SH, PGs and K_{ATP} channel opening were also evaluated to elucidate the mechanism of action of risperidone in its gastroprotective effect against CRS.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats (180–220 g) were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University (B.H.U.). The animals were housed in polypropylene cages at an ambient temperature of 25 ± 1 °C and 45–55% RH, with a 12:12 h light/dark cycle. The animals had free access to commercial food pellets (Doodh dhara Pashu Ahar, India) and water unless stated otherwise. Experiments were conducted between 09:00 and 14:00 h. "Principles of laboratory animal care" (NIH publication number 85-23, revised 1985) guidelines were followed.

2.2. Drugs, reagents and solvents

Indomethacin, glibenclamide, L-NAME (L(-G)-nitro-L-arginine methyl ester) and NEM (nethylmaleimide) were purchased from Sigma–Aldrich (St. Louis, MD, USA). All the other reagents and solvents used were of analytical grade.

2.3. Effect of single treatment of risperidone in CRS-model

The animals were divided into six groups of six animals each. Control and CRS group rats received the 0.3% carboxymethylcellulose (CMC) (3 ml/kg) suspension as vehicle. Other four groups received single treatment of risperidone (0.03, 0.1, 0.3 and 1 mg/kg, *p.o.*) (ARIS-0.03, ARIS-0.1, ARIS-0.3 and ARIS-1.0, respectively). The doses were selected according to Ishida-Tokuda et al. [11]. All groups were then subjected to 2 h CRS after 1 h of repeated treatment of drug/vehicle administration. All animals were sacrificed and stomachs were excised for ulcer scoring as described by Sairam et al. [21].

2.4. Effect of repeated treatment of risperidone in pyloric ligation (PL) and CRS model

In PL method, rats were divided into seven groups of six animals each. To one group vehicle (0.3% CMC, 3 ml/kg) was administered orally and to the other four groups risperidone at a dose of 0.03, 0.1, 0.3 and 1 mg/kg, was administered orally for 21 consecutive days, respectively (RRIS-0.03, RRIS-0.1, RRIS-0.3, RRIS-1.0). Other one group was treated with sulpiride (10 mg/kg, *p.o.*) for 5 consecutive days [22]. Last group was treated with ketanserin (10 mg/kg, *p.o.*) [23]. After 1 h of vehicle/drug administration on final day, animals were anaesthetized using pentobarbitone (35 mg/kg, *i.p.*), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed with interrupted sutures. After 4 h, stomachs were excised out for ulcer scoring as described by Sairam et al. [21] and contents were collected for the estimation of acidity, volume and pH of gastric content as previously described by Debnath et al. [24].

In CRS method, control and CRS rats received the vehicle (0.3% CMC, 3 ml/kg) for 21 days. Other four groups received repeated oral treatment of 0.3% CMC suspension of risperidone at a dose of 0.03, 0.1, 0.3 and 1 mg/kg for 21 consecutive days, respectively (RRIS-0.03, RRIS-0.1, RRIS-0.3, RRIS-1.0). Other one group was treated with sulpiride (10 mg/kg, *p.o.*) for 5 consecutive days [22]. Last group was treated with ketanserin (10 mg/kg, *p.o.*) [23]. All groups except control group were then subjected to 2 h CRS [21] on final day after 1 h of drug/vehicle administration. Lastly, all animals were sacrificed and stomachs were excised for ulcer scoring and hexosamine estimation.

2.5. Histological studies

For histological examination, the stomach tissues were excised and rinsed with ice-cold saline solution (0.9% sodium chloride) to remove blood and debris adhering to tissues. The tissues were then fixed in 10% formalin for 24 h. The fixative was removed by washing through running tap water overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 5 μm thickness and stained with haematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details.

2.6. Estimation of gastric hexosamine level

In order to investigate the possible effect of risperidone on hexosamine, a marker of gastric adherent mucus, gastric mucosa was scraped and homogenized in ice-cold saline. The hexosamine content was estimated according to the method of Dische and Borentrend [25]. Briefly, about 0.5 ml of the hydrolyzed fraction of scrapped mucosa of stomach was taken. To this 0.5 ml of acetyl/acetone reagent was added. Then for 1 ml of mixture, 0.1 ml of sodium carbonate (0.5 N) was added. The mixture was heated in boiling water bath for 20 min and then cooled under running tap water. About 1.5 ml of 90% glacial acetic acid was added and allowed to stand for 30 min. The color intensity was measured in spectrophotometer at 530 nm against blank prepared by using distilled water instead of hydrolysate. Hexosamine content was determined from the standard curve prepared by using D-glucosamine hydrochloride and concentration was expressed in mg/g of tissue.