Protective Effect of Furoxan Against Immobilization Stress-Induced Gastric Damage in rats

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الخلاصـــة

اكتشف حديثا ان اوكسيد النتريك من الوسائط الأساسية في الآلية الدفاعية فـــي المعـــدة وذلك من خلال قابليته على زيادة كل من الامداد الدموي للغشاء المخاطي وإنتـــاج المخـــاط وتثبيط الاستجابة الالتهابية للنسيج. لذلك كان الهدف من هذه الدراسة هـــو فحـــص الفعاليـــة الدفاعية المعدية للفوركسان (المانح لمجموعة اوكسيد النتريك) ضد الأذى المعـــدي المحـــدث بواسطة الإجهاد المقيد للحركة. لقد احدث الأذى المعدي فـــي مجـــاميع حيوانـــات الـــسيطرة (المعالجة مسبقا بمحلول الملح الفسلجي أو الحامل- بولي اثيلين كلايكول) والمعرضة للاجهاد المقيد الحركة ولفترة 6 ساعات. تم تقيم الأذى المعدي بواسطة قياس مساحة الأذى. لقد آدت المعالجة مسبقا بالفوركسان (بجرعة 2ملغم / كغم وعن طريق الفم) قبل 6 أيـــام مـــن بدايـــة الكرب الى التقليل المعنوي من مساحة الأذى المعدي المحدث بواسطة الاجهاد المقيد للحركـــة حيث انه لم يلاحظ وجود أي أفه في المعدة مقارنة مع مجاميع السيطرة. كما أكدت النتائج ان الفور كسان المانح لمجموعة اوكسيد النتريك لم يحدث أي تخريش موضعي للمعدة مع توفيره وقاية معدية ضد الكرب المحدث للاذى المعدي. نستنتج ان اوكسيد النتريك المتحرر من مركب الفوركسان قد اظهر تأثيرات مفيدة وذلك عن طريق تعزيز القابلية الدفاعية للغشاء المخـــاطي ضد الاجهاد المقيد للحركة المحدث للاذى المعدي.

ABSTRACT

Nitric oxide (NO) has been recently recognized as a fundamental mediator in gastric defence mechanism because of its ability to increase

gastric mucosal blood flow, mucus production, and to inhibit tissue inflammatory responses. The aim of this study was to investigate the gastroprotective activity of furoxan (NO- donor group) against gastric damage induced by immobilization stress. In the present study gastric damage elicited in the control animals (pretreated with saline or polyethylene glycol-vehicle) by 6-h restraint stress. Gastric damages were quantitated by measuring the area of damage. Furoxan (2mg/kg P.O.) pretreatment once a day for 6 consecutive days before the onset of stress, resulted in significantly reduced the area of gastric damage induced by immobilization stress which is no lesion was observed in the stomach in compared with controls. These results suggest that, the furoxan (NO-donor group) neither had a topical irritating action on the stomach and provided gastric protection against stress-induced gastric damage. we conclude, that the NO released from furoxan was exert beneficial effects on gastric mucosa by enhancing the mucosal defensive ability against stress-induc gastric damage.

INTRODUCTION

Immobilization stress-induced gastric damage is supported by considerable evidence (1,2,3). The role of endogenous corticosterone in stress – induced gastric damage has been debated by Weisis, Murphy et al (4,5) found that the degree of ulceration after stress correlated positively with the level of plasma corticosterone. Also consistent with findings of Al-Mohaisen et al, (6) that increase plasma corticosterone level 3- to 4-fold after single acutly immobilization stress (2-h), and these novel studies suggest that immobilization stress influence the hypothalamic-pituitary-adrenal axis in rats by elevating the level of plasma corticosterone. Thus, on the basis of thesis studies, the corticosterone increase during immobilization stress was considered to be an ulcerogenic. So that gastric damage produced by immobilization stress mainly attributable to the elevating the level of the plasma corticosterone during stress leading to suppression of gastric cytoprotective properties prostaglandin biosynthesis (PGs) (7,8) because of increase corticosterones enhancing the production of protein called (lipocortin), which inhibits the enzyme phospholipase A_2 in the cell membranes and inhibition of induction of cyclooxygenase activity, thereby retarding release of arachidonic acid from cellular phospholipids thereby inhibiting prostaglandin (PGs) formation in the stomach (9,10). This contention has been supported by findings of Hirata et al. and Takeuchi et al (7,12), that the stress ulcer may be related to a deficiency of endogenous PG_s.

Several components of gastric mucosal defense are influence or mediated by PGs, including maintenance of mucosal blood flow, mucus and bicarbonate secretion, epithelial cell turn over and repair and mucosal

immunocyte function (9,10,13,14). Therefor, it is possible that inhibition of (PGs) biosynthesis during stress lead to a reduction in the ability of the gastric mucosa to defend itself against stress leading to gastric damage.

Recently, a series of new compounds have been synthesized as (NO) nitric oxide donor drugs (NODD) like furoxan (15,16,17,18). NO has well–document protective effects in the stomach (19,20,21). The mechanism through which NO is capable of protecting the stomach, through its is ability to increase gastric mucosal blood flow, mucus production and inhibit tissue inflammatory responses. (20,21, 22,23, 24).

Recently devised approach to developing with a number of NO– releasing derivatives, so-called "NO-NSAIDs (NO-non-steroidal anti-inflammatory drugs (25,26), have been shown to be safe and effectively diminished gastrointestinal toxicity in compared with conventional NSAIDs, such as in a model of hypothermia–induced gastric damage, pre-treatment with indomethacin or aspirin significantly worsened the severity of the lesions observed whilst NO – aspirin had no pro-ulcerogenic activity (27).

The present study was performed to evaluate whether or not a furoxan (No donor group) would be capable of conferring protection to the gastric mucosa against stress- induced gastric damage.

MATERIALS AND METHOD

Male albino Wister rats 150-250 g in weight used. They were housed under standard condition with 10/14-h light/dark cycle and 20 °C room temperature food and water were available adlib.

This study was designed to investigate the protective activity of furoxan (NO donor group) against stress-induced gastric damage. For this study 18 rats were randomly divided in to three group of (n=6) each. Rats in the Group 1 were pretreated orally with furoxan (2mg/kg) was prepared as a suspension in a vehicle containing polyethylene glycol 400 (PEG 400;vehicle). The adequacy of the dose level/regimen has been established by the results of preliminary experiments. Control rats in the group 2 pretreated with vehicle (PEG 400;vehicle). An additional control group of animals in the group 3 were pretreated with saline was also used. Rats in the group (1,2,3) were orally pretreated with furoxan (2mg/kg) or vehicle or saline by gavage needle (volume 2ml/kg) once a day for 6 consecutive days before the onset of stress. On the 6 th day, the last pretreatment was performed 1 h before starting the stress. Briefly, the immobilization stress was applied by placing the rat on the a restraint board for (6 hr) as described previously by Lee et al, (1) (Fig 1). At the end of 6-hr stress, animals were killed by cervical dislocation, and the stomachs were removed, inflated by injecting 10 ml of 1% formalin for 10 min to fix the

tissue walls and opened along the greater curvature. The area (square millimeters) of gastric damage developed in the glandular mucosa of stomach was measured under a binocular-dissecting microscope, although the gastric damag they are commonly referred to as "stress ulcers"(28).

The following drugs was used in the present study Furoxan: 5,7-(4-hydroxyphenelamin-4,6 dinitrobenzofuroxan) (Kazan Chemical Co., Russia), polyethylene glycol 400 (Fluka AG, Buchs, Germany).

Statistical Analysis

Data are presented as the mean ± S.E. from 6 rats per group. Statistical analyses by one-way analysis of variance following the least significant difference test (29). The of significant was at $p < 0.01$.

RESULTS

The immobilization stress procedure significantly produced in the control group (saline-pretreated rats) typical largely gastric damage in the glandar mucosa of the stomach, the area of the gastric damage was 38.3±2.3 mm^2 (Fig.2) .

Comparison of the results of two control groups (saline or vehicle - pretreated rats) showed that the polyethylene glycol 400 vehicle give orally (volume 2ml/kg) for 6 consecutive days before the onset stress in itself decreased the area of the stress – induced gastric damage (area of the gastric damage was 36.9±1.7 mm^2) which is less than that observed in rats pretreated with saline (area of the gastric damage 38.3±2.3 mm^2) (Fig.2) .

Gastric damage induced by immobilization stress was prevented by furoxan (NO donor group), which has been pretreatment with furoxan orally at dose of 2mg/kg for 6 consecutive days before the onset of the acute ulcerogenic stress procedure resulted in a significant ($P<0.01$) reduction the extent of gastric damage which is no lesion was observed in the stomach, in compared with rats pretreated with saline or vehicle (Fig.2).

DISCUSSION

In the present study, the gastric damage evoked by 6-h immobilization-stress in control (pretreated with saline or vehicle) animals confirm the previous reports of the ulcerogenic response to stress (1,2,3). The gastric damage produced by immobilization stress mainly attributable to the elevating the level of the plasma corticosterone (4,5,6) leading to suppression of gastric cytoprotective properties prostaglandin biosynthesis (PGs) (7,8,9,10,11,12), since the gastric mucosal defense are mediated by

PGs, including maintenance of mucosal blood flow, mucus and bicarbonate secretion, epithelial cell turn over and repair and mucosal immunocyte function (9,10,13,14). Therefor, it is possible that inhibition of PG_s biosynthesis during stress lead to a reduction in the ability of the gastric mucosa to defend itself against stress leading to gastric damage. This contention has been supported with recent findings of Hirata et al. and Takeuchi et al (7,12), that the stress ulcer may be related to a deficiency of endogenous gastric cytoprotective PG_s and caused gastric damage .

In the present study, administration of furoxan (NO donor group) orally (2mg/kg) for 6 consecutive days before the induction of immobilization stress significantly reduced gastric damage. These results suggest that the protective effect of furoxan may be attributable to the NO released from this compound. The mechanism through which NO releasing from furoxan is capable of protecting the stomach, through its is ability to increase gastric mucosal blood flow, mucus production, and to inhibit tissue inflammatory responses. (20,21,22,23, 24) . This contention has been confirm and supported with recent findings of Ukawa et al.(27) that NO-releasing drugs reduction of severity of stress-induced gastric damage, which has been in a model of hypothermia–induced gastric damage, pretreatment with indomethacin or aspirin significantly worsened the severity of the the lesions observed whilst NO – aspirin had no pro-ulcerogenic activity. Thus also furoxan (NO donor group) in the present study may reduction of severity of stress-induced gastric damage by similar mechanism.

Also as mention above suppression of cytoprotective properties prostaglandin biosynthesis (PGs) formation in the stomach mucosa during stress which participate is a critical step in the pathogenesis of the stress-induced gastric damage (7,8,12). A recent report has shown interestingly, that nitric oxide (NO) like PG_s (30,31,32), since furoxan (NO donor group) (15,16,17,18) . It is conceivable that NO releasing from furoxan as a fundamental mediator in gastric defense mechanisms may replaces the lost PG_s during stress leading to prevent gastric damage .

We also proposed anothers mechanism for the protective effect of furoxan (NO donor group) against stress-induced gastric damage. Since NO-mediated mucosal vasodilation (23) to counteract the vasoconstrictor effect resulting from suppression of cytoprotective properties prostaglandin biosynthesis (PGs) formation in the stomach during stress, a vasodilator effect of NO released following furoxan (NO donor group) administration most probably plays a significant part in minimizing the ulcerogenic potential of these stress . Several lines of evidence support this proposal (a vasodilator effect of NO). For example, in anaesthetized rats, flubiprofen constricted mesenteric post-capillary venues by 16.6% whilst NO- flubiprofen dilated these vessels by 6.7% (25) .

This contention also consistent with the finding of MacNaughton et al (19) that NO, an endothelium-derived relaxing factor (vasorelaxant activity), may play regulation of gastric mucosal defence. It is therefore conceivable that gastric mucosal blood vessels dilate after oral ingestion of furoxan (NO donor group) because they are exposed to much higher concentrations of NO than other vascular beds lead to also counteract the vasoconstrictor effect resulting from suppression of cytoprotective properties prostaglandin biosynthesis (PGs) formation in the stomach during stress

The results of the present study show that the furoxan (NO donor group) is totally devoid of topical irritant action, is not ulcerogenic, does not potentiate gastric ulcerogenic response to stress and is rather protective of the stomach. Thus we conclude that these experiments clearly indicated that the furoxan (NO donor group) can provide gastroprotection against stress-induced gastric damage.

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Fig . 1. Immobilization stress was applied by placing the rat on the a restraint board for (6 hr) as described previously by Lee et al, (1) . At the end of 6-hr stress, animals were killed by cervical dislocation, and the stomachs were removed, inflated by injecting 10 ml of 1% formalin for 10 min to fix the tissue walls and opened along the greater curvature. The area (square millimeters) of gastric damage developed in the glandular mucosa of stomach was measured under a . binocular-dissecting microscope .

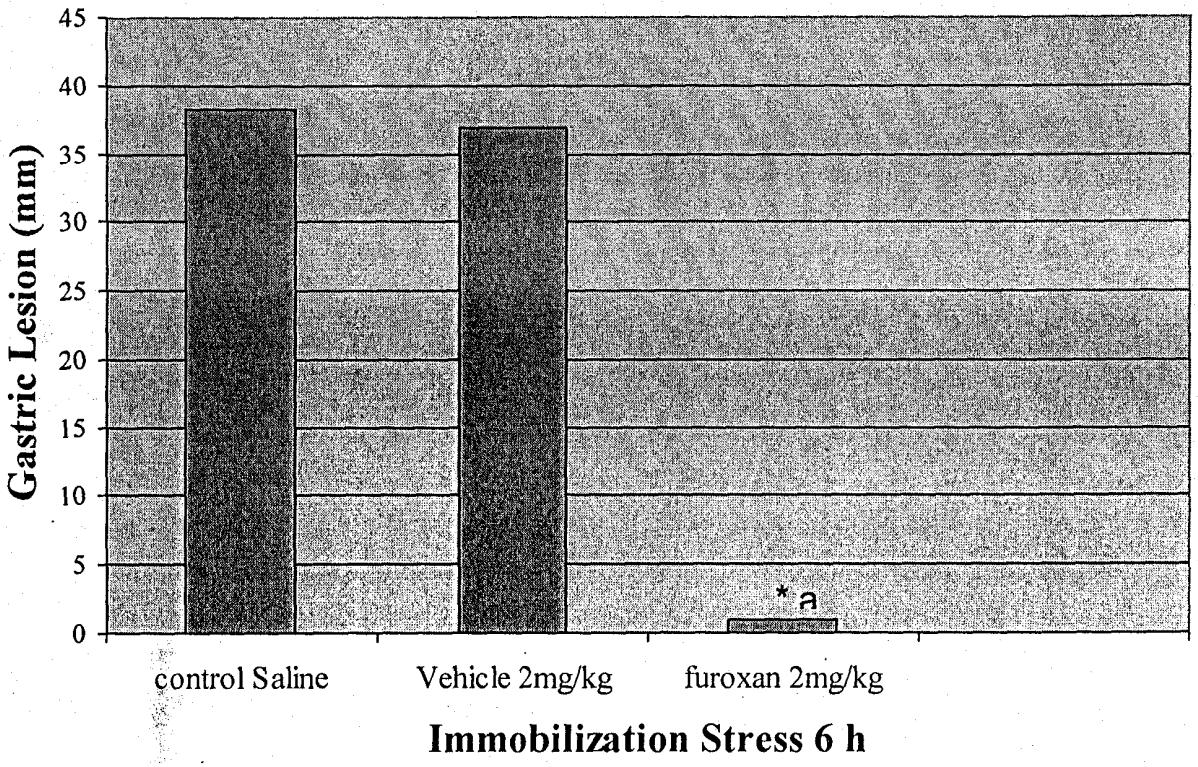

Fig. 2. Effects of furoxan, vehicle (PEG 400), or saline on gastric damage induced by immobilization-stress for 6-h. in rats. Furoxan (2mg/kg), vehicle (2ml/kg- PEG 400), or saline (2ml/kg) were administered orally for 6 consecutive days before the onset of stress. Data are presented as the mean ± S.E. from 6 rats. Statistically significant difference at * $P<0.01$, a $P<0.01$ compared with corresponding saline – treat group and vehicle – treat group.