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Adenosine Triphosphate-Induced Gastric Cytoprotection Against Ulcerogenic Effects of Hypothermic Restraint Stress and Diclofenac in Rats

Afaf A. Ein Shoka

College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

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The Protective effect of adenosine triphosphate (ATP) on gastric ulcer induced in rats has been studied. Gastric ulceration was induced by hypothermic restraint stress or diclofenac sodium. Gastric acid secretion and mucosal injury produced by the hypothermic restraint stress was greater as compared with those produced by diclofenac sodium. ATP significantly reduced area of injury, however, increased cyclic adenosine monophosphate (cATP) content. Administration of dipyridamole along with ATP did not change the total lesion area in both models when compared to ATP alone. Aminophylline antagonized the protective effect of ATP on the injured area. Famotidine was found to be effective in reducing gastric acid output as well as the total injured area without any change in cAMP content when given along with ATP.

Key words : Gastric ulceration, Hypothermic restraint stress ulcer, Adenosine triphosphate (ATP), Cyclic adenosine monophosphate (cAMP), Dipyridamole, Aminophylline, Famotidine, Diclofenac sodium

INTRODUCTION

The existence of pathological relationships between adenine nucleotides and gastric ulceration or gastric acid secretion has been mentioned in various publications (Bieck *et al.*, 1973; Csalay and Csakuary, 1976). Other reports attributed gastric cellular deterioration in ulcers to depletion of adenine nucleotide reserve in gastric mucosal tissues (Gyton, 1976; Rainsford, 1989).

Although adenine nucleotides are known to have vasodilating properties and to enhance tissue oxygenation (Talaat *et al.*, 1961), and in spite of the protection against incidence of peptic ulceration (Pfeiffer and McPherson, 1976), which was reported after vascular enhancement of gastric tissues, yet neither any of the adenine nucleotides had been ever thought to be suitable in gastric cytoprotection, nor gastric cytoprotection had been mentioned as a property of adenine nucleotides. Adenosine triphosphate (ATP), being an endegenous compound is unlikely to produce toxic effects if it is used in high doses. So the role of an appropriate dose of such compound on experimentally induced gastric mucosal ulceration has been investigated. Besides, modulation of some gastric cytoprotective drugs by ATP has been tested in this study.

Correspondence to : Afaf A. Ein Shoka, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

MATERIAL AND METHODS

Ulcer induction and drug regimen

A total of 66 adult male Wister albino rats (weighing 180-220 g) were used in this study. All rats were fed on standard chew diet and were allowed free access to water. Twenty hours before drug administration, the rats were deprived of food with water *ad lib.* They were randomly divided into two main groups. The first group was then classified into six subgroups where each consisted of 6 rats. The first and second subgroups were used as control and injected with normal saline solution equivalent to those used for drug administraion. The third subgroup was intravenously infused with adenosine triphosphate, ATP (Boehringer Manheim, Germany) in a dose of 1.5 mg/kg/minute over a period of 30 min. The fourth subgroup was similarly infused with ATP, then injected i.p. with dipyridamole (Boehringer, Mannheim, Germany) in a dose of 10 mg/kg. The fifth subgroup was also infused with ATP then injected i.p. with aminophylline (Sigma, England) in a dose of 50 mg/kg. The sixth subgroup was similarly

Table I. Effect of hypothermic restraint stress or diclofenac sodium on gastric acid output, gastric damage and gastric mucosal cAMP

Treatment	Gastric Volume, ml	Titrable Acidity, mEq/L	Gastric acid, output, mEq	Total lesion area, mm^2	Gastric Mucosal cAMP content, pmol/g
Contol (normal)	3.10 ± 2.65	38.50 ± 2.65	135.30 ± 11.71	00.00	325.50 ± 32.20
Hypothermic restraint stress method	4.75*± 0.33	43.75*± 3.15	225.25*± 21.56	7.57* ± 0.26	496.50* ± 41.90
Diclofenac	4.50*± 0.22	49.20*± 3.71	215.20*± 14.81	5.57*+ ± 0.35	381.60*+ ± 22.90

*Significantly diffeent from control (normal) at $p<0.05$.
+Significantly different from hypothermic restraint stress method at $p<0.05$.

infused with ATP then injected i.m. with famotidine (MSD, Merch Sharp and Dohme B.V. Haarlem-Netherlands) in a dose of 15 mg/kg. With the exception of the first subgroup, all other subgroups were subjected for hypothermic restraint stress ulcer induction by the method of Levine (Levine, 1971), where the animals were immobilized in restraint cages and placed in a ventillated refrigerator for a couple of hours at 4℃, then sacrificed by cervical dislocation.

The second group was classified, treated in the same way as the first group with the exception that ulcer was induced by subcutaneous administration of diclofenac sodium (Ciba Geigy, Basle, Swizerland) in a dose of 100 mg/kg, and the rats were sacrificed after 6 hours.

Determination of gastric volume, total acidity, and gastric acid output

After sacrificing the animal, the stomach was removed and excised along the greater curviture and the gastric juice was collected, measured and centrifuged. The gastric total acidity was determined by titration with 0.01N sodium hydroxide to pH 8 and the total gastric acid output was calculated as the product of volume of gastric juice and concentration of acid.

Measurements of lesion areas

The stomach was rinsed in normal saline and examined by a steriomicroscope (Zeiss SR) attached to microprocessor linked planimeter (Morphomat-30, Zeiss) whereupon the glandular portion was clearly visualized at the lower magnification. The image was traced by a digitizing cursor and the total area of ulcerations was recorded using an Epso R×80 printer.

Determination of cAMP contents

A homogenate of gastric mucosa in ice cold water containing 4 mM EDTA was prepared using a Sorval omni mixer (Sorvall Co., France). Extraction of 1 ml of the homogenate with 2 ml of trichloroacetic acid (12%), centrifugation and neutralization of the extract with Tris buffer afforded a solution in which cAMP was determined by the radioimmuno assay technique (cAMP kits, Radiochemical Center, Amersham, England).

Statistical Analysis

The data were analysed using ANOVA with the Tukey's Studentized Range (HSD) Test where appropriate, accepting $p<0.05$ as significant.

RESULTS

The results of ulcer induction in rats by means of hypothermic restraint stress or diclofenac sodium are shown in Table I. There was a significant increase in the gastric volume and total gastric output which were consistant with the total injured mucosal area. There was a significant increase in gastric acid output produced by diclofenac sodium compared to that produced by the hypothermic restraint stress. On the other hand, total lesion area and gastric mucosal cAMP content produced by hypothermic restraint method were significantly greater than that produced by diclofenac sodium method.

Table II shows the effect of ATP pretreatment on gastric acid output, the injured area and gastric mucosal cAMP content in rats subjected to restraint gastric ulceration and concomitantly treated with dipyridamole, aminophylline or famotidine.

Pretreatment with ATP (Table II) reduced the injured area and increased the gastric mucosal cAMP content with a slight increase in gastric acid output.

Co-administration of dipyridamole and ATP (Table II) resulted in an increase in gastric mucosal cAMP content without any change in total lesion areas as compared to pretreatment with ATP alone.

Administration of aminophylline to rats in presence to ATP (Table II) significantly reversed ATP actions on total lesion area and gastric mucosal cAMP content with a significant reduction in gastric acid output.

Administration of ATP in association with famotidine produced significant reduction in gastric volume, titrable acidity, and total acid output. The total area of lesions was likewise decreased whereas the gastric cAMP content was normalized

Table II. Effect of adenosine triphosphate (ATP) pretreatment on gastric acid output, gastric mucosal damage, and gastric mucosal cyclic adenosine monophosphate (cAMP) content in adult male wister albino rats subjected for restraint gastric ulceration, and concomitantly treated with dipyridamole, aminophylline or famotidine

Treatment	Gastric Volume, ml	Titrable Acidity, mEq/L	Gastric acid output, mEq	Total lesion area, mm^2	Gastric Mucosal cAMP content, pmol/gm
Control (Restraint ulcer)	4.75 ± 0.33	43.75 ± 3.15	225.25 ± 21.56	7.57 ± 0.26	496.50 ± 41.90
ATP	4.50 ± 0.35	39.60* ± 3.21	191.20* ± 16.32	3.49* ± 0.23	589.70* ± 45.90
ATP + Dipyridamole	5.25*± 0.31	46.50* ± 4.31	247.56* ± 17.47	3.15* ± 0.21	615.80* ± 46.70
ATP + Aminophylline	4.75 ± 0.24	36.25* ± 2.95	125.60* ± 12.44	7.65^{+} ± 0.35	485.30^{+} ± 38.70
ATP + Famotidine	1.90*± 0.27	21.75*$^{+}$ ± 3.12	55.43*$^{+}$ ± 3.75	2.76*$^{+}$ ± 0.18	331.30*$^{+}$ ± 29.60

*Significantly different from control (Restraint ulcer) at $p<0.05$.
$^{+}$Significantly different from ATP at $p<0.05$.

Table III. Effect of adenosine triphosphate (ATP) pretreatment on gastric acid output, gastric mucosal damage, and gastric mucosal cyclic adenosine monophosphate (cAMP) content in adult male wister albino rats subjected for diclofenac sodium gastric ulceration, and concomitantly treated with dipyridamole, aminophylline or famotidine

Treatment	Gastric Volume, ml	Titrable Acidity, mEq/L	Gastric acid output, mEq	Total lesion area, mm^2	Gastric Mucosal cAMP content, pmol/gm
Control (Diclofenacsodium)	4.50 ± 0.22	49.20 ± 3.71	215.20 ± 14.81	5.57 ± 0.35	381.60 ± 22.90
ATP	4.75* ± 0.41	48.55 ± 3.96	229.67* ± 13.75	3.12* ± 0.18	550.50* ± 24.60
ATP + Dipyridamole	5.50* ± 0.32	51.62*$^{+}$ ± 4.66	223.63 ± 14.59	2.70* ± 0.15	573.60* ± 23.90
ATP + Aminophylline	3.25*$^{+}$ ± 0.22	47.35* ± 3.79	162.39*$^{+}$ ± 12.37	5.15^{+} ± 0.26	397.50^{+} ± 31.70
ATP + Famotidine	1.75*$^{+}$ ± 0.25	19.85*$^{+}$ ± 3.74	75.28*$^{+}$ ± 4.47	2.10*$^{+}$ ± 0.11	356.50*$^{+}$ ± 32.90

*Significantly different from control (Diclofenac sodium) at $p<0.05$.
$^{+}$Significantly different from ATP at $p<0.05$.

The effect of ATP pretreatment on gastric acid output, gastric mucosal damage, and gastric mucosal cAMP content on rats subjected to diclofenac sodium gastric ulceration, and concommitantly treated with dipyridamole, aminophylline or famotidine is shown in Table III.

Adenosine triphosphate pretreatment decreased total lesion area and increased cAMP content with a slight increase in gastric acid output.

Administration of ATP in combination with dipyridamole produced a significant increase in gastric volume, titrable acidity and gastric mucosal cAMP content as compared to treatment with ATP alone.

Concomitant administration of ATP and aminophylline increased gastric volume and gastric acid output and significantly reversed ATP actions including gastric mucosal cAMP content and total lesion area.

Administration of ATP in association with famotidine significantly reduced gastric acid output, total lesion area, and gastric mucosal cAMP content as compared to ATP treatment per se.

DISCUSSION

The results of this study showed that ulcer induced in rats by hypothermic restraint stress or diclofenac sodium is accompanied by an increase in total gastric output, and this is in accordance with Mozsik *et al.* (1976). and Di Murro *et al.* (1988).

The increase of gastric mucosal cAMP content in restraint gastric ulceration method is in agreement with Costa *et al.* (1975). who found that cold stress caused an increase in cAMP in the rat adrenal medulla. Csalay and Csakvary (1976), and Biek *et al.* (1973). observed that ulcerated gastric mucosa was deficient with high energy phosphate content (ATP) due to its transformation to cAMP. Hence, it is probable that cAMP may have a role in gastric acid secretion and peptic ulcer induction (Alonso and Harris, 1965; Shaw and Ramwell, 1968).

Gastric ulceration produced by diclofenac sodium is in accordance with the reports of Ligumsky *et al.* (1970) and Beck *et al.* (1990) where gastric mucosal damage was not accompanied by any change in gastric mucosal cAMP content. Moreoever, diclofenac sodium was found to produce colonic ulceration in human being (Carson *et al.*, 1990). In addition, diclofenac sodium as a nonsteriodal antiinflammatory drug, inhibits prostaglandin synthesis leading to increased leukotriene synthesis which may participate in gastric inflammation and mucosal damage (Bennett, 1989).

ATP is known to exert a potent vasodilating effect and it induces high blood and tissue oxygenation, protects against shivering of a subject exposed to cold

stress and prevent tissue stress (Gyton, 1976). This might explain the observable reduction of toal lesion area in rats subjected for resistant gastric ulceration, as gastric ulceration might be caused by severe vasoconstriction and/or tissue anoxia (Pfeiffer and McPherson, 1976). The increase in mucosal cAMP as a result of ATP pretreatment might have been due to stimulated transformation of ATP to such cyclic nucleotide through the metabolic sequences.

The central effect of ATP (Stolk *et al.*, 1974) is likely a prophylactic endeavor of tissue necrosis or ulceration. Hence ATP, may be of clinical value as an adjunct therapy in gastric ulcer. This is postulated by the statement of Cornet (Cornet, 1973) that ATP reduced the response of biological systems to stress.

Dipyridamole did not reduce the injured area of the gastric mucosa as compared to pretreatment with ATP alone. On the other hand, the increased gastric mucosal content of cAMP under the influence of ATP administration was promoted by dipyridamole. This may be due, in part, to inhibition of metabolism and transport of adenosine and adenine dinucleotides, where dipyridamole inhibits adenosine uptake by the erythrocytes and other cells, besides inactivation of cyclic nucleotide phosphodiesterase (Goodman and Gilman, 1985).

Aminophylline reversed ATP actions on total lesion area and gastric mucosal cAMP content. Being an adenosine receptor antagonist (Goodman and Gilman, 1985), aminophylline was expected to evoke such reversion of ATP actions.

Inhibition of gastric acidity by the H_2-blocker, famotidine, may potentiate the protective ability of ATP against ulcerogenicity produced by either method mentioned previously. Meanwhile, famotidine seems to inhibit the transformation of such high energy phosphate compound into cAMP in the gastric mucosa, which, as mentioned previously, could have a role in prophylaxis against gastric ulcerogenicity.

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