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Effect of Catecholamine on Indomethacin-Induced Ulceration and Apoptosis in Rat's Stomach

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ABSTRACT

In the present study, the effects of pre-treatment with catecholamine on indomethacin-induced ulcer were studied. Rats were exposed to various treatments with epinephrine and dopamine 30 minutes before ulcer was induced using NSAID (indomethacin). Experimental ulceration was induced in fasted rats using Indomethacin (40mg/kg.p.o). Four hours later after indomethacin administration, the stomachs were opened under thiopentane anesthesia and the ulcer area scored by planimetry. Sections of the stomachs were prepared for histology and stained for apoptotic cell count. Acid secretion was also studied in the control and treated animals by pylorus ligation technique. Indomethacin treatment resulted in the formation of ulcer with ulcer index of 5.0±0.5 while the pre-treatment with catecholamine significantly reduced ulceration episodes (epinephrine: ulcer index=3.0±0.7, dopamine: ulcer index=2.0±0.7, $p<0.05$). Total Apoptotic score in the indomethacin-induced rats' stomachs was 19.5±0.38. This was reduced to 4.3±1.70 and 1.3±0.33 in the epinephrine and dopamine treated rats respectively. Administration of catecholamine prior to indomethacin resulted in a significant reduction in total stomach acidity from 2.381±0.042 (indomethacin alone) to 1.581±0.12 (epinephrine-treated) and 1.358±0.12 (dopamine-treated) $p<0.05$. However, dopamine was found to have more inhibitory influence compared to epinephrine. The results suggest an effective drug combination therapy clinically for ulcer patients placed on NSAID especially indomethacin.

Keyword: Ulcer, catecholamine, apoptosis, total acid output, indomethacin, pylorus ligation technique.

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INTRODUCTION

Peptic ulcers and erosions are probably the results of an imbalance between the aggressive and defensive mechanism in gastric duodenal mucosa[1,2,3] Acute gastric mucosal lesions and hemorrhages do frequently occur in patients and experimental animals under various stress situations and after administrations of ulcerogenic drugs[4,5] However, in the last few years, it has been shown that the mucosal defensive factors play important role in protecting the stomach from injurious influences and they can be impaired under many circumstances.

Gastric ulcers are most often localized on the lesser curvature of the stomach. The ulcer is a round to oval parietal defect, 2 to 4 centimeters with a smooth base and perpendicular but elevated borders characterized by inflammatory surrounding[6]. Apoptosis is a highly regulated process that is of critical importance for homeostasis of organisms, functioning to eliminate superfluous cells[7]. Apoptosis has an essential function in maintain the integrity of the gastric intestinal mucosa. Its deregulation is associated with the occurrence of lesion such as atrophic gastritis, peptic ulcer, intestinal metaplasia and stomach tumorigenesis[8].

Gastric epithelial homeostasis is maintained by a balance between cell proliferation rate and programmed cell death or apoptosis, and an imbalance of these two processes leads to increased proliferation of the gastric mucosa and may enhance the effect of carcinogens on DNA increasing the risk of mutational changes and the development of gastric cancer[9]. Several works are available in literature on the role of sympathetic nervous system or its neurotransmitters (catecholamine) in the maintenance of gastrointestinal integrity. Although the reports are conflicting, many agree that catecholamine inhibit gastric acid secretion[10, 11] and inhibit the formation of experimental ulcers in several animal species[12]. In this study, the effects of pre-treatment of exogenous catecholamine on experimental ulcerations were further explored and studied with special emphasis on the role of gastric secretion and the role of apoptosis.

MATERIALS AND METHOD

The experiment was carried out on male Wister rats weighing about 150 to 250g. The rats were raised in the animal house of the department of Basic Medical Sciences of the University of Ilorin, Nigeria and fed on grower's pellet. The animals were kept at a temperature of 24^{o}C-27^{o}C and 60-65% humidity with 12 hours light and dark cycle. The experimental protocols and procedures used in this study were approved by Ethical Committee, University of Ilorin, Nigeria and conform to the guideline of the care and use of animals in research and teaching (*NIH Publication No. 85-93, revised 1985).*

Animal Grouping

The rats were deprived of food 24 hours before the experiment but allowed water ad libitum and were divided into four (4) groups A, B, C, and D as follows;

Group A: (n=5, those not treated at all)

Group B: (n=5, those treated with indomethacin only)

Group C: (n=8, those treated with epinephrine, followed by indomethacin 30 minutes later.)

Group D: (n=8, those treated with dopamine, followed by indomethacin 30 minutes later.)

Drug Administration

A. Indomethacin-induced ulceration. Indomethacin (Strides, Belgium) dissolved in 1% sodium hydrogencarbonate was administered orally (40mg/kg, Elegbe & Bamgbose, 1976) to the fasted rats 30 minutes before the surgery in group B while it was given to group C and D 30 minutes after the administration of test drugs (catecholamine) and were then placed under anesthesia 4 hours after gastric secretion and ulcer was monitored and studied thereafter.
B. Group C was treated with epinephrine (Tonogen R, Ritcher Gedeon, Budapest hungary) intraperitoneally at 0.4mg/kg b.w. (Nagy et al, 1976) 30 minutes before the administration of indomethacin
C. Group D was treated with dopamine (Tonogen R, Ritcher Gedeon, Budapest Hungary) intraperitoneally at 0.4mg/kg b.w. (Nagy et al, 1976), 30 minutes before the administration of Indomethacin.

Experimental Procedure

The animals were anaesthetized with thiopentane at a dose of 6ml/kg b.w. A tracheal canullae was inserted via incision on the neck to ensure normal spontaneous breathing throughout the period of experiment. Abdominal incision through the linea alba to expose the stomach and a semi-transection made at the junction of the pylorus and the duodenum. A pylorus canullae was inserted and tied to collect gastric juice. The specimens were kept warmed by a 40 watts electric lamp to prevent hypothermia. After 4 hours, the animals were sacrificed, the stomachs excised, washed in normal saline to remove any debris, pinned on a cork board and examined for the severity of intraluminal bleeding.

Gastric lesions were evaluated by examining the inner surface with magnifying lens and for ulcer scoring. Ulcers were independently assessed and scored by two observers using the method of Rao[13] as follows;

0= no ulcer (normal stomach)

1= up to 5 petechial hemorrhages with erosion of depth 1mm

3= up 10 petechial hemorrhages with erosion of depth above 1mm. lesions were prepared into slides.

Apoptotic Counting

The stomachs were removed and fixed in 10% formalin and processed for routine staining. Apoptotic counting was done using a microscope (x40, x100, and x400). The apoptotic cells were identified by use of formalin fixed biopsy specimens by use of Hematoxylin and Eosin staining and counting was by serology. The mean number of apoptotic cells were counted in complete, well oriented gastric glands.

Measurement of Gastric Acidity

Samples of gastric content were centrifuged for 10 minutes at a speed of 2000rev/min. The supernatants of each sample were titrated with 0.01M NaOH to a pH of 7. The pH of the gastric juice was measured using an electrometric pH meter. The acid content was expressed in mmol/L using the formula;

$$MaVa/MbVa;$$

Where Ma= concentration of acid, Va= volume of acid, Mb= concentration of base and Vb= volume of base.

Statistical Analysis

Comparisons between treatments were done using student's paired T-test and ANOVA. The results were expressed as the mean, standard error of mean and differences were considered significant at $p< 0.05$.

RESULTS AND DISCUSSION

Acute gastric mucosal and hemorrhages do frequently occur in patients and experimental animals under various stress situations and after administration of the ulcerogenic drugs[14, 4]. This was carried out on animals in group two (control B) and the results showed intensive ulcerations on the glandular stomach as revealed by the results in table 1.

Table 1: Showing the total acid output and the number of apoptotic cells of the rats' stomach.

Group	Treatment	Total acid output (mmol/l)	Number of apoptotic cells
A	Control(untreated)	1.32 ± 0.222	1.10 ± 0.1
B	Indomethacin-treated only	2.381 ± 0.042*	19.50 ± 0.38*
C	Pre-treated with epinephrine	1.581 ± 0.121*	4.30 ± 1.70*
D	Pre-treated with dopamine	1.358 ± 0.241*	1.30 ± 0.33*

Results are presented in mean ±SEM $p< 0.05$.

The investigation on total acid output revealed (table 1) that the total acid output was significantly increased from 1.327± 0.22mmol/L in the normal control (untreated) group to 2.381± 0.042mmol/L in the indomethacin alone group. ($p\leq 0.05$). The total gastric output in the group treated with epinephrine (figure B) showed a significant reduction in gastric output from 2.381±0.042mmol/L to 1.581± 0.121mmo/L when compared with the group treated with indomethacin alone while the output in the group pre-treated with dopamine (figure B) showed a significant decrease as well from 2.381±0.042mmol/L to 1.385±0.241mmol/L when compared with the group treated with indomethacin alone (figure B,$p\leq 0.05$).

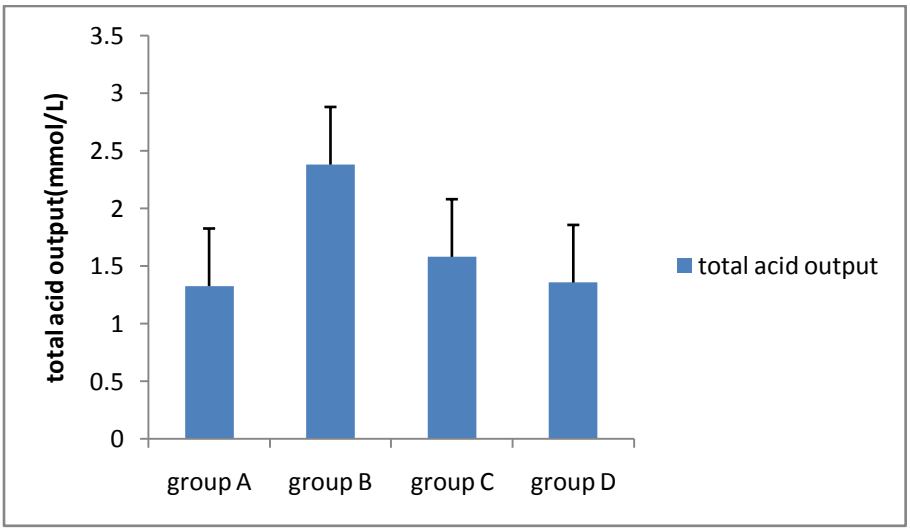

Figure B: Bar chart showing the total acid output (mmol/L) across the groups

Ulcer incidence was also verified in the study and the results showed that ulcer score in the pre-treated group with epinephrine was reduced from 5.0±0.5mm^2 in indomethacin-induced group to 3.0±0.7mm^2 while the ulcer score in the pre-treated group with dopamine was equally reduced from 5.0±0.5mm^2 in the indomethacin-induced group to 2.0±0.7mm^2. The extrapolations from the study revealed that the reduction in the gastrointestinal dysfunction by ulcer was favorably achieved with the introduction of epinephrine and dopamine. It could be further explained that the energy-supplying biochemical alterations are reduced. The catecholamine is able to reduce the activity of membrane/Na^+K^+-dependent/ATPase from rat's gastric mucosa[15]. This enzyme has an important role in the extreme hyper secretion and ulcer formation in pylorus-occluded rats. It has been established that catecholamine have a biphasic effect on gastric ulceration. The ulcerogenic action is due to its alpha action and anti-ulcerogenic effect is due to tachyphylaxis. Nagy et al submitted that the inhibition of a gastric secretion by epinephrine can be explained with its inhibitory effect on membrane ATPase activity

Apoptotic cell study revealed (figure A) that the result of indomethacin on apoptosis was compared with the control (untreated) group and was found to be significantly higher,(the number of apoptotic cells in the indomethacin-induced group was 19.5±0.38 while it was 1.01±0.1 in the normal untreated group). The same was also compared with the test (drug) groups and was equally found to be significantly higher ($p \leq 0.05$). (The number of apoptotic cells was significantly reduced from 19.5±0.38 in indomethacin-induced group to 4.3±1.70 in the epinephrine group and 1.30±0.33 in the dopamine group). In other word, the investigation revealed lower apoptotic indices in both epinephrine and dopamine groups compared to the indomethacin alone which was higher- that is the more the ulcer incidence, the higher the apoptotic index. There are also similar reports on duodenal ulcer[16] (Ashktorab et al, 2003) and gastric ulcer[17] (Santoch, 2006) both showing increased apoptotic index that was significantly reduced after H. pylori eradication.

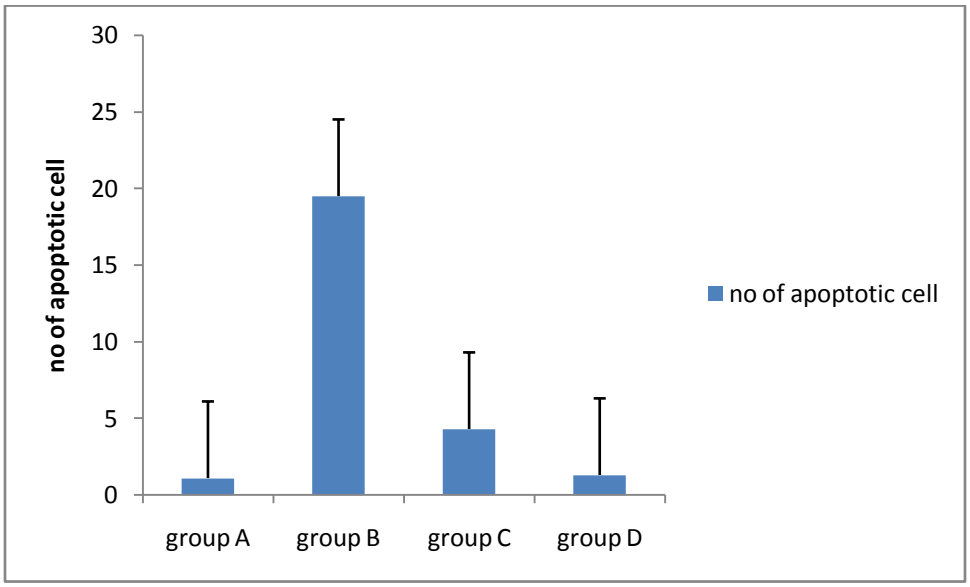

Figure A: Bar chart showing number of apoptotic cells across the groups.

Samples of micrographic plates (plate A, B, C, and D) showed varying degree of severity of ulcer incidence and dead (apoptotic cells) cells. The dead cells were those whose nuclear materials had been destroyed by the ulcerogenic drug administration while the healthy ones still retained theirs. Plate A showed a large concentration of active cells characterized by dark semi round cells because they were not treated with the drugs while plate B showed a huge number of less active or dead cells characterized by faintly-colored round cells as a result of drug administration while plate C and D showed a more improved number of active dark-colored cells despite drug administration displaying some inhibitory tendencies of the cathecolamine pre-treatment.

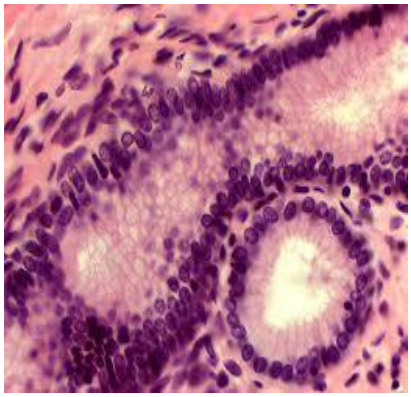

Plate A: control (untreated)

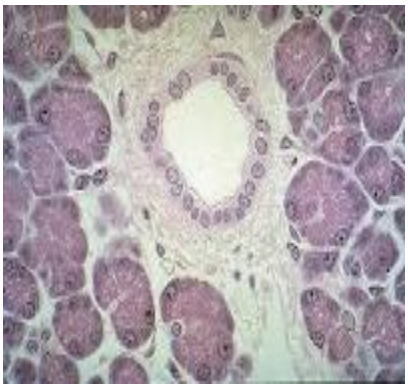

Plate B: indomethacin group

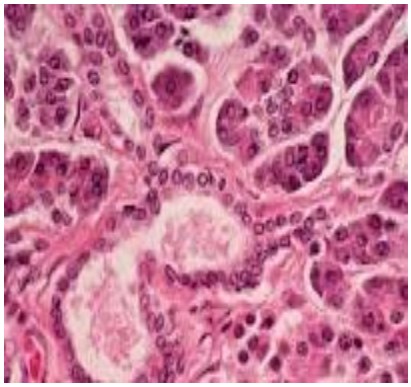

Plate D: epinephrine group

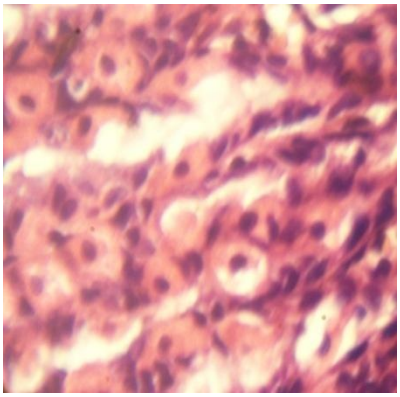

Plate D: dopamine group

CONCLUSION

Summarily, the effects of exogenous catecholamine on indomethacin-induced ulceration and apoptosis in rats' stomach have been studied with the conclusion that pre-treatment with catecholamine in regulated doses and in form of prophylaxis could have a far reaching effect in preventing episodes of gastrointestinal sores via interference on cellular mechanism of membrane/Na^+K^+ dependent/ATPase system.

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