Original Article

Biochemistry

Effects of Ibuprofen on Gastric Mucosa of Albino Rats and the Protective Role of L-Arginine, A Morphological Study Under Light Microscope

1. Santosh Kumar 2. Ghulam Mujtaba Kolachi 3. Kiran Mehtab

1. Asstt. Prof. of Anatomy, SMC, SMU, Karachi 2. Assoc. Prof. of Anatomy, DMC, DUHS, Karachi
3. Asstt. Prof. of Community Medicine, SMC, SMU, Karachi

ABSTRACT

Objective: To observe the effects of Ibuprofen on the gastric mucosa of albino- -rats and the protective role of L-Arginine (if any) under light microscope

Study Design: A prospective experimental study.

Place and Duration of Study: This study was conducted at the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi from 01.04.2008 to 31.05.2008.

Materials and Methods: This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 45 albino rats of either sex between 90-120 days were selected and were divided into three groups, 'A', 'B' and 'C', containing 15 animals each and were further sub-divided into three sub-groups containing 5 animals, according to time of sacrifice, i.e. 4, 6, and 8 weeks respectively. Group 'A' served as control. Group 'B' received ibuprofen, 70 mg/kg body weight and group 'C' received ibuprofen, 70mg and L-Arginine 300 mg, per kilogram body weight per day with feed. Animals were sacrificed at their respective time. The abdomen was opened, stomach was removed and opened along the greater curvature, divided into cardiac, body and pyloric parts, which in turn were fixed in Buffered neutral formalin for 24 hours. Tissues were processed in ascending strength of alcohol, cleared in xylene and infiltrated and embedded with paraffin. Five micron thick sections were made on the rotatory microtome and were stained with Haematoxylin and eosin, Periodic Acid Schiff Orange-G and Alcian blue-Periodic Acid Schiff.

Results: Group A: No ulceration and erosion was found and no abnormality was noted in gastric mucosa **Group B:** With regards to ulcers and erosion the results were moderately significant ($P<0.001$) in B1 and B2 and highly significant ($P< 0.0001$) in B3 when compared to control. Mucosal thickness was decreased, in B1 results were moderately significant ($P<0.001$) and in B2, B3 results were highly significant ($P<0.0001$). **Group C:** No difference in results was noted when compared to group A.

Conclusion: The damaging effects of Ibuprofen on gastric mucosa are minimized when given with L-Arginine.

Key Words: Ibuprofen. L-Arginine, Haematoxylin and eosin

INTRODUCTION

Gastric ulcer disease remains widespread; a stressful lifestyle and non-steroidal anti-inflammatory drugs make significant contributions to this pathological situation (Filaretova et al., 2007). Acute gastritis and peptic ulceration are caused by the heavy use of the non-steroidal anti-inflammatory drugs, particularly aspirins (Bagshaw et al., 1987; Kumar et al, 2003). Non-steroidal anti-inflammatory drugs are one of the most frequently used medicines worldwide. The major clinical problem encountered with them is gastrointestinal complications. In the USA, about 16,500 people per year die as a result of non-steroidal anti-inflammatory drugs associated gastrointestinal complications (Mizushima, 2008). Ibuprofen, the most commonly used non-steroidal anti-inflammatory drug in the United States, was the first member of the propionic acid class of non-steroidal anti-inflammatory drug (Gilman et al., 2006). Propionic acid derivatives include Ibuprofen, Naproxin, Fenoprofen, Ketoprofen, Flurbiprofen and Oxaprozin. These agents offer significant advantages over the aspirin and indomethacin because they are better tolerated as anti-inflammatory drugs (Kato, 2002; Hatazawa et al., 2006). These non-steroidal anti-inflammatory drugs act by inhibiting the enzyme cyclooxygenase which catalyzes the conversion of the arachidonic acid to prostaglandins (Yip-Schneider et al., 2001; Takeeda et al., 2004; Underwood, 2004). Thus drugs that inhibit the prostaglandins secretions decrease the mucus secretion and predispose it to the development of the acid-peptic diseases (Gilman et al., 2006).

L-Arginine is an essential amino acid for the infants and the children and it is semi essential in the adults. Although the arginine is made by the liver as a step in the synthesis of the urea, children cannot produce arginine rapidly enough to support growth requirements (Nakaki, 1994; Jimenez et al., 2004). It is one of the most metabolically versatile amino acids and serves as

a precursor for the synthesis of urea, nitric oxide, polyamines, proline, glutamate, and creatinine (Wu and Maoris, 1998). Nitric oxide synthesized from L-Arginine plays an important role in the gastric mucosal integrity by interacting with endogenous prostaglandins (Takeuchi et al., 1993). Endogenous prostaglandins play a protective role on endotoxin-induced gastric mucosal micro circulatory disturbance and mucosal damage (Pique et al., 1998).

Oral L-Arginine was shown to have the effects to ameliorate ischemia reperfusion injury of the intestine and to protect the barrier function of the intestinal mucosa. This might be related to an increase in the nitric oxide level in intestinal mucosa resulting in maintenance of a stable Endothelin/nitric oxide ratio (Chen et al., 2005; Hung, 2006). Nitric Oxide synthesized from L-arginine has been shown to increased gastric mucosal blood flow, and inhibition of its synthesis has been shown to delay the healing of gastric ulcers (Lazaratos et al., 1995). It has many effects on the mammalian functions including promotion of wound healing, stimulation of the immune system and analgesia (Nakaki, 1994). This study is designed to observe the protective role of L-Arginine (if any) against the mucosal damage caused by ibuprofen.

MATERIAL AND METHODS

This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 45 healthy and active adult albino rats of either sex between 90-120 days were selected for present study. The animals were divided into three groups, A, B and C, containing 15 animals each and were further sub-divided into three sub-groups containing 5 animals each according to time of sacrifice, i.e. 4, 6, and 8 weeks respectively. Group 'A' served as control. Group 'B' received ibuprofen (available in the market as "Brufen" by Bayer Laboratories, Karachi Pakistan) at the dose of 70 mg per kilogram body weight per day orally with feed (Dokmeci et al., 2007) and L-Arginine as "Arginine", General Nutrition Corporation, Pittsburg, USA. The dose of the L-Arginine was 300 mg per kilogram body weight per day with feed (Takeuchi et al., 1993).

The animals were sacrificed at the end of their respective period of treatment under the ether anaesthesia. Their abdomen was opened with a long midline incision. The stomach was removed and opened along the greater curvature with an incision extending from cardiac end to the pyloric end and the contents of the stomach were noted for color, consistency, and blood. The stomach was stretched, fixed and cleaned and dipped in normal saline very gently. The mucosa was observed grossly for color and hemorrhagic spots and then under dissecting microscope for color, blood vessels and hemorrhagic areas and the number of erosions/ulcers. Stomach was divided into cardiac, body and pyloric parts and was fixed in Buffered neutral formalin for 24 hours. After that tissues were processed in ascending strength of alcohol, cleared in xylene and infiltrated and embedded with paraffin. Five micron thick sections were made on the rotatory microtome and were stained with Haematoxylin and eosin, for general morphology and morphometric study which was done under the light microscope under 8x ocular and 40x objective. Sections from body were also stained with Periodic Acid Schiff Orange-G for the mucus content of the surface mucus cells and the mucus neck cells and was observed in all parts of stomach under 8x ocular and 40x objective, and was graded as follows: Mild (++) secretions in the basal part of the cells. Moderate secretions (+++) extending upto the middle part of the cells. Marked (++++) secretions extending upto the apical part of the cells. The cardiac and pyloric parts were also stained with combined Alcian blue-Periodic Acid Schiff technique. Randomly selected every seventh stained section, in three fields were studied for morphology and morphometery. The statistical significance of difference of various quantitative changes between the groups was evaluated by student "t" test. The difference was regarded statistically significant if the 'P' value was equal to or less than 0.05. All calculations were done by utilizing computer software SPSS.

RESULTS

Group-A: The animals were healthy and active. On gross examination the external surface of the stomach was shinny and glistening with no dilated blood vessels. All stained sections showed normal histological architecture.

The mean values of number of erosions/ulcers of stomach and mucosal thickness are shown in table 1 and 2.

The mucous content of the surface mucous cell in subgroups A1, A2 and A3 of cardiac and pyloric parts was same mild (++). The mucus of the surface mucus cell in all subgroups-A1, A2, and A3 of body of stomach were same mild (++) respectively.

Group-B: The animals were weak, sluggish in activities and on the gross observation of the external surface of stomach were dull, slightly red and blood vessels were dilated in all subgroups. In H&E stained sections the lining epithelium was disrupted, exfoliated, ulcers and erosions were present. In combined Alcian blue-PAS stained sections the surface mucus cells were less in number and elongated in appearance and enucleated and nuclei were visualized in the lumen. In the PAS orange-stained sections, the mucous neck cells were distorted in shape and the parietal cells were small in size.

The mean values of number of erosions/ulcers of stomach (Table 1 and Fig.1& 2) in subgroups B1, B2

and B3 were 6±0.31, 6±0.63, and 9±0.63 respectively. The results are moderately significant in subgroup B1 (P< 0.001) and highly significant (P<0.0001) in subgroups B2 and B3 when compared to control.

Table No.1: Mean* value of Erosions / Ulcers recorded in the Stomach of Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Erosions of ulcers recorded in Stomach		
			4th Week	6th Week	8th Week
A	A1	Control	0		
	A2			0	
	A3				0
B	B1	Ibuprofen	6±0.31		
	B2			6±0.63	
	B3				9±0.63
C	C1	Ibuprofen + L-Arginine	0		
	C2			0	
	C3				0
D	D1	L-Arginine	0		
	D2			0	
	D3				0

***Mean±SEM**

The mean values of mucosal thickness of the parts of stomach are shown in Table 2 and Graph 1and 2. Mucosa thickness was decreased in all parts of stomach. In cardiac part in B1 it was moderately significant (P< 0.001) while in B2 and B3 it was highly significant (P<0.0001) when compared to control. In body of and pyloric part of stomach in all subgroups results are moderately significant (P< 0.001) when compared to control.

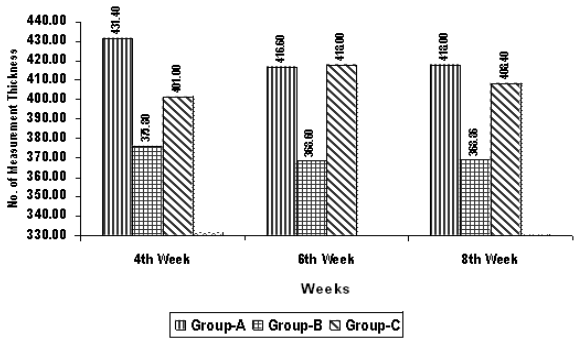
The mucous content of the surface mucous cell in subgroups B1, B2 and B3 of cardiac and pyloric parts were same marked (++++), while in body of stomach was moderate (+++) same as in control animals respectively.

Group- C: The animals were healthy and active and on gross examination of stomach, the surface appeared smooth, shiny and few blood vessels were observed. The H&E stained sections revealed the normal architecture of the stomach. In all subgroups the surface lining cells were composed of simple columnar epithelium lying on the basement membrane similar to control animals. The ulcers/erosions were not visualized(Table 1).

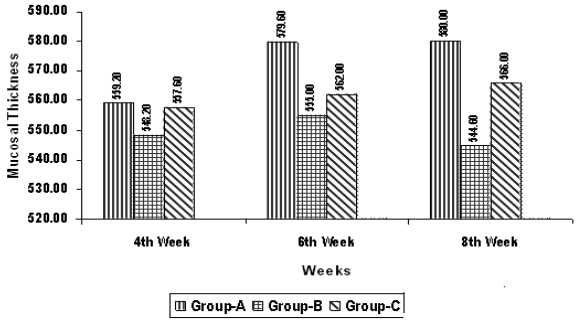
Table No.2: Mean* value Mucosal Thickness (µm) of different parts of Stomach In Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Measurement thickness of cardiac part of stomach		
			4th Week	6th Week	8th Week
A	A1	Control	431.40±12.36		
	A2			416.60±2.655	
	A3				418.00±2.565
B	B1	Ibuprofen	375.80±2.28		
	B2			368.60±3.14	
	B3				368.86±2.276
C	C1	Ibuprofen + L-Arginine	401±6.20		
	C2			418±4.06	
	C3				408.40±2.65
Groups	Sub-groups	Treatment Given	Measurement thickness of body part of stomach		
A	A1	Control	560.00±1.516		
	A2			566.00±1.816	
	A3				572.00±1.414
B	B1	Ibuprofen	446.00±1.549		
	B2			441.00±1.095	
	B3				431.00±2.701
C	C1	Ibuprofen + L-Arginine	558.00±2.042		
	C2			562.00±2.042	
	C3				553.00±5.612
Groups	Sub-groups	Treatment Given	Measurement thickness of pyloric part of stomach		
A	A1	Control	559.20±2.49		
	A2			579.60±1.63	
	A3				580.40±2.46
B	B1	Ibuprofen	548.20±2.10		
	B2			555.00±3.22	544.60±2.03
	B3				
C	C1	Ibuprofen + L-Arginine	557.60±2.50		
	C2			562.00±2.54	566.00±1.80
	C3				

*Mean±SEM



Graph No. 1: Mucosal Thickness of Cardiac Part of Stomach in different Groups of Albino Rats



Graph No.2: Mucosal thickness of the pyloric part of stomach in different groups of albino rats

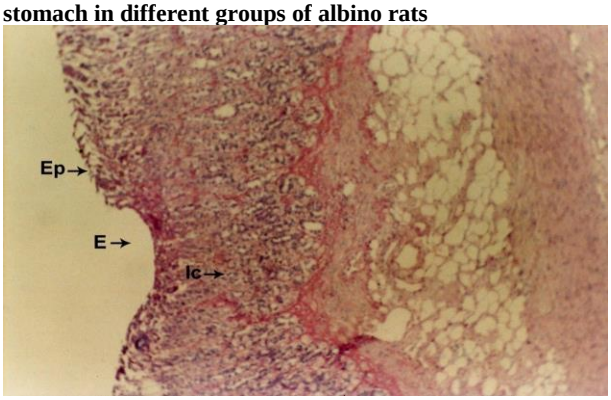


Figure No.1: Shows the mucosal changes in the cardiac part of the stomach in the ibuprofen treated group (time and magnification)

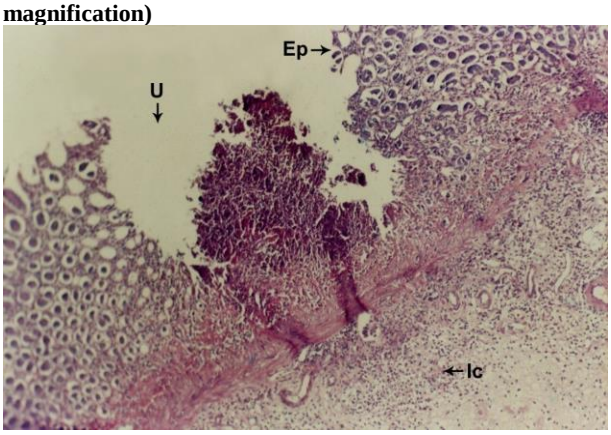


Figure No.2: Shows the mucosal changes in the pyloric part of the stomach in ibuprofen treated group

Few inflammatory cells were observed in the lamina propria and dilated blood vessels were present near the muscularis mucosae in subgroup C1. The muscularis mucosae, submucosa and serosa were visualized as normal in appearance. In combined Alcian Blue-PAS stained and PAS orange-G stained sections all subgroups were normal in appearance similar to control. The mean values of mucosal thickness are shown in Table 2 and Graph 1and 2. The mean values of mucosal thickness of the cardiac part when compared with the subgroups (B1, B2 and B3) animals, the result are significant ($P<0.05$). When compared with control the results are insignificant ($P>0.05$). The mean values of the mucosal thickness of body of the stomach when compared with the subgroups (B1, B2 and B3) the result are significant ($P<0.05$) and insignificant ($P>0.05$) when compared with control. The mean values of the mucosal thickness in the pyloric part are insignificant ($P>0.05$) in B1, significant ($P<0.05$) in B2 and are moderately significant ($P<0.000$) in B3. Insignificant ($P>0.05$) results are obtained when compared with control.

The mucous content of the surface mucous cell in subgroups C1, C2 and C3 of cardiac part moderate (+++) similar to control, while in pyloric and body of stomach was mild (++) from the control animals respectively.

DISCUSSION

Ibuprofen is a commonly used non-steroidal anti inflammatory drug that produces gastric mucosal injury and inhibition of nitric oxide synthase. (Abraham et al., 2005). Nitric oxide is produced from the amino acid L-arginine via the catalytic action of nitric oxide synthase (Garaliene, 2006).

The number of erosion/ulcers was recorded under the dissecting microscope, in group-B animals, the number was increased significantly from 4th to 8th week, and the findings of presence of ulcers over gastric mucosal surface are in agreement with the findings of Tanaka (2002), who used nonsteroidal anti-inflammatory drugs. Kato (2002) used indomethacin and rofecixib; and Jimenez et al (2004) used Ibuprofen in their experimental studies on rat and measured the size of lesion under dissecting microscope but they did not count. In groups-C no erosions/ulcers were found. Bagshaw et al (1987) studied the aspirin-induced chronic gastric ulcer in rat.

In the present study the decrease in mucosal thickness in group-B could be attributed to the injury caused by Ibuprofen, which resulted in demolition phase at 4th week of treatment, at 6th week extensive exfoliation of the surface epithelial cells and at 8th week damage of mucosa was more extensive with surface ulceration. Depending on the severity of injury, the mucosal response varies from vasodilatation and edema of the lamina propria, to erosion and hemorrhage. These agents (NSAIDs) cause a prompt exfoliation of surface epithelial cells with diminished extensive mucus

secretion such that the protective barrier against acid attack may be compromised. Many of their effects are probably mediated by an inhibition of prostaglandin synthesis as suggested by Underwood (2004). These findings are in conformity with those of Hatazawa et al (2006), who observed the effects of Indomethacin on small intestine in rats and reported that the damage on day-1 was deep in the mucosa, whereas the epithelial cells were totally denuded and severe edema was observed in the submucosa. Hung (2006) observed that necrotic cell-injury was found in both epithelial layers and lamina propria. The observations of present study in group-C showed that the protective effect of L-Arginine significantly increased mucosal thickness from 4 to 8 weeks in all parts of the stomach. Takeuchi et al (1993) reported that nitric oxide synthesized from L-arginine plays an important role in the gastric mucosal integrity by interacting with endogenous prostaglandins. The endogenous prostaglandins play a protective role on endotoxin-induced gastric mucosal microcirculatory disturbance and mucosal damage (Pique 1998). Endogenous prostaglandins are involved in adaptive gastric protection against acute injury, and cyclooxygenase -1 is responsible for the production of PGs (Takeeda et al., 2004). According to Jimenez et al (2002), role of L-Arginine in ibuprofen-induced oxidative stress in gastric mucosa was to considerably reduced the gastric lesions and hemorrhagic. Takeuchi et al (1993) reported that L-arginine exhibited over 80% reduction in the HCl-induced gastric lesions.

In the group-C, the surface epithelial cells were significantly increased. It might be due to the increased in blood circulation and mucosal barrier; the findings are confirmed with Kumar et al (2004). Takeeda et al (2004), in their study concluded that endogenous prostaglandins derived from both COX-1 and COX-2 are involved in the mucosal defense of the inflamed stomach, partly by decreasing acid secretion and contribute to maintaining the mucosal integrity under such conditions.

The mucus content of the surface mucus cells, mucus neck cells, which protect the gastric wall from the auto digestion, also neutralize the acid chyme. In the present study the changes occur in mucus cells as a reaction to injury by ibuprofen in group B. In group C the mucus content of the mucus cells was similar to normal due to the increased secretion of the prostaglandins.

CONCLUSION

Based on present study, it is concluded that Ibuprofen induced gastric mucosal damage could by minimized by simultaneous treatment with L-Arginine.

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Address for Corresponding Author:
Dr. Santosh Kumar
Assistant Professor of Anatomy
Sindh Medical College, SMU, Karachi.
E-mail: santosh.kumar@duhs.edu.pk
Cell: 03332317920