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PERI-OPERATIVE EFFECTS OF ROFECOXIB (SELECTIVE CYCLO-OXYGENASE-2-INHIBITOR) ON COLONIC ANASTOMOSIS IN RATS

By
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Rofecoxib (selective cyclo-oxygenase-2-inhibitor) had a wide range of surgical therapies especially (analgesic) in the peri-operative period. Its effects on the intestinal wound healing is unknown. The aim of the studied was to investigate effects of Rofecoxib on colonic anastomosis in rats in the first post-operative week.
The study included 40 male rats divided into two groups, studied, 20 and control 20. In both, resection-anastomosis of left colon with interrupted single layer of vicryl 6/0 was done. The control group received distilled water and the studied group received Rofecoxib pre-operatively and during the first post-operative week.
Bursting pressure of the anastomosis in the studied group decreased from 116.40 + 10.20mmHg preoperatively to 41.60 + 3.00 mmHg and 49.40 + 5.79 mmHg respectively at 3 and 7 days post-operatively (P<0.001) compared to 124.05 + 11.15 mmHg, 107.70 + 12.90 mmHg and 114.55 + 12.91 mmHg respectively in the control group at the same intervals. Epithelialization of grade I with numerous inflammatory cells was noted in the anastomosis in the studied group at the third post-operative day, while epithelialization of grade III and less numerous inflammatory cells together with reformed lamina propria and congested blood vessels were noted at the seventh post-operative day.
Incidence of colonic of fistula was 33% in the studied group versus 9% in the control group at the seventh post-operative day. While mortality was 25% in the studied group compared to 9% in the control group at the seventh post-operative day. No fistula or mortality occurred on the third post-operative day.
In conclusion, administration of Rofecoxib significantly impaired healing (decreased cellular proliferation and collagen deposition) and decreased bursting pressure of colonic anastomosis in rats during the first post-operative week. Rofecoxib increased incidence of fistula in this period.

Key words: Peri-operative effects-Rofecoxib-colonic anastomosis-rats.

INTRODUCTION

Anastomotic dehiscence in the colon is associated with high morbidity and mortality rates. In elective surgery, clinically detected leakage is reported to occur in up to 11 percent[1,2]. Which becomes higher in emergent resection-anastomosis[3]. At least one third of the mortality rates following colo-rectal surgery is attributed to anastomotic leakage[4,5].

The danger of leakage is greatest from the fourth to seventh days when tensile strength of the wound would rise rapidly. By one week the anastomosis resists bursting more strongly than the more normal surrounding tissue[6]. Leakage is about as likely to occur few millimeters from the anastomosis as it is in the anastomosis itself[7].

Intestinal wound healing nearly occurs in a stepwise process including: (i) coagulation and inflammation. (ii) fibroplasia and matrix deposition (ii) angiogenesis and epithelization (iv) collagen maturation and wound contraction[8].

The fibroblast is the workhorse for intestinal wound repair. It is the cell responsible for the formation of all connective tissue components in the healing wound including collagen[9].

Epithelialization is a prominent process in intestinal wound healing. While the basal cells at the wound margin multiply in a horizontal direction, the basal cells behind this margin assume a vertical growth column characteristic of a normal epithelial barrier[10]. An intestinal wound remains in the inflammatory phase of healing with no effective collagen production until it has sutured to an epithelial element [11].

Selective inhibitors of the inducible form of cyclo-oxygenase (cox-2)[12] are of potential benefit in the peri-operative period for both their analgesic effect without causing significant gastrointestinal tract toxicity [13] and perhaps anti-neoplastic actions in prevention of cancer colon (inhibition of angiogenesis)[14,15]. However, the effects of these drugs on intestinal wound healing are unknown.

The aim of this study was to investigate the effects of Rofecoxib (selective cyclo-oxygenase-2-inhibitor) on the healing of colonic anastomosis in rats in the third and seventh days post-operatively

MATERIALS AND METHODS

Fourty-Sprague-Dawly male rats weighing 250-280 grams, 8-10 weeks of age were obtained from the animal house at Faculty of Medicine, Mansoura University. The animals were kept in cages (ten in each cage) at room temperature and were observed for two weeks prior to surgery to exclude any disease. Animals were fed alibitum standard rat chow and all had free access to water through the experiment. The weight of the animals were calculated before surgery, three and seven days post-operatively. The animals were divided into two groups each was 20 in number. Guidelines of Mansoura University for the care of laboratory animals were followed. The animals were kept fasting for 3 days pre-operatively (free access to water only). Animals were anaesthetized with thiopentone sodium 50mg/Kg intra-peritoneally[16].

Abdomen was shaved and the skin was sterilized with Tincture Iodine and Ethyl alcohol. Midline incision was done. Identification of the left colon and a segment of 2cm of it was resected above the peritoneal reflection and re-anastomosis was done by interrupted single layer of vicryl 6/0 (A. B. Care, autosuture, USA) at 1.5mm distances and a rubber drain was left intra-peritoneally to be removed on the seventh post-operative day. The control group received 5ml distilled water intra-peritoneally preoperatively and daily postoperatively for seven days. The studied group received Rofecoxib (10mg/Kg)[15] preoperatively and daily for seven days post-operatively as follows: Rofecoxib 12.5mg tablets, (October Pharma S.A.E. 6th of October City, Egypt) were rinsed in 12.5ml distilled water till it became clear then a dose of 5ml volume was injected intra-peritoneally (Figs. 1,2,3,4).

Eight rats from each group were anaesthetized and re-opened three days post-operatively and the following was done: (i) clinical evaluation for fistulae occurrence, (ii) bursting pressure was measured in situ (mmHg) manometrically using 18(F) abocath connected to mercury pressure gauge at the anastomotic site with two mosquito forceps on both sides of the anastomosis, (iii) the anastomotic sites with 2cm from each side were resected for histological examination and the rats were killed. On the seventh post-operative day, 9 rats of the studied group, and 11 of the control groups were dealt with as was done on the third post-operative day as three rats of the studied group and one rat of the control group died on the seventh post-operative day

Histological evaluation of colonic anastomosis segment was done through paraffin sections preparation stained with:

(1) Haematoxylin and Eosin[14] stain to demonstrate degree of healing, fibroblast infiltration, capillaries formation.

(2) Mallory trichrome stain[15] to study collagen deposition in healed anastomotic area.

The mucosal injury and healing were scored according to Shah et al. (1997)[16]

Grade	*Epithelialization*	*Capillaries*	*Fibroblasts*	*Collagen*
1	1/3 wound	one/mm^3	1-2/mm^3	Few
2	2/3 wound	2-4 / mm^3	3-6/mm^3	Moderate
3	All	>5 1mm^3	>6/mm^3	Marked

Statistical methods:

Mean standard deviation and standard error of mean were used to test for association between different categorical variables. Mann-Whitney u test was used to test for difference in quantitative variables between the two groups. Chi-square test was used to test for difference in the frequency of fistula. P was considered significant if less than 0.05. These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows 7.5 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study included fourty male rats which were divided into two groups control, 20 and studied 20 at the animal house of Faculty of Medicine, Mansoura University started at 5th August 2003.

Weight of the rats is shown in (Table 1) in both groups pre-operatively, three and seven days post-operatively.

Mean bursting pressure in the studied group decreased from 116.40 + 10.20 mmHg pre-operatively to 41.60 + 3.00 mmHg and 49.40 + 5.79 mmHg respectively at 3 and 7 days post-operatively (P<0.001) compared to 124.05 + 11.15 mmHg, 107.70 + 12.90 mmHg, 114.55 + 12.91 mmHg in the control group, preoperatively, 3 and 7 days post-operatively respectively (Table 2), (Fig. 5).

Incidence of fistula was 9% and 33% at 7 days post-operatively in the control and studied group respectively, while no fistulae occurred in either group on the third post-operative day. Mortality rate at 7 days post-operatively in the control and the studied group was 9% and 25% respectively, while no mortality occurred in either group on the third post-operative day (Table 3) (Fig. 6).

As regards wound healing and collagen deposition it was of grade II in the studied group (Figs. 7,8) but of grade I in the studied group (Figs. 9,10) on the third post-operative day. In the control group it was of grade II (Figs 11,12) while in the studied group it was of grade III (Figs. 13,14) on the seventh post-operative day

Inflammatory cells were less numerous in the control group (Figs. 7,11) than in the studied group (Figs. 9,13) on the third post-operative day.

Also in the studied group (Fig. 9) on the third post-operative day more than the same group (Fig. 13) on the seventh post-operative day.

In spite of this fibroblasts and blood capillaries were numerous in the control group (Figs. 7,11) more than the studied group both on the third day (Fig. 9) and seventh day (Fig. 13) post-operatively

Table (1): ***Weight of rats preoperatively (wp) and at 3 days (w3) and 7 days (w7) post-operatively.***

Group		*WP*	*W3*	*W7*	*WP vs W3*	*WP vs W7*
	Mean	262.50	241.50	242.55	<0.001	<0.001
Control	SD	36.26	34.68	32.13		
	Standard error of mean	8.11	7.76	7.18		
	Mean	262.50	234.30	238.25	<0.001	<0.001
Studied	SD	36.11	35.17	33.46		
	Standard error of mean	8.07	7.86	7.48		
P		9.989	0.602	0.718		

VS = versus

Table (2): ***Bursting pressure (mmHg) preoperative (BPP) and at 3 days (BP3) and 7 days (BP7) post-operatively in the studied and the control group.***

Group		*BPP*	*BP3*	*BP7*	*BPP vs BP3*	*BPP vs BP7*
	Mean	124.05	107.70	114.55	<0.001	<0.001
Control	SD	11.15	12.90	12.91		
	Standard error of mean	2.49	2.88	2.89		
	Mean	116.40	41.60	49.40	<0.001	<0.001
Studied	SD	10.20	3.00	5.79		
	Standard error of mean	2.28	0.67	1.28		
P		0.024	<0.001	<0.001		

VS = versus

Table (3): ***Incidence of fistula and mortality at 3 and 7 days post-operatively.***

	Third day		*Seventh day*	
	Control group (n = 20)	Studied group (n = 20)	Control group (n = 12)	Studied group (n = 12)
Fistula	0	0	1 (9%)	4 (33%)
Mortality	0	0	1 (9%)	3 (25%)

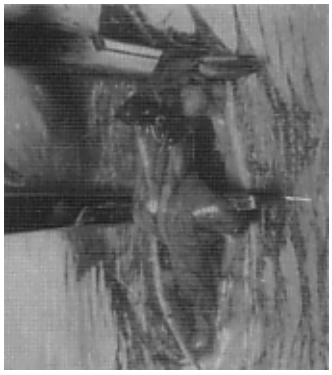

Fig.(1): Colonic anastomosis

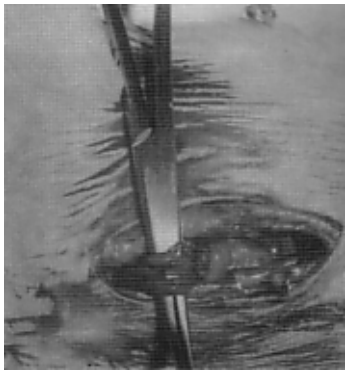

Fig. (2): Finishing the anastomosis

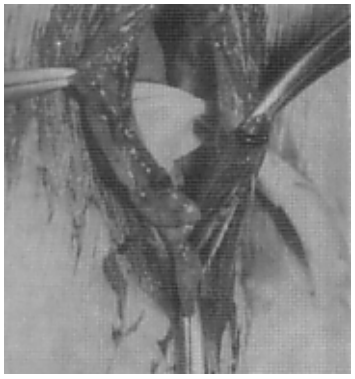

Fig.(3): Intra-peritoneal drain

Fig. (4): Rofecoxib injection

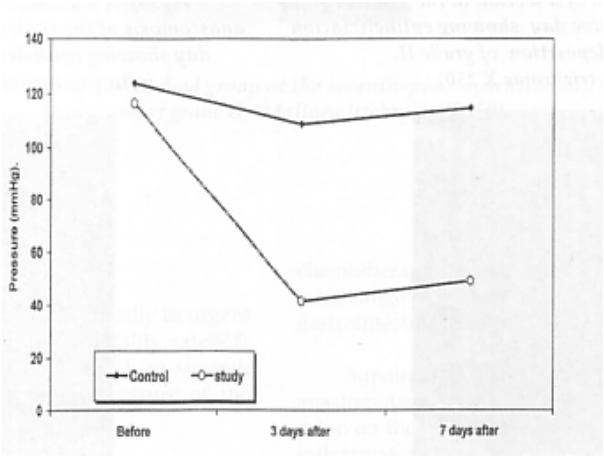

Fig (5) Bursting pressure before and after operation in study and control groups

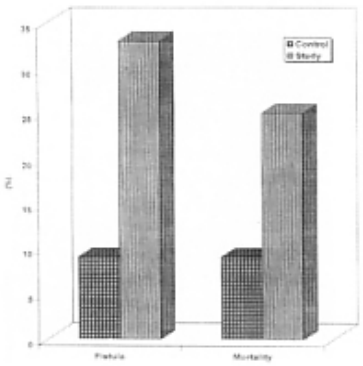

Fig. (6): Frequency of fistula and mortality in the seventh post operative day in the studied and control groups

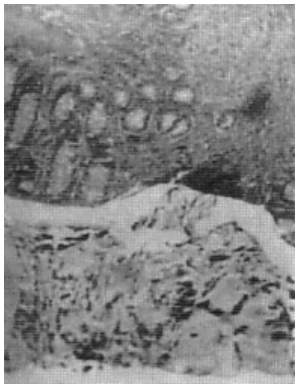

Fig.(7): A photomicrograph of a section in colonic anastomosis in the control group at the third post – operative day showing healed epithelium of grade II, (arrow), few fibroblasts . in reconstructed lamina propria. (Hx, E X 250)

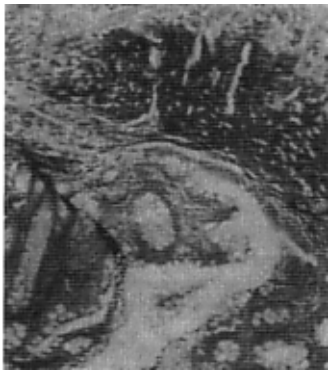

Fig. (8): A photomicrograph of a section in the control group at the third post – operative day showing epitheliziation and collagen deposition of grade II. (Mallory trichrome X 250)

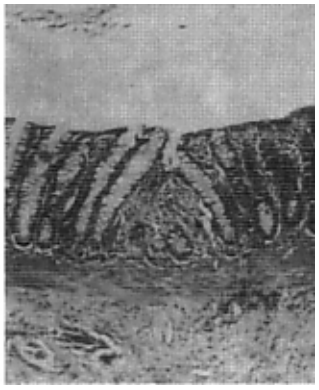

Fig.(9): A photomicrograph of a section in colonic anastomosis of the studied group at the third–post–operative day showing epitheliziation of grade I with numerous inflammatory cells (arrows). (Hx, E X 250)

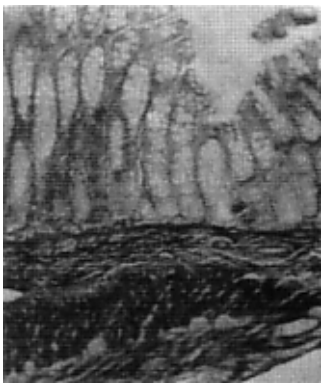

Fig.(10): Colonic anastomosis of the studied group at the third–post–operative day showing epitheliziation and collagen deposition of grade I In the healed area (Arrow). Normal collagen is noted (N) (Mallory trichrome X 250).

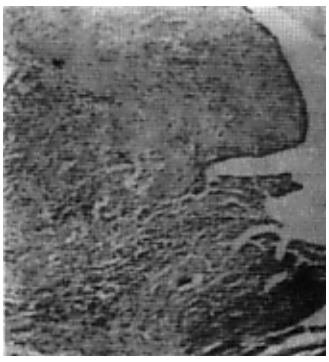

Fig.(11):A photomicrograph of colonic anastomosis of the control group at the seventh post–operative day group showing epitheliziation of grade III , reformed lamina propria and congested blood vessels (V). (Hx, E X 250)

Fig.(12): Colonic anastomosis of the control group at the seventh–post–operative day showing healing epithelium, collagen deposition of grade III (Mallory trichrome X 250).

Fig.(13): A photomicrograph of a section in colonic anastomosis of the studied group at the seventh post–operative day showing healed epithelium of grade II, III, reformation of lamina propria with few small crypts (C), less numerous inflammatory cells (Arrow) (Hx, E X 250)

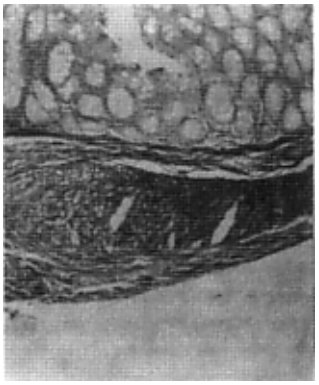

Fig.(14): Colonic anastomosis of the studied group at the seventh–post–operative day showing collagen deposition of grade II, (Mallory trichrome X 250).

DISCUSSION

Leakage of left colonic anastomosis especially in urgent situations is associated with about 30% mortality rate[6,7,8]. The danger of leakage is greatest from fourth to seventh post-operative days due to low bursting pressure of the anastomotic site[9].

Cyclo-oxygenase-2-inhibitor (Rofecoxib) has selectivity used for inhibition of cox-2 at least 5 times potency that for cox-1. It was used for relief of post-operative pain, it has anti-pyretic action by blocking of cytokine induced prostaglandins synthesis in the hypothalamus, has anti-platelet function [13], and it was used as potential target for chemotherapy in colo-rectal cancer [14]. Rofecoxib has this wide range of surgical therapies without causing significant gastrointestinal tract toxicity [12].

Bursting pressure is suitable for measuring of anastomotic strength of early anastomotic healing in the colon on the third and seventh post-operative day [20]. Acute inflammatory response and associated neutrophil recruitment in the anastomosis does not negatively affect the healing in the rat colon [21].

In our study epithelization of grade I with numerous

inflammatory cells were noted in the anastomosis on the third post-operative day, while epithelization of grade III and less inflammatory cells were noted in the anastomosis in the seventh post-operative day together with reformed lamina propria and congested blood vessels indicating the deleterious effects of Rofecoxib.

Histological evaluation of the colonic anastomosis in rats received Rofecoxib revealed more extended inflammation, lower proliferation rate, thin mucosal layer but however, impairment of the reparative colonic epithelium proliferation was essentially the most significant effect[18,19].

Collagen deposition and degradation were estimated by measuring hydroxyproline in the anastomosis[20,,23,25]. Collagen deposition was of grade I and III in the study group in our study on the third and seventh post-operative days respectively. This may points to decreased collagen deposition in the anastomosis in the rats received Rofecoxib. The use of collagen biometrial was advised in gastro-intestinal anastomosis[28,29].

Different techniques for experimental colonic anastomosis were described[23,24,25,26]. In our study single interrupted layer with 1.5mm distances was associated with a fistula rate of 9% and 33% in the control and studied groups. The surgical technique was standardized in both control and studied groups and fistulae occurred on the seventh post-operative day only. The incidence of fistula was 50% in another study[15]. Decrease bursting pressure at the third and seventh post-operative days was associated with impaired colonic epithelization, less inflammatory cells and low collagen deposition in our study.

Mortality rate in the study group was 25% compared to 9% in the control group. This may be due to occurrence of fistulae which was correlated also with low bursting pressure and impaired healing of the anastomosis.

In conclusion, administration of Rofecoxib significantly impaired healing (decreased cellular proliferation and collagen deposition) and decreased bursting pressure of colonic anastomosis in rats during the first post-operative week. Rofecoxib increased the incidence of fistula formation in this period.

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