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Anticancer Activity of Selective Cyclooxygenase-2 Inhibitor with Conventional NSAIDs

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Cyclooxygenase-2 (COX-2) is frequently detected in colon cancer and is believed to play a crucial role in colorectal carcinogenesis. The incidences of colorectal cancer were 0 % in group A, group B was taken as 100 %, 91 % in group C, 46 % in group D, 71 % in group E and 15 % in group F (p = 0.001, ANOVA). Compared with AOM controls, treatment with etoricoxib 10 mg/kg/day also showed lower ACF count and liver dysplasia. Although there is significantly higher COX-2 expression than their adjacent normal tissues, suggesting that the chemo preventive effect of etoricoxib may be mediated by a COX independent pathway. Treatment with etoricoxib (5 mg/kg) had no significant effect on number of ACF, treatment with etoricoxib (10 mg/kg) reduced colorectal cancer incidence and growth in rats.

Key Words: Etoricoxib, Anticancer, Colorectal lesion, Cyclooxygenase-2 (COX-2).

INTRODUCTION

Colorectal cancer is the third leading cause of cancer related death worldwide. Notwithstanding the global declining incidence of colorectal cancer, mortality is still rising in Asian countries. Despite progresses in operative techniques and supporting treatment during the last decades, its prognosis is still relatively poor as only 40-50 % survive for a 5 years follow-up period[1]. It is therefore of great importance to explore the possibilities of efficient prevention to reduce morbidity and mortality. To date, there is no effective measure to prevent development of colorectal cancer. Although life style changes and food habits are the most important factor for colorectal carcinogenesis. Since the observation from the Physician's Health Study that usage of aspirin may reduce the risk of colorectal cancer, intense interest has been directed towards investigation of the anticancer properties of aspirin and non-steroidal antiinflammatory drugs (NSAIDs). Celecoxib, a COX-2 inhibitor, has been shown to reduce polyposis formation in a cohort of patients with familial adenomatous polyposis syndrome. COX-2 expression is upregulated in *H. pylori* induced mucosal inflammation. It is frequently expressed in colorectal cancer as well as in premalignant colorectal lesions. Inhibition of COX-2 *in vitro* results in growth inhibition of colorectal cancer cells. Furthermore, the use of COX-2 inhibitors has been shown to suppress the growth of colorectal cancer xenografts in nude mice[2]. Unlike colorectal cancers, however, there are a lack of animal and human data demonstrating the effectiveness of COX-2 inhibition and NSAIDs in the prevention of colorectal cancer.

Increased understanding of the etiology of colon cancer has been observed from the studies of AOM induced neoplasia[3]. AOM is a powerful colon, liver and kidney carcinogen in rodents[4-6].

EXPERIMENTAL

Carcinogen (Azoxymethane) (CAS:25843-45-2) was obtained from Sigma, St Louis, MO. Test drug (etoricoxib) was purchased from Sun Pharma, Ahmedabad while, Standard drug (Doxorubicin Hcl inj) obtained from Cadila Health Care.

Preparation of drug: For the induction of colorectal tumors, Azoxymethane is prepared according to the standard method[7]. A fresh solution was prepared once a week. Animals were weighed weekly and azoxymethane dosage was adjusted accordingly, *i.e.*, 15 mg/kg body weight. The solution was injected subcutaneously once a week for 2 weeks. Eight weeks after the last injection, the animals were sacrificed.

Animals: Virus-free adult male inbred male Wistar rats (> 100 g) were taken from the Laboratory Animal bread center, School of Pharmaceutical Sciences, SOA University, Bhubaneswar. All the experimental procedures and protocols used in this study were reviewed and approved *via* the Approval No. 17/09/IAEC/SOAU by the Institutional Animal EthicalCommittee (IAEC) of School of Pharmaceutical Sciences, SOA University, Bhubaneswar, constituted in accordance with the guidelines of the CPCSEA, Government of India.

Anticancer activity: Ten 13 weeks-old inbred male Wistar rats were taken and housed in wire cages to minimize coprophagy and maintained in an air-conditioned environment of 23 ± 2 ºC with a 12:12 h light-dark cycle. Rats were taken and separated by weight into six groups and started on their experimental doses. Rats were weighed weekly and average diet intake/cage of six rats was recorded at weeks 1, 2, 3, 4, 5, 6, 10 and 14 whilst on the experimental doses. After 4 weeks on the different experimental doses rats were s.c. injected once a week for 2 weeks with AOM (Sigma, St Louis, MO) dissolved in normal saline at a dose of 15 mg/kg body weight. All experimental procedures using animals were approved by the Animal Ethics Committee and by the SOA University.

Eight weeks after the second AOM infusion all rats were sacrificed by decapitation. The intestinal tract was removed and the colon (cecum to anus) was removed and rinsed with saline, opened longitudinally, flattened onto blotting paper and fixed in 10 % buffered formalin overnight[7]. The tissue was divided into two equal segments (proximal and distal) between the peyers patch and the herring bone and stained with 0.2 % methylene blue in phosphate-buffered saline for 20 min. Aberrant crypts were distinguished from the surrounding normal crypts by fulfilling the following criteria: increased size, slit like lumen, thicker cell wall and raised crypt. Using a light microscope (50 × magnification), the number of aberrant crypts and foci were recorded for the proximal, transverse and distal colon.

Sections from liver, proximal, transverse and distal colon were removed and fixed in 10 % neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for light microscopic evaluation.

RESULTS AND DISCUSSION

The mean body weights at the beginning of the experiment were 110 g and at end of etoricoxib treatment (after 14 days) the body weights of rats were as follows:

Control, 125 ± 1.41; AOM (15 mg/kg), 137 ± 1.69; etoricoxib (5 mg/kg), 132 ± 1.84; etoricoxib (10 mg/kg), 127 ± 1.71; etoricoxib (20 mg/kg), 128 ± 2.04; doxorubicin (1 mg/kg), 126 ± 2.36. As we have reported, rats on all treatment groups didn't find the body weight differences at the beginning of the experiment, *i.e.*, initial stage of the drug treatment, from first week to fourth week no significance difference of body weight was noted. Etoricoxib (5 mg/kg) ($p < 0.05$) and AOM (15 mg/kg) ($p < 0.01$) gained more weight than rats on the other four groups shown in Table-1. During the first 48 h period following the administration of AOM, 2 rats in the AOM group and 1 rat in the etoricoxib (20 mg/kg) groups developed diarrhea and died. Deaths were attributed to acute AOM toxicity. A small decrease in the body weight was observed in all rats receiving AOM (Fig. 1). However, no group differences were noted in the body weights on 10th week of study but on the day of sacrifice AOM group ($p < 0.01$) and etoricoxib (5 mg/kg) group ($p < 0.05$) showed significance difference in body weight (Table-1). Similarly, no differences were noted in the liver or kidney weights among the three treatment groups.

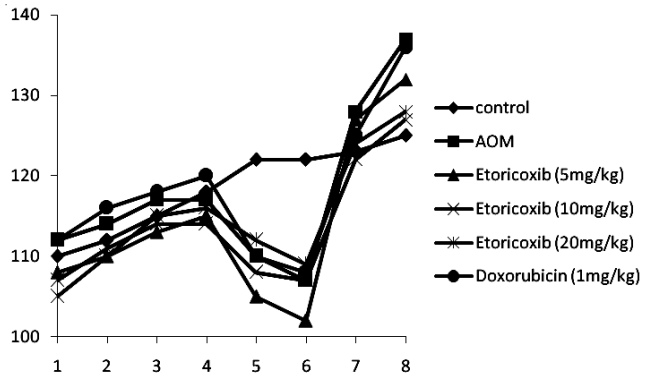


Fig. 1. Comparison of body weight of AOM treated rats (14 week)

The histopathologic grading of the H and E liver sections of 14 weeks post-AOM treatment are summarized in Table-2. Statistical analysis revealed significant differences between rats treated with different doses of etoricoxib with respect to the animals treated with doxorubicin exhibiting each of the reactive and severe liver dysplasia (Fig. 2). Differences in the extent of liver dysplasia between rats on AOM and etoricoxib (10 mg/kg) were compared by using Fisher's exact test. A statistically significant difference is observed. However, between

TABLE-1
EFFECT ON BODY WEIGHT OF STANDARD (DOXORUBICIN) AND DIFFERENT DOSES OF TEST (ETORICOXIB) TREATMENT ON AOM TREATED RATS

Group	Week							
	1	2	3	4	5	6	10	14
Control	110 ± 4.6	112 ± 2.4	115 ± 4.3	118 ± 2.9	122 ± 1.23	122 ± 1.23	123 ± 1.82	125 ± 2.01
AOM (15 mg/kg)	112 ± 4.5	114 ± 1.8	117 ± 6.6	117 ± 3.1	110 ± 2.58**	107 ± 1.78***	128 ± 1.98	137 ± 1.84**
Etoricoxib (5 mg/kg)	108 ± 3.8	110 ± 2	113 ± 5.13	115 ± 3.2	105 ± 1.52***	102 ± 1.12***	127 ± 2.22	132 ± 1.65*
Etoricoxib (10 mg/kg)	107 ± 2.1	111 ± 2	114 ± 4.4	114 ± 5.1	108 ± 2.47**	107 ± 1.96***	122 ± 1.41	127 ± 2.63
Etoricoxib (20 mg/kg)	105 ± 2.9	110 ± 4.2	115 ± 3.5	116 ± 4.3	112 ± 2.32**	109 ± 2.68***	124 ± 2.42	128 ± 2.08
Doxorubicin (1 mg/kg)	112 ± 2.9	116 ± 4	118 ± 5.5	120 ± 4.4	110 ± 1.93**	108 ± 1.36***	125 ± 1.63	126 ± 2.38
F Value	0.664	0.663	0.13	0.297	7.85^b	14.37^b	1.42	4.54^b

Values are expressed as means ± SEM (n = 6). Data were analyzed by using one-way ANOVA followed by Dunnet's test. (F-value denotes statistical significance $^a p < 0.05$, $^b p < 0.01$). t-Value denotes statistical significance at *p < 0.05, **p < 0.01, ***p < 0.001, respectively in comparison to control group.

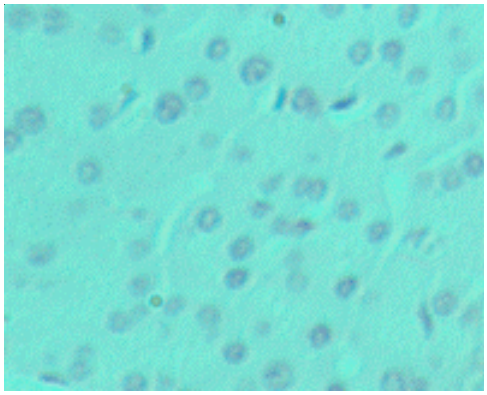
TABLE-2
EFFECTS OF STANDARD (DOXORUBICIN) AND DIFFERENT DOSES OF TEST (ETORICOXIB) ON AZOXYMETHANE-LIVER HISTOLOGY

Group	No. of animals	Liver dysplasia (%)		
		Reactive	Mild	Severe
Control	6	0	0	0
AOM (15 mg/kg)	6	1(17)	1(17)	4(67)
Etoricoxib (5 mg/kg)	6	1(17)	1(17)	4(67)
Etoricoxib (10mg/kg)	6	3(50)	2(33)	1(17)*
Etoricoxib (20mg/kg)	6	2(33)	2(33)	2(33)
Dixorubicin (1mg/kg)	6	5(83)	1(17)	0

Number in () indicates percentage of rats on specified dosing in liver dysplasia category. AOM and etoricoxib (10 mg/kg) group were compared by using Fisher's exact test. *$p < 0.05$.

rats on the AOM and etoricoxib (10 mg/kg) group, with rats in the AOM and etoricoxib (5 mg/kg) group having a greater proportion of severe liver dysplasia. Etoricoxib (5 mg/kg) treated rats that were infused with AOM had the highest levels of severe dysplasia (67 %) as compared to rats on etoricoxib (10 mg/kg), etoricoxib (20 mg/kg) and doxorubicin (1 mg/kg) group (17, 33 and 0 %, respectively). Rats treated with etoricoxib (10 mg/kg) and etoricoxib (20 mg/kg) groups had higher levels (33 %) of mild dysplasia than rats on AOM (15 mg/kg) group, etoricoxib (5 mg/kg) and doxorubicin (1 mg/kg) group *i.e.*, (17 %). AOM (15 mg/kg), etoricoxib (5 mg/kg) treated rats that were infused with AOM had the lowest percentage of reactive liver dysplasia (17 %) compared to 33, 50 and 83 % for rats on etoricoxib (20 mg/kg), etoricoxib (10 mg/kg) and doxorubicin (1 mg/kg) group, respectively.

The distribution and frequency of ACF in rat groups 1-6 are shown in Table-3. There were few or no ACF in the colons of rats who did not receive AOM injection (groups 1) as expected. For the rats treated with AOM, the frequency of ACF in etoricoxib (5 mg/kg) and etoricoxib (20 mg/kg) groups were higher in the proximal, middle and distal colon Fig. 3. In comparison with all the groups, those given etoricoxib (10 mg/kg) administration to rats has a significant reduction in the frequency of ACF incidence in the whole colon ($p < 0.001$) in Table-3. We observe a significant decrease in the number of ACF 46 and 15 % in the etoricoxib (10 mg/kg) and doxorubicin (1 mg/kg) group, respectively where as etoricoxib (5 mg/kg) 91 % and etoricoxib (20 mg/kg) 71 % did not show significant difference in the number of ACF.



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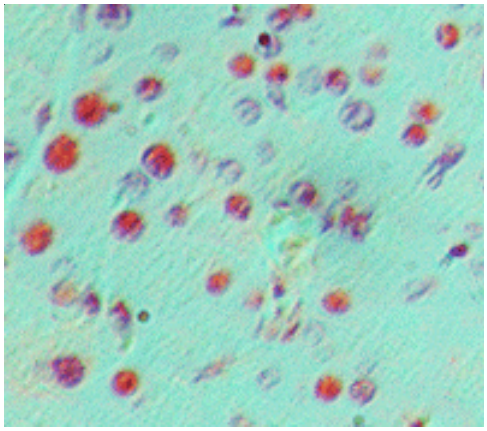
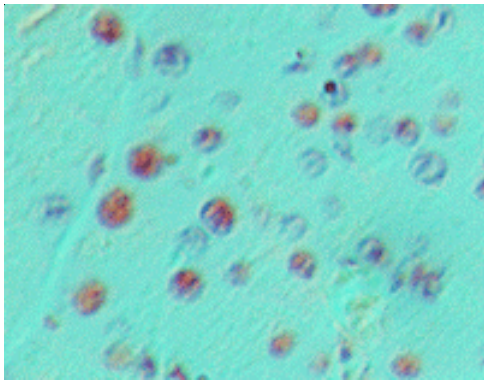


AOM (15 mg/kg) treatment



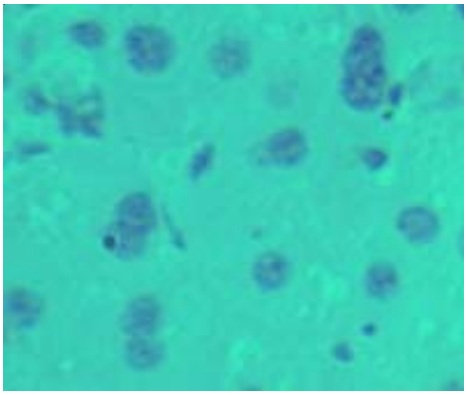
Etoricoxib (10 mg/kg) treatment

Fig. 2. Photomicrograph demonstrating liver dysplasia (400 ×)

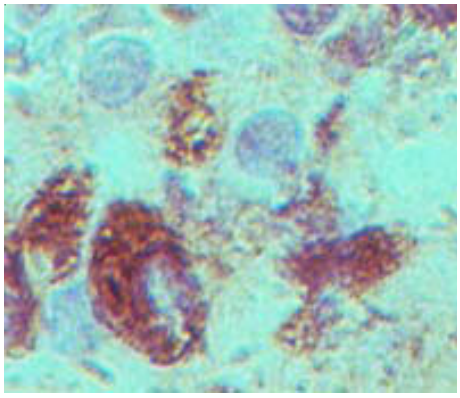
TABLE-3
EFFECT OF STANDARD (DOXORUBICIN) AND DIFFERENT DOSES OF TEST (ETORICOXIB) TREATMENT ON THE NUMBER OF ACF

Group	Dose and route	Number of ACF			Total No. of ACF
		AC	TC	DC	
Control	–	0	0	0	0
AOM	15 (mg/kg) S.C.	48 ± 1.29	76 ± 2.112	34±2.206	158
Etoricoxib	(5 mg/kg) P.O.	43 ± 2.80 (89 %)	69 ± 2.851 (90 %)	32±1.966 (94%)	144 (91 %)
Etoricoxib	(10 mg/kg)P.O.	24 ± 2.75*** (50 %)	29 ± 2.160*** (38 %)	21 ± 1.932*** (61 %)	73 (46 %)
Etoricoxib	(20 mg/kg) P.O.	37±2.60** (77 %)	43 ± 1.966** (56 %)	33 ± 1.549 (97 %)	113 (71 %)
Doxorubicin	(1 mg/kg) P.O.	7±1.54*** (15 %)	12 ± 1.095*** (15 %)	5 ± 0.632*** (14 %)	24 (15 %)
F Value		87.66[b]	248.82[b]	88.88[b]	

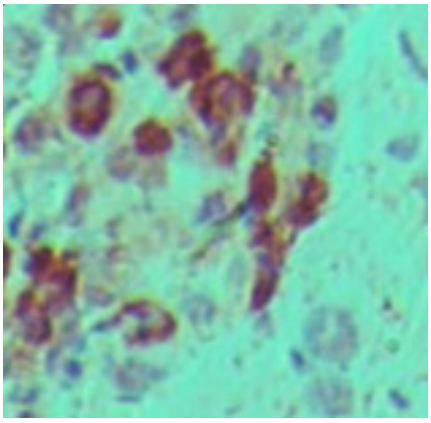
Values are expressed as means ± SEM (n = 6). Data were analyzed by using one-way ANOVA followed by Dunnet's test. (F-value denotes statistical significance [a]$p < 0.05$, [b]$p < 0.01$). t-Value denotes statistical significance at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, respectively in comparison to AOM (15 mg/kg) treated group.



Control



AOM (15 mg/kg) treatment



Etoricoxib (10 mg/kg) treatment

Fig. 3. Photomicrograph demonstrating changes in colon (400 ×)

The finding that AOM-induced cancers are less frequent in germ-free animals and animals treated with antibiotics suggests an important role for the colonic flora in AOM-induced carcinogenesis. Similar data is not available for human colon cancer. For further exploration of these issues, male inbred wister rats were administered a chemical carcinogen, AOM, for 14 weeks, to evaluate the anticancer activity of etoricoxib with respect to its antimicrobial effect in the intestinal flora[7-9]. Recently, COX-2 and Bcl-2 were found to be coexpressed in the glandular corpus epithelium of rats treated with MNNG. This up regulated expression is associated with cell proliferation, atrophy and intestinal metaplasia of the stomach. It is therefore logical to anticipate that treatment with etoricoxib (COX-2 inhibitor) may have an antiproliferative and hence chemopreventive effect on AOM induced colorectal and liver cancer[3].

In conclusion, treatment with etoricoxib (5 mg/kg) had no significant effect on number of ACF and liver dysplasia, treatment with etoricoxib (10 mg/kg) reduced colorectal cancer incidence and growth as well as liver dysplasia significantly in rats. Etoricoxcib significantly inhibit the microorganism in the intestinal flora. Thus it is postulated that the antimicrobial effect of etoricoxcib has potentiate the anticancer activity in AOM induced cancer model in wister rat.

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