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Original

Effect of different non-steroidal anti-inflammatory drugs, aspirin, nimesulide and celecoxib on the disaccharide hydrolases and histoarchitecture of the rat intestinal brush border membrane

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to cause gastrointestinal damage. New anti-inflammatory drugs have been developed in an attempt to improve their gastrointestinal side effect profile which however failed to do so. Therefore, the objective of the present study was to compare the effect of three different NSAIDs, aspirin, nimesulide and celecoxib on the intestinal brush border membrane (BBM) marker enzymes and correlate these alterations to the histoarchtecture of the intestine using electron microscopic study. Female Wistar rats were divided into four different groups viz: Group I (Control), Group II (aspirin treated), Group III (nimesulide treated) and Group IV (celecoxib treated). The Group II, III and IV received the corresponding drugs dissolved in water orally at a dose of 40 mg/kg body weight, while the control received the vehicle only. After 28 days, all the treatment groups demonstrated significant alterations in the activities of intestinal disaccharide hydrolases and alkaline phosphatase in both the crude homogenates and BBM preparations as well. The histopathological observations also showed considerable changes in the intestinal mucosa. It was suggested that NSAIDs like aspirin, nimesulide and celecoxib pose intestinal side effects due to initial changes in the enzymatic composition of the intestinal apical membranes. It was further concluded that newly discovered NSAIDs such as celecoxib has better safety profiles but studies are still required to comment decisively on the suitability of various NSAIDs depending upon their cyclooxygenase enzyme specificity.

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Key words: *Non-steroidal anti-inflammatory drugs. Membrane disaccharidases. Intestinal brush border membrane.*

EFECTO DE DIFERENTES FÁRMACOS ANTI-INFLAMATORIOS NO ESTEROIDEOS, ASPIRINA, NIMESULIDA Y CELECOXIB, SOBRE LAS HIDROLASAS DE DISACÁRIDO Y LA HISTO-ARQUITECTURA DE LA MEMBRANA DEL BORDE EN CEPILLO INTESTINAL DE LA RATA

Resumen

Es sabido que los fármacos anti-inflamatorios no esteroideos (AINE) causan daño gastrointestinal. Los nuevos fármacos anti-inflamatorios se han desarrollado con la esperanza de mejorar su perfil de efectos adversos gastrointestinales, lo que sin embargo no se ha logrado. Por lo tanto, el objetivo de este estudio fue comparar el efecto de tres AINE distintos, aspirina, nimesulida y celecoxib, sobre las enzimas marcadoras de la membrana del borde en cepillo (MBC), y correlacionar estas alteraciones con la histo-arquitectura del intestino utilizando la microscopia electrónica. Se dividió a ratas hembra Wistar en cuatro grupos distintos: Grupo I (Control), Grupo II (tratado con aspirina), Grupo III (tratado con nimesulida) y Grupo IV (tratado con celecoxib). Los grupos II, III y IV recibieron por vía oral el fármaco correspondiente disuelto en agua, a una dosis de 40 mg/kg de peso corporal, mientras que el grupo control sólo recibió el vehículo. Tras 28 días, todos los grupos de tratamiento mostraron alteraciones significativas en las actividades de las disacaridasas intestinales y la fosfatasa alcalina tanto en las preparaciones homogéneas crudas como en las preparaciones de MBC. Las observaciones histopatológicas también mostraron cambios considerables en la mucosa intestinal. Se sugería que los AINE como la aspirina, nimesulida y celecoxib acarrean efectos adversos debidos a cambios intestinales en la composición enzimática de las membranas intestinales apicales. Se concluye, además, que los nuevos AINE como el celecoxib poseen mejores perfiles de seguridad pero aún son necesarios estudios para poder opinar de forma decisiva sobre la idoneidad de los diversos AINE dependiendo de su especificidad por la enzima ciclooxigenasa.

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Palabras clave: *Fármacos anti-inflamatorios no esteroideos. Disacaridasas de membrana. Membrana del borde en cepillo intestinal.*

Introduction

Non-steroidal anti inflammatory drugs (NSAIDs) - the most tolerated and widely prescribed drugs in the treatment of inflammation and pain[33] act by inhibition of the enzyme cyclooxygenase (Cox). But, these broad ranges of beneficial effects attributed to them come at the cost of various side effects such as ulcers and other serious gastrointestinal (GI) complications.[23, 25] Due to the use of NSAIDs at a large scale, these GI toxicities account for the most prevalent drug-associated health risks. These side effects are due to the non-specific inhibition of the two isoforms Cox-1 and Cox-2 that are the constitutive and inducible forms of the enzyme, prostaglandin synthase. Cox-2 is responsible for inflammation whereas Cox-1 is required for normal homeostasis.[7] Since the discovery of these two iso-enzymes and their characterization established search for new NSAIDs with varying Cox-2 specific inhibition and thus different levels of GI tolerability have gained pace.

Selective Cox-2 inhibitors (such as celecoxib) exhibit 100-1000 fold selectivity for Cox-2 with minimum side effects as compared to the other relatively classical NSAIDs such as nimesulide and aspirin.[10, 12] Numerous reports suggest that both aspirin and nimesulide pose dose-related GI toxicities.[21, 22] Although celecoxib a recent and widely prescribed NSAID is considered safer, it is also found to be associated with a number of side effects such as gastroenteritis, hemorrhoids, dysphagia etc.[20, 28]

Therefore, studies are still warranted to evaluate the safety profile of various NSAIDs belonging to different classes, so as to comment decisively on the desirability of these drugs which can help in better management of the patients suffering from inflammatory conditions.

It has been hypothesized that these drugs may cause intestinal toxicity by initial local alterations in the enzymatic composition of brush border membrane (BBM) lining the GI tract upto different levels. Membrane bound proteins are important constituents of the BBM, as they contain disaccharidases viz: sucrase, lactase and maltase and also binds the alkaline phosphatase, dipeptidases, enterokinases etc.[13, 14] Moreover, it also contains the transport proteins as well as the Na^+/K^+ ATPase responsible for maintaining Na^+ ion gradient required for active inward transport of products of digestion such as glucose and amino acids.[6, 30]

The present study was designed to compare the effects of three different NSAIDs viz: aspirin, nimesulide and celecoxib (which represent different classes of Cox inhibitors) on the enzyme composition (intestinal BBM associated disaccharidases and alkaline phosphatase) of the brush border membranes. The study was further extended to report the alterations brought about by these at ultra-microscopic levels using histological parameters by electron microscopy.

Materials and methods

Animals: The female Wistar rats (175-200 g) were procured from the Central Animal House of the Panjab University. The principles of animal care as laid down by the National Institute of Health (NIH publication no. 23-85, revised in 1985) were strictly followed in the maintenance of the animals. Animals were acclimatized for a period of one week prior to any treatment. They were fed with standard pellet diet and water ad libitum. This diet constitutes crude protein 24%, other extract 4%, crude fibre 4%, ash 8%, calcium 1%, phosphorus 6% and nitrogen-free extract 50%, vitamin B_{12}, thiamine, riboflavin, pentotothenic acid, niacin, pyridoxine, choline chloride and folic acid. The mineral content of this diet was determined earlier by neutron activation method[19] and it was found not to be deficient in any mineral.

The animals were randomly segregated into four groups. Group I served as control, Group II was administered aspirin (40 mg/kg)[24] orally, Group III nimesulide (40 mg/kg)[27] and Group IV celecoxib (40 mg/kg)[27] orally for 28 days. The drugs were dissolved in water and the controls were administered equal amount of it as vehicle. The dosage was chosen so as to compare the toxic effects of the equal but clinically safer concentrations of these drugs. In this regard, it is important to note that NSAID-induced gastric mucosal lesions are time and dose dependent.[9] On the 29th day after overnight fasting, animals were sacrificed under an overdose of ether anaesthesia. In order to avoid diurnal variation in the different parameters, animals were sacrificed uniformly around 8.00 am throughout the study. Intestine was removed, divided into duodenum, jejunum, ileum and colon, washed with pre-cooled physiological saline, weighed separately and a 10% homogenate of different segments prepared. A small aliquot used for enzyme estimations and rest is used for the preparation of BBM as described.

Preparation of Intestinal Brush Border Membrane: Intestinal BBM was isolated by the method of Schmitz et al.[26] A known weight of each portion of the intestine was minced and 10% (w/v) homogenate was prepared in chilled 1mM Tris-50 mM mannitol buffer (pH-7.4) in a motor driven homogenizer at 4 ^{0}C. The homogenate was filtered through two layers of cheese cloth. To the filtrate, anhydrous $CaCl_2$ was added with constant stirring on a magnetic stirrer to a final concentration of 10 mM and left for 10-15 minutes in cold. Later it was centrifuged at $2,000 \times g$ for 10 minutes at 4 ^{0}C. The supernatant was recentrifuged at 42,000 xg for 20 minutes. The pellet obtained was suspended in 20 vol. of 50 mM sodium maleate buffer (pH 6.5-6.8) and recentrifuged at 42,000xg for 20 minutes. The final pellet was suspended in 50 mM sodium maleate buffer (pH 6.5-6.8) containing 0.02% sodium azide. The final membrane preparation obtained was similar to the P_2 fraction of Schmitz et al.[26] and used for various biochemical studies.

Table Ia

Effect of aspirin, nimesulide and celecoxib on sucrase and lactase activities in various segments of rat intestinal homogenates

| | Enzyme activity (μmoles/100 mg protein/min) | | | | | | | |
| | Sucrase | | | | Lactase | | | |
Intestinal segment	Control	Aspirin	Nimesulide	Celecoxib	Control	Aspirin	Nimesulide	Celecoxib
Duodenum	38.4 ± 0.2	24.3 ± 0.33**	21.7 ± 0.23	38.3 ± 0.27	7.9 ± 0.09	9.6 ± 0.13	6.9 ± 0.12***	9.2 ± 0.21**
Jejunum	26.2 ± 0.46	36.1 ± 0.35	40.8 ± 0.39**	27.3 ± 0.4	8.1 ± 0.12	12.6 ± 0.11***	21.1 ± 0.24	13.2 ± 0.13**
Ileum	27.2 ± 0.39	24.1 ± 0.47**	24.4 ± 0.39	24.0 ± 0.16	6.7 ± 0.13	9.3 ± 0.09	12.1 ± 0.15	4.1 ± 0.09
Colon	14.3 ± 0.37	13.6 ± 0.2**	12.3 ± 0.5**	20.9 ± 0.19**	4.3 ± 0.24	8.6 ± 0.26	4.2 ± 0.11	4.5 ± 0.13

Table Ib

Effect of aspirin, nimesulide and celecoxib on maltase and alkaline phosphatose activities in various segments of rat intestinal homogenates

| | Enzyme activity (μmoles/100 mg protein/min) | | | | | | | |
| | Maltase | | | | Alkaline phosphatase (μmoles/mg protein/min) | | | |
Intestinal segment	Control	Aspirin	Nimesulide	Celecoxib	Control	Aspirin	Nimesulide	Celecoxib
Duodenum	44.5 ± 0.35	50.9 ± 0.18***	52.2 ± 0.25***	50.2 ± 0.12**	1.52 ± 0.12	1.85 ± 0.06***	1.28 ± 0.26	1.07 ± 0.07**
Jejunum	44.7 ± 0.11	49.0 ± 0.31**	45.8 ± 0.08**	42.4 ± 0.33**	0.85 ± 0.17	1.71 ± 0.09***	1.47 ± 0.26**	0.628 ± 0.009*
Ileum	32.0 ± 0.43	40.1 ± 0.34	34.7 ± 0.25*	32.4 ± 0.22**	0.30 ± 0.06	0.87 ± 0.09	1.32 ± 0.06	0.607 ± 0.098**
Colon	33.1 ± 0.07	21.4 ± 0.19	30.0 ± 0.34	42.7 ± 0.10	0.25 ± 0.10	0.67 ± 0.08***	0.49 ± 0.47*	0.41 ± 0.09*

Values are mean ± SD of four observations.
*, **, ***, Represent p < 0.05, p < 0.01, p < 0.001 respectively.

Intestinal enzyme assays

Assay of disaccharidases: The activities of sucrase, lactase and maltase were determined in homogenate and BBM preparation as well by measuring the D-glucose liberated from the respective disaccharide sugar substrate using a glucose oxidase-peroxidase enzymatic system (GOD-POD) of Dahlqvist.[8]

Assay of alkaline phosphatase: Alkaline phosphatase activity was assayed in both the homogenates and the BBM according to the method of Bergmeyer[3] by measuring the liberated inorganic phosphate from the phosphate monoester substrate, p-nitrophenyl phosphate.

Protein Estimation: Total protein content in the BBM was determined by the method of Lowry[17] using BSA as the standard.

Scanning electron microscopic study: The intestine of control as well as tests (aspirin, nimesulide, celecoxib) animals was opened in the middle and the epithelium exposed, fixed on hard sheet in 25% glutaraldehyde phosphate buffer (pH 7.4). The fixed intestinal epithelium were dehydrated with ascending series of acetone and treated with amyl acetate (100%). These samples were then subjected to critical point drying, and coated with gold palladium (fine coat ion sputter JFC-1100) material. Different images for treated and control groups were viewed. The method had been based on the method as described by Baccethi.[2]

Statistical analyses: Mean values ± standard deviations were calculated and Student's *'t'* test was used to establish the validity of the results.

Results

Table Ia demonstrates a significant decrease in the activity of sucrase in the duodenal, ileal and colonic homogenates of aspirin treated animals while jejunum exhibited a non-significant increase. In case of Nimesulide treated animals a significant increase in sucrase activity can be seen in the jejunal homogenates whereas a non-significant decrease in the duodenal and ileal segments and a fairly significant decrease in sucrase activity were observed in the colonic homogenates. Celecoxib treatment led to a significant increase in the colonic homogenates while other intestinal segments did not show any considerable change. A statistically significant increase in the lactase activity can be demonstrated in all the homogenates of the aspirin treated animals. Nimesulide treated jejunal and ileal segments exhibited an increase whereas the duodenal and colonic parts demonstrated a decrease. The duodenal and jejunal homogenates of celecoxib group showed an increase whereas ileal and colonic segments showed a non-significant change in the lactase activity.

Table Ib shows that for maltase, an increase in the activity was recorded in the duodenal, jejunal and ileal

	Enzyme activity (μmoles/100 mg protein/min)							
Intestinal segment	*Sucrase*				*Lactase*			
	Control	*Aspirin*	*Nimesulide*	*Celecoxib*	*Control*	*Aspirin*	*Nimesulide*	*Celecoxib*
Duodenum	84.8 ± 0.39	151.9 ± 0.39	147.6 ± 0.25	89.3 ± 0.33**	40.07 ± 0.16	9.6 ± 0.11	9.3 ± 0.15	15.8 ± 0.24**
Jejunum	143.9 ± 0.36	211.5 ± 0.41	160.3 ± 0.37	107.0 ± 0.38**	36.1 ± 0.08	33.09 ± 0.16	25.03 ± 0.25	11.7 ± 0.11**
Ileum	144.4 ± 0.36	139 ± 0.3**	209.5 ± 0.41	73.2 ± 0.056**	12.0 ± 0.2	22.8 ± 0.11	30.7 ± 0.56	6.8 ± 0.11
Colon	61.4 ± 0.5	98.3 ± 0.19**	97.8 ± 0.29	60.9 ± 0.49	4.8 ± 0.1	7.2 ± 0.15	5.3 ± 0.07**	2.6 ± 0.09

	Enzyme activity (μmoles/100 mg protein/min)							
Intestinal segment	*Maltase*				*Alkaline phosphatase (μmoles/mg protein/min)*			
	Control	*Aspirin*	*Nimesulide*	*Celecoxib*	*Control*	*Aspirin*	*Nimesulide*	*Celecoxib*
Duodenum	426.5 ± 0.24	430.6 ± 0.27	432.1 ± 0.2	464.6 ± 0.25***	0.95 ± 0.3	1.01 ± 0.6*	2.64 ± 0.21*	0.96 ± 0.53
Jejunum	254.7 ± 0.27	296.8 ± 0.11	286.6 ± 0.42	351.7 ± 0.19***	1.60 ± 0.69	2,07 ± 0.05	2.48 ± 0.29*	1.98 ± 0.12
Ileum	170.1 ± 0.11	223.6 ± 0.42	234.3 ± 0.15	222.3 ± 0.22	0.54 ± 0.13	0.98 ± 0.007**	3.4 ± 0.9**	0.92 ± 0.03
Colon	104.5 ± 0.2	114.1 ± 0.34	114.2 ± 0.07**	116.5 ± 0.7**	0.41 ± 0.03	1.34 ± 0.36**	2.5 ± 0.13	0.41 ± 0.04

Values are mean ± SD of four observations.
*, **, ***, Represent $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively.

segments of both the aspirin and nimesulide treated animals, whereas the celecoxib treated animals demonstrated an increase in the duodenal and colonic segments only. Similarly, for alkaline phosphatase an increase in the activity was seen in all the segments in both aspirin and nimesulide treated groups whereas the celecoxib treated animals did not show any change. There was an increase in the ileal and colonic parts whereas the duodenal and colonic segments exhibited a decrease.

The drug treatments also produced changes in the specific activities of these enzymes in the BBM of various intestinal segments. Table IIa & b show an increase in the sucrase, maltase and alkaline phosphatase activities in all the membrane preparations of aspirin and nimesulide groups, whereas celecoxib treated group demonstrated a decrease in the sucrase activity in all segments except in jejunum. For maltase this group exhibited an increase in activity in all the segments. As far as BBM associated lactase is concerned both aspirin and nimesulide followed the same pattern, notably a decrease in the duodenal and jejunal segments and an increase in the ileal and colonic preparations. The celecoxib group demonstrated an overall decrease in BBM preparations of all the segments. There was an increase in the activity of alkaline phosphatase in all the segments and in all the treatment groups.

Electron microscopy (fig. 1a-d) shows the alterations in the intestinal surface following the different treatments. All the treated groups showed a decrease in microvilli and crypt number. The disruptions in the epithelial lining was also observed but was maximum in nimesulide treated group. The villi of nimesulide group also formed a dense and elaborate network like sieve. Numerous fragmental protrusions extended and formed a network in case of the celecoxib treated animals. Abnormally tall villi were also observed in nimesulide and celecoxib treated animals.

Discussion

Any alteration in the biochemical and biophysical composition of the intestine in the presence of the NSAIDs may affect its functional and dynamic aspect. In the present study significant alterations in the intestinal disaccharidases and alkaline phosphatase was noticed in the animals treated with the NSAIDs. The decrease in the activity of sucrase and simultaneous increase in the activities of maltase in the corresponding homogenates of different intestinal segments following various NSAID treatments directs that a definite kind of biophysical and biochemical correlation exists between the two enzymes. This is in confirmation with the fact that a sucrase-isomaltase complex exists in the intestine which is synthesized as a single chain enzymatically active polypeptide.[11] So, in this case the increase in the maltase activity clearly shows activa-
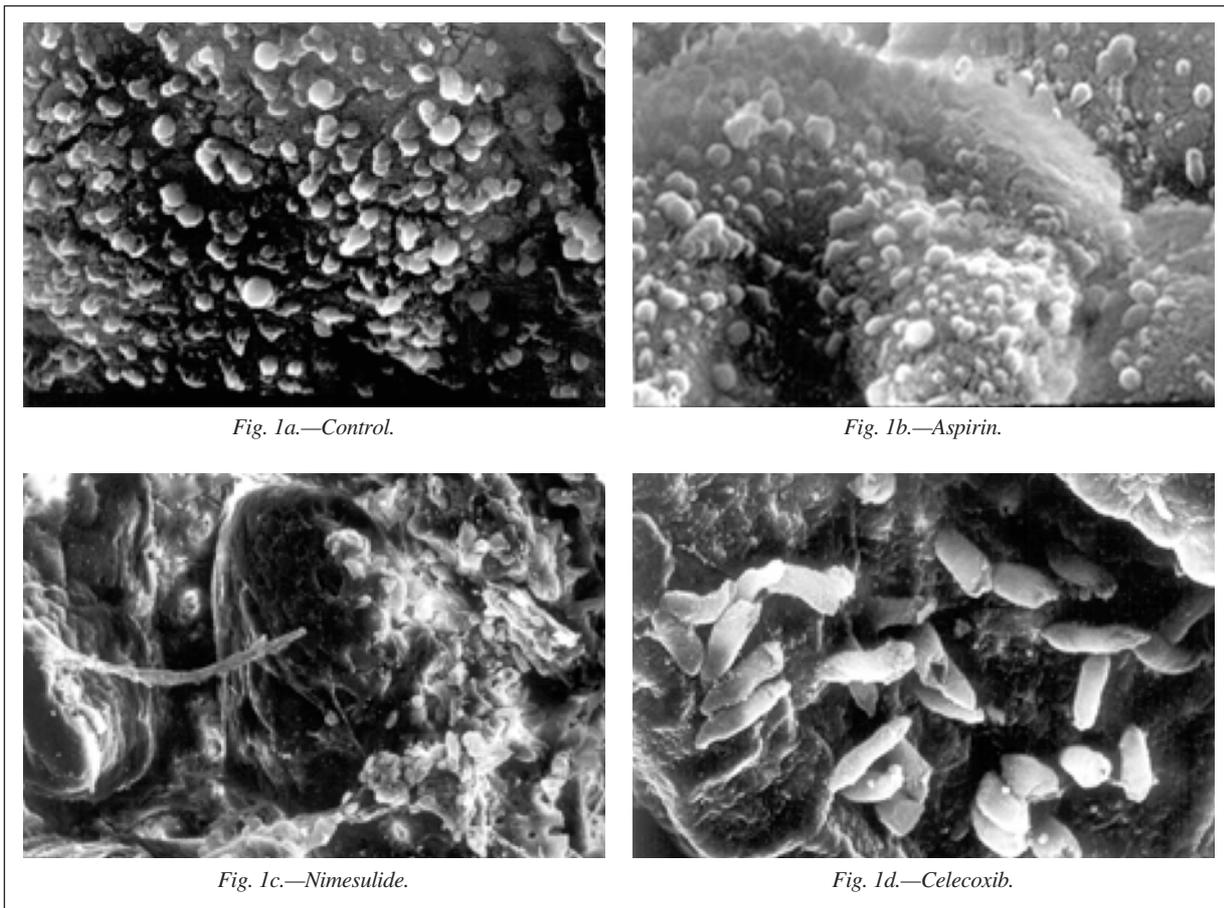
Fig. 1a.—Control.

Fig. 1b.—Aspirin.

Fig. 1c.—Nimesulide.

Fig. 1d.—Celecoxib.

Fig. 1 (a-d).—Scanning Electron micrographs of the intestine following aspirin, nimesulide and Celecoxib treatments, respectively as compared to control.

tion of an additional activated mechanism to fulfil the nutritional needs in response to decrease in the activity of its other counter part i.e. sucrase. Timofeeva et al.[31] have also described a rise in disaccharidases in the intestine of protein deficient animals.

Similarly, an overall increase in the lactase and alkaline phosphatase activities in both homogenates and BBM preparations as observed in response to various drug treatments can be due to an increase in the number of molecular enzyme proteins. Reports exist wherein response to deficiency of one protein or protein malnutrition can lead to an increase in the activities of other proteins.[18,29,32] A significant increase in the activities of disaccharidases and alkaline phosphatase as observed in our study was also observed in the animals treated with various drugs[4,5] and thus are indicative of intestinal dystrophy.

The decrease in the activities of various enzymes in the different intestinal segments may be due to a diminished efficiency in mRNA translation, an increase in protein degradation, and/or a partial inactivation of the disaccharidase active site[16] by these drugs. These data suggest that the decrease is likely due to a decline in enzyme protein rather than an inactivation of the disaccharidase activity. Studies in pancreatectomized rats[1] and in a mouse model of exocrine pancreatic insufficiency[15] showed that the

presence of pancreatic proteases may result in a lower disaccharidase activity.

The changes in the histoarchitecture of intestine following treatment with different classes of NSAIDs demonstrated that these drugs reduced the number of vili, microvilli and crypts as compared to the control animals. A decrease in the number of crypts and villi which are the symptoms of mucosal inflammation has been studied in response to the chemotherapy.[34] This reduced number of membranous invaginations (villi) may lead to decrease in the absorption of nutrients. We have already reported that NSAIDs such as aspirin and nimesulide bring about significant alterations in the membrane associated disaccharidases and changes in the absorption of nutrients (glucose and histidine) in the animals treated with aspirin and nimesulide,[24] Kaushal and Sanyal (in press).

Thus, the administration of different classes of Cox inhibitors like aspirin, nimesulide and celecoxib when used in animal studies at the clinically safer doses have also caused structural and functional changes in the intestinal brush border membrane as evident by the alterations in the biochemical and biophysical state of the tissue.

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