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Original

Effects of non steroidal anti-inflammatory drugs on the antioxidant defense system and the membrane functions in the rat intestine

P. Nair, S. Singh Kanwar and S. Nath Sanyal*

Department of Biophysics. Panjab University. Chandigarth. India.

Abstract

In the present study the effects of two cycloxygenase-2 (COX-2) selective inhibitors, celecoxib and nimesulide as compared to a non-selective COX inhibitor, aspirin was studied in the rat intestine. Female Wistar rats weighing between 150-175 g were divided into four groups having 8 animals each as follows: Group 1(Control), Group 2- Aspirin (40 mg/kg), Group 3- Nimesulide (10 mg/kg) and Group 4- Celecoxib (10 mg/kg). After 35 days of treatment the animals were sacrificed, intestine removed and the effects on the antioxidant defense system, membrane composition and functions along with the membrane specific enzymes were studied in different regions of the intestine. The study showed a significant increase in the lipid peroxide levels as TBA-reactive substance as well as the conjugated dienes, except for celecoxib treated group which showed a decrease. Significant decrease was also observed in the level of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione-s-transferase and catalase activities for aspirin and nimesulide group while Celecoxib caused an increase in glutathione reductase (GR). Aspirin and nimesulide exhibited an increase in the brush border membrane (BBM) bound enzyme activities like sucrase, lactase, maltase and alkaline phosphatase in the small intestine while celecoxib showed decrease in lactase, maltase and alkaline phosphatase. The phospholipid content increased only for aspirin treated group while cholesterol decreased in all the treatment groups. Also celecoxib treatment brought about an increase in glycolipid content. The membrane fluidity was studied by the rotational diffusion of 1, 6, diphenyl, 1, 3, 5 hexatriene (DPH) incorporated in the membrane and the fluorescence polarization (p), fluorescence anisotropy(r), anisotropy parameter $[r_0/r -1]^{-1}$ and order parameter $[S^2 = (4/3r – 0.1)/ r_0]$ were recorded. No significant change in the fluorescence parameters were observed in the BBM and the liposomes made from the

EFECTOS DE LOS FÁRMACOS ANTIINFLAMATORIOS NO ESTEROIDEOS SOBRE EL SISTEMA DE DEFENSA ANTIOXIDANTE Y LAS FUNCIONES DE MEMBRANA EN EL INTESTINO DE RATA

Resumen

En el presente estudio se comparaban los efectos de dos inhibidores selectivos de la ciclo-oxigenasa-2 (COX-2), celecoxib y nimesulide, con los de un inhibidor no selectivo de la COX, la aspirina, en el intestino de la rata. Se escogieron ratas Wistar hembra, con un peso de 150-175 g, y se las dividió en 4 grupos, con 8 animales cada uno, como sigue: Grupo 1 (Control), Grupo 2 – Aspirina (40 mg/kg), Grupo 3 – Nimesulide (10 mg/kg), y Grupo 4 – Celecoxib (10 mg/kg). Tras 35 días de tratamiento, se sacrificó a los animales, se extirpó el intestino, y se estudiaron los efectos sobre el sistema de defensa antioxidante, la composición de la membrana y sus funciones, además de las enzimas de membrana específicas, en diferentes regiones del intestino. El estudio mostró un aumento significativo de las concentraciones de peróxido lipídico como sustancia TBA reactiva así como de dienos conjugados, excepto para el grupo tratado con celecoxib que mostró un descenso. También se observó un descenso significativo en las actividades de glutatión reducido (GSH), de superóxido dismutasa (SOD), de glutatión-s-transferasa y de catalasa en los grupos tratados con aspirina y con nimesulide, mientras que el grupo tratado con celecoxib tuvo un aumento de la glutatión reductasa (GR). La aspirina y el nimesulide mostraron un aumento de las actividades de las enzimas ligadas a la membrana de borde en cepillo (BBM) como la sucrasa, lactasa, maltasa y fosfatasa alcalina. El contenido en fosfolípidos aumentó sólo en el grupo tratado con aspirina mientras que el colesterol disminuyó en todos los grupos de tratamiento. En el grupo de celecoxib también se objetivó un aumento en el contenido en glucolípidos. Se estudió la fluidez de la membrana mediante difusión rotacional del 1,6-difenil-1,3,5-hexatrieno (DPH) incorporado a la membrana y se registraron la polarización de fluorescencia (p), la anisotropía de fluorescencia (r), el parámetro de anisotropía $[r_0/r -1]^{-1}$ y el parámetro de orden $[S^2 = (4/3 r – 0,1)/ r_0]$. No se observaron cambios significativos en los parámetros de fluorescencia en la BBM ni de los li-

Correspondence: Dr. S. N. Sanyal.
Dept. of Biophysics
Panjab University. Chandigarth
India 160 014
E-mail: sanyalpu@gmail.com

BBM lipids for the treatment groups. These results indicate that celecoxib may be accepted as a safer drug in terms of overall gastro-intestinal toxicity as compared to the aspirin and nimesulide.

Key words: *Non-steroidal anti-inflammatory drugs. Oxidative stress. Intestinal membrane. Rat.*

posomas formados a partir de los lípidos de la BBM en ninguno de los grupos tratados. Estos resultados indican que se puede considerar al celecoxib como un fármaco más seguro en cuanto a toxicidad gastrointestinal general en comparación con el nimesulide y la aspirina.

Palabras clave: *Fármacos antiinflamatorios no esteroideos. Estrés oxidativo. Membrana intestinal. Rata.*

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to relieve pain and symptoms of arthritis and soft tissue inflammation. The use of NSAIDs is limited by their tendency to cause mucosal damage in the gastrointestinal tract. Aspirin, nimesulide and celecoxib belong to three different groups of NSAIDs with difference in cycloxygenase (COX) inhibition wherein nimesulide and celecoxib act as COX-2 selective inhibitor while aspirin as a non selective inhibitor for COX enzyme. NSAIDs act to block the first phase of prostaglandin synthesis by binding to and inhibition of COX conversion of arachidonic acid (AA) to PGG_2[1]. Aspirin is an exception where it inhibits due to irreversible acetylation of cycloxygenase component of COX, leaving peroxidase activity unaffected[2].

Aspirin relieves the pain by inhibiting the synthesis of prostaglandins that prevents the sensitization of pain receptors by different stimulants, which appear to mediate pain response[3-5]. Clinical and epidemiological evidence suggest that aspirin produces dose related gastrointestinal toxicity[6-8] that is sometimes fatal[8]. Although nimesulide also inhibits inflammation, various non-prostaglandin mechanisms have been proposed to explain its mode of action: inhibition of 1) histamine release and activity[9], 2) cytokine release[10]. In clinical trials it has shown adverse reaction in gastrointestinal system but is reversible upon cessation of the drug[11-13]. Celecoxib as compared to these two drugs is highly COX-2 selective and has the same mode of action. Patients with significant risk factors for cardiovascular events (e.g. hypertension, hyperlipidaemia, diabetes mellitus, and smoking) should only be treated with celecoxib after careful consideration. Studies have shown that celecoxib inhibits the progression of colon tumor in human and animal models and inhibits the *in vitro* growth of several other tumor cell types[14-16]. Gastrointestinal (GI) problems are far less common with celecoxib than with other NSAIDs. This is due to the fact that celecoxib is highly COX-2 selective and therefore less likely to cause gastric damage. Celecoxib can lead to liver damage. Other rare but serious side effects of celecoxib include allergic reactions and kidney problems.

All the three drugs have been shown to cause intestinal toxicity at different levels. Toxicity may be due to initial biochemical modifications in the brush border membrane or due to alteration in the intestinal mucosal architecture. In the present experimental work COX-2 selective inhibitors (nimesulide and celecoxib) and non-selective COX inhibitors (aspirin) were studied for their influence on the oxidative stress status, membrane functions, lipid profile and lipid fluidity in rat intestine.

Materials and methods

Animals and treatment

Female Wistar rats weighing between 150-175 g were obtained from the Central Animal House, Panjab University, for the experimental work, strictly in accordance with the guidelines as outlined by the institutional ethics committee. After acclimatizing for 1 week, animals were divided into four groups having 8 animals each as follows: Group 1(Control), Group 2- Aspirin (40 mg/kg), Group 3- Nimesulide (10 mg/kg) and Group 4- Celecoxib (10 mg/kg). After 35 days of treatment the animals were sacrificed under an overdose of ether anesthesia. In order to avoid diurnal variation, the animals were uniformly sacrificed around 8 a.m. throughout the study. From each animal intestine was removed and divided into duodenum, jejunum, ileum and colon. Each segment was flushed with ice cold saline, weighed and proceeded for the reported parameters.

A 10% homogenate of the intestinal segments was prepared in chilled 1mM Tris-50 mM Mannitol buffer (pH 7.4). The homogenate was centrifuged at 1,000 g for 10 min at 4° C. Pellet was discarded and the supernatant used for various biochemical estimations. A portion of 1,000g supernatant was again centrifuged at 10,000 g for 20 min to obtain post mitochondrial supernatant (PMS) which was further used for biochemical estimations. The brush border membrane (BBM) of different segments of rat intestine was isolated using the method of Schmitz et al (1973)[17]. The 10% homogenate was passed through two layers of cheese cloth. Anhydrous $CaCl_2$ was added with constant stirring (10 mM final conc.) to the above filtrate & left for 10-15 min in cold. It was centrifuged at 2,000 g for 10 min at 4° C. The supernatant obtained was centrifuged at 42,000 g for 20 min. The pellet was suspended in 20 vol. of 50 mM sodium maleate buffer (pH 6.5-6.8). The supernatant was discarded. The suspended pellet was centrifuged at 42,000 g for 20 min. The pellet obtained was suspended in 50 mM sodium maleate buffer (pH 6.5-6.8) containing 0.02% sodium azide (NaN_3). The final membranes so obtained were used for prepa-

ring liposomes from the extracted lipids and for various biochemical as well as fluorescence studies. The BBM prepared above was free from mitochondria, microsomes, lysosomes, basolateral membranes and nuclei as assessed by the marker enzymes.

Disaccharidase assays: Sucrase, Lactase and Maltase

The activities of these 3 enzymes were determined by measuring the D-glucose liberated from the respective sugar substrate using Glucose oxidase-Peroxidase enzymatic system (GOD-POD)[18].

Alkaline phosphatase assay

Alkaline phosphatase activity was assayed according to the method of Bergmeyer[19] where p-nitrophenyl phosphate was used as the substrate. Alkaline phosphatese acts on this by hydrolyzing it to yield p-nitrophenol. The yellow colour was measured at 410 nm.

Lipid peroxide (LPO)

Lipid peroxide formation was assayed by the method of Wills[20]. Since malonyldialdehyde is a degradation product of peroxidised lipids, the development of pink colour with the absorption characteristics (Absorption maximum at 532 nm) as a TBA-MDA chromophore has been taken as an index of lipid peroxidation.

Glutathione estimation

Glutathione content was estimated according to the method of Ellman[21]. In this method 5, 5-Dithiobis 2-Nitrobenzioc acid (DTNB) is reduced by –SH groups to form 1 mole of 2-nitro-5-mercapto benzoic acid per mole of SH.

Glutathione S- transferase (GST)

The enzyme was assayed by the method of Habig et al[22]. GST catalyses the formation of the glutathione conjugates of CDNB which absorb maximum at 340 nm and have an extinction coefficient of 9.6 m M^{-1} cm^{-1}.

Glutathione reductase (GR)

The enzyme was assayed by the method of Massey and Williams[23]. The utilization of NADPH is directly related to the activity of GR.

Catalase

Catalase was estimated in an U. V. spectrophotometer by the method described by Luck[24]. H_2O_2 was used as substrate. The absorption of H_2O_2 solution is measured at 240 nm on decomposition of H_2O_2 with catalase absorption that decreases with time which is recorded and from this decrease in optical density, the enzyme activity calculated.

Superoxide dismutase (SOD)

Superoxide dismutase assay was performed according to the method of Kono et al[25]. The reduction of Nitro Blue Tetrazolium (NBT) to a blue color formation mediated by hydroxylamine hydrochloride was measured under aerobic conditions.

Extraction of lipids

Lipids were extracted from the BBM following the method of Folch et al[26]. Membrane suspension (150-200 mg protein) was mixed in a flask with 20 vol of chloroform: methanol (2:1 v/v) and left for 15 min at 45° C. The contents were mixed thoroughly and filtered through a Whatman No1 filter paper into a graduated cylinder. The residue left on the filter paper was then washed three times with 10 ml of chloroform: methanol (2:1). Then, 0.2 vol KCl (0.9%) was added (20% of total volume) to the extract. The contents were mixed vigorously and allowed to stand in cold overnight so as to separate the aqueous and lipid layers distinctly well. Upper aqueous phase was removed with Pasteur pipette and the lower layer washed three times with 2 ml chloroform: methanol: 0.9% KCl 3: 48:47 v/v. The washed lower layer was transferred to a round bottom flask and evaporated to dryness at a temp below 45° C while the upper aqueous layer was added each time to the previously separated upper phase. To the residue, 5 ml of chloroform: methanol: water, 64:32:4 v/v was added and evaporated to dryness. This was repeated three times. The dried lipid was redissolved in chloroform and filtered again. The filtrate was evaporated in a rotary evaporator under reduced pressure and at a temp slightly less than 45° C. A known volume of chloroform: methanol (2:1 v/v) was added to redissolve the lipids in a tightly closed container and used as such for various lipid estimations.

Estimation of total Lipids

Total lipids were estimated following the method of Fringes and Dunn[27] measuring the coloured complex with a phosphate ester of vanillin (colouring reagent).

Estimation of cholesterol

In the presence of H_2SO_4 and Glacial acetic acid, cholesterol forms a colored complex with $FeCl_3$ that can be measured colorimetrically at 540 nm[28].

Estimation of Glycolipids

Estimation of hexose unit found in conjugation with lipids in the glycolipids was done by the method of Dubious et al[29].

Estimation of phospholipid phosphorus

Inorganic phosphorous estimation was done in the phospholipids after digestion according to the method of Ames (1966)[30].

Estimation of ganglioside-sialic acid

Sialic acid was estimated by the method of Warren[31]. Sialic acid (N acetyl neuraminic acid) is oxidized with Sodium periodate in conc Orthophosphoric acid. The periodate oxidation product is coupled with Thiobarbituric acid and resulting chromophore is extracted in Cyclohexanone.

Conjugated diene estimation

Conjugated diene content in the sample was estimated following the method as described by Lakshmi and Balasubramanian[32]. 50 μl of redissolved lipids in 2:1 Chloroform: Methanol mixture was taken in different test tubes and dried completely at 37° C. To this 1 ml of sodium maleate buffer was added and conjugated diene content was estimated in them by measuring the absorbance at 233 nm, and the amount calculated using the molar extinction coefficient of 2.52×10^4 M^{-1}cm^{-1}.

Liposomes preparation

Liposomes were prepared from the extracted lipids by the method of Schachter and Shinitzky[33]. Extracted lipid was suspended in sodium maleate buffer to a final concentration of approximately 0.3 mg/ml and the mixture was sonicated for 10 min using a bath type sonicator. Thereafter, the suspensions were centrifuged for 10 min at 50,000 g in a Beckmann Coulter ultra centrifuge. The supernatant liposomes were tested for membrane fluidity with the fluorescent probe (1, 6-diphenyl-1, 3, 5-hexatriene) as described for the membranes in the following.

Fluorescence studies

The lipid-soluble fluorescent probe, 1, 6-Diphenyl-1, 3, 5-hexatriene (DPH) was used in the fluidity studies. For this a stock solution of 2 mM probe in tetrahydrofuran (THF) was prepared and stored being protected from light at room temp. Aqueous suspension of DPH was prepared freshly each time. A small volume of DPH solution in THF was injected with rapid stirring into 1,000 volumes of sodium maleate buffer at room temperature. The suspension was stirred for at least 2 hr after which no odor of THF was detected and the suspension showed negligible fluorescence. In a typical experiment BBM (100-200 μg protein) and liposomes were incubated in 2 ml of sodium maleate buffer containing 1 μM DPH suspension for 2-4 hr at 37° C. Thereafter, estimations of fluorescence intensity (F), fluorescence polarization (p) and fluorescence anisotropy (r) were made with an excitation wavelength of 365 nm and emission wavelength of 430 nm using a Perkin Elmer Luminescence Spectrometer LS 55. Anisotropy parameter $[r_o/r -1]^{-1}$ was then calculated using r_o value for DPH as 0.362[34]. Also the order parameter was calculated using the relationship $S^2 = (4/3 r - 0.1)/ r_o$[35].

Statistical analysis

Statistical analysis of the data was performed by analysis of variances (one way ANOVA) following one way ANOVA post-Hoc test using least significance difference (LSD).

Results

A highly significant increase in the lipid peroxidation was observed in the duodenum region of the aspirin treated group whereas celecoxib showed a fairly significant decrease (table I). A fairly significant increase was reported in the jejunum and ileum segment of the aspirin treated group. No alteration in lipid peroxides was found in the colon region following any of the treatments.

Table I
Effect of aspirin, nimesulide and celecoxib on lipid peroxidation in intestinal homogenate

Group	Lipid peroxidation (nmoles MDA/min/mg protein)			
	Duodenum	*Jejunum*	*Ileum*	*Colon*
Control	46.302 ± 10.003	64.810 ± 21.902	98.426 ± 19.460	106.983 ± 31.527
Aspirin	104.06 ± 11.66***	93.632 ± 26.003*	123.067 ± 7.206*	100.52 ± 16.170
Nimesulide	52.806 ± 2.683	78.484 ± 14.360	116.160 ± 11.424	94.03 ± 7.751
Celecoxib	29.329 ± 3.953*	83.195 ± 9.987	82.591 ± 10.81	81.28 ± 9.128

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
*p < 0.05, **p < 0.01, ***p < 0.001 in comparison to control.

In the duodenum region of intestine of aspirin treated group a significant decrease in the reduced glutathione level was observed whereas a significant increase recorded in the celecoxib (table II). In the jejunum region aspirin treated group showed a significant decrease ($p < 0.01$) while celecoxib caused a significant increase. Aspirin treated group showed a fairly significant decrease whereas nimesulide showed no significant change and celecoxib a non significant increase in the glutathione level in the ileum region. Colon region showed a significant decrease in the glutathione level for the aspirin and nimesulide treated groups.

Aspirin treatment brought about a significant decrease in GST activity in the duodenum region while nimesulide administration caused a significant increase (table III). Jejunum showed a highly significant increase for the celecoxib treated group. A highly significant decrease was observed in the ileum region of both aspirin and nimesulide treated groups, respectively. The colonic segment also showed a highly significant decrease in the enzyme activity for the aspirin and nimesulide treated groups.

Aspirin and celecoxib treated groups witnessed a fairly significant decrease in the SOD level in the duodenum segment (table IV). Jejunum region showed a fairly significant decrease in the aspirin treated group. SOD level in the other two treated groups was found to

be normal as compared to the control in the jejunum region. The ileum region followed the same trend as in the jejunum in all the treated groups. The colon region observed a highly significant decrease for the aspirin treated group. A fairly significant decrease was also reported in the same region for the nimesulide treated group.

Table V shows the effects of aspirin, nimesulide and celecoxib on the intestinal catalase activity. Duodenum region showed a fairly significant decrease in catalase in case of the aspirin treated group. A fairly significant decrease in the enzyme activity was found in the ileum region of aspirin as well as that of the nimesulide treated groups. The colon region was reported to have a fairly significant decrease for aspirin and nimesulide.

A fairly significant decrease in the Glutathione reductase activity was found in the duodenum region of intestine in the case of aspirin treated group while a fairly significant increase was found in the celecoxib treated group (table VI). Aspirin treatment brought about a fairly significant decrease in the jejunum region of intestine. For ileum, enzyme activity was reduced fairly significantly for the aspirin treated group.

Aspirin administration brought about a fairly significant increase in the sucrase activity in the duodenum and jejunum and a highly significant increase in the ileum region (table VII). The sucrase activity remained unaf-

Table II

Effect of aspirin, nimesulide and celecoxib on glutathione (GSH) levels in intestinal homogenate

Group	Reduced glutathione (nmoles/mg protein)			
	Duodenum	Jejunum	Ileum	Colon
Control	90.800 ± 13.250	88.213 ± 11.789	100.26 ± 13.051	104.600 ± 5.242
Aspirin	59.452 ± 7.127**	63.185 ± 8.435**	73.067 ± 15.189*	88.799 ± 6.984**
Nimesulide	84.820 ± 3.428	92.130 ± 12.403	97.375 ± 12.094	93.552 ± 5.743*
Celecoxib	115.401 ± 5.917**	112.570 ± 9.747*	105.541 ± 9.796	115.082 ± 7.213

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

Table III

Effect of aspirin, nimesulide and celecoxib on glutathione S-transferase in intestinal homogenate

Group	Glutathione S-transferase (nmole CDNB conjugate/min/mg protein)			
	Duodenum	Jejunum	Ileum	Colon
Control	0.352 ± 0.0455	0.281 ± 0.0380	0.464 ± 0.049	0.453 ± 0.048
Aspirin	0.201 ± 0.0871**	0.232 ± 0.012	0.187 ± 0.002***	0.206 ± 0.072***
Nimesulide	0.322 ± 0.028	0.302 ± 0.018	0.392 ± 0.053*	0.334 ± 0.037 **
Celecoxib	0.493 ± 0.0796**	0.442 ± 0.0651***	0.497 ± 0.016	0.415 ± 0.050

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

M. J. Martínez y cols.

Table IV
Effect of aspirin, nimesulide and celecoxib on superoxide dismutase in intestinal homogenate

Group	Super Oxide dismutase (units/mg protein)*			
	Duodenum	Jejunum	Ileum	Colon
Control	2.313 ± 0.170	2.653 ± 0.283	3.716 ± 1.722	2.506 ± 0.174
Aspirin	1.946 ± 0.0378*	1.920 ± 0.537*	1.773 ± 0.116*	1.950 ± 0.234***
Nimesulide	2.243 ± 0.277	2.476 ± 0.083	3.079 ± 0.764	2.113 ± 0.078*
Celecoxib	2.64 ± 0.0916*	2.736 ± 0.100	3.173 ± 0.149	2.484 ± 0.118

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.
* One unit of SOD activity denotes the inverse of amount of protein (mg) required to inhibition of the reduction rate of NBT by 50%.

Table V
Effect of aspirin, nimesulide and celecoxib on catalase in intestinal homogenate

Group	Catalase (nmoles of H_2O_2 decomposed/min/mg protein)			
	Duodenum	Jejunum	Ileum	Colon
Control	0.341 ± 0.0317	0.281 ± 0.0565	0.304 ± 0.107	0.380 ± 0.0434
Aspirin	0.228 ± 0.0435*	0.288 ± 0.0751	0.208 ± 0.028*	0.229 ± 0.0125**
Nimesulide	0.326 ± 0.0301	0.282 ± 0.109	0.400 ± 0.043*	0.222 ± 0.0566**
Celecoxib	0.377 ± 0.0562	0.168 ± 0.0358	0.311 ± 0.031	0.310 ± 0.0750

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

Table VI
Effect of aspirin, nimesulide and celecoxib on glutathione reductase in intestinal homogenate

Group	Glutathione Reductase (nmolesmin/mg protein)			
	Duodenum	Jejunum	Ileum	Colon
Control	0.170 ± 0.0110	0.209 ± 0.0503	0.196 ± 0.018	0.194 ± 0.030
Aspirin	0.129 ± 0.0306 *	0.134 ± 0.017*	0.141 ± 0.015*	0.169 ± 0.048
Nimesulide	0.174 ± 0.0134	0.172 ± 0.014	0.175 ± 0.042	0.156 ± 0.017
Celecoxib	0.219 ± 0.0304*	0.173 ± 0.052	0.159 ± 0.033	0.159 ± 0.030

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

fected in all the regions of small intestine of nimesulide and celecoxib treated groups.

Duodenum region witnessed a highly significant increase in the lactase activity in the aspirin treated group whereas a highly significant decrease was observed in celecoxib (table VIII). A significant rise in the lactase activity was seen in the jejunum region for the aspirin treated group. In the ileum a fairly significant increase was recorded in all the treated groups.

Celecoxib group brought about a highly significant decrease in the maltase activity in the duodenum region (table IX). In the jejunum fairly significant increase was found for the aspirin treated group while the nimesulide and celecoxib showed a significant decrease in the same region. Nimesulide and celecoxib treatment brought about a highly significant decrease in the maltase activity in the ileum region.

Table VII
Effect of aspirin, nimesulide and celecoxib on sucrase activity in intestinal homogenate

| Group | Sucrase (μmoles/min/mg protein) | | |
	Duodenum	Jejunum	Ileum
Control	0.531 ± 0.067	0.378 ± 0.0306	0.246 ± 0.026
Aspirin	0.760 ± 0.194*	0.453 ± 0.040*	0.361 ± 0.035***
Nimesulide	0.593 ± 0.041	0.384 ± 0.024	0.257 ± 0.047
Celecoxib	0.504 ± 0.033	0.370 ± 0.035	0.239 ± 0.013

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *p < 0.05, **p < 0.01, ***p < 0.001 in comparison to control.

Table VIII
Effect of aspirin, nimesulide and celecoxib on lactase activity in intestinal homogenate

| Group | Lactase (μmoles/min/mg protein) | | |
	Duodenum	Jejunum	Ileum
Control	0.210 ± 0.016	0.210 ± 0.033	0.079 ± 0.012
Aspirin	0.257 ± 0.018***	0.326 ± 0.079**	0.113 ± 0.017*
Nimesulide	0.212 ± 0.018	0.219 ± 0.010	0.117 ± 0.027*
Celecoxib	0.117 ± 0.009***	0.215 ± 0.017	0.124 ± 0.032*

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *p < 0.05, **p < 0.01, ***p < 0.001 in comparison to control.

Table IX
Effect of aspirin, nimesulide and celecoxib on maltase activity in intestinal homogenate

| Group | Maltase (μmoles/min/mg protein) | | |
	Duodenum	Jejunum	Ileum
Control	2.768 ± 0.130	2.707 ± 0.161	3.117 ± 0.151
Aspirin	2.925 ± 0.128	2.917 ± 0.097*	3.052 ± 0.178
Nimesulide	2.585 ± 0.185	2.462 ± 0.115**	2.057 ± 0.151***
Celecoxib	2.112 ± 0.310***	2.456 ± 0.102**	1.995 ± 0.093***

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *p < 0.05, **p < 0.01, ***p < 0.001 in comparison to control.

A highly significant increase in the alkaline phosphatase activity in the duodenum region was found for the aspirin treated group (table X). Nimesulide showed a fairly significant increase while celecoxib showed a fairly significant decrease in the same intestinal region. Jejunum witnessed a highly significant increase for the aspirin treated group whereas a fairly significant decrease was found for the celecoxib. In the ileum a highly significant increase in the enzyme activity was recorded in favor of the aspirin and nimesulide treated groups, respectively, while celecoxib showed a highly significant decrease. Alkaline phosphatase activity remained unaffected in the colon region of intestine of all the treated groups.

Table XI shows the results of the effects of aspirin, nimesulide and celecoxib administration on the total lipids in the intestinal BBM. A highly significant decrease in total lipids was observed in the case of aspirin treated group. A fairly significant increase in the phospholipid composition in the intestinal BBM was also

Table X
Effect of aspirin, nimesulide and celecoxib on alkaline phosphatase activity in intestinal homogenate

Group	Alkaline phosphatase (nmoles/min/mg protein)			
	Duodenum	Jejunum	Ileum	Colon
Control	0.487 ± 0.017	0.242 ± 0.023	0.163 ± 0.019	0.0608 ± 0.012
Aspirin	0.572 ± 0.020***	0.550 ± 0.060***	0.235 ± 0.011***	0.053 ± 0.010
Nimesulide	0.517 ± 0.013*	0.241 ± 0.028	0.202 ± 0.015*	0.0465 ± 0.008
Celecoxib	0.453 ± 0.022*	0.181± 0.012*	0.095 ± 0.029***	0.0540 ± 0.011

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

Table XI
Effect of aspirin, nimesulide and celecoxib on lipids profile in the intestinal BBM

Group	Lipids profile				
	Total lipids (µg/mg protein)	Phospholipids (µg/mg protein)	Cholesterol (µg/mg protein)	Glycolipids (µg/mg protein)	Gangliosides (ng/mg protein)
Control	0.396 ± 0.0208	160.670 ± 13.636	15.730 ± 1.135	2.217 ± 0.218	118.227 ± 48.359
Aspirin	0.230 ± 0.030***	195.04 ± 17.839*	9.233 ± 0.421**	2.355 ± 0.184	110.857 ± 41.807
Nimesulide	0.340 ± 0.0264	175.297 ± 23.684	9.090 ± 3.442***	1.826 ± 0.111	129.220 ± 25.441
Celecoxib	0.346 ± 0.0598	136.721 ± 15.577	7.94 ± 2.304***	3.872 ± 0.555***	151.895 ± 44.360

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

recorded. However, a significant decrease in cholesterol level was seen in the aspirin treated group. A highly significant decrease was observed in the case of nimesulide and celecoxib. The glycolipid composition showed a highly significant increase in the intestinal BBM of the celecoxib treated group. No significant change was observed in the case of other treated groups. Also, for the ganglioside composition no significant change was observed.

The occurrence of conjugated dienes is a mark of lipid peroxidation in the membranes. A fairly significant increase in the diene formation was noticed in the intestinal BBM of the aspirin treated group while no alterations observed in nimesulide and celecoxib (table XII). Aspirin treatment produced the highest absorbance corresponding to the conjugated dienes followed by nimesulide while celecoxib produced the decrease in absorbance when compared to the control (fig. 1).

The treatments of NSAIDs produced no significant alterations in the fluorescence studies related to the parameters like fluorescence polarization, anisotropy and subsequently no change in the calculated anisotropy parameter and the order parameter values observed in the intestinal BBM (table XIII) and liposomes (table XIV). The fluorescence intensity was found to be maximum in aspirin treated BBM and greater than control. Whereas, nimesulide treated BBM resulted in lesser

Table XII
Effect of aspirin, nimesulide and celecoxib on the formation of Conjugated dienes in intestinal BBM

Group	Conjugated dienes (µmoles dienes/mg protein)
Control	1.563 ± 0.1399
Aspirin	1.954 ± 0.1539*
Nimesulide	1.350 ± 0.2173
Celecoxib	1.8212 ± 0.290

Statistical analysis: Values are mean ± SD of 6-8 independent observations.
 *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to control.

intensity peak than control while celecoxib BBM showing the peak corresponding to the least intensity among all the treatments (fig. 2).

Discussion

Results from the present studies have shown that an increase in lipid peroxidation occurs in different intestinal segments in NSAIDs treatment except in celecoxib. Earlier studies have also reported an increase in MDA levels during NSAIDs treatment[36]. Celecoxib showed a decrease in the lipid peroxidation in the duodenum
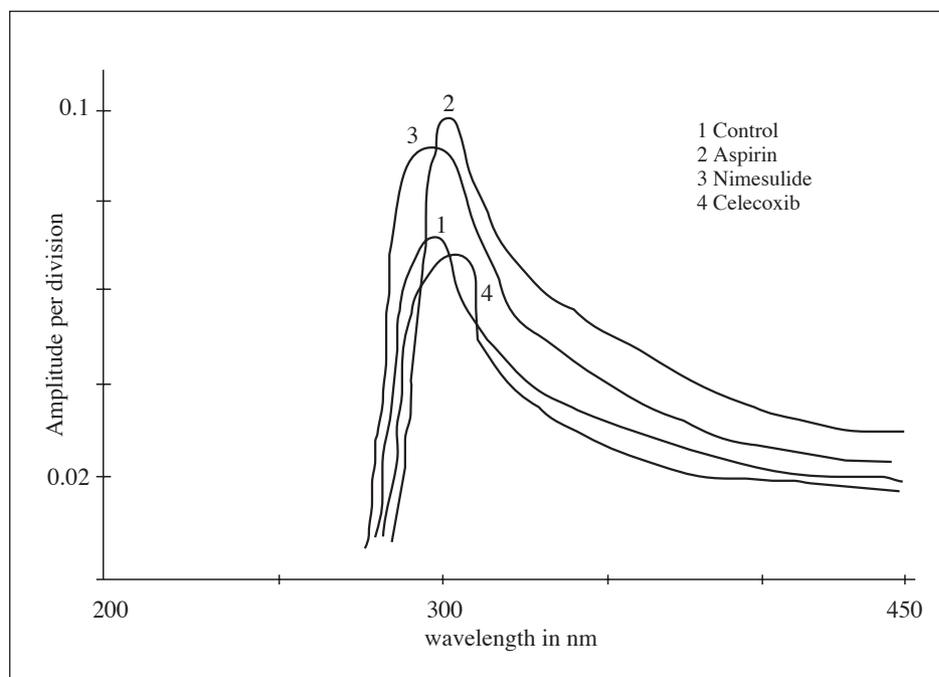
Fig 1.—Effect of aspirin, nimesulide and celecoxib on the absorbance spectra of conjugated dienes of intestinal BBM.

Table XIII

Effect of aspirin, nimesulide and celecoxib on fluorescence polarization and anisotropy in BBM

Group	Fluorescence polarization (p)	Anisotropy (r)	Anisotropy parameter (r⁰/r-1)	Order parameter (S)
Control	0.376 ± 0.015	0.292 ± 0.011	4.16 ± 0.89	0.890 ± 0.023
Aspirin	0.390 ± 0.019	0.275 ± 0.036	2.84 ± 1.46	0.851 ± 0.078
Nimesulide	0.408 ± 0.021	0.314 ± 0.024	5.08 ± 1.37	0.933 ± 0.047
Celecoxib	0.371 ± 0.041	0.317 ± 0.025	6.06 ± 2.32	0.939 ± 0.050

Statistical analysis: Values are mean ± SD of 6-8 independent observations.

Table XIV

Effect of aspirin, nimesulide and celecoxib on fluorescence polarization in liposomes made from BBM lipids

Group	Fluorescence polarization (p)	Anisotropy (r)	Anisotropy parameter (r⁰/r-1)	Order parameter (S)
Control	0.293 ± 0.012	0.270 ± 0.019	2.96 ± 0.90	0.843 ± 0.040
Aspirin	0.310 ± 0.018	0.284 ± 0.074	3.56 ± 0.44	0.874 ± 0.015
Nimesulide	0.312 ± 0.019	0.288 ± 0.015	3.88 ± 0.96	0.882 ± 0.031
Celecoxib	0.302 ± 0.013	0.280 ± 0.009	3.37 ± 0.49	0.865 ± 0.019

Statistical analysis: Values are mean ± SD of 6-8 independent observations.

region suggesting that the lower dose regimen to be safer against oxidative stress. The decrease in lipid peroxidation following celecoxib administration can be attributed to its high COX-2 selectivity as the later is known to induce lipid peroxidation in cellular systems. The level of glutathione is considered a critical determinant for the threshold of tissue injury. The importance of GSH is emphasized further by a study that showed a substantially disruptive effect on the mucosal architecture of pharmacological inhibition of GSH synthesis[37]. In the present study, no decline in GSH was observed in all the intestinal regions following nimesulide and celecoxib
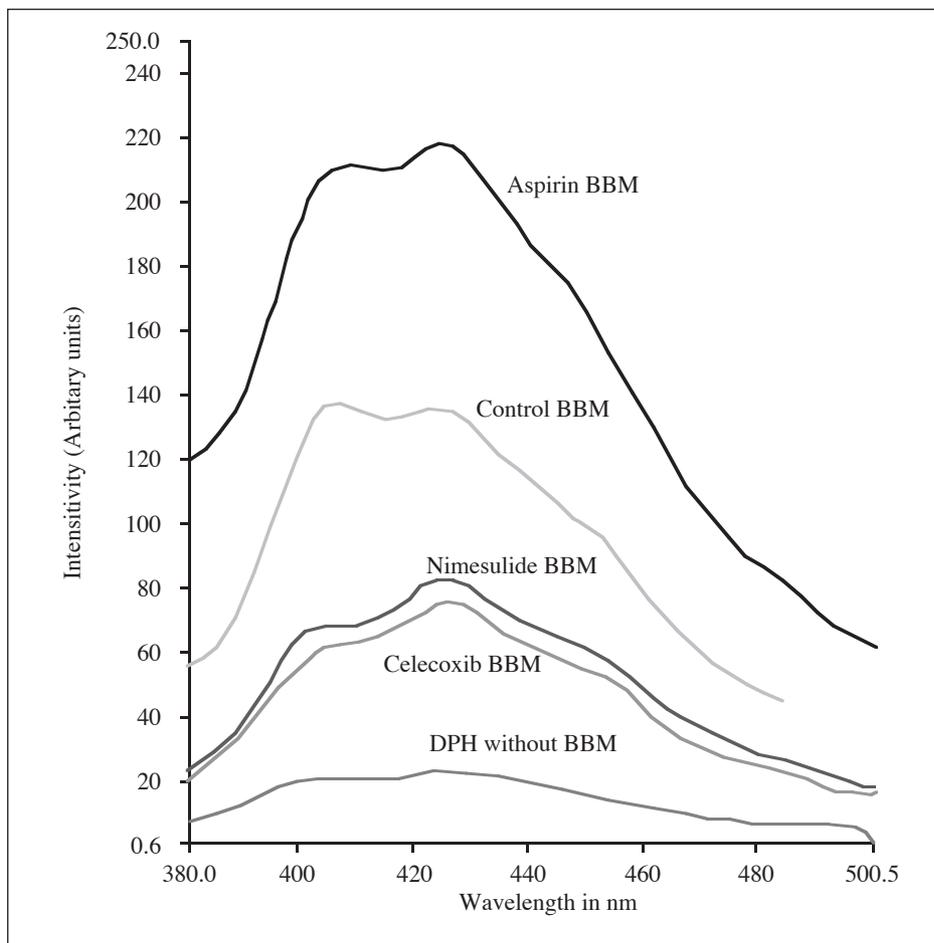
Fig 2.—Effect of aspirin, nimesulide and celecoxib on the DPH fluorescence spectra of intestinal BBM.

treatment while an enhanced level observed following aspirin treatment. This decrease in GSH levels attributes to the fact that GSH is being consumed in response of an increased lipid peroxidation during these drugs administration. Previous works have also reported a decrease in the GSH levels[37,38] in intestinal tract following certain COX-2 non selective NSAIDs administration at higher doses. These results suggest that at a higher dose of COX-2 non selective NSAIDs, there is a greater risk of oxidative stress and hence intestinal injury. GST has GSH as its substrate and the decline in GST activity is in response to the dearth of substrate as GSH was also found to be low in small intestine as well as colon regions following aspirin and nimesulide treatment. However, celecoxib resulted in an increase in the GST activity.

Most of the NSAIDs are known to scavenge free radicals while certain NSAIDs like indomethacin have been reported to induce free radicals generation[39]. High level of COX-2 induced prostaglandins is known to be associated with the oxidative stress as COX-2 over expression induces the generation of free radicals and lipid peroxide formations. SOD and catalase are both antioxidant enzymes that function as blockers of free radical process[40]. In various previous studies SOD activity has been shown to decrease significantly in the GI mucosa after the administration of indomethacin[38,41], diclofenac[42] and ibuprofen[43]. Results of the present work have shown aspirin to decrease the level of SOD in all the small intestine regions as well as the colon. Further, the increase in the level of SOD in celecoxib treatment can be due to the highly COX-2 inhibitory nature and free radical scavenging capabilities of celecoxib that protects the cellular environment from reactive oxygen species by increasing the enzyme synthesis. Catalase activity was found to be decreased in the small intestine for aspirin and nimesulide treated groups.

Various reports have shown a decreased GR activity in the GI mucosa involving NSAIDs administration[44]. GR activity was found to be decreased in all the small intestinal regions in the aspirin treated group. Further, celecoxib caused an increased GR activity in duodenum region while causing no alterations in other small intestinal regions and colon. The increase in GR activity at low dose regimen of celecoxib can be seen as its protective mechanism whereby it increases the GR synthesis that helps in reducing the oxidized substrate (GSSG) by converting it into the reduced form (GSH) and thereby acting as an antioxidant.

The increased enzyme activities of the disaccharidases in small intestinal homogenates for aspirin and nimesulide group might be due to an increase in the number of molecular enzyme proteins. A rise in disaccharidases in the protein malnourished rats has also been described earlier[45,46]. The increased activity of disaccharidases was attributed to the high amount of carbohydrates present in a protein deficient diet. The substrate itself has a role to play in the stimulation of disaccharidase activities[47-51]. Mahmood et al[52] reported that there is a two to three fold elevation in the sucrase and maltase activity in the protein calorie malnourished intestine (PCM) in undernourished monkeys as compared to the control animals. There was a decrease in lactase and maltase activity for the celecoxib dose in the present investigation. The alkaline phosphatase activity was however, increased for aspirin and nimesulide treated groups in the small intestine regions. A previous study by Singh et al[53], reported an increase in the serum alkaline phosphatase activity after aspirin treatment and linked such increase with the hepatotoxic effects caused by aspirin. Thus, the increase in the alkaline phosphatase enzyme can be attributed to the self-protective mechanism of the system against the cytotoxicity of aspirin and nimesulide. Futhermore, the decline in alkaline phosphatase activity observed in small intestine following celecoxib treatment strongly favours its cytoprotective role.

Alterations in the lipid or protein composition may change the membrane fluidity, which is determined by lipid-protein interactions. Membrane fluidity is directly linked with membrane functions[54]. Aspirin and celecoxib treated groups showed no changes in total lipid or lipid: protein ratio as such. The total phospholipid content was found to be significantly increased in the nimesulide treated group. This increase in the phospholipid content under the effect of NSAIDs is supported by the fact that NSAIDs also increase the membrane fluidity[55]. Also changes in phospholipid content in the intestinal BBM can affect the membrane bound enzymes and permeability of the membrane to ions[56]. Further, increased phospholipid content makes the membrane more susceptible to peroxidation induced damages. Celecoxib did not alter the phospholipid content. Cholesterol content also decreased in all of the treatment groups. In a previous study[57] it was reported that decrease in cholesterol phospholipid ratio in the intestinal BBM indicates an increase in fluidity. Celecoxib treatment has brought about an increase in glycolipids also. However, ganglioside composition noticed no significant changes for the treatment groups. Conjugated dienes are a measure of lipid peroxidation. The increase in amount of these in the present study in aspirin treatment indicates the extent of lipid peroxidation.

Previously fluorescence polarization methods have been applied increasingly to the study of biological membranes[58-60]. The particular usefulness of these methods stems from the fact that polarization of the fluorescence of a molecule depends upon the rate of molecular rotation of the lipid[61]. Binding of a fluorophore to biological macromolecule or membrane can be monitored by an increase in the polarization of fluorescence[61]. Similarly, since the rotation rate depends on the resistance offered by the microenvironment to the motion of the probe, fluorescence polarization provides an estimate of the environmental resistance which is interpretable as an apparent microviscosity and ultimately as a measure of fluidity[62]. In the present study no significant change in the fluidity parameters were observed in the BBM and the liposomes for the treatment groups. This indicates that aspirin, nimesulide and celecoxib at their respective doses don't affect the fluidity of the membrane as such.

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