The effect of prostaglandin synthase inhibitor, aspirin on the rat intestinal membrane structure and function

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Abstract

Aspirin at a dose of 50 mg/kg body weight was found to decrease the activity of the rat intestinal brush border membrane (BBM) - associated enzymes such as the sucrase, lactase, maltase and alkaline phosphatase. Aspirin treatment also led to a decrease in the microviscosity in the native as well as the benzyl alcohol treated membrane which might be due to the lipid peroxidative damage in the membrane. Physical correlation of the membrane oxidative damage was evident as the Fourier Transformation Infra Red (FTIR) study of the Aspirin treated membrane, which include an increased proportion of gauche to trans conformer, shift in the methylene C-H asymmetric and symmetric stretching frequencies, C = O double bond stretching, NH bending, antisymmetric (N)-CH3 bending, C-N stretching and antisymmetric CNC stretching while there was no change in the CH2 wagging and twisting as well as in NH-bending amide bond I and II. Aspirin treatment also caused an alteration in the glucose and histidine transport, as evident by a decreased Vmax value while the apparent Km remaining unchanged in the control and Aspirin-treated animals confirming that there was no change in the substrate affinity constant of the membrane transport proteins for the glucose and the basic amino acid, although the rate of transport decreased considerably. There was a decrease noted in the energy of activation of glucose and histidine transport when studied at different temperature but no change in the temperature of phase transition in the BBM with Aspirin treatment, thus implying that perhaps the thermotropic phase transition in the membrane may have relatively little effect on the transport processes. The result suggests an underlying molecular mechanism indicating the implied membrane damage by Aspirin, an important member of the non-steroidal antiinflammatory drug (NSAID) family which could possibly through an oxidative damage may lead to an altered molecular structure, physical state and biological functions of the intestinal membrane.

(Nutr Hosp. 2010;25:290-298)
DOI:10.3305/nh.2010.25.2.4598

Key words: Aspirin. Transport studies. FTIR. Disaccharidases. Pyrene fluorescence.
Introduction

Despite the introduction of many new drugs, aspirin (acetyl salicylic acid) the first among the non-steroidal anti-inflammatory drugs (NASIDs) is still the most widely prescribed analgesic, antipyretic and anti-inflammatory agent.\textsuperscript{1-3} Recent studies have shown that the long-term intake of aspirin in humans leads to protection against the development of colorectal cancer as well as malignancies in other tissues.\textsuperscript{4-6} In addition, aspirin has been demonstrated to inhibit chemically induced carcinogenesis in various animal models.\textsuperscript{7-9}

The protective effects of aspirin against carcinogenesis have been presumably attributed to its ability to inhibit inflammation.\textsuperscript{10} The anti-inflammatory action of aspirin is believed to result from its non-specific inhibition of cyclooxygenase (COX)\textsuperscript{11-13} by acetylating the amino terminal of serine on the active site of the enzyme thereby reducing the level of prostaglandin production. Moreover, it has got the ability of scavenging free radicals which further increases its anti-inflammatory efficacy.\textsuperscript{14} Also, salicylic acid the metabolite of aspirin exhibits anti-inflammatory action by inhibiting the migration of leucocytes besides the aggregation of human neutrophils which is induced by some chemotactants. Aspirin has been reported to inhibit the growth and size of intestinal tumor, probably by inhibiting the activation of NF-κB, a transcription factor critically involved in the production of inflammatory cytokines and the subsequent inflammatory response.\textsuperscript{15,16} The most spectacular effect of aspirin is to cause inhibition of platelet aggregation and therefore, the drug of choice for patients suffering from cardiovascular diseases, such as angina pectoris, coronary artery diseases, myocardial infarction and stroke.\textsuperscript{17-19}

Besides its wide range of uses, it has certain adverse effects however, probably arising out of the deficient cytoprotective role of the prostaglandins, such as the stomach irritation, gastrointestinal disturbances, ulcerative alteration of certain enzyme levels and other biochemical parameters.\textsuperscript{17,18} Keeping in view the above, therefore, in the present study the intestinal membrane structure and function have been studied in rats in aspirin treatment.

Materials and methods

Animals and drug treatment: Male Wistar rats weighing between 100-125 g were procured from the central animal house, Panjab University. They were housed in individual cages and maintained on rat pellet diet and water \textit{ad libitum}. The animals were kept individually in polypropylene cages under hygienic conditions, and ambient light and temperature, strictly in conformation with the guidelines as laid down by the institutional ethics committee. After acclimatization for one week, the animals were divided into two groups, control and treated, comprising six animals each. Treated group of animals were given aspirin dissolved in water orally at the dose of 50mg/5 ml H\textsubscript{2}O/kg body weight while the control received water only. The treatment was discontinued after 28 days and on 29th day after overnight fasting, the animals were sacrificed under an overdose of ether anesthesia. In order to avoid diurnal variation in the biochemical parameters, nutrient uptake and enzymatic analysis, the animals were sacrificed uniformly around 9AM throughout the study. From each animal the intestine was removed, divided into duodenum, jejunum, ileum and colon, washed with chilled normal saline, wet weight of the tissue recorded and proceeded for the above mentioned parameters.

Preparation of intestinal brush border membrane (BBM): Intestinal BBM of different segments, i.e., duodenum, jejunum, ileum and colon were isolated following the method of Schmitz et al. (1973).\textsuperscript{20} The 10% (w/v) homogenate of the tissue in 1mM Tris-50mM Mannitol buffer (pH 7.4) at 4°C was passed through two layers of cheese cloth. To the filtrate, anhydrous CaCl\textsubscript{2}, was added with constant stirring on a magnetic stirrer to a final concentration of 10 mM and left for 10-15 min in cold. Later it was centrifuged at 2,000 x g for 10 min at 4°C. The supernatant was re-centrifuged at 42,000 x g for 20 min. The pellet was suspended in 20 vol of 50mM sodium maleate buffer (pH 6.5-6.8) and re-centrifuged at 42,000 x g for 20 min, the final pellet obtained was suspended in 50 mM sodium maleate buffer containing 0.02% sodium azide. The final membrane preparation obtained was similar to the P\textsubscript{2} fraction of Schmitz et al. (1973)\textsuperscript{20} and essentially free from mitochondria, microsomes, lysosomes, basolateral membranes and nuclei as assessed by marker enzyme assays. Also, purity of the isolated BBM was assessed by enrichment of the marker enzymes that showed 20-25 fold purification in the activity of alkaline phosphatase and the disaccharidases.

Assay of Disaccharidases: Sucrase, lactase and maltase activity were determined by measuring the D-glucose liberated from the respective sugar substrates and then using a glucose oxidase-peroxidase enzymatic system (GOD-POD) as described earlier.\textsuperscript{21}

Assay of Alkaline phosphatase: Alkaline phosphatase activity was assayed according to the method of Bergmeyer (1963)\textsuperscript{22} by measuring the liberated p-nitrophenol from the phosphate mono-ester substrate, p-nitrophenyl phosphate.

Protein estimation: Protein concentration was determined by the method of Lowry et al. (1951)\textsuperscript{23}, using bovine serum albumin as the standard.

Measurement of membrane microviscosity using pyrene as an extrinsic fluorophore: Pyrene fluorescence excimer (dimer) formation was used to study the lateral diffusion in the membrane as described by us earlier.\textsuperscript{24} A 100 μl of membrane preparation was taken and added 1ml stock solution (5 mM in acetone) of pyrene and 100 μl of sodium maleate buffer. The tubes were incubated at 37°C for 1 h, vortexed in between and then centrifuged at 10,000 x g for 1 h. To the pellet was added sodium maleate buffer till the solution was clear. Finally, the flu-
orescence intensity was read in a fluorimeter (ELICO CL 53, Vardhman Scientific Agencies, India) using a primary filter of 365 nm while the secondary filter used were 515 and 475 nm for the excimer and monomer fluorescence, respectively. The viscosity was calculated from the monomer/excimer fluorescence intensity ratio using the following relationship:

$$\frac{E/M (\text{excimer fluorescence})}{(\text{monomer fluorescence})} = \frac{(\text{Pyrone}) \cdot TK/\eta}{(\text{Pyrone})}$$

Where T is the absolute temperature in Kelvin, K is the Boltzmann constant (1.38062 x 10^-23j/k) and η is the microviscosity, while the pyrene concentration was kept at 5 mM.

**FTIR studies in the intestinal BBM:** A 100 µl aliquot of the membrane preparation was taken, and to it 1 ml of sodium maleate buffer (pH 6.5-6.8) was added. The tubes were centrifuged for 10 min at 10,000 x g. The precipitates were dried and mixed with KBr in the ratio of 5:95. The mixture was passed at a pressure of 10-15 tonnes with the help of a hydraulic pressure machine. The FTIR spectra were recorded in these pellets in the range of 450-4,000 cm^-1 in a Perkin-Elmer instrument.

**Transport studies:** Everted sac technique was used to study the intestinal transport. A section of the intestine is turned inside out and tied at each end. This everted sac is immersed in buffered ionic medium containing the metabolite and change in the concentration of the molecule is measured after incubation. By turning the intestine inside out transport is now from a large volume to a small volume inside the everted gut.

**Glucose transport and estimation:** An increase in the appearance of histidine from the medium in the sac is taken as an indication of amino acid transport across the membrane. Injected was 1 ml of KRB buffer into all the sacs and they were placed in KRB containing 10, 20, 30, 40 and 50 mM histidine for 30 min. The solution was taken out of the sacs after the designated time interval in a standard assay system and 2 ml of acetic acid was added. It was kept in boiling water bath for 10 min to deproteinize the solution. To this was added 0.4 ml of sodium nitrite added to the tubes. Tubes were shaken left for 5 min and 0.6 ml of sodium carbonate added into all the tubes and incubated for 30 min. L-histidine was measured by reducing it with diazotized sulphanilic acid, producing a colored compound that is read at 498 nm. Blank and standard histidine samples were also run simultaneously. The effect of temperature was studied by immersing the sacs in KRP containing 40 mM L-histidine for 30 min at 4, 20, 37 and 50°C. To study the effect of time, sacs were immersed in the medium containing 40 mM L-histidine for different time intervals: 30, 45, 60 and 120 min.

**Results and discussion**

The present study was carried out to investigate the effects of aspirin on the intestinal functions of rats such as the brush border membrane disaccharidases and alkaline phosphatase activities as well as the intestinal transport of D-glucose and L-histidine. The functional changes of the intestinal membrane were correlated with the physical characteristics such as the membrane fluidity study by pyrene excimer formation and the analysis of the functional groups by FTIR study.

**Changes in specific activity of the enzyme:** Table I shows the results of the effect of aspirin administration orally for 28 days in duodenal, jejunal, ileal and colonic homogenates, which demonstrate a significant alteration in the specific activities of the sucrase, lactase, maltase and alkaline phosphatase in the treated animals as compared to the control. Aspirin treatment resulted in a highly significant decrease (p < 0.001) in the sucrase activity in duodenal, jejunal and colonic homogenates and a significant decrease in ileal homogenate (p < 0.01). Lactase showed a highly significant decrease (p < 0.001) in jejunal and significant decrease in the duodenal homogenate (p < 0.01), and fairly significant decrease (p < 0.05) in ileal and colonic homogenates. For maltase, a

Effect of aspirin on enzymes in intestinal homogenates and the isolated brush border membrane

<table>
<thead>
<tr>
<th>Intestinal segment</th>
<th>Sucrase</th>
<th>Lactase</th>
<th>Maltase</th>
<th>Alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.076 ± 0.002</td>
<td>0.049 ± 0.003***</td>
<td>0.032 ± 0.001</td>
<td>0.021 ± 0.004**</td>
</tr>
<tr>
<td>BBM</td>
<td>2.006 ± 0.109</td>
<td>0.579 ± 0.078**</td>
<td>0.476 ± 0.111</td>
<td>0.884 ± 0.100***</td>
</tr>
</tbody>
</table>

| Homogenate         | Control | Treated | Control | Treated |
| Jejunum            | 0.131 ± 0.015 | 0.066 ± 0.002** | 0.063 ± 0.006 | 0.033 ± 0.002** |
| BBM                | 0.146 ± 0.019 | 0.128 ± 0.012 | 0.110 ± 0.013 | 0.105 ± 0.010 |

| Homogenate         | Control | Treated | Control | Treated |
| Ileum              | 0.100 ± 0.020 | 0.048 ± 0.006** | 0.054 ± 0.006 | 0.02 ± 0.005** |
| BBM                | 0.345 ± 0.028 | 0.73 ± 0.093** | 0.145 ± 0.008 | 0.257 ± 0.033** |

| Homogenate         | Control | Treated | Control | Treated |
| Colon              | 0.026 ± 0.003 | 0.006 ± 0.0005** | 0.026 ± 0.003 | 0.016 ± 0.004** |
| BBM                | 0.139 ± 0.014 | 0.111 ± 0.018 | 0.034 ± 0.005 | 0.027 ± 0.004 |

Table I

Values are expressed as mean ± SD of four observations. *p < 0.05, **p < 0.01, ***p < 0.001, represents the comparison between the control and treated groups.

highly significant decrease (p < 0.001) was recorded in duodenal, jejunal and colonic homogenates whereas in ileum, the enzyme activity showed a decrease which was not statistically significant. A highly significant decrease (p < 0.001), in the activity of alkaline phosphatase was observed in duodenal, jejunal and colonic homogenates while the ileal tissue showed a fairly significant decrease (p < 0.05).

Aspirin treatment also produced changes in the specific activities of various enzymes in the brush border membranes (BBM) of various intestinal segments. The result shows that it significantly decreased the sucrose activity in duodenal BBM (p < 0.001) and also a decrease in jejunal and colonic BBM which was however not statistically significant. In ileal BBM a highly significant elevation in sucrose activity (p < 0.001) was observed. Similarly lactase activity showed a highly significant decrease (p < 0.001) in duodenal BBM and a much significant decrease in jejunum and colon. Lactase level was found to be significantly increased (p < 0.01) in ileal BBM. Aspirin treatment to animals significantly inhibited the activity of maltase in duodenal and colonic BBM (p < 0.01). The decrease in jejunum was found to be statistically non-significant, while the ileal BBM showed a highly significant increase in maltase activity (p < 0.001). The alkaline phosphatase activity was found to be decreased in duodenum (p < 0.001), jejunum (p < 0.01) and also in colon (p < 0.05), while the enzyme activity was significantly increased in ileal BBM (p < 0.01). The comparison of the data in table I also shows the presence of these enzymes in greater amount in the partially purified brush border membranes as expected which shows considerable enrichment of the activity. The enzyme activities also showed similar pattern of alteration in BBM as observed in the homogenates after aspirin treatment. The observed decrease in the activity of the enzymes might be due to either reduced substrate affinity (kinetic effect) or specific modulation in protein molecules number or activity (metabolic effect). On the other hand a rise in BBM enzyme in the ileum may indicate to a metabolic shift in absorption and digestive activities in preference to jejunum as earlier kinetic evidences suggest a close functional link between the carrier mediated sugar transport system and the disaccharide hydrolases, as these are more enriched in the jejunum.

Studies on the pyrene fluorescence excimer formation in the intestinal BBM: The fluorescent aromatic hydrocarbon, pyrene has been solubilized and incorporated in the biological membranes, which appears to be located in the hydrocarbon core of the membrane. Steady state fluorescence measurements were performed at 27°C at an angle of 90° to the exciting beam in the fluorimeter and E/M ratios were calculated by comparing the fluorescence intensity at 515 nm to that 475 nm using 360 nm as the exciting wavelength. Microviscosity of the membrane was calculated from thereon and the inverse of microviscosity is taken as the fluidity. In a membrane suspension, the dimer (excimer) formation is independent of total pyrene concentration in the sample. Table II shows the E/M ratios in native membranes of control and treated rats, and benzyl alcohol treated membranes in control and treated rats, respectively. Native membrane of treated rats showed an increase in E/M ratio in all the intestinal segments except in the jejunum. The increased value of E/M ratio leads to a decrease in microviscosity which in turn indicates an elevation in membrane fluidity. Similarly in case of benzyl alcohol treated membranes, BBM of aspirin treated rats showed an increased E/M ratio except in jejunum. A decrease in microviscosity was observed in all the intestinal segments except jejunum resulting from an elevation in E/M ratios. In ileum and colon fluidity was highly increased. However, a small decrease and large reduction in fluidity was observed in duodenum and jejunum, respectively.
Considerable evidence exists that many function of biological membranes are influenced by composition and physical state of membrane lipids and the resultant membrane fluidity. Lipid protein interaction of BBM of rat small intestinal epithelial cells has also been examined by Brasitus et al. (1979) where the membrane fluidity has been characterized as the rotational motional freedom of the lipid molecules or substitue thereof in the bilayer. Moreover a number of studies have revealed that a large number of plasma membrane activity including certain transmembrane transport processes such as the sodium dependent D-glucose which transport appears to be influenced by the lipid composition and fluidity of the membrane.

In study with benzyl alcohol treated membranes, increased lateral diffusion of pyrene in the membranes might have resulted due to partial lipid removal and thus more motional freedom of the probe in the hydrocarbon phase. Aliphatic as well as aromatic alcohols can modify some properties of lipid bilayer as phase transition temperature and certain structural and mobility parameters as shown by ESR and NMR measurements. Effects of benzyl alcohol and some short chain alkanols in altering lipid phase fluidity in the biological membrane have also been demonstrated earlier. Similarly, the efficiency of pyrene excimer formation has been shown to increase linearly with the increase of isomyl alcohol concentration in the membrane up to approximately 50 mM.

Infrared spectroscopy study of the BBM: Infrared spectroscopy is a method of physicochemical analysis which had been employed here to study the macromolecular composition and organization in the biomembranes. The IR spectra may give unequivocal structural information; quite often the absence of band is as informative as the presence of a particular one. The instruction which may exist between membrane lipids and intrinsic proteins and the degree to which the intrinsic proteins can perturb a lipid bilayer have been the subject of many studies. Infrared spectroscopy, both difference infrared and Fourier transform provide new powerful non-perturbing tool operating on an entirely different time scale than NMR spectroscopy for studying the conformation of membranes. The IR measurements were carried out using a Perkin Elmer spectrophotometer and scans were computer averaged in the region of 400-3,500 cm\(^{-1}\). In all the cases the maximum noise suppression of the instrument was used together with the wide slit-width to reduce the scanning time.

The IR spectra of the different intestinal BBM of the control animals are shown in figure 1 (a-h). Administration of aspirin caused the appearance of new peaks or disappearance of the peaks which were present in the control animals. Also, change in the peak height and shifting in the wave numbers have been observed. Aspirin treated duodenal BBM showed 32 peaks as compared to 26 in the control. Jejunal BBM of the treated animals exhibited 28 peaks against 22 in the control group. Ileal BBM showed 21 peaks in the treated groups while 25 peaks in the control. In colonic BBM, 26 peaks were noticed in the treated animals as compared to the 29 in the control group.

In duodenal BBM of aspirin treated rats changes in wave number were noticed as compared to the control at 3,395 cm\(^{-1}\) to 3,397 cm\(^{-1}\) which corresponds to OH-stretching (R-OH), NH\(_2\) stretching (R-NH\(_2\)) and NH-stretching (R-NH-R). Similarly, changes in wave number were noticed at 2923 cm\(^{-1}\) due to anti symmetry stretching (\(-\text{CH}_2\)\(_2\)) changes in wave number 1,921 cm\(^{-1}\) due to C=O double bond stretching (R-C=O), changes in wave number 1,291 cm\(^{-1}\) due to C=O double bond stretching R-C=O, NH-bending amide I bond R-NH\(_2\), 1,560 cm\(^{-1}\) (anti symmetry and symmetry C=O double bond stretching R-CO-O, NH-bending amide II bond R-NH\(_2\) and 1,523 cm\(^{-1}\) (symmetry NH\(_2\)- bending R-NH\(_2\), NH-bending R-NH\(_2\)). Changes in wave number 1,421 cm\(^{-1}\) were due to CH\(_2\)-bending of \(\alpha\)-methylene group –CH\(_2\)-CO-O-R, and asymmetry and symmetry CO=O double stretching R-CO-O, while symmetry bending \(-\text{CH}_2\)-antisymmetry and symmetry C=O double bond stretching R-CO-O, asymmetry and symmetry C=O double stretching R-CO-O and OH-bending R-OH were responsible for change in wave number 1374. Also, changes in the wave number at 1,220 cm\(^{-1}\) resulted due to \(-\text{CH}_2\)- wagging and \(-\text{CH}_2\)-twisting (\(-\text{CH}_2\)\(_2\)) OH-bending (R-CO-OH, R-OH) and

### Table II

<table>
<thead>
<tr>
<th>Intestinal segment</th>
<th>Native membrane</th>
<th>Benzyl alcohol treated membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Excimer/monomer</td>
<td>Microviscosity</td>
</tr>
<tr>
<td></td>
<td>(EM)</td>
<td>(η)</td>
</tr>
<tr>
<td>Duodenum</td>
<td>Control</td>
<td>1.572</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>3.492</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Control</td>
<td>2.196</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>2.510</td>
</tr>
<tr>
<td>Ileum</td>
<td>Control</td>
<td>1.757</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>2.437</td>
</tr>
<tr>
<td>Colon</td>
<td>Control</td>
<td>1.875</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0.480</td>
</tr>
</tbody>
</table>

The results are mean of two independent observations run in duplicate and η = X x 10\(^{-23}\)
Fig. 1.—The Fourier Transform Infrared spectra (FTIR) of intestinal brush border membranes of control and aspirin treated rats. Control duodenum (a), aspirin duodenum (b); control jejunum (c), aspirin jejunum (d); control ileum (e), aspirin ileum (f); control colon (g) and aspirin colon (h).
antisymmetry PO₂ double bond stretching (R-O-PO₂-O-R). Besides changes in the wave number, aspirin treatment also resulted in the change in the peak height at the following: 3,395 cm⁻¹, 2,923 cm⁻¹, 2,853 cm⁻¹ [(-CH₂-) symmetry stretching], 2,361 and 2,359 cm⁻¹ (C-X stretching, X = C or N) 1,921, 1,830, 1,795 and 1,773 cm⁻¹ (C = X, stretching, X = C, N, O), 1,718 & 1,702 cm⁻¹ (C = O double bond stretching R-CO-OH), 1,684, 1,653 cm⁻¹, 1,560 cm⁻¹, 1,542 cm⁻¹ (NH-bending amide II bond R₂NH), 1,523 cm⁻¹, 1,458 cm⁻¹ [antisymmetry bending C-H bending (-CH₂-) ], 1,421 cm⁻¹, 374 cm⁻¹ and 670 cm⁻¹ (totally, symmetrical C-N- stretching, gauche dq-isotope).

In jejunal BBM, after aspirin treatment the changes in the different numbers observed were at 2,922, 2,853, 1,656 and 671 cm⁻¹ while the peak height showed considerable alteration at 2,922, 2,852, 1,656 and 671 cm⁻¹. Ileal BBM showed changes in wave number at 3,395, 2,340, 1,752, 1,721, 1,705, 1,687, 1,656, 1,625, 1,477, 1,461, 1,377, 1,053 cm⁻¹ while noticeable alteration in the peak height at 3,395, 2,361, 2,340, 1,752, 1,721, 1,705, 1,687, 1,656, 1,552, 1,339, 669, 520, 469 and 419 cm⁻¹.

Colonic BBM showed marked alterations in the wave number shift at 34,334, 2,341, 1,868, 1,793, 1,772, 1,651, 1,558, 1,457, 1,397, 520, 469 & 419 cm⁻¹ while the peak height changed were discernable at 2,361, 2,341, 1,845, 1,793, 1,772, 1,651, 1,558, 1,542, 1,523, 1,339, 669, 520, 469 and 419 cm⁻¹.

The proportion of gauche to trans conformations and therefore the static order of lipid acyl chains can be measured by shifts in the methylene C-H asymmetric and symmetric stretching frequencies. The presence of high intrinsic protein concentrations within the lipid bilayer structure introduces considerable amino acid side chain contribution to the –C-H bonds. High frequency shoulder on the C-H stretching bonds is attributed to the high levels of intrinsic proteins present. Uptake of end-product nutrients: The uptake studies of end products of digestion such as glucose and amino acid like histidine were carried out in jejunal segments in both the control and aspirin treated animals. Orally
administered aspirin at a dose level of 50 mg/kg body weight for 28 days resulted in an increase in the uptake of end product nutrients. Moreover, there was an increase in the uptake of glucose and histidine when studied at an increasing substrate concentration in the treated animals as compared to the control (fig. 2a, b).

The uphill movement of these nutrients particularly across the BBM depends heavily on the carrier molecules (transport proteins) and the increase in the uptake could be attributed to the substrate affinity constant ($K_m$) of the protein. To probe such possibility the Michaili's-Menten parameters ($K_m$ and $V_{max}$) were studied and the Lineweaver-Burk plot clearly showed that there was no change in $K_m$ and $V_{max}$ value between the control and aspirin treatment (fig. 2 c, d) which may lead to the speculation of other reason for such metabolic increase, such as the uptake of glucose and histidine, possibly due to membrane lipid effect.

Uptake of nutrients in the intestinal segments showed the dependence on temperature of incubation. Though there was an increase in glucose and histidine transport in the treated animals with every temperature (4, 20, 37 & 50 °C) but the optimum temperature (37°C) reflected no change in both the transport processes even after aspirin administration. Moreover, uptake also undergoes changes in relation to temperature resulting in the non-linearity (break) of the Arrhenius plot in control as well as treated groups, although showing close parallelism and near proximity of these lines (fig. 3 a, b).

Ducis and Koepsell (1983) concentrated on the lipid composition required for sodium dependent D-glucose transport in the reconstituted liposomes and concluded that in addition to cholesterol the presence of PE and sphingomyelin enhanced the transport activity further. This observation seems to be in tandem with our results, as aspirin treatment increases the fluidity, causes partial lipid removal or removal of cholesterol which enhances the glucose and histidine transport. Moreover, studies have suggested that alteration of membrane fluidity may influence the uptake of sodium dependent D-glucose into rat small intestine and renal BBM vesicle. Increase in transport can be the consequences of increase in cell number or ability of aspirin to enhance the absorption capacity of the enterocytes by induction of specific carrier proteins.

It has been established that a temperature dependent change in the physical state of the lipid can influence certain membrane activities carried on by the proteins. Since Arrhenius plots reveal a proximity between the control and aspirin treated animals in glucose and histidine transport, a close similarity is therefore expected in the transport process in the two membranes. Non-linearity in Arrhenius plots indicates that the proteins involved in both kinds of transport may experience temperature induced changes in the membrane, namely fluidity and therefore may be termed as the membrane intrinsic proteins. Break in the Arrhenius plot was observed at the same temperature, which rules out the possibility of any effective denaturation of the enzymes involved between the fluid and ordered lipid domains of the membrane. Also no change in transition temperature ($T_c$) was observed which evaded any chance of aspirin binding to the membrane lipid bilayer but possibly altering the phase transition or melting process of the membrane lipids. In addition, the unaltered transition temperature and a negligible decrease in activation energy ($E_a$) in the aspirin treated animals reflects only small alteration in the energy requirements of carrier proteins for binding of substrate molecules.

The treatment of aspirin for 28 days in male Wistar rats has caused structural and functional changes in intestine as evident by alteration in the enzyme levels, increase in E/M ratio leading to enhanced fluidity, physical changes as determined by IR spectra along with changes in glucose and histidine uptake. Discontinuities in Arrhenius plots represent several phenomena such as lipid phase transition from gel to liquid crystalline state, a lateral lipid phase separation and the interaction between the boundary lipid phase associated with membrane protein and the bulk lipid phase.
Phase transition in small intestine and colonocyte membranes have also been examined by assessing the temperature dependence of enzymatic and transport activities where it is suggestive that the breakpoint temperature is determined by the lipid immediately surrounding the protein called the annular lipid rather than by the bulk lipid phase of the membrane.

References