Alterations in the intestinal glycocalyx and bacterial flora in response to oral indomethacin

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs), used extensively in clinical medicine, tend to cause adverse effects in the gastrointestinal tract. Earlier work has shown that oral administration of indomethacin produced oxidative damage in the small intestine and attenuation of the glycocalyx layer of the mucosa. The present study assessed, in greater detail, the alterations produced in the glycocalyx of rat small intestinal mucosa in response to indomethacin, with specific reference to surfactant-like particles (SLP) and brush border membranes (BBM). Changes in gut flora in response to the drug were also studied, as it has been shown that luminal bacteria play a role in the pathogenesis of NSAID-induced intestinal damage. The levels of sugars such as sialic acid, fucose, hexosamine and hexose were increased in SLP and decreased in the BBM following indomethacin treatment, with the effects being maximal 24 h after the administration of the drug. The composition of lipids in the SLP was also found to be altered. There was a significant increase in the number of bacteria in the luminal contents of the small intestine and caecum in these animals, as compared with controls. The number of bacteria adherent to the intestinal mucosa was also significantly higher in the drug-treated group. In vitro studies revealed that there was an increased tendency for bacteria to adhere to SLP isolated from indomethacin-treated rats. These results suggest that alterations in glycosylation of SLP and BBM in response to indomethacin, along with qualitative and quantitative changes in the luminal bacterial flora, may facilitate translocation of bacteria into the mucosa. These changes may contribute to the enteropathy observed as a result of NSAID treatment.

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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) exert analgesic, antipyretic and anti-inflammatory activities. They act by suppressing prostaglandin synthesis through inhibition of the enzyme,
cyclooxygenase (COX) (Vane, 1971). The resultant decrease in levels of prostaglandins are also known to produce the side effects of these drugs. However, inhibition of prostaglandins alone does not explain the mechanism of pathogenesis of the adverse effects of NSAIDs. Other mechanisms that are known to be involved in NSAID-induced tissue damage include uncoupling or inhibition of oxidative phosphorylation (Somasundaram et al., 1997) and oxidative stress (Basivireddy, Vasudevan, Jacob, & Balasubramanian, 2002; Vaananen, Meddings, & Wallace, 1991). Earlier work with the small intestine has shown that treatment with indomethacin, a commonly used NSAID in toxicity studies, resulted in free radical-induced damage to enterocytes, with the villus tip cells being particularly susceptible to these effects (Basivireddy et al., 2002). Such changes were associated with infiltration of neutrophils into the small intestine and activation of xanthine oxidase (XO), resulting in oxidative stress in the mucosa with concomitant decrease in the levels of antioxidant enzymes. These effects were also associated with evidence of mitochondrial dysfunction. In addition, indomethacin administration was found to produce attenuation of the small intestinal glycocalyx layer, as shown by light microscopy. It also resulted in alterations in the composition and function of the intestinal brush border membranes (BBM) (Basivireddy, Jacob, Ramamoorthy, Pulimood, & Balasubramanian, 2003).

In the mucosa of the small intestine, a thick, jelly-like viscous layer coats the surface of the brush border membranes (BBM). This layer, referred to as surfactant-like particles (SLP) or the mucus or glycocalyx, prevents direct contact between the BBM and the luminal contents (Alpers, Zhang, & Ahnen, 1995; Engle, Grove, Bocich, Mahnood, & Alpers, 1995; Mack, Ahre, Hyde, Wei, & Hollingsworth, 2003). Glycoproteins and glycolipids are the main components of SLP and they contain a high content of sugars. SLPs are secreted initially into the basolateral space. They then migrate to the luminal surface through the tight junctions and form a continuous coat over the BBM (Engle et al., 1995). This layer contributes to the lubrication and hydrophobicity of the mucosal surface (Butler, Lichtenberger, & Hills, 1983). SLPs also act as a barrier against auto-digestion and ulceration of the mucosa and as a vehicle for secretion of enzymes like alkaline phosphatase (Alpers et al., 1995).

Various glycoconjugates are expressed on the surface of enterocytes. Cell-surface glycoconjugates have been implicated in a variety of intercellular interactions (Peters, Barber, & Grant, 1982). Mucosal glycosylation is also known to play an important role in host-bacterial interactions. The sugar epitopes of glycoproteins and glycolipids act as attachment sites for normal flora and also serve to trap and expel pathogens (Bourlioux, Koletzko, Guarner, & Braesco, 2003; Moncada, Kamnanadiminti, & Chadee, 2003). The integrity of this layer is, thus, important for normal functioning of the gastrointestinal tract.

Studies have suggested that luminal bacteria may also play a role in NSAID-induced intestinal injury. It has been shown that such damage is less in germ-free or antibiotic-treated rats than in normal rats, suggesting a role for luminal bacteria in the pathogenesis of the process (Satoh, Guth, & Grossman, 1983). Many factors determine bacterial virulence or their ability to cause infection and disease. These include adherence factors, ability of the bacteria to invade tissue, bacterial toxins and enzymes and anti-phagocytic factors (Brooks, Butel, & Morse, 2001). There is little information available on the effects of indomethacin on the glycocalyx and how it interacts with luminal bacteria to produce NSAID-induced intestinal damage.

In view of our earlier studies that showed that the glycocalyx of the small intestine was attenuated by the administration of indomethacin, this study looked at the effect of the drug on the glycocalyx of the intestine, with specific reference to the composition of the SLP and BBM. Changes in bacterial flora in the small intestine and caecum were also studied to gain better understanding into the mechanisms by which bacteria may contribute to NSAID-induced damage.

2. Methods

Bovine serum albumin (BSA), 1-cysteine hydrochloride, O-dianisidine, D-fucose, D-galactose, glucosamine, galactosamine, glucose oxidase, indomethacin, lipid standards, poly-L-lysine, sodium metaperiodate, sodium arsenite, thio-barbituric acid and D-mannose and were obtained from Sigma Chemical Co., St. Louis, USA. Polyethylene glycol (PEG) 4000 was obtained from Fluka AG, Switzerland. All other chemicals used were of analytical grade.
2.1. Animals

Male albino rats (200–250 gm) were used for the experiments. The animals were housed in a registered animal house, exposed to 12 h light-dark cycles and fed with standard rat chow and water ad libitum. All the procedures performed on the animals were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.2. Protocol for administration of indomethacin

Male rats were dosed with indomethacin (40 mg/kg) (Basivireddy et al., 2002, 2003) by gavage. Control animals received an equal volume of the vehicle for the drug (5% sodium bicarbonate). Twelve and 24 hours later, the animals were killed by cervical dislocation, their abdomens opened immediately and the entire length of the small intestine and caecum removed. These time intervals after administration of the drug were chosen for study, as preliminary experiments had showed significant effects of indomethacin consistently at these time periods. Animals were fasted for 12 h before sacrificing.

2.3. Isolation of surfactant-like particles (SLP) and brush border membranes (BBM)

SLP was isolated as described (Eliakim, DeSchryver-Kecskemeti, Noger, Stenson, & Alpers, 1989), with slight modifications. Briefly, the small intestine was removed, opened along its antimesenteric border and washed with physiological saline. The thin viscous layer on the surface of the mucosa was scraped gently with filter paper (Whatmann No. 3) to remove the gelatious SLP. The filter paper was then soaked in 2 ml of PBS pH 7.4, centrifuged at 3000 rpm for 10 min and the supernatant collected. This procedure was repeated twice and the supernatants collected were pooled to obtain SLP. After removal of the SLP, the intestinal mucosa was scraped with a glass slide and the scrapings obtained were used for isolation of BBM as described earlier (Prabhu & Balasubramanian, 2001). Purity of the isolated BBM in different experimental groups was checked by enrichment of the marker enzymes, alkaline phosphatase (Dorai & Bachhawat, 1977), sucrase and maltase (Dahlqvist, 1968). Protein was estimated using bovine serum albumin as standard (Lowry, Rosebrough, Farr, & Randall, 1951).

2.4. Estimation of sugar content in SLP and BBM

Since the glycocalyx is rich in glycoconjugates, the content of sugars in this layer and in the BBM were assessed. Sialic acid contents in SLP and BBM were estimated as described (Serifer & Gerstenfeld, 1962). Hexose and fucose contents of SLP and BBM were also determined as described, with slight modifications (Djurdjic & Mandic, 1990). Briefly, to 200 μl of sample corresponding to 10–20 μg protein, 1 ml of a cooled mixture, consisting of six parts of concentrated sulphuric acid and one part of water, was added. This was heated for 3 min in a boiling water bath and then immediately cooled in ice. To this 0.2 ml of CPS reagent (containing 1% of l-cysteine-HCL and 0.075% of phenol) was added. The mixture was kept in an ice bath for 60 min and then its absorbance read at 398 nm for fucose and 490 nm for hexoses. Total hexosamines were estimated using Ehrlich’s reagent as described (Ludowieg & Bennamman, 1967).

2.5. Lipid analysis of SLP

The lipid content of the SLP was next estimated to detect any alterations that may have occurred in response to indomethacin. Lipids in SLP were extracted as described (Bligh & Dyer, 1959). Neutral lipids were separated on silica gel G plate using the solvent system hexane: diethyl ether: acetic acid (80:20:1, v/v). Spots corresponding to standard were identified by iodine exposure and eluted. Cholesterol, cholesteryl ester (Zlatkis, Zak, & Boyle, 1953), diacylglycerol and triacylglycerol (Snyder & Stephens, 1959) were quantitated as described. Individual phospholipids were separated on silica gel H plate using the solvent system chloroform: methanol: acetic acid: water (25:14:4:2, v/v) and quantitated by phosphate estimation after acid hydrolysis (Bartlett, 1959).}

2.6. Isolation and quantitation of bacteria from intestinal and caecal contents and mucosa

Preliminary microbiological studies were carried out to assess the effect of indomethacin on bacteria.
in the caeca of experimental rats. Caecal contents from drug-treated and control animals were serially diluted in sterile saline and cultured on plates to detect aerobes and anaerobes. The aerobes studied were *E. coli*, enterococci, klebsiella, staphylococci, *Proteus vulgaris* and *citrobacter*. Anaerobes cultured were *prevotella*, *propionibacteria*, *bacteroids*, *peptostreptococci* and *veillonella*. The media used for aerobic culture included blood agar, *McConkey* agar and *rugosa* SL agar, while for anaerobic culture, *neomycin* blood agar, 5% sheep blood agar without nutrient agar base, *veillonella* agar and *rugosa* SL agar were used. The numbers of bacteria grown were quantitated by counting colony-forming units (cfu) and expressing them as cfu/g of ceacal contents.

A piece caecal mucosal tissue (approximately 1 cm² in size) was homogenized in 1 ml of sterile saline. The homogenate was subjected to quantitative culture analysis as described above and the numbers of bacteria grown were expressed as cfu/cm² tissue. The small intestine was also harvested, under sterile conditions, from sacrificed animals. The lumen of the small intestine was infused with 15 ml of sterile saline. The contents of the lumen were then collected into a sterile tube. This fluid was then serially diluted in saline and cultured on plates. The numbers of bacteria grown were quantitated by counting colony-forming units (cfu) per milliliter of intestinal washing.

### 2.7. Assessment of bacterial adherence in vitro

*E coli* strains were isolated from caecal specimens from control animals, grown on *McConkey’s* media. They were identified using standard microbiological techniques. These organisms were used for in vitro assays to measure bacterial adherence, as described below.

*E. coli* isolated from control caecal contents were tested for their ability to adhere to microtitre plates coated with SLP or BBM isolated from control and indomethacin-treated rats. SLP or BBM were layered on poly t-lysine (PLL)-coated microtitre plates and bacterial adherence was studied as follows. Briefly, 10 µg PLL in PBS was added to each well and allowed to stand at room temperature for 30 min. The unbound PLL was removed from the wells by gently washing with PBS and drying at room temperature for 1 h. The wells were then loaded with either SLP or BBM corresponding to 30 µg of protein and allowed to stand at room temperature for 20 min. Unbound material was washed off with PBS. It was found from that this resulted in coating of approximately 40–50% of the SLP and 20–30% of BBM of the added material in each well. The percentage of sample bound was calculated from measurements of the protein content in the bound and unbound fractions of the samples (data not shown). To each of these wells, 0.1 ml of *E coli* corresponding to 3 × 10⁶ bacteria, isolated from the caecal content of control rats, was added and incubated for 30 min at room temperature. Following this, the supernatant in the wells, containing unbound bacteria, was collected, serially diluted and quantitated by culturing on MacConkey agar plates. Colony counts were done and percentage of bound bacteria was calculated using the following formula:

\[
\text{Bound bacteria} = \frac{(\text{Total number of bacteria}) - \text{(unbound bacteria)}}{\text{(Total number of bacteria)}} \times 100
\]

*E. coli* bound to the wells was also quantitated using crystal violet staining (Merritt, Gaind, & Anderson, 1998). For this, following removal of the unbound bacteria, microtitre wells were washed with PBS, 0.1 ml of 10% formalin added and allowed to stand for 5 min at room temperature. The formalin was washed out using PBS and then 0.1 ml of 1% crystal violet was added to each well. The dye solution was removed after 3 min and the wells washed twice with PBS. The wells were then dried, 0.3 ml of 95% ethanol added to each well, kept for 5 min and then read at 540 nm. The optical density (OD) measured indicated the degree of bacterial adherence.

### 2.8. Testing of monosaccharides as adherence blocking agents

Since the glycocalyx is rich in sugars and bacteria are known to interact with the intestinal mucosa via
glycoconjugates, an attempt was made to determine the nature of sugars that were important in these interactions. d-Mannose, d-glucose, d-galactose, d-fucose, glucosamine, and galactosamine were each added to bacterial suspensions separately to obtain a final concentration of 2.5% (w/v) and incubated for 1 min prior to addition to SLP or BBM coated microtitre plates (Schaeffer, Amandsen, & Jones, 1980). Quantitation of bacteria that adhered to the plates was done as described above.

2.9. Statistical analysis

Data were analyzed by the Mann Whitney U-test to detect significant differences between the groups. Data at 12 and 24 h were compared with corresponding controls. A p-value of less than 0.05 was accepted as statistically significant. Statistical analysis was done using the statistical package for social sciences (SPSS) (version 11) software.

3. Results

3.1. Effect of indomethacin on sugar content in SLP and BBM

Fig. 1 shows the content of various sugars in isolated SLP from control and experimental animals at 12 and 24 h after oral administration of indomethacin. Significant increases in sialic acid, fucose, hexoses and hexosamines were seen after indomethacin treatment at both time periods, with levels higher at 24 h. These changes denoted significant changes in the content of sugars of the glycocalyx of the intestinal mucosa.

Fig. 2 shows the sugar content of BBM from control and drug-treated animals. In contrast to the findings in SLP, a significant decrease in the content of sugars was seen in indomethacin-treated BBM, with levels at 24 h being lower than those at 12 h. These alterations in the BBM denoted that the glycoconjugates in this layer were also affected by the administration of indomethacin.

3.2. Effect of indomethacin on lipid content in SLP

Fig. 3 shows the content of neutral lipids in SLP after administration of indomethacin as compared with control. A significant decrease in the contents of triacylglycerol (TAG), diacylglycerol (DAG), cholesteryl ester and free cholesterol and an increase in monoacylglycerol were seen after administration of indomethacin. There was a significant decrease in the content of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), with corresponding increases in the levels of lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE), after drug-treatment (Fig. 4A–D). These changes were more marked at 24 h than at 12 h after administration of the drug. The cholesterol/phospholipid (C/P) ratio was found to be significantly decreased both at 12 and 24 h after indomethacin treatment (Fig. 4E).
Fig. 2. Content of various sugars in BBM from control and experimental animals, 12 and 24 h after oral administration of indomethacin: sialic acid (A), fucose (B), hexoses (C) and hexosamines (D). Each value represents mean ± S.D. of duplicate estimations from six different animals. *p < 0.05 as compared with corresponding control group.

3.3. Effect of indomethacin on intestinal bacteria

Preliminary studies showed that there were significant increases in the number of *E. coli* grown from caecal contents from indomethacin-treated animals, both at 12 and 24 h, as compared with control animals at corresponding time periods (Table 1). Significant increases in numbers were also seen in the numbers of staphylococci, prevotella and peptostreptococci 24 h after the drug, but not 12 h later (Table 1). There were no significant changes in any of the other organisms grown consistently (Table 1). Growth of other organisms, such as enterococci, klebsiella, proteus and citrobacter, that occurred was found to be inconsistent.

Culture of homogenates of caecal tissue showed that the organism that was grown most consistently was *E. coli*. Significant increases were seen in the numbers of *E. coli* grown from tissue from drug-treated rats, as compared with that from control animals (Fig. 5). These increases were seen both at 12 and 24 h after administration of indomethacin. There were no significant increases in the numbers of other enterobacteriae and anaerobes grown (data not shown).
Analysis of bacteria in the small intestine from rats 12 and 24 h after treatment with indomethacin showed a significant increase in the number of *E. coli* grown, as compared with that from control animals (Fig. 6). This was seen in cultures of both intestinal washings and mucosal homogenate, the latter being a measure of bacteria adherent to the mucosa. Growth of other organisms, such as enterococci and anaerobes, was inconsistent and no significant increases were found in their numbers (data not shown).

Bacterial adherence to SLP was assessed in vitro by interaction of *E. coli* isolated from normal animals with the SLP or BBM, from control and indomethacin-treated animals, which were coated on microtitre wells. Bacterial adherence was quantitated by two different methods, namely sub-culturing of the unbound bacteria in the supernatant in the wells and by the ability of the adherent bacteria on the microtitre wells to bind to crystal violet dye. As shown in Fig. 7A and B, a greater degree of *E. coli* binding occurred to SLP isolated from animals treated with indomethacin. In the case of the BBM, however, the number of *E. coli* binding to the BBM was lower in the drug-treated group as compared with control BBM (Fig. 7C and D). Since the degree of *E. coli* binding was higher in the case of SLP isolated from indomethacin-treated rats, further characterization of the specificity of binding was done by incubating the *E. coli* with different sugars prior to SLP binding. It was observed that *E. coli* binding to SLP was inhibited 80–90% in the presence of mannose, galactose, fucose and galactosamine whereas this inhibition was not seen in the presence of glucose or glucosamine (Fig. 8). This indicated that mannose, galactose, fucose and galactosamine may play a role in mediating interactions between luminal bacteria and the glycoconjugates.

### Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>Control 12h</th>
<th>Indom 12h</th>
<th>Control 24h</th>
<th>Indom 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>4.11 (0.82)</td>
<td>6.5 (0.17)</td>
<td>4.6 (0.13)</td>
<td>9.4 (0.12)</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>2.08 (0.19)</td>
<td>3.56 (1.42)</td>
<td>2.28 (0.23)</td>
<td>7.04 (2.13)</td>
</tr>
<tr>
<td>Prevotella</td>
<td>6.46 (0.34)</td>
<td>7.49 (1.67)</td>
<td>6.66 (1.20)</td>
<td>8.07 (1.39)</td>
</tr>
<tr>
<td>Propionibacteria</td>
<td>7.73 (0.98)</td>
<td>8.09 (1.36)</td>
<td>7.90 (1.07)</td>
<td>8.24 (1.64)</td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>5.82 (1.02)</td>
<td>5.72 (1.12)</td>
<td>5.76 (1.13)</td>
<td>7.98 (1.55)</td>
</tr>
<tr>
<td>Veillonella</td>
<td>6.49 (1.27)</td>
<td>5.70 (1.36)</td>
<td>6.16 (1.58)</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as the logarithm of colony forming units (±S.D.) per gram caecal contents.*

## 4. Discussion

Normally, the gastrointestinal tract has a highly evolved mucosal defense system, which includes both non-immune (mucus, peristalsis, epithelial surfactants) and immune (secretory IgA) mechanisms (Otte, Kiehne, & Herzig, 2003; Wallace & Granger, 1996). The coating of SLPs on the surface of BBM in the small intestine is one such mechanism that forms a barrier against the damaging effect of the contents of the intestinal lumen (Einerhand et al., 2002). These contents include bile, enzymes and bacteria. In its native state, SLPs form an elastic gel with high viscosity (Bell et al., 1985). Substances such as bile salts, ethanol, NSAIDs and other stresses can severely reduce the hydrophobicity of this mucus gel layer (Hills, 1996; Lichtenberger et al., 1995). When this barrier is disturbed, the glycoconjugates are no longer able to form a gel, they become soluble and the coating is then unable to carry out its normal physiological function (Bell et al., 1985). This would result in increased permeability of the intestinal mucosa, leading to entry of noxious substances from the lumen into the mucosa. One of the events resulting from this is translocation of bacteria or endotoxins from the gut lumen into the mucosa and into the circulation (Marshall, Christou, & Meakins, 1993), with resultant mucosal damage and systemic sepsis, respectively.

Mucus cells, particularly those on the surface epithelium, appear to be responsible for the synthesis and secretion of SLP (DeSchryver-Kecskemeti et al., 1989). The functions of SLP are determined by the presence of sugars such as sialic acid, fucose, hexose and hexosamines (Sharma & Schumacher, 2001). These sugars, in combination with lipids such as phospholipids, help maintain the integrity of this layer. The integrity of this phospholipid-rich layer
in the mucosa has been shown to be regulated, in part, by prostaglandins (Scheiman, Kraus, Bonville, Weinhold, & Boland, 1991; Willemsen, Koetsier, van Deventer, & van Tol, 2003).

In the present study, indomethacin treatment resulted in alterations in the sugar content of the SLP and BBM. Specifically, this involved an increase in the sugar content in SLP and a decrease in BBM. These changes were maximal at 24 h following indomethacin treatment. In vitro studies to measure bacterial adherence to SLP coated on microtitre plates showed increased adherence of normal *E. coli* on SLP isolated 24 h after indomethacin treatment. This adherence was shown to be sugar-specific, since mannose, galactose, fucose and galactosamine were able to inhibit it whereas glucose and glucosamine did not have any effect. This suggests that alterations in glycosylation tended to increase adherence of *E. coli* to the glyocalyx. Interactions between bacteria and tissue cell surfaces in the adhesion process are complex. Several factors such as surface hydrophobicity, net surface charge, bacterial ligands and host cell receptor interactions play important roles. Bacteria
Fig. 6. Number of colony-forming units of *E. coli* (log transformed data) grown from small intestinal washings and mucosal homogenate from rats in control and experimental groups, 12 and 24 h after oral administration of indomethacin.* p < 0.05 when compared with control group.

also have specific surface molecules that interact with host cells. These include fimbriae that help mediate adherence of bacteria to host cell surfaces. Fimbriae help adherence to epithelial cell receptors that contain specific sugars (Makrides & MacFarlane, 1983). The changes seen in the sugar content of the SLP and BBM may thus be responsible for the alterations in bacterial adherence to the SLP, an observation that is corroborated by other studies (Hynes, Teneberg, Roche, & Wadstrom, 2003; Mack et al., 2003).

Earlier work has shown that generation of oxygen free radicals contribute to indomethacin-induced alterations in the intestinal mucosa (Basivireddy et al., 2002).

Fig. 7. Quantitation of *E. coli* bound to SLP and BBM in vitro. SLP and BBM were isolated from control and experimental animals, 12 and 24 h after oral administration of indomethacin. Colony forming units (cfu) (A) and crystal violet staining (B) by SLP. Colony forming units (cfu) (C) and crystal violet staining (D) by BBM. Each value represents mean ± S.D. of six separate experiments with triplicate adherent study. * p < 0.05 when compared with control group.

2003). It is possible that the changes seen in the sugar content of SLP and BBM in this study may be brought about by the oxidative stress produced as a result of indomethacin treatment. Reactive oxygen species are known to be secretagogues and may modulate the activity of glycosyltransferases or glycosidases, with resultant changes in glycosylation (Melissa & Rabb, 2002). Studies have also shown a role for oxidants in modulating the mucosal barrier, by effecting alterations in surface viscosity of the mucosa and thereby facilitating bacterial binding and translocation into the mucosa (Mojzis, Hegedušova, & Mirošay, 2000; Tatsumi, Kodama, Kashima, Okkuma, & Kuriyama, 1992).

The changes seen in the lipid content of SLP were similar to those seen in the BBM (Basivireddy et al.,...
2003). It has been shown that in indomethacin-induced damage, PLA2 activation occurs as a result of the generation of free radicals, resulting in release of arachidonic acid and lysophospholipids. Similar findings have been shown to occur in the mucosa of the gall bladder, where release of arachidonic acid and lysophospholipids occurs through activation of PLA2. Both of these products have been shown to act as potent glycoconjugate secretagogues (LaMont, Turner, DiBenedetto, Handin, & Schafer, 1983; LaMorte et al., 1986).

Studies have also shown that aspirin, an NSAID, can destabilize the gastric mucosa by disrupting phospholipid (Lichtenberger et al., 1995). Such changes are likely to contribute to disruption of the integrity and function of SLP (Mojzis et al., 2000; Tatsumi et al., 1992).

Resident intestinal microflora include both aerobes and anaerobes. The nature and number of these are stable under normal circumstances. In the normal situation, most of the luminal bacteria are non-adherent and they are swept away by mucus and other fluids that bathe the tissue surface. Bacterial adherence to the mucosa may be considered to be pathological and is accomplished by specific adhesins that are present on the outer surface of the bacteria (Grange & Mouricout, 1996). There is considerable experimental evidence to show that luminal bacteria contribute to the development of NSAID-induced intestinal damage (Mesarangi et al., 1992; Satoh et al., 1983). However, the precise mechanisms by which they do so, are not clear. Our results show that indomethacin administration can result in qualitative and quantitative changes in the bacterial population in the small intestine and caecum. Bacteria cultured from the mucosal homogenates represent the organisms that were adherent to the mucosa. Changes in host-microbial interactions were seen, as indicated by increased bacterial adherence to the intestinal and caecal mucosa. Disturbances in normal intestinal flora have been shown to result in bacterial overgrowth with pathogenic bacteria as main colonizers (Freestone et al., 2002). Other studies have also shown that administration of indomethacin have produced marked increases in intestinal flora of experimental rats (Honda, Matsumoto, & Kuroki, 1999; Kent, Cardelli, & Stamler, 1969; Komikata, Tanaka, Miyazawa, & 2002; Tanaka, Hase, Miyazawa, & 2002). Under such pathological conditions, attachment of bacteria to specific receptors on the surface of epithelial cells that are rich in sugars (such as sialic acid, hexose, fucose and hexosamines) takes place, facilitating their translocation into the mucosa (Grange & Mouricout, 1996; Helander, Hansson, & Svennerholm, 1997). We postulate that the changes seen in the content of sugars of the glycocalyx of the rat small intestinal mucosa may contribute to the increased bacterial adherence seen, with subsequent bacterial translocation and the development of enteropathy.

In summary, this study has shown significant alterations in the content of sugars and lipids in SLP and BBM of the small intestine following indomethacin treatment. This was associated with qualitative and quantitative alterations in the intestinal luminal bacteria, with an increased tendency for bacteria to adhere to the SLP. This suggests that alterations in the mucosal glycocalyx and luminal bacteria may be responsible for translocation of luminal bacteria into the mucosa and the subsequent development of NSAID-induced enteropathy.
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