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Short term effects of indomethacin on rat small intestinal permeability. Role of eicosanoids and platelet activating factor

F Mion, J-C Cuber, Y Minaire, J-A Chayvialle

Abstract

Short term effects of indomethacin on intestinal permeability were studied on a model of rat isolated vascularly perfused terminal ileum. The objectives of this study were (a) to assess the effects of indomethacin on intestinal permeability and histology; (b) to assess the effects of prostaglandins, leukotrienes, and platelet activating factor (PAF) on the same parameters; (c) to evaluate the role of these inflammation mediators on indomethacin induced permeability modifications. Intravascular administration of 1·25 and 2·5 mM indomethacin induced a significant increase of ^{51}Cr-EDTA transfer rate. Histological analysis showed only mucosal oedema. Pretreatment with 16,16 dimethyl-prostaglandin E_2 did not reverse these changes. Intravascular administration of PAF, leukotrienes B_4 and D_4 provoked a significant rise in ^{51}Cr-EDTA transfer rate and intraluminal protein leakage, with an intense vascocongestion of the mucosal capillaries. These changes were completely prevented by perfusion of the respective specific antagonists (BN52021 for PAF, LY255,583 for leukotriene B_4 and MK571 for leukotriene D_4). None of these three antagonists, however, or MK886, a selective 5′-lipo-oxygenase inhibitor, could reverse the indomethacin induced permeability changes. Indomethacin induced increased intestinal permeability at these high concentrations does not seem to be a result of changed prostanoid or PAF metabolism. Alternative mechanisms of the initial damage of non-steroid anti-inflammatory drugs should be sought.

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In humans, longterm treatment with non-steroidal anti-inflammatory drugs (NSAIDs) can induce an increase in small intestinal permeability.[1 2] This phenomenon is supposed to be an early event occurring after NSAID administration, leading to intestinal inflammation by eliciting the toxic action of exogenous compounds such as food and bacteria[3 4] on a damaged intestinal mucosa. The exact mechanisms, however, leading to increased permeability remain largely unknown.

NSAIDs are potent inhibitors of cyclo-oxygenase, and the decreased production of endogenous prostaglandins could favour increased permeability. It has been shown that exogenous prostaglandins can decrease indomethacin induced permeability changes,[5] although no clear relation between cyclo-oxygenase inhibition and the occurrence of intestinal lesions has been established.[6] Alternatively, increased local production of leukotrienes could lead to increased intestinal permeability[7 8]: NSAIDs may increase leukotriene synthesis by diversion of the arachidonic acid cascade towards the lipo-oxygenase pathway.[7]

Platelet activating factor (PAF), the most potent ulcerogenic agent, may be another contender for increased intestinal permeability, by induction of dramatic changes in the mucosal vasculature[9] or through its action on neutrophils.[10] Its local production may be stimulated by NSAIDs, either directly or through the development of an inflammatory response.[11]

The aims of this study were to show in an ex vivo model of isolated vascularly perfused ileum of the rat, the effects of indomethacin on small intestinal permeability, and to assess the role of prostaglandin E_2, leukotriene B_4, leukotriene D_4, and PAF in the indomethacin induced changes.

Materials and methods

MATERIALS

The following reagents were purchased: indomethacin powder, 16,16-dimethyl prostaglandin E_2 (5 mg/ml ethanol), leukotrienes B_4 and D_4 (in ethanol solution), PAF (L-A-phosphatidyl-cholin, B-acetyl-gamma-O-alkyl) (2 mg/ml chloroform), from Sigma Chemical; bovine serum albumin 30% (BSA), from BioMérieux, France; Azonutril 25 (a mixture of amino acids) from Laboratoire Roger Bellon, France; ^{51}Cr-EDTA complex (^{51}Cr-EDTA in 5 μM EDTA, pH=7·0, SA=300 μCi/μL) from New England Nuclear. The following antagonists were provided: LY 255,583 (a specific leukotriene B_4 receptor antagonist) by Lilly Laboratories[12]; MK-571 (a specific leukotriene D_4 receptor antagonist) by Merck Sharp and Dohme Research Laboratories[13]; MK-886 (a selective leukotriene synthesis inhibitor) by Merck Frosst Centre for Therapeutic Research[14]; and BN 52021 (a specific PAF receptor antagonist) by Institut Henri Beaufour.[15]

SURGICAL PREPARATION

The surgical steps of the rat isolated vascularly perfused ileum have been reported in detail.[16] Male Wistar rats (250–350 g) were anaesthetised with pentobarbital-sodium (50 mg/kg, intraperitoneal). After opening the abdomen by a midline incision, the right and middle colic arteries were tied and cut off. A loop of terminal

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ileum (10 cm length) was isolated: both ends of the loop were catheterised with Silastic tubing and the lumen of the intestine flushed twice with 10 ml prewarmed isotonic saline. A metal cannula (0·6 mm inside diameter, 0·8 mm outside diameter) was quickly (within 30 s) inserted in the superior mesenteric artery, and a Silastic one (0·9 mm inside diameter, 1·2 mm outside diameter) was introduced into the portal vein. The vascular perfusion was immediately started at a rate of 1·5 ml/min. The perfused solution was a Krebs-Henseleit solution (pH 7·4) containing 25% washed bovine erythrocytes, 3% BSA, 5 mM glucose, and 1% azonutril. This mixture was continuously gassed with 95% oxygen/5% carbon dioxide and warmed at 37°C. The vascular perfusion pressure was permanently monitored from a T connector located in the arterial perfusion system. The ileal loop was then transferred into a temperature stable (37°C) plastic box filled with isotonic saline. Intestinal lumen was continuously perfused with isotonic saline at a flow rate of 0·5 ml/min. Both vascular and luminal perfusions were performed without recirculation (open system) and the effluents were collected as five minute fractions throughout the study. Perfusion time was limited to 120 minutes for all experiments.

EXPERIMENTAL DESIGN

Pharmacological manipulations of the isolated ileum were always started after a 20 minute control basal period. Indomethacin, eicosanoids, and their various antagonists were given in the vascular perfusion system either as a constant perfusion or as a bolus through a catheter close to the arterial inflow. The doses chosen were derived from previously reported studies. All drugs were dissolved in a 3% BSA enriched Krebs-Henseleit solution just before they were given. Indomethacin was first dissolved in dimethyl sulphoxide (90 mg indomethacin in 400 µl), neutralised with 1 N sodium hydroxide, and brought to the final concentration with 3% BSA enriched Krebs-Henseleit solution.[17] For each drug studied, control experiments consisted of administration of the drug solvent (Krebs-Henseleit solution plus 0·4% dimethyl sulphoxide for indomethacin, 0·1% ethanol for leukotrienes and dimethyl prostaglandin E_2, 0·05% chloroform for PAF, 0·1% mannitol for BN52021).

INTESTINAL PERMEABILITY STUDIES

The vascular to ileal lumen passage of albumin (as a marker of vascular permeability)[18] was estimated by measuring the amount of protein present in the luminal effluent/unit of time.[19] The vascular to lumen ^{51}Cr-EDTA transfer rate (R) has been well established as a marker of epithelial permeability.[20] It was assessed by the percentage of radioactivity detected in the luminal effluent when ^{51}Cr-EDTA was infused in the vascular system at a constant rate (about 10 000 cpm/ml of vascular perfusate).[21] The percentage was calculated as the following ratio:

$$R=(C_{lum}\times Q_{lum}\times 100)/(C_{vasc}\times Q_{vasc})$$

where C_{lum} and C_{vasc} represent cpm/ml of ^{51}Cr-EDTA in the luminal effluent and the vascular perfusate respectively; Q_{lum} and Q_{vasc} represent the luminal and vascular perfusion rates (0·5 and 1·5 ml/min respectively).

VIABILITY STUDIES

Oxygen and glucose consumption have been previously assessed in this model.[16] Epithelium injury was assessed by measuring lactate dehydrogenase release in the vascular and luminal effluents. In preliminary studies, we found that lactate dehydrogenase release was considerably increased after a 10 minute period of ischaemia and reperfusion of the ileal loop (data not shown); we therefore used this parameter as a marker of epithelial integrity. The vascular perfusion pressure (mean (SEM)) remained stable (45 (5)) mm Hg throughout control experiments.

HISTOLOGICAL ANALYSIS

At the end of the perfusion experiments, perfused intestinal loops were removed, fixed in Bouin solution, embedded in paraffin wax, and stained with haematoxylin and eosin for histological analysis.

STATISTICAL ANALYSIS

The data in all figures are presented as mean (SEM) values. Results were compared using the Student's *t* test for unpaired values, with $p<0·05$ as the significance threshold.

Results

INTRAVASCULAR PERFUSION OF INDOMETHACIN

Permeability effects – In control experiments, permeability remained stable throughout the 120 minute perfusion (luminal protein concentration less than 100 µg/ml; ^{51}Cr-EDTA R=0·2% (0·1), n=7); there was no increase in vascular lactate dehydrogenase release over time (lactate dehydrogenase=16·4 (1·4) IU/l at the beginning of the perfusion, compared with lactate dehydrogenase=22·0 (2·6) after two hours; $p>0·1$). Indomethacin effect was assessed for increasing concentrations (from 0·1 mM to 2·5 mM). Intestinal permeability changes were detectable only in the presence of 1·25 and 2·5 mM indomethacin (Fig 1). There was a selective increase in ^{51}Cr-EDTA transfer rate, starting 30 minutes after the beginning of indomethacin administration. The amount of protein present in the luminal effluent was unchanged (<100 µg/ml throughout the experiments). An increase in lactate dehydrogenase release in the vascular effluent was not seen after 80 minutes perfusion of 2·5 mM indomethacin (lactate dehydrogenase =20·8 (2·4) IU/l at the end of the experiment period, not significantly different from controls, n=6).

Histological modifications – In the control experiments, histological examination of ileal loops after 120 minutes perfusion showed a normal pattern except for a slight oedema of the

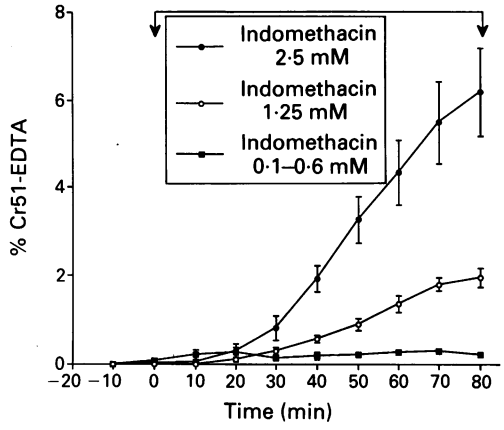

Figure 1: Effects of indomethacin (0·1–2·5 mM) on blood to lumen ^{51}Cr-EDTA permeability of rat ileal loops. Indomethacin was given intravascularly. A significant increase ($p<0·05$) was seen for the highest concentrations of indomethacin (1·25 and 2·5 mM), 30 minutes after the start of the perfusion.

lamina propria (Fig 2), as previously described.[15] After vascular perfusion of 1·25 and 2·5 mM indomethacin, histological analysis essentially showed appreciable oedema of the lamina propria as well as the submucosa; lymphatic dilation was also noticeable (Fig 3).

16,16-DIMETHYL PROSTAGLANDIN E_2

PERMEABILITY EFFECTS

Intravascular or intraluminal administration of 2·5 μM 16,16- dimethyl prostaglandin E_2 did not produce any ^{51}Cr-EDTA transfer rate increase or histological changes (data not shown). Furthermore 16,16-dimethyl prostaglandin E_2 did not significantly reduce the indomethacin induced increase of ^{51}Cr-EDTA transfer rate, when given 20 minutes before and during 2·5 mM indomethacin perfusion (^{51}Cr-EDTA R=3·0% (0·2) at the end of the perfusion (n=4), compared with a value of 4·3% (0·7) in experiments (n=6) with 2·5 mM indomethacin alone, p=0·19).

PAF INDUCED INTESTINAL ACTIONS

Intravascular perfusion of PAF (3·2 pM) provoked a rapid and significant increase in protein leakage from the vascular bed to ileal lumen (547 (72) μg/ml 20 minutes after PAF was given compared with <100 μg/ml after PAF solvent perfusion, $p<0·01$), with a concomitant rise of the perfusion pressure (+27 (5) mm Hg compared with +5 (2) mm Hg in control experiments, $p<0·01$). There was also a significant increase of ^{51}Cr-EDTA vascular to lumen transfer rate (Fig 4), while no increase in vascular lactate dehydrogenase release was noted. These effects were completely prevented by 0·1 mM BN 52021, starting 10 minutes before the PAF infusion (Fig 4).

The histological pattern was different from indomethacin induced changes: there was a striking dilatation of mucosal capillaries, filled with erythrocytes (Fig 5). Mucosal lymphatic capillaries were also dilated, with moderate submucosal oedema. Histological changes were completely prevented by the vascular perfusion of BN 52021 (data not shown).

EFFECTS OF LEUKOTRIENE B_4 AND LEUKOTRIENE D_4

Vascular administration of a bolus of 0·5 μg leukotriene D_4 increased rapidly and significantly protein leakage (465 (120) μg/ml 10 minutes after leukotriene D_4 injection, com-

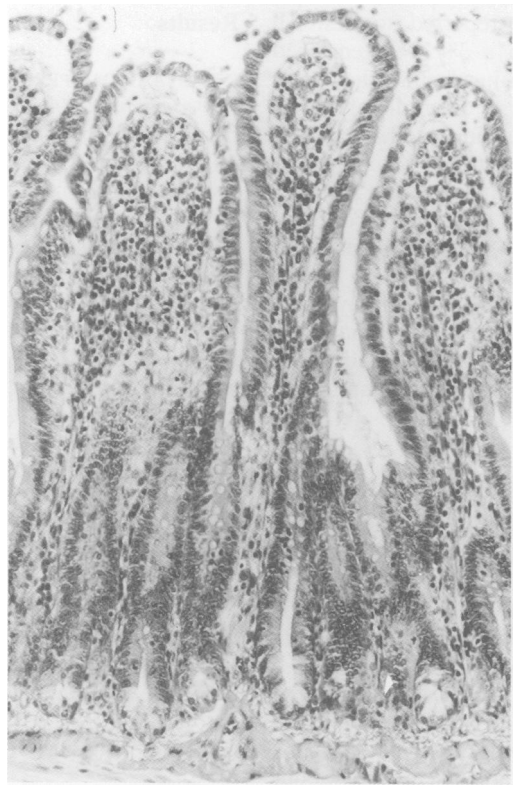

Figure 2: Light microscopic view of an ileal loop section after a 120 minute control perfusion experiment. Note the normal appearance of the villi and the submucosa, and the moderate oedema of the lamina propria. (Haematoxylin and eosin staining, original magnification ×60).

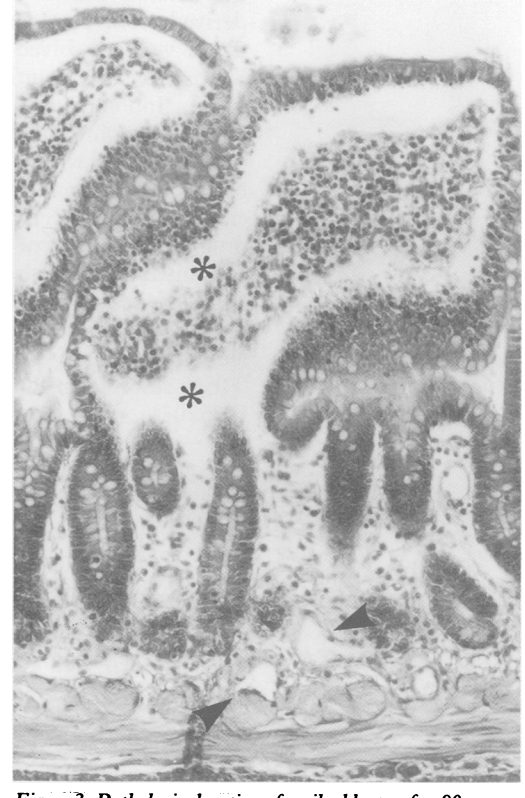

Figure 3: Pathological section of an ileal loop, after 80 minutes of vascular perfusion of 2·5 mM of indomethacin. There is an important mucosal oedema (asterisk), associated with dilated lymphatic capillaries (arrow). (Haematoxylin and eosin staining, original magnification ×60).

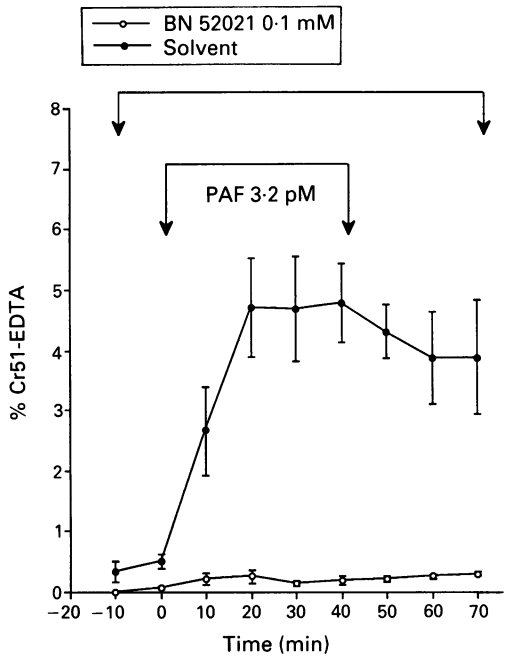

Figure 4: Blood to lumen ^{51}Cr-EDTA permeability changes induced by PAF (3·2 pM). A rapid permeability increase was seen 10 minutes after the start of vascular administration of PAF. This effect was completely prevented by the perfusion of a specific PAF receptor antagonist (BN52021, 0·1 mM), starting 10 minutes before the PAF infusion. (n=6 for both experiments; p<0·001.)

pared with <100 μg/ml after leukotriene D_4 solvent injection, p<0·03), ^{51}Cr-EDTA vascular to lumen permeability (^{51}Cr-EDTR R=2·95% (1·16), 10 minutes after leukotriene D_4 injection, compared with 0·2% (0·1) after solvent injection, p<0·05, n=4) and perfusion pressure (+22 (3) mm Hg compared with 5 (3) mm Hg for controls, p<0·05). No increase of vascular lactate dehydrogenase release over time was seen. Histological changes were similar to those seen after PAF administration: considerable dilatation of mucosal vascular and lymphatic capillaries were the most striking changes. Complete prevention of increased permeability (^{51}Cr-EDTA R=0·3% (0·1) 10 minutes after leukotriene D_4 injection, protein leakage <100 μg/ml), increased perfusion pressure (+6 (3) mm Hg after leukotriene D_4 injection), and of histological changes was obtained when 0·1 mM MK571, a selective antagonist of leukotriene D_4, was perfused 10 minutes before and after leukotriene D_4 administration.

Higher doses of leukotriene B_4 were needed to obtain similar changes: 1 μg bolus of leukotriene B_4 induced an increase of protein leakage from the vascular sector into the lumen (230 (95) μg/ml 10 minutes after leukotriene B_4 injection, compared with <100 μg/ml after solvent injection, p<0·05), ^{51}Cr-EDTA transfer rate (^{51}Cr-EDTA R=1·5% (0·9) 10 minutes after leukotriene B_4 injection, compared with 0·2% (0·1) for controls, p<0·05, n=4), increased perfusion pressure (+12 (5) mm Hg compared with +4 (2) mm Hg after solvent injection), and mucosal vascular congestion. The administration of 0·1 mM LY255,583, a selective antagonist of leukotriene B_4, completely prevented those acute changes.

EFFECTS OF SPECIFIC ANTAGONISTS OF LEUKOTRIENES AND PAF ON INDOMETHACIN INDUCED PERMEABILITY INCREASE

When perfused 10 minutes before and during 2·5 mM indomethacin administration, none of the antagonists (BM 52021, LY255,583, MK571 and MK886) significantly decreased indomethacin induced ^{51}Cr-EDTA blood to lumen permeability increase: ^{51}Cr-EDTA R was 4·5% (0·5) after 80 minutes of indomethacin perfusion with 0·1 mM BN52021, 3·8% (0·1) with 0·1 mM LY255,583, 4·8% (0·5) with 0·1 mM MK571, and 3·7% (0·7) with 0·1 mM MK886, compared with a value of 4·3% (0·7) with 2·5 mM indomethacin alone (n=5 for each antagonist, p>0·1). Neither vascular to lumen protein leakage or vascular lactate dehydrogenase release was increased (<100 μg/ml in each case). The indomethacin induced rise of perfusion pressure was not corrected by any of the four antagonists used.

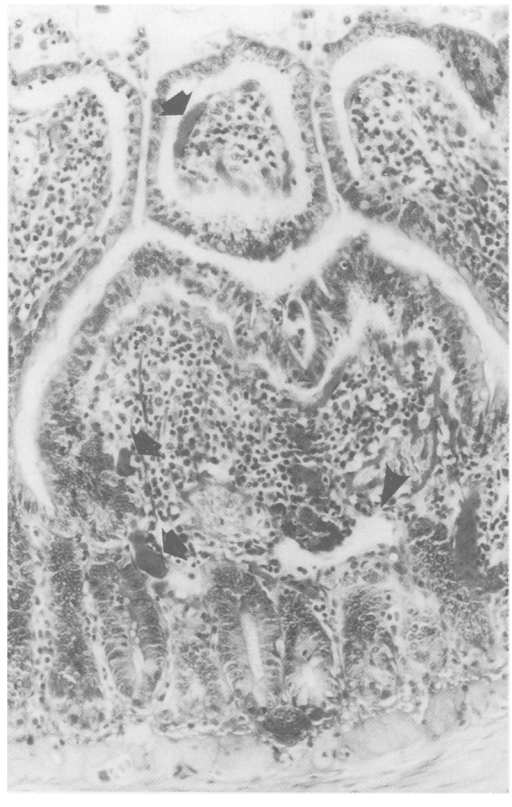

Figure 5: Pathological section of an ileal loop, after a vascular perfusion of 1·6 ng/ml platelet activating factor (PAF). A considerable congestion of mucosal vascular capillaries, filled with erythrocytes, can be seen (arrows). Lymphatic capillaries are also dilated (arrow head). (Haematoxylin and eosin staining, original magnification ×60).

Discussion

In the isolated vascularly perfused small bowel model, we have shown that high doses of indomethacin rapidly increased vascular to lumen ^{51}Cr-EDTA permeability. Although these high concentrations of indomethacin will probably not be achieved in humans, the doses usually given in animal models to induce intestinal lesions are much higher than those used in clinical practice.[3,4] This high dose requirement is probably partly a result of interspecies variations of sensibility to NSAIDs. Our results are in accordance with those of Krugliak *et al*[22]: these authors found an increase of PEG 400 intestinal absorption only when 2·5 and 5 mM indomethacin were given.

In our model, indomethacin did not increase protein loss through the intestinal mucosa, a process essentially related to changes of the microvascular endothelium.[18,23] The intestinal permeability of hydrophilic macromolecules is limited by the integrity of the mucosal vasculature, the 'aqueous pores' of the luminal mem-

brane of enterocytes, and the intercellular tight junctions.[24] Our results show an increased epithelial permeability without apparent damage to the mucosal capillaries. There was probably not a change in enterocytes because there was no release of intracellular enzymes such as lactate dehydrogenase, and histological examination did not show enterocyte injury.

These results suggest that inhibition of endogenous prostaglandins was not the first stage of indomethacin induced permeability increase, as administration of exogenous synthetic analogues of prostaglandin E_2 did not prevent the increased vascular to lumen ^{51}Cr-EDTA transfer rate. Similar findings have been made in humans.[25] The dose of dimethyl prostaglandin E_2 given was selected from previous studies,[21 22] and should have been adequate to reverse indomethacin induced permeability changes, if these changes are a result of decreased prostaglandin synthesis. Furthermore, if cyclo-oxygenase inhibition was the main factor for the permeability increase, this effect would have been seen for much lower concentrations of indomethacin: in vitro, this drug inhibits cyclo-oxygenase at concentrations as low as 30 μM.[26]

Thus, other mechanisms had to be found to explain the effects seen of high concentrations of indomethacin on small intestinal permeability. We showed that leukotrienes B_4 and D_4, as well as PAF, could increase vascular to lumen ^{51}Cr-EDTA permeability. The mechanisms seemed to be different, however, from indomethacin induced changes, because of the dramatic vascular modifications, including major congestion of mucosal capillaries and increased vascular to lumen protein loss, seen with leukotrienes and PAF. It was noteworthy that leukotriene D_4 was more potent than leukotriene B_4, a finding consistent with the more specific action of leukotriene D_4 on the tone and permeability of microvasculature.[27] The effects of PAF, leukotriene B_4, and leukotriene D_4 on small intestinal permeability were specific for each molecule, as they could be prevented by the administration of their respective antagonists. These antagonists did not prevent, however, indomethacin induced permeability changes, suggesting that leukotrienes and PAF were not responsible for the early functional intestinal changes induced by the high doses of indomethacin used in this study.

Neutrophil accumulation in the intestinal mucosa has been recently shown to play an important part in the development of indomethacin induced intestinal ulcers.[8 10 28] This mechanism probably does not account for the present findings, because the suspension of washed erythrocytes used as perfusate contained less than 500 leucocytes/mm^3, and because no accumulation of polymorphonuclear cells was seen in the wall of intestinal loops at the end of perfusion experiments. We believe neutrophil accumulation to be only a part of the inflammatory response developed as a consequence of the initial indomethacin induced increased intestinal permeability.

From these data, inhibition of endogenous prostaglandins as well as enhanced production of leukotrienes and PAF probably do not explain the early permeability changes induced by indomethacin. Alternative mechanisms may be inferred from the ability of the molecule to act on other cellular enzymatic processes.[29] Firstly, indomethacin, being a weak acid and a lipophilic molecule, can interfere directly with cell membranes to modify their functional properties. Secondly, indomethacin has recently been shown to change the cellular synthesis of adenosine triphosphate through the anaerobic glycolytic pathway[30]: this also may increase the epithelial tight junction permeability, known to be energy dependent.[31 32] Thirdly, indomethacin can induce the release of oxygen derived free radicals[33]: these very unstable components may cause lipid peroxydation, producing functional changes of the intestinal barrier.

In conclusion, high concentrations of indomethacin can rapidly and selectively induce an increase of the small intestinal epithelial permeability, without any microscopical damage to enterocytes or mucosal vasculature. From our data, this effect does not seem to result from inhibition of endogenous prostaglandins synthesis alone, or from increased leukotriene and PAF production. Instead, indomethacin induced permeability changes may be a result of the synergistic action of the drug on several different metabolic processes. This would imply that any treatment strategy aimed at a single mechanism might fail to restore normal intestinal permeability.

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