Vascular anatomy defines sites of indomethacin induced jejunal ulceration along the mesenteric margin

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Abstract

Background—Indomethacin induces ulceration in the rat jejunum with sparing of the ileum. The ulcers localise between vasa recta along the mesenteric margin of the bowel, observations that have not been fully explained.

Aim—To examine the relationship between the localisation of experimental ulcers and the vascular anatomy of the rat small intestine.

Methods—The normal vascular anatomy of the rat jejunum and ileum was studied and compared using arterial carbon ink perfusion. The anatomical localisation of early and advanced lesions induced by indomethacin was examined with particular reference to the vasculature. Mucosal injury induced by feeding vessel ligation for 24 hours or brief ischaemia-reperfusion injury was examined. The existence of anatomically sensitive sites to indomethacin was tested in a two dose study.

Results—In the rat jejunum, poorly vascularised sites along the mesenteric margin were highly susceptible to indomethacin induced injury, such sites being absent from the ileum. Villous contraction was a feature of both early indomethacin injury and ischaemia-reperfusion injury in the rat jejunum. Twenty four hour ligation of jejunal vasa brevia selectively induced ischaemic Injury along the mesenteric margin. Two doses of indomethacin to rats did not induce greater injury than a single dose.

Conclusions—Results support the hypothesis that the rat jejunum possesses vascularly compromised sites along the mesenteric margin that are susceptible to indomethacin induced injury. Indomethacin may cause ischaemia-reperfusion injury selectively at these sites.

Keywords: indomethacin; blood supply; small intestine; ulcers; rat; vascular anatomy; ischaemia

Jejunal ulceration induced by a single oral dose of the non-steroidal anti-inflammatory drug (NSAID) indomethacin to rats is one of several chemically induced experimental models of inflammatory bowel disease. It has been noted that a most interesting and consistent feature of these NSAID induced experimental lesions is their propensity to occur along the mesenteric margin of the jejunum and more specifically, at sites supplied by short feeding vessels (vasa brevia, VB) located between the long feeding vessels (vasa recta, VR). It is of interest that in Crohn’s disease small intestinal ulcers also tend to arise along the mesenteric margin where blood vessels pass through the muscularis propria, an acknowledged, but largely ignored observation.

Early anatomical studies of the human gastrointestinal tract identified the existence of long (VR) and short (VB) feeding vessels that supplied the antimesenteric and mesenteric margins of the bowel wall respectively. While the mesenteric margin is known to be preferentially ulcerated in the indomethacin/rat model this predilection still lacks an explanation.

In this paper we have compared the normal vascular anatomy of the rat jejunum with the ileum (sensitive and resistant sites of indomethacin induced ulceration respectively) and examined the anatomical relationship between the localisation of lesions and the vascular anatomy. We also tested the hypothesis that the predilection for such lesions to arise at anatomically susceptible sites is explained by the existence of critically perfused areas in the bowel wall which would be more prone to ischaemic injury.

Materials and Methods

MATERIALS

Male Sprague-Dawley rats (240–270 g) were kept under standard laboratory conditions and had free access to food at all times. Indomethacin was purchased from the Sigma Chemical Company (St Louis, Missouri, USA) and black carbon ink from Pelikan (Hanover, Germany). Experiments were performed under a project licence approved by the Home Office (UK).

METHODS

Vascular anatomy of the normal rat small intestine

After terminal anaesthesia (2% halothane and fentanyl 0.15 mg/kg intraperitoneally) rats underwent midline laparotomy. Heparin (100 U) was injected into the inferior vena cava and the rats were killed by cardiac exanguination. The gastrointestinal tract was then arterially perfused with carbon ink for visualisation of the vascular anatomy. Briefly, the abdominal aorta was cannulated with 5 gauge (French) polyethylene intravenous tubing and the portal vein ruptured to permit perfusate outflow. The Aorta was perfused with heparin-saline followed by carbon ink until the entire bowel had turned black.
Segments of mid-jejunum (20 cm) and terminal ileum (10 cm) were removed and divided into two, one half being opened longitudinally along the mesenteric margin and the other opened along the antimesenteric margin. The tissues were pinned onto cork and immersion fixed overnight. The tissues were then dehydrated in absolute ethanol and cleared to transparency in methylsalicylate using the method of Spalteholtz. Small segments (2 cm) of jejunum and ileum were left unopened for the examination of intact tissue. The vascular anatomy was examined by dissection microscopy, focusing primarily on the feeding vessels of the bowel wall and the submucosal plexus. Distances (mm) between feeding vessels were measured using a ruler. It was possible to trim away small sections of cleared tissue from the main specimen for direct visualisation under a light microscope by securing the piece with a glass cover slip. A calibrated point counting eyepiece graticule (25 µm intervals) was used to make vascular measurements.

To quantify the vascularity of the mesenteric and antimesenteric halves of the rat jejunum and ileum, segments of cleared tissues from each rat were examined by light microscopy as described above. A line (AC) between two consecutive VR, not separated by a VB, passing from the mesenteric margin to the antimesenteric margin was visualised using the eyepiece graduated scale (fig 1). The line was bisected into mesenteric (AB) and antimesenteric halves (BC). All arteries transecting the line in each half were scored 1, 2, or 3 according to outer diameter (score 1 = 25–50 µm; score 2 = 50–75 µm; score 3 = 75–100 µm). A total score for each half was determined by summing the scores. For each rat, five consecutive lines were assessed in this way and the mean score was calculated for the mesenteric and antimesenteric halves. The mean (SEM) score in each half was determined for the group of rats (n=5).

Indomethacin induced jejunal ulceration
Two groups of rats (n=5) received an oral dose of indomethacin 10 mg/kg as a sodium bicarbonate 1.5% aqueous solution. In one group the rats were terminally anaesthetised four hours after indomethacin and the entire small intestine was examined externally for early lesions. After terminal cardiac exanguination, the small intestine was perfused arterially with carbon ink as described in the previous section. The jejunum (defined as the segment of small intestine beginning 10 cm distal to the pylorus and ending 20 cm from the ileocaecal valve) and terminal ileum (defined as the terminal 10 cm of intestine), were opened longitudinally along the antimesenteric margin, pinned onto cork, immersion fixed overnight, and cleared to transparency using the method described earlier. The position of each indomethacin induced early mucosal lesion and its relationship with the vasculature was noted by both dissection microscopic examination and light microscopy. Smaller pieces of tissue from the main specimen were placed on a glass slide and covered with a glass cover slip. Areas of normal mucosa from antimesenteric and mesenteric sites were also examined. Lesions and normal areas were examined histologically by trimming them from the main specimen, washing in chloroform overnight, and processing into paraffin wax for routine histology.

In the other group the rats were killed 24 hours later and the small intestine examined externally for lesions followed by longitudinal opening along the mesenteric margin for assessment of advanced mucosal ulceration.

Ligation of vasa recta and vasa brevia
Experiments involving extramural ligation of rat VR and VB feeding vessels were performed to establish which parts, if any, of the bowel wall would ulcerate after prolonged (24 hours) ischaemia. Ligation of VB for a shorter period followed by reperfusion was carried out to
establish whether villous shortening is a feature of ischaemia-reperfusion injury.

Six rats were anaesthetised and laparotomy was performed. Under dissection microscopic guidance, five consecutive VR and three consecutive VB (about 5 cm away from the ligated VR) along the same side of the jejunum and distal ileum were ligated with 6–0 nylon sutures prior to penetration of the muscularis propria. The laparotomy was closed and rats were allowed to recover. The rats were killed by anaesthetic overdose 24 hours later followed by opening of the small bowel longitudinally along the antimesenteric margin. The ligated segments of the small intestine were pinned onto cork boards and immersion fixed overnight followed by macroscopic examination. The entire ligated segments were trimmed for routine histological assessment. Six other rats underwent sham operation without ligation and were treated and examined in the same way.

Twelve other rats were anaesthetised and laparotomised as above. In six of these, the blood flow through a single VB was temporarily arrested by applying traction to a 6–0 suture tied loosely around the extravascular aspect for 35 minutes. Blood flow was then restored for 15 minutes and the experiment was terminated for macroscopic and histological assessment of the mucosa. The other six rats represented sham operated controls that were treated in the same way except that no traction was applied to the ligature. The segment of bowel supplied by the ligated VB (about 0.3 mm either side of the sutured vessel) was fixed in formalin and trimmed into four segments for histology. Villous height was assessed by measuring the crypt height:villous height ratio using a calibrated eyepiece graticule. The ratio was assessed in four villi situated within the mucosa supplied by the ligated vessel and four villi within the antimesenteric margin. The ratio was also measured in equivalent regions of the bowel wall of sham operated rats. The mean ratio for each rat was assessed at each site and the results expressed as mean (SEM) for the group (n=6).
10 mg/kg 24 hours after the first followed by histological assessment four hours later. The aim of administering two doses of indomethacin was to determine whether the jejunum possesses anatomically defined areas susceptible to indomethacin injury. If such areas exist, the first dose would preferentially identify these sites, and a second dose should theoretically not produce further lesions. This experiment relies on the ability to differentiate histologically between late ulcerative lesions (focal but complete mucosal loss) arising from the first dose and early preulcerative changes (villous shortening, epithelial and microvascular injury, and superficial villous necrosis) attributed to the second dose.

STATISTICS
The data were analysed using Wilcoxon rank sum test. An associated probability of 5% or less was considered significant.

Results
VASCULAR ANATOMY OF THE RAT JEJUNUM
The arterial blood supply of the rat jejunum and terminal ileum was identified as arising from the mesenteric branch of the abdominal aorta. Within the mesentery the mesenteric artery divided into about 20 terminal branches that formed a continuous peri-intestinal marginal artery running parallel, and close, to the mesenteric margin of the bowel wall. Arising from the marginal vessel were two types of feeding arteries that supplied the bowel wall and were distinguished by their length, outer diameter, and point of insertion into the bowel wall. If the cross section of the bowel wall is considered to represent a clock face, with the mesenteric and antimesenteric margins representing 6 and 12 o’clock respectively, the first type of vessel consisted of VR (diameter 200–250 µm) that branched directly from the peri-intestinal marginal artery and alternately pierced the bowel wall at approximately 5 and 7 o’clock to supply the anterior and posterior walls (figs 1 and 2). Usually accompanied by a vein, they entered the muscularis obliquely, passing through the latter for about 0.2–0.3 cm and bifurcated within the submucosa at 3 and 9 o’clock. The two branches arising from this bifurcation passed over to the antimesenteric side where they united with equivalent branches from the opposite side of the bowel wall, forming the submucosal collateral plexus (fig 1).

The second type of feeding vessel was shorter and of lower calibre (VB diameter 100–200 µm) arising from either the marginal artery directly or from the extramural aspect of VR, the latter type of VB being much shorter and of a lower calibre than the former (figs 2 and 3). VB entered the bowel wall at 6 o’clock, the point of entry sited between the point of entry of consecutive VR. A significant difference between the jejunum and ileum was the greater distance between consecutive jejunal VR on the same side of the bowel wall compared with the equivalent ileal distance (VR-VR distance jejunum = 3.1 (0.2) mm versus ileum = 2.0 (0.04) mm; p<0.05). This difference made the ileum appear much more vascular than the jejunum and intervening jejunal VB supplied a larger area of bowel wall along the mesenteric margin.
After bifurcation, VR formed an extensive submucosal plexus of vessels that consisted of collateral connections within the antimesentericpectofofthejejunalandilealwall(fig 4). In the jejunum, however, this submucosal plexus did not extend to the mesenteric margin of the bowel; here, the underlying mucosa was supplied either by VB or small blind ending branches of VR as the latter passed through the muscularis propria (fig 5A). Only a few connecting arteries existed between jejunal VR within the mesenteric side of the bowel wall giving an overall impression that the jejunal antimesenteric margin was more vascular than the mesenteric margin. This was not the case in the ileum where many such connecting arteries were apparent (fig 5B). The vascularity score of the jejunal mesenteric margin was significantly lower than that of the antimesenteric side (jejunal mesenteric score = 3.9 (0.4) versus antimesenteric = 8.6 (0.6); p<0.05). There was no such vascular difference in the ileum at these sites (ileal mesenteric score = 6.5 (0.6) versus antimesenteric = 6.6 (0.2)).

Small arterioles branched from the submucosal plexus to supply the muscularis propria, pericryptal areas, and individual villi. Apart from the main peri-intestinal marginal artery there were no intercommunicating vessels between adjacent VR or VB outside the bowel wall.

INDOMETHACIN INDUCED JEJUNAL LESIONS

Indomethacin, four hours after dosing, induced multiple lesions in the mid-jejunum of all animals, no lesions being evident in the duodenum and terminal ileum. Externally, both the appearance and localisation of these early lesions were similar to those of the advanced lesions. After carbon ink perfusion they were easily identified as black, round, and oval shaped punched out mucosal depressions, measuring approximately 0.2–0.4 cm in diameter (fig 6). On examination of the cleared specimen, most lesions (95 (5)% were localised preferentially to the mesenteric margin between VR (fig 7). VB were noted to supply the larger areas (about 30–50 villi) of mucosa affected by indomethacin. The territory defined for smaller lesions was supplied by up to three small branches of VR, each small lesion being situated at the interface of these vessels (fig 8).

Histologically, four hour lesions showed severely contracted and superficially necrotic villi with ectatic surface capillaries filled with ink. Villi within the central core of the lesion were the most severely damaged (fig 9), peripheral villi showing milder changes that included villous contraction, epithelial loss, and superficial capillary distortion with little necrosis. The mucosa of both the antimesenteric margin and mesenteric margin between lesions showed mild villous shortening and superficial capillary distortion, the severity of which increased the nearer the mucosa to lesions.

Indomethacin, 24 hours after dosing, induced visible external lesions (brown circular and oval shaped spots) confined to the jejunum, most of which (97.4 (0.4)%) were located along the mesenteric margin between VR. Each of these lesions corresponded to deep oval shaped mucosal ulcers arranged along the mesenteric margin, and separated by a segment of normal mucosa. Histological examination showed focal mucosal loss with neutrophil infiltration of the ulcer base and muscularis propria.

LIGATION OF VASA RECTA AND VASA BREVIA

Twenty four hours after ligation of the feeding vessels, four of the six rats developed small single mucosal ulcers (0.5 mm) directly overlying the ligated jejunal VB. These small lesions were situated along the mesenteric margin with
spared of the antimesenteric margin. No lesions were noted in the ileum or in the segment of jejunum in which the VR were ligated. Histologically, all lesions showed both focal mucosal necrosis and neutrophil infiltration at the mesenteric margin (fig 10), microscopic appearances that were quite similar to those noted 24 hours after oral indomethacin. No lesions were identified in the other two rats. None of the sham operated rats developed lesions.

Sham ligation had no significant morphological effect on the jejunal mucosa but ligation–reperfusion of a single jejunal VB caused significant focal villous shortening within the adjacent mucosa along the mesenteric margin (fig 11; mean (SEM) villous height:height ratio in VB ligated rats, mesenteric villi = 0.87 (0.05) versus antimesenteric = 0.67 (0.06), p<0.05; in non-ligated sham rats, mesenteric villi = 0.65 (0.07) versus antimesenteric villi = 0.64 (0.05)). The villous shortening was confined to the area of the bowel wall considered to be supplied by the ligated vessel while the surrounding mucosa serving as internal control was normal.

**SUSCEPTIBILITY OF ANATOMICALLY DEFINED JEJUNAL SITES TO INDOMETHACIN**

A single dose of oral indomethacin 10 mg/kg to rats induced histological changes in approximately half of the jejunal sections studied, four and 24 hours after dosing; the remaining half from each group were normal (table 1). Early changes (four hours) consisted of villous shortening, superficial villous necrosis, and neutrophil infiltration. Late changes consisted of full thickness mucosal necrosis and neutrophil infiltration. No early histological changes were seen in the rats receiving two doses of indomethacin; late changes were observed in 38.4% of the sections and 61.2% were normal. There was no significant difference in the number of late lesions in rats receiving either one or two doses of indomethacin.

**Discussion**

In this study of the vascular anatomy of the rat small intestine, we have identified a site along the mesenteric margin of the jejunum that is susceptible to both early and late phases of indomethacin induced injury. The vascularity of this site was significantly lower than the equivalent site along the antimesenteric margin, giving the clear impression that the arterial blood supply of the mesenteric margin mucosa might become preferentially compromised during an ischaemic insult to the small intestine. An equivalent avascular site was not identified in the rat terminal ileum, a section of the small bowel that is indomethacin resistant.

Mucosal ulceration only occurred at the jejunal mesenteric margin following ligation of jejunal VB while ligation of jejunal VR and ileal VB and VR failed to induce ischaemic injury. This is strong evidence that the mesenteric marginal site supplied by VB might represent a critically perfused part of the jejunum that is not served by a collateral blood supply and is therefore susceptible to ischaemia. Evaluation of the normal vascular anatomy would suggest that jejunal VB are effectively end arteries.

The observation that a second dose of indomethacin to rats failed to cause additional injury to the jejunum may be interpreted as exhaustion of anatomically susceptible sites by the first dose, leaving no sites available for the second dose to damage.

The observation by others that parenteral, as well as oral, dosing with indomethacin is able to induce jejunal lesions in the rat, and the preference for lesions to arise at anatomically defined sites in this study, suggest that a systemic effect of the drug is likely to play an important role in the pathogenesis of lesions. However, exposure of the jejunal mucosa to both luminal and systemic indomethacin is necessary for the early reduction in villous blood flow as demonstrated in ex vivo videomicroscopic experiments in rats. Luminal bacteria, food, and bile acids are believed to play important secondary roles in the genesis of lesions in this animal model. Bile duct ligation of rats prior to administration of either oral or parenteral indomethacin prevents jejunal ulceration. Indomethacin is known to undergo extensive enterohepatic cycling; the protective effect of bile duct ligation would therefore imply that systemic levels of indomethacin alone are insufficient to cause inflammation and necrosis of the mucosa.

**Table 1 Evaluation of indomethacin susceptible sites in the rat duodenum**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Early changes</th>
<th>Late changes</th>
<th>No abnormality</th>
</tr>
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<tbody>
<tr>
<td>4 h (1 dose)</td>
<td>58.8 (10.4)</td>
<td>0</td>
<td>41.4 (11.0)</td>
</tr>
<tr>
<td>24 h (1 dose)</td>
<td>0</td>
<td>47.4 (9.7)</td>
<td>52.6 (9.7)</td>
</tr>
<tr>
<td>24 + 4 h (2 doses)</td>
<td>38.4 (7.3)</td>
<td>61.2 (7.3)</td>
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Two groups of rats received a single oral dose of indomethacin 10 mg/kg; the jejunum was assessed histologically four and 24 hours later for early and late changes. A third group received a second dose of indomethacin 24 hours after the first dose and the jejunum was assessed for histological changes four hours later. Early (4 h) histological changes were defined as focal villous shortening, superficial villous necrosis and neutrophil infiltration. Late (24 h) histological changes were defined as focal full thickness mucosal necrosis and neutrophil infiltration.
Inhibition of prostaglandin synthesis by NSAIDs was thought to play a role in gastrointestinal injury caused by this class of drug. However, Whittle observed a dissociation of the appearance of the damaging effects of indomethacin and prostaglandin synthesis that does not support such a role for prostaglandins.1,2 Furthermore, not all NSAIDs that inhibit small intestinal prostaglandin synthesis cause ulceration at this site.3

Neutrophil recruitment by endothelial cell adhesion has also been proposed to play a primary role in NSAID damage to the gut4 but recent studies have questioned the idea that NSAID gastrointestinal injury is neutrophil dependent.5,6,7 It is certainly difficult to explain the distribution of intestinal lesions on the basis of the studies claiming prostaglandin inhibition and neutrophil recruitment to be primary pathogenic mechanisms operating in this situation.

Precisely how indomethacin might influence blood flow to the small intestine is not known, but intravenous administration of indomethacin has resulted in a rapid splanchnic bloodflow to the small intestine is not known, inhibition and neutrophil recruitment to be the basis of the studies claiming prostaglandin inhibition and neutrophil recruitment to be primary pathogenic mechanisms operating in this situation.

By reducing jejunal blood flow, ulcerogenic doses of indomethacin might cause a generalised ischaemia of the small intestine that is sufficient to cause macroscopic damage at "watershed" sites. The VB ligation studies presented in this paper and the superior mesenteric artery ligation experiments of Boros et al, also performed in the rat,8,9 suggest that villous shortening is a physiological response to mucosal ischaemia-reperfusion. Support for the involvement of vasoconstriction in this situation is the observation that the β₂ adrenoceptor agonist CL316243, known to have vasodilator activity in the gastrointestinal tract,10 is a potent inhibitor of indomethacin induced jejunal ulceration in the rat.11 Reversal of early histological villous shortening, a preulcerative phase of injury thought to result in disruption of villous blood flow, was noted to be associated with CL316243 protection in that study.12 Ex vivo videomicroscopy has shown slowing, and finally stopping of villous blood flow, 20 minutes after combined parenteral and luminal administration of indomethacin to the jejunal mucosa of anaesthetised rats, changes that could only be recorded in villi located between VR.13

An alternative explanation for the focal nature of the lesions might involve contraction of the muscularis propria. Contraction of the muscularis propria has been implicated by Takeuchi et al14 and Mersereau and Hinche15 to play a role in NSAID induced gastric ulceration in the rat. They noted that lesion formation correlated with an increase in gastric smooth muscle contraction and motility; both lesion formation and muscular contraction were prevented by the spasmylytic muscarinic antagonist, atropine. Whether muscular contraction and motility are factors in the pathogenesis of NSAID injury to the small intestine has yet to be determined, but β₂ adrenoceptor agonists, known to have spasmylytic activity in the rat small intestine,16 and atropine, are both inhibitors of indomethacin induced jejunal ulceration in the rat.17,18

The predilection of the mesenteric margin to form lesions specifically at a site supplied by primary arterial branches suggests a role for "watershed" areas. In conclusion, we have identified a defined anatomical site along the mesenteric margin of the rat jejunum that is susceptible to both the early and late phases of indomethacin induced ulceration. The vascularity of this site is significantly lower than the equivalent area along the antimesenteric margin and ligation studies suggest that the blood supply to it does not communicate with the submucosal plexus of vessels that exists in the rest of the bowel. This site seems to represent a critically perfused area of bowel that could be more prone to ischaemia and reperfusion injury.

12 Kelly D, Piasecki C, Anthony A, Pounder RE, Wakefield AJ. Early indomethacin lesions in rat jejunum: reduced focal


