Recovery of ischaemic injured porcine ileum: evidence for a contributory role of COX-1 and COX-2

A T Blikslager, D N Zimmel, K M Young, N B Campbell, D Little, R A Argenzio

Background: We have previously shown that the non-selective cyclo-oxygenase (COX) inhibitor indomethacin retards recovery of intestinal barrier function in ischaemic injured porcine ileum. However, the relative role of COX-1 and COX-2 elaborated prostaglandins in this process is unclear.

Aims: To assess the role of COX-1 and COX-2 elaborated prostaglandins in the recovery of intestinal barrier function by evaluating the effects of selective COX-1 and COX-2 inhibitors on mucosal recovery and eicosanoid production.

Methods: Porcine ileal mucosa subjected to 45 minutes of ischaemia was mounted in Ussing chambers, and transepithelial electrical resistance was used as an indicator of mucosal recovery. Prostaglandins E, and E2 (PGE) and 6-keto-PGF1α (the stable metabolite of prostaglandin I2 [PGI2]) were measured using EUSA. Thromboxane B2 (TXB2, the stable metabolite of TXA2) was measured as a indicator of COX-2 activity.

Results: Ischaemic injured tissues recovered to control levels of resistance within three hours whereas tissues treated with indomethacin (5×10−6 M) failed to fully recover, associated with inhibition of eicosanoid production. Injured tissues treated with the selective COX-1 inhibitor SC-560 (5×10−4 M) or the COX-2 inhibitor NS-398 (5×10−4 M) recovered to control levels of resistance within three hours, associated with significant elevations of PGE and 6-keto-PGF1α compared with untreated tissues. However, SC-560 significantly inhibited TXB2 production whereas NS-398 had no effect on this eicosanoid, indicating differential actions of these inhibitors related to their COX selectivity.

Conclusions: The results suggest that recovery of resistance is triggered by PGE and PGI2, which may be elaborated by either COX-1 or COX-2.

SMALL INTESTINE

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Materials and Methods

Experimental animal surgeries

All studies were approved by the North Carolina State University Institutional Animal Care and Use Committee. Animals were 6–8 week old crossbred (Duroc×Yorkshire×Landrace) pigs of both sexes purchased from North Carolina State University Porcine Educational Unit. Animals were acclimatized to the holding facilities at the College of Veterinary Medicine for at least three days. Pigs were housed singularly and maintained on a commercial pelleted feed. Pigs were held off feed for 24 hours prior to experimental surgery. All experiments were performed by the same team to minimise bias.

Abbreviations: COX, cyclo-oxygenase; Isc, short circuit current; Jms, mucosal to serosal flux; NSAIDs, non-steroidal anti-inflammatory drugs; PGE, prostaglandin E1 and E2 (PGE) and 6-keto-PGF1α (the stable metabolite of prostaglandin I2 [PGI2]); R, transepithelial electrical resistance; PD, potential difference; TXB2, thromboxane B2; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; PBS, phosphate buffered saline.
variability. A physical examination was performed on all ani-
mals by veterinary investigators (Blikslager, Zimmel) to
ensure the good health of the animals on the day of
experimental surgery. General anaesthesia was induced
with xylazine (1.5 mg/kg intramuscularly), ketamine (11 mg/kg
intramuscularly), and pentobarbital (15 mg/kg intravenously)
and was maintained with intermittent infusion of pentobarbi-
tal (6–8 mg/kg/h). Pigs were placed on a heating pad and ven-
tilated with 100% O<sub>2</sub> via a tracheotomy using a time cycled
ventilator. The jugular vein and carotid artery were cannu-
lated, and blood gas analysis was performed to confirm
normal pH, and partial pressures of CO<sub>2</sub> and O<sub>2</sub>. Lactated
Ringer's solution was administered intravenously at a mainte-
nance rate of 15 ml/kg/h. Blood pressure was continuously
monitored via a transducer connected to the carotid artery.
The ileum was approached via a ventral midline incision. Ileal
segments were delineated by ligation the intestinal lumen at
10 cm intervals, and subjected to ischaemia by clamping the
local mesenteric blood supply for 45 minutes. Following the
ischaemic period, pigs were killed and intestinal loops were
resected.

**Ussing chamber studies**

The mucosa was stripped from the seromuscular layer in oxy-
genated (95% O<sub>2</sub>/5% CO<sub>2</sub>) Ringer's solution, and mounted in a
3.14 cm<sup>2</sup> aperture Ussing chambers, as described in previous
studies. Tissues were bathed on the serosal and mucosal sides
with 10 ml Ringer's solution. The serosal bathing solution
contained 10 mM glucose and was osmotically balanced on
the mucosal side with 10 mM mannitol. Bathing solutions
were oxygenated (95% O<sub>2</sub>/5% CO<sub>2</sub>) and circulated in water
jacketed reservoirs. The spontaneous potential difference (PD)
was measured using Ringer-agar bridges connected to calomel
electrodes, and PD was short circuited through Ag-AgCl elec-
trodes using a voltage clamp that corrected for fluid resistance.
Transepithelial resistance (Ωxcm<sup>2</sup>) was calculated from spon-
taneous PD and short circuit current (I<sub>s</sub>). If spontaneous PD
was between −1.0 and 1.0 mV, tissues were current clamped at
±100 µA for five seconds and the PD recorded. I<sub>s</sub> and PD were
recorded every 60 minutes for 240 minutes.

**Chemicals**

Tissues treated with COX inhibitors were bathed in Ringer's
containing the appropriate concentration of indomethacin
(Sigma Chemical Co., St Louis, Missouri, USA), NS-398 (ICN
Pharmaceuticals, Costa Mesa, California, USA), or SC-560
(Cayman Chemical Co., Ann Arbor, Michigan, USA) to prevent
prostaglandin production while stripping mucosa from the
seromuscular tissues. The appropriate COX inhibitors were
so also added to the serosal and mucosal bathing solutions prior
to mounting tissues in Ussing chambers.

**Eicosanoid analyses**

Samples were taken from the serosal bathing solutions of tis-
uses after 60 minutes and 240 minutes of the experiment and
were immediately frozen in liquid N<sub>2</sub>. Samples were stored at
−70°C prior to eicosanoid analysis. Samples were analysed for
concentrations of PGE<sub>2</sub>, 6-keto-PGF<sub>1α</sub> (the stable metabolite of
prostaglandin I<sub>1</sub> (PGI<sub>1</sub>)), and thromboxane B<sub>2</sub> (TXB<sub>2</sub>, the stable
metabolite of TXA<sub>2</sub>) using commercial ELISA kits according to
the manufacturer's instructions (Biomedical Technologies Inc.,
Stoughton, Massachusetts, USA).

**Morphometric measurements**

Tissues were taken immediately after ischaemia and following
the 240 minute recovery period for histological evaluation.
Tissues were sectioned (5 µm) and stained with haematoxylin
eosin. For each tissue, three sections were evaluated by an
investigator blinded to the treatment group. Four well oriented
villi were identified in each section. Morphometric measure-
ments were performed as previously described. The height of
the villus, and the width at the midpoint of the villus, were
obtained using a light microscope with an ocular micrometer.
In addition, the height of the epithelial covered portion of each
villus was measured. The surface area of the villus was calcu-
lated using the formula for the surface area of a cylinder. The
formula was modified by subtracting the area of the base of
the villus, and multiplying by a factor accounting for the vari-
able position at which each villus was cross sectioned. In
addition, the formula was modified to take into account the
hemispherical nature of the villous tip. The percentage of the
villous surface area that remained denuded was calculated from
the total surface area of the villus and the surface area of the
villus covered by epithelium. Per cent denuded villous sur-
face area was used as an index of epithelial restitution.

**Isotope flux studies**

To assess mucosal to serosal flux of mannitol, 0.2 µCi/ml of
[<sup>3</sup>H]<sub></sub>mannitol was added to the mucosal solution of tissues.
Following a 15 minute equilibration period, standards were
taken from the bathing reservoirs. Subsequently, three one
hour fluxes were performed by taking samples from the sero-
sal bathing reservoirs. Samples were collected in scintillation
vials and assayed for β emission (counts/minutes). Mucosal
to serosal fluxes of mannitol (J<sub>ms</sub>) were determined using
standard equations.

**Gel electrophoresis and western blotting**

Control and ischaemic injured mucosa was stripped in
oxygenated Ringer's solution containing either no treatment
or indomethacin, as described for the Ussing chamber experi-
ments. Approximately half of each piece of tissue was then
snap frozen whereas the remaining tissue was recovered for
240 minutes in oxygenated Ringer's prior to snap freezing.
Tissues were stored at −70°C prior to preparation for sodium
dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-
PAGE), at which time they were thawed to 4°C. Tissue portions
(1 g) were added to 3 ml of chilled RIPA buffer (0.15 M NaCl,
50 mM Tris (pH 7.2), 1% deoxycholic acid, 1% Triton X-100,
0.1% SDS), including protease inhibitors. The mixture was
homogenised on ice, centrifuged at 4°C, and the supernatant
savad. Protein analysis of extract aliquots was performed (D<sub>2</sub>
protein assay; Bio-Rad, Hercules, California, USA). Tissue
electrodes (amounts equalised by protein concentration)
were mixed with an equal volume of 2x SDS-PAGE sample buffer
and boiled for four minutes. Lysates were loaded on a 10%
SDS-polyacrylamide gel and electrophoresis was carried out
according to standard protocols. Proteins were then transferred
to a nitrocellulose membrane (Hybond ECL; Amersham Life
Science, Birmingham, UK) using an electroblotting minitrans-
fer apparatus according to the manufacturer's protocol. Mem-
branes were blocked at room temperature for 60 minutes in
Trio buffer saline plus 0.05% Tween-20 (TBST) and 5% dry
powdered milk. Membranes were washed twice with TBST and
incubated for one hour in primary antibody (COX-1 or COX-2,
affinity purified goat polyclonal antibodies; Santa Cruz
Biotechnology, Inc., Santa Cruz, California, USA). After wash-
ing three times for 10 minutes each with TBST, membranes
were incubated for 45 minutes with horseradish peroxidase
conjugated secondary antibody. After washing three addi-
tional times for 10 minutes each with TBST, the membranes
were developed for visualisation of protein using an alkaline
phosphatase conjugate substrate kit (Bio-Rad).

**Immunohistochemistry**

Tissues were fixed in 10% neutral buffered formalin, routinely
processed for paraffin embedding, and cut into 5 µm sections.
Following placement on slides, sections were deparaaffinised
and rehydrated. Slides were subsequently incubated in 3%
H<sub>2</sub>O<sub>2</sub>, washed, and subjected to protease digestion for 10 min-
utes. Slides were washed in phosphate buffered saline (PBS)
and incubated with normal goat serum (Biogenex, San
Ramon, California, USA) for 20 minutes. Slides were incubated for one hour with either rabbit antisheep COX-1 polyclonal antibody or rabbit antihuman COX-2 polyclonal antibody (Alexis Co., San Diego, California, USA). This step was not performed on negative control slides. Slides were washed four times in PBS between 20 minute incubations with biotinylated goat antirabbit antibody and streptavidin labelled peroxidase (Biogenex). Slides were then placed in 3-amino-9-ethylcarbazole, washed in distilled water, counterstained with 0.5% methyl green for 30 seconds, and mounted.

**Data analysis**

All data were analysed using a statistical software package (Sigmastat; Jandel Scientific, San Rafael, California, USA). Data are reported as mean (SEM) for a given number (n) of animals for each experiment. The statistical significance level selected for all tests was p<0.05. Prior to ANOVA, data were analysed to determine if they were normally distributed and had equal variance (Levene median test). If data failed either of these analyses, ANOVA on ranks was performed. All data were analysed using one way ANOVA at each time point to

**Figure 1** (A) Electrical responses of ischaemic injured porcine ileal mucosa to treatment with cyclooxygenase (COX) inhibitors. Forty five minutes of ischaemia resulted in baseline transepithelial resistance (R) ∼50% that of control. Untreated ischaemic injured tissues recovered control levels of R within 180 minutes whereas tissues treated with the non-selective COX inhibitor indomethacin (5×10⁻⁶ M) did not fully recover. However, ischaemic injured tissues treated with the selective COX-2 inhibitor NS-398 (5×10⁻⁶ M) or selective COX-1 inhibitor SC-560 (5×10⁻⁶ M) recovered levels of R not significantly different from control within three hours. *p<0.05 versus control. Significance was determined by one way ANOVA, n=12. (B) Mucosal to serosal fluxes (J_m) of mannitol across control and ischaemic injured tissues during a 240 minute recovery period. J_m mannitol in ischaemic injured tissues was significantly greater than control during the first flux period (60–120 minutes) regardless of treatment. While J_m mannitol recovered to levels not significantly different from control by 240 minutes in untreated ischaemic injured tissues or those treated with NS-398 (5×10⁻⁶ M) or SC-560 (5×10⁻⁶ M), J_m mannitol in indomethacin treated tissues remained greater than control levels throughout the recovery period. *p<0.05 versus control tissues. Significance was determined using one way ANOVA, n=8.

**Figure 2** Histological appearance of ischaemic injured porcine ileal mucosa. (A) Ischaemia for 45 minutes resulted in lifting and sloughing of epithelium from the tips of villi. (B) After a 240 minute in vitro recovery period, villi contracted and epithelial restitution was complete. (C) Treatment of tissues with indomethacin (5×10⁻⁶ M) had no observable effect on restitution in tissues recovered in vitro for 240 minutes. (D) Similarly, tissues treated with NS-398 (5×10⁻⁶ M) had evidence of villous contraction and complete epithelial restitution. 1 cm bar=100 µm.
determine if there were statistically significant differences between treatments. Tukey’s test was used to determine differences among treatments following ANOVA, unless ANOVA on ranks was performed, in which case a Student-Newman-Keuls test was performed.

RESULTS

Effect of COX inhibitors on recovery of mucosal resistance, permeability, and morphology

Ischaemic injured porcine ileum recovered to control levels of transepithelial electrical resistance (R) within 180 minutes whereas R in ischaemic injured tissues treated with the non-selective COX inhibitor indomethacin (5×10⁻⁶ M) remained below that of control levels throughout the experiment (fig 1A). In contrast, the selective COX-2 inhibitor NS-398* (5×10⁻⁶ M) or the selective COX-1 inhibitor SC-560** (5×10⁻⁷ M) did not impair recovery of R compared with ischaemic controls. Similarly, mucosal to serosal fluxes of mannitol, a relatively small macromolecule (4 Å stokes radius) that traverses tissues via the paracellular space, decreased to control levels in untreated ischaemic injured tissues and in tissues treated with NS-398 or SC-560 within 180 minutes, whereas in tissues treated with indomethacin, mannitol fluxes remained significantly elevated above control levels for the duration of the 240 minute recovery period (fig 1B).

Histological evaluation of tissues immediately following the 45 minute ischaemic period revealed sloughing of villous tips (fig 2A) which amounted to denudation of 16.5 (2.2)% of the villous surface area. Blinded evaluation of tissues at the end of the 240 minute recovery period showed 0.0 (0.0)% denudation as a result of epithelial restitution, regardless of treatment (fig 2B–D). The degree of villous contraction was not significantly different among treatment groups (data not shown). Treatment of control tissues with COX inhibitors had no significant effect on R levels or histological appearance of tissues over the 240 minute recovery period (data not shown).

Eicosanoid levels in COX inhibitor treated tissues

To determine if the differences in recovery of R and permeability shown with indomethacin, NS-398, or SC-560 could be associated with a different profile of eicosanoid production, we measured tissue production of PGE, 6-keto-PGF₉α (the stable metabolite of PGF₁α), and TXB₂ (the stable metabolite of TXA₂). The latter has been used as a specific indicator of COX-1 activity. Accordingly, TXB₂ levels were elevated in ischaemic injured tissues throughout the recovery period whereas tissues treated with 5×10⁻⁷ M indomethacin recovered to the same extent as untreated ischaemic tissues. Significance was determined by one way ANOVA following two way ANOVA on repeated measures, n=8.

As shown in fig 3A, indomethacin and whole blood.

Figure 4

Electrical responses of ischaemic injured porcine ileum recovered to control levels of transepithelial electrical resistance (R) that remained significantly below untreated ischaemic tissue levels throughout the recovery period whereas tissues treated with 5×10⁻⁷ M indomethacin recovered to the same extent as untreated ischaemic tissues. *p<0.05 versus untreated ischaemic tissues.

Figure 3

Eicosanoid levels in control and ischaemic injured tissues before and after a 240 minute recovery period. (A) Prostaglandin E, and E₃ (PGE) levels showed significant reduction compared with untreated tissues whereas SC-560 had no significant effect on PGE production at 240 minutes. (B) 6-keto-PGF₉α (the stable metabolite of prostaglandin I₃, [PGI₃]) levels showed trends similar to those of PGE levels, including significant inhibition at 240 minutes by indomethacin and NS-398. (C) Levels of thromboxane B₂ (TXB₂, the stable metabolite of TXA₂) were measured as a potential indicator of COX-1 activity. Accordingly, TXB₂ levels were elevated to the same degree in control tissues, ischaemic injured tissues, and tissues treated with the COX-2 inhibitor NS-398 at both time periods. However, tissues treated with indomethacin had no significant elevations in TXB₂ during the recovery period, and tissues treated with SC-560 had levels significantly below those of untreated tissues at both 60 minutes and 240 minutes. Significance was determined using one way ANOVA at each time period, n=8. Treatments with different letters at each time period were significantly different from one another.
Figure 5  Elaborated eicosanoid levels in response to varying doses of indomethacin. Prostaglandin E (PGE) (A) and 6-keto-PGF1α (B) levels were significantly elevated in tissues treated with 5x10⁻⁶ M indomethacin but fully inhibited by 5x10⁻⁷ M and 5x10⁻⁸ M indomethacin. (C) There were no significant elevations in thromboxane B2 (TXB2) regardless of the dose. *p<0.05 versus control tissue at 60 minutes. Treatments with different letters at 240 minutes were significantly different from one another. Significance was determined using one way ANOVA, n=8.

Figure 6  Electrical responses of ischaemic injured porcine ileal mucosa to varying doses of NS-398. Tissues treated with 5x10⁻⁴ M NS-398 had significantly depressed levels of transepithelial electrical resistance (R) compared with tissues treated with either 5x10⁻⁵ M or 5x10⁻⁶ M NS-398. *p<0.05 versus untreated ischaemic tissues. Significance was determined using one way ANOVA, n=8.

NS-398 only partially inhibited production of PGE and 6-keto-PGF1α and had no inhibitory effect on TXB2 production. In contrast with these results, the selective COX-1 inhibitor SC-560 did not significantly inhibit PGE or 6-keto-PGF1α at 240 minutes but significantly inhibited TXB2 production.

Response to varying doses of indomethacin and NS-398

The results shown in fig 3 suggested that the differences in eicosanoid production in the presence of the different COX inhibitors might be due to a relative difference in sensitivity of the two COX enzymes to the inhibitors. To test this possibility, we performed experiments in which the doses of indomethacin or NS-398 were serially diluted or concentrated, respectively. Although a 10-fold dilution of indomethacin (5x10⁻⁷ M) inhibited recovery of R to the same extent as the original dose, a 100-fold dilution (5x10⁻⁸ M) permitted tissues to recover to levels similar to those of untreated ischaemic injured tissues (fig 4). This recovery of R at the 100-fold dilu-

COX western blots

Although the above experiments suggested that a difference in sensitivity of COX-1 and COX-2 for the inhibitors could exist, possible changes in the concentration of active enzyme during the 240 minutes of the experiment could make such an interpretation difficult. Therefore, the relative concentrations of COX-1 and COX-2 protein were determined by western blot on control and ischaemic injured tissues prior to and following the 240 minute recovery period. COX-1 protein levels were similar in control and ischaemic injured tissues prior to recovery (0 minutes; fig 7) whereas a 100-fold increase in concentration of NS-398 (5x10⁻⁴ M) inhibited recovery of R and completely inhibited production of all three eicosanoids.

COX immunohistochemistry

To determine the location of the two COX enzymes in this tissue, we performed immunohistochemical analyses immediately after ischaemic injury and following the recovery period. Tissues stained with secondary antibody alone were negative for stain uptake whereas tissues exposed to COX-1 or COX-2 antibody revealed the presence of these proteins in ischaemic injured tissues (fig 9). COX-1 was noted in intestinal crypt epithelial cells
of the COX enzymes may result in compensatory upregulation of activity of the other COX isofrom. Therefore, it is difficult to apply information from such COX knockout models to wild-type animals that may express both COX enzymes.

**DISCUSSION**

Although previous studies have evaluated the importance of COX-1 and COX-2 in mucosal repair and the importance of COX-1 and COX-2 in maintenance of mucosal barrier function; it has been difficult to develop a clear understanding of the relative roles of these enzymes. One reason for this is the inherent complexity of the models used. For example, knockout of one of the COX enzymes may result in compensatory upregulation of activity of the other COX isofrom. Therefore, it is difficult to apply information from such COX knockout models to wild-type animals that may express both COX enzymes.

However, not all studies using COX null animals agree on compensatory increases in eicosanoids, which compounds the difficulty of interpreting COX knockout studies. On the other hand, in wild-type animals, interpretation of the relative production of eicosanoids such as PGE by the two COX enzymes is hampered by the fact that both COX-1 and COX-2 are expressed at significant levels throughout the recovery period commencing immediately following the injurious event. Furthermore, it appears that TXB2 is produced by COX-1 in ischaemic injured tissues but there was marked expression of COX-2 in ischaemic injured tissues. Comparison of tissues immediately following ischaemia and after the recovery period revealed little difference in expression. Indomethacin appeared to slightly decrease the expression of COX-2. STD, COX-1 (70 kDa) or COX-2 (72 kDa) were detected in other tissues and may result from a link between COX enzymes and specific eicosanoid production has been observed in other tissues and may result from a link between COX enzymes and specific eicosanoid production.

The results of our study are consistent with the hypothesis that PGE and PGI2 produced by either COX-1 or COX-2 are capable of triggering full recovery of mucosal barrier function. Evidence that elaboration of these eicosanoids by COX-1 can stimulate recovery includes: (1) ischaemic injured tissues treated with the selective COX-2 inhibitor (NS-398) made a full recovery at doses as high as 5 × 10^-5 M; (2) at doses of NS-398 that allowed mucosal recovery, there was no inhibition of TXB2 production unless it was given at very high doses. Such a link between COX enzymes and specific eicosanoid production has been detected in other tissues and may result from a link between specific COX isofroms and TXA2, synthase.

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indomethacin with corresponding lower concentrations of PGE (<20 pg/ml) do not allow recovery. Similarly, $10^{-7}$ M NS-398 inhibits full recovery (fig 6) in the presence of 55 (14) pg/ml PGE whereas lower doses of NS-398 with corresponding higher concentrations of PGE (>110 pg/ml) allow full recovery. Thus we propose that a minimal concentration of $\sim 110$ pg/ml PGE by 240 minutes is required for full recovery in this model.

Some additional findings related to eicosanoid production (fig 3) suggest that COX-1 is responsible for a greater proportion of prostanoid elaboration during the early phase of mucosal recovery whereas COX-2 may be responsible for greater prostanoid production during the latter period of mucosal recovery. For example, PGE, 6-keto-PGF$_{1\alpha}$ and TXB$_{2}\alpha$ were all significantly inhibited by SC-560 and indomethacin at the 60 minute time period but not by NS-398. On the other hand, PGE and 6-keto-PGF$_{1\alpha}$ were significantly inhibited by NS-398 and indomethacin at the 240 minute time period but not by SC-560. However, data on SC-560 suggest that at $5\times 10^{-7}$ M, this agent was not fully inhibiting COX-1. Thus although TXB$_2$ was significantly inhibited by SC-560, it was not fully inhibited. It is therefore possible that at a dose of SC-560 that was high enough to fully inhibit TXB$_2$, significant inhibition of PGE and 6-keto-PGF$_{1\alpha}$ would be detected that should correspond to that fraction of these prostanoids that were not inhibited by NS-398.

A recent study has shown that both COX-1 and COX-2 contribute to homeostasis of the mucosal barrier. For example, COX-1 or COX-2 null mice demonstrated increased colonic mucosal ulceration in response to dextran sodium sulphate compared with wild-type controls. Furthermore, there was an additive increase in susceptibility to dextran sodium sulphate induced mucosal injury in COX-1 null mice treated with NS-398. Whether such changes in mucosal ulceration resulted from changes in mucosal susceptibility to dextran sodium sulphate induced injury or differences in the rate of mucosal recovery was not clear. Studies on recovery of pre-existent injury in wild-type animals do not provide firm conclusions on the role of COX isoforms. For example, NS-398 retarded gastric ulcer repair in mice, and exacerbated colonic inflammation in rats, but these findings were not compared with the effects of a relatively non-selective COX inhibitor such as indomethacin or with a selective COX-1 inhibitor. In studies where comparisons between selective COX-2 inhibitors and non-selective COX inhibitors were performed, both classes of drugs were shown to inhibit repair of gastric ulcers to a similar extent, suggesting that COX-2 rather than COX-1 eicosanoids are required for recovery of ulcerated gastric mucosa. However, the relative roles of COX-1 and COX-2 could not be distinguished because there were no experiments in which COX-1 was selectively inhibited. We approached this problem by comparing the effects of selective inhibitors of COX-1 or COX-2 with that of the non-selective COX inhibitor indomethacin. In so doing, we came to the conclusion that both COX-1 and COX-2 play a role in mucosal reparative events in ischaemic injured small intestine.

The finding that indomethacin showed evidence of COX-1 selectivity at low doses was not entirely unexpected as many of the so-called non-selective COX inhibitors show some degree of specificity for COX-1. In particular, indomethacin and aspirin show a relatively high degree of specificity for COX-1 compared with agents such as ibuprofen, which essentially inhibits both COX enzymes to the same degree. None the less, indomethacin appears to be a potent COX inhibitor as at doses as low as $5\times 10^{-7}$ M, indomethacin inhibited all eicosanoid production. However, some degree of selectivity for COX-1 became evident at a dose of $5\times 10^{-7}$ M.

The mechanism by which COX elaborated eicosanoids stimulate recovery of ischaemic injured porcine epithelium remains to be fully elucidated but it appears to involve closure of dilated paracellular spaces rather than an effect on epithelial restitution. Such a mechanism would explain the lack

Figure 9 Immunohistochemical analysis of ischaemic injured tissues before and after a 240 minute recovery period. For purposes of comparison, paired photomicrographs are presented for tissues stained with only the secondary antibody or for tissues also treated with anti-cytochrome 1 (COX-1) or anti-COX-2. (A) Tissues treated only with secondary antibody showed the presence of only the counterstain whereas tissues additionally treated with anti-COX-1 immediately following ischaemia (B) showed COX-1 protein localised to crypt epithelium. (C, D) Similar results for COX-1 staining were noted after the 240 minute recovery period. (E) Tissues treated only with secondary antibody showed the presence of only the counterstain whereas tissues additionally treated with anti-COX-2 immediately following ischaemia (F) showed COX-2 protein localised to sloughing villous epithelium and lamina propria mononuclear cells. (G, H) Similar results for COX-2 staining were noted after the 240 minute recovery period except that COX-2 was localised to repairing epithelium (1 cm bar=50 μm).
of differences among the various treatments when evaluating histological indices of restitution in the present study (fig 2). In fact, in previous studies, we have shown that epithelial restitution in this model is near complete within 60 minutes and a time when there is little evidence of recovery of transepithelial resistance. Recovery of resistance is subsequently associated with closure of inter-epithelial spaces within restituted epithelium. Thus restitution is likely a critical initial step in the repair process prior to recovery of paracellular resistance. The rapidity of restitution shown in our model is similar to other ex vivo models. For example, in guinea pig ileum, treatment with detergent (Triton-X 100) resulted in sloughing of epithelium from the tips of villi that was able to restitute within 60 minutes following detergent washout. The rate of epithelial restitution in ex vivo model systems may be more rapid than in in vitro models of restitution because of the absence of concurrent reparative mechanisms in vitro. For example, villous contraction in ex vivo models dramatically reduces the denuded surface area that remains to be restituted such that far fewer cells are required to rescale a defect. We have previously documented significant decreases in villous height during recovery of ischaemic injured porcine ileum as evidence of this mechanism.

The fact that either COX-1 or COX-2 elaborated eicosanoids appeared equally capable of stimulating mucosal recovery is somewhat puzzling given the immunohistochemical localisation of these enzymes. COX-2 was expressed within repairing epithelium and adjacent mononuclear cells but COX-1 was localised to crypt epithelium, which raises the question as to how prostanooids released by crypt epithelium might stimulate recovery of injured villous epithelium. We are confident of our immunohistochemical results because COX-1 has been localised to crypt epithelium in the mouse, and humans, and COX-2 has been localised to repairing epithelium and subepithelial mononuclear cells in patients with Crohn’s disease. One possible integrative signalling mechanism whereby prostanooids released by crypt epithelium might stimulate recovery of villous epithelium is the enteric nervous system, particularly as it is now well established that certain prostagonoids, notably PGE2 and PGL2, are powerful neuromodulators. However, a full understanding of such mechanisms will require further study.

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