

# Mucosal adaptation to indomethacin induced gastric damage in man – studies on morphology, blood flow, and prostaglandin E<sub>2</sub> metabolism

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## Abstract

The effect of 28 days' continuous administration of oral indomethacin on gastroduodenal morphology, gastric mucosal blood flow, and gastric mucosal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) metabolism in man was studied to define further the mechanisms of mucosal injury induced by indomethacin. Indomethacin caused acute gastroduodenal damage in all cases, which was maximal at 24 hours of administration. With continued intake, mucosal adaptation occurs resulting in resolution of endoscopic mucosal damage. At the time of maximal mucosal damage, gastric mucosal blood flow was significantly reduced compared with values before treatment ( $p < 0.001$  in fundus and  $p < 0.002$  in antrum), with good correlation between the severity of damage and the magnitude of the reduction in blood flow ( $r = 0.76$ ). Mucosal recovery was associated with a return of the blood flow to normal. PGE<sub>2</sub> in mucosal homogenate was significantly reduced by indomethacin in both the fundus ( $p < 0.01$ ) and antrum ( $p < 0.01$ ) after 24 hours but there was no correlation between the magnitude of this reduction and the severity of mucosal damage ( $r = -0.34$ ). Despite mucosal recovery by 28 days, PGE<sub>2</sub> values remained significantly below those before treatment in both the fundus ( $p < 0.01$ ) and antrum ( $p < 0.01$ ). The PGE<sub>2</sub> degradation capacity was not influenced by indomethacin. In conclusion, mucosal adaptation to acute damage by indomethacin occurs in man and seems independent of local PGE<sub>2</sub> metabolism.

It is well known that non-steroidal anti-inflammatory drugs (NSAIDs), extensively used as simple analgesics and in rheumatic diseases, cause gastrointestinal injury, particularly in the stomach and proximal small bowel.<sup>1-3</sup> The damage produced can result in serious complications such as ulceration, bleeding, and perforation, which can be life threatening.<sup>4-6</sup>

How NSAIDs cause gastroduodenal damage is unknown, although the depletion of mucosal prostaglandins, which reduces the competence of the mucosal defence mechanisms, is thought to be of major importance.<sup>7-9</sup> Support for this mechanism is provided by the findings that NSAIDs reduce many aspects of mucosal defence such as epithelial HCO<sub>3</sub> secretion,<sup>10</sup> mucus synthesis and secretion,<sup>11,12</sup> and surface hydrophobicity<sup>13</sup> – effects largely prevented by treatment beforehand with prostaglandin. In addition to their effects on mucosal defence, NSAIDs also reduce the integrity of the 'mucus

cap', produced in response to superficial injury, which is important in providing a suitable environment for epithelial repair to take place.<sup>14</sup>

The effects of NSAIDs on mucosal blood flow, a vital component of mucosal defence and repair, remain controversial. Ashley *et al*<sup>15</sup> have shown that aspirin causes a reduction in gastric mucosal blood flow at the sites of damage, with augmentation of blood flow elsewhere, and an overall increase in gastric blood flow. Main and Whittle<sup>16</sup> observed reduced basal gastric mucosal blood flow in rats after doses of indomethacin that inhibited prostaglandin formation, while Kaufman<sup>17</sup> found a decrease in basal gastric mucosal blood flow in conscious dogs given indomethacin. Studies in man by Konturek<sup>18</sup> have suggested that indomethacin reduces basal gastric mucosal blood flow. These and other findings suggest that endogenous prostaglandins contribute to the maintenance of basal gastric mucosal blood flow in animals and man.<sup>19</sup>

The incidence of serious side effects with NSAIDs, however, is low considering the large numbers of these drugs prescribed.<sup>20</sup> Since endoscopic studies have shown acute gastric mucosal damage in most subjects during the first week of NSAID administration,<sup>1,21</sup> these rather contradictory observations suggest that tolerance develops during the course of continued NSAID intake. This tolerance or adaptation of the gastric mucosa to a variety of damaging agents has been well documented in animal studies,<sup>22</sup> and more recently with aspirin<sup>23</sup> and indomethacin<sup>24</sup> in man. The mechanisms whereby the mucosa develops tolerance to damage, however, remain uncertain.

The aims of our studies were thus threefold: firstly, to document the morphological changes occurring in gastroduodenal mucosa during 28 days of treatment with the NSAID indomethacin; secondly, to measure mucosal blood flow in the gastric mucosa during this period; and thirdly, to measure gastric mucosal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) metabolism during this 28 day period.

## Methods

### SUBJECTS

Studies were carried out on 14 healthy volunteers (eight men and six women) with a mean age of 23 years (range 19–26). Five volunteers were smokers, smoking less than 20 cigarettes daily, and they did not change their smoking habits during the study. Alcohol was allowed during the study and subjects followed their normal drinking habits (all subjects consumed less than

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80 g of alcohol per week). No subject had a previous history of gastrointestinal disease or had taken aspirin or any other NSAID in the previous three months.

All subjects gave written informed consent to the studies and ethical approval was given by the Salford Area Health Authority Ethical Committee.

#### ENDOSCOPY

Standard upper gastrointestinal endoscopy was performed by one investigator throughout (CJS). A 1% lignocaine hydrochloride throat spray was applied before introduction of the endoscope and no sedation was used. All endoscopies were performed between 11 am and 12 mid-day, subjects having had a very light breakfast at 7 am on the morning of endoscopy.

#### STUDY DESIGN

Subjects underwent endoscopy at entry to the study. Oral indomethacin (50 mg three times daily) was then taken for 28 days continuously. The indomethacin was taken with meals and with a light breakfast at 7 am on the morning of endoscopy. Endoscopy was repeated at 24 hours, seven days, and 28 days while on the indomethacin and was performed between 11 am and 12 noon. At each endoscopy, mucosal damage was graded and mucosal blood flow measured in the fundus and antrum of the stomach using a laser Doppler technique. At the end of each endoscopy three biopsy specimens were taken from normal looking areas of both the antrum and fundus of the stomach. These specimens were immediately placed in polyethylene vials, frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$  for later measurement of PGE<sub>2</sub> metabolism.

Before each endoscopy, subjects were questioned directly about gastrointestinal symptoms and in addition kept a detailed written record of the duration, time of day, and severity of any such symptoms between endoscopies. Ten ml of blood were taken randomly from each subject during the study for later detection of indomethacin to confirm compliance.

Three further subjects were used as controls. These subjects underwent endoscopy with mucosal grading, measurement of mucosal blood flow, and PGE<sub>2</sub> metabolism as above over a 28 day period but received empty indomethacin capsules. The endoscopist was blind to the capsule content.

#### MORPHOLOGY

At endoscopy, gastric and duodenal integrity

TABLE I Variation in mucosal blood flow measured by laser Doppler flowmetry in the antrum of stomach in six subjects measured over 10 seconds

Subject no	Blood flow range at single point (n=3)	Mean (range) blood flow (n=6, arbitrary units)
1	39–42	40 (36–44)
2	46–50	46 (44–48)
3	41–43	39 (37–42)
4	38–43	42 (40–44)
5	46–49	51 (48–52)
6	52–54	50 (46–53)

was graded and recorded according to a standardised scoring system as previously described.<sup>24</sup> The entire stomach and duodenal cap were examined in proximal to distal manner before measurement of blood flow in order to eliminate any errors that might have been caused by a misinterpretation of artifacts from either the endoscope or the laser Doppler probe.

#### MEASUREMENT OF MUCOSAL BLOOD FLOW

Gastric mucosal blood flow was measured using the technique of laser Doppler flowmetry (LDF). The operating principle of LDF has been fully described elsewhere<sup>25</sup> but briefly is based on the principle that light scattered by moving red blood cells undergoes a shift in its frequency, the mean Doppler shift providing an estimate of blood flow. Mucosal blood flow measured by LDF correlates well with flow measured by other methods.<sup>26 27</sup>

In the laser Doppler flowmeter used in the present study (Periflux PF2, Perimed Ltd, Stockholm, Sweden) light from 2 mW He-Ne laser is transmitted down an optical fibre (diameter 0.7 mm) contained in a PF109 endoscopic probe (Perimed Ltd, Sweden) of outside diameter 2.5 mm. The probe is inserted down the biopsy channel of an Olympus Q10 endoscope (Keymed, Southend-on-sea, UK). It became apparent in early experiments that the distance between the probe tip and the end of the endoscope was critical as the magnitude of the laser Doppler readings varied according to this. To obviate this problem the probe was marked in such a way that it always protruded 3 cm from the end of the endoscope. After diffuse scattering of the incident laser light, a portion of the backscattered light is picked up by two further fibres (diameter 0.7 mm) contained in the endoscopic probe and transmitted to the flowmeter. The signal is processed giving a low noise output which corresponds to the tissue blood flow beneath the probe. A continuous recording of blood flow is obtained using a chart recorder with a paper speed of 1 mm/second. Throughout the studies the upper limit of the signal processor was set to 12 kHz with a constant gain of three and a time constant of 1.5 seconds.

Measurements of blood flow were made under direct vision in the gastric fundus and antrum for areas that looked endoscopically normal, the tip of the probe being gently abutted against the mucosa by advancing the whole endoscope and not the probe alone. Particular care was taken not to dimple the mucosa. A valid reading was one where a constant reading was obtained for at least five seconds uninterrupted by any movement artifact of the probe head relative to the tissue or by any loss of optical coupling due to peristalsis. A minimum of three such readings was taken at each site and the mean was calculated and recorded.

Studies on the reproducibility of the laser Doppler technique were performed on six subjects attending for routine endoscopy. These subjects had blood flow measured at six points in the antrum and six in the fundus of the stomach over a 10 second period as described above. In addition, blood flow was measured at one par-

ticular point over 10 seconds on three occasions, withdrawing the probe from the mucosa between measurements. Results are given in Table I and show the good reproducibility of the technique in each subject.

#### PGE<sub>2</sub> METABOLISM

Biopsy specimens were processed and PGE<sub>2</sub> content and degradation rate in mucosal homogenates (calculated as the rate of formation of 13,14-dihydro-15-keto-PGE<sub>2</sub> from radiolabelled PGE<sub>2</sub>) were measured as previously described.<sup>28</sup> Briefly, biopsy specimens were homogenised and filtered through a nylon mesh. The protein content of the homogenate was measured by the method of Lowry *et al*<sup>29</sup> and ranged between 0.1 and 0.4 mg/ml. For the measurement of PGE<sub>2</sub>, a 200 µl portion of the homogenate was immediately added to 3 mol/l citric acid to prevent any possible enzymatic conversion. To this was added 800 µl of standardised buffer containing <sup>3</sup>H-PGE<sub>2</sub> (7000 dpm) as a recovery marker. This was followed by immediate extraction into 3 ml diethyl ether. Degredation was determined by incubating 200 µl of homogenate at 37°C with 800 µl of buffer containing 5 µmol/l PGE<sub>2</sub>, 0.18 µCi <sup>3</sup>H-PGE<sub>2</sub>, and 2.5 mmol/l NAD<sup>+</sup> as a cofactor. Assays were stopped after 30 minutes with 3 mol/l citric acid and were immediately extracted into 3 ml diethyl ether. Blanks were simultaneously prepared in which citric acid was added before the tissue homogenate. All assays and blanks were prepared in duplicate.

Extraction of PGE<sub>2</sub> and the degradation product 13,14-dihydro-15-keto-PGE<sub>2</sub> was by thin layer chromatography. For each assay, 200 µl of the ether phase were plated, dried, and run in the organic phase of ethyl acetate/iso-octane/acetic acid/water (110:50:20:100). For assay of degradation, the region corresponding to 13,14-dihydro-15-keto-PGE<sub>2</sub> was scraped off and counted in phase contrast scintillation fluid. The region corresponding to PGE<sub>2</sub> was similarly scraped into glass vials and the silica washed to elute the PGE<sub>2</sub>, which was measured by radioimmunoassay (Dupont PGE<sub>2</sub> [<sup>125</sup>I] RIA kit). Recovery of PGE<sub>2</sub> was over 80%. Degradation rates were calculated as the difference between the timed assay and the blank value. Results are expressed as pmol PGE<sub>2</sub>/mg protein/minute.

#### STATISTICAL ANALYSIS

Results are calculated as mean (SEM) and differences compared statistically using a Student's *t* test for paired data.

### Results

#### MORPHOLOGY (FIG 1)

After 24 hours' indomethacin, all subjects had evidence of mucosal damage that was similar in severity in both the stomach and the duodenum (mean scores: 1.5 (0.25) in the stomach and 1.0 (0.3) in the duodenum, *n*=14, *p*=0.10 for difference between gastric and duodenal damage (unpaired *t* test)). By day 7 the damage had resolved significantly (mean scores: 0.7 (0.22) in

the stomach and 0.57 (0.2) in the duodenum, *n*=14, *p*<0.01 for improvement in gastric damage and *p*<0.05 for improvement in duodenal damage between 24 hours and day 7). Two subjects with persistent headache and nausea were withdrawn from the study. By day 28, in all but two subjects who had persistent grade 2 gastric or duodenal damage, macroscopic mucosal damage had resolved (*n*=12). No subject experienced gastrointestinal symptoms during the study and indomethacin was detected in the serum of all subjects. At no time was there any mucosal damage in the three control subjects. There was no relation between the endoscopic severity of gastric damage and alcohol intake.

#### BLOOD FLOW (FIG 2)

Fundal mucosal blood flow was significantly higher than that in the antrum (*p*<0.001). Gastric mucosal blood flow was significantly

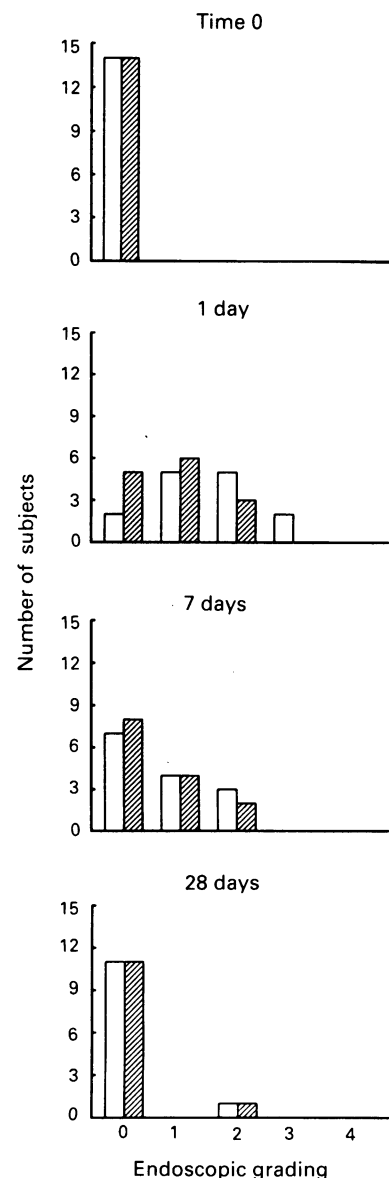


Figure 1: The effect of oral indomethacin (50 mg three times daily) on gastric (open) and duodenal (hatched) morphology over 28 days of continuous administration in 14 healthy volunteers (*n*=12 at 28 days).

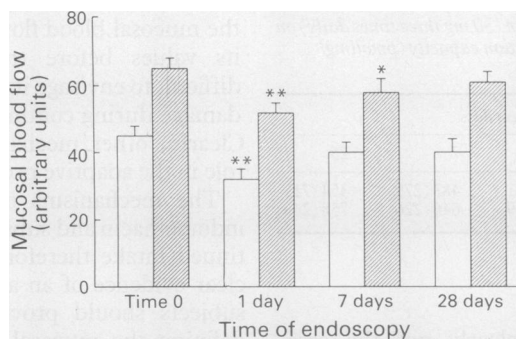


Figure 2: Gastric mucosal blood flow measured in fundus (hatched) and antrum (open) during 28 days' continuous administration of indomethacin (50 mg three times daily) in 14 healthy volunteers ( $n=12$  at 28 days). \* $p<0.05$ , \*\* $p<0.01$  compared with baseline blood flow.

reduced in the fundus ( $p<0.001$ ) and antrum ( $p<0.002$ ) of the stomach after 24 hours' indomethacin intake – the time of maximal mucosal damage. By day 7 blood flow had increased: it was still significantly less than the value before treatment in the fundus ( $p<0.02$ ) but not in the antrum ( $p=0.08$ ). By day 28, the time of endoscopic mucosal recovery, blood flow was not significantly different to that before treatment. There was no change in blood flow in the three control subjects over the 28 days of the study.

To determine the correlation between the severity of mucosal damage and the change in blood flow, it was necessary to regrade the endoscopic damage. This was done by using the original grading criteria but grading damage separately for fundus and antrum on a scale of 0 (normal) to 4 (severe damage ± ulceration). This is because the original grading system did not differentiate between antral and fundal damage. There is a good correlation ( $r=0.76$ ) between the severity of mucosal damage and the percentage change in mucosal blood flow after 24 hours of indomethacin intake, the time of maximal mucosal damage (Fig 3).

#### PGE<sub>2</sub> METABOLISM (FIG 4)

PGE<sub>2</sub> in mucosal homogenate was measured in 10 of our volunteers. Total PGE<sub>2</sub> content was significantly higher in the antrum than in the fundus of the stomach (antrum, mean (SEM): 83 (21) and fundus: 36 (11) pmol/mg of protein,  $n=10$ ,  $p<0.05$  (unpaired  $t$ )). After 24 hours' indomethacin, PGE<sub>2</sub> content was significantly reduced from values before treatment in both the fundus and antrum (antrum: 30 (9) and fundus: 21 (7) pmol/mg of protein,  $n=10$ ,  $p<0.01$  for reduction in both fundus and antrum). By day 7, when endoscopic damage was improving, PGE<sub>2</sub> content remained significantly reduced compared with values before treatment (mean (SEM) 43 (13) in antrum and 24 (8) in fundus,  $n=10$ ,  $p<0.01$  for both fundus and antrum). In spite of mucosal recovery by day 28, PGE<sub>2</sub> content remained significantly reduced (36 (16) in the antrum and 22 (12) in the fundus,  $n=10$ ,  $p<0.01$  for difference between fundal and antral values between values before treatment and 28 days). There was no correlation between the fall in PGE<sub>2</sub> content and the severity of the mucosal damage after 24 hours' indomethacin, the time of maximal damage ( $r=-0.34$ ). In the three control

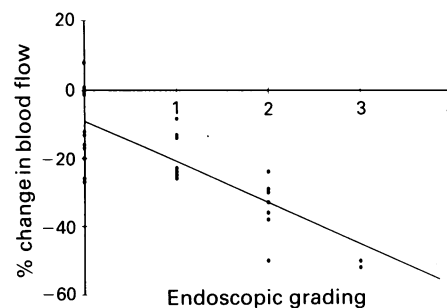


Figure 3: Correlation between severity of mucosal damage in fundus and antrum of stomach and % change in mucosal blood flow after one day of indomethacin treatment (50 mg three times daily) in 14 healthy volunteers.  $r=0.76$ .

subjects, there was no change in the PGE<sub>2</sub> content of mucosal homogenates during the study period.

During the 28 days of the study there was no significant change in mucosal PGE<sub>2</sub> degradation capacity (Table II).

#### Discussion

Our results confirm previous findings<sup>24</sup> that indomethacin (50 mg three times daily) produces acute gastroduodenal damage in all subjects and that with continued administration endoscopic damage resolves. This adaptation to damage appears at day 7 and is complete by day 28, although not all subjects exhibit complete resolution of injury. Two of our volunteers had persistent grade 2 mucosal damage at day 28, although in both of these it was less than that after 24 hours of drug administration. It is unclear why mucosal damage failed to resolve in these two subjects, both were non-smokers, drank less than 40 g of alcohol per day, and had no other features to differentiate them from other volunteers.

Adaptation includes healing of the acute injury and enhancement of mucosal defence to prevent further damage during continued exposure to the damaging agent and has been well described in a variety of animal models and to a range of injurious agents.<sup>22</sup> This phenomenon was initially attributed to increased generation of 'protective' mucosal prostaglandins,<sup>22</sup> although recently this has been questioned.<sup>30</sup>

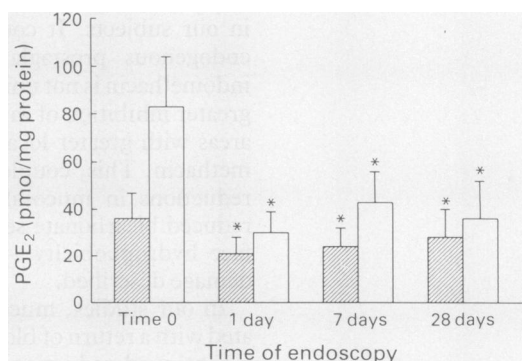


Figure 4: Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) content of biopsy specimen homogenate from the fundus (hatched) and antrum (open) of the stomach during 28 days' continuous administration of indomethacin (50 mg three times daily) in 10 healthy volunteers. \* $p<0.01$  compared with baseline values.

TABLE II Effect of indomethacin (50 mg three times daily) on gastric prostaglandin E<sub>2</sub> degradation capacity (pmol/mg/minute). (Values, mean (SEM))

	Time 0	Treatment days		
		1	7	28
Fundus	325 (45)	346 (86)	483 (279)	354 (71)
Antrum	511 (122)	543 (119)	646 (226)	758 (263)

n=10.

With NSAIDs this is clearly not the case. Because of the cyclo-oxygenase inhibitory action of these drugs (responsible for their anti-inflammatory properties) mucosal prostaglandins are reduced, as shown in our results and also those of others.<sup>31-33</sup> During treatment with indomethacin, PGE<sub>2</sub> remains significantly reduced from values beforehand, despite endoscopic evidence of mucosal recovery. It would therefore seem that adaptation occurs independently of mucosal PGE<sub>2</sub> metabolism. We did not measure PGE<sub>2</sub> synthesis directly but clearly the reduction in PGE<sub>2</sub> content of mucosal homogenates with indomethacin is a result of reduced synthesis, as PGE<sub>2</sub> degradation capacity was not affected by indomethacin in our studies. The finding of a lack of correlation between the magnitude of the reduction in PGE<sub>2</sub> and mucosal damage, coupled with the data showing mucosal recovery despite continued reduction in PGE<sub>2</sub> provides further evidence of a cause and effect relationship between inhibition of mucosal prostaglandins by NSAIDs and gastric mucosal damage.<sup>31-33</sup>

How indomethacin causes the initial damage and the subsequent adaptation with continued intake remain unknown. Previous work involving histological studies has suggested that indomethacin induced injury is focal, with little in the way of diffuse change.<sup>24,34</sup> We have shown a reduction in the gastric mucosal blood flow with indomethacin, which confirms earlier findings by Konturek *et al.*<sup>18</sup> In addition, we have found a good correlation between the severity of the mucosal damage and the magnitude of the reduction in mucosal blood flow. It has been suggested that endogenous prostaglandins contribute to the maintenance of basal gastric mucosal blood flow in animals and man.<sup>19</sup> Thus, reduction of endogenous prostaglandins by indomethacin's cyclo-oxygenase inhibitory action may explain the reduction in gastric mucosal blood flow seen in our subjects. It could be hypothesised that endogenous prostaglandin reduction by oral indomethacin is not uniform, possibly because of greater inhibition of mucosal cyclo-oxygenase in areas with greater local concentrations of indomethacin. This, coupled with more generalised reductions in mucosal defence – for example reduced bicarbonate secretion and reduced surface hydrophobicity – may result in the focal damage described.

In our studies, mucosal recovery was associated with a return of blood flow to normal despite a continued reduction in PGE<sub>2</sub>. How mucosal blood flow regulates itself in this situation remains unclear but an increase in blood flow would enhance mucosal defence and repair of injury and contribute to the adaptive process. As

the mucosal blood flow was not increased above its values before treatment, however, it is difficult to envisage its role in preventing further damage during continued exposure to the drug. Clearly, other mechanisms play an important role in the adaptive process.

The mechanisms of the initial damage by indomethacin and subsequent adaptation to continued intake therefore remain unknown. The clear evidence of an adaptive response in most subjects should provide further stimulus for defining the mucosal mechanisms responsible for it.

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