Enantiomers of flurbiprofen can distinguish key pathophysiological steps of NSAID enteropathy in the rat

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Abstract

Background—Non-steroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal damage by a non-prostaglandin (PG) dependent “topical” action and by inhibiting cyclooxygenase.

Aims—to discriminate between these two effects by studying some key pathophysiological steps in NSAID enteropathy following administration of (R)- and (S)-flurbiprofen, the racemic mixture, and an uncoupler, dinitrophenol.

Methods—The effects of dinitrophenol, racemic, (R)-, and (S)-flurbiprofen on mitochondria were assessed in vitro and on key pathophysiological features of small intestinal damage in vivo (ultrastructure by electron microscopy, mucosal prostanooid concentrations, intestinal permeability, inflammation, and ulcer count) in rats.

Results—All the drugs uncoupled mitochondrial oxidative phosphorylation in vitro, caused mitochondrial damage in vivo, and increased intestinal permeability. Dinitrophenol and (R)-flurbiprofen caused no significant decreases in mucosal prostanooid concentrations (apart from a decrease in thromboxane (TX) B, concentrations following (R)-flurbiprofen while racemic and (S)-flurbiprofen reduced mucosal prostanooids significantly (PGF, TXB, and 6-keto-PGF, concentrations by 73–95%). Intestinal inflammation was significantly greater following administration of (S)-flurbiprofen and racemate than with dinitrophenol and (R)-flurbiprofen. No small intestinal ulcers were found following dinitrophenol or (R)-flurbiprofen while both racemic and (S)-flurbiprofen caused numerous ulcers.

Conclusions—Dinitrophenol and (R)-flurbiprofen showed similarities in their actions to uncouple mitochondrial oxidative phosphorylation in vitro, alter mitochondrial morphology in vivo, increase intestinal permeability, and cause mild inflammation without ulcers. Concurrent severe decreases in mucosal prostanooids seem to be the driving force for the development of severe inflammation and ulcers.

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Keywords: non-steroidal anti-inflammatory drug; enteropathy; flurbiprofen

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for symptomatic relief of pain and inflammation, but there is concern over their gastrointestinal side effects which affect both the gastroduodenal and the small intestinal mucosa. It is widely believed that this toxicity is a consequence of inhibition of cyclooxygenase 1 (COX-1) while the analgesic and anti-inflammatory therapeutic effects are due to COX-2 inhibition. Selective COX-2 inhibition is therefore thought to be desirable therapeutically. However most conventional NSAIDs have equal or greater, depending on the assay system, in vitro inhibitor activity for COX-1 than COX-2 and when given at full therapeutic doses, inhibit both enzymes.

There is, however, circumstantial evidence that COX-1 inhibition by itself may not be the only factor in the development of the gastrointestinal damage. Firstly, much larger doses of aspirin (aspirin doses of 1024 mg/kg produce small bowel lesions in about 30% of fasted animals) are required to cause consistent small bowel damage than the dose required for effective COX inhibition in the rat. Also, despite comparable and effective inhibition of gastric COX activities induced by rectal administration of a variety of NSAIDs in rats some fail to cause gastric damage. Secondly, there is no clear correlation between inhibition of COX and intestinal damage, unlike the anti-inflammatory actions of NSAIDs. Thirdly, COX-1 knockout mice have less than 1% of normal mucosal prostaglandin concentrations yet do not spontaneously develop gastrointestinal lesions.

Another aspect of the gastrointestinal damage by NSAIDs relates to the “topical” effect(s) of the drugs. This term is used for the non-prostaglandin mediated effect of NSAIDs which occurs when high concentrations of the drugs are in contact with the mucosa, following ingestion or via biliary excretion, or during drug absorption. The topical effect can be documented in man by endoscopy where short term tolerability to NSAIDs can be enhanced by changing the drug formulation or route of administration (enteric coating, NSAID-phospholipid formulation, rectal administration, etc.). abolishing gastric acid secretion, and by rendering some NSAIDs non-acidic by forming an ester linkage to a moiety that contains or does not contain nitric oxide.

The “ion trapping hypothesis” provides a basis for the “topical” action of NSAIDs but...
not the mechanism.\textsuperscript{24, 25} One suggestion is that uncoupling of oxidative phosphorylation may be the biochemical mechanism underlying the “topical” toxicity of NSAIDs.\textsuperscript{26–28} Most conventional NSAIDs and acidic pro-NSAIDs (fenbufen and sulindac) uncouple mitochondrial oxidative phosphorylation.\textsuperscript{29, 30} Furthermore, there are some indications that non-acidic NSAIDs (such as nitric oxide) and COX inhibition (local and systemic action leading to a decrease in mucosal prostaglandin production).\textsuperscript{31, 32} The involvement of COX-2 in inflammation (n=8); and (c) the susceptibility of NSAID damage to the chemiosmotic coupling mechanism between electron transfer and oxidative phosphorylation.\textsuperscript{33} The animals were killed by cervical dislocation and placed in ice cold homogenising solution (75 mM sucrose, 225 mM mannitol, 10 mM 4-morpholine-propanesulphonic acid (MOPS), 1 mM EDTA, and 5 mg/ml bovine serum albumin (BSA) at pH 7.4), cut finely, and washed twice with homogenising solution to remove excess blood. The liver was then homogenised in a Potter-Elvjehm homogeniser. The last centrifugation step was repeated and the mitochondrial pellet was removed and washed with ice cold homogenising buffer. The isolation procedure takes some 45 minutes with the homogenate being kept between 0 and 4°C. The mitochondrial protein concentration was determined using Pierce’s BCA protein assay kit (Pierce, Illinois, USA), with BSA as the standard protein. The homogenate was then centrifuged at 10 000 g for another 10 minutes, after which the mitochondrial pellet was removed and resuspended in 40 ml homogenising solution. The last centrifugation step was repeated and the mitochondrial pellet resuspended in 1–2 ml of homogenising buffer. The isolation procedure takes some 45 minutes with the homogenate being kept between 0 and 4°C. The mitochondrial protein concentration was determined using Pierce’s BCA protein assay kit (Pierce, Illinois, USA), with BSA as the standard protein. Materials and methods

ANIMALS AND DRUG DOSES

Separate groups of male Sprague-Dawley rats, weighing 200–250 g, were used to determine: (a) the effect of the drugs on mitochondria in vitro (six experiments at each concentration) and in vivo (n=4); (b) mucosal prostaglandin (PG) and thromboxane (TX) concentrations (n=8); (c) intestinal permeability (n=8); (d) inflammation (n=8); and (e) 25 hour and seven day ulcer count (n=8).

For the in vivo experiments animals received single doses of 0.5 ml of 3.0 mM DNP (higher doses were associated with appreciable mortality), racemic, (R)-, or (S)-flurbiprofen by gavage (10 mg/kg each: 1 ml of 12 mM solution, with a final concentration of dimethyl sulphoxide (DMSO) less than 5%, to a 250 mg rat). The enantiomers were obtained from Boots Pharmaceutical Company (Nottingham, UK) and DNP and racemic flurbiprofen from Sigma Chemical Company (Dorset, UK). The same dose of racemic, (R)-, and (S)-flurbiprofen was given (mg/kg) as we sought to achieve a similar topical effect with all drug forms.

The (R)- and (S)-flurbiprofen were 99.7% and 99.6% enantiomerically pure, respectively, and the racemate was a 1:1 mixture of each. Animals given the vehicle (1 ml of a 5% solution of DMSO) acted as controls.

MITOCHONDRIAL EXPERIMENTS

In vitro Preparation of mitochondria—Coupled mitochondria were obtained as previously described.\textsuperscript{34} The validity of using rat liver mitochondria as surrogate for monitoring chemiosmotic uncoupling in the intestine is consistent with the accepted universality of the chemiosmotic coupling mechanism between electron transfer and oxidative phosphorylation.\textsuperscript{35} The animals were killed by cervical dislocation and the liver rapidly dissected and placed in ice cold homogenising solution (75 mM sucrose, 225 mM mannitol, 10 mM 4-morpholine-propanesulphonic acid (MOPS), 1 mM EDTA, and 5 mg/ml bovine serum albumin (BSA) at pH 7.4), cut finely into approximately 1 cm² pieces with scissors, and washed twice with homogenising solution to remove excess blood. The liver was then homogenised in the same solution and homogenised in a Potter-Elvjehm homogeniser by six strokes of a rotating Teflon pestle. The homogenate was then centrifuged at 500 g for 10 minutes to remove excess blood, nuclei, and cell debris. The supernatant was centrifuged at 11 000 g for another 10 minutes after which the mitochondrial pellet was removed and resuspended in 40 ml homogenising solution. The last centrifugation step was repeated and the mitochondrial pellet resuspended in 1–2 ml of homogenising buffer. The isolation procedure takes some 45 minutes with the homogenate being kept between 0 and 4°C. The mitochondrial protein concentration was determined using Pierce’s BCA protein assay kit (Pierce, Illinois, USA), with BSA as the standard protein. Measurement of oxygen consumption—Oxygen consumption, phosphate to oxygen utilisation ratio (P/O ratio) and respiratory control ratios were measured using a Clarke type oxygen electrode (Rank Brothers, Cambridge) as described by Chance and Williams.\textsuperscript{36} The ele-
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or more severe evidence of uncoupling,12 42 drial changes. Mitochondria may show subtle changes of mitochondria (PGE) were only accepted if this assessment was descriptive and quantitative.

The drugs were dissolved in DMSO, but the final concentration of DMSO in the chamber never exceeded 5% vol/vol. Control experiments used solvents only.

In vivo

The in vivo effects of DNP, the individual enantiomers, and racemic flurbiprofen on mitochondrial morphology were assessed by electron microscopy. The drugs were given by gastric gavage after an overnight fast and animals remained fasting. Two hours later an abdominal incision was made, under anaesthesia (Hypnoval-Hypnorm), the stomach was opened, and a catheter placed in the second part of the duodenum. The whole of the small intestine was flushed, avoiding distension, with a solution of gluteraldehyde (3.0% vol/vol) in 0.1 M sodium phosphate buffer, pH 7.4–7.4. A 1 cm length of jejunum (20 cm distal to the ligament of Trietz) was then placed in glutaraldehyde for three days and processed for electron microscopy. Samples were cut ultrathin with an Ultratome-Richart Ultracut-E and examined with a Joel 1200 cm electron microscope in transmission mode. Regions of well oriented cuts that had at least 10 consecutive enterocytes for analysis were examined. Assessment was descriptive and quantitative. For the latter 10 enterocytes were examined from each animal with reference to mitochondrial changes. Mitochondria may show subtle or more severe evidence of uncoupling,13 14 with a spectrum of distension, ballooning, and vacuolisation with disrupted outer and inner mitochondrial membranes. For the purpose of this study the more subtle criteria for uncoupling (elongation associated with mild swelling of mitochondria) were only accepted if this occurred in a cluster of five or more adjacent mitochondria. The results are expressed as percentage of cells containing abnormal mitochondria to the nearest decade. All samples were coded and the morphological assessment was performed with no knowledge of treatment.

PROSTANOID DETERMINATION

Extraction

Animals were fasted overnight and throughout, but had access to water. At 8.00 am they received the drugs; five hours later they were stunned by a blow to the back of the head and killed. A part of the small bowel, approximately 20 cm distal to the ligament of Trietz, was snap frozen and removed for prostanooid extraction and quantification. This time point had been decided on from previous studies using indomethacin and other NSAIDs43 and preliminary studies with the isomers of flurbiprofen where intestinal prostanooids were measured one, five, and 24 hours after drug administration. The one and five hour results show comparable decreases in mucosal prostanooid concentrations, but the five hour data give more consistent results. There is variable, but substantial recovery at 24 hours (data not shown).

Pieces were thawed (kept at 0°C) and individual segments homogenised with a cold Silverson homogeniser in 2 ml ice cold acidified methanol/water (1:1 vol/vol adjusted to pH 3 with formic acid) for 30 seconds. The homogenate was transferred to an extraction tube and the homogenate head was washed with a further 2 ml of acidified cold methanol/water which was added to the extraction tube. The homogenate was extracted with chloroform (8 ml) and the mixture centrifuged at 2700 g for 20 minutes at room temperature. The separated lower chloroform layer was evaporated to dryness using a Gyravap at 37°C and stored at −20°C until analysis when the extract was reconstituted in 2 ml of ethanol/phosphate buffer saline (1:9 vol/vol) at the time of radioimmunoassay. The extraction efficacy of this procedure is in excess of 90% for each of the prostanooids.

Radioimmunoassay

Eicosanoids were determined in duplicate by radioimmunoassay using suitable dilutions of prostaglandin (PG) E, thromboxane (TX) B, or 6-keto-PGF antiserum (Sigma Chemical Company, Poole, Dorset), titrated PGE, or TXB, or 6-keto-PGF antiserum standard (Amersham International, Amersham).44 45 Assay sensitivities were 10 pg, and the intra-assay and interassay coefficients of variation ranged from 2 to 5% depending on the prostanooid measured. As the PGE antisera did not distinguish between PGE, or PGE, the results are expressed as PGE.

The immunological cross-reaction specificities of the antisera were:

- PGE antisera: PGE, 100%; PGE, 100%; PGE, 0.35%; PGE, 0.3%; PGA, 0.4%; PGB, 0.3%.
- TXB antisera: TXB, 100%; PGF, <0.1%; PGE, <0.01%; 6-keto-PGF, <0.01%; PGA, <0.1%; PGB, <0.1%.
- 6-keto-PGF antisera: 6-keto-PGF, 100%; PGE, 4.0%; PGE, 23.0%; PGE, 7.0%; PGA, 0.2%; PGB, 0.6%; TXB, 0.6%.

Scintillation counts were measured using a Packard 2200 CA fitted with a Securia program.

INTESTINAL PERMEABILITY

After an overnight fast and 20 hours after receiving the drugs, 10 µCi (approximately 50 nmol) of chromium-51 labelled EDTA (Amersham International) in a volume of 0.5 ml in
water was administered by gavage (without anesthesia), followed by 1 ml of water. The animals were placed in individual metabolic cages for five hours for collection of urine and had access to water. Laparotomy was then performed under anesthesia and the bladder emptied by puncture. The total five hour radioactivity excreted in the urine was determined, together with that of standards, in a Wallac 1284 gamma counter for five minutes; this gives a minimal detectable activity of less than 0.01% of the administered dose.  

GRANULOCYTE MARKER PROTEIN

Granulocyte marker protein (GMP) was determined from daily faecal samples. Collections were made for three days prior to gavage with the drugs. Faecal collection was continued for a further seven days after drug administration.

The stools were stored at −20°C and thawed at the time of assay. Aliquots (1 g) were suspended in 4 ml of faecal extraction buffer which was homogenised, then centrifuged at 45 000 g for 20 minutes at 4°C. The supernatant was used to determine the granulocyte marker protein concentrations by enzyme linked immunosorbent assay (ELISA) as described previously. Microtitre plates were coated by adding 200 µl of an IgG fraction of rabbit anti-GMP diluted 1/1000 in phosphate buffered saline (PBS) to each well. The plates were then covered with Mylar foil and stored at 4°C until required. Granulocyte marker standards, ranging from 2.34 to 75 ng/ml, were prepared by diluting purified granulocyte marker in the assay buffer. The intra-assay and interassay coefficients of variation are 13% and 10%, respectively. The granulocyte marker protein is stable and not significantly degraded by bacteria for at least a week at room temperature.

MACROSCOPIC DAMAGE

At 25 hours the anaesthetised animals underwent laparotomy and the intestinal mucosa was exposed by a cut through the antimesenteric side of the intestine. The number and size of ulcers (pointed if less than 5 mm or longitudinal if more than 5 mm) were recorded for each animal. The assessor was not aware of the treatments.

STATISTICAL ANALYSIS

The SYSTAT statistical package was used for calculations. Results are expressed as mean (SE). Statistical differences between groups were assessed by the non-parametric Mann-Whitney test and differences in sequential studies (GMP excretion) by the paired Student’s t test using Bonferroni’s correction.

Results

MITOCHONDRIAL FUNCTION

Figure 1 shows that DNP was an effective uncoupler of oxidative phosphorylation in vitro. Individual enantiomers and racemic flurbiprofen increased mitochondrial respiration by two- to threefold from the baseline concentration (p<0.01) at concentrations between 0.25 and 1.0 mM, but at no concentration was there a significant difference between racemic, (R)-, and (S)-flurbiprofen. Stimulation of respiration was followed by a progressive decrease in oxygen consumption with further increases in drug concentration; this is indicative of inhibition of electron transport along the
respiratory chain. This pattern of respiratory response to increasing concentrations of an agent is characteristic of the group of drugs known as respiratory uncouplers.49

No electron microscopy abnormalities were detected in the control animals and their quantitative mitochondrial score was 0–5%. By comparison the quantitative mitochondrial damage score was 30, 30, 30, and 40% for DNP; 40, 60, 60, and 90% for (R)-flurbiprofen; 50, 50, 50, and 60% for (S)-flurbiprofen; and 20, 60, 60, and 60% for the racemate. All differed significantly (p<0.01) from control, but there was no significant difference (p>0.05) among the flurbiprofen treated groups.

INTESTINAL PROSTANOID CONCENTRATIONS

Figure 2 shows the mucosal concentrations of PGE, TXB₂, and 6-keto-PGF₁α from the different groups of animals and the percentage change from control concentrations five hours after their administration. DNP and (R)-flurbiprofen did not alter prostanoid concentrations significantly (p>0.1) apart from TXB₂ concentrations following (R)-flurbiprofen which were significantly (p<0.05) decreased from control concentrations. There were no significant differences between (S)-flurbiprofen and the racemate; both significantly (p<0.01) decreased mucosal PGE by 90–95%, TBX₂ by 78–82%, and 6-keto-PGF₁α by 73%–79% from controls. Prostanoid concentrations following (R)-flurbiprofen were significantly higher (p<0.05) than those following (S)-flurbiprofen and the racemate.

INTESTINAL PERMEABILITY

Figure 3 shows that intestinal permeability to ⁵¹Cr-EDTA was significantly (p<0.01) increased at 1–6 and 20–25 hours after administration of DNP. Similarly the individual enantiomers and racemic flurbiprofen increased intestinal permeability significantly (p<0.01) at both time points. There was no significant (p>0.05) difference in the urinary excretion of ⁵¹Cr-EDTA between the groups 1–6 hours after administration of the drugs or at 20–25 hours.

Permeability returned towards the predose values by day 7 following the drugs, reaching control concentrations in the case of DNP and racemic flurbiprofen.

Figure 3 Intestinal permeability following DNP, racemic, (R)-, and (S)-flurbiprofen. **p<0.01 (differences from control; Mann-Whitney).

Figure 4 Effects of racemic, (R)-, and (S)-flurbiprofen on faecal excretion of granulocyte marker protein (GMP). Arrows indicate when the drugs were administered. **Differed significantly from baseline values (p<0.01; Student’s t test with Bonferroni’s correction).
INTESTINAL INFLAMMATION

Figure 4 shows that the faecal excretion of the granulocyte marker protein remained low after administration of solvent and increased significantly (p<0.01) on the first and/or second day following DNP, racemic, (R)-, and (S)-flurbiprofen administration. In all cases the increased excretion was transient with a return towards control values within two days. The intensity of the inflammation was significantly (p<0.01) greater following the administration of racemic and (S)-flurbiprofen than DNP or (R)-flurbiprofen as assessed by the cumulative excretion of the granulocyte marker protein (less the mean baseline excretion) on days 1 and 2 after administration of the drug.

INTESTINAL ULCERATION

No ulcers were seen at 24 hours or at seven days after vehicle, DNP, or (R)-flurbiprofen. By contrast the groups gavaged with racemic or (S)-flurbiprofen developed both pointed (mean 12 (2) and 12 (1), respectively) and longitudinal (mean 10 (1) and 10 (3), respectively) small intestinal ulcers at 24 hours. Seven days post-dose there were on average 9 (2) and 3 (1) longitudinal ulcers in the (S)-flurbiprofen and racemate groups, respectively, but no pointed ulcers.

Discussion

Previous in vitro studies indicated that increasing concentrations of acidic NSAIDs uncouple mitochondrial oxidative phosphorylation and then inhibit mitochondrial respiration, a response characteristic of the so called inhibitory uncouplers.59 The lack of stereoselectivity in mitochondrial damage in the present study, which has also been shown with other chiral compounds,51 suggests the independence of uncoupling of mitochondrial oxidative phosphorylation from COX inhibition. Although DNP increased mitochondrial respiration to the greatest extent and at a lower concentration than racemic, (R)-, or (S)-flurbiprofen in vitro, it was associated with significantly less in vivo changes to mitochondria. There are a number of possible reasons for this apparent discrepancy. The drugs were given in different quantities (0.5 ml of 3 mM DNP and 1 ml of 12 mM flurbiprofen) and there may be differences in pharmacokinetic factors (rate and site of absorption) between DNP and flurbiprofen due to their different physicochemical properties.

The in vivo electron microscopy changes of mitochondria following DNP and flurbiprofen were nevertheless almost identical to those reported to be caused by aspirin in mouse and canine stomach,52 53 and alcohol and bile acids, both of which are potent uncouplers of oxidative phosphorylation, in mouse stomach.54 Whether the concentrations required for uncoupling to occur in vitro (0.03–1.0 mmol)35 36 37 are achieved in vivo within the intestinal mucosa following therapeutic oral administration of NSAIDs has not been determined directly, but a mechanism for NSAID accumulation within epithelial cells during absorption has been proposed previously by the “ion trapping” hypothesis.55 DNP and (R)-flurbiprofen did not alter mucosal prostaglandins appreciably from control concentrations, apart from the decrease in TXB2, concentrations following the latter. The stereoselective inhibition of COX by (R)-flurbiprofen in vivo is somewhat less impressive than that observed in vitro,56 58 but is nevertheless in agreement with some ex vivo rat and human data.59 60 The reason for the difference between the in vitro and in vivo stereoselectivity of (R)-flurbiprofen may be due to the slight chiral inversion of the (R)- to the (S)-enantiomer in vivo57 or indeed the minute quantities of (S)-flurbiprofen (0.3%) in the (R)-flurbiprofen preparation.58 In contrast racemic and (S)-flurbiprofen decreased prostaglandin concentrations significantly and equally despite the fact that the latter only contained half as much of the drug active for COX inhibition. The explanation for this may be our use of the high doses of flurbiprofen in these experiments, conventionally used for producing consistent small intestinal damage in the rat, that are many times higher than that required for effective COX inhibition (ceiling effect).

The five hour urinary excretion of 51Cr-EDTA following gavage is widely used as a marker of the integrity of the gastrointestinal mucosa from the stomach to caecum.61 62 63 It seems likely that it is the small intestine which is the main determinant of the five hour urine excretion of 51Cr-EDTA in healthy64 and diseased rats.65 66

Racemic, (R)-, and (S)-flurbiprofen all increased intestinal permeability to a similar degree, in agreement with that previously described with enantiomers of etodolac,66 flurbiprofen, ketoprofen, and ibuprofen, but had differential effects on mucosal prostaglandins. Furthermore, the effect of DNP on intestinal permeability conforms to the findings of increased paracellular permeability in human epithelial monolayers after application of agents that similarly interfere with ATP production.67 Collectively this suggests that the increase in intestinal permeability following flurbiprofen is a consequence of uncoupling (topical effect) rather than the consequence of a decrease in mucosal prostaglandins, as suggested directly and indirectly by a number of studies.11 12 16 23 24 25

The immediate consequence of increased intestinal permeability is uncertain, but we have suggested that it may be a central mechanism in the development of intestinal inflammation in man, including NSAID enteropathy.71 72 The faecal excretion of the granulocyte marker protein has previously been validated73 as a marker of intestinal inflammation. The intensity of the inflammation following DNP and (R)-flurbiprofen was comparable and significantly less than that associated with the (S)-enantiomer and racemate. If the inflammation is a response to the increase in intestinal permeability, this implies that a concomitant decrease in physiological
mucosal prostaglandin concentrations may be associated with an increased intensity of the inflammatory response. Furthermore, as DNP and \((-\)-flurbiprofen did not cause ulcers, while increasing intestinal permeability to a similar extent to that caused by \((S)-\)flurbiprofen and racemate, it seems that a significant decrease in mucosal prostaglandins may be the driving force in the development of ulcers; this observation may relate to the known effects of prostaglandins on the microcirculation. 8 75 79

In summary, our main aim was to exploit the different properties of the \((R)-\) and \((S)-\) enantiomers of flurbiprofen to dissociate their uncoupling topical effect from their effect on COX and to compare the effect of the \((R)-\)enantiomer with that of the uncoupler DNP. The results show that racemic and \((S)-\)flurbiprofen caused similar pathophysiological changes to other conventional NSAIDs in respect of uncoupling in vitro and in vivo, and intestinal permeability, prostasoid, inflammatory, and ulcerative changes. 22 32 \((R)-\)flurbiprofen had a similar effect on mitochondria and intestinal permeability, but a significantly smaller effect on mucosal prostasoids and intestinal inflammation; it was not associated with small bowel ulcers. By comparison there was a close similarity between the changes induced by \((R)-\)flurbiprofen and those of DNP, except that the latter had no significant effect on mucosal prostasoids. These findings conform to the suggestion 22 32 30 that the mitochondrial effects of NSAIDs may lead to increased intestinal permeability and low grade inflammation. Profound decreases in mucosal prostasoids seem to be instrumental in increasing the severity of the intestinal inflammation and in the development of ulcers. The implications are that both actions (uncoupling and inhibition of COX) are important in the pathogenesis of flurbiprofen induced intestinal injury.

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