Selective inhibition of fatty acid oxidation in colonocytes by ibuprofen: a cause of colitis?

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Abstract
Ibuprofen is associated with initiation or exacerbation of ulcerative colitis. As ibuprofen selectively inhibited fatty acid oxidation in the liver or caused mitochondrial damage in intestinal cells, its effect on substrate oxidation by isolated colonocytes of man and rat was examined. Ibuprofen dose dependently (2-0-7.5 mmol/l) and selectively inhibited \(^{14}\text{CO}_2\) production from labelled n-butyrate in colonocytes from the proximal and distal human colon (n=12, p<0.001). Glucose oxidation was either unaltered or increased. Because short chain fatty acid oxidation is the main source of acetyl-CoA for long chain fatty acid synthesis, the inhibition of prostaglandin synthesis by ibuprofen in the colonic mucosa could also occur at this level. Because the concentrations of ibuprofen that can be attained in the human colon are not known, conclusions drawn from current dosages are tentative. The inhibition of fatty acid oxidation by ibuprofen may be biochemically implicated in the initiation and exacerbation of ulcerative colitis, manifestation of which would depend on the ibuprofen concentrations reached in the colon.

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Keywords: ibuprofen, colitis, colonocytes, fatty acid oxidation.

Non steroidal anti-inflammatory drugs (NSAIDs) can have deleterious effects on the intestinal mucosa. In the colon, these drugs can produce colitis de novo or cause exacerbation of quiescent ulcerative colitis.\(^1\)\(^-\)\(^3\) NSAIDs produce permeability changes in the colonic mucosa independent of colitis,\(^4\) or cause colonic bleeding\(^5\) with or without strictureting and diaphragm formation.\(^6\)\(^-\)\(^7\) Even though the inhibition of cyclo-oxygenases and therefore prostaglandin production has been invoked as a causative factor of damage,\(^1\)\(^3\) there also seems a lack of correlation between reduced mucosal synthesis of prostaglandins and the degree of mucosal injury.\(^8\)

The epithelial barrier of the colonic mucosa in health is maintained by nutrition from the lumen through n-butyrate\(^6\)\(^-\)\(^11\) derived from bacterial fermentation of starches. Fatty acid oxidation of n-butyrate in colonocytes governs the supply of acetyl-CoA for oxidative and synthetic functions of epithelial cells, such as lipogenesis,\(^12\) mucus synthesis, and processes of detoxification.\(^13\) Inhibition of n-butyrate oxidation is associated with formation of ulcerative colitis\(^14\)\(^-\)\(^16\) and in the defunctioned colon low concentrations of n-butyrate result in diversion colitis\(^17\) which is remedied by re-introduction of luminal short chain fatty acids (SCFAs).\(^18\)

Ibuprofen inhibits SCFA oxidation in isolated mitochondria of mouse liver,\(^19\) an action which in whole animal studies\(^20\)\(^-\)\(^28\) was confirmed to be selective for SCFA and long chain fatty acid oxidation, without affecting glucose oxidation. As NSAIDs may uncouple oxidative phosphorylation in mitochondria and reduce adenosine triphosphate (ATP) production\(^21\)\(^-\)\(^24\) it remained unclear whether the effect of ibuprofen on the colonic mucosa was due to a selective action on fatty acid oxidation or to generalised mitochondrial damage. Because ibuprofen produces colitis\(^1\)\(^-\)\(^28\) independent of bacterial activity,\(^29\) the aim of the present study was to establish the action of ibuprofen on fatty acid and glucose oxidation in isolated colonocytes of healthy rat and man. The effect of ibuprofen was studied with a view to comparing changes observed in healthy colonocytes with those observations made in colonocytes affected by ulcerative colitis.\(^14\)\(^-\)\(^16\)

Methods

Tissue collection and cell preparation
Colonocytes were prepared from female, Sprague-Dawley rats\(^30\) weighing between 130 and 200 g. Animals were bred in the animal houses of the University of Adelaide, kept on a timed cycle, and used in the fed state, as this produced the optimal yield of cells. Animals were killed by stunning/cervical fracture and the colon was removed and flushed clear of luminal contents with water at 22°C.

Human colonocytes were prepared from unaffected segments of colon obtained from surgical specimens of patients undergoing colectomy for colorectal cancer. The warm ischaemia time of the colectomy specimens was less than three minutes. The colonic specimen was washed clear of debris with water, cut open, and pinned to assist removal of mucosal strips from the muscularis propria with scissors. Strips were taken at least 10 cm from tumour tissue.

Isolated colonocytes were prepared and tested for viability as previously described.\(^30\) Aliquots of 1 ml of cells were used for experimental procedures.

Reagents and preparation
Sodium n-butyrate and D-glucose were obtained from BDH (Melbourne, Australia),
n-[1-14C] butyrate from DuPont NEN Research (North Sydney, Australia), and [6-14C] glucose was purchased from Amersham (Sydney, Australia). Ibuprofen as a 50%/50% mixture of S(+-)-ibuprofen and R(-)-ibuprofen was obtained from the Department of Clinical Pharmacology, Queen Elizabeth Hospital, Woodville, Australia, and was originally a gift from the Boots Company PLC (Nottingham, England). Ibuprofen, 2.0-7.5 mmol/l, was dissolved in 0.1 M NaOH followed by addition of HCl to bring the pH back to 7.4. Enzymes and co-factors for enzymatic estimation of metabolites were obtained from Boehringer Corporation (North Ryde, Australia) and Sigma Chemical Co (St Louis, MO, USA).

**TABLE 1 Acetocetate and lactate production by isolated rat colonocytes in the presence of glucose or n-butyrate with 5-ASA or ibuprofen. (Values, mean (SEM); number of cultures in brackets)**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Drug and dose (mmol/l)</th>
<th>n-butyrate (5 mmol/l)</th>
<th>D-glucose (5 mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetocetate</td>
<td>Control</td>
<td>2.72 (0.29) (6)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>2.0</td>
<td>1.93 (0.30) (6)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.61 (0.21) (6)**</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>0.28 (0.10) (5)**</td>
<td>–</td>
</tr>
<tr>
<td>Lactate</td>
<td>Control</td>
<td>Ibuprofen</td>
<td>6.33 (0.58) (6)</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>–</td>
<td>6.43 (0.68) (6)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>–</td>
<td>5.90 (0.54) (6)**</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>–</td>
<td>3.00 (0.47) (6)**</td>
</tr>
<tr>
<td>5-ASA</td>
<td>2.0</td>
<td>–</td>
<td>5.38 (0.43) (6)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>–</td>
<td>5.20 (0.13) (6)</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>–</td>
<td>4.52 (0.40) (6)**</td>
</tr>
</tbody>
</table>

*Student’s paired t test, p<0.01 compared with control acetocetate; **p<0.001 compared with control acetocetate; ***p<0.005 compared with control lactate; ****p<0.05 compared with control lactate.

**CELL INCUBATIONS**

Cell suspensions, 1 ml representing 5-14 mg dry weight of epithelial cells, were incubated for 40 minutes in conical flasks equipped with a glass centre well and stoppered with Subaseals (William Freeman, UK). Gas exchange was O2 and CO2 (19:1, vol/vol). Incubations were performed at 37°C in 1 ml of physiologic saline containing 2.5-5% (wt/vol) bovine serum albumin, dithiothreitol 1 mmol/l, and substrates at 5 mmol/l. The specific activity of [1-14C] n-butyrate was 1900 cpm/μmol, and that of [6-14C] glucose was 1700 cpm/μmol. Ibuprofen, at concentrations of 2.0-7.5 mmol/l, was incubated simultaneously with radioactive substrates. The incubation was stopped by adding 0.5 ml of 10% perchloric acid and the protein precipitate was centrifuged after cooling with ice for two hours. The supernatant was neutralised to pH 7.4 with 20% potassium hydroxide.

**METABOLIC PRODUCT ANALYSIS**

Acetocetate and lactate concentrations were measured enzymatically from neutralised extracts of cells according to Bergmeyer. The effect of ibuprofen on metabolism analysis was checked and no adverse or synergistic reactions were detected. 5-ASA interfered with the estimation of acetocetate, which was not therefore measured. Standards of metabolites (lactate, acetocetate) were included in each assay: the coefficient of variation between standards was less than 1%. 14CO2 were measured as previously described.

**CALCULATION AND STATISTICAL ANALYSIS**

Results of metabolic products were expressed per g dry weight, which was obtained by drying 1 ml of cell suspension to constancy at 90°C and corrected for the dry weight of the albumin contained in the medium. 14CO2 generation from fatty acid glucose was calculated from the specific activities and trapped 14CO2 in NaOH. Observations obtained from the same tissue or animals on which parallel experiments were performed were subject to Student’s paired t test. The 0.05 probability level was taken to indicate a significant difference in observations.

**Results**

Initial experiments were undertaken with isolated colonocytes from the whole colon of isolated cells were reduced to 2.0-7.5 mmol/l as isolated cells are more sensitive to drug dosages than whole organs. The luminal concentrations of ibuprofen that can be attained in the human colon have not been determined and would depend, among other things, on absorption in the small intestine and transit time. A dose comparison was made with 5-ASA even though a 2.0-7.5 mmol/l concentration of ibuprofen may only be achieved at the mucosal level in the upper gastrointestinal tract.
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Figure 2: $^{14}$CO$_2$ production by human colonocytes from [6-$^{14}$C] glucose in the presence of 2.0 and 7.5 mmol/l of 5-ASA and ibuprofen in the proximal and distal human colons. Observations are mean (SEM) of six colons at each site, with parallel experiments from the same batch of cells. Student’s paired t test: *=p<0.05 compared with control; **=p<0.05, 2.0 mmol/l ibuprofen compared with 7.5 mmol/l ibuprofen; ***=p<0.025 and ****=p<0.01 compared with control.

Figure 3: $^{14}$CO$_2$ production by human colonocytes from [1-$^{14}$C] n-butyrate in the presence of 2.0 and 7.5 mmol/l of 5-ASA and ibuprofen in the proximal and distal human colon. Observations are mean (SEM) of six colons at each site with parallel experiments from the same batch of cells. Student’s paired t test: *=p<0.01 and **=NS compared with control; ****=p<0.001, 2.0 mmol/l ibuprofen compared with 7.5 mmol/l; ****=p<0.025 and *****=NS compared with control.

the rat to establish effects of dosage changes with ibuprofen and 5-ASA. Ibuprofen dose dependently decreased both n-butyrate and D-glucose oxidation to CO$_2$. Reduction of $^{14}$CO$_2$ production from 1.68 (0.12) to 0.36 (0.11) µmol/min/g (dry weight) with [1$^{-}$14C] n-butyrate occurred at the 7.5 mmol/l concentration of ibuprofen (Fig 1), which reflected a 79% reduction of fatty acid oxidation, while reduction of glucose at that concentration of drug was 60% from 0.60 (0.04) to 0.24 (0.06) µmol/min/g (dry weight). Ketogenesis and lactogenesis were reduced in parallel with the reduction of $^{14}$CO$_2$ (Table I). 5-ASA led to a slight reduction in both butyrate and glucose oxidation, a reduction that at 7.5 mmol/l was significantly different from that produced by ibuprofen (p=<0.01 for glucose and p=<0.001 for n-butyrate).

Human colonocytes were only exposed to drug concentrations of 2.00 and 7.5 mmol/l with both glucose and n-butyrate, each drug exposure comprising cells from the same batch of cell isolates. Unlike findings with rat colonocytes, 5-ASA did not reduce either n-butyrate or glucose oxidation in the proximal or distal human colon, but ibuprofen at 7.5 mmol/l selectively diminished fatty acid oxidation
(Figs 2 and 3) and ketogenesis (Table II). It is noteworthy that ibuprofen stimulated glucose oxidation, particularly in the distal colon (Fig 2). Lactogenesis was significantly reduced in the presence of 5-ASA (Table II), a feature that was not paralleled by a reduction of \(^{14}\)CO\(_2\) from the respective substrates.

### Discussion

The detrimental change in fatty acid but not glucose oxidation induced by ibuprofen in human colonocytes suggests maintenance of the Krebs cycle and therefore mitochondrial function in cells. 5-ASA did not change fatty acid oxidation of colonocytes in line with observations made by Ireland and Jewell.\(^{15}\) The effect of ibuprofen on fatty acid oxidation in human colonocytes was analogous to that observed by Freneaux et al\(^{18}\) with isolated liver mitochondria and in intact animals. As both ketone body and CO\(_2\) production were equally reduced in colonocytes, a change within the \(\beta\)-oxidation pathway rather than the Lyven (ketone body) pathway or Krebs cycle must be involved. Damage to oxidative phosphorylation could, however, have occurred in rat colonocytes as both glucose and fatty acid oxidation were impaired by ibuprofen. Such damage usually occurs in experimental animals at higher doses of drugs.\(^{23}\)

The biochemical mechanisms whereby NSAIDs cause mucosal damage have received considerable review.\(^{33} 34\) Aspirin\(^{35}\) and ibuprofen\(^{36}\) at dosages much lower than those currently used inhibit cyclo-oxygenase activity which reduces the synthesis of prostaglandins that are generally considered to be pro-inflammatory. Prostaglandins and 12 lipoxigenase activity in ulcerative colitis are increased in colonic epithelial cells.\(^{37}\) With regard to the mucosal toxicity of NSAIDs, Whittle and Vane\(^{33}\) proposed a two-step damaging process – an initial step of topical irritation possibly related to uncoupling of oxidative phosphorylation\(^{21-24}\) followed by a step in which loss of prostaglandins represented a loss of cytoprotection and therefore an additive effect to mucosal damage. Present observations with fatty acid oxidation in human colonocytes indicate that very high dosages of ibuprofen limit the supply of acetyl-CoA from fatty acid oxidation for lipid and prostaglandin synthesis. Mucosal damage, therefore, may not always be through loss of cytoprotection of reduced prostaglandins but perhaps via metabolic damage through changes in fatty acid oxidation. A precise relationship between drug dosages, mucosal injury, impaired fatty acid oxidation, and reduced mucosal prostaglandin synthesis in colonocytes needs to be established by further experimentation as was done in gastric mucosal injuries.\(^{38}\)

The reduction of butyrate oxidation by 79% by ibuprofen in human colonocytes reflects an overall decrease of 55% of cell energy as butyrate normally supplies 70% of cellular oxidation\(^{9-11}\) in colonocytes. Because both lipogenesis\(^{12}\) and efficient absorption are dependent upon butyrate oxidation, any impairment of fatty acid oxidation can explain changes of membrane permeability\(^{4}\) observed with ibuprofen. The mechanism proposed for ibuprofen damage in the colon is in line with the evolution of ulcerative colitis that is related to impairment of fatty acid oxidation.\(^{14-16}\) A reduction in fatty acid oxidation suggests a mechanism whereby ibuprofen can both exacerbate and cause ulcerative colitis.

Ibuprofen reduces the growth of colonic polyps and tumours.\(^{39} 40\) This effect may depend upon the inhibition of butyrate oxidation because \(n\)-butyrate normally promotes the growth of colonic tumour cells to a more differentiated form.\(^{41}\) The proposed explanation would relate to metabolic changes in mitochondria and cytoplasm of colonocytes rather than nucleic acids, implying that neoplastic lesions may be diminished by change in fatty acid oxidation but not eradicated. This is in keeping with the effects of ibuprofen noted in the formation of experimental colon cancer.\(^{39}\)

The observations now made with ibuprofen on fatty acid oxidation cannot be extended to other NSAIDs until these have been individually investigated. Nevertheless changes in fatty acid oxidation with ibuprofen may explain a number of clinical and experimental observations with regard to colonic mucosal disease, especially ulcerative colitis. The dose dependent metabolic changes observed in the colonocytes indicate that continued use of

| Table II: Acetoacetate and lactate production by isolated human colonocytes in the presence of glucose or n-butyrate with 5-ASA or ibuprofen. (Values, mean (SEM); number of colons in brackets) |

<table>
<thead>
<tr>
<th>Metabolite formation (nmol/min/g dry wt)</th>
<th>Proximal colon</th>
<th>Distal colon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolite</strong></td>
<td><strong>Drug and dose (mmol/l)</strong></td>
<td><strong>n-butyrate (5 mmol/l)</strong></td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>Control</td>
<td>2.57 (0.48) (6)</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>Lactate</td>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>5-ASA</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Student's paired t test, p<0.001 compared with acetoacetate control; **p<0.01 compared with lactate control.
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ibuprofen is possible in colonic disease but at a lower dosage range.

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