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Aspirin changes the secretion rate and amino acid composition of human small intestinal mucin in subjects with ileal conduits

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The effect of aspirin on the rate of secretion and amino acid composition of human ileal mucin was studied, using subjects with ileal conduits as a model system in which mucin secreted from the ileal conduit tissue is flushed out in the urine and can be measured and analysed. Aspirin (600 mg per day, administered orally) increased the daily mucin output by 37–104% in subjects by days 3 or 4, but thereafter the mucin output declined to below the baseline level by day 10. Mucin samples, purified from the ileal conduit urine during the control period and during aspirin administration, were compared. There were no discernible changes in the degree of polymerisation or the density, but during aspirin administration the amino acid composition was significantly changed, and in particular threonine and proline were enriched. One possible explanation, consistent with the compositional analyses, is that the N- and C-terminal regions of the mucin subunits have been cleaved off and lost during aspirin administration. The observed changes in mucin secretion may have implications for the mechanism of the toxic effects of aspirin on the small intestine by altering the barrier properties of the mucus layer.

Keywords: mucin secretion, aspirin, NSAID, ileal conduit, glycoprotein, cytoprotection

Abbreviations: EGF, epidermal growth factor: NSAID, non-steroidal anti-inflammatory drug.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin are amongst the most widely prescribed drugs in medicine. Their uses include the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout, dysmenorrhoea, renal and biliary colic, and migraine. They are also used as general analgesics and antipyretics. In addition, aspirin is increasingly used to reduce clotting in vascular degenerative diseases of heart and brain.

A protective mucosal defence system of mucus, surface-active phospholipids, bicarbonate and a comprehensive blood supply protects the stomach against endogenous and exogenous noxious substances. NSAIDs including aspirin are exogenous agents which impair gastric and duodenal cytoprotective mechanisms in a dose dependant manner, and increase the risk of damage to the mucosa by acid and pepsin to produce gastritis, gastric erosions and peptic ulceration. NSAIDs inhibit cyclo-oxygenase and hence impair endogenous prostaglandin formation [1, 2]. Prostaglandins increase bicarbonate and mucus secretion, and contribute to epithelial integrity [3, 4]. It is likely that the prostaglandins increase the production of surface-active phospholipids [5]. In addition prostaglandins are potent inhibitors of the release from mast cells of pro-ulcerogenic inflammatory mediators such as histamine, tumour necrosis factor and platelet-activating factor [6]. Aspirin appears to have a local irritant effect on the stomach in addition to its effect on cyclooxygenase activity [7].

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Epidermal growth factor (EGF) increases mucus production [8] as well as gastric blood flow [9]. Aspirin (and indomethacin) inhibits EGF secretion in human duodenal tissue [10]. Aspirin also increases neutrophil adherence and activation [11, 12] leading to tissue damage and bleeding.

While the roles of NSAIDs in gastro-duodenal mucosal damage are widely investigated, their deleterious effects on cytoprotection in the small and large intestines are not so well researched. Clinical observation recognises disturbances in small and large intestinal function during NSAID treatment. These include increased intestinal permeability [13], bleeding, protein loss, strictures, inflammation, leucocyte localisation and tissue damage, and less frequently ulcers and perforations. The pathogenesis of NSAID-induced small intestinal damage is unclear at present. It is likely to be preceeded by increased intestinal permeability, exposing the mucosa to luminal irritants [14]. This is followed by localisation of neutrophils to the area of inflammation, and tissue damage.

We have developed a model for studying the secretion of mucin by human small intestinal tissue, using subjects with ileal conduits formed to provide a urinary reservoir after total cystectomy [15]. The conduit retains its blood and nerve supply but is isolated from continuity with the remainder of the gastrointestinal tract. Mucus secretion from the ileal conduit is flushed by the urine into the collecting bag, and mucin measured. Mucin present in the urine is not degraded by enzymic or bacterial action. The ileal conduit model therefore is an ideal system for studying the effect of aspirin on small intestinal tissue mucin secretion in humans, without the confounding effects of luminal digestion products. Urine from control persons without ileal conduits gives no detectible ELISA reactivity in the mucin assay, indicating that glycoproteins present in the urine, entering the conduit from the kidney, including Tamm-Horsfall glycoprotein are not reactive [15]. Mucins are the major structural component of the primary mucus barrier that covers the digestive tract surfaces, and little research has been carried out on how NSAIDs affect the protective mucus layer in the human small intestine.

In the present study we have examined the effect of oral aspirin administration on ileal conduit mucin secretion. The aspirin will be delivered systematically to the conduit after absorption. In addition it may also act on the conduit through urinary excretion since aspirin is eliminated by the kidneys. In this manner the model simulates the physiological *in vivo* situation where both the systemic and luminal sites of action of aspirin have been implicated. The results show that aspirin clearly affects mucin secretion by the small intestinal tissue, though in a manner we did not predict.

Materials and methods

Aspirin

Soluble aspirin (300 mg tablets) was obtained from Reckitt and Colman, Auckland, New Zealand.

Subjects

Subjects who had had ileal conduits for more than 6 months were invited to take part in the study. The experimental protocol was approved by the Auckland Hospital Research Ethics Committee and written informed consent was obtained.

The ileal conduit model and urine collection

Subjects with ileal conduits secrete small intestinal mucus from the conduit pouch into their urine. Ileal conduits constitute a useful model for studying factors that alter small intestinal mucin secretion, since the urine of normal persons contains no mucin recognised by the ELISA system we have used to measure ileal mucin [15].

Subjects collected their total 24 h urine output, and kept it at 4 °C in the presence of 3 mM sodium azide to minimize growth of microorganisms. Each morning the previous 24 h urine collection was transported to the laboratory, processed as previously described, and the mucin content measured in diluted aliquots by ELISA (sensitivity 0.3–3.0 ng mucin protein) [15].

Management of subjects

The effect of aspirin on small intestinal mucin output from the ileal conduit was studied in four subjects. During a period of 3–5 days (the pre-experimental control period) the normal rate of 24 h mucin secretion was established by measuring the volume and mucin concentration in the urine. During the following 5 or 12 days (the experimental period) each subject took 600 mg aspirin orally per day (300 mg after breakfast and 300 mg after dinner) and the output of mucin was measured in the urine. Finally during the next 3–5 days no aspirin was taken (the post-experimental control period) and the mucin output measured. Subjects maintained their normal diets and daily activities during the period of urine sampling. No side effects of aspirin were reported.

Mucin assay

A sandwich ELISA was used to measure human small intestinal mucin in urine samples, as previously described [15]. The anti-mucin antibody, used in the ELISA, was an IgG-enriched fraction of rabbit anti-serum. The anti-serum was raised against isolated post-mortem human ileal mucin which had been separated from the non-mucin antigenic contaminant which bands at a slightly higher density on CsCl gradients [16].

Gel exclusion chromatography

Sepharose CL-2B chromatography was used to determine whether there were marked differences in molecular sizes of mucin(s) in the ileal conduit urine, from subjects during aspirin administration compared to the control period. Urine (3 ml) was chromatographed on a Sepharose CL-2B column (1.6 cm × 36 cm) and eluted with 0.1 M sodium phosphate buffer, pH 7, containing 50 mM NaCl and 3 mM sodium azide.

Mucin isolation and purification

Mucin was purified from 24 h urine collections as previously described [15]. Briefly, the urine was centrifuged to remove any precipitate (this contains negligible mucin [15]), and the urine then concentrated by hollow fibre dialysis (100 kDa cut-off). The concentrated mucus was chromatographed on a Sepharose CL-4B column, and the excluded mucin-containing material ultracentrifuged in a CsCl density gradient for 48 h. Fractions containing mucin were desalted and freeze-dried.

Amino acid composition of purified mucin samples

The amino acid composition of mucin was analysed, after hydrolysis and addition of a norleucine internal standard, on a polystyrene sulfonic acid resin using a Beckman 119BL amino acid analyser and a ninhydrin detection system. Phenylalanine and tyrosine estimations are interfered with in this analysis by amino sugars, and tryptophan is destroyed.

Statistical analyses of data

The significance of the changes in amino acid composition, after aspirin administration, were analysed using a pooled means *t*-test with two subjects as strata. Because tests were performed on 15 amino acids, and the percentage composition values will thus be linked, the *p* values were determined both from the raw data and after analysis involving the Bonferroni correction [17] using PROC MULTTEST [18]. The Bonferroni correction is extremely conservative.

Results

Assay of daily mucin secretion from the ileal conduit tissue

Subjects maintained their regular diet and activities during the study, and were in good health. Each subject collected his or her 24 h urine output, and this was transfered to the laboratory where the quantity of ileal conduit mucin in the urine was quantified by ELISA following suitable dilution.

Pre-experimental control period

Previous work showed that the daily urinary output of mucin from a subject hardly fluctuates under normal conditions [15]. The basal rate of mucin secretion was measured for a period of 3–5 days before aspirin was administered (Fig. 1). For each subject the output stayed within the expected narrow range. The lowest mucin output was observed in subject S (0.85 mg protein of mucin per day) and the highest was in subject K (7.6 mg protein of mucin per day). The mucin secreted was not related to total urine volume (unpublished data).

Experimental period of aspirin intake

After the per-experimental control period subjects took 600 mg of aspirin per day for 5 days. Ileal conduit mucin output increased gradually during the initial 3 or 4 days of aspirin administration, to levels higher than those measured during the pre-experimental control period (Fig. 1). Having reached a peak, daily mucin output began to decline below the peak during days 4 or 5. The highest mucin daily outputs were 37% (subject C), 104% (subject K), 61% (subject M) and 64% (subject S) above the average daily output from the pre-experimental period.

Post-experimental control period

The subjects stopped taking aspirin, and their daily levels of ileal conduit mucin output were measured for a further 3–5 days. In three subjects the outputs were not significantly different from those of the pre-experimental control period (Fig. 1), but in subject K the levels appeared slightly higher.

Prolonged aspirin administration

A study on aspirin administration was carried out using subject M, in which aspirin administration lasted 12 days instead of the usual 5 days (Fig. 2). After an initial pre-experimental daily mucin output measurement of 1 day (the basal output was already known) aspirin was taken during this prolonged experimental period. As expected the mucin output rose to a peak by the third day, and then started declining. By days 10 to 12 of the experimental period mucin output was below the baseline level. After aspirin intake ceased, the mucin output in the post-experimental period climbed back to the pre-experimental baseline level after 3 further days.

Gel chromatography of mucin

Sepharose CL-2B molecular exclusion chromatography was performed on urine samples to determine whether there were large changes in the molecular size of the mucin secreted during aspirin administration. The urine samples were taken for chromatography on the final day of the pre-experimental control period and on the fourth day of the aspirin administration period during the study referred to in Fig. 1, subject C. In both urine samples the bulk of the mucin eluted in the excluded volume of the column (Fig. 3). Thus there was no indication that mucin

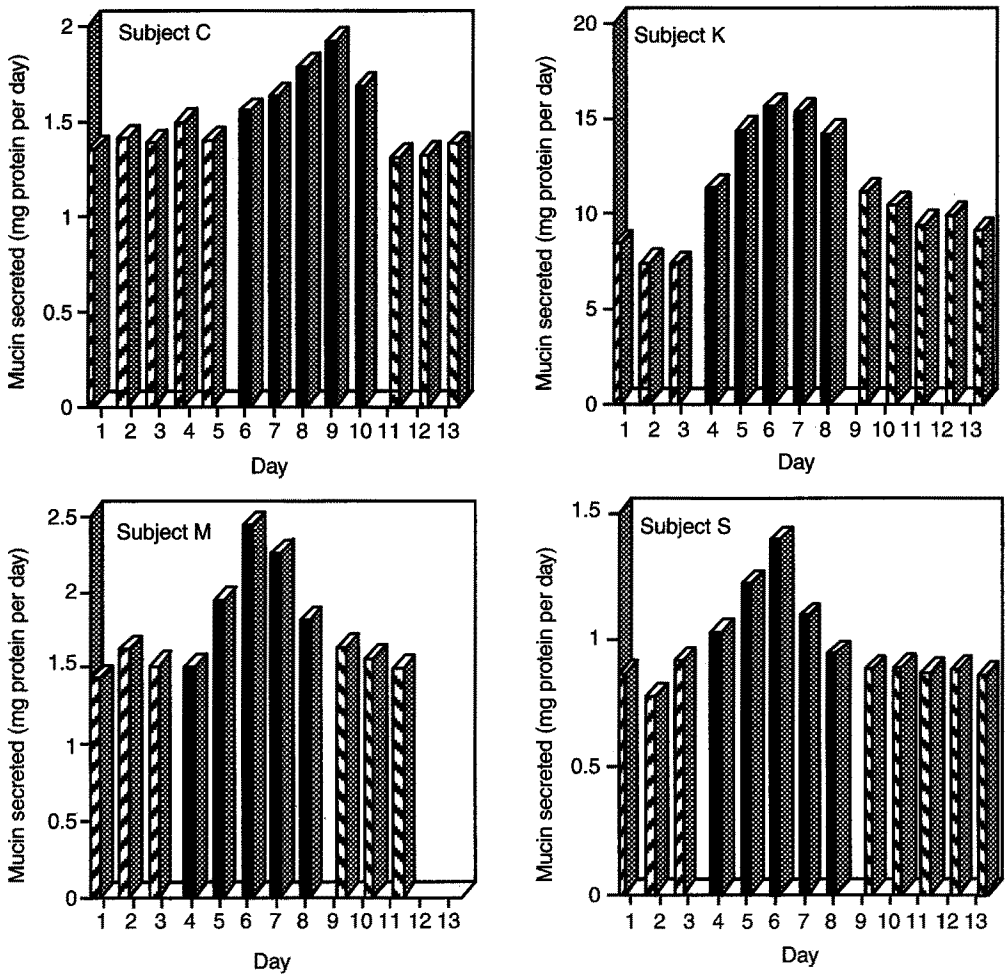

Figure 1. The effect of aspirin on daily mucin secretion from ileal conduits. (_) The pre-experimental baseline output of mucin was established during the first 3–5 days, during which no aspirin was given. (■) Aspirin (600 mg per day) was administered orally for the next 5 days. (▨) The last 3–5 days were a post-experimental control period, when no aspirin was given.

produced during the aspirin administration period was less polymerised. A second study on urine samples from subject K gave similar results (data not shown).

CsCl density gradient centrifugation

Mucin was isolated from the daily output of ileal conduit urine from subject C on the last day of the pre-experimental control period and on the fourth day of aspirin intake. The urine was concentrated using hollow fibre dialysis, chromatographed on Sepharose CL-4B, and the void volume peak centrifuged on a CsCl gradient for 48 h. The mucin present in density fractions was assayed after dialysis to remove CsCl (Fig. 4). Both mucin samples peaked at a density of 1.42 $g\,ml^{-1}$, showing that aspirin administration did not cause large changes in the density of the secreted mucin. A similar result was obtained with another subject (Si), from whose daily urine output the mucin was isolated on the last day of the pre-experimental period and on the third day of the aspirin administration period (data not shown).

Amino acid compositions of ileal conduit mucins

The amino acid compositions of mucins, isolated from the urine of the two subjects above, were determined in duplicate after purification as described above and freeze-drying of the mucin-containing fractions (Table 1, columns i and iii). During the pre-experimental control period the threonine, proline and serine contents of mucins were 22.0, 13.2 and 7.2% for subject C, and 17.2, 10.1 and 9.1% for subject Si, respectively. Another subject (K) from whom the composition of ileal conduit mucin has been previoulsy published [15] showed values of 23.8, 14.0 and 10.4% for these amino acids. By way of a comparison we show the theoretical amino acid composition of MUC2 mucin, deduced from the published gene sequence on the basis that no post-translational cleavage except the loss of the signal sequence occurs and that MUC2 mucin contains 50 tandem-repeat sequences (Table 2, column i).

During the aspirin administration period the threonine content of the mucin increased markedly to 37.8% in

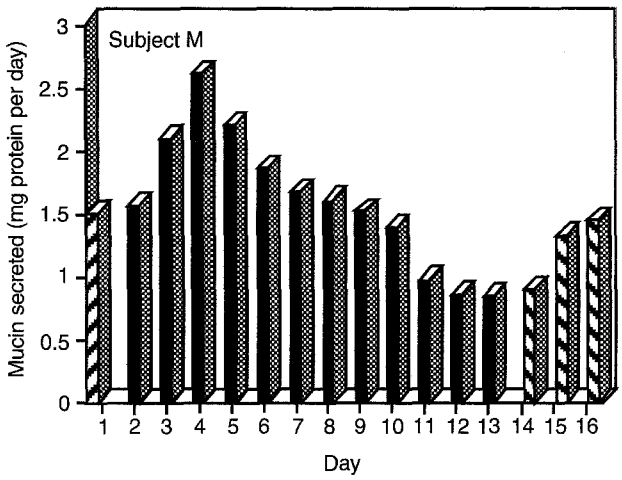

Figure 2. The effect of aspirin on daily mucin secretion from ileal conduits. ( ) The pre-experimental baseline output of mucin was monitored during the first day, during which no aspirin was given. The baseline level for subject M was already known from the experiment in Fig. 1. (■) Aspirin (600 mg per day) was administered orally for the next 12 days. (▨) The last 3 days were a post-experimental control period, when no aspirin was given.

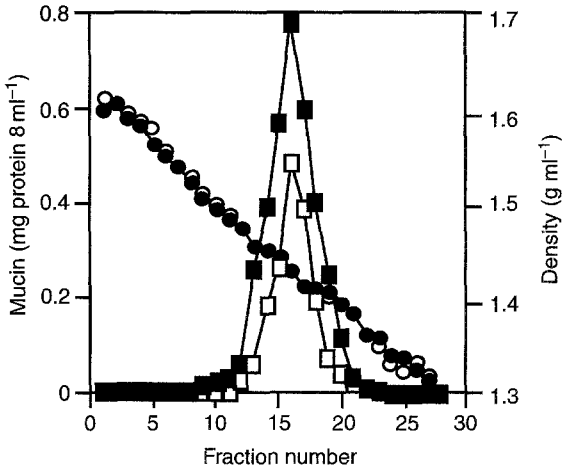

Figure 4. CsCl density gradient centrifugation of ileal conduit mucin. Ileal conduit urine 24 h collections from subject C were concentrated by hollow fibre dialysis, and the mucin purified by Sepharose CL-4B column chromatography. The mucin was then centrifuged for 48 h at 100 000 × **g** on a CsCl density gradient. (○, ●) Density of fractions. (□, ■) Mucin. Open symbols indicate the pre-experimental control period. Closed symbols indicate the fourth day of aspirin administration.

subject C and to 25.6% in subject Si. The significance of the increase was $p = 0.004$ from the raw data, and $p = 0.055$ using the Bonferroni correction. The other amino acids to show an increase were proline (to 15.2 and 13.4%) ($p = 0.024$ from the raw data, and $p = 0.359$ using the Bonferroni correction) and methionine which is difficult to accurately measure. Twelve other amino acids decreased (the p values using the raw data are given, followed by the p values using the Bonferroni correction): aspartate + asparagine ($p = 0.0002$ and 0.0035), serine ($p = 0.0019$ and 0.029), glutamate + glutamine ($p = 0.0007$ and 0.010), glycine ($p = 0.0001$ and 0.001), alanine ($p = 0.0001$ and 0.001) valine ($p = 0.007$ and 0.103), leucine ($p = 0.002$ and 0.031), lysine ($p = 0.002$ and 0.029), histidine ($p = 0.013$ and 0.19) and arginine ($p = 0.05$ and 0.669). The changes in the remaining amino acids were either not estimated or not significant.

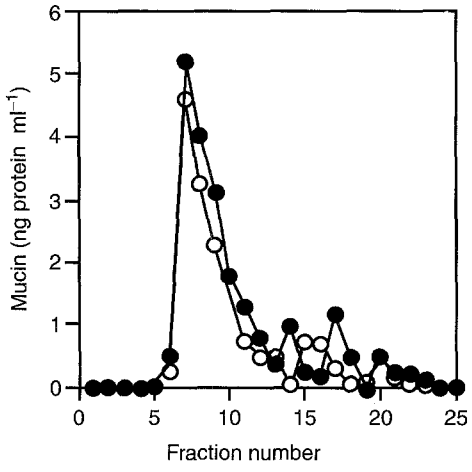

Figure 3. Sepharose CL-2B chromatography of ileal conduit mucin. A 3 ml urine sample from subject C was chromatographed, and mucin measured in eluted fractions. (○) Sample from the pre-experimental control period. (●) Sample from the fourth day of aspirin administration.

Discussion

The effect of aspirin, a commonly used NSAID, on the secretion of small intestinal mucin has been studied using subjects with ileal conduits as a model. Oral administration of 600 mg of aspirin per day caused a consistent increase in small intestinal mucin secretion. This rise in mucin secretion reached a maximum daily output between 37% and 104% above the normal baseline level of secretion by the third or fourth day. This was followed by a decline in mucin secretion below the maximum rate by the fourth or fifth day of aspirin administration. In a longer term experiment, in which the aspirin was taken by the subject for twelve days, the mucin secretion decreased to 56% of the pre-experimental control rate by days 11 and 12, and returned to the pre-experimental control levels on the third day after stopping aspirin administration.

This consistent finding that aspirin caused an initial increase in mucin secretion levels was unexpected, in view of the evidence that aspirin inhibits biosynthesis of mucin in the stomach [23] and gallbladder [24]. It was

Table 1. Amino acid compositions of ileal conduit mucins. Mucins were purified from the pre-experimental control period (columns i and iii) and from the fourth day (column ii) and the third day (column iv) of the aspirin administration period, using subjects C (columns i and ii) and Si (columns iii and iv). The amino acid compositions are given in residues per 100 residues.

	Amino acid composition (residues per 100 residues)			
Amino acid	*(i) Subject C. Control period*	*(ii) Subject C. Aspirin administration period*	*(iii) Subject Si. Control period*	*(iv) Subject Si. Aspirin administration period*
Asp + Asn	6.75 ± 0.17	4.47 ± 0.31	8.98 ± 0.16	6.29 ± 0.09
Thr	22.01 ± 0.28	37.77 ± 3.93	17.23 ± 0.31	25.59 ± 0.28
Ser	7.23 ± 0.12	5.61 ± 0.41	9.06 ± 0.18	7.29 ± 0.06
Glu + Gln	8.79 ± 0.04	5.72 ± 0.40	8.91 ± 0.08	7.79 ± 0.15
Pro	13.21 ± 0.78	15.22 ± 1.29	10.11 ± 0.07	13.44 ± 0.08
Gly	9.32 ± 0.04	5.37 ± 0.21	10.22 ± 0.25	7.79 ± 0.04
Ala	5.20 ± 0.09	2.65 ± 0.04	5.81 ± 0.19	4.70 ± 0.04
Cys (1/2)	2.46 ± 0.04	2.03 ± 0.24	1.61 ± 0.22	1.54 ± 0.12
Val	6.50 ± 0.25	5.14 ± 0.30	6.51 ± 0.01	5.90 ± 0.01
Met	–	0.50 ± 0.07	0.36 ± 0.03	0.74 ± 0.04
Ile	3.72 ± 0.13	3.57 ± 0.25	3.41 ± 0.05	3.60 ± 0.02
Leu	4.28 ± 0.01	3.13 ± 0.12	5.60 ± 0.31	4.69 ± 0.04
Tyr	2.00[a]	2.00[a]	2.0[a]	2.0[a]
Phe	1.90[a]	1.90[a]	1.9[a]	1.9[a]
Lys	3.52 ± 0.05	2.28 ± 0.25	3.37 ± 0.00	3.50 ± 0.07
His	1.25 ± 0.05	1.28 ± 0.05	2.07 ± 0.03	1.66 ± 0.05
Arg	1.88 ± 0.39	1.28 ± 0.10	2.86 ± 0.16	1.58 ± 0.50
Trp	–[a]	–[a]	–[a]	–[a]

[a]Phenyalanine and tyrosine contents were taken to be 2.0 and 1.9 residues per 100 residues, using a previous analysis [15], as measurements of these amino acids were interfered with by amino sugars using this separation system and the ninhydrin detection method. Tryptophan was not detected, but should be less than one residue per 100 residues.

therefore appropriate to examine the properties of the mucin being secreted during normal conditions and during aspirin administration.

The ELISA assay was sufficiently sensitive that urine samples could be chromatographed on Sepharose CL-2B directly, and mucin measured in the fractions. If the mucin had been in subunit form then a peak of mucin in the included column volume would have been seen. In fact both the urine from the pre-experimental period and the aspirin administration period gave a single large peak at the excluded volume. Thus we obtained no evidence that mucin polymerization had been affected by the aspirin.

Daily urine collections from the pre-experimental period and the aspirin administration period were concentrated, chromatographed on Sepharose CL-4B and the density of the mucin measured in a CsCl density gradient. Again no evidence was obtained that there was any difference between the densities of the mucin samples.

The amino acid compositions of mucin secreted during the pre-experimental control period and during aspirin administration were examined. There was a large increase in the threonine content in the latter period, and a small increase in the proline content. With minor exceptions the other amino acids showed decreasing contents. The most obvious explanation for this phenomenon is that the tandem repeat region of the mucin molecule has been enriched during the aspirin administration period.

Mucin from the *MUC2* gene is thought to be a major part of small intestinal mucin secretion. We have previously published the composition of an ileal conduit mucin from subject K [15]. It is interesting to now compare this amino acid analysis of subject K's mucin with the amino acid composition deduced from the subsequently-published gene sequence of *MUC2* [20]. As shown by Toribara *et al.* [19] there are two common alleles of *MUC2*, representing genes with 50 and 100–115 tandem-repeat sequences. In Table 2, column i, the deduced amino acid composition of a MUC2 mucin is shown in which the mucin genes are assumed to be homozygous for a 50 tandem-repeat mucin, and have undergone only signal peptide cleavage. The published composition for the three most common amino acids for subject K's mucin (threonine 23.8%, proline 14% and serine 10%) [15] are close to the values predicted from the gene sequence (threonine 25.8%, proline 14.2% and serine 7%). The composition of another ileal conduit mucin, from subject G, was strikingly close to that predicted for a MUC2 mucin in which the subject's

Table 2. The theoretical composition of MUC2 protein deduced from the gene sequence, assuming 50 tandem repeat sequences [19]. In column i the composition was compiled on the basis that no post-translational cleavage occurs, except loss of the signal sequence [20]. In column ii the composition was compiled on the basis that the first two D-domains (N-terminal 757 amino acids) and the putative link-glycopeptide (C-terminal 694 amino acids) have been cleaved and then lost after post-translational modification [20, 21, 22].

Amino acid	*(i) MUC2 assuming no post-translational cleavage except signal peptide loss*	*(ii) MUC2 after loss of 2 D-domains and link-glycopeptide*
Asp + Asn	5.46	3.80
Thr	25.8	36.80
Ser	7.0	6.71
Glu + Gln	6.39	4.65
Pro	14.2	18.82
Gly	5.70	4.58
Ala	3.06	1.59
Cys (1/2)	5.34	3.38
Val	5.62	4.58
Met	1.23	1.20
Ile	3.54	4.11
Leu	4.35	2.44
Tyr	2.18	1.40
Phe	1.91	1.16
Lys	2.74	1.82
His	1.91	1.01
Arg	2.16	1.16
Trp	0.79	0.78

mucin contains 100 tandem repeat regions (33.1% compared to 32.3% threonine) (Roberton AM, Rabel B, Tasman-Jones C, Lee SP, unpublished results).

The mucins from the two subjects studied in the present work were also analysed. Subject C's mucin (Table 1) contained 22.0% threonine, 13.2% proline and 7.2% serine, which is close to that of subject K [15] and to the theoretical values predicted for a MUC2 mucin with 50 tandem repeats (Table 2, column i). The measured amino acid composition of subjects K, G and C are thus consistent with MUC2 being the predominant mucin expressed in their ileal conduit mucins. Subject Si shows rather lower values for threonine (17.2%) and proline (10.1%), though serine (9.1%) was higher than the predicted value. We speculate that the ileal conduit mucin from subject Si may contain a proportion of other mucins (such as MUC3) in addition to MUC2 mucin, since other known mucins have a lower threonine content in their tandem-repeat regions [25, 26].

A mechanism is needed to explain the increased threonine (increased to 37.8% in subject C and 25.6% in subject Si) and proline contents (increased to 15.2% and 13.4%) found in the mucin of subjects treated with aspirin (Table 1). One possible explanation is that the tandem repeat region of MUC2 mucin has been enriched by post-translational modification during secretion in the presence of aspirin. Gum *et al.* [20] have predicted that hydrolytic cleavage of the two D domains at the N-terminus of MUC2 mucin may occur, and Xu *et al.* [21, 22] have shown that a C-terminal region analogous to the putative link-glycopeptide may be cleaved at the C-terminus. When both these modifications are applied to the gene sequence of *MUC2*, and these terminal regions are lost, a hypothetical MUC2 protein is obtained with the composition shown in Table 2, column ii. (An alternative mechanism is that these cleavages occur in the normal mucin too, but the disulphide bonding of the cleaved terminal regions to the main chain is decreased during aspirin administration, leading to their loss, i.e. aspirin may affect disulphide bond formation.) The predicted threonine, proline and serine contents of the mucin deduced from the gene seqeunce are 36.8%, 18.8% and 6.7%. This compares with values of 37.8%, 15.2% and 5.6% for subject C after aspirin administration, and 25.6%, 13.4% and 7.3% for subject Si. The values for subject C are comparable. Subject Si also showed the increased threonine and proline and decreased serine, but as speculated above, subject Si may also secrete significant proportions of other mucin types in addition to MUC2.

The mechanism by which aspirin causes an increase in mucin secretion in small intestinal tissue is unknown. One possible explanation is that aspirin sets up a local reactive response, causing a discharge of stored preformed mucus. When this depletes the goblet cells, the rate of new synthesis in the presence of aspirin might be expected to be lower than the basal rate of synthesis seen in the absence of aspirin. This would explain the lower than baseline output seen in days 10–12 (Fig. 2). It would also be consistent with the evidence that aspirin decreases mucin biosynthesis in the stomach [23] and gallbladder [24], and inhibits the cyclo-oxygenase involved in prostaglandin synthesis [27].

In making the measurements of daily mucin secretion, we are making the assumption that the binding of the mixed anti-ileal mucin antibody for the ileal conduit mucin added to the ELISA is unchanged for the control period and the aspirin administration period. If the mucin undergoes post-translational modification, as suggested by our composition studies, then the ELISA will only be accurate and dependable if the major epitopes are from the region which remains (including the tandem repeat region). This possible problem associated with the use of immuno-assay methods for measuring mucin will apply to many other studies that have used these methods to quantitate the effects of stimulatory and inhibitory effectors on mucin secretion.

The ileal conduit model is proving to be a useful model for studying the effect of pharmacological agents on the secretion of small intestinal mucin. We have shown that orally-administered Misoprostol, a prostaglandin E_1 analogue, stimulated the secretion of ileal conduit mucin and also changed its amino acid composition (Roberton AM, Rabel B, Tasman-Jones C, Lee SP, unpublished results), and this will be described in a separate study. Unplanned changes in the lifestyle of subjects during the mucin measurement period can affect the results markedly, and it is important that subjects do not deviate from their normal diet and drug intake. For example, an episode of high alcohol intake by one subject greatly reduced the mucin output; and in another subject, treatment with the steroidal drug prednisone for an asthma attack increased the mucin secretion almost three fold during the pre-experimental control period. However, with good patient compliance, reliable data on mucin secretion and its composition can be obtained. In addition to its usefulness in studying the effects of drugs, the model clearly could be adapted to study the effects of lifestyle variables; for example, effects of dietary components, diurnal variation, or fasting on ileal mucin secretion.

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References

1. Whittle BJR (1983) *Br J Pharmacol* **80**: 545–51.
2. Ivey KJ (1988) *Am J Med* **84**: Suppl. 2A: 41–48.
3. Macherey HJ, Sprakties G, Petersen K-U (1993) *Am J Physiol* **264** (Gastrointest. Liver Physiol 27): G126–36.
4. Lee SP, Nicholls JF, Roberton AM (1987) *Eur J Clin Invest* **17**: 1–6.
5. Kao Y-CJ, Lichtenberger LM (1993) *Gastroenterology* **104**: 103–13.
6. Hogaboam CM, Bissonnette EY, Chin BC, Befus AD, Wallace JL (1993) *Gastroenterology* **104**: 122–29.
7. Levi S, Shaw-Smith C (1994) *Br J Rheumatol* **33**: 605–12.
8. Romano M, Kraus ER, Boland CR, Coffey RJ (1994) *Ital J Gastroenterol* **26**: 223–28.
9. Hui WM, Chen ZW, Kung AWC, Cho CH, Lum CT, Lam SK (1993) *Gastroenterology* **104**: 1605–10.
10. Zandomeneghi R, Serra L, Pavesi C, Baumgartl U, Poppi C, Montanari P (1992) *Digestion* **51**: 37–41.
11. Wallace JL, McKnight W, Miyasaka M, Tamatani T, Paulson J, Anderson DC, Granger DN, Kubes P (1993) *Am J Physiol* **265** (*Gastrointest. Liver Physiol* 28): G993–98.
12. Konturek JW, Dembinski A, Stoll R, Konturek M, Domschke W (1993) *Scand J Gastroenterol* **28**: 767–71.
13. Bjarnason I, Williams P, Smethhurst P, Peters TJ, Levi AJ (1986) *Gut* **27**: 1292–97.
14. Bjarnason I, Hayllar J, MacPherson AJ, Russell AS (1993) *Gastroenterology* **104**: 1832–47.
15. Roberton AM, Rabel B, Harding CA, Tasman-Jones C, Harris PJ, Lee SP (1991) *Am J Physiol* **261** (*Gastrointest. Liver Physiol.* 24): G728–34.
16. Rabel B, Cornish J, Lee SP, Harris PJ, Tasman-Jones C, Roberton AM (1994) *Biochem Mol Biol Int* **32**: 995–1002.
17. McArdle BH (1987) *Comp Biochem Physiol* **87A**: 979–82.
18. SAS Institute Inc. (1992) SAS Technical Report P-229, SAS/STAT In *Software: Changes and Enhancements, Release 6.07.* pp. 620. Cary, NC: SAS Institute Inc.
19. Toribara NW, Gum JR, Culhane PJ, Lagace RE, Hicks JW, Petersen GM, Kim YS (1991) *J Clin Invest* **88**: 1005–13.
20. Gum JR, Hicks JW, Toribara NW, Siddiki B, Kim YS (1994) *J Biol Chem* **269**: 2240–46.

21. Xu G, Huan L, Khatri I, Sajjan US, McCool D, Wang D, Jones C, Forstner G, Forstner J (1992) *Biochem Biophys Res Commun* **183**: 821–28.
22. Xu G, Huan L-J, Khatri IA, Wang D, Bennick A, Fahim REF, Forstner GG, Forstner JF (1992) *J Biol Chem* **267**: 5401–7.
23. Kent PW, Allen A (1968) *Biochem J* **106**: 645–58.
24. Rhodes M, Allen A, Lennard TWJ (1992) *Gut* **33**: 1109–12.
25. Gum JR (1992) *Am J Respir Cell Mol Biol* **7**: 557–64.
26. Jass JR, Roberton AM (1994) *Pathol Int* **44**: 487–504.
27. Hawkey CJ, Rampton DS (1985) *Gastroenterology* **89**: 1162–88.