Comparison of leukocyte excretion and blood loss in inflammatory disease of the bowel

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Abstract

Clinical relapse of inflammatory bowel disease is characterised by increased neutrophil migration into the intestine. The site of the neutrophil chemoattractant(s), whether luminal or mucosal, may be important since, on contact with a chemoattractant, neutrophils cause indiscriminate damage to their immediate surroundings by generating reactive oxygen species and by lysosomal enzyme release. If this happens within the mucosa, inflammation should correlate significantly with tissue damage as assessed by bleeding, but if it occurs within the intestinal lumen, the inflammation would be disproportionately greater than the bleeding such as is seen in classical exudation. Intestinal inflammation and bleeding were quantitated with the simultaneous use of indium-111 labelled neutrophils (four day faecal excretion of indium-111) and chromium-51 labelled red cells in patients with ulcerative colitis (n=12), Crohn's disease (n=15), and NSAID induced enteropathy (n=34). Intestinal inflammation and blood loss correlated significantly (Spearman) in patients with ulcerative colitis (20-3% v 6-5 ml/d (median) r: 0.85, p<0.001) and NSAID enteropathy (1-6% v 1-9 ml/d, r: 0.60, p<0.01) but not in Crohn's disease (17-0% v 2-1 ml/d, r: 0.38, p>0.1). For a given indium-111 excretion, patients with ulcerative colitis had significantly greater (p<0.01) blood loss than patients with Crohn's disease. These results suggest that the predominant site of neutrophil chemoattractants may be within the mucosa in ulcerative colitis and NSAID enteropathy and within the lumen in Crohn's disease.

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The study of humoral and cellular immunopathogenesis of inflammatory bowel disease has received much interest. The most characteristic feature of clinical relapse is the increased flux of neutrophils into the intestine. Understanding the movement of neutrophils and the mechanisms through which they mediate tissue injury is fundamental to elucidating the pathogenesis of relapse.

Although controversial, it now seems likely that the striking 'in vitro' findings of reduced neutrophil mobilisation and altered function in patients with inflammatory bowel disease are not intrinsic to the cell but reflect neutrophil conditioning by different inflammatory milieu. Study of indium-111 labelled neutrophils shows close similarities between ulcerative colitis and Crohn's disease in respect of the quantity and kinetics of neutrophil migration from blood to lumen across the intestinal mucosa. The question then arises as to the site and nature of the neutrophil chemoattractant. The latter has been extensively studied by Stenson, who suggested a two stage pathogenic framework for inflammatory bowel disease. He proposes that there is an as yet unknown triggering event which initiates an early neutrophil influx. Subsequent to this there is an amplified second phase of neutrophil chemotaxis which is much greater than the original. There is compelling evidence to suggest that the second amplification stage is mediated by leukotriene B4, a lipo-oxygenase product released by the neutrophils themselves.

It is clear that whatever the primary or secondary chemoattractant is the neutrophils destined for it will respond in a predictable fashion. On contact with the chemoattractant they form oxygen reactive species and there is release of lysosomal enzymes after phagocytosis. Both of these mechanisms may destroy the chemoattractant but will also cause unavoidable non-specific damage in the immediate vicinity of the neutrophil.

The site of neutrophil chemoattractants may therefore be an important determinant of the secondary damage (as assessed by blood or protein loss) caused by neutrophils. This is particularly well demonstrated in the experimental animal where the emigration of neutrophils through the intestinal mucosa in response to a luminal chemoattractant does not cause tissue damage while intra-cutaneous inoculation of the same substance does.

In an attempt to localise, in vivo, the site of neutrophil chemoattractants in patients with ulcerative colitis and Crohn's disease, we used a dual isotope method to quantitate simultaneously intestinal inflammation and blood loss. We argued from the above that the two should correlate closely if the neutrophils are seeking a mucosal chemoattractant, less so if the chemotactant is in the lumen, in which case the inflammation should be disproportionately greater than the blood loss. Patients on non-steroidal anti-inflammatory drugs (NSAID) acted as a control group. There is some evidence to suggest that the neutrophil chemoattractant in NSAID enteropathy may be a metronidazole sensitive microbe.

Subjects and methods

Patients were recruited from gastroenterology and rheumatology outpatient clinics at Northwick Park Hospital. All patients gave fully informed consent to the studies which were approved by the Harrow Health Authority Ethical Committee.
INFLAMMATORY BOWEL DISEASE

Twelve patients with ulcerative colitis (eight pancolitis and four left sided colitis; age 22–70 years; five were on sulphasalazine and two on prednisolone 10 and 30 mg) and 15 patients with Crohn’s disease (one of whom had undergone ileal resection) (six ileal and pancolitis, one left sided colitis, six ileal and two small intestinal; age 18–70 years; 13 were on no treatment, one on 10 mg prednisolone and one on sulphasalazine) were studied. There were no significant differences in haemoglobin; white cell and platelet counts; erythrocyte sedimentation rate; or clinical disease activity between patients with ulcerative colitis and those with Crohn’s disease. After the indium-111 and 51-chromium excretion studies all patients had a full colonoscopy or double contrast barium enema to confirm the diagnosis and to evaluate the extent of disease.

PATIENTS WITH RHEUMATOID ARTHRITIS

Thirty four patients with rheumatoid arthritis were studied. The patients had been on treatment for more than six months and although the type of NSAID varied, all had been established on one drug for at least six weeks. Twenty patients were on concomitant, second line agents (nine colchicine, six gold im, five penicillamine); but we have previously shown that these drugs do not affect the frequency or severity of the small intestinal inflammation caused by NSAIDs.17 18

INDIUM LABELLING STUDIES

An indwelling catheter was placed in an antecubital vein. Six millilitres of blood were drawn into a syringe containing 11 ml of acid citrate dextrose (National Institutes of Health formula A), dispensed into two sterile polyethylene tubes, and allowed to sediment for one hour at room temperature. The supernatant was removed and centrifuged at 100 g for five minutes. The resultant supernatant was removed immediately and resup at 300 g to yield a cell free plasma. The pellet from the 100 g centrifugation was resuspended and incubated for 10 minutes at room temperature in 0-1 HEPES saline buffer (pH 7-4) containing 20 mM HEPES in 0-8% sodium chloride, 4-4 mM tropolone and 300 μCi (11 MBq) indium-111 chloride (Amersham International). Five millilitres of cell free plasma were added to the cell suspension and centrifuged at 100 g for five minutes. The suspension containing the unlabelled indium-111 was poured off and the labelled cells were resuspended in 6 ml of cell-free plasma. Five millilitres (7–9 MBq) were injected and the rest used for standards. The labelling efficiency by this technique averages 84% (range 74–93%).2 The leukocytes maintain their integrity and function during this procedure.3 Abdominal scintigrams were obtained at one to four hours and 20 hours after injection of the labelled cells, to localise the site and extent of disease using an IGE 400 AT gamma camera with a STAR computer at the appropriate channel settings.

Individual faecal excretions were collected over a four day period in 500 ml polythene clip top containers. Standards (0-1 ml aliquots of the labelled cells) were made up to 50 ml with water and distributed over a fixed amount of filter paper in an identical container. Both samples and standard were individually counted in a high resolution bulk sample counter as previously described.22 Counts were made for 20 seconds, which allows the measurement of 0-01% of the injected dose with a counting accuracy of ±4%. Each count was then corrected for any chromium-51 crossover into the indium-111 channel which is negligible if performed on completion of the study. Normal excretion values had been obtained in patients with the irritable bowel syndrome (n=14) who excreted 0-5% (mean) with an upper limit of normal at 1%.

BLOOD LOSS STUDIES

At the same time as blood was obtained for leukocyte labelling, 10 ml were dispensed into 40 ml of citrate-phosphate-dextrose solution containing trisodium citrate dihydrate 3 (w/v); sodium dihydrogen phosphate 0-015 (w/v); dextrose 0-2 (w/v) at pH of 6-9. The solution was centrifuged for 1000 g for 10 minutes, the supernatant was discarded and 1-5 μCi/kg body weight of disodium (51) chromate was added dropwise to the pellet of red cells while mixing gently. The pellet was allowed to stand at room temperature for 15 minutes to allow binding to occur and unbound 51-chromium was then removed by washing with 0-9% saline. The final pellet was made up to 10 ml with saline before injection into the patient.

Individual faecal excretions were made for five days in conjunction with the indium-111 neutrophil studies. Standards were made up by drawing 5 ml of venous blood daily for four days after injection of the labelled red cells. Four ml were distributed over a fixed amount of filter paper in a faecal collection container. Once the indium-111 counting was completed the samples were stored for four to six weeks to allow decay of the indium-111. Each count was corrected for any possible spillover of residual indium-111. The chromium-51 activity in the stools was correlated with the chromium-51 activity in blood from the previous day which allows quantitation of intestinal blood loss. The upper normal limit of intestinal blood loss is 1-0 ml/ day. Using a 300 μCi (11 MBq) injection of indium-111 and a 100 μCi (4 MBq) injection of chromium-51 the estimated radiation dose received by the patient during these studies is 7-2 milli Sieverts (effective dose equivalent).

STATISTICAL ANALYSIS

Wilcoxon’s rank sum test was used for non-parametric data to analyse the mean values, which are given as median and interquartile range. Spearman’s rank correlation coefficient was used to correlate between inflammation and bleeding. The sign test was used to assess the differences in red cell loss (using the square root data) between patients with ulcerative colitis and those with Crohn’s disease for a given level of inflammation.
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### Inflammation and blood loss in inflammatory bowel disease and NSAID induced enteropathy

<table>
<thead>
<tr>
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<th>111-In leukocytes (n&lt;1%)</th>
<th>51Cr red blood cells (n&lt;1 ml/d)</th>
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</thead>
<tbody>
<tr>
<td>Ulcerative colitis</td>
<td>20-3% (8-3-53-1%)</td>
<td>6-5 ml/d (1-8-29-2 ml/d)</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>17-0% (12-1-22-0%)</td>
<td>2-1 ml/d (0-7-5-5 ml/d)*</td>
</tr>
<tr>
<td>Patients on NSAIDs</td>
<td>16% (0-7-3-9%)**</td>
<td>1-9 ml/d (0-5-3-8 ml/d)**</td>
</tr>
</tbody>
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Values represent median (interquartile range).
* Differences significantly from ulcerative colitis (p<0.05); ** Differences significantly from ulcerative colitis and Crohn’s disease (p<0.05); *** Differences significantly from ulcerative colitis but not from Crohn’s disease (p<0.05).

Results

The Table shows that there was no significant difference in the four day faecal excretion of indium-111 between patients with ulcerative colitis or Crohn’s disease. Figure 1 shows that there is a significant correlation between the inflammation and blood loss in patients with ulcerative colitis (r: 0.85, p<0.002) but not in those with Crohn’s disease (r: 0.38, p>0.1). At a given faecal excretion of indium-111 (% dose) patients with ulcerative colitis have significantly greater blood loss (p<0.01) than patients with Crohn’s disease.

When patients with small and large bowel Crohn’s disease were analysed separately there was no significant difference between the faecal excretion of indium-111 leukocytes and chromium 51 red cells, and the lack of significant correlation between the two parameters was evident in both subgroups (small bowel Crohn’s (r= -0.08, p=0.80) and large bowel Crohn’s (r=0.39, p=0.30)). Moreover, for a given faecal excretion of indium-111, patients with both small and large bowel Crohn’s disease bleed significantly (p<0.02) less than patients with ulcerative colitis.

The Table shows that patients on NSAIDs had significantly less inflammation than patients with inflammatory bowel disease but the blood loss was similar to that of patients with Crohn’s disease. Figure 2 shows that there is a significant correlation (r: 0.60, p<0.01) between inflammation and blood loss in patients taking NSAIDs.

### Discussion

The techniques of indium-111 labelled neutrophils and chromium-51 red cells are well described and are accurate, specific, and sensitive methods for quantitating intestinal inflammation and blood loss. The indium-111 leukocyte technique is particularly suitable for the present study since it utilises the homing properties of neutrophils in response to specific chemotractants. Its simultaneous use with labelled red cells shows the specificity of the technique and the selectivity of neutrophil migration to the intestine in these disorders.

Thus, as neutrophils are normal constituents of blood it would seem possible that the faecal excretion of indium-111 simply reflected non-specific intestinal bleeding or oozing (for example, vascular injury due to eroding ulcers). If this was indeed the case, then each 1% of injected neutrophils excreted in faeces should be associated with equivalent blood loss. However, 1% of the circulating blood volume in an average person (five litres) is 50 ml. Clearly, the excretion of the neutrophils is considerably greater than could be accounted for simply by bleeding. Of the various neutrophil functions – namely adherence, aggregation, orientation, locomotion, and chemotaxis – few are better defined and understood than the sequence of events when the neutrophil comes into contact with a chemotactrant. 11-26 On contact free radical species are generated via nicotinamide adenine dinucleotide phosphate linked oxidase, flavoproteins, cytochrome b, and myeloperoxidase. Concomitant phagocytosis internalises the chemotactrant for further degradation with subsequent lysosomal enzyme release. Both mechanisms, however, cause indiscriminate damage in the immediate vicinity of the neutrophil. The idea that damage relates to the localisation of the chemotactrant, whether mucosal or luminal, is supported by data in the experimental animal. 18, 27

Although the pathogenesis of the three disorders is uncertain, that of NSAID enteropathy is probably best understood. 18, 28-30 NSAIDs cause direct cellular damage during drug absorption by several interacting actions on intermediary metabolism. 29 The mucosa is further compromised because of the effect of NSAIDs on cyclooxygenase, effectively preventing the production of reparative prostaglandins. 20 Damaged intercellular junctions allow increased permeation of luminal substances, which by itself seems to be insufficient to elicit detectable neutrophil chemoattraction. Indeed inflammation is only consistently evident after six months of NSAID ingestion and may be due to a metronidazole sensitive microbe. 29 The driving force for inflammation then is a combination of drug and luminal induced mucosal damage. Although not directly comparable (because of the different range of indium-111 faecal excretion) it seems that there is similar blood loss in NSAID treated patients and patients with Crohn’s disease, although the inflammatory activity was 10 fold higher in the uptake of free iron by neutrophils.
7 Curran FT, Allan RN, Keighley MKD. Superoxide produc-
8 Verspaget HW, Mieremet-Coms MAC, Weterman JT, Pena
AS. Partial defect of neutrophil oxidative metabolism in
9 O’Morain C, Segal AW, Walker D, Levi AJ. Abnormalities of
neutrophil function do not cause the migration defect in
10 Wandall JH. Neutrophil granulocyte function. Danish Med
11 Stenson WF. Archidonic acid metabolites in inflammatory
12 Lobos EA, Sharon P, Stenson WF. Chemotactic activity in
inflammatory bowel disease: role of leukotrienes B4 and
1989; 320: 865–76.
15 Bellamy JEC, Nielsen NO. Immune mediators of damage by
neutrophils into the lumen of the small intestine. Infect
16 Bjarnason I, Zanelli G, Smith T, Poult P, Williams P, Smethurst 
17 Bjarnason I. Non-steroidal anti-inflammatory drug-induced
Recent advances in Gastroenterology – 7. Edinburgh: 
18 Bjarnason I, Hayllar J, Smethurst P, Price AB, Gumpel MJ. 
Metronidazole reduces intestinal inflammation and blood
loss in non-steroidal anti-inflammatory drug induced enter-
19 Harvey RF, Bradshaw JM. A simple index of Crohn’s disease
S, et al. Blood and protein loss via small intestinal inflamma-
tion induced by non-steroidal anti-inflammatory drugs. 
21 Mollison PL, Vesil N. The use of the isotope 11Cr as a label for
22 Scott JT, Porter IH, Lewis SM, Dixon ASJ Studies of 
gastrointestinal bleeding caused by corticosteroids, 
23 Segal AW, Jones OTG. Novel cytochrome b in phagocytic 
24 Bellavite P. The superoxide-forming enzyme system of 
25 Segal AW. The electron transport chain of the mitochondrial 
oxidase of phagocytic cells and its involvement in the 
molecular pathology of chronic granulomatous disease. In: 
Peters TJ, ed. The cell biology of inflammation in the 
26 Sartor RB, Crommarije WJ, Powell DW, Schwall JH. 
Granulomatous enterocolitis induced in rats by purified 
27 Bjarnason I, Peters TJ. Intestinal permeability, Non-steroidal 
anti-inflammatory drug enteropathy and inflammatory 
bowel disease. Gut 1989; 30 (Festschrift); 22–8.
28 Bjarnason I, Smethurst P, Walker F, McElmack SC, Pearson 
P, Macpherson A, Mennies IS. Glucose and citrate reduces the 
permeability changes caused by indomethacin. Gastro-
29 Bjarnason I, Smethurst P, Fenn CG, Lee CF, Mennies IS, Levi 
AJ. Misoprostol reduces indomethacin induced changes in 
human small intestinal permeability. Dig Dis Sci 1989; 34: 
407–11.
30 Sharon P, Stenson WF. Enhanced synthesis of leukotriene by 
colonic mucosa in inflammatory bowel disease. Gastro-
31 Stenson WF. Eicosanoids in inflammatory bowel disease 
with special reference to leukotriene B4. In: Peters TJ, ed. The 
cell biology of inflammation in the gastrointestinal tract. Hull: 
32 Lindner AE, Marshak RH, Wolf BS, Janowitz HD. 
33 Dvorak AM. Ultrastructural pathology of Crohn’s disease. In: 
Williamson CN, ed. Inflammatory bowel disease – basic 
research and clinical implications. Toronto: MTP Press, 1987: 
3–41.
34 Moil Kawasaki T. Nutritional management of inflamma-
35 Rhodes RT, Rock BJ, Beaton FB. Cysteine in acute inflammatory 
disease? The role of nutrition, elemental and exclusion diets. 
36 Jegasothy BV, Lovell MGW. Split ileostomy for Crohn’s 
colitis. In: Bayless TM, ed. Current management of inflamma-
37 Rutgeerts P, Goboes K, Peeters M, Hiele M, Penninckx F, 
Aerts R, et al. Effect of faecal stream diversion on recurrence 
38 Rutgeerts P, Peeters M, Hiele M, Kerremans R, Penninckx F, 
Aerts R, et al. A placebo controlled trial of metronidazole for 
recurrence prevention of Crohn’s disease after resection of the 
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