Gut, 1992, **33**, 320–323

Intestinal permeability in patients with Crohn's disease and their first degree relatives

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Abstract

It has been reported that intestinal permeability to polyethylene glycol 400 is increased in patients with Crohn's disease and their apparently unaffected first degree relatives. Because of the implications that these findings have for the aetiology of Crohn's disease these studies were repeated. Patients with Crohn's disease (n=28) and 32 first degree relatives from 11 families underwent a polyethylene glycol 400 (PEG400) intestinal permeability test and a hyperosmotic (1500 mosmol/l) absorption/permeability test using 3-0-methyl-D-glucose, D-xylose, L-rhamnose, lactulose, and ^{51}chromium labelled ethylenediaminetetraacetate. The five hour urine excretion of polyethylene glycol 400 did not differ significantly between controls (n=25) and first degree relatives, 25·5 (3·3)% *v* 24·6% (4)% (mean (SD)) p>0·1, respectively. Patients with small bowel involvement excreted significantly less (p<0·01) polyethylene glycol 400 (16·3 (4·6)% than controls while those with Crohn's colitis did not (26·4 (3·9)% p>0·1). The permeation of the monosaccharides in patients with Crohn's disease and their first degree relatives did not differ from normal subjects. The permeation of lactulose and ^{51}chromium ethylenediaminetetraacetate was not significantly altered in first degree relatives but was significantly increased in the patients, as was the lactulose/L-rhamnose urine excretion ratio which is a specific measure of small intestinal permeability. These studies show normal absorption and permeability in first degree relatives of patients with Crohn's disease. A genetically determined abnormality of intestinal permeability is not likely to be an important aetiological factor in Crohn's disease.

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Accepted for publication
2 July 1991

Although the aetiology of Crohn's disease is unknown, it is likely that the disease represents the final expression of a variety of genetic and environmental factors.[1 2] The aetiological factors are largely unknown and may fundamentally differ from factors which can cause relapse of the disease such as non-steroidal antiinflammatory drugs, alcohol, intestinal infections and psychological stress.[3] Despite extensive study no correlation has been found between Crohn's disease and a number of genetic markers.[4-6] The familial clustering of Crohn's disease is nevertheless well established and a propositus has about 10% chance of a 1st degree relative having or developing either Crohn's disease or ulcerative colitis.[6 7]

The recent findings of increased intestinal permeability to polyethylene glycols [PEG400] (mean Mol wt 400) in patients with Crohn's disease and 66% of their apparently unaffected relatives is particularly important.[8] It implies an inheritable genetic defect with direct relevance to the pathogenesis of the disease. Such a defect would lead to disease only if associated with a separate genetically determined or acquired derangement of immune responsiveness.[9-11]

In view of the important implications of these findings we sought to confirm the results by studying patients with Crohn's disease and their first degree relatives. Two test solutions were used, one containing PEG400 and another containing 3-0-methyl D-glucose, D-xylose, L-rhamnose, lactulose and ^{51}chromium labelled ethylenediaminetetraacetate (^{51}CrEDTA) with a hyperosmotic stress to assess the integrity of the various permeation pathways used by these probes.

Methods

SUBJECTS

Twenty five healthy causcasian volunteers acted as controls. Twenty eight patients with Crohn's disease were studied. Table I shows the clinical details. Twelve patients with Crohn's disease (11 families) had two or more first degree relatives available and willing to take part in these studies. Thirty two apparently healthy first degree relatives were studied (representing 85% of those potentially available). Relatives under 18 were excluded and also subjects on non-steroidal antiinflammatory drugs.

PROCEDURES

Each subject was tested twice within a week, first with PEG400 and then with a combined absorption permeability test. All fasted from midnight until 10 am at whch time normal food and fluids were allowed. At 8 am the test solution was ingested. Complete urine collections were made for five hours (8 am to 1 pm) into a container containing 1 ml (10% w/v) of sodium ethyl mercurithiosalicylate (Thiomersal) as preservative for the sugar markers.

Test solution A contained: PEG400: (5 g), (BDH chemicals Ltd, Poole Dorset UK) in 100 ml water (125 mosmol/l).

Test solution B contained; 3-0-methyl-D-glucose: (0·2 g) (Sigma Chemical Co, Poole, Dorset UK) To assess active carrier mediated transport[12 13]; D-xylose: (0·5 g) (Sigma Chemical Co) To assess passive carrier mediated transport[12 13]; L-rhamnose: (1 g), To assess small pore permeation.[12 13]

Lactulose: (5 g), (lactulose syrup, Duphar Laboratories Ltd, West End Southampton, UK):

TABLE I *Clinical details of patients with Crohn's disease*

	Sex	Age	Site of disease	Length of history (yr)	Surgery	Previous treatment	Treatment of time of study
1	M	28	Small bowel	9	–	P, ED, SP	Nil *
2	M	45	Small bowel	30	SBR	Nil	Nil
3	F	33	Small bowel	8	–	Nil	Nil *
4	M	28	Ileal	New	–	Nil	ED
5	F	28	Ileal	New	–	Nil	Nil
6	M	55	Ileal	20	–	SP	Nil
7	F	24	Ileal	New	–	Nil	Nil
8	M	18	Ileal	1	–	P	ED *
9	M	60	Ileal	30	ITA	Nil	Nil
10	M	58	Ileal	20	ITA	P, SP	Nil
11	F	54	Ileal	30	TC	P, ED	Nil
12	F	40	Ileal	11	TC	ED, SP	Nil *
13	M	62	Ileocolonic	2	–	Nil	Nil
14	F	30	Ileocolonic	6	Sub TC	P, ED, SP	Nil *
15	F	27	Ileocolonic	2	–	P, ED, SP	Nil *
16	F	21	Colonic	New	–	Nil	Nil *
17	M	21	Colonic	1	–	P, SP	P
18	M	33	Colonic	14	–	SP	–
19	M	28	Colonic	3	–	P, ED, SP	Nil *
20	F	22	Colonic	3	–	ED	Nil
21	F	38	Colonic	New	–	Nil	Nil
22	M	44	Colonic	16	–	SP	Nil
23	M	28	Colonic	New	–	Nil	Nil
24	F	27	†	13	ITA	P	Nil *
25	F	28	†	7	ITA	P, ED	Nil
26	F	26	†	8	ITA	P, ED	Nil *
27	M	35	†	5	TC	P, ED, SP	Nil *
28	F	55	†	20	TC	Nil	Nil *

ED= elemental diet, ITA=ileotransverse anastomosis, P=prednisolone, SP=sulphapyridine, SBR=partial small bowel resection, TC=total colectomy, *=family studied, †=no evidence of recurrent disease.

To assess large pore permeation[12 13]; ^{51}CrEDTA: (100μCi); (Amersham International, Amersham, Buckinghamshire, UK): To assess large pore permeation,[12 13] with glycerol: (6·0 ml) as an osmotic filler to make a final osmolality of 1500 mosmol/l.

MARKER ANALYSIS

Analysis of PEG400

The total five hour urine collection is diluted to 500 ml with water. Five millilitres of this diluted urine is mixed with 50 μl 4% (v/v) tetraethylene diethylglycol (Sigma Chemical Co,) as internal standard. The sample is then rotary mixed with 1 g amberlite MBI resin (BDH Chemicals Ltd) for 10 minutes. Another 1 g resin is added and mixed for a further 10 minutes. The sample is then centrifuged at 2000 *g* for 10 minutes and 20 μl of the supernatant is analysed by high pressure liquid chromatography (HPLC) for the separate components of polyethylene glycol.

The high pressure liquid chromatography system used consists of a 5 μm Hypersil ODS column (25 cm×5 mm) (Runcorn, Cheshire, UK) through which 30% methanol in 0·1 M amonium acetate is pumped at a rate of 1 ml/min. The eluent is then passed through a refractive index detector (R1-4, Varian $0·5\times10^{-5}$ RIU/FS Varian instrument group, Walnut Creek, Ca, USA) attached to a pen chart recorder (Philips PM 8251 recorder, Cambridge, UK).

Using this method nine ethylene glycol polymers are derived. Only the five species (PEG polymers 3–7, mol wt 282–458) that give the clearest definition are calculated. The method is sensitive with a minimal level of detection of 5 mg PEG400/l urine.

ANALYSIS OF SUGAR MARKERS

Urinary lactulose was estimated as by Noon *et al.*[14] Urine was desalted by shaking with Duolite MB5113 mixed anion-cation exchange resin (BDH Chemicals Ltd) in the H^+, acetate form and run in parallel with appropriate standard applications, on 46 cm×56 cm sheets of Whatman no 3 chrome paper (Whatman Laboratory Products Ltd, Springfield Mill, Maidstone, Kent, UK) with butan -1-ol/ethylacetate/pyridine/water (30:30:30:15 by vol: 20 hour descending). After 4-aminobenzoic/orthophosphoric acid colour reaction, the separated zones were measured with a recording and intergrating densitometer (chromosan 200, Joyce-Laebl and Co Ltd, Team Valley, Gatehead, Durham, UK). Lactulose concentrations were calculated by comparison of test with standard peak areas assuming a linear relationship below 20 μg/zone.

A modified thin layer chromotographic technique was used for estimating urine 3-0-methyl-D-glucose, D-xylose and L-rhamnose.[15 16] This involves measurement of peak heights by scanning densitometry incorporating an arabinose internal standard to overcome errors of application. Sugar separation was achieved by multiple development on half plates (10 cm×20 cm) of plastic backed silica gel 60 (Merck, Darmstadt, Germany) using three consecutive ascending runs (8·5 cm each) with ethylacetate/pyridine/acetic acid/water (75:15:10:10:, by vol). The layers were dried for at least 30 minutes between each run, and for four hours to remove pyridine before performing a 4-aminobenzoic acid/orthophosphoric acid colour reaction at 120–130°C for 10 minutes. After localisation chromatograms were kept refrigerated in polyethylene envelopes and exposure was minimised during scanning. Peak heights were measured and corrected to a constant internal standard value. Test concentrations were then divided by interpolation from a standard L-rhamnose D-xylose, 3-0-methyl-D-glucose concentration curves from the same chromatograms.

The chromatographic procedures have a minimum level of detection of below 0·1 mmol/l for the sugars and recovery above 90%. The coefficient of variation without replication lies between 3 and 8% over the test range of sugar concentration.

TABLE II *Five hour urine excretion of sugars and ^{51}CrEDTA*

	3-0-methyl-D glucose	D-xylose	L-rhamnose	Lactulose	^{51}CrEDTA	Lactulose/ L-rhamnose
Control	39·0 (8·3)%	24·7 (5·8)%	9·4 (2·6)%	0·37 (0·21)%	0·57 (0·25)%	0·039 (0·020)
1st degree relatives	39·8 (11·8)%	24·4 (6·4)%	10·7 (4·3)%	0·53 (0·31)%	0·74 (0·31)%	0·050 (0·017)
Crohn's disease	33·3 (11·4)%	20·8 (7·7)%	8·1 (4·4)%	0·93 (0·67)%*	1·84 (1·68)%*	0·144 (0·121)*

Value represents mean (SD) urine excretion (% dose) in five hours *differs significantly from control ($p<0·05$).

ANALYSIS OF ^{51}CrEDTA

Five millilitre aliquots of measured urine volumes were counted in a Wallac LKB 1280 gamma counter for five minutes with 5 ml of a 1:500 dilution of the appropriate stock test

solution. Sensitivity was 0·03% of the administered dose per litre of urine, and the precision between 1·0 and 5·0% depending on the level of activity.

STATISTICAL ANALYSIS

Wilcoxon's rank-sum test was used to assess significances between groups.

Results

Figure 1 shows the five hour urine excretion of PEG400. The mean (SD) excretion from controls 25·5 (3·3%) did not differ significantly ($p>0·1$) from patients with Crohn's disease, 21·6 (7·2)% or their first degree relatives 24·6%. Figure 2 shows the PEG400 results from the patients with Crohn's disease when grouped according to disease location. Patients with small intestinal involvement have significantly reduced permeation of PEG400 (16·3 (4·6))% while patients with Crohn's colitis only, are mostly within the normal range.

Table II shows the five hour urine excretion of 3-0-methyl-D-glucose, D-xylose, L-rhamnose, lactulose and ^{51}CrEDTA. The urine excretion of monosaccharides in patients with Crohns disease and their first degree relatives did not differ significantly ($p>0·1$) from controls. The first degree relatives had excretion values of lactulose and ^{51}CrEDTA which did not differ significantly from controls but patients with Crohn's disease had significantly increased permeation of both markers. The lactulose/L-rhamnose urine excretion ratio, a specific measure of intestinal inflammation largely unaffected by other determinants of single marker permeation rates,[12] is shown in Table II. Patients with Crohn's disease have significantly increased intestinal permeability but first degree relatives did not differ significantly from controls. Two relatives with increased intestinal permeability had a high alcohol intake.

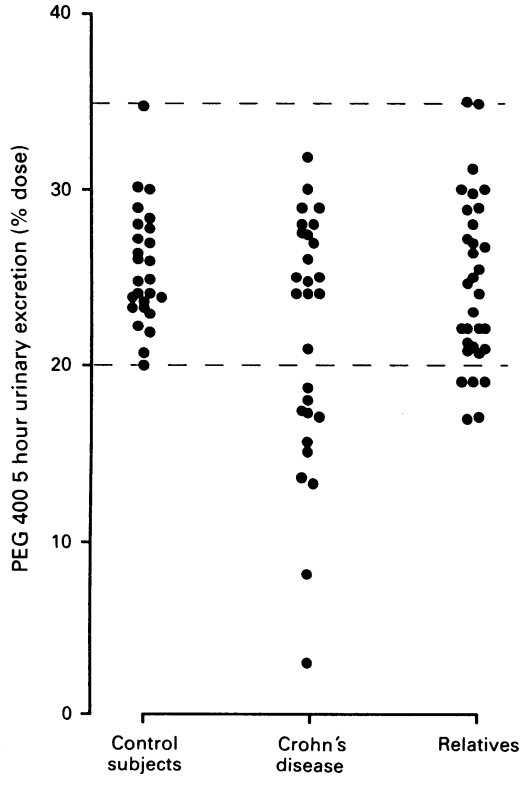

Figure 1: Five hour urine excretion (% dose) of polyethylene glycol 400.

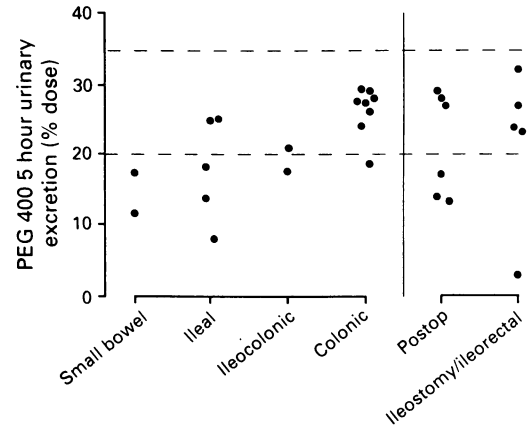

Figure 2: Polyethylene glycol 400 permeation in relation to disease location.

Discussion

The intestinal barrier function is thought to be relevant to the aetiology and pathogenesis of many intestinal diseases.[10-13] While the precise mechanism is uncertain it is suggested that disruption of the barrier function could allow the permeation of luminal antigens and predispose to disease by immunological mechanism. The question is, to what extent does the permeation of PEG400, L-rhamnose, lactulose and ^{51}CrEDTA reflect disruption of the intestinal barrier to such constituents? While this has not been systematically investigated, available data suggest that the permeation of ^{51}CrEDTA correlates with plasma levels of IgA immune complexes in patients with IgA nephritis.[17] Moreover in the experimental animal there is a significant correlation between ^{51}CrEDTA permeation rates on the one hand and bacterial chemotactic peptide and ovalbumin permeation, suggesting that water soluble macromolecules do share the ^{51}CrEDTA permeation pathway.[18,19] Comparative studies and analysis of marker permeation in disease shows that the permeation of lactulose mirrors that of ^{51}CrEDTA.[15] L-rhamnose, however, permeates the intestinal mucosa much more efficiently and behaves much like D-xylose in disease, implying that it uses a different permeation pathway to that of ^{51}CrEDTA and lactulose. This assumption indeed underlies the principle of differential urine excretion of orally administrated test probes as a specific measure of intestinal permeability, which has widespread acceptance in theory as well as in practice.[12] The permeation of these markers in the current study conforms to that found previously in patients with Crohn's disease[20-23] and their first degree relatives.[24,25] Furthermore, the hyperosmolar test used in our study would 'stress' a marginally abnormal mucosa and exaggerate any borderline abnormality among the first degree relatives.

Polyethylene glycol 400 was claimed to be an

ideal intestinal permeability probe[26] but it is clear that it does not conform to the principle of differential urine excretion of orally administered test substances. The various polymers share a permeation pathway which is distinct and of much greater capacity than that used by lactulose and ^{51}CrEDTA.[12 13 15] Although there is concern about the completeness of urine excretion of PEG400 after intravenous administration, the main difficulty has been to explain why PEG400 permeates the intestinal mucosa 50-100 times more efficiently than ^{51}CrEDTA and lactulose, despite similar molecular size and aqueous solubility.[15] PEG400 differs markedly in shape from ^{51}CrEDTA and lactulose and behaves much more like L-rhamnose and mannitol in response to physiological stresses and in disease suggesting that it uses a different permeation pathway to ^{51}CrEDTA and lactulose.[15 27] The precise anatomical correlation of these physiologically defined pathways are, however, unknown.

In the present study, patients with Crohn's disease involving the small intestine had significantly reduced permeation of PEG400 in agreement with two other studies.[28 29] Hollander *et al*, however, found the permeation of PEG400 to be significantly increased both in patients with Crohn's disease and their first degree relatives.[8] The reasons for these discrepancies are not immediately obvious but it is clear that the possible effect of intestinal resection was not a factor in our patients.[9] Hollander *et al* administered PEG400 with a light test meal but this is unlikely to account for the differences. The most striking difference between these two studies is the excretion of PEG400 in controls. While our controls excreted 20–37% of the orally administered test dose the corresponding range reported by Hollander *et al* is approximately 1·3–8·0% with a mean excretion of 3·8%. These low control excretion values have not been reported by any other group.[26 28–30]

These studies show normal intestinal permeation and permeability to test markers in first degree relatives with Crohn's disease. A genetically determined abnormality of intestinal permeability inferred by the findings of Hollander *et al* does not appear to be an important aetiological factor in Crohn's disease.

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