Impaired intestinal barrier function measured by differently sized polyethylene glycols in patients with chronic renal failure

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Abstract

The intestinal mucosa plays a fundamental role as the site for absorption of nutrients, and as an important barrier from potentially harmful agents in the intestinal lumen. Little is known of the permeability properties of the intestinal mucosa in uraemic patients. The intestinal permeability to differently sized polyethylene glycols (PEGs; range 326-1254 daltons) was studied in nine patients with chronic renal failure (24 hour endogenous creatinine clearance 5-27 ml/minute). The maximum 24 hour urinary recovery of PEGs was decreased in the uraemic patients but relatively more of the larger than the smaller PEGs were found in these patients. The results suggest a reduced urinary recovery of PEGs caused by renal dysfunction but also a relatively increased intestinal permeability to larger PEGs in the uraemic patients.

Although the intestinal membranes are the site of absorption of nutrients, they also provide a barrier that prevents potentially harmful agents from leaving the intestinal lumen.¹ However, infections such as rotavirus, and villous atrophy caused in disorders such as coeliac disease, can lead to altered barrier properties.²⁻⁴ Arthritis in association with chronic inflammatory bowel diseases has been thought to depend on absorption of immunogenic substances via the altered gut mucosa.⁵ Furthermore, several potentially toxic substances are produced by the bacterial flora in the intestinal lumen and these may accumulate after absorption in the uraemic state.⁶⁻⁹

Histological changes, including reduction of villous height, increased crypt depth, and infiltration of inflammatory cells and functional changes such as decreased activity of dipeptidases and increased activities of disaccharidases, have been shown in the intestine in patients with chronic uraemia.¹⁰⁻¹³

Although proteinaceous macromolecules are thought to be digested in the lumen and further broken down during the absorption process, some material may enter the bloodstream.¹⁴ Several studies have indicated that even larger molecules and particles can be transmitted across the intestinal membranes by persorption.¹⁵

Several inert or weakly metabolised molecules have been used to assess intestinal permeability, including ⁵¹Cr-EDTA, urea, mannitol, rhamnose/lactulose, and polyethylene glycols.¹⁶⁻¹⁸

We have recently investigated the intestinal permeability to differently sized polyethylene glycols (PEGs) (range 326-1162 daltons) and

their recovery in urine after intravenous injection in rats with chronic uraemia.¹⁹⁻²⁰ Firstly, we found that the intestinal permeability to the larger PEG molecules (range 546-1162 daltons) increased in the uraemic rats. Secondly, intestinal permeability decreased in normal and uraemic rats fed a low protein diet. The investigation also showed that intravenously administered PEGs were excreted without glomerular exclusion in the size range used (326-1162 daltons) in both normal rats and those with chronic uraemia.

This study aimed to investigate the intestinal permeability to differently sized polyethylene glycols (range 326-1162 daltons) in nine patients with chronic renal failure.

We found an over all reduction in intestinal permeability of PEGs but a relatively increased permeability to larger PEG molecules (414-898 daltons) in patients with chronic uraemia. The permeability profiles of the uraemic patients and normal subjects were also compared with the results of computer simulations of a multicompartment model, focussing on the effects of reduced renal excretion capacity.

Patients and methods

PATIENTS

The uraemic group consisted of nine patients with stable chronic renal insufficiency without overt uraemic symptoms. None had undergone gastrointestinal surgery or had any history of gastrointestinal disease. The patients were in hospital during the study and most were on an unrestricted protein intake diet. One patient had a terminal uraemia and was on a moderately protein restricted diet of approximately 40 g per day (patient 9 in Table I). Daily energy intake varied between 1800-2200 kcal. Phosphate binders, vitamin B, and calcium carbonate were given and antihypertensives were added when needed. No patient was treated with glucocorticoids or non-steroidal anti-inflammatory drugs (NSAIDs). Further patient data are given in Table I. The control group comprised six healthy volunteers (four women and two men) with no history of renal or gastrointestinal disease (Table I). The study was approved by the local ethical committee.

PEG TEST

A mixture of PEG 400 and PEG 1000 (range 326-1244 daltons) (100 mg PEG 400 and 2.5 g PEG 1000 dissolved in 10 ml of water, obtained as Macrogolum from Apoteksbolaget, Stock-

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TABLE 1 Details of uraemic patients and control subjects

Patient	Sex	Age (yrs)	Diagnosis	Serum creatinine ($\mu\text{mol/l}$)	Serum urea (mmol/l)	24 hr creatinine clearance (ml/min/1.73m^2)
1	M	35	CGN	274	20	27
2	M	73	HN	355	23	18
3	M	55	CGN	451	27	16
4	F	75	NSC	413	41	12
5	M	54	CGN	796	31	11
6	M	82	NSC	446	33	10
7	F	51	PC	542	22	9
8	M	63	CGN	667	30	8
9	F	66	CPN	586	45	5
Mean		62		503	30	13
Range		35–82		274–796	20–45	5–27
Controls:						
Mean		31		83	5	99
Range		23–41		63–99	3–7	69–124

CGN=chronic glomerulonephritis; HN=hydronephrosis; NSC=nephrosclerosis; PC=polycystic disease; CPN=chronic pyelonephritis.

holm, Sweden) was given orally in 150 ml of water after overnight fasting. Ingestion of food and drugs was not allowed during the first two hours. Urine was collected over six, 24, and 48 hours. The urine was frozen at -20° for further analysis of PEGs. When the urinary recovery is discussed below it refers to the 24 hour recovery unless otherwise stated.

ANALYSES OF PEGS

Six, 24, and 48 hour samples of urine were analysed. Since the urine contained visible flocculated material which seemed to interfere with the separation and analysis of PEGs according to a previously published procedure,²¹ the extraction of PEG from the urine was modified. Thus, 6 ml samples and controls with known amounts of PEGs in duplicates were mixed with 1.5 ml trichloroacetic acid (TCA 50%) for 10 minutes at room temperature and centrifuged for 10 minutes at 3700 rpm ($\approx 1150 g$) in a Wifug Doctor Centrifuge (Wifug, Stockholm, Sweden). Then 7 ml of the combined supernatants of the duplicates were neutralised to around pH 7 with 0.5 mol/l NaOH, as measured with pH indicator paper. Some 6 ml was then mixed in a vortex mixer with 3.7 g Amberlite MB-3 mixed anionic and cationic resin (BDH Ltd, England), inverted repeatedly for 30 minutes at 37°C , mixed with new resin, and inverted for another 30 minutes at 37°C . A 2 ml sample was freeze dried overnight, dissolved in methanol (42%) and water (58%), filtered through a $0.45 \mu\text{m}$ filter, and analysed with high performance liquid chromatography (HPLC; HSRI 931, Tecator, Sweden, equipped with reverse phase C-8 column, Lichrosorb RP-8 Fertigkolonnen, Merck AG, Darmstadt, West Germany). The pressure was about 20 MPa and the flow rate (waste mode) around 40 ml/hour. By comparing the peak height of each PEG molecular weight species in the urine with the amount given orally, the percentage recovery of each PEG molecular size was calculated.

CHARACTERISTICS OF THE MUCOSAL BARRIER

Based on the recovery of different sized PEGs in each patient, the barrier was characterised in the following ways. Firstly by the breakpoint (dalton) – that is the PEG size where the recovery

Figure 1: Graphic representation of the multicompartment model, where k_{ij} represent the rate constant from compartment i to j .

(R) (%) = $(R_{\text{max}} - R_{\text{min}})/2 + R_{\text{min}}$. R_{max} and R_{min} are the maximum and minimum urinary recoveries of the molecules used; this parameter describes the PEG size where 50% of the intestinal filtering is expressed.²² Secondly by the slope of the recovery values for increasing PEG size, since the intestinal exclusion of molecules because of size was roughly linear and inversely proportional to the molecular weight. To describe the correlation between urinary recovery and molecular weight, a linear correlation coefficient was calculated by using the eight PEG sizes symmetrically surrounding the breakpoint. This was done because the urinary recovery patterns, as a result of the relative low recovery, did not fit well with a previously described model.²³

COMPUTER SIMULATIONS OF URINARY RECOVERY IN RENAL FAILURE

The passage of molecules from the intestine to the urine was approximated with a multicompartment model (Fig 1), and simulated for different critical parameters, using Stella software (High Performance System Inc, Lyme, New Hampshire, USA) and a Macintosh SE computer. The transit times between the intestinal segments were described by ramp functions. During the first 30 minutes, absorption was assumed to occur from a segment corresponding to the jejunum, and from 30 to 60 minutes the intestinal contents were transported further into the ileum. Absorption from the ileum took place only between 60 and 105 minutes, when the transport to the colon started. Absorption from the colon alone occurred between 135 and 360 minutes, when the simulation ended. The rate

TABLE II Mucosal barrier characteristics calculated from the urinary recovery of polyethylene glycols (PEGs) in the uraemic and control groups. Values are mean (SEM)

Barrier characteristics	Controls (n=6)	Uraemic (n=9)	Significance (p<)
Breakpoint (Da PEG)*	541 (60)	688 (25)	0.05
Regression coefficient†	3.92 (0.98)	1.18 (0.42)	0.01

*Breakpoint Da PEG, is the PEG molecular weight at which 50% of the intestinal filtering has occurred.

†The regression coefficient is based on a linear approximation of the recovery of molecules according to molecular weight for the eight molecules surrounding the breakpoint of each individual.

Figure 2: Twenty four hour urinary recovery of polyethylene glycols (PEGs) in the control group (open bars) and in the uraemic group (closed bars). The recovery is significantly higher for all molecular weight species in the control group ($p < 0.05$ – 0.001).

Figure 3: (A) Six hour (open bars) and 24 hour (closed bars) urinary recovery of polyethylene glycols (PEGs) in the control group. Only small quantities of small molecules (range 326–546 daltons) are excreted between 6 and 24 hours. (B) Six hour (open bars) and 24 hour (closed bars) urinary recovery of PEGs in three uraemic patients (nos 7–9 in Table I). Small quantities are excreted between 6 and 24 hours.

constants (k_{41} , k_{42} , k_{43}) for the absorption of different sized PEG molecules from the jejunum, ileum, and colon, respectively, were calculated from exponential functions applied to steady state intestinal perfusion data in man.² The rate constants describing exchange with the extravascular space and renal clearance were taken from earlier results obtained in pigs.²⁴ With regard to the aims of the present investigation, the simulations were performed by changing the rate constant describing the passage from blood to urine between 20% to 150% of the basal value – the value of the rate constant k_{64} for each molecular weight species of PEG was changed between $0.2 \cdot k_{64}$ and $1.5 \cdot k_{64}$.

LABORATORY TESTS AND STATISTICS

Serum and urine creatinine and serum urea concentrations were determined by routine methods at the Department of Clinical Chemistry, University Hospital, Linköping, Sweden. The results are given as mean (SEM) and the statistical calculations were performed by Student's *t* test for unpaired data.

Results

The 24 hour urinary recovery of PEGs in the uraemic and control groups are shown in Figure 2. The reduced recovery of PEGs in the uraemic group is statistically significant for all molecular weight species ($p < 0.05$ – 0.001). The six and 24 hour urinary recovery in the control group and in three uraemic patients (patients 7–9) are shown in Figures 3A and B. As the Figures indicate, only minor quantities of the smaller molecules (326–414 daltons PEG) are excreted between six and 24 hours, particularly in the control group (Fig 3A). This pattern was not so striking in the three uraemic patients (Fig 3B). Negligible amounts of PEGs were, however, detected in 24–48 hour urine samples in both the control and in the uraemic groups.

Table II shows the calculated permeability characteristics of the two groups. The breakpoint was significantly shifted towards a larger value – that is to the right in Figure 1 – in the uraemic group ($p < 0.05$). Moreover, the regression coefficient was lower in the uraemic group ($p < 0.01$).

The relative urinary recovery of the molecules, compared with the recovery of 326 daltons is shown in Figure 4, suggesting a relative increased recovery of larger PEGs in the uraemic patients. The simulated urinary recovery of PEG 400 (range 282–590 daltons) with various renal function is displayed in Figure 5A. The results suggest that the over all urinary recovery of molecules is decreased when the renal excretion capacity is reduced. Furthermore, the simulation also suggests that there would be a relatively increased recovery of the larger molecules when the renal clearance is reduced (Fig 5B). The difference was much less pronounced, however, than in Figure 4. The breakpoint did not change significantly solely by reducing the renal function. It varied only between 397–408 daltons in Figure 5A, when the renal excretion was altered from 150 to 20%.

Figure 4: Relative 24 hour urinary recovery of polyethylene glycols (PEGs) in the control group (open bars) and in the uraemic group (closed bars). The recovery of 326 dalton PEG in each group has been set to 1.0.

Discussion

The urinary recovery of PEGs given orally depends on several factors, the absorptive surface area, the intestinal transit time, the permeability of the intestinal membranes, and renal excretion – that is, renal function.^{16–18} The relative recovery of molecules differing in size is thought to depend mainly on the permeability of the intestine, provided the transit time is not greatly affected.^{22–25}

The reduced over all recovery of the PEGs in the uraemic group (Fig 2) was probably primarily the result of the impaired renal function. The relative recovery of the individual molecules, however, was not identical in the two groups. The breakpoint – that is the molecular size where 50% of the intestinal filtering is expressed – shifted significantly towards a larger value, 698 (23) daltons in the uraemic group compared with

540 (60) daltons in the healthy subjects (Table II). This indicates that relatively more larger molecules were recovered in the uraemic patients than in the control subjects. The relative urinary recovery of the molecules, compared with that of 326 dalton PEG, is shown in Figure 4. For comparison the recovery of 326 dalton has been set to 1.0 in both groups.

The urinary recovery of orally given PEGs was inversely proportional to molecular weight (Fig 2). The intestinal exclusion due to the size of the probes probably does not show a truly linear dependence, but it seems reasonable to use a linear approximation to describe the data for the limited size range of probes used. A gut mucosa with an increased selectivity will give a comparatively low recovery of large molecules and hence a steeper slope of the curve, resulting in a larger negative regression coefficient. On the contrary, a less selective gut, that allows larger molecules to pass more freely, would give a flatter curve and then a comparatively smaller value of the regression coefficient. Using this approach, we interpret the smaller regression coefficient in the uraemic group as a sign of a reduced intestinal selectivity towards the larger PEGs (Table II).

An explanation for the increased breakpoint and the smaller regression coefficient in the uraemic patients could be a higher relative excretion of the larger PEGs in the uraemic patients compared with subjects with normal renal function.

The opposite was found, however, when PEGs were given intravenously to uraemic rats. The overall 24 hour recovery was decreased for all molecular weight species as could be expected because of the impaired renal function.²⁰ But the urinary recovery of the smaller molecules (326–590 daltons) was relatively higher than the recovery of the larger ones (634–1162 daltons). The opposite was found in the control rats, in which the urinary recovery of the smaller molecules was lower than the recovery of the larger PEGs.²⁰ Furthermore, the urinary recovery of the larger PEGs (590–1162 daltons) increased in direct proportion to the molecular weight in both

Figure 5: (A) Computer simulated 6 hour urinary recovery of different sized polyethylene glycols (PEGs) in subjects with 150% of normal renal excretion (□), 100% of normal renal excretion (□), reduced renal excretion of 50% (▨) and reduced renal excretion of 20% (■). (B) Simulated relative 6 hour urinary recovery in the four groups with different renal excretion. 150% (□) of normal renal excretion, 100% (□), 50% (▨) and 20% (■). The recovery of 282 dalton PEG has been set to 1.0 in each case.

normal and uraemic rats, which was probably the result of a larger distribution volume of the smaller tracers.²⁰ This suggests that a simple comparison of ratios between larger and smaller molecules might not completely compensate for the reduced renal function in uraemic subjects.²⁰ Moreover, intravenous administration of PEGs suggested that there was no glomerular exclusion of PEGs up to 1162 daltons.²⁰ Neither have previous studies shown any reduced renal excretion of PEG 400, 600, or 1000.²⁶⁻²⁹

Although there was no sign of altered intestinal transit time in the uraemic subjects, these changes could affect the urinary recovery of PEGs.¹⁶ However, a recent study has shown that moderate changes in gastric emptying time and intestinal transit time have a negligible effect on the relative recoveries of di- and monosaccharides.²⁵ In another study the authors were unable to show a correlation between the absorption of polyethylene glycols and the gut transit time.³⁰ These results should also have a bearing on the relative recovery and the breakpoint of differently sized PEGs.

Differences in the distribution volume of the molecules in the two groups could cause disparity in the urinary recovery between uraemic and control subjects. Previous studies have indeed shown an increased distribution volume of the smaller PEGs compared with the larger molecules.^{24,29} However, this is an unlikely explanation of the difference, since there was a negligible increase in recovery between six and 24 hours in the control group. Moreover, the breakpoint – that is the molecular size where 50% of the intestinal filtering is expressed – was very similar for the six hour and 24 hour urinary recovery (531 (53) and 539 (49) daltons PEG, respectively). Delayed urinary excretion of the smaller molecules in the control subjects, as indicated in Fig 3A, compared with the uraemic patients (Fig 3B) would be more likely to increase the breakpoint in the control subjects.

The mean age was higher in the uraemic than in the control subjects (Table I), which may have influenced the permeability tests. Earlier studies using sugars as markers have shown, however, that increased age does not change intestinal permeability.^{31,32} In several independent studies using PEGs as probes, the permeability characteristics have been shown to be remarkably similar in different adult control groups, irrespective of age and sex.^{33,34} Only in 6–8 year old children was a slightly higher recovery observed.²¹ Furthermore, in the present study, there was no correlation between age and the breakpoint value. The only correlation seen was between creatinine clearance and the breakpoint – that is, the lower the clearance the larger the breakpoint ($r=0.55$).

The experimental data obtained from the two groups have also been compared with a mathematical multicompartiment model simulating urinary recovery of PEG 400 (282–590 daltons) in subjects with normal and reduced renal excretion of PEGs. The simulation shows that the overall recovery of the molecules should decrease when the renal function is reduced (Fig 5A). Furthermore, the relative recovery of the larger molecules should increase compared with the

smaller ones at the same time (Fig 5B). The recovery patterns of the PEGs described by mathematical simulation agree with the recovery pattern found in the uraemic patients and in the control group (Figs 2, 4, and 5A and B). The breakpoint, however, varies only between 397–408 daltons (data not shown), when the renal excretion capacity is reduced from 150% to 20% in the mathematical model. In addition, the changed relative recovery of the individual molecules was much less pronounced in the mathematical model (Fig 5B) than in the data from the experimental groups (Fig 4). When looking at the results obtained by the mathematical model it must be remembered that the model is based on data obtained from perfusion studies in man² and from results gained after intravenous administration of PEGs in pigs.²⁴

The proposal that there is increased intestinal permeability (larger average pore size³⁵) in the uraemic patients is a logical one supported by several observations: (1) increased breakpoint in the uraemic group; (2) smaller regression coefficient in the uraemic group; (3) relatively higher urinary recovery of the larger molecules in the uraemic group. The results from intravenous injection of PEGs in rats with chronic uraemia also suggest that the mucosal membranes have different permeability, which shows up in the urinary recovery of PEGs.²⁰

These findings suggesting an increased permeability to larger molecules, agree with recent observations in rats, where increased intestinal permeability was shown in acute uraemia^{36,37} and in those with chronic renal failure.¹⁹ Moreover, increased permeability of the blood brain barrier towards inert molecules in chronic uraemic rats has been shown by other investigators.³⁸

Shortening of the villi, elongation of the crypts, and infiltration of lamina propria with inflammatory cells have previously been seen in patients with chronic renal failure.¹⁰⁻¹³ Indeed, transmigration of polymorphonuclear cells has been observed to increase the permeability of larger molecules, probably via paracellular pathways.³⁹ Increased intestinal permeability has also been shown in mucosal inflammation, as in Crohn's disease.^{40,41} Moreover, several studies have suggested that the bacterial flora of the gut is altered in chronic uraemia and that bacteria associated materials could be implicated in the pathogenesis of this disorder.^{6-9,42,43} In fact, enterotoxin from *Escherichia coli* and synthetic peptides which mimic bacterial peptides are known to increase the intestinal permeability to large molecules – for example dextran 3000.⁴⁴⁻⁴⁷ These observations may explain the impaired gut barrier in uraemic subjects.

An alternative explanation is the accumulation of harmful, endogenous low molecular weight substances in serum in the uraemic state.^{9,48} These uraemic toxins could also affect the intestinal integrity and allow larger molecules to pass more freely over the mucosal barrier than under normal conditions.

In conclusion, the results suggest that despite the reduced overall recovery there is an increased leakage of the larger PEG molecules (414–898 daltons PEG in the test mixture) in chronic uraemic patients. An explanation could be open-

ing of the paracellular pathways, particularly the tight junctions – for example by bacterial peptides or oligopeptides accumulated in plasma, or both. A possible effect is an impaired barrier function in the intestinal wall towards potential toxic substances in the intestinal lumen. The results encourage further efforts to identify and isolate the causative agent(s) and to delineate the precise mechanisms of the altered mucosal barrier properties in chronic uraemia.

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