韓國營養學會誌 29(2) : 153~158, 1996

Intestinal Permeabilities of Polyethylene Glycols (330-1122 D) in the *In Situ* Perfused Rat

Meehye Kim

Department of Pharmacy, University of Manchester, Manchester M13 9PL, U.K.

ABSTRACT

Polyethylene glycols (PEGs) are hydrophilic molecules that have been used to characterize intestinal permeability via the paracellular pathway. Using a mixture of PEGs (400, 600 and 1000), containing oligomers in the molecular weight range 330 to 1122 D, the molecular weight permeability dependence in the jejunum of the rat small intestine was examined, employing an *in situ* recirculation perfusion technique. Individual oligomers were determined by HPLC with refractive index detection. In the range studied, a distinct molecular weight cut-off was not apparent. Corrected for the length of jejunum used in the study, over the molecular weight range 330 to 1122 D, the apparent permeability (P_{app}) of PEG ranged from $4.92 \pm 0.02 \times 10^{-5}$cm/sec (mean ± SEM, n=5) to $0.28 \pm 0.02 \times 10^{-5}$cm/sec. Also, it was observed that the apparent permeability was inversely proportional to approximately MW^2. The results in this study suggest that molecular weight is an important factor in determining the intestinal permeability. (*Korean J Nutrition* 29(2) : 153~158, 1996)

KEY WORDS : intestinal permeability · polyethylene glycol · small intestine · rats.

Introduction

The intestinal epithelium demonstrates differential properties, providing both barrier and transport functions with respect to luminal molecules. Intestinal permeability relates to the selective ability of the intestinal epithelium. A variety of compounds have been used to delineate the permeability characteristics of mucosal membranes in health and disease[1-8] These include urea, erythritol, mannitol, lactulose, inulin , and creatinine[3)9)10)].

The effect of the form of protein in enteral feedings on their efficacy and tolerance has been a controversial issue for many years. It was observed that amino acids presented to the gastrointestinal tract as small peptides (dipeptide and tripeptide) are absorbed more efficiently than are the equivalent free amino acids[11)]. During hypermetabolic stress, critical illness(i.e., after trauma, post-surgery, sepsis), and diseases of the gastrointestinal tract, there may be an absorptive benefit to peptide-based feeding solutions[12)]. Fogel et al(1975)[13)] also reported that amino acid absorption is decreased after jejunoileal bypass in man. However, dipeptide absorption remains intact in these patients. The reason why peptide-based enteral feeding is superior to amino acid-based diets or intact protein in critically ill patients still remains unknown. This might be due to changes in intestinal permeability in these patients.

A new approach to the measurement of intestinal permeability using low molecular weight polyethylene glycol(PEG) in man and animals has been developed by many investigators[1)2)7)8)]. Chadwick and his colleagues[1)2)] introduced a mixture of low molecular weight polyethylene glycols as 'ideal' probe molecules for measuring intestinal permeability : Polyethylene glycol is non-toxic, not degraded by intestinal bacteria, not metabolized during or after passage through the intestinal wall, and rapidly excreted in the urine. The dif-

Accepted : February 2, 1996

ferent-sized molecular compounds cross the intestinal epithelium at different rates, allowing characterisation of the passive permeability properties of the mucosa. The PEG method offers a simple, harmless and reproducible method to measure intesinal permeability properties. The PEGs also appear to be good markers because of their high solubilities and low toxicities The current interest is in paracellular absorption, which is thought to be an important mechanism for absorption of a wide variety of small polar molecules such as peptides. With the aim of delineating aspects of the influence of molecular size on paracellular absorption, a range of small molecular weight polyethylene glycols (MW 330-1122 D) have been studied in the *in situ* perfused rat. The objective of this study was to assess the permeability profile as a function of molecular weight or size in the small intestine.

Materials and Methods

1. Surgical Procedures

After an overnight fast, five male rats(CD-Crl : CD (SD)BR, Charles River Breeding Laboratories, Ltd. U. K.) weighing 250-300g were anaesthetized(18mg/kg B.W.) by i.v. pentobarbital(Sagatal ; May & Baker, U. K.) injection and body temperature was maintained at 37℃ using an electric heating pad. Following leparotomy, the jejunum was exposed and the ligament of Treitz localized. A 20cm length of proximal jejunum distal to the ligament of Treitz with intact mesenteric vasculature were cannulated at both ends and returned to the peritoneal cavity without disrupting the blood flow. The length of jejunal segment used was measured using a standard 20cm silk thread. Anaethesia was maintained by administration of i.v. pentobarbital (6mg/kg B.W.) every 15min throughout the experimental period.

2. Experimental Protocol

Before the experiment, 20mL of the appropriate perfusing solution was placed in reservoir(pH 7.4). After 15min equilibration period, a 200μL perfusate sample was withdrawn from the reservoir at the first 10min and then every 30min for 3hr. At the end of the experiment the animals were killed by anaesthetic overdose and the perfused intestinal loops were removed. The results were standardized per cm of tissue length.

3. Perfusion Details

The following was applied to jejunum in each animal using an *in situ* perfusion technique : The cannulae from the intestinal loop were connected by teflon tubing to the perfusing solution reservoir maintained at 37℃. A peristaltic perfusion pump(Anachem, U.K.) recirculated solution through intestinal loop at a flow rate of 0.5mL/min(a rate chosen based on preliminary experiments). The perfusing solution was gassed with O_2 95% : CO_2 5%(V/V) additionally containing 5g/L of polyethylene glycol(PEG) 4000 with 5μCi ^{14}C-PEG 4000(Amersham, U.K) as a non-absorbable marker for fluid transport. A mixture of 5% polyethylene glycols(PEG 400/600/1000 ; 1/2/20 ratio) in isotonic electrolyte solution(Table 1) was perfused through the jejunum at 37℃. The ratio of the batches of PEG was chosen to provide similar peak heights of the oligomers after HPLC.

4. Sources of Chemicals

Polyethylene glycols(PEGs) with average molecular weights of 400, 600, and 1000 D were obtained from BDH, U.K. ^{14}C-PEG 4000 was purchased from Amersham International, U.K. Unless otherwise stated all other chemicals used were of analytical grade and all solvents for HPLC analyses were HPLC grade.

5. Analytical Methods

High-performance liquid chromatography(HPLC) has been used to separate and quantitate the individual oligomers of a broad spectrum of PEGs[14]. The individual PEGs in perfusate samples were separated using a 5μm reverse-phase column(Spherisorb, C8, 250×4.6mm) with 42% methanol as eluent and analyzed with refractive index detection(Model 410 differential refractometer ; Waters and Associates, Milford, MA, USA). The refractometer was programmed to maintain a constant temperature of 30℃. Each perfusate sample was subjected to a direct assay without extraction. Assay standards were prepared by spiking known quantities of PEG 400, 600, and 1000 into isotonic perfusate solution. A calibration curve for each oligomer was obtained by linear regression analysis of peak height against the known total polymer concentration. The peak height for each oligomer in the unknown sample was calculated and converted to the relative amount of PEG present using

Table 1. Composition of isotonic electrolyte perfusion solution (m mol/L)

Sodium Chloride	25	Sodium Sulphate	40
Potassium Chloride	10	Sodium Bicarbonate	20
Mannitol	80	PEG 4000	1.25×10^{-3}

the regression equation. Perfusate samples were dissolved in 4mL of scintillation fluid(Opti Phase 'Hisafe' II ; LKB Scintillation Products, U.K.) in polythene vials prior to counting ^{14}C activity in liquid scintillation spectrophotometer(1218 RackBeta Instrument, LKB Wallac, Helsinki, Finland).

6. Calculations

Percentage absorption of each oligomer was assessed by decreases in the concentrations of oligomers in the reservoir, with correction of any water movement by ^{14}C-PEG 4000 activity. A control experiment without the intestine indicated that there was no loss of compound during 3 hr period of the study. As a reasonable approximation loss of each oligomer from the reservoir occurred monoexponentially. Apparent intestinal permeability per unit length was calculated in order to determine the intrinsic absorption potential of PEGs in jejunum of the small intestine. A simple relationship relating the first-order absorption rate constant(k) to permeability coefficient was used to calculate the apparent permeability(P_{app}) of each oligomer, as follows ;

$$P_{app}(cm \cdot sec^{-1}) = \frac{K \cdot V}{A} \qquad (1)$$

where V is the volume of the reservoir and A is the estimated diffusional area of intestinal lumen.

Results

Fig. 1 demonstrates jejunal absorption of PEG oligomers(330-1122 D) after recirculation for 3hr in perfused rats. The percent absorbed of the PEGs over the 3hr period decreased with increasing molecular weight. Absorption ranged from 45%(MW 330 D) to 3.3%(MW 1122 D) in the jejunum. Net water absorption during the 3hr period of the study was 1.1 ± 0.24mL in the jejunum. The absorption was only 6% of the total volume of the reservoir.

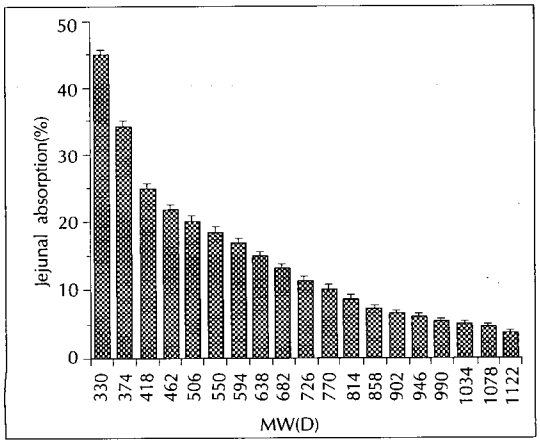

Fig. 1. Intestinal absorption of PEG oligomers(330-1122 D) after recirculation for 3 hr in perfused rats. Values are means ± SEM for five rats.

Table 2 shows the apparent permeability of PEG oligomers in perfused rats. Corrected for the length of jejunum used in the study, over the molecular weight range 330 to 1122 D, the apparent permeability(P_{app}) of PEG ranged from $4.92 \pm 0.02 \times 10^{-5}$cm/sec(mean ± SEM, n=5) to $0.28 \pm 0.02 \times 10^{-5}$cm/sec.

A logarithmic plot of the apparent permeability against molecular weight is shown in Fig. 2. The apparent permeabilities of PEGs are inversely proportional to $MW^{2.1}$ in jejunum.

Discussion

Polyethylene glycol(PEG) has been widely used as an ideal probe to study intestinal permeability via the paracellular pathway[1)2)15)]. Earlier studies with polyethylene glycols were restricted to PEG 400[1)2)], which comprise primarily of oligomers between 242-594 D. However, recent studies have shown that intestinal absorption of higher molecular weight oligomers occurs even up to PEG 2000, although beyond PEG 1000 very little molecular weight dependency is seen[16)]. Most studies have involved measures of percent absorbed as a function of molecular weight. The current study is concerned with intestinal permeability of oligomers of PEG up to 1000 along the small intestine. By mixing commercial sources of PEG 400, 600 and 1000 it was possible to study simultaneously all the oligomers between 330 and

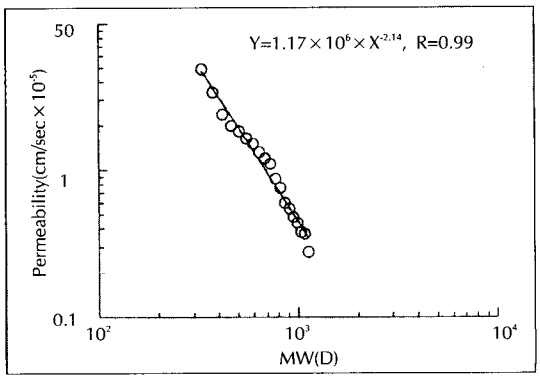

Fig. 2. Intestinal permeability-molecular weight profiles(log-log plot) for PEG oligomers(330-1122 D) in perfused rats.

Table 2. Apparent intestinal permeability of PEG oligomers in perfused rats.

MW(D)	Jejunum ($cm/sec \times 10^{-5}$)
330	4.92 ± 0.02*
374	3.42 ± 0.15
418	2.40 ± 0.09
462	2.00 ± 0.05
506	1.84 ± 0.04
550	1.65 ± 0.02
594	1.52 ± 0.02
638	1.33 ± 0.03
682	1.21 ± 0.05
726	1.11 ± 0.05
770	0.88 ± 0.04
814	0.76 ± 0.05
858	0.60 ± 0.03
902	0.55 ± 0.02
946	0.48 ± 0.02
990	0.44 ± 0.03
1034	0.38 ± 0.04
1078	0.37 ± 0.05
1122	0.28 ± 0.02

*Values are means ± SEM for five rats.

1122 D. Good resolution of each oligomer was achieved by HPLC.

Increasingly, many small polar molecules are thought to be absorbed via the paracellular pathway[17]. In an attempt to define quantitatively the factors controlling paracellular absorption, studies have been undertaken in the perfused rat intestinal preparation as a model of events in man. It is believed that the results in rat are likely to have application to man[18].

As a reasonable approximation loss of each oligomer from the reservoir occurred monoexponentially (Data not shown). Based on this observation, the value of the first-order absorption rate constant(k) was calculated. In this study, assuming that the segment is a distended uniform cylinder, the radius of the jejunum was 0.19cm. This corresponded to internal surface area of $24cm^2$ for jejunum. Substituting this information into Equation 1 allowed the estimation of the apparent permeability.

The absence of a cut-off phenomenon over the molecular weight range studied(Fig.2) suggests that a similar mechanism of permeation exists for both small and large molecules with movement through aqueous channels between cells. The results in this study(Table 2 and Fig. 2) showed that intestinal permeability(P) was inversely proportional to MW^2($P \sim 1/M^n$, n=2). Czaky(1987)[19] reported that intestinal permeability was negatively associated with molecular weight($P \sim 1/M^n$, n=1). In the present study, it was observed that intestinal permeability was more sensitive to MW changes($P \sim 1/M^2$) compared to the data shown by Czaky(1987)[19]. However, Hollander et al(1988)[20] reported that intestinal permeability was better correlated with the smallest cross-sectional diameter of the probes than with molecular weight.

The perfusate medium(Table 1) containing mannitol was chosen to minimise the net movement of water across the small intestine. This objective was achieved, with only a 6% net absorption of water occurring over the 3 hr period of study. Addition of glucose has been known to cause greater water absorption in intestine[21]. Nonetheless it is important to correct concentration in the reservoir for water movement when calculating rate of solute absorption, especially for poorly absorbed compounds. We used ^{14}C-PEG 4000 as a nonabsorbable marker for fluid transport.

The hypothesis that protein hydrolysates will provide an absorptive advantage over free amino acids in patients with reduced absorptive area or severe exocrine pancreatic insufficiency still remains unproved. Widespread use of hydrolysate-based diets is unjustified in the majority of patients, on the grounds of superior absorption characteristics. There may be some groups with severely impaired small bowel function who may benefit from use of a dipeptide- and tripeptide-based diet, rather than those which are currently available[12][13]. This benefit may result from the changes in intestinal permeability of patients who have impaired bowel functions. Characterization of the intestinal permeability using an ideal marker(e.g. PEGs) would provide an useful base to determine the differences of nutrient absorption in health and disease.

In conclusion, it was the first time to observe that intestinal permeability was decreased inversely with MW^2 rather than MW^1. The results in this study suggest that molecular weight or size is a important factor in determining intestinal permeability. It should be further investigated whether the intestinal permeability is changed in patients with critical illness or diseases of gastrointestinal tract.

Literature cited

1) Chadwick VS, Phillips SF, Hofmann AF. Measurement

of intestinal permeability using low molecular weight Polyethylene Glycols (PEG 400). I. Chemical analysis and biological properties of PEG. *Gastroenterology* 73 : 241-246, 1977a

2) Chadwick VS, Phillips SF, Hofmann AF. Measurement of intestinal permeability using low molecular weight Polyethylene Glycols (PEG 400). II. Application to normal and abnormal permeability states in men and animals. *Gastroenterology* 73 : 247-251, 1977b
3) Loehry CA, Kingham J, Baker J. Small intestinal permeability in animals and man. *Gut* 14 : 683-688, 1973
4) Tagesson C, Sjodahl R, Thoren B. Passage of molecules through the wall of the gastrointestinal tract. I. A simple experimental model. *Scand J Gastroenterol* 13 : 519-524, 1978
5) Bjarnason I. Intestinal permeability. *Gut Suppl* 1 : S18-S22, 1994
6) Bode C, Vollmer E, Hug J, Bode CH. Increased permeability of the gut to polyethylene glycol and dextran in rats fed alcohol. *Ann NY Acad Sci* 625 : 837-840, 1991
7) Oliva A, Armas H, Farina JB. HPLC determination of polyethylene glycol 400 in urine ; oligomeric profile in healthy and celiac disease subjects. *Clin Chem* 40 : 1571-1574, 1994
8) Peeters M, Hiele M, Ghoos Y, Huysmans V, Geboes K, Vantrappen G, Rutgerts P.Test conditions greatly influence permeation of water soluble molecules through the intestinal mucosa : need for standardisation. *Gut* 35 : 1404-1408, 1994
9) Bright-Asare P, Binder HJ. Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. *Gastroenterology* 64 : 1-8, 1973
10) Fordtran JS, Rector FC, Soter MF, Kinney J. Permeability characteristics of the human small intestine. *J Clin Invest* 44 : 1935-1944, 1965
11) Heimburger DC. Peptides in clinical perspective. *Nutr in Clin Pract* 5 : 225-226, 1990
12) Zaloga GP. Physiological effects of peptide-based enteral formulas. *Nutr in Clin Pract* 5 : 231-237, 1990
13) Fogel MR, Ravitch MM, Adibi SA. Free amino acid and dipeptide absorption in the jejunum of patients with jejuno-ileal bypass for obesity. *Gastroenterology* 68 : 894, 1975
14) Tagesson C, Sjodahl R. Passage of molecules through the wall of the gastrointestinal tract. Urinary recovery of different-sized Polyethylene Glycols after intravenous and intestinal deposition.*Scand J Gastroenterol* 19 : 315-320, 1984
15) Ma TY, Hollander D, Krugliak P, Katz K. PEG 400, a hydrophilic molecular probe for measuring intestinal permeability. *Gastroenterology* 98 : 39-46, 1990
16) Donovan MD, Flynn GL, Amidon GL. Absorption on polyethylene glycol 600 through 2000 : The molecular weight dependence of gastrointestinal and nasal absorption. *Pharmaceut Res* 7 : 863-868, 1990
17) Riley SA, Kim M, Sutcliffe F, Kapas M, Rowland M, Turnberg LA. Effects of a non-absorbable osmotic load on drug absorption in healthy volunteers. *Brit J Clin Pharmac* 34 : 40-46, 1992
18) Kim M, Rowland M. Absorption of hydrophilic drugs from the rat small intestine. First Annual Meeting of the U.K. Association of Pharmaceutical Scientists. York Abstract : P32. Apr.14-16, 1992
19) Czaky TZ. Intestinal permeation : an overview. *Handbook Exp Pharmacology* 70 : 50-89, 1987
20) Hollander D, Ricketts D, Boyd CAR. Importance of 'probe' molecular geometry in determining intestinal permeability. *Can J Gastroenterology* 2 (Suppl. A) : 35A-38A, 1988
21) Rainbird AL, Low AG, Zebrowska T. Effects of guar gum on glucose and water absorption from isolated loops of jejunum in conscious growing pigs. *Br J Nutr* 52 : 489-498, 1984

=국 문 초 록=

장내 관류된 동물에서 Polyethylene Glycols에 의한 장내 투과율 (Intestinal Permeability) 측정에 관한 연구

김 미 혜

영국 Manchester 대학교, 약학과
Department of Pharmacy, University of Manchester, Manchester M13 9PL, U.K

최근에 Polyethylene glycol(PEG)은 intestinal permeability을 측정하는데 좋은 지표물질(marker)로서 사용되어지고 있다. 본 연구는 330-1122 D 범위의 PEGs(400, 600, 1000)의 혼합물(1 : 2 : 20 ratio)을 사용하여 분자량과 permeability의 관계를 검토하고자 살아있는 동물의 소장(공장)내에서 *in situ* recirculation perfusion technique을 이용해 시도되었다. 각각의 PEG oligomers등은 refractive index detection에 의한 HPLC로 분석하였다. 실험에 사용된 공장의 길이를 고려했을때 PEG의 apparent permeability(PEGs 분자량 범위 : 330-1122 D)는 $4.92 \pm 0.02 \times 10^{-5}$cm/sec(mean ± SEM, n=5)에서 $0.28 \pm 0.02 \times 10^{-5}$cm/sec이었다. 또한 apparent intestinal permeability가 (분자량)^{2}에 역비례함을 보였다. 본 연구결과로 부터 분자량이 intestinal permeability에 영향을 미치는 중요한 요인임이 시사된다.