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Effect of bioadhesive polymer on phenol red absorption in normal and ulcer rats

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Abstract

The influence of a bioadhesive polymer, poly(acrylic acid) crosslinked with 2,5-dimethyl-1,5-hexadiene (PADH) on the absorption of a poorly absorbable water-soluble dye (phenol red), was studied in normal and ulcer rats. The urinary recovery after oral administration of phenol red solution was significantly increased in normal and indomethacin-ulcer rats pre-administered with bioadhesive polymer. The percentage of urinary recovery in normal, ulcer, normal pre-administered polymer, and ulcer pre-administered polymer rats were 2.7 ± 0.2, 8.3 ± 0.8, 22.6 ± 1.5, and $27.2 \pm 1.7\%$, respectively. The results indicated that PADH can enhance the absorption of water soluble compound but through a different mechanism when compared with sparingly soluble compounds. The polymer has the ability to attach to the mucin/epithelial cell surface, as determined by visual inspection of the rat stomach, however it could only detain a low percentage of phenol red in the polymer as confirmed by the in-vitro affinity study under gastric condition. These findings suggested that the enhancement of absorption of phenol red by the bioadhesive polymer is essentially by the increase of permeability of gastrointestinal (GI) membrane to the transport of phenol red through thinning or damage of the protective mucosal layer. © 1997 Elsevier Science B.V.

Keywords: Phenol red absorption; PADH bioadhesive polymer; Bioadhesion; Increased permeability

1. Introduction

Phenol red (Phenolsulfonphthalein), a water-soluble dye used as often as volume change indicator, is a well known poorly absorbed compound from the rat stomach and small intestine (Schanker et al., 1957, 1958). Studies conducted by Nakamura et al. (1976a,b) suggested the very low affinity to the GI mucosa and the poor lipoid solubility as possible reasons for the poor absorbability of this compound. Many workers had studied the enhancement of phenol red absorption in recent years. One of the known paths is by increasing its permeability to the intestinal mucosa. Surfactants have often been used to provide

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this effect (Feldman et al., 1970; Swenson et al., 1994). The suggested mechanism of action is based on the change in the permeability of GI membrane to the transport of the drug. Nakamura et al. (1982) provided further evidence of the increased permeability of this compound to the GI barrier via mucosal damage.

In the last decade many researchers have shown interest in using bioadhesive polymers as absorption enhancers of drugs in various drug delivery systems. These polymers have been shown to prolong the residence time of the delivery system by adhering to the mucus layer at the site of absorption, thereby increasing the contact time and the intimacy of contact of the drug with its absorbing membrane. The above studies (Longer et al., 1985; Tur et al., 1997) were mainly conducted on dosage forms containing compounds which are sparingly soluble in water consequently the increase in GI transit time would increase the period for the drug to dissolve. However, no study has been done on the effect of bioadhesive polymer on water soluble compounds in solution forms which have bioavailability problem. The aim of this experiment is to study the effect of bioadhesive polymer PADH on the absorption of water soluble phenol red in a solution form and its possible mechanism(s) of enhancement of absorption in rat.

2. Materials and methods

2.1. Materials

Phenol red, acrylic acid, benzoyl peroxide were purchased from E. Merck (Darmstadt, Germany). Indomethacin and carboxymethyl cellulose sodium salt (CMC) were from Sigma (St. Louis, MO). Sodium hydroxide was from R and M (Essex, UK), magnesium sulfate heptahydrate from BDH Chemicals (Poole, UK) and 2,5 dimethyl-1,5-hexadiene from TCI (Kasei, Japan). All other chemicals were at least of analytical grade.

2.2. Synthesis of the bioadhesive polymer

Polymer of acrylic acid cross-linked (0.3%) with 2,5-dimethyl-1,5-hexadiene (PADH) was synthesized according to the method of Ch'ng et al. (1985). The polymer was extensively washed with distilled water and then dried in a hot air oven at 90°C for 24 h before being ground to the required size of 400–630 μm.

2.3. Absorption studies in rats

Eighty female rats of the Sprague-Dawely strain, weighing between 150 and 200 g, were fasted for 24 h in individual wire-bottom cylindrical stainless steel cages with water allowed ad libitum. The animals were randomly divided into four groups (each of 20 rats) namely: (A) normal rats; (B) indomethacin-induced ulcer rats; (C) normal rats pre-administered with polymer; and (D) indomethacin-induced ulcer rats pre-administered with polymer. Groups (A) and (C) received intragastric treatment with 4 ml of saline whereas for groups (B) and (D), 100 mg/kg of indomethacin freshly suspended in 1% CMC was given. The latter dose was known to be ulcerative after 15 h of starvation, with water allowed ad libitum (Nakamura et al., 1982). After 15 h, the Groups (A) and (B) were given 4 ml of saline and Groups (C) and (D) were administered with 150 mg bioadhesive polymer (PADH) in capsule (# 4) and 4 ml saline. Both administrations were given by using a modified plastic syringe, under light ether anesthesia. Two hours later, all the rats received an oral dose of 2 μmol of phenol red in 2 ml normal saline and placed in a metabolic cage where the urine was collected quantitatively for 8 h. The volume of the urine was measured by using a calibrated cylinder, and the phenol red content of the voided sample was determined.

2.4. Assay of phenol red in urine

Phenol red in the urine samples was assayed colorimetrically using the method reported by Nakamura et al. (1976a, 1982). One ml of the voided urine sample was alkalinized with 5 ml of 1N NaOH and the absorbance of the solution was

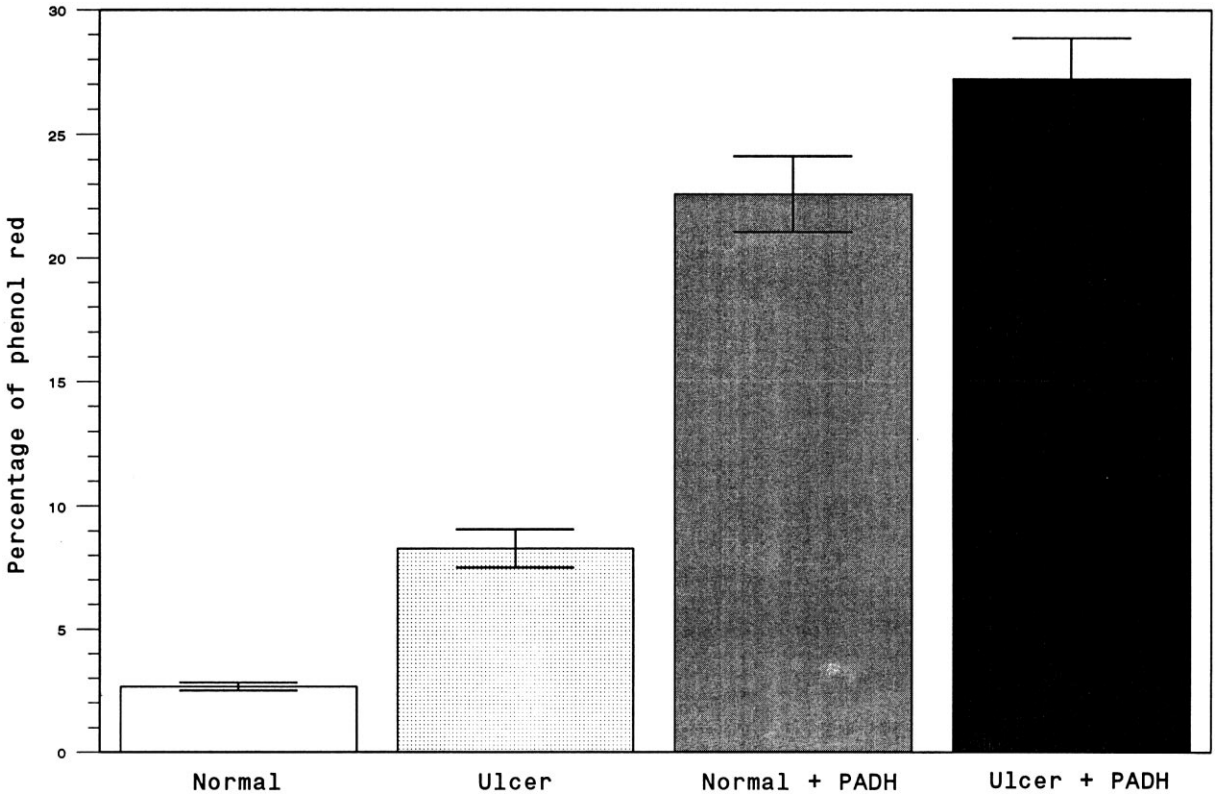

Fig. 1. Urinary recovery of phenol red after 8 h in experimental rats. Each bar represents the mean ± S.E. of 20 animals.

determined at 560 nm using a U-2000 spectrophotometer (Hitachi, Tokyo, Japan). The total amount of phenol red excreted was determined by means of a standard curve constructed from five known concentrations of phenol red ranging from 5 to 25 μg/ml in normal rat urine.

2.5. Statistical analysis

Statistical analysis was performed on the mean data using one way analysis of variance (ANOVA) followed by extended Tukey's test at an associated p level of < 0.05.

2.6. In-vivo bioadhesion study

In this study, a capsule containing 150 mg PADH, previously stained with methylene blue, was given to each of the normal and ulcer rats. After 2 h, the rat was sacrificed with an over dose of ether. The stomach was excised, opened along the greater curvature and the mucosal surface was examined. The extent of adhesion of the polymer to the stomach was evaluated visually and then photographed.

2.7. Phenol red affinity study

The binding ability of phenol red to the bioadhesive polymer was investigated under gastric condition. PADH (150 mg) was dispersed in 10 ml of simulated gastric fluid containing 2 μmol of phenol red. The ability of the polymer to absorb the dye was determined by measuring the decrease in the absorbance of the solution from its initial value at 505 nm using spectrophotometer model U-2000 (Hitachi, Tokyo, Japan).

Table 1
Analysis of variance (ANOVA)

Source of variance	SS	DF	MS	*F*	*P*
Treatment	8078.025	3	2692.675	92.793	<0.001
Residual	2205.395	76	29.018		
Total	10 283.420	97			

3. Results

3.1. Urinary recovery of phenol red

Fig. 1 shows the urinary recovery of phenol red, expressed as percent of dose, after an oral administration of phenol red solution to normal and ulcer rats in the presence or absence of a bioadhesive polymer. The percentage of urinary recovery of phenol red in 8 h from normal, ulcer, normal pre-administered polymer and ulcer pre-administered polymer rats were 2.7 ± 0.2, 8.3 ± 0.8, 22.6 ± 1.5 and $27.2 \pm 1.7\%$, respectively. Statistical analysis using one way analysis of variance (Table 1) indicated significant differences ($p < 0.001$) between the treatment groups. A posterior comparison among the means using the Tukey's test (Table 2) revealed that the urinary recovery of phenol red in the four groups was significantly different.

The urinary recovery of phenol red was significantly increased in ulcer rats. Our results showed that creating mucosal damage results in an approximately three-fold increase in the absorption of the drug across the GI membrane.

Table 2
Statistical analysis using Tukey's test

Group comparison[a]	Mean difference	Critical value	*P*
A vs. B	5.607	4.507	0.001
A vs. C	19.932	4.507	0.001
A vs. D	24.537	4.507	0.001
B vs. C	14.325	4.507	0.001
B vs. D	18.930	4.507	0.001
C vs. D	4.605	4.507	0.050

[a] A, normal; B, ulcer; C, normal/PADH; D, ulcer/PADH.

Similarly, oral pre-administration of a bioadhesive polymer markedly enhanced the absorption of this poorly absorbable compound ($p < 0.001$). The presence of the polymer resulted in an approximately eight and three-fold increase in the drug absorption in normal and ulcer rats respectively. The rank order of drug absorption was (from highest to lowest):

$$\text{Ulcer} + \text{PADH} > \text{normal} + \text{PADH} > \text{ulcer} > \text{normal rats.}$$

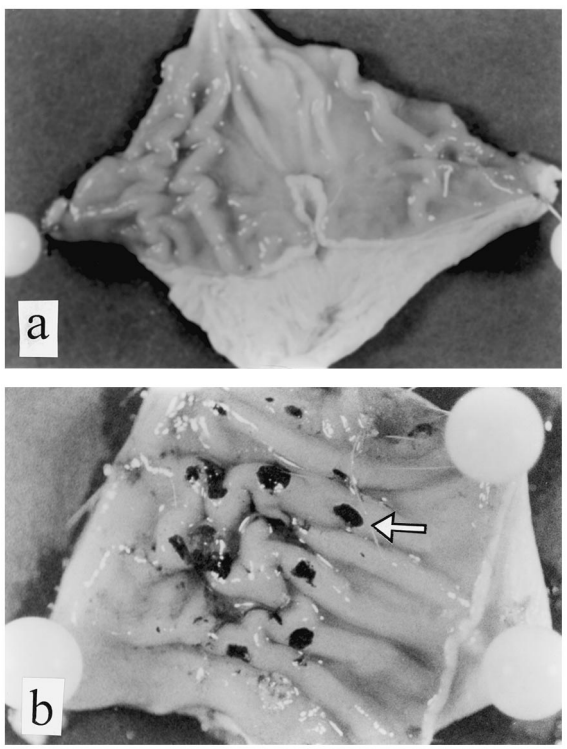

Fig. 2. Representative photographs of gastric mucosa 15 h after an oral administration of indomethacin suspension (b) compared to that administered saline as a control (a).

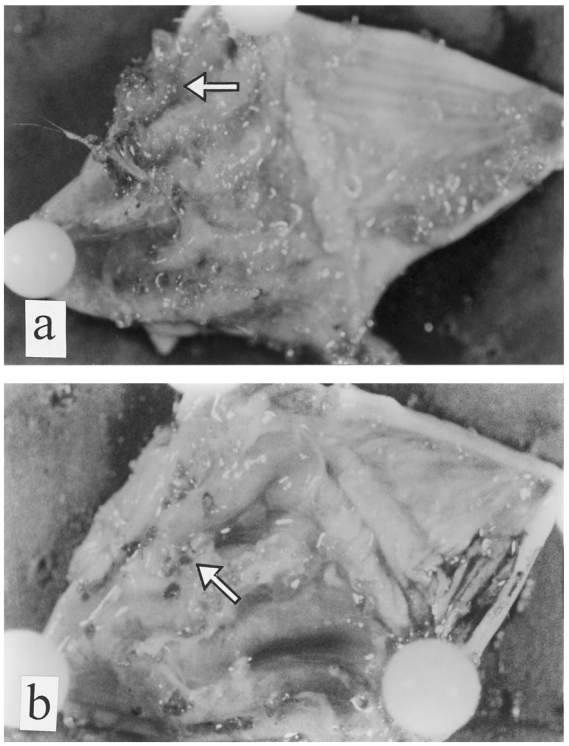

Fig. 3. Representative photographs of gastric mucosa taken after 2 h of an oral administration of a bioadhesive polymer to normal (a) and ulcer rats (b).

3.2. Bioadhesion studies

Oral administration of indomethacin suspension to normal rat produced severe lesions in rat's stomach. The ulcer appeared as dark-red elongated bands throughout the funds (Fig. 2b). Examination of the rats stomach after oral administration of a bioadhesive polymer revealed that the polymer particles adhered strongly to the mucus and to the exposed surface tissue (Fig. 3a,b). The adhesion was so strong that they could not be removed from the stomach even by rinsing the gastric mucosa with distilled water.

3.3. Phenol red affinity study

Phenol red showed only slight affinity to the polymer under gastric condition (Fig. 4). Results indicated that the polymer had the ability to attract only 17% of the phenol red during the course of the study.

4. Discussion

The result of in-vitro affinity study indicates that only about 17% of phenol red can be absorbed onto the polymer and even taking into account a possible dynamic equilibrium of phenol red between the polymer and the surrounding solution is existed will not be able to explain the huge increase in absorption if the mechanism is solely due to the prolonged GI transit of phenol red loaded bioadhesive polymer (Tur et al., 1997). We believe in the case of absorption of phenol red from a solution and other highly water soluble compounds, the main mechanism of enhanced absorption is the alteration of GI membrane permeability although the increase in GI transit may also contribute to the enhancement, however, the contribution is minor. The adhesion of polymer particles to the mucin layer and the subsequent dislodgment from this lining will affect the continuity and the thickness of the mucin layer and consequently it will also affect the rate and the amount of absorption of phenol red. It is known that one of the main functions of mucin layer is to control the diffusion process of molecules across this viscous layer. If this layer is damaged, destroyed or thinned as happened in the above experiments, the absorption of even hardly absorbable phenol red is greatly increased. The treatment of fasted rats with indomathacin can only produce a limited number of lesions in the stomach (Fig. 2b), consequently it can only increase three folds in the absorption of phenol red when compared with normal rats (Fig. 2a). In comparison, the polymer can interact with a larger surface area of the stomach lining (Fig. 3a) hence producing a greater eight-fold increase in absorption. The result from the group with indomethacin-induced ulcer rats pre-administered with polymer shows that the enhancement (27.2%) is slightly less than the summation of the above two effects (30.9%). This small difference can be resulted from the slight retardation of diffusion process of phenol red by the polymer which covers the ulcer (Fig. 3b).

The above results are in good agreement with the results obtained by Nakamura et al. (1982) and other researchers (Feldman et al., 1970;

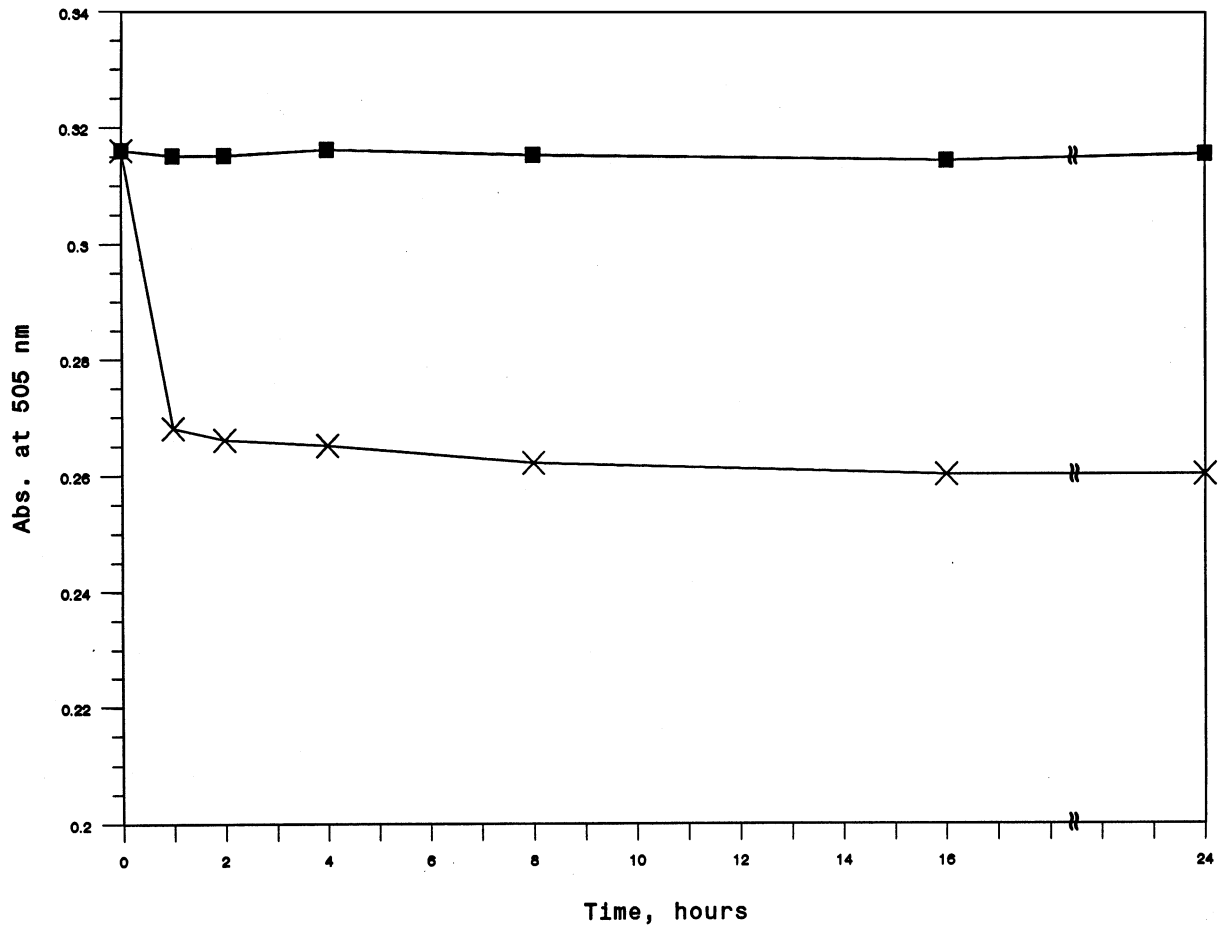

Fig. 4. Affinity of phenol red to the bioadhesive polymer (×) relative to that of control (■).

Swenson et al., 1994), however, it is worth mentioning that the use of classical enhancers such as surfactants and bile salts are frequently limited by the potential toxicity and acute local damage to the intestinal wall. Illum et al. (1994) had studied the use of mild bioadhesive polymer, chitosan as nasal permeation enhancer for peptide drugs and they reported that only small changes occurred on the integrity of the rat nasal mucosa after using chitosan. In our study, the polymer causes a significant change in the permeation of phenol red through the GI wall, possibly through the thinning or damage of the mucus layer when the polymer particles attach and detach from the layer. Furthermore, the polymer was found to have good ability to bind with the exposed cell surface of the ulcerated stomach hence offering some protection to the ulcer by decreasing the diffusion process and minimizes the direct contact of harmful substances to the exposed tissue. These findings may offer the possibility of using polymers as an alternative to the classical permeability enhancers.

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