Intestinal Permeability Enhancement: Structure-Activity and Structure-Toxicity Relationships for Nonylphenoxypolyoxyethylene Surfactant Permeability Enhancers

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INTRODUCTION

Non-ionic surfactants have been reported to be effective enhancers of the permeability of the walls of the alimentary tract (1–3). Studies generally indicate that non-ionic surfactants of intermediate polarity are most effective as permeability enhancers. Studies of the toxicity of non-ionic surfactants have generally shown that the polar non-ionics are nontoxic, while non-ionics of intermediate polarity exhibited some toxicity (4–7). We have recently demonstrated that permeability enhancement by surfactants is generally associated with transient superficial damage to the intestinal wall, and that this damage is reversible (8). In the present report, we describe an investigation of the structure-activity and structure-toxicity relationship for a homologous series of nonylphenoxypolyoxyethylene (NP-POE) surfactants. NP-POE-9, -10.5, and -20 (HLB < 17) increased the permeability of the intestinal wall to the polar drug phenol red, in a rat intestinal perfusion model. Perfusion with these surfactants also resulted in mucosal histological damage, and release of biochemical damage markers into the lumen. The more polar NP-POE-30, -50, -100 (HLB > 17) did not enhance phenol red permeability, and did not acutely damage the intestinal wall. These data indicate a correlation between intestinal permeability enhancement and acute damage to the intestinal wall, for enhancers of the NP-POE class.

EXPERIMENTAL

Materials

Nonylphenoxypolyoxyethylenes were obtained from Rhone-Poulenc (Princeton, NJ). The manufacturer's designations for NP-POE-9, -10.5, -20, -30, -50, and -100 are Igepal CO-630, 710, 850, 880, 970 and 990, respectively. These surfactants are polydisperse and the numerical designations -9, -10.5, etc. represent the average number of POE groups per molecule. Phenol red (sodium salt) was from Sigma Chemical Co. (St. Louis, MO).

Methods

Rat intestinal perfusions were carried out as previously described (8). Briefly, phenol red (0.25 mg/ml) was co-perfused for 1 hr with various NP-POEs at a surfactant concentration of 1% (w/v). The absorption rate constant K_A was calculated from:

$$K_A = \frac{Q(1 - C_o/C_i)}{V}$$

where Q is the measured flow rate (~0.2 ml/min), V is the volume of the perfused intestinal segment (2.77 cc), C_i is the ingoing phenol red concentration, and Co is the outgoing phenol red concentration. K_A was determined for the last three 15 min intervals during the perfusion, and was averaged for each perfusion experiment. Plasma phenol red concentrations were determined in a separate group of rats at the end of a 1 hr perfusion, in which the starting perfusate phenol red concentration was 2 mg/ml. Phenol red was assayed using HPLC, as previously described (8).

Lactate dehydrogenase and lipid phosphate were assayed in perfusates as previously described (8). Intestinal segments were prepared for histological evaluation as previously described (8). Various measures of histological abnormality were quantitated on an arbitrary scale of 0–3, with 0 indicating no effect and 3 indicating an extensive effect. This evaluation was carried out by an experienced veterinary pathologist in a blinded fashion.

RESULTS

Absorption Enhancement

Table I presents phenol red absorption rate constants, obtained by rat intestinal perfusion of phenol red with members of a homologous series of NP-POEs. K_A was significantly greater than control when phenol red was co-perfused with NP-POE-9, -10.5, -20, or -30 ($p < 0.01$). NP-POE-50 and -100 were ineffective permeability enhancers. Table I also presents steady state phenol red plasma levels at the end of a 1 hr perfusion. These data demonstrate that NP-POE-9, -10.5, and -20 were effective permeability enhancers while NP-POE-30, -50, and -100 were not. The fold-increase in plasma phenol red concentration in the presence of the enhancing NPPOE's (e.g. NP-POE-10.5) is much larger than the fold-increase in K_A (Table I). While this difference might result from artifactually low K_A's due to surfactant-induced transintestinal water flux (9–11), we consider this explanation to be unlikely, based on the good reproducibility of the K_A data. Phenol red is known to exhibit saturable biliary extraction and hepatic metabolism (17), and the observed superproportionality in drug plasma level could be due to saturation of these clearance pathways in the presence of the permeability enhancers. It is also possible that the NP-POE

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Table I. Effects of 1% Nonylphenoxypolyoxyethylene Surfactants (NP-POE) on the Absorption of Phenol Red, in Rat Intestinal Perfusions. Absorption Rate Constant K_A is Averaged Over 2nd, 3rd and 4th 15 min Interval of a 1 hr perfusion. Plasma Phenol Red Concentration was Measured at the End of a 1 hr Perfusion

Treatment	HLB†	n_A*	K_A ($\times 10^3$ min^{-1})	n_B**	Plasma phenol red (micro gm/ml)
Control	—	8	2.3 ± 0.4	8	0.35 ± 0.12
NP-POE-9	13.4	4	5.4 ± 0.4	4	22 ± 4
NP-POE-10.5	14.0	6	9.6 ± 2.0	5	31 ± 10
NP-POE-20	16.3	5	9.9 ± 5.0	4	25 ± 8
NP-POE-30	17.4	4	4.0 ± 0.7	4	0.24 ± 0.15
NP-POE-50	18.3	4	2.7 ± 1.3	4	0.15 ± 0.05
NP-POE-100	19.1	4	2.8 ± 0.7	4	0.42 ± 0.14

* Number of rats averaged for K_A measurement.
† Hydrophile-lipophile balance value
** Number of rats averaged for plasma phenol red measurement.

surfactant is itself absorbed, and interferes in some way with phenol red clearance mechanisms.

Intestinal Wall Damage

Table II presents the quantities of LDH and lipid phosphate released into the intestinal lumen during a 1 hr coperfusion of phenol red with NP-POEs. Significant quantities of LDH were released during perfusions with NP-POE-9, -10.5, and -20. LDH was not released into the lumen during perfusions with the more polar homologs NP-POE-30, -50, and -100. Similar effects were observed for release of membrane phospholipid into the lumen (Table II). For example, the micromoles lipid phosphate released during perfusions with NP-POE-20 differed from control at the $p < 0.01$ level.

Histological evaluation was performed on segments of intestine which were perfused for 1 hr with phenol red in the absence and presence of various NP-POEs. Cross sections of intestine perfused with phenol red exhibited well aligned viable epithelial cells, with some typical sloughing of mature cells at the villous tips. For intestines perfused with phenol red and 1% NP-POE-20, large quantities of mucus and debris were observed, villi were contracted and swollen, and many epithelial cells were rounded and poorly aligned. Cross sections of intestine perfused with phenol red and 1% NP-POE-30 were similar to the control phenol red perfusion, and did not exhibit the abnormalities seen as a result of perfusion with NP-POE-20.

Histological sections were scored for a variety of abnormalities on a scale from 0 (no effect) to 3 (severe effect). Scores for individual animals are presented in Table III, as well as an average total score for each surfactant treatment. Mucus/debris refers to the presence of basophilic material and lysed cells in the lumen. Villous shortening refers to apparent retraction of villi. Erosion refers to loss of epithelium, exposing the lamina propria, without ulceration. Swollen epithelial cells are an indication of cytoplasmic fluid gain. Flat epithelial cells refers to cells which are short, and have spread laterally in an apparent attempt to cover voids in the epithelium. [Goblet cells] (concentration of goblet cells) is an indication of relative loss of columnar epithelial cells. In general, goblet cells appeared to be more resistant than the absorptive enterocytes to surfactant-induced damage.

The data in Table III indicate that NP-POE-9, -10.5, and -20 cause significant acute damage to the intestinal epithelium. NP-POE-30 and -50, on the other hand, exhibit no significant local toxicity. This striking structural dependence of local toxicity is similar to that observed for the two above described biochemical damage markers and for phenol red permeability enhancement.

Table II. Biochemical Markers of Intestinal Wall Damage. Total Units of Lactate Dehydrogenase (LDH) and Total Lipid Phosphate Released into the Perfusate were Measured During a 1 hr Rat Intestinal Coperfusion of Phenol Red (0.25 mg/ml) with Various Nonylphenoxypolyoxyethylene (NP-POE) Surfactants (1%, w/v)

Treatment	n*	LDH (units)	Lipid phosphate (micromoles)
Control	8	3 ± 1.0	0.17 ± 0.17
NP-POE-9	4	26 ± 4	0.65 ± 0.23
NP-POE-10.5	6	31 ± 9	1.16 ± 0.33
NP-POE-20	5	19 ± 13	0.74 ± 0.34
NP-POE-30	4	1.19 ± 0.48	0.13 ± 0.12
NP-POE-50	4	0.83 ± 0.47	0.18 ± 0.14
NP-POE-100	4	1.39 ± 0.14	0.23 ± 0.05

* Number of rats averaged.

DISCUSSION

The use of non-ionic surfactants as intestinal permeability enhancers has been widely studied (reviewed in 12, 13). In the present study, we have directly compared permeability enhancement and local toxicity for a series of homologous NP-POE non-ionic detergents. Figure 1 presents the correlation of phenol red absorption rate constant K_A with LDH released into the perfusate, for each individual perfusion experiment. It is clear that the phenol red absorption rate is correlated with release of the biochemical damage marker, for the studied series of homologous NP-POEs and mixtures ($R^2 = 0.77$). Figure 2 presents the correlation of phenol red K_A with lipid phosphate released into the perfusate, for each individual perfusion experiment. The phenol red absorption rate is correlated with this second marker of intestinal wall damage ($R^2 = 0.71$). Furthermore, histological evaluation of surfactant-perfused intestines demonstrates that those homologs which enhance phenol red permeability also cause acute morphological damage of the intestinal epithelium. These observations are consistent with a recent study which surveyed a variety of anionic and non-ionic surfactants (8). The association of permeability enhancement with local toxicity suggests that the observed increased drug transport occurs through damaged epithelial regions. This is not proven by the present work, and the possibility remains that permeation enhancement and local toxicity are independent sequelae of the interaction of non-ionic surfactants with the intestinal epithelium, although we consider this latter interpretation less likely.

The sharp distinction in the behavior of NP-POE-20 and NP-POE-30 is striking. It is unlikely that the observed differences are due to the higher molar concentration of NP-

Table III. Histological Evaluation of Rat Small Intestinal Mucosa after 1 hr Perfusion with Phenol Red in the Absence and Presence of Various Nonylphenoxypolyoxyethylene Surfactants (NP-POE) (1%, w/v). See Text for Details.

	Score * for treatment with					
	Control	NP-POE-9	NP-POE-10.5	NP-POE-20	NP-POE-30	NP-POE-50
(# rats)	(2)	(3)	(2)	(2)	(2)	(1)
Mucus/debris	1, 1	3, 2, 3	2, 2	3, 2	0, 1	1
Villous shortening	0, 0	1, 1, 1	1, 1	2, 1	0, 0	0
Erosion	0, 0	1, 2, 0	3, 3	2, 2	0, 0	0
Swollen epithelial cells	0, 0	1, 1, 1	1, 0	0, 0	0, 0	0
Flat epithelial cells	0, 0	1, 1, 1	0, 1	1, 1	0, 0	0
[Goblet cells]	0, 0	1, 1, 1	1, 1	2, 2	0, 0	0
Total	1	8, 8, 7	8, 8	10, 8	0, 1	1
Average total score	1	7.6	8	9	0.5	1

* Scores range from 0 (no effect) to 3 (severe effect).

POE-20 compared to NP-POE-30. While the relative molar concentrations of NP-POE-20 and NP-POE-30 differ by only 33%, the ratio K_A(NPPOE-20)/K_A(NPPOE-30) was 2.5. The NP-POE-20/NP-POE-30 effect ratios for LDH and lipid phosphate were 16 and 5.7, respectively. These ratios are larger than would be predicted if the only difference between NP-POE-20 and NP-POE-30 was molecular weight.

Table I lists the HLB values (hydrophile-lipophile balance; 14, 15) for NP-POEs. It appears that a break point in efficacy and acute toxicity occurs at HLB ~17 for surfactants of the NP-POE structural class. Survey of the published literature on permeability enhancement and toxicity generally indicates a significant decrease in the efficacy or toxicity of non-ionic surfactants as the polarity is increased past some threshold HLB, although the threshold HLB is not necessarily 17 for all structural classes. Polysorbate 80, for example, has HLB = 15, but is an ineffective permeability enhancer (8). Macek and Krzeminski (6) reported studies of the toxicity of alkylphenoxy-POE and POE-ether surfactants in bluegill sunfish. In these studies, surfactants with HLB $\leq$ 14.2 were toxic, while those with HLB $\geq$ 17.4 were not. In another example, Ichikawa *et al.* (1) studied various polyoxyethylene ethers as enhancers of rectal insulin absorption. POE-ethers with HLB value between 12.5 and 14.9 were effective absorption enhancers. POE-ethers with HLB $\geq$ 15.7 or $\leq$9.4 were ineffective. Florence (16) has pointed out that a surfactant with a medium chain-length alkyl group will be capable of penetrating the membrane lipid bilayer. Because of its aqueous solubility, a medium chain surfactant has a greater monomer concentration (higher CMC) than a surfactant with a longer alkyl chain. Increasing the length of the alkyl chain may improve membrane penetration, but this

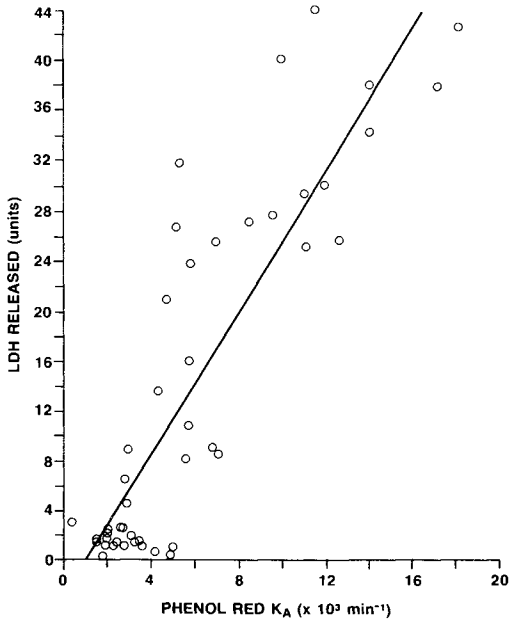

Fig. 1 Relationship between phenol red K_A and LDH released into the lumen, for 1 hr rat intestinal perfusions with phenol red and 1% (w/v) NP-POE. The NP-POE consisted of fractions with 9, 10.5, 20, 30, 50 or 100 POE units, or mixtures of NP-POE-10.5 and NP-POE-50.

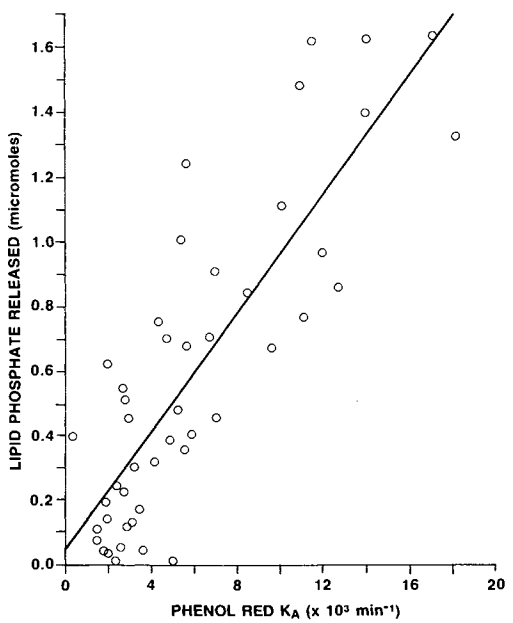

Fig. 2 Relationship between phenol red K_A and lipid phosphate released into the lumen, for 1 hr rat intestinal perfusions with phenol red and 1% (w/v/) NP-POE. The NP-POE consisted of fractions with 9, 10.5, 20, 30, 50, or 100 POE units, or mixtures of NP-POE-10.5 and NP-POE-50.

effect may be offset by a lower monomer concentration. Highly polar surfactants, e.g., NP-POE-100, may be so water-soluble that they fail to effectively partition into (and remain in) bilayer membranes.

In summary, NP-POE surfactants with HLB < 17 are effective permeation enhancers in the rat intestinal perfusion model. This permeation enhancement is associated with acute intestinal wall damage reflected by both biochemical and histological markers. NP-POE surfactants with HLB > 17 do not enhance permeability and do not cause intestinal wall damage in the perfusion model. While these studies permit comparison of the relative acute toxicity of homologous non-ionic surfactants, a true measure of toxicity can only be obtained via chronic oral toxicology testing in appropriate species.

NOTATION

hydrophile-lipophile balance value (HLB); lactate dehydrogenase (LDH); nonylphenoxy(polyoxyethylene)$_9$ (NP-POE-9).

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