Cancer Chemother Pharmacol (2001) 47: 27–33
DOI 10.1007/s002800000192

ORIGINAL ARTICLE

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The absorption of retinoic acids from the gastrointestinal tract is dependent upon chemical structure

Received: 11 February 2000 / Accepted: 20 July 2000 / Published online: 25 November 2000

Abstract *Purpose*: The gastrointestinal permeability of a number of retinoic acids was determined in order to evaluate whether the gastrointestinal membrane was able to distinguish between retinoids in which the polyene chain was present in several different isomeric forms. In addition, the structure of the six-membered ring was varied in order to determine which portion of the molecule was most important for its recognition by the membrane. The role of bile salt micelle composition in the intestinal absorption of retinoids was also evaluated. *Methods*: In situ perfused rat intestinal segment preparations ($n = 78$) were used, and the retinoids were each perfused at a concentration of approximately 1 μg/ml in either simple micelles of sodium taurocholate (10 m*M*) or mixed micelles of sodium taurocholate/egg phosphatidylcholine (10 m*M*/10 m*M*). The flow rate of the perfusate was either 0.1 or 0.35 ml/min. *Results*: For each retinoid, the mixed micelles were associated with a higher degree of retinoid uptake into the jejunal cells than were the simple micelles. In addition, the permeability was higher when the perfusate flow was greater, indicating that the aqueous boundary layer of the intestine contributes to the resistance to the disappearance of the retinoid from the intestinal lumen. Retinoid structure was also found to have a significant effect on the permeability in the mixed micelle systems at both low and high flow rates, but not with simple micelles. The structure of the six-membered ring was not a major determinant of the permeability. However, the permeability of the retinoids with the polyene chain in the 13-*cis* position was significantly greater than when the chain was all-*trans* or in the 9-*cis* position. *Conclusions*: The isomeric position of the polyene chain and the presence of phospholipid in the micellar vehicle have a significant influence on the membrane transport of the retinoic acids.

Key words Retinoic acid · Absorption · Gastrointestinal · Micelles · Membrane transport

Introduction

In recent years there has been a resurgence of interest in the clinical use of the retinoids in cancer prevention and chemotherapy. All-*trans*-retinoic acid (ATRA) is used successfully for treatment of acute promyelocytic leukemia [1, 5, 32, 38]. 13-*cis*-Retinoic acid (13CRA) has shown potential in the treatment [9] and chemoprevention of head and neck squamous cell carcinoma [17] and has been used in phase I trials in pediatric patients with neuroblastoma following bone marrow transplantation [19]. A retinamide, fenretinide, has also shown promise in the chemoprevention of breast and other cancers [18].

For use in chemoprevention, retinoid administration will be long-term, and the most likely route of administration will be oral. However, the oral bioavailability of the retinoids in general has been shown to be quite variable [2]. With the observation that the bioavailability of retinoids is generally increased in the presence of food [7, 8, 11, 15], and the indication that dietary retinol absorption is influenced by the composition of bile salt/fatty acid mixed micelles [16], it was hypothesized that micellar systems enhance the bioavailability of the retinoids. Further, this enhancement was thought to be structurally specific, with certain chemical structures allowing better partitioning into the mixed micelle.

From initial studies, several results are apparent. The most important finding is that the gastrointestinal membrane itself appears to distinguish between chemical

Portions of this work were presented at the 89th Annual Meeting of the American Association for Cancer Research, 28 March to 1 April 1998, New Orleans, La. (Proc Am Assoc Cancer Res 39:2128, 1998).

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structures. For example, in retinoids whose only structural difference is the hydrophilic group at the end of the polyene chain distal from the six-membered ring, the ethyl ester is clearly absorbed better than either the carboxylic acid or the amide [28]. In addition, this difference in absorption is not predictable from retinoid physicochemical characteristics such as the micelle/water partition coefficient or the aqueous solubility [22]. Indeed, the difference in absorption is most marked in an experimental set-up in which the gastrointestinal membrane is perfused at high perfusate flow rates [28].

In order to extend these findings, a series of retinoid carboxylic acids (Fig. 1) were studied to determine whether the gastrointestinal membrane was able to distinguish between retinoids in which the polyene chain was present in several different isomeric forms. In addition, the structure of the six-membered ring was varied in order to determine which portion of the molecule was most important in its recognition by the membrane.

Materials and methods

Chemicals

Retinoid acids, including ATRA, 13CRA, 9-*cis*-retinoic acid (9CRA), acitretin (ETA), 13-*cis*-acitretin (cETA), MTTO, and 13-*cis*-MTTO were received as gifts from Hoffmann-LaRoche (Nutley, N.J.). Several are shown in Fig. 1. Sodium taurocholate (NaTC) was purchased from Sigma Chemical Co. (St. Louis, Mo.) and was recrystallized by a modification of a published procedure [29]. Egg phosphatidylcholine (PC) was purchased from Avanti Polar Lipids (Alabaster, Ala.) and stored at −20 °C under nitrogen and protected from light. HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) was obtained from Sigma. All other reagents were reagent grade or better. All procedures were carried out under yellow light to minimize photodegradation of the retinoids.

The retinoids were perfused in two different micellar systems: simple micelles (10 m*M* NaTC) or mixed micelles (10 m*M* NaTC and 10 m*M* PC). The TC concentration was within the range observed physiologically [27], and these micellar systems are well characterized by a published phase diagram [26]. The final concentration of each retinoid in each micellar solution was targeted to be 1 μg/ml because the retinoids are completely solubilized at this concentration (unpublished data) [13, 22].

A solution of simple micelles of 10 m*M* NaTC containing a final concentration of approximately 1 μg/ml retinoid was prepared. A weighed amount of NaTC was dissolved in methanol in a flask. An aliquot of retinoid stock solution was added and the solvent was removed by evaporation. Isotonic 10 m*M* HEPES buffer solution, pH 6.5 (adjusted with 0.1 *N* NaOH) was added to the dried mixture of retinoid and NaTC. The contents were gently swirled and sonicated for 20 min. The clear simple micellar solution was allowed to equilibrate at room temperature overnight prior to use in intestinal perfusion studies.

A mixed micelle solution of 10 m*M* PC and 10 m*M* NaTC containing approximately 1 μg/ml of retinoid was prepared by the simultaneous lyophilization of PC with the retinoid, followed by the addition of the bile salt dissolved in buffer. Egg PC in chloroform was placed in a flask to which an appropriate aliquot of the retinoid stock solution was added. NaTC was dissolved in isotonic HEPES buffer, pH 6.5. Addition of NaTC did not alter the pH of the solution. The bile salt solution was added to the freeze-dried mixture and then gently swirled and sonicated. In some cases, the solution was warmed to 50 ºC to aid in clarification of the micellar solution. The solution was allowed to equilibrate overnight prior to use in intestinal perfusion studies.

Perfusion studies

Male Sprague-Dawley rats, weighing 250–300 g ($n = 78$) were purchased from Harlan (Madison, Wis.), and allowed to acclimate for at least 48 h in the animal facilities, receiving rat chow and water. The rats were fasted for 15–18 h prior to the perfusion studies with water allowed ad libitum. Each rat was anesthetized with an intraperitoneal injection of 55 mg/kg pentobarbital (Nembutal sodium solution; Abbott Laboratories), and an incision was made along the abdominal midline. The small intestine was exposed and a segment of jejunum was cannulated, with the L-shaped glass inlet cannula secured into the jejunum at the ligament of Trietz. The outlet cannula was placed approximately 12–18 cm downstream from the inlet cannula. The vasculature remained intact. The segment was slowly flushed with 12 ml prewarmed saline, then gently flushed with air. The inflow cannula was then attached to a syringe on a Harvard compact infusion pump (Harvard Apparatus, South Natick, Mass.) with PE-50 tubing.

The retinoid solution in simple or mixed micelles was then perfused through the segment at the appropriate rate for 120 min. A sample of the perfusate solution was taken immediately prior to the experiment and frozen for subsequent determination of the inflow concentration. The perfusate entering the segment was kept warm during the experiment with the aid of a heating pad placed

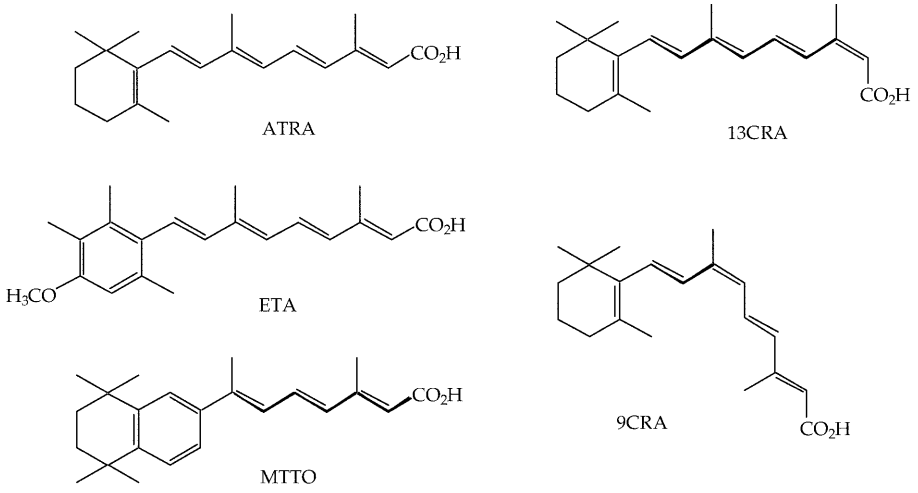

Fig. 1 Chemical structures of selected retinoic acids

around the syringe delivering the perfusate. Anesthesia was maintained with intraperitoneal doses of 15 mg/kg pentobarbital as needed. The body temperature was monitored throughout the perfusion with a rectal thermometer (Curtin Matheson Scientific, Eden Prairie, Minn.) and maintained at approximately 37 ºC with a heating pad and lamp. The perfused segment was kept moist with normal saline during the experiment. Outflow samples were collected in ten-minute intervals in preweighed glass vials, then weighed and frozen at −20 ºC until analysis. At the end of the experiment a sample of the solution remaining in the syringe was taken and frozen for subsequent determination of whether the retinoid had degraded during the experimental procedure. Samples were analyzed for retinoid concentration, NaTC concentration, and phospholipid (PC) concentration.

Each retinoid was perfused at a low flow rate (0.1 ml/min) and a high flow rate (0.35 ml/min) in both simple and mixed micelles. In general, 12 rats received a given retinoid at one of the various combinations of flow rate and perfusate composition. These micelle compositions had previously been shown by histological examination to have no effect on the integrity of the intestinal epithelium [28].

Analytical methods

Inflow and outflow samples were handled as reported previously [28]. Briefly, the outflow samples (500 µl) were placed in silanized glass centrifuge tubes and treated with twice the volume of acetone in order to precipitate the phospholipids. An appropriate internal standard was then added. The tubes were vortex-mixed for 1 min and then centrifuged at 1300 *g* (Clinicool, Damon/IEC, Needham Heights, Mass.) at 4 ºC for 15 min. The supernatant from each tube was transferred to amber autoinjection vials for HPLC analysis. Preliminary studies indicated that some of the retinoids bound nonspecifically to plastic. Hence the use of any plastic material that might be in direct contact with the retinoids was avoided.

The retinoids were analyzed by HPLC methods reported previously [22] with modification. The HPLC setup consisted of a Waters (Milford, Mass.) Model 6000A pump, a WISP 710B autoinjector module, a Shimadzu (Kyoto, Japan) SPD-6A UV variable wavelength detector, and a Shimadzu Chromatopac C-R6A integrator. The mobile phase consisted of acetonitrile in an ammonium acetate buffer, and was pumped at a flow rate of 1.5 ml/min. Detection was carried out at 350 n*M* and the elution was isocratic. The details of the individual HPLC methods for the retinoids are shown in Table 1.

TC was quantitated by HPLC methods reported previously [28]. The PC levels in the intestinal perfusion outflow samples were quantitated by a previously reported method [6] with modification.

Data analysis

A steady-state analysis was used to determine the effective permeability (P_{eff}) of the retinoids. P_{eff} represents the permeability of the retinoid as it disappears from the intestinal lumen, presumably into the epithelial cells lining the gut wall. P_{eff} is related to the ratio of the inflow concentration (C_{in}) of the retinoid and its outflow concentration (C_{out}) at steady-state [14]:

$$\frac{C_{out}}{C_{in}} = \exp\left[\frac{P_{eff}(2\pi rL)}{Q}\right] \quad (1)$$

where the radius of the gut lumen is represented by r (estimated at 0.18 cm [14]), L is the length of the intestinal segment (cm), and the Q is the bulk fluid flow rate through the intestine. C_{in} and C_{out} were quantitated by HPLC and C_{out} was corrected gravimetrically for net water flux [28]. If the net water flux was ±25% for the majority of the fractions in a given experiment the data were discarded from further consideration. The retinoid concentration in the syringe at the end of the experiment was not significantly different from that prior to the experiment, so the C_{in} was calculated as the average of these two determinations. The fraction C_{out}/C_{in} represents the concentration of the drug leaving the perfused segment normalized to the concentration entering the segment, i.e. the fraction remaining. L was determined by measuring the length of the segment at the end of the experiment after resection. Q was determined by the slope of the plot of volume remaining in the syringe as a function of time. The effective permeability was calculated by rearrangement of Eq. 1. This analysis carries with it the assumptions that the perfused segment is a uniform, right cylinder with constant radius and permeability across the length of the segment [14].

Analysis of variance (ANOVA) was used to determine the influence of flow rate, perfusate composition, and chemical structure on the intestinal permeability of the retinoids. The effect of perfusion flow rate and micelle type on the calculated P_{eff} values for individual retinoids was analyzed by two-way ANOVA with a *P*-value < 0.05 being considered significant. This two-way ANOVA was not possible for 13-*cis*-MTTO because this compound was only evaluated in four rats, one at each combination of flow rate and micelle composition. An additional factor, the retinoid structure, was included in a three-way ANOVA for the analysis of pooled data from all retinoids.

In order to determine more unambiguously which structural attribute of the retinoid molecule was the most important in determining its permeability, the effects of side-chain position (i.e. all-*trans* vs 13-*cis*-, vs 9-*cis*) and ring structure (aromatic vs cyclohexenyl vs arotinoid) were explored. Scheffé's post hoc analysis allowed these analyses to be stratified by flow rate and micelle type.

Results

Figure 2 illustrates the fraction of the drug remaining in the perfusate in rats receiving 9CRA in simple or mixed micelles at the high flow rate (0.35 ml/min). It is clear that the mixed micelles were associated with a higher degree of retinoid uptake into the jejunal cells than were the simple micelles. Table 2 indicates that this was a

Table 1 HPLC systems used for retinoid acids

Analyte	C_{18} column length (cm)[a]	% ACN/% buffer	Analyte retention time (min)	Internal standard (retention time, min)
ATRA	25	80/20[b]	10.3	ETA (5.6)
13CRA	25	80/20[b]	8.1	ETA (5.6)
9CRA	25	80/20[b]	10.6	MOE (6.1)
ETA	15	–[c]	2.6	Retinyl acetate (7.9)
cETA	25	75/25[d]	4.4	ETA (6.6)
MTTO	15	80/20[b]	7.9	ETA (5.3)
cMTTO	25	80/20[b]	7.1	ETA (5.6)

[a] Supelco C_{18}, 4.6 mm ID, 5 µm particle size
[b] Buffer is 1.5% acetic acid in water
[c] Mobile phase: 840 ml acetonitrile, 160 ml water, 0.8 g ammonium acetate, 10 ml glacial acetic acid
[d] Buffer is 0.4% ammonium acetate in water

general finding for the retinoids in the current study, in agreement with previous reports [28, 39]. Two-way ANOVA indicated that the permeability of each of the retinoids was greater when perfused in mixed micelles than in the simple micelles. In addition, the permeability was higher when the perfusate flow was greater, indicating that the aqueous boundary layer of the intestine was the rate-limiting barrier to the disappearance of the retinoid from the intestinal lumen [14, 16]. When the data in Table 2 were subjected as a whole to three-way ANOVA, retinoid structure was also found to have a significant effect on permeability.

Post hoc analysis indicated that for the simple micelle systems, permeability was not influenced by structure at either flow rate. However, in the mixed micelle systems, structural effects were significant at both the low and high flow rates. ANOVA indicated that the structure of the six-membered ring was not a major determinant of permeability. However, the isomeric position of the carboxylic acid had a significant effect on permeability. The permeability of the retinoids with the polyene chain in the 13-*cis* position was significantly greater than when the chain was all-*trans* or in the 9-*cis* position. There was no significant difference between the permeabilities of the 9-*cis* and the all-*trans* acids.

The permeability of the intestine to both the NaTC and PC was also analyzed. The jejunal permeability of TC was significantly affected by the flow rate, with greater permeabilities at high flow rates (Table 3). However, the permeability of TC was the same no matter what type of micelle (simple or mixed) was present. It appeared that the type of retinoid that was carried by the micelles had an effect on the TC permeability (data not shown), but this finding was due mainly to the ETA in mixed micelles at the high flow rate. The TC permeability for the ETA animals was the highest of all, and all statistical significance appeared to be due to the differences between ETA and the other retinoids.

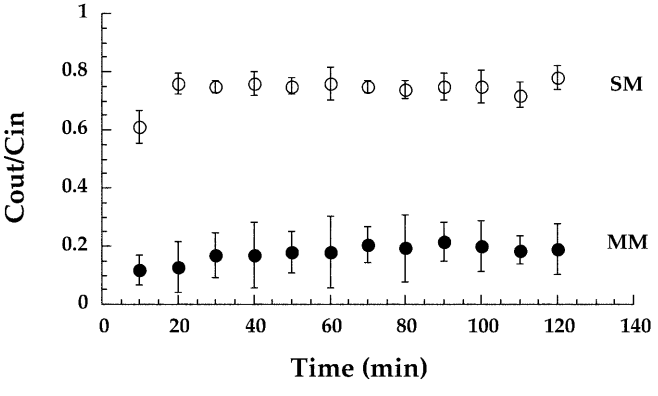

Fig. 2 The fraction of 9CRA remaining in the perfusate (C_{out}/C_{in}) as a function of time and perfusate composition at a flow rate of 0.35 ml/min. The simple micellar perfusate made up of 10 mM TC is designated *SM* and the mixed micellar perfusate made up of PC/TC (10 mM/10 m*M*) is designated *MM*. Data are presented as means ± SD ($n = 3$)

Table 2 Apparent permeability ($P_{eff} \times 10^3$, cm/min) of retinoids in the jejunum. Values are means ± SD ($n = 3$ unless otherwise indicated). Two-way ANOVA performed on P_{eff} for each individual retinoid (except 13-*cis*-MTTO) indicated that both perfusion flow rate and micelle type were significant effects at $P < 0.05$. Three-way ANOVA performed on P_{eff} for all retinoids indicated that perfusion flow rate, micelle type and retinoid structure were significant effects at $P < 0.05$

	ATRA		ETA		MTTO		13CRA		c-ETA		13c-MTTO		9CRA	
	Simple micelles	Mixed micelles	Simple micelles	Mixed micelles	Simple micelles	Mixed micelles	Simple micelles	Mixed micelles	Simple micelles	Mixed micelles	Simple micelles	Mixed micelles	Simple micelles	Mixed micelles
Low flow rate (~0.1 ml/min)	4.38 ±1.94	20.19 ±8.95	3.44 ±1.19	15.37 ±3.92	3.06 ±0.83	24.43 ±6.04	2.89 ±1.23	28.63 ±10.61	4.89 ±0.81	39.04 ±8.79[a]	3.94[b]	30.2[b]	3.37 ±0.32	15.98 ±5.15
High flow rate (~0.35 ml/min)	5.74 ±2.44	42.13 ±11.16	10.44 ±1.16	22.31 ±7.30	4.02 ±0.51	43.04 ±2.23	9.04 ±4.96	58.81 ±18.97	6.49 ±0.35	102.38 ±40.02[a]	5.63[b]	43.57[b]	5.14 ±0.75	29.33 ±8.35

[a] $n = 4$
[b] $n = 1$

Table 3 Apparent permeability ($P_{eff} \times 10^3$, cm/min) of taurocholate in the jejunum. Values are means ± SD ($n = 18$ unless otherwise indicated). Two-way ANOVA performed on P_{eff} indicated that perfusion flow rate was a significant effect at $P < 0.05$

	Simple micelles	Mixed micelles
Low flow rate (~0.1 ml/min)	0.47 ± 0.55	0.67 ± 1.56
High flow rate (~0.35 ml/min)	1.63 ± 2.39[a]	2.08 ± 1.98[b]

[a] $n = 17$
[b] $n = 19$

In contrast to the findings of previous studies, the P_{eff} of PC was not shown to be dependent upon flow rate (Table 4). As in the TC permeability case, ANOVA indicated that the type of retinoid that was carried by the micelles had an effect on PC permeability, but once again this finding was due mainly to the ETA in mixed micelles at the high flow rate.

Discussion

It has long been understood that the absorption of lipophilic compounds found in the intestine is aided by solubilization in bile salt mixed micelles. The formation of the micelles is mediated by the bile salt solubilization of lipids [4]. This process allows more lipophile to be in solution in smaller aggregates than would be in a normal aqueous environment [22] and thus increases the rate of passive diffusion to the apical membrane of the intestine. Bile salts and lipids are known to influence the intestinal absorption of retinol [16]. Therefore this study was undertaken to further explore the role of bile salt micelles on the intestinal absorption of structurally related retinoic acids.

The absorption of retinol from the gastrointestinal tract appears to be relatively well characterized. In the rat, there is a saturable component to the transport at low intestinal concentrations (30–300 n*M*), and at higher concentrations (450–2700 n*M*), a passive diffusion mechanism appears to predominate [16]. Furthermore, the saturable transport system appears to be quite structurally specific [10]. Modifications on the ring structure of retinol have no influence on the transport, but the structure of the hydrophilic portion of the molecule appears to confer selectivity. Retinol and 3-dehydroretinol competitively inhibit the transport of [^{3}H]-retinol. However, 13-*cis*-retinol, retinaldehyde [10] and ATRA do not have an effect on the uptake of retinol in the rat gut [30].

That there might be a structural specificity in the absorption of therapeutic retinoids has been suggested in previous work with the aromatic retinoic acid, ETA and its ethyl ester (ET) and ethyl amide (MOE) forms. In that work, at high flow rates and in mixed micelles, ET showed the greatest permeability, followed by ETA and MOE. The difference between the permeability of ET and MOE is not predictable based upon physicochemical properties such as aqueous solubility or micelle/water distribution coefficient [22], and the possibility that a specific carrier system exists for the therapeutic retinoids was raised [28].

Table 4 Apparent permeability ($P_{eff} \times 10^3$, cm/min) of phosphatidylcholine in the jejunum. Values are means ± SD

Low flow rate (~0.1 ml/min)	0.76 ± 1.55 ($n = 18$)
High flow rate (~0.35 ml/min)	1.65 ± 1.58 ($n = 19$)

The present study corroborates the finding of differences in intestinal permeability within a series of retinoic acids, even though the physicochemical properties of these acids are not likely to be sufficiently different to account for the marked differences [22]. There appear to be several noteworthy findings. First, for each retinoic acid there was an increase in permeability with flow rate. In modeling absorption in the present experimental system it was assumed that the drug faces two resistances to diffusion out of the gastrointestinal lumen [14]. These resistances are arranged in series. The first is the aqueous boundary layer, a stagnant water layer lining the apical membrane of the gastrointestinal lumen. The second is the resistance of the biomembrane. It is assumed that if a drug is highly lipophilic, the aqueous boundary layer constitutes the rate-limiting barrier to absorption. Thus if the flow rate through the lumen is increased, the aqueous layer will be "thinned", and the barrier to lipophilic drug absorption will be decreased. Therefore, at higher flow rates, the permeability of a lipophilic drug will be increased [4]. For all the compounds of interest, in both the simple and mixed micelles, an increased flow rate led to an increased permeability of the retinoid. This is in agreement with previous reports [16, 28]. However, the retinoids are not solubilized directly in an aqueous system. The poor water solubility requires that the retinoids be almost completely solubilized in micelles [13, 22]. Absorption of the retinoids from the simple micelles is consistent with the aqueous boundary layer dominating the observed permeability.

The mixed micelles are larger than the simple micelles [21], thus for the same aqueous boundary layer thickness, the mixed micelles should be transported to the biomembrane more slowly than the simple micelles. However, at both high and low flow rates, the mixed micelle systems are associated with higher permeabilities for the retinoids than are the simple micelles. The precise molecular events that occur at the enterocyte surface during the uptake of micelle-solubilized solutes remain unclear. In studies of the interactions between TC/cholesterol/dipalmitoyl-PC mixed micelles incubated with rabbit brush-border membrane vesicles (BBMV) [33], it was concluded that the mixed micelles first interact with the membrane bilayer, then the micellar components are integrated into the bilayer. This interaction appears to be associated with proteins on the apical side of the membrane [24, 36, 37]. These proteins are required for the exchange of PC between mixed lipid micelles or

vesicles and BBMV [36]. Furthermore, this interaction is accompanied by the partial release of the contents carried by the donor [37]. The protein-mediated exchange of PC appears to take place in both rabbit and human BBMV systems [24], and in the rabbit is associated with three lipid exchange proteins of 13, 22, and 100 kDa in size [24]. If there is a specific group of proteins with which PC interacts, then the interaction of the PC/TC mixed micelles with the enterocyte membrane will be more favorable than that for the simple TC micelles. Thus, it is conceivable that the retinoids associated with the mixed micelle may be internalized within the enterocytes during the protein-mediated uptake of PC.

It has been shown that addition of mucin to NaTC/egg PC mixed micelles causes an increase in the diffusivity of the mixed micelle [23]. This may be a result of the phospholipid preferentially binding to the mucin glycoprotein. Therefore, in the gastrointestinal tract, as the micelles pass through the mucin, some of the phospholipid may bind to mucin, thus decreasing the phospholipid content of the micelle. This depletion of the phospholipid content would cause a decrease in size of the micelle (and an increase in its diffusivity) as the bile salt/PC ratio increases. This may also facilitate mucin removal, which would change the character of the aqueous boundary layer. Although this dynamic process clearly complicates the interpretation of the present data, the fact remains that the PC-containing mixed micelles are increasing the permeability of the intestine to the retinoic acids.

In addition to the above effects, at a given micelle composition and flow rate, there are significant differences in retinoic acid permeability for different retinoid structures. This is of particular note at the high flow rate with mixed micelles. It can be speculated first that there is a structurally specific carrier system in the membrane for the retinoic acids, apparently distinct from that reported for retinol [30]. There are some data indicating that the oral bioavailability of ATRA is decreased at higher doses, and is also decreased with chronic dosing, possibly due to the downregulation of the absorption process [12]. This implies a carrier-mediated absorption process. The present work indicates that the isomeric conformation of the polyene chain has a significant effect on permeability. The permeability of the retinoids with a 13-*cis* conformation was significantly greater than when the chain was either the all-*trans* or 9-*cis* conformation. The structure of the six-membered ring system was less important.

It should be noted that PC has been reported to be a substrate for the human MDR-1 p-glycoprotein (p-gp) [3], an efflux pump that is expressed in a variety of tissues, including the jejunum [34]. P-gp also appears to play a role in the development of resistance to ATRA in leukemic cells [20, 25], although others have shown that p-gp will not affect the cellular accumulation of ATRA [31]. In the present context, these finding are suggestive of an interaction between PC and certain retinoids at the intestinal membrane. This remains speculative at the present time.

Despite the complexity of the system, two things remain clear. First, the gastrointestinal tract is differentially permeable to the retinoic acids, and second, the presence of mixed micelles enhances their permeability. Because of the importance of ATRA and related compounds in cancer chemoprevention and chemotherapy, a mechanistic understanding of the absorption process is clearly needed.

Acknowledgement This work was supported by PHS grant RO1-CA-55493.

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