Effect of Oleic Acid Vesicles on Intestinal Absorption of Carboxyfluorescein in Rats

Masahiro Murakami,1,2 Hiroshi Yoshikawa,1 Kanji Takada,1 and Shozo Muranish1

Received April 19, 1985; accepted September 10, 1985

The effect of oleic acid vesicles (ufasomes) on the intestinal absorption of entrapped carboxyfluorescein (CF) was investigated by an in situ closed-loop method in rats. Entrapment of CF in ufasomes enhanced the absorption of CF at the earlier stage following intraduodenal administration, and the threshold concentration of the fatty acid for promoting the absorption of CF was approximately 8 mM. The absorption of CF from the large intestine was promoted much more effectively than from the small intestine. These studies suggest that ufasomes have potential as carriers for the oral administration of poorly absorbable drugs.

KEY WORDS: oleic acid; ufasome; promotive absorption; intestinal absorption; drug carrier; 6-carboxyfluorescein.

INTRODUCTION

Liposomes are versatile biological vesicles that can encapsulate a variety of substances and macromolecules (e.g., polypeptides, proteins). Attention has focused on the potential application of liposomes to oral delivery of drugs that are not normally absorbed from the gastrointestinal tract because of their high hydrophilicity, large molecular weight, or instability in the lumen (1–3). However, it is still ambiguous whether liposomes directly increase the absorption rate of these drugs or only protect them from chemical or enzymatic degradation in the lumen. In our preceding report (4), it was suggested that liposomes composed of egg phosphatidylcholine do not substantially promote the absorption of carboxyfluorescein (CF), a water-soluble (i.e., poorly absorbable) model dye, from the rat small intestine. Recent reports (5,6) indicate that liposomes do not permeate the intestinal mucosa in their intact form. It is, therefore, important to develop vesicles with suitable efficiency as oral drug carriers.

We previously reported the marked effect of lipid-surfactant mixed micelles on the intestinal absorption of poorly absorbable drugs, such as gentamicin, streptomycin (7), heparin (8,9), and interferon (10), in rats. As Muranushi et al. (11) pointed out, lipids enhanced the intestinal absorption of these drugs are the fusogenic lipids as defined by Akhong et al. (12). On the other hand, unsaturated long-chain fatty acids may form closed vesicles with a bilayer-like structure, so-called “ufasomes” (13). On the basis of these considerations, we have investigated the usefulness of ufasomes as potential oral drug carriers.

Carboxyfluorescein (CF) was used as a model compound for poorly absorbable drugs. Because most of the absorbed CF is excreted into bile (unpublished data), and in order to avoid decomposition of ufasomes by bile acids in the intestinal lumen, bile fistula rats were used. As a typical unsaturated fatty acid, oleic acid was chosen in view of its low sensitivity for peroxidation, which renders the membrane of ufasomes leaky (14).

MATERIALS AND METHODS

Materials. CF was purchased from Eastman Kodak Co. (Rochester, N.Y.). Oleic acid of high-purity grade (at least over 99%) was kindly supplied by Nippon Oil & Fats Co. Ltd. (Tokyo). All other chemicals and solvents were of reagent grade.

Preparation of Ufasomes. Oleic acid ufasomes containing CF (CF/ufasomes) were prepared according to the method of Gebicki and Hicks (15) with some modification. Oleic acid was dissolved in chloroform to 4% (w/v) and stored at −20°C. For standard preparations, 1 ml of the stock oleic acid solution was placed in a test tube and evaporated to dryness with a stream of nitrogen at 40°C. After the addition of sufficient 1 N NaOH to neutralize the fatty acid, residual film was sonicated with an Ohtake Model 5202 sonicator (Ohtake Seisakusho Co., Ltd., Tokyo) at 15 W for 2 min under nitrogen in an ice-water bath to hydrate with 1 ml of 2% (w/v) Tris–HCl-buffered CF solution of pH 8.5. The resulting suspension of ufasomes was then applied to a coarse Sephadex G-25 column (18 × 2 cm) to remove the excess CF unentrapped within the ufasomes. The void-volume fraction was collected using the same buffer as eluent.

In Vitro Stability of Ufasomes. CF/ufasomes in suspension with pH 8.5 Tris–HCl buffer were incubated in a water bath at 37°C. One-milliliter aliquots of the suspension were
Fig. 1. Gel filtration pattern of ufasomes. (a) Elution chromatogram of CF/ufasomes for the standard preparation. ND < 0.01 mM. (b) CF/ufasomes prepared with oleic acid at 40 mg (——), 10 mg (---.), and 5 mg (---.). Chromatography was performed on a coarse Sephadex G-25 column at a flow rate of ca. 20 ml/hr (2 ml/tube).

periodically taken and immediately ultrafiltered using an Amicon MPS-1 micropartition system with a YMT membrane (Amicon Ltd.) at 700g for 8 min. The filtrates and another aliquot of the suspension were used for CF assay as described below.

Absorption Experiments in Rats. Absorption experiments were performed in male Wistar albino rats (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan) weighing 230–280 g by the in situ closed-loop method as described previously (4). Animals were fasted for 16 hr prior to experiments (but given water ad libitum) and anesthetized intraperitoneally with sodium pentobarbital (32 mg/kg of body weight) during the experiments. The intestine was exposed through the midline incision, and an intestinal loop was prepared by cannulation of 3-cm silicon tubing (i.d., 3 mm; o.d., 5 mm) at the proximal and distal ends of the small intestine or the entire large intestine (colon and rectum). The bile duct was cannulated with polyethylene tubing (i.d., 0.5 mm; o.d., 0.8 mm), and bile was removed from the body during the experiments. The test solution warmed to 37°C, was introduced into the intestinal loop, which was closed by clipping with a forceps at the cannulated position of each tubing. The administered volume was 5 ml for the small intestine and 2.5 ml for the large bowel. The dose of CF was 1 mg/kg of rat body weight. Blood samples were collected periodically via a polyethylene catheter (i.d., 0.5 mm; o.d., 0.8 mm) placed into the carotid artery.

In Situ Stability of Ufasomes. At the predetermined time after the administration of the test solution, the remaining solution in the lumen was thoroughly collected by forcing it out with syringe air. The collected solution was ultrafiltered under the conditions described above, and its pH was measured.

Analytical Methods. The fluorescence of CF in the diluted samples was measured with a Hitachi Model 650-10S fluorescence spectrophotometer (Hitachi Ltd., Tokyo) at 520 nm at an excitation wavelength of 490 nm. For measurement of the total fluorescence, to 0.1 ml of the ufasome suspensions 0.1 ml of 12.5% (w/v) Triton X-100 solution was added to release all of the CF and then diluted with the appropriate buffer volume.

Determination of the concentration of CF in rat plasma was carried out as previously reported. Briefly, 50 μl of plasma was mixed with 0.1 ml of 12.5% (w/v) Triton X-100 and 3 ml of 1 N HCl. To the resulting mixture 6 ml isoamyl alcohol was added, and CF was extracted. After centrifugation, 5 ml of the isoamyl alcohol was pipeted, 4 ml of pH 10 Na₂CO₃–NaHCO₃ buffer solution was added, and CF was reextracted into the buffer phase. The aqueous phase was fluorometrically analyzed. Determination of oleic acid was carried out with a commercial kit for the measurement of nonesterified fatty acids by the ACS-ACOD method (NEFA Kit-U, Nippon Shoji Kaisha Ltd., Osaka, Japan).

Fig. 2. Release of CF from ufasomes in vitro. The percentage of CF remaining in the ufasomes is plotted. Vertical bars represent the standard deviations of the means for three determinations.