[Chem. Pharm. Bull. 34(10)4382—4384(1986)]

Effect of Storage on the Ability of Lipid–Surfactant Mixed Micelles to Promote Enteral Absorption of Bleomycin in Rats

HIROSHI YOSHIKAWA,* KANJI TAKADA and SHOZO MURANISHI

Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

(Received January 16, 1986)

The effect of storage on the activity of lipid–surfactant mixed micelles (MM), a powerful gastrointestinal absorption enhancer, was investigated in rat large intestine. The MM composed of linoleic acid and polyoxyethylated (60 mol) hydrogenated castor oil were stored for 6 months with shielding from light and oxygen under refrigeration; they potentiated the absorption of poorly absorbable bleomycin as effectively as freshly prepared MM.

Keywords——enteral absorption promoter; storage effect; lipid–surfactant mixed micelle; bleomycin; poorly absorbable drug

Lipid–surfactant mixed micelles (MM) have been investigated in our laboratory as a non-toxic absorption promoter in the alimentary canal. MM is very effective in promoting the absorption of various poorly absorbable small-[1,2] and macro-molecular drugs such as bleomycin,[3] heparin[4-6] and interferons.[7-10] It is essential to assure the stability of the adjuvant and of its effect in order to design suitable dosage forms. In this paper, we report the effect of storage on the activity of MM to promote the absorption of poorly absorbable bleomycin (approximate M_r 1500) in the rat large intestine.

Experimental

Materials——Bleomycin (BLM) was kindly supplied by Nippon Kayaku Co., Ltd., Tokyo, Japan. Linoleic acid and oleic acid (99.0%, Nippon Oil & Fats Co., Ltd., Tokyo, Japan) and HCO60 (polyoxyethylated (60 mol) hydrogenated castor oil, Nikko Chemicals Co., Ltd., Tokyo, Japan) were used as components of MM.

Preparation and Storage of the Test Solutions——MM solution was prepared by dispersing linoleic acid or oleic acid (0.5% (w/v)) and HCO60 (0.8% (w/v)) in degassed distilled water, followed by sonication at 37 °C with a sonicator (model 5202, Ohtake Seisakusho Co., Ltd., Tokyo, Japan). MM solution was preserved at 4 °C with shielding from light and under nitrogen gas in ampules until required. Test solutions were prepared by dissolving BLM (2.5 mg/ml) in fresh MM solution and in stored MM solutions for various periods (1 week, 1 month and 6 months), just before administration.

Absorption Experiment——Male Wistar rats (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan) weighing 300—350 g were given a pellet diet (CE2; Clea Japan Co., Ltd., Tokyo, Japan) and water *ad libitum*. The rats (not fasted) were anesthetized by intraperitoneal injection of pentobarbital, and a midline incision was made. A closed loop of the entire large intestine (colon and rectum) was prepared by ligation, with silicone cannulas into the proximal end of the colon and the distal end of the rectum. The intestinal contents were removed by slow infusion of saline solution (37 °C) into the loop. Once the intestinal content was flushed out, a gentle stream of air was applied to aid in the removal of residual fluids. Two milliliters of test solution containing BLM (dose, 5 mg/rat) at 37 °C was introduced into the loop of the large intestine. A polyethylene catheter (i.d., 0.5 mm, o.d., 0.8 mm, Dural Plastics & Eng., Pty., Dural Australia) was inserted into the carotid artery and blood samples were collected periodically. Plasma was separated by centrifugation at 15000 *g* for 2 min and immediately immersed in an ice bath.

Pretreatment——Pretreatment with MM solution was performed according to the following procedure. The MM solution stored for various periods (not containing BLM) was first introduced into the loop of the large intestine. After 2 h, the MM solution in the intestine was entirely removed with the aid of air supplied through a

syringe and the intestine was washed out with saline solution at 37 °C. Immediately after washing, 2 ml of saline solution containing BLM (2.5 mg/ml) was infused into the loop. Plasma samples were collected as described above.

Analytical Method for BLM——An antimicrobiological assay was used for the determination of BLM in the plasma samples. The disc plate method using *Bacillus subtilis* ATCC 6633 as a test microorganism was employed.

Transmittance of MM Solution——Transmittance of MM solution was measured as an index of the stability of MM at 640 nm and 37 °C using a spectrophotometer (model 200-20, Hitachi Ltd., Tokyo, Japan).

Results and Discussion

The administration of BLM alone into the loop of the rat large intestine resulted in a low blood level of the drug (0.5—0.8 μg/ml for 6 h). Fresh MM composed of linoleic acid and HCO60 greatly promoted the BLM absorption as shown in Fig. 1, but fresh MM composed of oleic acid + HCO60 showed a smaller enhancement (blood BLM level, 1—10 μg/ml for 6 h). Therefore, linoleic acid was chosen as the lipid component of MM in the storage experiment.

Figure 1 also shows the plasma BLM levels after administration of BLM into the large intestine with MM (linoleic acid + HCO60) stored for various periods. In each case, there was a quick increase of BLM evel and a relatively rapid blood clearance during the first 3 h, and tailing of the disappearance curve was observed from 3 to 6 h after administration. The peak BLM levels were detected at 30 min (MM solution stored for 1 week, 1 month and 6 months) or 1 h (no storage), and no significant differences of peak BLM levels were detected. The area under the plasma concentration–time curve (AUC) of BLM up to 6 h, calculated from the average level (Fig. 1), was 96.8 (no storage), 102.5 (storage period, 1 week), 91.3 (1 month) or 122.4 $\mu g \cdot h \cdot ml^{-1}$ (6 months). These data indicated that the promotive ability of MM was maintained during storage for at least 6 months. We previously investigated by the same method the maintenance of promotive ability of stored MM on interferon absorption from rat large intestine[11] and the data mostly agreed well with the results on BLM in this study.

The physico-chemical stability of stored MM was studied by measuring the transmittance of MM at 640 nm as an index of lipid solubilization. As shown in Table I, the percentages of transmittance of fresh MM and 6-months-stored MM are 87.7% and 89.6%,

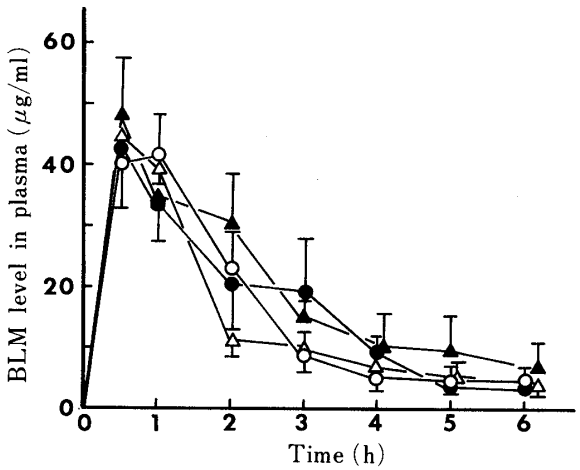

Fig. 1. Plasma Concentrations of BLM after Administration of BLM with Mixed Micelles Stored for Various Periods into the Lumen of Rat Large Intestine

○, no storage; ●, stored for 1 week; △, 1 month; ▲, 6 months. Each point represents the mean ± S.E. of 4—6 experiments.

TABLE I. Effect of Storage on the Transmittance of MM Solution

MM	Fresh	After storage (6 months)
Percentage of transmittance at 640 nm	87.7%	89.6%

Transmittance of MM solution was measured at 37 °C.

respectively, so that the solubilized state of MM was scarecely changed during the storage period.

Pretreatment of the large intestinal mucosa with stored MM solution (6 months) did not enhance BLM absorption (not shown), and the effect of stored MM as an absorption promotor is considered to be a temporary and reversible action just like that of MM immediately after preparation.[2,9] Muranushi *et al.*[12] suggested that the incorporated lipid (*e.g.* linoleic acid) of MM causes disorder in the intestinal membrane interior (increase of membrane fluidity), possibly by interaction of the lipid of MM with the polar head groups of membrane phospholipid, and consequently the membrane permeability is enhanced. Therefore, the fact that the pretreatment with MM had no effect indicates that the actions of MM on intestinal mucosal membrane (increase of fluidity and interaction with membrane phospholipid) occur only while MM is actually present. The results obtained in this study demonstrate that MM composed of linoleic acid and HCO60 maintains its absorption-promotive action on the mucosa of the large intestine, as well as its lack of toxicity, during prolonged storage. We expect that the results presented in this paper will aid the development of dosage design for absorption-promoter adjuvants.

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