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Effects of polyoxyethylene cetyl ether on the absorption of 3′,4′-dideoxykanamycin B from rat rectum

Seishi Tsuchiya, Yukihiko Aramaki, Shigenari Ozawa * and Hideo Matsumaru **

Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03 (Japan)

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Summary

The effects of the surface-active agent, polyoxyethylene cetyl ether (POEC), on the rectal absorption of 3′,4′-dideoxykanamycin B (DKB) and on the rectal surface were examined in rats. With the in situ perfusion method, the absorption of this antibiotic was dependent on the drug concentration, but not on the surface-active agent concentration. In the in vivo study, the suppository of emulsified base (o/w type) was favorable to increased absorption of the drug and its extent of bioavailability was about 50% as compared with intramuscular administration.

To investigate the effect of POEC on the rectal surface, the release of protein, sugar, phospholipid and enzymes into the perfusate was determined. This experiment suggests that the enzymatic composition of rectal mucosal membrane is different from that of small intestine. Using scanning electron microscope, it was observed that the mucous on the rectal surface was only washed off by the perfusion of POEC.

These results suggest that the epithelial cells are not seriously damaged by POEC and that POEC is a useful additive to promote the absorption of DKB from suppositories.

Introduction

The availability of drugs is markedly influenced by the route of administration. It is well known that when some drugs (such as insulin (Shichiri et al., 1978), certain

* Present address: Kissei Pharmaceuticals, Matsumoto, Nagano 399-65, Japan.
** Present address: Nippon Zenyakukogyo, Koriyama, Fukushima 969-05, Japan.

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aminoglycoside antibiotics (Touitou et al., 1978), and lidocaine (DeBoer et al., 1980)) are orally administered, low absolute bioavailability is obtained because of digestive degradation, poor absorbability from the gastrointestinal tract, or first-pass metabolism. Though there are some limitations, rectal administration of drugs is a good route because it partially avoids the disadvantages cited.

To enhance absorbability of drugs after rectal administration, certain surface-active agents (Shichiri et al., 1978; Nishihata et al., 1980a; Walters et al., 1981; Brookes and Marshall, 1981; Stanzani et al., 1981) or adjuvants (Nishihata et al., 1980b; Nishihata et al., 1981; Yoshioka et al., 1982) are often added to the suppository base. Recently, to investigate the development of an alternative route of drug administration and increase bioavailability, a non-ionic surface-active agent, polyethylene glycol monocetyl ether, was added to a suppository containing insulin, heparin and gentamicin (Touitou et al., 1978). The rectal absorption of drugs was enhanced when the surface-active agent was added to the suppository base, but little information is available concerning the effects on the rectal mucosal membrane.

In the present experiment, we investigated, by in vivo absorption and in situ perfusion of rat rectum, the effects of a non-ionic surface-active agent, polyoxyethylene cetyl ether, on the rectal absorption of an aminoglycoside antibiotic, 3',4'-dideoxykanamycin B, which was poorly absorbed after oral administration. Furthermore, in the perfusion experiment, the release of protein, sugar, phospholipid and enzymes into the perfusate in rat rectum was measured, and scanning microscopical investigation was carried out before and after the perfusion of rat rectum with solution containing the surface-active agent, to determine the degree of damage to the rectal membrane.

Materials and methods

Materials

3',4'-Dideoxykanamycin B (DKB; 712 μg/mg) and polyoxyethylene cetyl ether (POEC) were kindly provided by Meiji Seika Kaisha (Tokyo) and Nikko Chemicals (Tokyo), respectively. The other reagents were of the best available grade. Male Wistar rats (200–250 g) were purchased from Tokyo Jikken Dobutsu (Tokyo).

Absorption of 3',4'-dideoxykanamycin B by in situ perfusion

Male Wistar rats, fasted with free access to water for 20 h before the experiment, were anesthetized with ethyl carbamate (100 mg/100 g of body weight). Each rectum was exposed and a glass cannula was inserted into the rectum from the lower part of colon. The other cannula was inserted through the anus. Thus, about 2 cm of the rectum was exposed to the perfusate. The rectum was perfused with phosphate-buffered saline (pH 7.4) containing various concentrations of DKB and/or POEC at the constant rate of 3.0 ml/min for 120 min (37°C). The blood samples were withdrawn from the leg vein through a heparinized polyethylene cannula (PE-50) at 0, 0.3, 0.6, 1.0, 1.5 and 2.0 h after the beginning of this experiment. The movement of water in the perfusate was corrected with blue dextran (0.5 mg/ml).

Effect of polyoxyethylene cetyl ether on rectal membrane

About 2 cm of each rectum was perfused by the same procedure as mentioned above, except for the conditions of perfusion. In this experiment, the rectum was perfused with the amphibian Ringer's solution (0.56 g of NaCl, 0.025 g of KCl, 0.03 g of $CaCl_2$ and 0.02 g of $NaHCO_3$ per liter of water) at the constant rate of 0.3 ml/min for 180 min. A 3 ml aliquot of the single-pass perfusate was collected for every 10 min. During the first 30 min, the rectum was perfused with the amphibian Ringer's solution, and the amphibian Ringer's solution containing 0.1% (w/v) POEC was perfused from 30 to 60 min. Then up to 180 min, the amphibian Ringer's solution without POEC was perfused again. The amphibian Ringer's solution was used to avoid the disturbance of phosphorus in the estimation of phospholipid.

Preparation of suppository and in vivo experiment

Three classes (oleaginous, emulsified and water-soluble) of cylindrical suppositories of 3 mm diameter were prepared by the fusion method and DKB was suspended in the vehicles by means of a glass homogenizer. Two kinds of the oleaginous suppositories consisted of 97% (w/w) of cocoa butter and 3% of DKB, and 97% of Witepsol W-35 and 3% of DKB. In the case of the emulsified base, the w/o type was composed of 87% of Witepsol W-35, 10% of POEC, and 3% of DKB, and the o/w type was 74% of Macrogol 4000, 3% of propylene glycol, 10% of Witepsol W-35, 10% of POEC, and 3% of DKB. The suppository of the water-soluble base was composed of 87% of Macrogol 4000, 10% of Macrogol 400, and 3% of DKB. These kinds of suppositories were administered at the dose of 5 mg DKB per kg body weight of the rat (5 mg/kg), which had been fasted for about 20 h before experiment. The concentration of DKB in the serum was measured immediately before dosing, and at 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 10.0 h after the administration of the suppository.

An amount of 5 mg/kg DKB was injected intravenously (i.v.) into the left side of the jugular vein and blood samples of 0.2 ml were obtained from the right side before dosing and after 2, 5, 10, 20, 30, 60, 120, 180 and 240 min. DKB was injected intramuscularly (i.m.) into the thigh with the amount of 5 mg/kg, and the blood samples were obtained from the jugular vein before dosing and at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 h post dosing.

Assay of 3',4'-dideoxykanamycin B concentration

The concentration of DKB in the serum was determined by the toblamycin radioimmunoassay kit (Monitor Science, U.S.A.) with the Aloka Autowell γ-system. JDC-751. The calibration curve of DKB agreed very closely with that of toblamycin (3'-deoxykanamycin B) determined by this kit, so the toblamycin radioimmunoassay kit was used to determine the concentration of DKB in this experiment. The concentration of DKB in the perfusate was assayed by the paper disc method with *Bacillus subtilis* ATCC 6633 as the test organism. The radioimmunoassay and the bioassay showed good correlation ($r = 0.973$, data not shown).

Protein, sugar and phospholipid concentration in perfusate

The protein concentration was determined by the method of Lowry (1951). The

quantitation of neutral sugar was by the method of Dubois et al. (1956). Phospholipid was measured using the phospholipid assay kit (Wako Pure Chemicals, Tokyo).

Assay of enzyme activities in perfusate

Sucrase activity was measured by the following procedure. To 0.1 ml of the perfusate, 0.2 ml of the substrate solution (1 mM sucrose in 100 mM malate buffer (pH 6.0)) was mixed, and then incubated at 37°C for 60 min. The concentration of glucose liberated by sucrase in the perfusate was determined by the mutarose-glucose oxidase method, using the Glucose C-Test Wako (Wako Pure Chemicals, Tokyo). The sucrase activity was presented as the amount of glucose liberated from sucrose as the substrate. The alkaline phosphatase activity was determined by the Kind-King method (1954) using phenylphosphate as the substrate. The lactic dehydrogenase activity was determined by the method of Cabaud and Wröblewski (1958) using pyruvic acid as the substrate. The β-glucuronidase activity was determined by the method of Loomis (1969), using *p*-nitrophenyl-β-D-glucuronide as the substrate.

Scanning electron microscopy

About 5 × 5 mm of each excised rectal section which was perfused with either the amphibian Ringer's solution only, or that containing 0.1% POEC for 30 min, was fixed with glutaraldehyde at pH 7.35 for 60 min, and then washed with water 3 times and postfixed with 1% OsO_4 in phosphate buffer (pH 7.5) for 120 min at room temperature. After the dehydration by the critical point drying using liquid CO_2, the rectum was stained with Au by JEOL ion sputter JFC-1100. The electron micrographs were obtained with the JEOL scanning microscope model JSM-T 200.

Results and discussion

Aminoglycoside antibiotics are usually administered intramuscularly because of their poor absorption from the gastrointestinal tract. In principle, the rectal route is a good alternative when there are difficulties with the oral or other route of drug administration. Recently, it was reported that some additives were used to increase the rectal absorption of drugs (Nishihata et al., 1980; Touitou et al., 1978; Nishihata et al., 1981; Yoshioka et al., 1982), and that surface active agents were one of the crucial factors. However, there is little quantitative information published concerning the effects of surface-active agents on the rectal surface. So, in this study, the effect of the non-ionic surface-active agent, POEC, on the absorption of DKB from the rectum and on the rectal surface, was investigated.

Effect of the DKB and POEC concentrations on the absorption of DKB from in situ perfusions

Using the in situ perfusion method, the effect of POEC concentration on the DKB absorption from rat rectum was examined. The results from 3 kinds of DKB concentrations perfused for 2 h are shown in Fig. 1. With all the concentrations of DKB, elevated serum levels were obtained when the perfusate contained POEC.

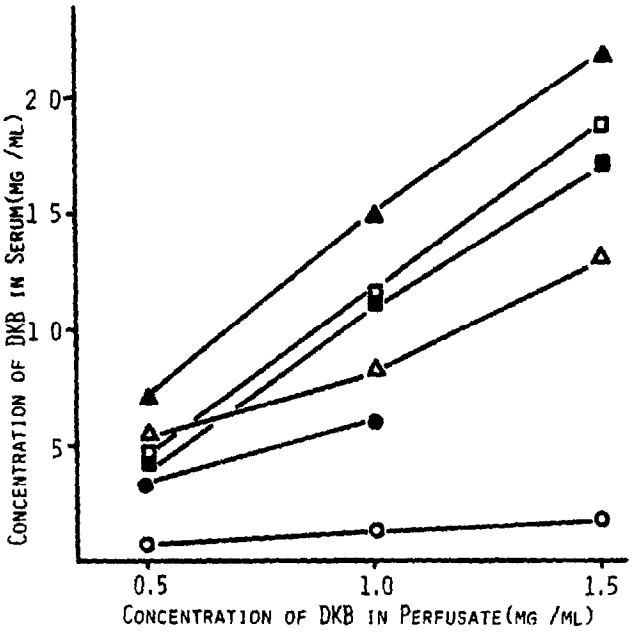

Fig. 1. Effect of DKB and POEC concentrations in perfusate on the rectal absorption in situ. POEC concentrations: ○———○, 0%; ●———●, 0.004%; △———△, 0.05%; ▲———▲, 0.5%; □———□, 1.0%; ■———■, 2.0%.

When 0.5% POEC was added, the maximum level of DKB was observed. The DKB concentration in the serum was not necessarily dependent on the concentration of POEC, but was almost dependent on the concentration of DKB in the perfusate.

Furthermore, the effect of perfusion time on DKB absorption from the rectum was examined using the perfusate with or without POEC. The serum levels of DKB

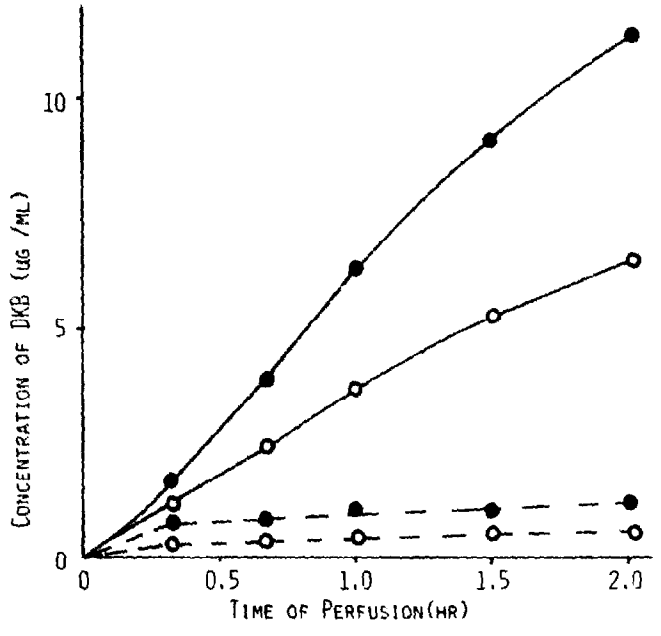

Fig. 2. Effect of perfusion time on the rectal absorption of DKB. DKB concentration; ●———●, 1.0 mg/ml; ○———○, 0.5 mg/ml. POEC concentration: ———, 0.5%; - - - - - -, 0%.

were almost proportional to the doses and were increased according to the perfusion time (Fig. 2). Without POEC in the perfusate, however, little DKB was absorbed.

These findings suggest that the absorption of DKB from the rectum may occur by passive transport when POEC is added. At higher concentrations of POEC (1 or 2%), however, the absorption of DKB was lower than that of 0.5% POEC. The rectal mucosal surface may undergo some alterations induced by the high concentrations of POEC.

Effect of suppository base

In the in vivo study, the effect of the base on DKB absorption from the suppositories prepared as described in Methods was investigated. As shown in Fig. 3, the maximum serum levels of DKB were reached within 30 min with the w/o and o/w type and were 9.7 mg/ml and 7.7 mg/ml, respectively. The areas under the curve (AUC) from the 5 kinds of suppositories were calculated by the trapezoidal rule, and then the extent of bioavailability was determined using the AUC of the emulsified (o/w type) suppository as the standard (Table 1). As shown in Table 1, the availabilities of the oleaginous or the water-soluble base were both 30–40% of the emulsified base.

Depending on the nature of the vehicles and of the drug substance, different release mechanisms can be considered. In the oleaginous vehicles, DKB, which is

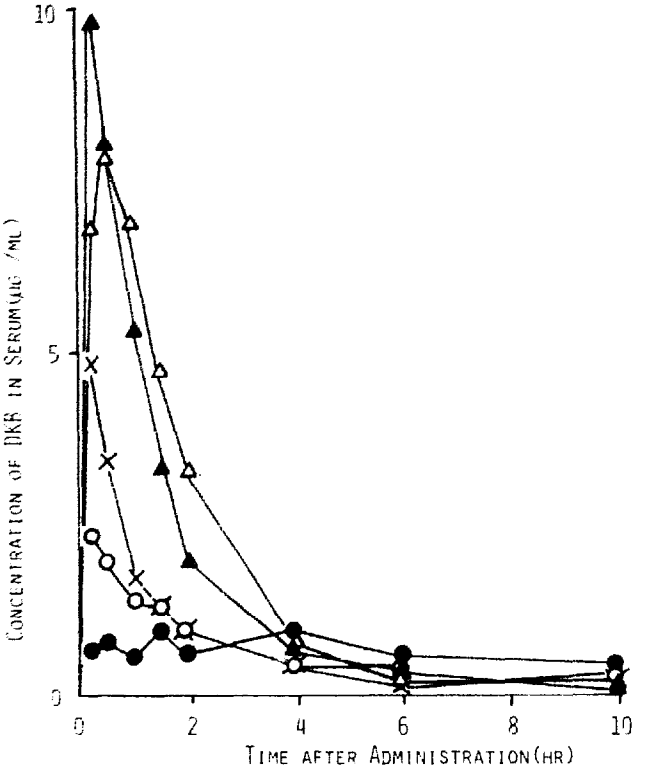

Fig. 3. Rectal absorption of DKB from various kinds of suppository bases. Five kinds of suppository bases were prepared as described in Methods, and 5 mg of DKB/kg of body weight was administered. ●——●, cocoa butter; ○——○, Witepsol W-35; ▲——▲, emulsified (w/o); △——△, emulsified (o/w); ×——×, water-soluble.

TABLE 1

ABSORPTION OF DKB FROM VARIOUS KINDS OF SUPPOSITORIES

Base	C_{max} (μg/ml)	AUC *	Extent of bioavailability (%)
Cocoa butter (oleaginous)	1.00	5.4 ± 1.5	31.78
Witepsol W-35 (oleaginous)	2.29	6.4 ± 0.4	37.69
w/o type (emulsified)	9.69	16.3 ± 1.2	96.45
o/w type (emulsified)	7.74	16.9 ± 1.6	100.00
Macrogol (water-soluble)	4.84	7.4 ± 1.5	43.49

* (μg/ml)×h. Mean ± S.D. (number of experiments, n = 3).

freely soluble in water and which has poor solubility in fat, will be suspended in them. The suspended drug may come into contact with the aqueous rectal fluid and dissolve in it, but the diffusion of the suspended drug in the melted vehicle may be slow and rate-determining.

In this experiment, excellent bioavailability was obtained from the emulsified-type base which contained POEC. This finding suggests an important role for the surface-active agent; e.g. facilitating the dispersion of DKB into the base or the aqueous rectal fluid and promoting the diffusion of DKB through the melted base and into the rectal membrane.

Blood concentration of DKB from the other two administration routes

As described above, the emulsified base caused increased rectal absorption of DKB. So the blood concentration–time curve of the DKB-suppository, which consisted of the emulsified base (o/w type) containing POEC, was compared with that of the i.m. or the i.v. administration of DKB (Fig. 4). The maximum serum level of the suppository containing 10% POEC was obtained within 30 min in a similar manner to that of i.m. injection.

The AUC obtained from each administration route was calculated and the extent of bioavailabilities of the rectal and the i.v. administration were calculated against the i.m. injection (Table 2). As shown in Table 2, the bioavailability of the suppository containing 10% POEC was approximately 50% relative to the i.m. injection.

From these results, it may be considered that, when a certain amount of POEC is added to the emulsified base of suppository, DKB is absorbed from the rectum as rapidly as after the i.m. injection. Previously, Nakazawa et al. (1977) reported that the minimum inhibitory concentration (MIC) of DKB was 1–2 μg/ml of serum. As shown in Fig. 4, the serum level of DKB above MIC was obtained when POEC was added to the base of suppository at 5 or 10%. The concentration of POEC in the suppository was considerably high, but when melted in the rectal lumen the surfactant must have been diluted by the secreted aqueous rectal fluid (Tsuchiya et al., 1977). Consequently, the addition of POEC was favorable in promoting the absorption of DKB from the rectum.

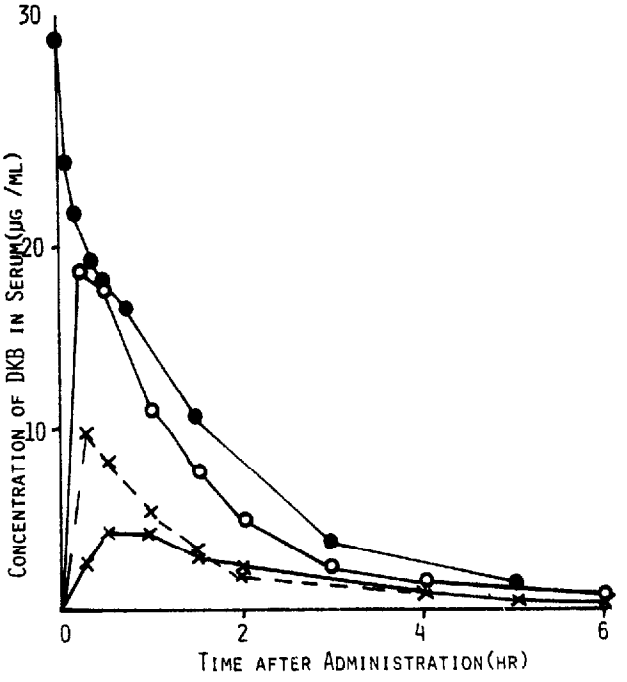

Fig. 4. DKB concentration in serum after administration of 3 dosage forms. ●———●, i.v. administration; ○———○, i.m. administration; ×———×, rectal administration (5% POEC, emulsified base); ×------×, rectal administration (10% POEC, emulsified base).

Effect of POEC on rectal surface — release of protein, sugar and enzymes

The rectal absorption of DKB was significantly increased by the surface-active agent, POEC. Thus POEC is a good additive for promoting the rectal absorption of DKB. However, the rectal surface may possibly be damaged by the surface-active agent. To clarify the effect of POEC on the rectal membrane, the release of membrane constituents, protein, sugar, phospholipid and enzymes into the perfusate was examined by the in situ rat rectum perfusion method. As shown in Fig. 5, protein and sugar was released in an almost similar manner. Soon after the amphibian Ringer's solution containing POEC was perfused, the release of protein

TABLE 2

BIOAVAILABILITY OF 3 DOSAGE FORMS OF DKB

Route of administration	AUC *	Extent of bioavailability (%)
Rectal (suppository, o/w type)		
10% POEC	16.30	53.42
5% POEC	11.80	38.65
Intravenous	40.67	133.21
Intramuscular	30.53	100.00

* (μg/ml)×h.

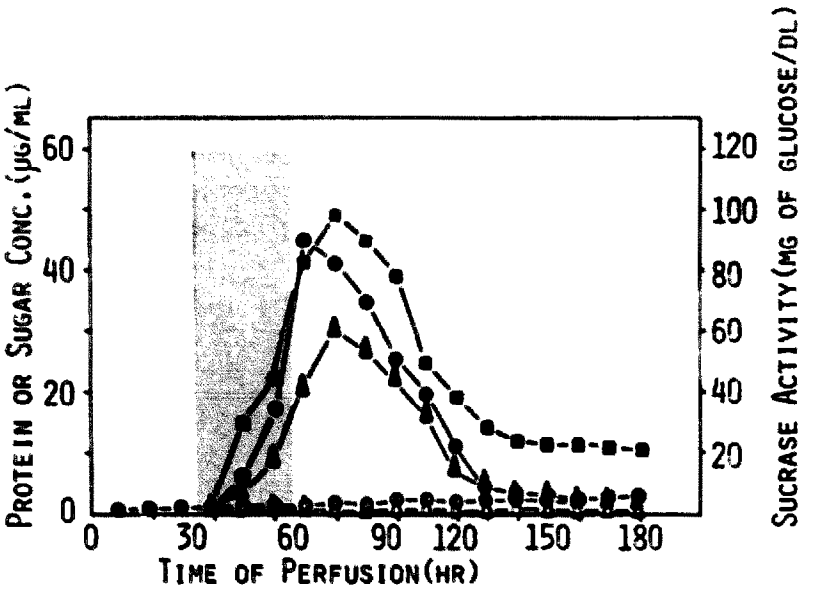

Fig. 5. Release of protein, sugar and sucrase into the perfusate of amphibian Ringer's solution containing 0.1% POEC. The perfusate was collected at every 10 min (3.0 ml). The dotted bar indicates the perfusion period with the amphibian Ringer's solution containing 0.1% POEC. Sucrase activity was shown as the amount of glucose liberated from sucrose as the substrate. ●———●, protein; ▲———▲, sugar; ■———■, sucrase. The open circle and triangle indicate the control values for protein and sugar, respectively, when perfused with amphibian Ringer's solution only.

and sugar began. The maximum release of these substances was obtained at 10 or 20 min after the time when the amphibian Ringer's solution containing POEC was displaced by the amphibian Ringer's solution again. Then the release of these substances decreased gradually and after 150 min, they reached the same level as the controls. Phospholipids could not be detected in either the control perfusate or that containing POEC.

Recently, to study enzymatic localization in the brush border membrane, Vasseur et al. (1978, 1979) perfused deoxycholate solution through rat jejunum in an in situ experiment. The results suggested that sucrase would be located in the surface of the brush border membrane and alkaline phosphatase was partially buried in the membrane. Using the same technique, the degree of influence of POEC on rat rectum was studied by measuring the activities of enzymes released into the perfusate. As shown in Fig. 5, only the activity of sucrase, which is considered to be localized at the mucous or membrane, was detected in the perfusate, but alkaline phosphatase and lactic dehydrogenase, which are considered to be the marker enzymes of membrane and cytosol, respectively, could not detected. In addition, lysosomal enzyme, β-glucuronidase, could not be detected in the perfusate. These results did not agree with those obtained by Vasseur et al. (1978) and suggested that the enzyme distribution in rectal membrane may be different from that in intestine.

Using 1.0% POEC or Triton X-100 solution, 10% homogenates of jejunum and rectum were prepared, and the activities of enzymes, such as alkaline phosphatase, sucrase and β-glucuronidase, were determined. As shown in Table 3, three enzyme

TABLE 3

ENZYME ACTIVITIES IN HOMOGENATES OF RECTUM AND INTESTINE

Enzymes	Rectum		Jejunum	
	POEC	Triton X-100	POEC	Triton X-100
Alkaline phosphatase	7.2	5.3	64.3	54.5
Sucrase	9.0	11.3	8.3	9.2
β-glucuronidase	0.58	0.70	1.95	1.13

Enzyme activities were defined as the units/mg protein. A unit of activity is defined as that amount which liberates 1 μmole of each substrate per minute at 37°C.

activities were detected in the homogenates even though some difference of the specific activities existed between rectum and intestine. In particular, the two tissues show marked differences in alkaline phosphatase activity.

It is generally considered that the mucous layer at the superficial structures of the gastric or the intestinal lumen is disrupted by surface-active agents, and this occurrence directly affects the plasma membrane (Walters et al., 1981; Vasseur et al., 1978). For example, low concentrations of bile salts changed the mucosal surface and the damaged plasma membrane, as indicated by the release of phospholipid and protein (Vasseur et al., 1978). In this experiment, using POEC, neither the release of phospholipid nor the subcellular marker enzymes (except sucrase) could not be detected in the perfusate. Therefore, it was concluded that unlike the case with bile salts, substantial damage to the plasma membrane of epithelial cells had not occurred upon treatment with POEC.

Observation by scanning electron microscopy

The effects of POEC on the rectal surface were examined histologically using the scanning electron microscope. On the rectal wall, which was perfused with the the amphibian Ringer's solution only for 60 min, the mucous secreted by the goblet cells into the rectal lumen, was clearly observed as shown in Fig. 6A. On the other hand, the mucous could not be detected on the surface of rectum which was perfused with the amphibian Ringer's solution containing 0.1% POEC for 30 min (Fig. 6B). The electron micrographs obtained at 5 h after the termination of the POEC showed that the mucous secretion had again returned to normal.

These results indicate that the rectal wall was not damaged seriously by the POEC solution. The reasons are as follows: (1) the release of phospholipid, which is one of the plasma membrane components, could not be detected in the perfusate; (2) the subcellular marker enzymes, alkaline phosphatase, lactic dehydrogenase and β-glucuronidase, could not be detected in the perfusate; and (3) from the scanning microscopy, the rectal surface mucous was only washed off by the POEC solution. Taking these points into the consideration, the added POEC affects the mucous of the rectal membrane surface, but the erosion is not deep, and the recovery occurs within a relatively short period.

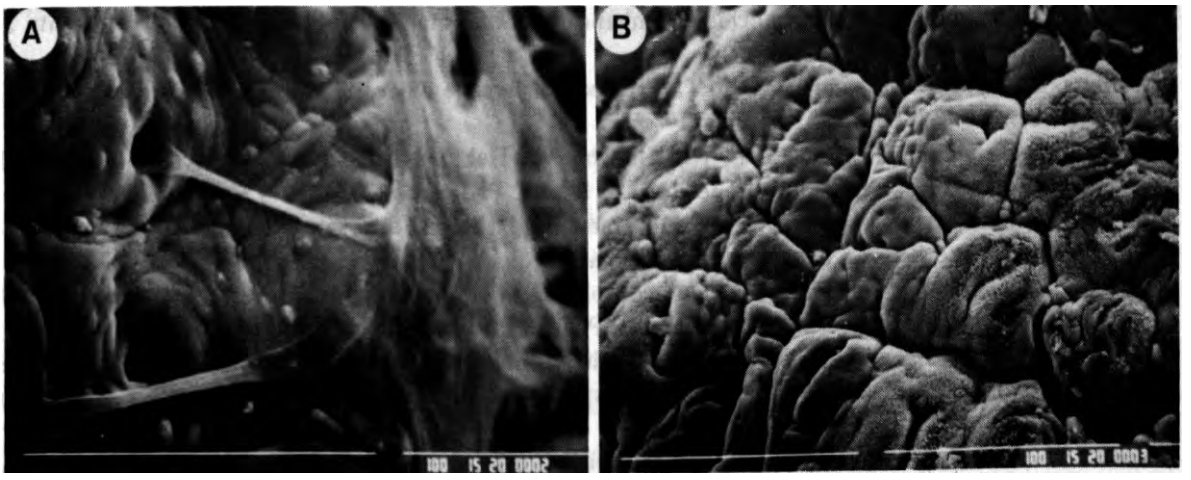

Fig. 6. Scanning electron micrographs of rat rectum perfused with amphibian Ringer's solution (A) and amphibian Ringer's solution containing 0.1% POEC (B). The white bar indicates 100 μm.

From these investigations, it appears that the addition of the non-ionic surface-active agent, POEC, to suppository bases may be useful in increasing rectal absorption of DKB.

Acknowledgement

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