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Research paper

Effect of viscous additives on drug absorption from the liver surface in rats using phenol red as a model

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Abstract

The purpose of this study is to obtain information that can be used to improve controlled release and residence time of drugs on the liver surface. Using carboxymethylcellulose sodium salt (CMC-Na) and polyvinyl alcohol (PVA), we examined the effect of viscous formulations on the absorption of phenol red as a model. In the presence of 3% CMC-Na or 15% PVA, the maximum plasma concentration of phenol red decreased after application to the rat liver surface using a cylindrical glass cell. The absorption ratios in 6 h calculated from the remaining amount of phenol red in the glass cell were 68.6, 60.5 and 48.7% (control: 73.1%) in the presence of 1 or 3% CMC-Na and 15% PVA, respectively. As a result of the reduction in the absorption ratio, the amount of phenol red excreted into the bile and urine in 6 h was decreased by the addition of the viscous additives. The decrease in absorption rate was characterized by a pharmacokinetic analysis of the plasma concentration profile. The change in absorption rate differed between the viscous additives, reflecting the result of the in vitro release experiment. Accordingly, the possibility that the drug absorption rate from the liver surface can be altered by viscous additives was suggested to have a promising prospect for therapeutic use.

Keywords: Viscous additive; Absorption; Liver surface; Phenol red; Pharmacokinetic analysis

1. Introduction

Normal treatment for liver diseases via intravenous and oral administration routes has been frustrated by inadequate distribution into the desired site in the liver as well as toxicity in other organs. Previously, we developed such direct routes as application to the liver surface and found it to be a useful method for drug delivery to the liver [1,2]. However, proper control of drug concentration and limited distribution around the applied area (especially liver surface) have to be attained in order to improve regional advantage of the drug in the liver. A controlled release pharmaceutical formulation for this purpose would help to decrease the number of applications and also the dose amount for injectable clinical use.

The administration conditions such as application volume and area, and binding to proteins existing in the peritoneal cavity are considered to influence drug absorption rates by the rat liver surface [3,4]. Also, viscosity of a drug solution is one of the most important physicochemical and pharmaceutical factors determining absorption rate of a drug. In ophthalmic therapy, polymers increasing vehicle viscosity have often been added to drug solutions to improve ocular bioavailability by prolonging contact of a drug with the eye tissues. While a relationship between formulation viscosity and ophthalmic bioavailability has been reported [5–7], there have been few reports discussing the reduction in absorption rate related to the physicochemical property [8,9]. The effect of viscosity on drug absorption across the surface membrane of a particular organ, including the liver, is thus far unknown.

In this study, we chose carboxymethylcellulose sodium salt (CMC-Na) and polyvinyl alcohol (PVA) as viscous additives. We examined the effect of these additives on drug absorption from the rat liver surface by utilizing a fast absorbing hydrophilic dye (phenol red) as a model, the absorption mechanism of which was previously determined [10].

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2. Materials and methods

2.1. Materials

Phenol red and PVA were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). CMC-Na (C-5013, high viscosity type) was obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade products.

2.2. Measurement of viscosity in buffer solution with viscous additives

We prepared CMC-Na and PVA solution (100 ml) with the indicated concentration in the isotonic phosphate buffer solution (pH 7.4). We measured the dynamic viscosity (mPa s) at 37°C with B8H type rotation viscometer.

2.3. Measurement of phenol red diffusivity from viscous solution

The diffusivity of phenol red in an isotonic phosphate buffer (pH 7.4) at 37°C in the presence or absence of 1% (w/v) CMC-Na or 15% (w/v) PVA was studied with chamber cells for equilibrium dialysis. The two chambers were separated into donor and acceptor sides using a Visking tube membrane (molecular weight cutoff 12 000–14 000). The membrane was washed three times for 2 min by boiling water, followed by equilibration with the isotonic phosphate buffer at 4°C for 24 h. Three milliliters of the mixture of phenol red (1 mg/ml) and viscous additive, and then buffer solution were added to the donor and acceptor sides, respectively. During dialysis at 37°C for 2 h, 50 μl of the solution in the receiver side was removed at 0, 15, 30, 60, 90 and 120 min, and the removed volume (50 μl) of the acceptor side has been replaced by 50 μl fresh buffer solution. Then, phenol red concentration in the acceptor side was measured.

2.4. Animal experiment

All animal procedures in the present study conformed to the Guidelines for Animal Experimentation in Nagasaki University.

Male Wistar rats (230–290 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the left femoral artery and common bile duct were cannulated with a polyethylene tube. A cylindrical glass cell (i.d. 9 mm, area 0.64 cm^2) was attached to the rat liver surface with adhesive chemical Aron Alpha (Sankyo, Tokyo, Japan). The phenol red solution (10 mg/ml × 0.2 ml) in an isotonic phosphate buffer (pH 7.4) containing 1 or 3% CMC-Na or 15% PVA was added to the glass cell.

Blood samples were collected from the heparinized cannula inserted into the femoral artery at 5, 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min, followed by centrifugation. Bile was collected at appropriate time intervals. The interval was 15 min at first 1 h, and was 30 min thereafter. At 6 h after the application, the urine remaining in the urinary bladder was collected with a syringe. In addition, the solution remaining in the glass cell was withdrawn.

2.5. Analytical methods

The concentrations of free phenol red in the plasma, bile, urine and solution recovered from the glass cell were determined spectrophotometrically at 560 nm after dilution with 1 N NaOH solution. The total concentration of free phenol red and its metabolite was similarly measured after subjection to acid hydrolysis [11]. The concentration of phenol red metabolite was estimated from the difference between these values.

2.6. Calculation of pharmacokinetic parameters

The absorption ratio in 6 h of phenol red was calculated from the remaining amount in the glass cell at 6 h after application to the rat liver surface.

The statistical moment parameters for the plasma concentration (C_p) profile of phenol red (AUC_p, MRT_p) were calculated as defined in Eqs. (1) and (2) by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation [12]. The difference of MRT_P values between i.v. administration and liver surface application corresponds to the mean time value for the absorption from the liver surface membrane (MAT).

$$AUC_p = \int_0^\infty C_p(t)dt \tag{1}$$

$$MRT_p = \frac{\int_0^\infty t \times C_p(t)dt}{\int_0^\infty C_p(t)dt} \tag{2}$$

Compartment model analysis of plasma concentration profile of phenol red after application to the rat liver surface was performed as follows. First, the plasma concentration profile of phenol red after i.v. administration was fitted to the biexponential equation described in Eq. (3), by the nonlinear least-squares method [13].

$$C_p(t) = \frac{D(\alpha - k_{21})}{V_c(\alpha - \beta)} e^{-\alpha \times t} + \frac{D(k_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta \times t} \tag{3}$$

Hybrid parameters α and β are defined as $\alpha + \beta = k_{12} + k_{21} + k_{el}$ and $\alpha \times \beta = k_{21} \times k_{el}$. D is the dose and V_c is the volume of the central compartment. k_{el} is the first-order elimination rate constant from the central compartment. k_{12} and k_{21} are the first-order transfer rate constants between the central and peripheral compartment. These parameters were substituted into the following Eq. (4) in order to determine the plasma concentration after application to the rat liver surface. The result for i.v. administration of phenol red has already been reported [10].

Next, in the same way, the plasma concentration profile of phenol red after application to the rat liver surface was

fitted in the two-compartment model with first-order absorption, by the non-linear least-squares method [13]. In this model, the plasma concentration profile can be described as follows:

$$C_p(t) = \frac{f \times D \times k_a}{V_c}\left\{\frac{k_{21} - k_a}{(\beta - k_a)(\alpha - k_a)}e^{-k_a \times t} + \frac{k_{21} - \alpha}{(\beta - \alpha)(k_a - \alpha)}e^{-\alpha \times t} + \frac{k_{21} - \beta}{(\alpha - \beta)(k_a - \beta)}e^{-\beta \times t}\right\} \quad (4)$$

k_a is the first-order absorption rate constant for absorption into the blood stream from the rat liver surface and f is the availability after application to the rat liver surface.

2.7. Statistical analysis

Statistical analysis was performed by applying unpaired Student's t-test. $P < 0.05$ was considered to be statistically significant. All results for animal experiments were expressed as the mean ± SE of at least four experiments.

3. Results

3.1. Viscosity of buffer solution with viscous additives

The viscosity (mPa s) at 37°C of the isotonic phosphate buffer (pH 7.4) containing 1% or 3% CMC-Na and 15% PVA is listed in Table 1. The viscosity of 3% CMC-Na was more than 100 times that of the 1% CMC-Na solution. The solution containing 15% PVA was as viscous as 1% CMC-Na.

3.2. Release of phenol red from viscous solution across semi-permeable membrane

We measured the cumulative in vitro release of phenol red at an initial concentration of 1 mg/ml in the presence of 1% CMC-Na and 15% PVA, in which the both solutions displayed almost equal viscosity (Table 1). Fig. 1 shows the in vitro phenol red release pattern across semi-permeable membrane. At 120 min after the in vitro release experiment, 15.7, 6.6 and 4.2% of applied phenol red diffused into the acceptor side in the control, 1% CMC-Na and 15% PVA conditions, respectively. The release rate of phenol red from the vehicle was reduced significantly by the viscous additives.

Table 1
Viscosity of CMC-Na and PVA

Viscous additive	Concentration (%)	Viscosity (mPa s)
Carboxymethylcellulose sodium salt (CMC-Na)	1	74
	3	8440
Polyvinyl alcohol (PVA)	15	53

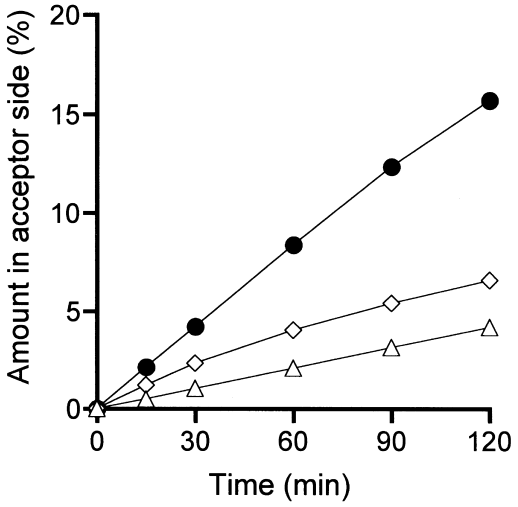

Fig. 1. Cumulative release profiles of phenol red at an initial concentration of 1 mg/ml through semi-permeable membrane from solution in the presence or absence of CMC-Na and PVA. Each point represents the mean of four experiments. (●) Control; (◇) 1% CMC-Na; (△) 15% PVA.

3.3. Effect of viscous additives on the absorption after application to the rat liver surface

In the absence or presence of the viscous additives (1 or 3% CMC-Na and 15% PVA), phenol red was found in the plasma after application to the rat liver surface utilizing a glass cell at a dose of 2 mg (Fig. 2). The phenol red metabolite could not be detected in the plasma. The maximum plasma concentration of phenol red was decreased by addition of the viscous additives, suggesting suppression of absorption rate from the rat liver surface. Also, addition of the viscous additives slowed down the elimination of phenol

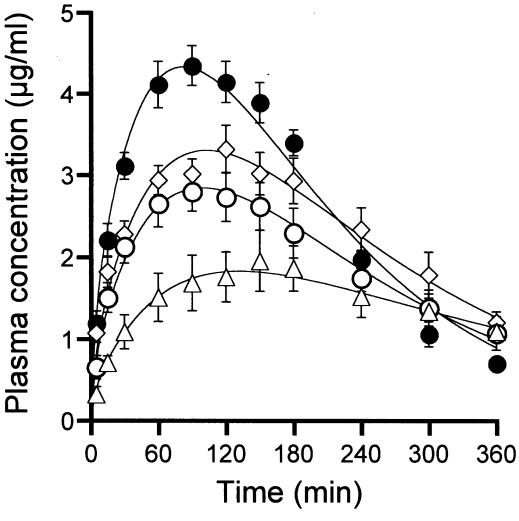

Fig. 2. Plasma concentration profiles of phenol red at a dose of 2 mg after application to the rat liver surface in the presence or absence of CMC-Na and PVA. Curves show simulated functions based on the pharmacokinetic parameters shown in Table 3. (●) Control; (◇) 1% CMC-Na; (○) 3% CMC-Na; (△) 15% PVA.

red from the plasma. In particular, 15% PVA seems to have a strong ability to reduce the absorption rate of phenol red.

After absorption from the rat liver surface, phenol red appeared in the bile as shown in Fig. 3. The metabolite (glucuronic acid conjugate) [11] was also excreted into the bile (Fig. 3B). The biliary excretion rate of free phenol red (Fig. 3A) and its metabolite (Fig. 3B) showed delayed patterns in the presence of 15% PVA, although less markedly in 1 or 3% CMC-Na. This finding might be due to a decrease in absorption from the rat liver surface.

Table 2 summarizes the recovery of phenol red in 6 h after application to the rat liver surface in the presence or absence of the viscous additives. The absorption ratio of phenol red in 6 h, calculated from the amount recovered from the glass cell, decreased with an increase in CMC-Na concentration, as 73.1, 68.6 and 60.5% of dose for the control, 1 and 3% CMC-Na, respectively. On the other hand, the absorption ratio of phenol red in 6 h was reduced to about two-thirds of the control condition in the presence of 15% PVA (48.7% of dose). The total biliary and urinary recoveries of free phenol red and its metabolite in 6 h were decreased by addition of CMC-Na, although the differences were not significant. A significant reduction was observed in the biliary excretion in the presence of 15% PVA.

The overall drug absorption process can be evaluated independent of the model with moment parameters. Area under plasma concentration curve (AUC_p) and mean residence time (MRT_p) are useful parameters for roughly evaluating drug absorbability from the rat liver surface. The moment parameters for infinite time of phenol red were calculated as shown in Table 3. The almost equal value of total AUC_p in each condition suggests that the addition of viscous additives does not alter the systemic extend of bioavailability of phenol red.

On the other hand, the MRT_P value of phenol red was prolonged by addition of the viscous additives, as compared with the control (Table 3). The prolonged MRT_P value is a reflection of the time required for the absorption process across the rat liver surface. The mean absorption time across the rat liver surface (MAT) of phenol red was calculated by subtracting the MRT_p (76.7 min) in i.v. administration from the MRT_p after application to the rat liver surface, as shown in Table 3. Addition of the viscous additives extended the MAT value of phenol red for over 100 min.

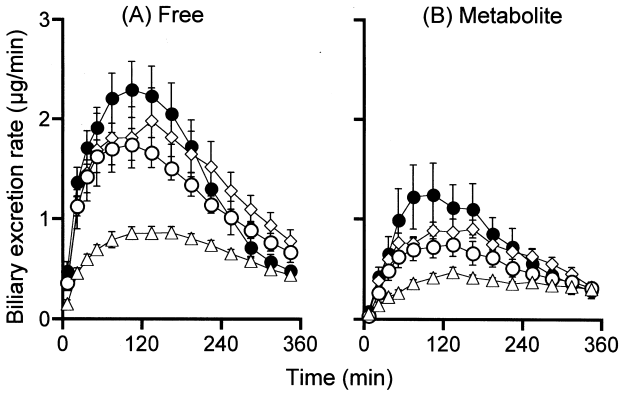

Fig. 3. Biliary excretion rate profiles of free phenol red (A) and its metabolite (B) at a dose of 2 mg after application to the rat liver surface in the presence or absence of CMC-Na and PVA. (●) Control; (◇) 1% CMC-Na; (○) 3% CMC-Na; (△) 15% PVA.

Moreover, we constructed a two-compartment model with first-order absorption to explain the in vivo behavior of phenol red after application to the rat liver surface. We performed model-fitting analysis of the plasma concentration of phenol red in the presence of the viscous additives. The fitting curves in Fig. 2 were drawn by using the obtained kinetic parameters k_a (Table 3). The experimental value and fitting curve agreed approximately. Therefore, it seems possible to use the pharmacokinetic model the same as the control condition even if viscous additives are added. The obtained k_a values of phenol red in the presence of the viscous additives were significantly smaller than that of the control (Table 3), supporting the possibility of reduction in the absorption rate.

4. Discussion

We chose CMC-Na and PVA as model viscous macromolecules in this study. CMC-Na, numerous price of carboxymethyl ether of cellulose sodium salt, becomes a paste-shaped colloid solution and displays high viscosity by dissolving in water. The viscous formulation using CMC-Na has been used for eye drops to improve efficacy of ophthalmic drugs. On the other hand, PVA produced by hydrolysis of polyvinylpyrrolidone has several residues of acetic acid. PVA solution has characteristics of viscous additive owing to its complexity in molecule residue.

When viscous additives were applied simultaneously to the rat liver surface employing a glass cell, an over 100-min prolongation was seen in MAT of phenol red describing mean absorption time across the rat liver surface (Table 3). This tendency was represented by a reduction in the k_a value with the pharmacokinetic analysis. In proportion to the reduction in the absorption rate, biliary excretion of phenol red was prolonged, which might lead to enhancement of drug availability in the liver.

Accordingly, the viscosity of the drug solution was found to be an important factor with respect to optimizing drug absorption from the liver surface. Drug concentration in the liver around the applied site could be retained by simultaneous administration of viscous additives. In addition, risk of systemic and/or local side effects can be overcome by adequate control of the pharmacological level of the drug, since rapid increase of phenol red concentration in the plasma was suppressed by addition of the viscous additives as shown in Fig. 2.

Reduction of drug diffusivity in the administered vehicle accompanying viscosity change should have resulted in sustained retention of phenol red in the glass cell. Drug

Table 2
Recovery (% of dose) of phenol red in 6 h at a dose of 2 mg after application to the rat liver surface in the presence or absence of CMC-Na and PVA[a]

Condition	Glass cell (free)	Bile			Urine		
		Free	Metabolite	Total	Free	Metabolite	Total
Control	26.9 ± 2.7	26.0 ± 3.6	13.3 ± 3.2	39.3 ± 5.5	13.9 ± 3.9	9.1 ± 2.2	23.0 ± 4.5
CMC-Na 1%	31.4 ± 2.6	25.5 ± 3.7	11.5 ± 1.1	37.0 ± 4.6	15.0 ± 2.6	6.2 ± 1.3	21.2 ± 3.5
CMC-Na 3%	39.5 ± 1.5**	21.9 ± 1.6	9.3 ± 1.5	31.2 ± 2.9	11.4 ± 2.2	9.1 ± 0.7	20.4 ± 1.8
PVA 15%	51.3 ± 5.4*	11.6 ± 0.7*	5.9 ± 0.4	17.5 ± 1.0*	14.8 ± 3.2	4.4 ± 0.6	19.2 ± 3.2

[a] Significantly different from control: $*P < 0.05$, $**P < 0.01$.

release rate appears to be modulated with varying viscous additive concentrations. Also, a large difference in alteration of the absorption rate across the rat liver surface was characterized by MAT and k_a values between CMC-Na and PVA. This can be explained by the result that PVA had a stronger effect on the drug diffusion in the vehicle compared to CMC-Na, judging from the in vitro release rate of phenol red across a semi-permeable membrane as shown in Fig. 1. PVA has numerous functional groups in the molecule, so the diffusion of phenol red in the vehicle could possibly be decreased as a result of interaction between PVP and phenol red.

This administration form would be performed through the peritoneal cavity around the liver surface in the case of clinical use. From the results obtained here, the drug availability should be increased by elevating vehicle viscosity of the dosing solution, and by giving drugs with selective affinity for the liver. Recently, implantable infusion pumps have been developed for treatment of several diseases, and endoscopic and laparoscopic operation techniques have made remarkable progress. These advanced medical technologies and pharmaceutical modifications should make possible the clinical application for a drug to the liver surface.

Intraperitoneal administration has been often utilized to increase antineoplastic drug exposure to tumors confined to the peritoneal cavity. However, most antineoplastic drugs in solution forms are rapidly cleared into the systemic circulation, as reviewed recently [14–16]. We suppose that the effective area for absorption became wide according to diffusion of drug solution in the peritoneal cavity. In addition, we have shown that the absorption rate from the peritoneal cavity in rats after i.p. administration to the liver surface was faster than that after i.p. administration to the distal small intestine [17]. Accordingly, it is necessary to understand the change in absorbability caused by dilution of drug solution with the serous fluid, binding of drug to the ingredient in peritoneal fluid or adhesion of drug to the peritoneal surrounding organ. Therefore, we should pay attention to application conditions such as volume and area on absorption from the liver surface, which we have already examined [4].

For i.p. administration to the liver surface, it seems necessary to increase the ability to attach to the liver surface with appropriate pharmaceutical modification. Adhesive formulation would enable us to control drug absorption precisely and to limit drug distribution in the liver, by increasing contact time with the membrane at the site of absorption. On the other hand, several pharmaceutical preparations such as liposome [18–20], microsphere [21,22] and carbon particle [23,24] have been used for controlled drug release in the peritoneal cavity. It is expected that the drug release can be controlled much more by a combination of these formulations and viscous additives. For example, if thermosensitive polymers exhibiting temperature-dependent reversible transition from liquid to semisolid had some interaction with the liver surface membrane, the injectable formulation would be useful to improve controlled drug release in the peritoneal cavity.

5. Conclusion

The drug absorption rate from the liver surface can be altered by viscous additives for controlled release. Not only reduction in drug release rate but also other factors were shown to be important and remain to be further clarified in the future.

Table 3
Pharmacokinetic parameters of phenol red at a dose of 2 mg after application to the rat liver surface in the presence or absence of CMC-Na and PVA[a]

Condition	AUC_p (μg/ml min)	MRT_p (min)	MAT_p (min)	k_a ($min^{-1} \times 10^{-3}$)
Control	1046.1 ± 47.8	171.2 ± 6.8	94.5 ± 6.8	8.66 ± 0.51
CMC-Na 1%	1184.1 ± 94.5	288.4 ± 31.6*	211.7 ± 31.6*	6.25 ± 0.69*
CMC-Na 3%	1059.8 ± 166.5	312.8 ± 42.3*	236.1 ± 42.3*	6.12 ± 0.80*
PVA 15%	1154.1 ± 213.2	568.1 ± 87.4**	491.4 ± 87.4**	2.94 ± 0.36**

[a] Significantly different from control: $*P < 0.05$, $**P < 0.01$.

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