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Vitamin K Prodrugs: 2. Water-Soluble Prodrugs of Menahydroquinone-4 for Systemic Site-Specific Delivery

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Purpose. The hydrochloride salts of the *N,N*-dimethylglycine esters of menahydroquinone-4 (1-mono, **1**; 4-mono, **2**; and 1,4-bis, **3**) were assessed *in vivo* as prodrug for the systemic site-specific delivery system of menahydroquinone-4 (MKH), the active form of menaquinone-4 (MK-4, vitamin $K_{2(20)}$).

Methods. The disposition of MK-4 and menaquinone-4 epoxide (MKO) following the intravenous administration of the prodrugs and MK-4 preparation solubilized with surfactant (H-MK-4) were studied in vitamin K cycle inhibited rats. The relative bioavailability of MKH after the administration of the prodrugs was assessed from the area under the plasma concentration of MKO vs. time curve (AUC_{MKO}). The specific delivery of MKH to its active site (liver) and coagulation activity after the administration of selected prodrug **1** were then compared with those of H-MK-4 in warfarin poisoned rats.

Results. All compounds showed linear pharmacokinetics, and significant bioavailability of MKH was also observed following the administration of **1** (188%), **2** (87%) and **3** (135%). Prodrug **1** caused the following increases; AUC^{liver} of MKO from 70.7 ± 5.77 (H-MK-4) to 167 ± 7.89 nmol · h/g, MRT^{liver} of MKO, from 3.87 ± 0.307 to 8.57 ± 0.432 h. The liver accumulation of intrinsic **1** reached a maximum (88% of dose) by 0.25 h. The rapid and liver-selective uptake and liver esterase mediated MKH regeneration characteristics of **1** enhanced the delivery of MKH to its active site and the selective advantage was increased 5.7 fold. The coagulation activity was extended 1.9 fold by **1** administration.

Conclusions. The results indicated that these highly water-soluble and liver-esterase hydrolyzable ester derivatives of MKH are potential candidates for parenteral prodrugs which can thus achieve the systemic site-specific delivery of MKH. Such effective and selective delivery of MKH to its active site can therefore lead to enhanced pharmacological efficacy and can also avoid the toxicity induced by the solubilizing agent used in the H-MK-4 preparation.

KEY WORDS: bioavailability; menahydroquinone-4; water-soluble prodrug; site-specific delivery; coagulation activity.

INTRODUCTION

The effective and selective delivery of the bioactive form of drugs to their site of action may lead to enhanced efficacy and reduced toxicity. Most drugs containing quinone functions undergo enzymatic reductive activation to a hydroquinone form during effective processing (1, 2). In addition, their pharmacological efficacy and toxicity are also expressed upon the enzyme-dependent reduction of quinone to hydroquinone.

Vitamin K is one such quinone drug, and its fully reduced form, vitamin K hydroquinone, is an essential cofactor in the biosyntheses of vitamin K-dependent proteins (reviewed in ref. 1). The bioreductive activation step of the vitamin consists of one-electron and two-electron reductions. The two-electron reduction pathway has generally been considered to predominate in the vitamin K cycle and is relatively non-toxic.

Warfarin and other coumarins block the vitamin K cycle by inhibiting of vitamin K epoxide reductase and the two-electron reduction which leads to anticoagulation (Fig. 1). Vitamin K can overcome the anticoagulation due to the formation of vitamin K hydroquinone via the one-electron reduction pathway, which is unaffected by coumarins (3, 4). However, the one-electron reduction generates semiquinones which can produce active oxygen species that result in oxidative stress such as menadione-induced hepatotoxicity (5-7). It appeared that the use of the quinone form of vitamin K in the treatment of coumarin anticoagulant poisoning might cause oxidative toxicity. Therefore, it would be most effective if the selective delivery of the hydroquinone to its active site could be carried out without the quinone reductive activation step.

Another delivery problem associated with vitamin K hydroquinone arises from the fact that vitamin K is practically insoluble in the aqueous media. The intravenous (iv) administration of the vitamin is frequently used in situations where a predictable alteration in the coagulation is to be achieved. However, the iv administration of the vitamin often produces an anaphylactoid reaction in certain individuals (8). These adverse reactions are believed to be related to the surfactant, polyoxyethylene hydrogenated castor oil (HCO-60), used in the parenteral dosage form.

In order to overcome the above mentioned delivery problems of the vitamin K hydroquinone, we have proposed the use of the ester prodrug approach for vitamin K hydroquinone (Fig. 1). The ester prodrug could improve the water solubility and, at the same time, achieve the systemic site-specific delivery of vitamin K hydroquinone without any bioreductive activation process.

In a previous paper (9), *N,N*-dimethylglycine esters (1-mono, **1**; 4-mono, **2**; and 1,4-bis, **3**, shown in Fig. 1) of menahydroquinone-4 (MKH), the active form of menaquinone-4 (MK-4), had been prepared and assessed as potentially useful prodrugs for parenteral use *in vitro*. Previous studies have shown that such compounds as hydrochloride salts could improve the water solubility and could also generate the active hydroquinone using esterase located in the active site (liver esterase) (9). These characteristics of the derivatives allow systemic site-specific delivery of the hydroquinone.

In this study, the disposition of MK-4 and menaquinone-4 epoxide (MKO) following iv administration of the esters in vitamin K cycle inhibited rats with warfarin was

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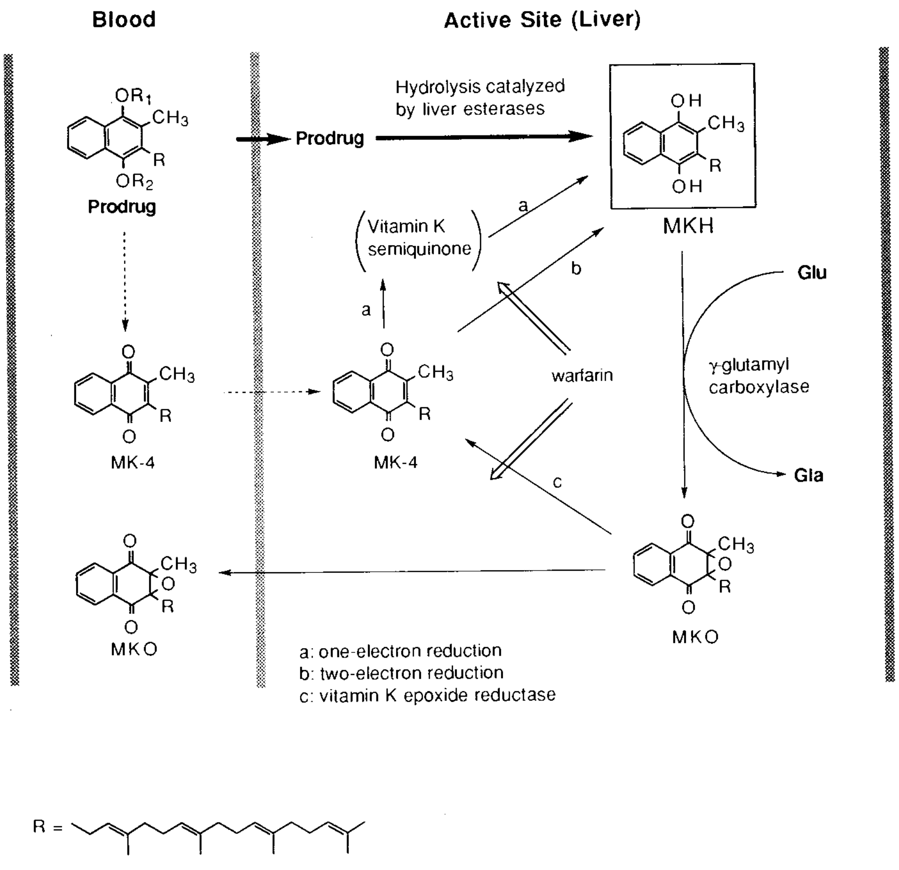

1: R_1 = $(CH_3)_2NCH_2CO$-, R_2 = H
2: R_1 = H, R_2 = $(CH_3)_2NCH_2CO$-
3: R_1 = R_2 = $(CH_3)_2NCH_2CO$-

Fig. 1. Schematic illustration of the prodrug concept for the systemic site-specific delivery of menahydroquinone-4.

first compared with that of the commercially available preparation of MK-4 in order to establish their utility as a prodrug for iv administration. In addition, the potential of selected prodrug 1 to achieve the systemic site-specific delivery of MKH and its coagulation activity were evaluated in warfarin poisoned rats, who were induced with hypoprothrombinemia while the vitamin K cycle was also inhibited.

MATERIALS AND METHODS

The hydrochloride salts of the *N,N*-dimethylglycine esters of MKH were synthesized in our laboratory using previously reported methods (9). The injection solution of MK-4 solubilized with HCO-60 (H-MK-4, Kaytwo®), MKO, warfarin (racemate) potassium, Hepaplastin® test were kind gifts from Eisai Co., Ltd. (Tokyo, Japan). All other chemicals were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Male Wistar rats, 280-320 g, were purchased from Charles River Japan (Atsugi, Japan).

Plasma Disposition Studies in Vitamin K Cycle Inhibited Rats

The rats were fasted for 16 h prior to the study and kept in coprophagy-preventing cages. Two hours before drug administration, the rats were intraperitonialy treated with warfarin potassium. The doses ranged from 0.01-8 mg/kg. The drugs were administered via the left femoral vein which was exposed by means of a small incision under light ether anesthesia. Blood (300 μl) was taken from the external jugular vein using heparinized syringes at 0.125, 0.25, 0.5, 1, 2, 4, 6, and 8 h. The plasma samples (100 μl) were added to 350 μl of methanol-ethyl acetate (4:1, v/v), vortexed for 2 min and then centrifuged at 3000 rpm for 5 min. The supernatant layer was determined by the HPLC method as mentioned below. Warfarin potassium and the esters were dissolved in water for injection and administered at 0.1 ml/100g of body weight except for 2 at 0.2 ml/100g. The doses of the drugs were 1, 2.5 and 5 mg/kg equivalent for MK-4.

To use the plasma MKO concentration as an indicator of MKH, the vitamin K cycle-inhibited state must be valid and reproducible. The dose effect of warfarin on AUC_{MKO} after the iv administration of H-MK-4 was preliminarily determined in the rats. The relationship between the warfarin dose and AUC_{MKO} gave a sigmoidal dose-dependent curve (data was not shown). A warfarin dose of over 1 mg/kg, the values of AUC_{MKO} were constant, which thus indicated a reproducible inhibited state of the vitamin K cycle. Similar dose-response relationships between the warfarin dose and the maximum plasma level of vitamin K epoxide have also been observed in man (10). Based on this finding, a warfarin dose of 1 mg/kg was chosen for the preparation of vitamin K cycle inhibited rats.

Disposition and Coagulation Activity Studies in Warfarin Poisoned Rats

The dose effect of warfarin potassium on the plasma coagulation activity was preliminarily determined for the warfarin poisoned rats, who were induced with hypoprothrombinemia while the vitamin K cycle was also inhibited. Sixteen hours before drug administration, the rats were intraperitonialy treated with warfarin potassium (5, 10 mg/kg) and kept in coprophagy-preventing cages, and fed sugar cubes during the study. H-MK-4 (5 mg/kg) was administered via the left femoral vein which was exposed by means of a small incision under light ether anesthesia. Blood (0.45 ml) was taken from the external jugular vein using syringes containing 3.2% sodium citrate at an appropriate time. The blood was centrifuged at 3000 rpm for 10 min. The coagulation activity of the plasma was determined by the method mentioned below. As can be seen in Fig. 2, the coagulation activities of all rats treated with warfarin decreased to below 5% and thereafter rapidly increased up to 60% at 3 h after H-MK-4 administration (5 mg/kg, iv). After 6 h, the activity again decreased. Similar coagulation activity-time profiles of 5 and 10 mg/kg warfarin treated rats indicated a valid and reproducible inhibited state of clotting factor synthesis. Based on these findings, a warfarin dose of 10 mg/kg was chosen for the preparation of warfarin poisoned rats.

The warfarin poisoned rats were prepared and the drugs (1 and H-MK-4) were administered according to the procedures mentioned above. The doses of the drugs were 5 mg/kg equivalent for MK-4. Under ether anesthesia, blood (4.5 ml) was taken from the abdominal artery using a syringe containing 0.5 ml of 3.2% sodium citrate and the liver was removed at 0.25, 0.5, 1, 2, 4, 6, and 12 h. Plasma was obtained by centrifugation at 3000 rpm for 10 min and an aliquot of plasma was used for an immediate analysis of the coagulation activity while other plasma was stored at −80°C until use for the HPLC analysis. The liver was homogenized with 3 volumes of 1.15% KCl solution using a POLYTRON homogenizer (Kinematica, Switzerland) and stored at −80°C until use for the HPLC analysis. The coagulation activity of the plasma was analyzed by the method mentioned below. The liver and plasma were determined by the HPLC method as mentioned below. Total plasma volume of 31.3 ml/kg of body weight is used for calculation of the plasma distribution of 1, MK-4 and MKO.

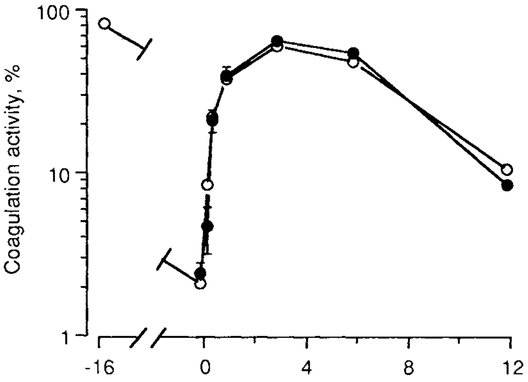

Fig. 2. The dose effect of warfarin potassium on the coagulation activity of H-MK-4 against hypoprothrombinemia induced by warfarin pretreatment in the rats. Key: (●) warfarin potassium 5 mg/kg; (○) warfarin potassium 10 mg/kg. Each point represents the mean ± SD of 3 rats. The dose of H-MK-4 is 5 mg/kg.

HPLC Analysis

Liver and plasma concentrations of 1, MK-4 and MKO were analyzed by the following HPLC method. The analysis of MK-4 and MKO was carried out using a modification of the HPLC method described by Haroon et al. (11). Liver and plasma samples (100 μl) were added to 350 μl of methanol-ethyl acetate (4:1, v/v), vortexed for 2 min and then centrifuged at 3000 rpm for 5 min. The supernatant layer was determined by the HPLC method as mentioned below. Unless otherwise noted, the HPLC system was used (Shimadzu Co. Ltd., Kyoto, Japan). The system consisted of a pump (LC-6A), an auto sample injector (SIL 9A), a diodearray detector (SPD-M10A), a fluorescence HPLC monitor (RF-540 equipped with 12 μl LC flow cell), a data analysis system class M10 and peak integrators (C-R7A). A reversed-phase column CAPCELL PAK C18 (4.6 × 200 mm, Shiseido, Tokyo, Japan) with a guard column Guard-PAK (Japan Waters, Tokyo, Japan) and a mobile phase of methanol containing 0.02 M acetic acid and sodium acetate at a flow rate of 1.0 ml/min were used. A 4.6 × 50 mm zinc column (Nacalai tesque, Kyoto, Japan) was placed in-line between the diodearray detector and the fluorometer. The eluent was photometrically monitored at 240 nm for 1 and was also fluorometrically monitored at a 320 nm excitation and a 430 nm emission for MK-4 and MKO. The quantitation of the compounds was achieved using the linear calibration curves of the peak area vs. concentration.

Coagulation Activity

The coagulation activity of the plasma was determined by the Hepaplastin® test. The coagulation time was measured by a coagulometer KC 1A (Amelung, Germany) and was converted to the coagulation activity by the use of a standard curve produced by determining the coagulation times of normal rat plasma diluted in plasma treated with barium sulfate, at a concentration 1-100%. The anticoagulated plasma had a prothrombin time >500 s, which indicated the absence of active vitamin K-dependent clotting factors.

Pharmacokinetic Analysis

The plasma and liver concentration versus time data were analyzed using the model independent and statistical moment methods (12, 13). Both the maximum concentration (C_{max}) and its corresponding time (t_{max}) were directly obtained from the observed data.

RESULTS AND DISCUSSION

To assess the esters as prodrugs of MKH, the hydroquinone levels in biological tissue must be measured. However, the accurate determination of MKH is impaired by its highly oxidative characteristics. It has been postulated that MKH is stoichiometrically metabolized to MKO concurrent with the γ-carboxylation process of glutamyl residue (Glu) to γ-carboxyglutamyl residue (Gla), and the back reconversion of MKO to MK-4 is inhibited under the vitamin K cycle inhibited condition with warfarin treatment (Fig. 1) (1). It thus appears that AUC_{MKO} can reflect the cumulative amount of MKH delivered to its site of action in the vitamin K cycle inhibited condition. It has also been shown that a good correlation is exhibited between the AUC of vitamin K_1 epoxide and vitamin K activity (coagulation time) in humans receiving coumarin therapy (14). Therefore, in this study, the disposition of MKH was first assessed using the plasma levels of MKO in rats in which the vitamin K cycle was inhibited by warfarin treatment.

Bioavailability of the Prodrugs

To assess the hypothesis that the esters **1-3** would be suitable water-soluble prodrugs of MKH for parenteral administration, the esters and H-MK-4 were intravenously administered to the vitamin K cycle-inhibited rats. The plasma concentration vs. time profiles of MKO and MK-4 are shown in Fig. 3. The rapid appearance of MKO and MK-4 in plasma after the prodrug administration indicated a regeneration of MKH and its pharmacological action *in vivo*. The pharmacokinetic parameters are listed in Table I.

In all cases the values of AUC_{MKO} and $AUC_{MK\text{-}4}$ were dose dependently increased in the dose range 1-5 mg/kg equivalent for MK-4. This finding indicated that there were no saturated processes during the regeneration of MKH from the prodrugs, the γ-glutamylcarboxylation and the reductive activation step (one-electron reduction) in the dose range tested.

Because the plasma MKO may reflect MKH which stimulates the γ-glutamylcarboxylase at the active site, the relative bioavailability for MKH (F_{MKH}) after the administration of the prodrugs relative to H-MK-4 administration was calculated using AUC_{MKO} as in Eqn. 1 and is shown in Table I.

$$F_{MKH} = \frac{AUC_{MKO,prodrug} \cdot D_{MK-4}}{AUC_{MKO,MK-4} \cdot D_{prodrug}} \qquad (1)$$

where $AUC_{MKO,MK\text{-}4}$ and $AUC_{MKO,prodrug}$ are the AUC_{MKO} values after the administration of H-MK-4 and the prodrug, respectively. $D_{MK\text{-}4}$ and $D_{prodrug}$ are doses of H-MK-4 and the prodrug, respectively.

Prodrugs **1** and **3**, but not **2**, showed an improvement in the bioavailability over H-MK-4 injection. This result clearly indicates that compound **1** might be the most useful candidate for the parenteral prodrug of MKH. Based on these findings, further studies were carried out using only prodrug **1**.

Disposition of the Selected Prodrug and H-MK-4 in Warfarin Poisoned Rats

The iv administration of vitamin K is frequently used in situations where a predictable alteration in the coagulation needs to be achieved, such as in coumarin poisoning. The potential of **1** to achieve a systemic site-specific delivery of MKH and its coagulation activity were evaluated in warfarin poisoned rats, with induced hypoprothrombinemia and an inhibited vitamin K cycle.

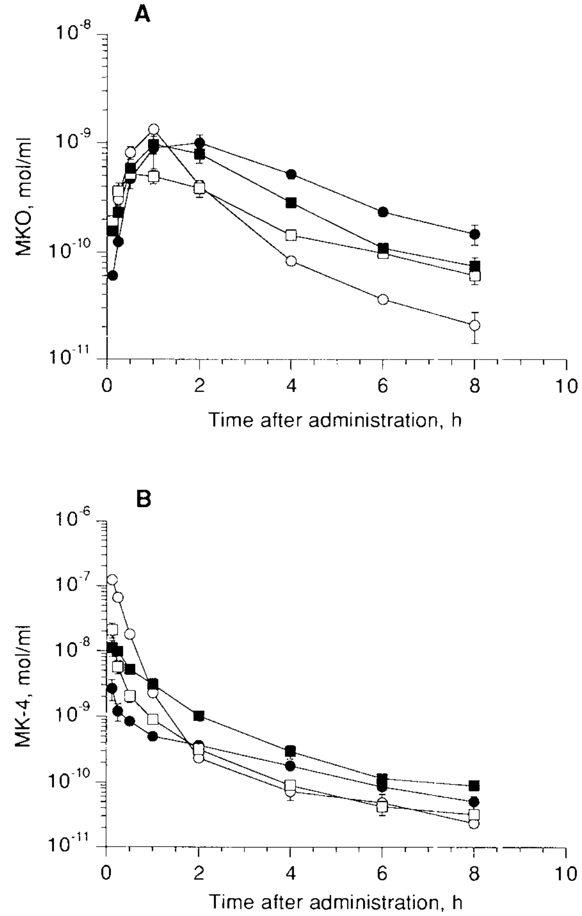

Fig. 3. Mean plasma concentration of MKO (A) and MK-4 (B) after the intravenous administration of the prodrugs and H-MK-4 in the vitamin K cycle inhibited rats. Key: (○) H-MK-4; (●) **1**; (□) **2**; (■) **3**. Each point represents the mean ± SD of 3 rats. The doses are 5 mg/kg equivalent for MK-4.

The time course of liver and plasma levels of **1**, MKO and MK-4 after iv administration of **1** and H-MK-4 in the warfarin poisoned rats are shown in Fig. 4. The plasma disposition profiles of MKO and MK-4 were similarly observed in the vitamin K cycle inhibited rats (Fig. 3), which thus indicated a complete inhibition of the vitamin K cycle.

As mentioned previously, since the quinone form of vitamin K (MK-4) is a nonactive compound which must be converted by the enzyme into an active species, MK-4 can thus be regarded as a prodrug for MKH. Therefore, a disposition of MKO in the liver is the most meaningful indicator for assessing the potential of vitamin K preparations for the MKH delivery system. The extent and the rate of delivery of MKH are assessed by using the pharmacokinetic parameters for MKO in the liver (Table II). The values of AUC_{MKO} and MRT_{MKO} of **1** were larger than that of H-MK-4, which indicated that the ester prodrug approach can make large extent and long duration of delivery of MKH to its active site. The

Table I. Pharmacokinetic Parameters for MKO and MK-4 in Plasma After the Intravenous Administration of the Prodrugs and H-MK-4 in Vitamin K Cycle Inhibited Rats[a]

	H-MK-4	Prodrugs		
		1	2	3
for MK-4				
C_{max} (nmol · ml^{-1})	107 ± 2.07	2.41 ± 0.826	20.8 ± 5.24	11.1 ± 3.03
t_{max} (h)	0.125	0.125	0.125	0.125
AUC_{MK-4} (nmol · h · ml^{-1})	31.7 ± 0.526	2.52 ± 0.387	6.01 ± 1.26	10.1 ± 1.15
MRT_{MK-4} (h)	0.338 ± 0.018	2.44 ± 0.032	0.924 ± 0.004	1.32 ± 0.063
for MKO				
C_{max} (nmol · ml^{-1})	1.92 ± 0.172	0.991 ± 0.131	0.518 ± 0.039	0.968 ± 0.180
t_{max} (h)	1	2	0.5	1
AUC_{MKO} (nmol · h · ml^{-1})	2.37 ± 0.115	4.45 ± 0.510	2.06 ± 0.322	3.20 ± 0.467
MRT_{MKO} (h)	2.14 ± 0.091	3.78 ± 0.404	3.93 ± 0.463	2.80 ± 0.066
F_{MKH}[b] (%)	100	188	87	135

[a] The values are the mean and S.D. of three rats at a dose of 5 mg/kg equivalent for MK-4.

[b] Calculated from eq. (1) using the mean values.

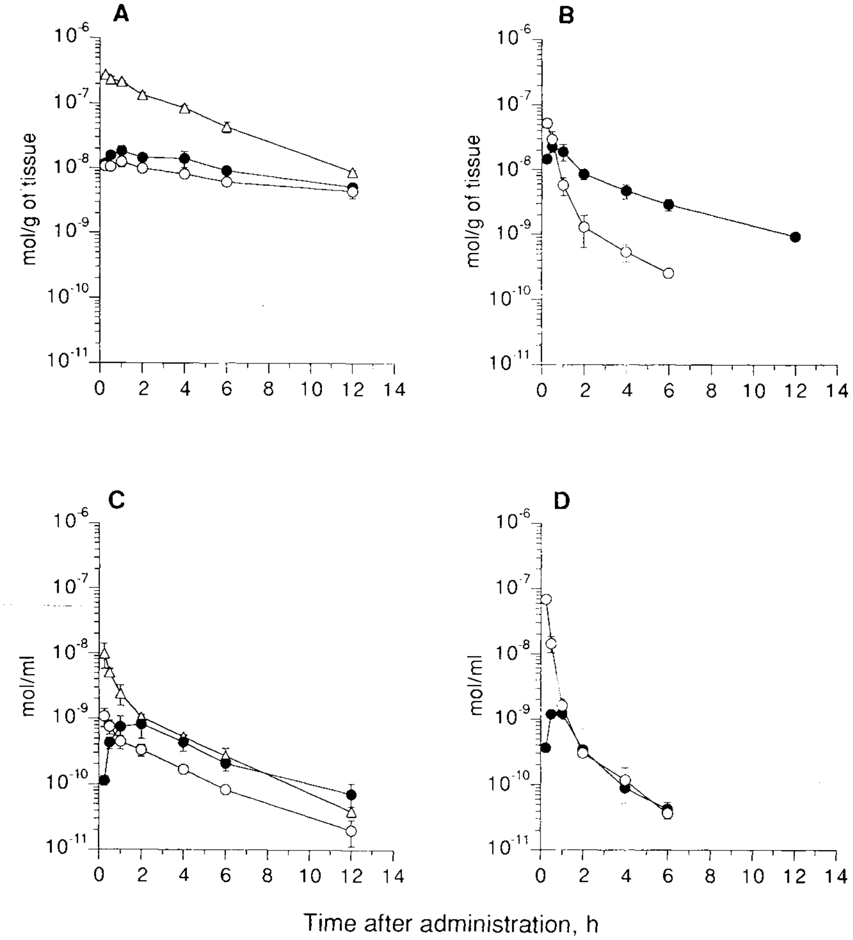

Fig. 4. Mean liver and plasma concentration of 1, MKO, and MK-4 concentration after the intravenous administration of 1 and H-MK-4 in warfarin poisoned rats. (A) The liver levels after 1 administration. (B) The liver levels after H-MK-4 administration. (C) The plasma levels after 1 administration. (D) The plasma levels after H-MK-4 administration. Key: (△) 1; (○) MK-4; (●) MKO. Each point represents the mean ± SD of 3 rats. The doses are 5 mg/kg equivalent for MK-4.

Table II. Pharmacokinetic Parameters in the Liver and Selective Advantage for Delivery of MKH to the Active Site After the Administration of 1 and H-MK-4 in the Warfarin Poisoned Rats[a]

	H-MK-4		Prodrug 1	
	Liver	Plasma	Liver	Plasma
for intrinsic 1				
C_{max}			276 ± 10.5 (nmol · g^{-1})	9.86 ± 4.06 (nmol · ml^{-1})
t_{max} (h)			0.25	0.25
AUC_1			918 ± 35.9 (nmol · h · g^{-1})	10.1 ± 1.07 (nmol · h · ml^{-1})
MRT_1 (h)			3.38 ± 0.117	2.02 ± 0.269
for MK-4				
C_{max}	52.3 ± 8.30 (nmol · g^{-1})	68.5 ± 7.13 (nmol · ml^{-1})	12.4 ± 2.14 (nmol · g^{-1})	1.08 ± 0.334 (nmol · ml^{-1})
t_{max} (h)	0.25	0.25	1	0.25
AUC_{MK-4}	33.3 ± 7.51 (nmol · h · g^{-1})	24.4 ± 3.31 (nmol · h · ml^{-1})	141 ± 24.2 (nmol · h · g^{-1})	2.19 ± 0.424 (nmol · h · ml^{-1})
MRT_{MK-4} (h)	0.338 ± 0.018	0.443 ± 0.030	10.7 ± 1.03	3.15 ± 0.340
for MKO				
C_{max}	22.3 ± 4.22 (nmol · g^{-1})	1.22 ± 0.232 (nmol · ml^{-1})	18.1 ± 3.56 (nmol · g^{-1})	0.824 ± 0.327 (nmol · ml^{-1})
t_{max} (h)	0.5	1	1	2
AUC_{MKO}	70.7 ± 5.77 (nmol · h · g^{-1})	2.49 ± 0.384 (nmol · h · ml^{-1})	167 ± 7.89 (nmol · h · g^{-1})	4.60 ± 1.33 (nmol · h · ml^{-1})
MRT_{MKO} (h)	3.87 ± 0.307	1.92 ± 0.245	8.57 ± 0.432	4.08 ± 0.611
Selective advantage[b]	1.0		5.7	

[a] The values are the mean and S.D. of three rats at a dose of 5 mg/kg equivalent for MK-4.

[b] Calculated from eq. (2) using the mean values.

MKH delivery characteristics of 1 resulted in an improved coagulation activity against the hypoprothrombinemia in the warfarin poisoned rats as shown in the next section.

The Assessment of the Prodrug as a Site-Specific Delivery System

For achieving systemic site-specific delivery of MKH via prodrug, site-selective uptake of the prodrug and site-selective activation of the prodrug are important criteria. It had been shown that the regeneration of MKH was catalyzed by both rat liver and rat plasma esterases (9). As can be seen in Fig. 4, 1 was rapidly eliminated from the plasma and accumulated in the liver and the maximum accumulation of 1 was achieved at 15 min after administration. The liver accumulation of 1, MK-4 and MKO were 88 ± 5.7, 3.4 ± 0.1, and 3.7 ± 0.3% of dose, respectively. The total recovery in the liver (1, MK-4 and MKO) was over 95% of dose. At this time, the plasma distribution of 1, MK-4 and MKO were only 2.7 ± 1.1, 0.3 ± 0.1 and 0.03 ± 0.01% of dose, respectively.

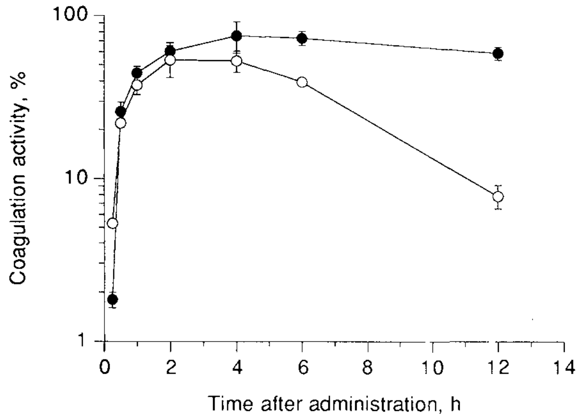

Fig. 5. The coagulation activity after the intravenous administration of 1 and H-MK-4 in warfarin poisoned rats. Key: (○) H-MK-4; (●) 1. Each point represents the mean ± SD of 3 rats. The doses are 5 mg/kg equivalent for MK-4.

The rapid and liver-specific uptake of 1 and the appearance of MKO and MK-4 in the liver clearly indicate that the regeneration of MKH might thus occur in the liver (an active site of MKH) while most of the MKH is delivered to the active site through the other route of the reductive activation pathway after the iv administration of 1. The plasma esterase reconversion characteristic is inconvenient for the specific delivery because it could be expected that resultant MKH might be easily oxidized to MK-4 in blood under oxidative conditions. However, the plasma distribution of 1 and MK-4 was very small. Therefore, it can be expected that the only a small amount of MKH may be delivered to the active site through the reductive activation pathway. These results thus support the initial hypothesis (Fig. 1) that an ester prodrug of MKH can achieve a selective delivery of the parent drug.

For the assessment of 1 as a site-specific delivery system for MKH, the selective advantage value (15) was also used. The liver level of MKO is a relative measure of the MKH which is delivered to the active site and is used as a cofactor for γ-glutamylcarboxylase. The selective advantage is defined as:

Selective advantage =

$$\left\{\frac{AUC^{\mathrm{Liver}}_{\mathrm{MKO,prodrug}}}{AUC^{\mathrm{Liver}}_{\mathrm{MKO,\ H\text{-}MK\text{-}4}}}\right\} \Big/ \left\{\frac{AUC^{\mathrm{plasma}}_{\mathrm{prodrug,prodrug}}}{AUC^{\mathrm{plasma}}_{\mathrm{MK\text{-}4,H\text{-}MK\text{-}4}}}\right\} \quad (2)$$

where $AUC^{\mathrm{Liver}}_{\mathrm{MKO,prodrug}}$ and $AUC^{\mathrm{Liver}}_{\mathrm{MKO,H\text{-}MK\text{-}4}}$ are AUC_{MKO} values in the liver after the administration of 1 and H-MK-4, respectively. $AUC^{\mathrm{plasma}}_{\mathrm{prodrug,prodrug}}$ and $AUC^{\mathrm{plasma}}_{\mathrm{MK\text{-}4,H\text{-}MK\text{-}4}}$ are *AUC* values of plasma 1 and MK-4 after 1 and H-MK-4 administration, respectively. The numerator is the relative exposure, as measured by *AUC*, to MKH at the active site after the administration of 1 and H-MK-4. The denominator is the relative drug exposure in the systemic circulation after the two administrations. A remarkable site-specific delivery of MKH was observed after the administration of 1; the selective advantage of 1 was 5.7 (Table II).

Coagulation Activity in Warfarin Poisoned Rats

Time course of coagulation activity after the administration of 1 and H-MK-4 in the warfarin poisoned rats is shown in Fig. 5. The coagulation activities of all rats rapidly increased and reached a maximum at 4 h after drug administration. The activity of 1 was maintained in 60% by 12 h, but the activity of H-MK-4 again declined from 4 h was under 10% at 12 h. The extent of the activity was assessed by the area under the effect vs. time curve ($\mathrm{AUEC}_{0\text{-}12\mathrm{h}}$) which was calculated from the temporary increase in the coagulation activity as a result of the drug administration using the linear trapezoidal method. The $\mathrm{AUEC}_{0\text{-}12\mathrm{h}}$ value of 1 (753 % · h) is 1.9 times larger than that of H-MK-4 (405 % · h). The enhanced and prolonged duration of the action observed in 1 administered rats was clearly a result of the enhanced delivery of MKH to its active site.

In conclusion, the animal experiments indicate that ester 1 may therefore be potentially useful prodrug of MKH for iv administration and can deliver MKH to the active site without incurring a reductive activation step. These effective and selective deliveries of MKH to its active site lead to an enhanced pharmacological efficacy and can also avoid the toxicity induced by the solubilizing agent (HCO-60) used in the MK-4 preparation. It appears that the reductive activation independent delivery of MKH might thus be able to avoid the oxidative toxicity in the treatment of hypoprothrombinemia induced in patients undergoing coumarin and cephalosporin therapies. This prodrug approach is also applicable to the site-specific delivery of the active form of phylloquinone (vitamin K_1), one of most widely used K vitamins. It appears that the reductive activation independent delivery of an active form (hydroquinone) of the quinone drug might thus be applicable for improving the toxicant effect of quinone anticancer drugs.

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