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 menahydroquinone-4 (MK-4, vitamin K200).

Methods. The disposition of MK-4 and menahydroquinone-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 (AUCMKO). The specific delivery of MKH to its active site (liver) and coagulation activity after the administration of selected prodrug I 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; AUCMKO of MKO from 70.7 ± 5.77 (H-MK-4) to 167 ± 7.89 nmol·h/g, MRTMKO 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 selective 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 menahydroquinone-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 menahydroquinone-4 epoxide (MKO) following iv administration of the esters in vitamin K cycle inhibited rats with warfarin was
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, Hepaplentin® 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 intraperitoneally 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 AUCMKO after the iv administration of H-MK-4 was preliminarily determined in the rats. The relationship between the warfarin dose and AUCMKO gave a sigmoidal dose-dependent curve (data was not shown). A warfarin dose of over 1 mg/kg, the values of AUCMKO were constant, which thus indicated a reproducible inhibited state of the vitamin K cycle. Similar dose-response relationships between the warfarin dose and