Water-Soluble Prodrug of Vitamin E for Parenteral Use and Its Effect on Endotoxin-Induced Liver Toxicity

Jiro Takata, a Sahe Ito, a Yoshiharu Karube, a,d Yoshiho Nagata, b and Yoshikazu Matsushima b

Faculty of Pharmaceutical Sciences, Fukuoka University, a 8-19-1 Nanakuma, Joohin-ku, Fukuoka 814-80, Japan and Kyoritsu College of Pharmacy, b 1-5-30 Shibakoen, Minato-ku, Tokyo 105, Japan.

Received August 14, 1996; accepted November 8, 1996

The acid salts of aminoalkanecarboxylic acid esters of d-α-tocopherol were in a previous in vitro study identified as prodrug candidates for a parenteral form of d-α-tocopherol. The disposition of d-α-tocopheryl N,N-dimethylaminoacetate hydrochloride (TDMA), the most potent candidate for the prodrug, after a single intravenous administration was investigated and compared with that of the d-α-tocopheryl acetate (TA) and dl-α-tocopherol, solubilized with HCO-60, in order to establish the utility as a prodrug for i.v. administration. The preventive effect of the prodrug against endotoxin (lipopolysaccharide (LPS))-induced liver lipid peroxidation was also investigated in mice. The plasma and liver levels of α-tocopherol (Toc) were increased rapidly after i.v. administration of the prodrug. The distribution of Toc and TDMA in the plasma and the liver at 1 h was as follows: 2.1 ± 0.2 (plasma, Toc), 2.0 ± 0.2 (plasma, TDMA), 32.8 ± 2.9 (liver, Toc), and 35.3 ± 6.5% of dose (liver, TDMA). The rapid and liver-selective uptake and liver-esterase specific regeneration characteristics of the prodrug enhance the delivery of Toc to liver. The liver availability of Toc after i.v. administration of TDMA, TA and Toc were 116, 50 and 100%, respectively. The elevation of liver lipid peroxide induced with LPS was significantly suppressed to a normal range by a single i.v. postadministration of TDMA (over 10 mg/kg equivalent for Toc). These results indicated that the water-soluble and liver-esterase hydrolysable derivative of Toc was a potential candidate for a parenteral prodrug which can thus achieve the systemic liver-specific delivery of Toc. Such effective and selective delivery of Toc into the liver can therefore lead to enhanced pharmacological efficacy against liver oxidative injury associated with free radicals.

Key words: vitamin E; d-α-tocopherol; water-soluble prodrug; endotoxin; liver-specific delivery; lipid peroxidation

α-Tocopherol (vitamin E, Toc), a biological antioxidant, is currently receiving attention concerning its efficacy in preventing and reducing the oxidative stresses resulting from ischemia and reperfusion, and in the treatment of toxicant protection, malabsorption disorders, hematologic disorders, cardiovascular disease and premature infants.

Toc is practically insoluble in water and is readily oxidized by atmospheric oxygen. Because of their high stability to oxidation, the ester derivatives of the vitamin (i.e., acetate and acid succinate) are commonly supplied for clinical use. When a rapid onset of action is required via parenteral administration, significant problems arise from the fact that the esters are characteristically insoluble in water as well as α-tocopherol. α-Tocopheryl acetate is solubilized by large amounts of surfactant in the parenteral formulation, and it has been confirmed that the hydrolysis of the acetate is the rate-limiting step in the course of the bioavailability of α-tocopherol. The use of surfactants in parenteral dosage forms generally induces toxicity such as the anaphylactoid reaction.

Because these delivery problems can primarily be attributed to the low water solubility of the ester and bioreconversion rate of the ester to Toc, it appears likely that the delivery characteristics of Toc can be improved by using the prodrug approach, i.e., development of derivatives possessing both a high water solubility and being capable of reverting rapidly to the parent drug following administration.

In the forgoing study, several aminoalkanecarboxylic acid esters of d-α-tocopherol (d-Toc) had been prepared and assessed as potentially useful prodrugs for parenteral use in vitro. Due to their facile liver enzymatic reversion to d-Toc and excellent solubility properties, d-α-tocopheryl N,N-dimethylaminoacetate hydrochloride (TDMA) was identified as the most promising prodrug candidate for the parenteral delivery form.

In this study, the disposition of the prodrug following intravenous (i.v.) administration in rats was first compared with that of the d-α-tocopheryl acetate (TA) and dl-α-tocopherol (dl-Toc) in order to establish the utility as a prodrug for i.v. administration. For treatment of oxidative injury, it is of interest to know if the prodrug can exhibit effectiveness against oxidative injury. For this purpose, the effect of the prodrug against endotoxin (lipopolysaccharide, LPS)-induced hepatotoxicity was also investigated.

MATERIALS AND METHODS

The TDMA was synthesized in our laboratory using a previously reported method. The injection solution of TDMA was solubilized in distilled water containing 10% propylene glycol and filtered through a membrane filter (0.22 μm). The injection solution of TA was solubilized in distilled water containing 10% polyoxyethylene hydrogenated castor oil (HCO-60) and 10% propylene glycol and used without filtration. The injection solution of dl-Toc, solubilized in 10% HCO-60 containing 10% propylene glycol, d-Toc and tocot were kindly given by Eisai Co., Ltd. (Tokyo, Japan). TA was purchased from Sigma Chemical Co. (MO, U.S.A.). HCO-60 was pur-
chased from Nikko Chemical (Tokyo, Japan). Endotoxin (LPS, *E. coli* 026:B6) was purchased from Difco Laboratories (MI, U.S.A.) and was dissolved in saline at the time of use. Male Wistar rats and male ICR mice were purchased from Charles River Japan (Yokohama, Japan). 2-Thiobarbituric acid (TBA) was purchased from E. Merck (Darmstadt, Germany). The BCA protein assay kit was purchased from Pierce (IL, U.S.A.). All other chemicals were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan).

**Disposition Studies in Rats** Male Wistar rats weighing 220—260 g were fasted for 16 h prior to use, but water was administered *ad libitum*. The rats were lightly anesthetized with ether and a small incision was made over the left femoral vein for i.v. administration. The injection solution of the drug was injected at a dose of 10 and 25 mg/kg (equivalent for Toc). 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, 8, and 24 h. The plasma was immediately separated by centrifugation at 5 °C and stored at −80 °C until 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.

**Pharmacokinetic Analysis** The plasma and liver concentration versus time data were analyzed using model independent and statistical moment methods. Both the maximum concentration (*C* max) and its corresponding time (*t* max) were directly obtained from the observed data. The systemic availability and liver availability for Toc after i.v. administration of the esters relative to Toc administration were determined from the ratio of AUC of Toc based on Eq. 1 and 2, respectively. The selective advantage value for Toc in the liver was calculated using Eq. 3.

\[
F_{\text{Plasma}} = \frac{AUC_{\text{Plasma}}}{AUC_{\text{Plasma, predrug}}} \cdot 100(\%)
\]

\[
F_{\text{Liver}} = \frac{AUC_{\text{Liver}}}{AUC_{\text{Liver, predrug}}} \cdot 100(\%)
\]

selective advantage = \frac{AUC_{\text{Liver}}}{AUC_{\text{Liver, predrug}}} \cdot \frac{AUC_{\text{Plasma}}}{AUC_{\text{Plasma, predrug}}}

where AUCPlasma, predrug, AUCLiver, predrug, AUCPlasma, Toc, predrug, AUCLiver, Toc, predrug, AUCPlasma, Toc, and AUCLiver, Toc are the AUC values for increased Toc in the plasma and liver after the administration of the esters and dl-Toc, respectively. AUCPlasma, predrug, AUCLiver, predrug is the AUC value for the esters. Dl-Toc and DL-Toc are doses of the esters and dl-Toc, respectively.

**HPLC Analysis** The plasma and liver concentrations of Toc, TA and intrinsic TDM were analyzed by the following HPLC method. To 200 μl sample of the plasma and the liver obtained was added 700 μl of ethanol containing 2 μg/ml tocloc. After vortex mixing for 2 min and centrifugation at 3000 rpm for 5 min, 50 μl of the supernatant was subjected to HPLC analysis. 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 a 12 μL LC flow cell), a data analysis system class M10 and peak integrators (C-R7A). A reversed-phase column Capcell Pak C18 (UG 120, 4.6×150 mm, Shiseido, Tokyo, Japan) and a mobile phase of methanol–acetonitrile (7:3, v/v) containing 0.02 m acetic acid and sodium acetate at a flow rate of 0.7 ml/min were used. The eluent was photometrically monitored at 283 nm and was also fluorometrically monitored at 298 nm excitation and 325 nm emission. The quantitation of the compounds was achieved using the linear calibration curves of the peak area vs. concentration.

**LPS-Induced Lipid Peroxidation** Male ICR mice, 5 weeks old, were used. It has been shown that the maximum liver lipid peroxidation induced by LPS was observed between 16 and 18 h after LPS treatment in rat6 and mice. Therefore, the endotoxemic mice were prepared by the administration of LPS according to the reported timing. The mice were intravenously administered with LPS (2.5 mg/kg). After 1 h LPS administration, TDMA was administered via the tail vein (doses were 0, 5, 10 and 25 mg/kg equivalent for Toc). Five mice were used for each group. Eighteen hours after LPS administration, blood was collected from the abdominal artery and the liver was removed under ether anesthesia. The liver was homogenized with 9 volumes of 1.15% KCl solution using a Polytron homogenizer and stored at −80 °C until analysis. Whole tissue homogenates were used for lipid peroxide assay and Toc assay.

**Assay for Tissue Lipid Peroxide Levels** Lipid peroxidation accumulation products were measured essentially according to the method of Ohkawa, evaluated as TBA-reactive substances (TBARS) and were described as nmol of malondialdehyde (MDA) per mg of protein. The TBA reagent was used as a 0.6% aqueous solution because of its low aqueous solubility at room temperature (under 0.8%). The absorbance at 535 nm was measured relative to a reference at 520 nm and 1,1,3,3-tetraethoxypropane was used as the MDA standard. The protein concentrations of the homogenates were determined by the BCA protein assay kit and bovine serum albumin was used as a standard.

**RESULTS AND DISCUSSION**

**Disposition of the Prodrug in Rats** For the purposes of developing the prodrug for parenteral use, a prodrug with high water solubility and high reconversion rate to Toc appeared most promising for further *in vivo* studies. It was previously observed that TDMA was soluble in water (over 100 μm) and that TDMA was converted into Toc catalyzed by esterases located in the liver as well as TA. Thus, TDMA (Fig. 1) was chosen to test the prediction that the aminooalkancarboxylic acid ester of Toc would be a suitable water-soluble prodrug of Toc for i.v. administration. It appeared that liver uptake of the prodrug and liver enzymatic activation characteristic of the prodrug are important criteria for achieving delivery of Toc via the prodrug. Therefore, the disposition of the intrinsic ester and Toc in plasma and liver after i.v. admin-
istration of TDMA was compared with that of the TA and dl-Toc, solubilized with HCO-60, in order to establish the utility for i.v. administration.

The plasma and liver profiles after i.v. administration of TDMA, TA, and dl-Toc in rats are shown in Figs. 2 and 3. The pharmacokinetic parameters for Toc and the intrinsic ester after the administration of TDMA, TA, and dl-Toc are summarized in Table 1. Following the i.v. administration, TDMA was more rapidly eliminated from the plasma and more extensively accumulated in the liver in comparison with TA and dl-Toc, and the maximum accumulation was achieved 30 min after administration. Increased Toc appeared within 15 min in the plasma and liver after TDMA administration, indicating a reconversion of TDMA into Toc in vivo. The relative systemic availability for Toc (F) after TDMA and TA administration are 26.3 and 12.1%, respectively. The liver level of Toc after TDMA administration was more rapidly increased and dramatically higher than that of TA and Toc administration for at least 24 h. TDMA showed an improvement in the liver availability over TA and dl-Toc administration; the relative liver availability of Toc (Fr) after TDMA, TA, and dl-Toc administration are 116, 49.7 and 100%, respectively.

As can be seen in Fig. 3, the total recovery in the liver achieved a maximum at 1 h after TDMA administration, and the liver and plasma distributions of Toc and TDMA were $32.8 \pm 2.9$ (Toc in liver), $35.3 \pm 6.5$ (TDMA in liver), $2.1 \pm 0.2$ (Toc in plasma), and $2.0 \pm 0.2\%$ (TDMA in plasma) of the dose. At this time, the distributions of Toc and TA after TA administration were $11.0 \pm 0.6$ (Toc in

![Chemical Structure of TDMA](image)

**Fig. 1.** Chemical Structure of TDMA

![Plasma Profile](image)

**Fig. 2.** Mean Levels versus Time Profiles in the Plasma and Liver after the Intravenous Administration of TDMA, TA, and dl-Toc in the Rats

**Key:** ■ Toc after TDMA administration; ○, TDMA after TDMA administration; ●, Toc after TA administration; O, TA after TA administration; ▲, Toc after dl-Toc administration. The doses are $25\text{mg}\text{kg}^{-1}$ equivalent for Toc. Each time point represents the mean ± S.D. of 3 rats, with each rat providing a single sample. Shadow areas represent control values.

| Table 1. Pharmacokinetic Parameters in Liver and Plasma after the Intravenous Administration of TDMA, TA, and dl-Toc in the Rats$^{a}$ |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Liver           | Plasma          | Liver           | Plasma          |
| **For TA or intrinsic TDMA** |                  |                  |                  |                  |
| $C_{\text{max}}$ ($\mu\text{mol} \cdot \text{ml}^{-1}$ or $\text{g}^{-1}$) | 0.048 ± 0.005   | 1.06 ± 0.105    | 0.802 ± 0.094   | 0.990 ± 0.069   |
| $t_{\text{max}}$ (h) | 0.5             | 0.25            | 0.5             | 0.25            |
| $AUC$ ($\mu\text{mol} \cdot \text{h} \cdot \text{ml}^{-1}$ or $\text{g}^{-1}$) | 0.625 ± 0.030   | 7.09 ± 0.317    | 9.15 ± 0.704    | 0.404 ± 0.058   |
| $MRT$ (h) | 8.57 ± 0.459    | 6.38 ± 0.034    | 7.58 ± 0.547    | 0.451 ± 0.012   |
| **For Toc** |                  |                  |                  |                  |
| $C_{\text{max}}$ ($\mu\text{mol} \cdot \text{ml}^{-1}$ or $\text{g}^{-1}$) | 0.649 ± 0.052   | 1.26 ± 0.067    | 0.357 ± 0.034   | 0.038 ± 0.004   |
| $t_{\text{max}}$ (h) | 4               | 0.25            | 4               | 0.25            |
| $AUC$ ($\mu\text{mol} \cdot \text{h} \cdot \text{ml}^{-1}$ or $\text{g}^{-1}$) | 8.39 ± 0.234    | 3.10 ± 0.088    | 4.17 ± 0.484    | 0.375 ± 0.055   |
| $MRT$ (h) | 7.58 ± 0.441    | 3.47 ± 0.225    | 8.18 ± 0.276    | 9.03 ± 0.564    |
| $F_{\text{rate}}$ (%) | 100             | 26.3            | 20.1            | 116             |
| $F_{\text{rate}}$ (%) | 100             | 49.7            | 49.7            | 116             |
| Selective advantage$^{b}$ | 1.0             | 2.1             | 0.2             | 8.9             |

$^{a}$ The values are the mean and S.D. of three rats at doses of $25\text{mg}\text{kg}^{-1}$ equivalent for \(\alpha\)-tocopherol.

$^{b}$—$^{d}$ Calculated from Eqs. 1, 2, and 3, respectively.

NII-Electronic Library Service
liver), 2.5 ± 0.4 (TA in liver), 1.06 ± 0.1 (Toc in plasma) and 41.7 ± 3.1% (TA in plasma) of the dose. The rapid and liver-specific uptake of TDMA and rapid and specific appearance of Toc in the liver clearly indicated that the regeneration of Toc might thus occur in the liver. It appeared that these characteristics of TDMA might allow liver specific delivery of Toc. For the assessment of TDMA as a liver-specific delivery system for Toc, the selective advantage value (Eq. 3) was also used. A remarkable liver-specific delivery of Toc was observed after the administration of TDMA; the selective advantage of TDMA, TA and dl-Toc were 8.9, 0.2 and 1, respectively.

The animal disposition studies clearly indicated that TDMA might be a useful candidate for the parenteral produg of Toc. In view of the pharmacokinetics, the rapid and selective delivery of Toc into the liver suggests that it may be a promising prodrug candidate for the prevention of liver oxidative injury associated with free radicals.

**Effect of Intravenous Postadministration of the Prodrug on LPS-Induced Liver Lipid Peroxidation**

Endotoxin (LPS) has been shown to induce many injurious reactions, among which endotoxin shock is the most important from the clinical viewpoint. Despite the rapid advances in the treatment of shock, mortality caused by endotoxin shock remains high and is mainly due to multiple organ failure. When the shock is severe and prolonged, it
is difficult to maintain or restore the liver functions. Metabolic events in animals suffering from the shock are regarded as secondary effects of decreased tissue perfusion, which leads to generalized ischemia and finally organ damage. The liver is especially susceptible to ischemia, which causes functional and structural damage in the liver by active oxygen generation. It has been shown that lipid peroxidation in the liver of mice is stimulated under endotoxemia and the endotoxin-induced liver oxidative stress can be reduced by the treatment with Toc. In these studies, Toc was administered in solution, solubilized by surfactants (e.g., HCO-60, polyoxyethylene sorbitan monoooleate (Tween 80)). These dosage regimens of Toc are not available for consideration in clinical use.

As mentioned above, because the prodrug can effectively and selectively deliver Toc to the liver, it can be expected that the prodrug administration might be effective for protection from endotoxin-induced liver oxidative toxicity. It has been shown that the carboxyesterases locate in the liver of mice. Thus, it can be also expected that TDMA might be able to act as the prodrug after i.v. administration in mice. To assess the pharmacological efficacy of the prodrug, therefore, the preventive effect of TDMA against the endotoxin-induced liver lipid peroxidation was studied in mice.

Figure 4 shows the effect of TDMA on the liver lipid peroxide levels (assessed as TBARS levels) and liver Toc levels in the endotoxemic mice. By comparison with the control groups, the liver TDMA level was significantly increased by ca. 3.5-fold at 18 h after LPS administration (2.5 mg/kg, i.v.). The increased ratio of liver TDMA was similar to the value reported. At this time, the liver Toc levels were significantly decreased under the control values. The elevated TDMA level was suppressed to the normal range by postadministration of TDMA at over 10 mg/kg doses equivalent for Toc but was not effective at a dose of 5 mg/kg equivalent for Toc. The liver Toc levels of all of the TDMA-administered endotoxemic mice were significantly higher than those of the control mice, indicating that the preventive effect of TDMA may be due to a high pharmacological tissue concentration of Toc, which after some time decreases below a critical threshold. The effective doses of TDMA are equivalent to 10—25 mg/kg for free Toc and less than 1/6 of the dose used by Sakaguchi et al. (60—68 mg/kg, i.m.). These results clearly indicate that a single i.v. postadministration of TDMA can protect the LPS-induced liver lipid peroxidation by increasing the Toc levels in the liver.

In conclusion, the animal experiments indicate that TDMA may therefore be a potentially useful prodrug of Toc for i.v. administration. The effective and selective delivery of Toc into the liver leads to an enhanced pharmacological efficacy against oxidative injury of the liver and can also avoid the toxicity induced by the solubilizing agent such as HCO-60 used in the parenteral formulation of Toc. It appears that the parenteral prodrug might thus be applicable for amelioration of the oxidative toxicant effect of Adriamycin. The studies on the amelioration of Adriamycin-induced toxicity will be reported in a future communication.

Acknowledgments We thank Eisai Co., Ltd., for the kind gift of d-a-tocopherol and related compounds. A part of this work was supported by a Grant-in-Aid for Scientific Research (No. 63771928) from the Ministry of Education, Science and Culture, Japan, granted to J.T., and the Science Research Promotion Fund of the Japan Private School Promotion Foundation, granted to Y.M.

REFERENCES

8) Sakaguchi O., Kanda N., Sakaguchi S., Hsu C.-C., Abe H.,


