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Research Article

An Excellent Delivery System for Improving the Oral Bioavailability of Natural Vitamin E in Rats

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Abstract. This study set out to develop a novel and stable nanoemulsion formulation of natural vitamin E with increased oral bioavailability. The natural vitamin E nanoemulsion was prepared by a modified emulsification technique. The physicochemical characteristics of natural vitamin E nanoemulsion were characterized and its pharmacokinetics study was performed as well. The experimental results showed droplet diameters ranging from 20 to 400 nm (average, 87.7 nm) with a negative electrostatic potential (−23.5±1.5 mv). The pharmacokinetics study of this nanoemulsion and corresponding soft capsule was carried out using noncompartment model method. Compared with the marketed soft capsule, the C_{max} of the natural vitamin E nanoemulsion was higher, while the T_{max} was shorter. Thus, plasma concentration–time profiles in rats dosed with nanoemulsion showed a 1.6-fold enhancement in the area under the curve of natural vitamin E compared with the marketed soft capsule. The antioxidative effects of the natural vitamin E nanoemulsion and the marketed soft capsule were also evaluated by the levels of superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration in serum and liver tissue. According to the SOD activity and the MDA concentration determined, the nanoemulsion was superior to the marketed soft as an antioxidative agent. The overall results demonstrated that the nanoemulsion drug delivery system could be a promising strategy for the delivery of natural vitamin E, which showed great potential for clinical application.

KEY WORD: Antioxidation; Nanoemulsion; Natural vitamin E; Oral bioavailability.

INTRODUCTION

Natural vitamin E is an essential nutrient derived from various crops, such as grains, nuts, and some vegetables. It is mainly used to treat custom miscarriage, male and female infertilities, and gynecopathies. It has been proved that the natural form of Vitamin E, D-alpha tocopherol, has better therapeutic effect than the synthesized form, a racemate of alpha tocopherol (1). At present, marketed formulations of natural vitamin E include soft capsule and tablet. However, the two dosage forms suffer from low oral bioavailability (2, 3). Hatanaka *et al.* reported that the oral bioavailability of synthesized α-tocopherol was increased by the introduction of nanoemulsion system (4).

As a drug delivery system, the nanoemulsion has many advantages such as increasing solubility and dissolution rate (5), enhancing drug stability (6), and improving diffusion across the intercellular space and mucosal (7). The nanoemulsion has its unique advantage in bioavailability enhancement. In recent years, much attention has been focused on lipid-based formulations to improve the oral bioavailability of drug compounds with poor water solubility, such as tamoxifen (8), candesartan cilexetil (9), and paclitaxel (10). The nanosized droplets leading to enormous interfacial areas associated with nanoemulsions would influence the transport properties of the drug, an important factor in sustained and targeted drug delivery (11). Nanoemulsions are heterogeneous systems composed of oil droplets dispersed in aqueous media and stabilized by surfactant molecules. Moreover, the nanoemulsion is kinetically stable without any apparent flocculation or coalescence during the long-term storage due to their nanometer-sized droplets. Furthermore, the Food and Drug Administration has approved nanoemulsions of water-insoluble drugs including Estrasorb®, Flexogan®, and Restasis for clinical application (12). However, no reports showed that the nanoemulsion could enhance the oral delivery of natural vitamin E to date. Thus, we are interested in designing and developing a novel nanoemulsion formulation to improve the bioavailability of natural vitamin E.

Therefore, we set out to prepare a stable nanoemulsion of natural vitamin E by a modified emulsification technique. The

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physicochemical properties of the novel formulations were characterized by assessing droplet size, zeta potential, stability, and morphology. Pharmacokinetic profiling of vitamin E after oral administration of the newly developed nanoemulsion or the markted soft capsule in rats was carried out for comparison. In addition, D-GalN was used to develop experimental oxidative damage rats. The anti-oxidative properties of newly developed natural vitamin E nanoemulsion were evaluated in the oxidative damaged rats.

MATERIALS AND METHODS

Chemicals

Natural vitamin E was purchased from Xinchang Medicine manufacturing factory (Zhejiang, China), medium-chain triglyceride (MCT) from Jiya Chemical company (Guangzhou, China). Egg lecithin (PL-100M) was provided by Q.P. Corp (Tokyo, Japan), and Poloxamer 188 was obtained from BASF (Ludwigshafen, German). The oleic acid was a gift sample from Malaysia. Polyvinylpyrrolidone was purchased from ISP (New Jersey, USA). D-GalN was obtained from Sigma-Aldrich. The marketed soft capsule was purchased from Liye Medicine manufacturing factory (Nanjing, China). All other chemicals were of analytical grade.

Preparation of Natural Vitamin E-Loaded Nanoemulsion Formulations

The natural vitamin E nanoemulsion formulation, shown in Table I, was prepared by a modified emulsification technique. Natural vitamin E, MCT, egg lecithin and oleic acid were dispersed in an adequate alcohol solution. Briefly, the aqueous phase, composed of poloxamer 188, PVP, and deionized water. Then, the oil phase, which consisted with natural vitamin E, MCT, egg lecithin, and oleic acid, was added to the aqueous phase under a moderate magnetic stirring. Both phases were heated up to 60°C separately. The mixture was then submitted to a high-shear mixer (FJ-200, Specimen Model Factory, China) for 3 min, which allowed the formation of a crude emulsion. After this, the mixture was intermittently sonicated with probe (JY92-II, Xinzhi Biotechnology, China) for 480 s to form nanoemulsion.

Measurement of the Size and Zeta Potential of Natural Vitamin E Nanoemulsion

The mean particle size of the natural vitamin E nanoemulsion was measured by particle analyzer (ZETASIZER-3000HSA, Malvern Instruments, UK). Prior to measurement, the natural vitamin E nanoemulsion was subject to tenfold dilution with deionized water. Diluted sample was carried out at 25°C.

The zeta potential of natural vitamin E nanoemulsion was analyzed using the particle analyzer. Zeta potential was calculated from the electrophoretic mobility by the Helmholtz–Smoluchowski equation. The measurement was carried out with diluted natural vitamin E nanoemulsion as described above. The measurement was repeated three times per sample.

Measurement of the Stability Constant

To estimate the physical stability of the natural vitamin E nanoemulsion, the stability constant (K_e) was determined (13). The smaller stability constant is required for the more stable nanoemulsion. The evaluation of stability constant was carried out as follows. Briefly, 5 ml of the nanoemulsion was added to a centrifuge tube and centrifuged at 4,000 rpm for 15 min. The supernatant liquid was discarded and 0.5 ml of the solution in the tube bottom was collected. Fifty microliter of the solution after centrifugation and nanoemulsion before centrifugation was added to the 10 ml volumetric flask separately. They were diluted with deionized water to constant volume.

Samples were performed on an ultraviolet spectrophotometer. Deionized water was as blank solution. Detection was recorded at wavelength of 500 nm. The absorbance of the dilution solution was determined. K_e was calculated using the equation below:

$$K_e = \frac{(A_0 - A)}{A_0} \times 100$$

where A_0 is the absorbance of the nanoemulsion before centrifugation and A is the absorbance of the solution after centrifugation. The measurement was repeated three times per sample.

Characterization by Transmission Electron Microscopy

In order to observe the morphology of the oil droplets in natural vitamin E nanoemulsion, transmission electron microscopy (TEM) analysis was carried out. The dilution factor was 10 times. The sample was negatively stained with 1.5% (*w*/*v*) phosphotungstic acid and allowed to dry. It was then visualized under an H-7650 transmission electron microscope (Hitachi, Japan) operating at 75 kV

Table I. Composition of Natural Vitamin E-Loaded Nanoemulsion formulations

Formulations	Compositions (g)					
	oleic acid	PVP	VE	MCT	Egg lecithin	poloxamer 188
F1	0.25	0.1	1	5	1.5	4
F2	0	0.1	1	5	1.5	4
F3	0.25	0	1	5	1.5	4

Table II. Physicochemical Character and Stability Constant of Natural Vitamin E Nanoemulsion (n=3)

	Size (nm)	Zeta potential (mV)	Stability constant (%)	Polydispersity index
F1	87.7±5.6	−23.5±1.5	28.63±0.45	0.284±0.054
F2	120.3±4.5	−14.2±0.9	82.92±0.86	0.312±0.032
F3	60.5±3.4	−20.5±0.8	92.76±0.75	0.254±0.012

Studies of the Preliminary Stability

Sterilization Stability

It was evaluated by monitoring change in the particle size, zeta potential, pH value, and drug concentration of natural vitamin E nanoemulsion pre and post flow steam sterilization at 100°C for 30 min.

Centrifugation Stability

It was evaluated by monitoring phase separation of natural vitamin E nanoemulsion after centrifugation at 4,000 rpm for 15 min.

Studies of Pharmacokinetics in Sprague–Dawley Rats

Animals and Drug Administration

Healthy male Sprague–Dawley rats, weighing 250±10 g, were purchased from Experimental Animal Center of China Pharmaceutical University. Prior to use, the rats were kept in a temperature and humidity controlled animal observation room (25°C, 55–60% air humidity) to adapt to the laboratory conditions for 5 days. All rats were kept for overnight fasting but allowed free access to water. The experiments were carried out in the compliance with conduct of the university's regulation and adhered to the principles of Institutional Animal Care and Use Committee Guidebook.

The rats were deprived of food but had free access to water 24 h before the experiment. Rats were randomly divided into three groups: nanoemulsion for intravenous injection group, nanoemulsion for oral administration group, and soft capsule for oral administration group. The rats were administered at a single dose of 40 mg/kg of natural vitamin E nanoemulsion and soft capsule respectively. About 0.5 ml blood sample from the jugular vein were collected into 1.0-ml heparinized plastic centrifuge tubes at immediately 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 h after oral administration. Meanwhile, 0.5 ml blood sample was collected into 1.0-ml heparinized tubes at immediately 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h after intravenous administration. Plasma were separated immediately by centrifugation at 4,000 rpm, 15 min and stored at below −20°C until analysis.

Determination of Natural Vitamin E Concentration in Plasma

All analyses of natural vitamin E were performed on a Shimadzu Promience high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan), which included the solvent delivery unit LC-20A with high-pressure flowline selection valves, an auto sampler SIL-20A, and column oven CTO-20A, connected with LC solution software. Natural vitamin E was detected using a Waters Symmetry C18 (4.6 mm×150 mm, 5 μm) column connected with a 4.6×20 mm guard column (Waters, USA), and its temperature was maintained at 30°C. One hundred microliter plasma samples were deproteinized by addition of 300 μl ethyl alcohol. The samples were then vortex mixed for 2 min, followed by addition of 500 μl hexane. After centrifugation of 10 min at 15,000 rpm, the supernatants were transferred into amber glass vials. The hexane extraction process was repeated. The organic portion was separated and evaporated to dryness under a gentle stream of nitrogen at room temperature. The residues were then reconstituted in 100 μl methanol before HPLC analysis. An aliquot (20 μl) samples were injected into HPLC column for analysis (14, 15).

HPLC analysis was performed. Standards and samples were separated using a mobile phase consisting of 100% methanol. The flow rate was set at 1 ml/min. Pharmacokinetic parameters were estimated using a noncompartment model method. After giving the same dose of oral and intravenous administration, the plasma bioavailability values (F) of natural vitamin E in nanoemulsion and soft capsule were calculated according to the following equation:

$$F = \frac{\mathrm{AUC_{oral}}}{\mathrm{AUC_{i.v.}}} \times 100\%$$

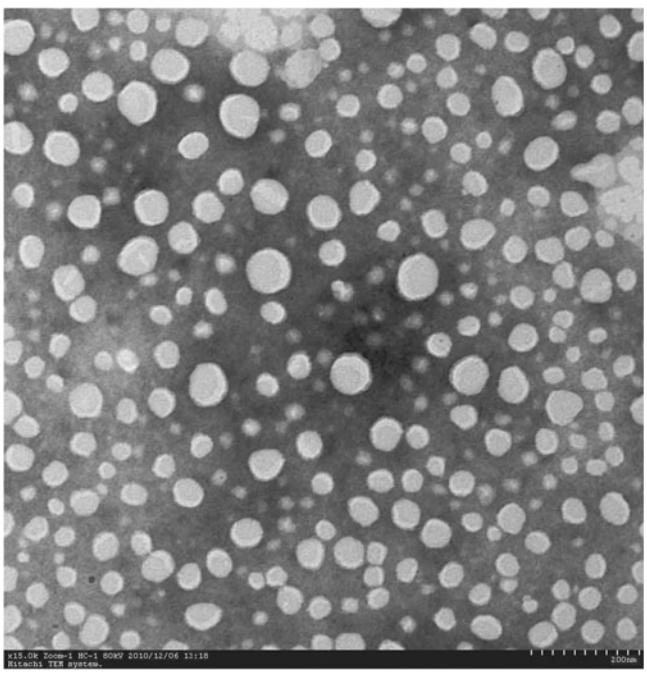

Fig. 1. Size distribution of natural vitamin E nanoemulsion

Table III. Comparision Between Pre and Post Sterilization (n=3)

	Size (nm)	Polydispersity index	Zeta (mv)	pH	Content (%)
Per sterilization	87.2±3.4	0.284±0.056	−23.53±2.31	6.97	99.52
Post sterilization	93.3±4.1	0.323±0.041	−22.3±3.15	6.79	100.4

Statistical Analysis

For statistical comparisons, all data were expressed as mean±standard deviation and were analyzed for statistically significant difference using independent sample *T* test.

Studies of Antioxidation in Rats

Oxidative Damage and Treatment Schedule

D-GalN has been used to develop experimental oxidative damage animal models. The study was performed on 20 male Sprague–Dawley rats weighting 250±10 g. The rats were fed with standard animal feeds along the period of the experiment, and were divided into four groups, which were normal group, model group, soft capsule group and nanoemulsion group. The rats of nanoemulsion and soft capsule group were orally administered with natural vitamin E nanoemulsion 80 mg/kg once a day for 7 days. Meanwhile, the rats of normal and model group were administered an equal volume of normal saline. On the seventh day of drug administration, expect for the normal group the others were all intraperitoneal injection with 10% D-GalN solution 500 mg/kg to establish experiment model of oxidative damage, and to observe the effects of natural vitamin E nanoemulsion and soft capsule on these models.

Biochemical Determinations

The superoxide dismutase (SOD) activity and the content of activity and malondialdehyde (MDA) in blood serum and liver tissue were detected by assay kits provided from Jiancheng Bioengineering Institute (Nanjing, China). The liver tissue for biochemical studies was prepared with normal saline.

Statistical Analysis

All data were expressed as mean±standard deviation and were analyzed for statistically significant difference using independent sample *T* test.

RESULTS AND DISCUSSION

Physicochemical Character and Stability Constant of Natural Vitamin E Nanoemulsion

The size, zeta potential, stability constant, and polydispersity index of natural vitamin E nanoemulsion were shown in Table II. The average particle size of F1, F2, and F3 were 87.7, 120.3, and 60.5 nm, respectively. Although the particle size of F3 was the smallest, the stability study demonstrated that F1 was more stable. This might be attributed to the enhanced viscosity of aqueous phase due to the use of PVP.

The zeta potentials of F1, F2, and F3 were −23.5±1.5, −14.2±0.9, and −20.5±0.8 mv, respectively. In this paper, we employed egg lecithin and poloxamer 188 as the primary emulsifier. The egg lecithin is a mixture of phospholipids with the major constituent being phosphatidylcholine and of the minor constituents being zwitterionic phospholipids such as phosphatidylserine, phosphatidylinositol, phosphatidyl glycerol, and phosphatidic acid that would contribute a negative charge. Meanwhile, the oleic acid molecules at the oil/water interface might not only increase the zeta potential, but also strengthen the molecular interactions between phospholipids and poloxamer emulsifiers (16). The results indicated that F1 was stable, and its size distribution was in a narrow scope. The following experiments were performed on F1.

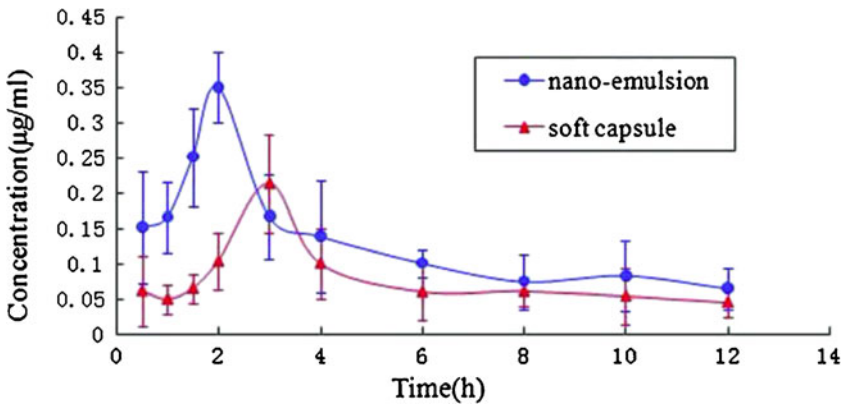

Fig. 2. TEM image of natural vitamin E nanoemulsion. *Bar* 200 nm

Characterization by Transmission Electron Microscopy

According to TEM images of the formulation, the prepared natural vitamin E nanoemulsion seemed to be well dispersed in water. All oil droplets were basically spherical in shape (shown in Fig. 1).

The Preliminary Stability of Nanoemulsion

The preliminary stability test showed that the particle size, zeta potential, pH value, and natural vitamin E concentration of natural vitamin E nanoemulsion were stable at sterilization (in Table III).

There was no phase separation during centrifugation stability study. The preliminary stability test showed that the drug content and droplet size of natural vitamin E nanoemulsion were stable at sterilization and after centrifugation.

Pharmacokinetics Behavior of Natural Vitamin E after Oral Administration

The plasma concentration–time profiles of natural vitamin E in rats following oral administration in dosage forms of both nanoemulsion and soft capsule were shown in Fig. 2, and the pharmacokinetic parameters were summarized in Table IV. A significant difference was observed between the pharmacokinetic profiles of the natural vitamin E nanoemulsion and that of the soft capsule. The results showed a higher C_{max} and a shorter T_{max} for the oral administration of the nanoemulsion than the soft capsule. The area under the curve (AUC) values of the natural vitamin E nanoemulsion and soft capsule were 1.482±0.03 and 0.907±0.02 μg hml^{-1}, respectively. Thus, there was a 1.6-fold enhancement in the AUC of natural vitamin E using the modified emulsification technique.

The oral bioavailability of natural vitamin E is not high (17). However, the nanoemulsion drug delivery system enhanced it. This was presumably achieved by the increase of drug solubility and the approach of drug emulsion particles to lymphatics (18).

Antioxidation of Natural Vitamin E after Oral Administration

The oxidative damage induced by D-GalN evoked a significant reduction of SOD activity and an increase of MDA content in the blood serum and liver tissue (shown in Figs. 3 and 4). D-GalN could lead to oxygen-derived free radicals released from activated hepatic-macrophages (19). It was reported that the increased production of ROS was the important reason of liver damage (20, 21).

Table IV. The main Pharmacokinetic Parameters of Natural Vitamin E in Rats (n=5)

Parameters	Unit	Nanoemulsion	Soft capsule
C_{max}	μg ml^{-1}	0.350±0.05*	0.213±0.07
T_{max}	h	2.0	3.0
$t_{1/2}$	h	6.90±0.2**	9.11±0.5
MRT	h	9.91±0.02**	13.04±0.5
AUC	μg hml^{-1}	1.482±0.03**	0.907±0.02
F	%	17.0±0.4**	10.4±0.2

*p<0.1, **p<0.01, compared with soft capsule group

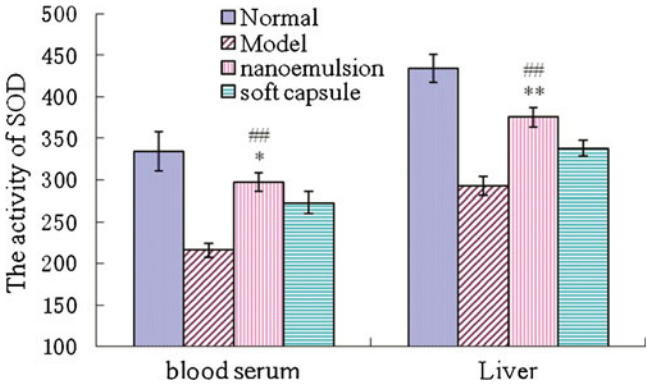

Fig. 3. Increase in plasma concentration of natural vitamin E after single oral administration of nanoemulsion formulation and soft capsule (n=5)

According to the results of this study, oral administration of the natural vitamin E nanoemulsion and the soft capsule had antioxidative effect. However, after oral administration of the natural vitamin E nanoemulsion and the soft capsule, the activity of SOD in liver tissue was 375.4±11.6 and 338±9.5 U $mgprot^{-1}$, respectively. After oral administration of the natural vitamin E nanoemulsion and the soft capsule, the content of MDA in liver tissue was 4.3±0.24 and 5.56±0.43 nmol $mgprot^{-1}$, respectively. It was clear that the antioxidation effect of the natural vitamin E nanoemulsion was more pronounced than the soft capsule (p<0.05).

CONCLUSION

In this study, a novel natural vitamin E-loaded nanoemulsion was designed to improve the oral bioavailability in rats. We have clearly demonstrated the potential utility of the nanoemulsion drug delivery system for formulating natural vitamin E to improve its oral bioavailability *in vivo*. The optimal formulation of the natural vitamin E nanoemulsion was successfully developed. The nanoemulsion is easily dispersed in water, forming nanosized emulsion with a diameter of 20–400 nm (averaged 87.7 nm) and with a negative potential (−23.5±1.5 mv). Pharmacokinetic studies in rats revealed significant increases of oral bioavailability and antioxidative efficacy compared with the soft capsule. Therefore, the nanoemulsion formulation could be a promising delivery option for

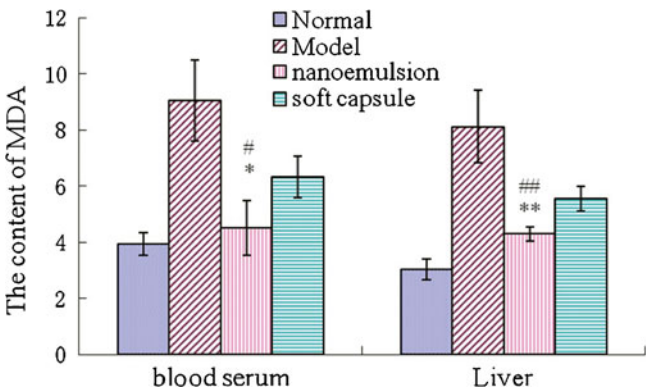

Fig. 4. The activity of SOD in blood serum and liver tissue. *p<0.1, **p<0.05 compared with soft capsule group; #p<0.05, ##p<0.01 compared with the model group

natural vitamin E, considering the improvements in its oral bioavailability and anti-oxidative effects.

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