Bioavailability Assessment of Vitamin A Self-Nanoemulsified Drug Delivery Systems in Rats: A Comparative Study

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Key Words

Vitamin A · Self-nanoemulsified drug delivery system · Bioavailability

Abstract

Objectives: To assess and compare the bioavailability of three different oral dosage forms of vitamin A in rats. The formulations included vitamin A self-nanoemulsified drug delivery (SNEDD) optimized formulation-filled capsule (F1), vitamin A SNEDD optimized formulation compressed tablet (F2) and vitamin A oily solution-filled capsules without any additives (control, F3). **Materials and Methods:** Bioavailability was assessed after a single oral dose of the three formulations using three groups of rats, each group comprising 6 rats. Blood samples were collected at baseline and over the next 8 h. Plasma was separated and extracted to obtain the drug, which was measured by HPLC. Statistical data analysis was performed using the Student t test and ANOVA with $p < 0.05$ as the minimal level of significance. **Results:** From the pharmacokinetic parameters, both F1 and F2 showed improved bioavailability compared to F3. The values of $AUC \pm SD$ were $3,080.7 \pm 190.2$, $2,137.1 \pm 130.5$ and $1,485.2 \pm 80.1$ ng·h/ml for F1, F2 and F3, respectively. The T_{max} was 1 h in case of F1 and F2 as compared to 1.5 h for F3. The $C_{max} \pm SD$ was 799.5 ± 48.5 , 656.2 ± 64.4 and 425.8 ± 33.1 for F1, F2 and F3, respectively. The increase in AUC , C_{max} and T_{max} was

significant ($p < 0.05$). The bioavailability calculated from the AUC for F1 and F2 relative to F3 was 207.4 and 143.8%, respectively. The bioavailability increased almost twofold and 1.4 times for F1 and F2, respectively. **Conclusions:** The study showed that the newly developed vitamin A SNEDD formulations increased the rate and extent of drug absorption compared to the oily drug solution. The present investigation demonstrated that vitamin A SNEDD optimized formulations, either as filled capsules or as compressed tablets, were superior to its oily solution with regard to their biopharmaceutical characteristics. Copyright © 2007 S. Karger AG, Basel

Introduction

Oral drug delivery has been a major route of drug administration for the treatment of many diseases. However, oral delivery of 50% of drugs is hampered because of the high lipophilicity of the drug itself. Every 4 out of 10 new candidate drugs have poor water solubility, and the oral delivery of such drugs is frequently associated with implications of low bioavailability, high intra- and intersubject variability, and lack of dose proportionality [1]. For this type of drugs, self-emulsifying drug delivery systems (SEDDS) represent a possible alternative. SEDDS are isotropic mixtures of natural or synthetic oil, surfac-

tant(s) with or without a cosurfactant. Upon mild agitation followed by dilution in organic media, such as gastrointestinal fluids, these systems can form fine oil-in-water emulsions [2]. Self-emulsifying formulations spread readily in the gastrointestinal tract, and the digestive motility of the stomach and the intestine provide agitation necessary for emulsifying [3]. However, studies have shown that the self-emulsification process is specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which self-emulsification occurs [4].

Numerous bioavailability studies [4, 5] carried out in animals and humans suggested that hydrophobic drugs are better absorbed when administered in self-dispersing lipid formulations. On the other hand, Charman et al. [2] reported that SEDDS improved the reproducibility of the plasma profile in terms of the maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) of the lipophilic compound WIN 54954, but there was no significant difference in the absolute bioavailability from the SEDDS formulations.

Vitamin A or all-*trans*-retinol acetate is a fat-soluble compound involved in several important biological functions including vision, growth, reproduction and the differentiation and maintenance of epithelial tissue [6]. In addition, it is a potent chemotherapeutic agent for treatment of acute promyelocytic leukemia and epithelial tumors [7–9]. The lipophilicity of vitamin A provides a challenge for its delivery by the oral route. Various attempts have been made for the preparation and bioavailability enhancement of vitamin A [10, 11].

The objectives of this study were to assess the bioavailability of vitamin A self-nanoemulsified drug delivery systems (SNEDDS), capsules and tablets previously developed in our laboratory [12, 13] and compare them with a capsule filled with an oily solution of vitamin A without any additives (blank).

Materials and Methods

Materials

Vitamin A acetate, vitamin A palmitate, soybean oil and talc powder were purchased from Sigma Chemical Co. (St. Louis, Mo., USA), and polyoxyl 35 castor oil (Cremophor EL) were obtained from BASF Corp. (Mount Olive, N.J., USA). Medium chain mono- and diglycerides (Capmul MCM-C8) were obtained from Abitec Corp. (Jamesville, Wisc., USA). Size 9 hydroxypropyl methylcellulose (HPMC) capsules were supplied by Shionogi Qualicaps (Whitest, N.C., USA). Avicel pH 105 was obtained from FMC Corp. (Philadelphia, Pa., USA). All chemicals were used as received.

Special Precautions

To avoid isomerization of vitamin A, all laboratory procedures involving the use of vitamin A were executed in a dimly lit environment.

Preparation of Dosing

Three dosage forms of vitamin A were used:

F1: vitamin A SNEDD optimized oily formulation, a mixture of vitamin A, soybean oil (16.17 mg), Cremophor EL (43.62 mg) and Capmul MCM C8 (42.53 mg) previously developed in our laboratory [12, 13]. The ingredients were mixed with vitamin A using a magnetic stirrer until a clear solution was obtained. The final preparation was filled in size 9 HPMC capsules.

F2: vitamin A SNEDD optimized oily formulation prepared as a tablet. This was done by mixing the F1 with Avicel PH105 as absorbent and 4% talc powder as lubricant to facilitate powder flow and tablet ejection. The mixture was then compressed at 3,000 lb, using a Carver press (model C, Carver Inc., Wabash, Ind., USA) attached to a semiautomatic compression assembly (model 2826, Carver Inc.) to obtain tablets.

F3: vitamin A oily liquid filled in size 9 HPMC capsules without any additives.

Animals and Dosing Procedures

Male Sprague-Dawley rats (Charles River Laboratories, Charlotte, N.C., USA) weighing 300–350 g were divided into three groups corresponding to the three formulations; each was comprised of 6 rats. Rats of group 1 received a single oral dose of F1, group 2 a single oral dose of F2, and group 3 a single oral dose of F3. The amount of vitamin A in each one of these formulations was adjusted to contain 7.5 mg/kg body weight. On the day of the experiment, animals were anesthetized by an intramuscular injection of a mixture of xylazine (20 mg/ml) and ketamine (100 mg/ml) solution in a ratio of 1:2. The initial dose was 0.3 ml/300 g body weight. Anesthesia was maintained with additional doses of the anesthetic solution as needed during the experiments. Blood samples (about 0.3 ml) were collected from the tail vein just before (0 h) and after dosing: 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h in a heparinized microcentrifuge tube, stored on ice until the plasma was separated by centrifugation (1,320 g for 5 min). The plasma was stored at -20°C until further analysis. All studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Sample Preparation

The method of sample preparation was a modification of Sharon et al. [14]. Briefly, 0.1 ml of plasma was placed in a 1.5-ml polypropylene microcentrifuge tube and 25 μl of the internal standard (vitamin A palmitate, 40 mg/l in 50% butanol/acetonitrile mixture) was added. To this mixture, 250 μl of the extraction solvent (butanol/acetonitrile mixture 1:1) was added. After vortex mixing for 60 s, 150 μl of dipotassium monohydrogen phosphate solution (1.2 mg/ml) was added and the vortex mixed for 30 s. The tubes were then centrifuged at 8,700 g for 2 min; 100 μl of the supernatant solution was injected directly onto the HPLC column.

Liquid Chromatography

The liquid chromatographic equipment (Waters, Boston, Mass., USA) included a model 6000A Solvent Delivery system, an

Fig. 1. Mean plasma concentration ($\pm$ SD) and time profiles of different vitamin A formulations after single oral dose administration.

Table 1. Pharmacokinetic parameters of different vitamin A formulations after single oral dose administration

Pharmacokinetic parameter	Formulation		
	F1	F2	F3
T_{max} , h	1	1	1.5
C_{max} , ng/ml	799.5 ± 48.6	656.25 ± 64.5	420.92 ± 26.9
K_{ab} , h^{-1}	1.53 ± 0.1	1.53 ± 0.1	1.27 ± 0.2
$T_{1/2 ab}$, h	0.45 ± 0.02	0.45 ± 0.2	0.56 ± 0.1
K_{el} , h^{-1}	0.30 ± 0.03	0.34 ± 0.4	0.44 ± 0.1
$T_{1/2 el}$, h	2.32 ± 0.2	2.03 ± 0.2	1.57 ± 0.2
Volume of distribution (V_d), liters	0.003 ± 0.0002	0.004 ± 0.0004	0.004 ± 0.0004
Total clearance rate, ml/min	0.01 ± 0.001	0.02 ± 0.001	0.03 ± 0.002
AUC (0- ∞), ng·h/ml	$3,080.7 \pm 190.2$	$2,137.1 \pm 130.5$	$1,485.2 \pm 80.1$
Relative bioavailability, %	207.4	143.8	—

autoinjector, a model 450 Wavelength Detector set at 324 nm and a Data Module recording integrator. Separation was allowed to occur on a 250 $\times$ 4.6 mm reversed-phase ODS-2 column (Waters Inc., Milford, Mass., USA) at ambient temperature. The mobile phase was acetic acid/water/acetonitrile (0.5/20.0/79.5% v/v) and used at a flow rate of 3.0 ml/min.

Statistical Data Analysis

Statistical data analysis was performed using the Student t test and ANOVA with $p < 0.05$ as the minimal level of significance.

Results

The limit of detection was 5 ng/ml. The observed retention times for vitamin A acetate and internal standard were 13 and 4 min, respectively. The pharmacokinetic

parameters and the plasma concentration-time profiles are shown in table 1 and figure 1, respectively. The vitamin A in F1 and F2 showed rapid absorption as indicated from T_{max} results. The observed T_{max} values were 1 h in F1 and F2 compared to 1.5 h in F3. The mean ($\pm$ SD) absorption rate constants of different formulations were 1.53 ± 0.1 , 1.53 ± 0.1 and 1.27 ± 0.2 h^{-1} for F1, F2 and F3, respectively. With respect to C_{max} , F1 and F2 had higher values compared to F3. The mean ($\pm$ SD) plasma peak concentrations were 799.5 ± 48.6 , 656.2 ± 64.5 and 420.9 ± 26.9 ng/ml for F1, F2 and F3, respectively. The difference between the groups, with regard to these parameters, was found to be significant at $p < 0.05$.

The area under the plasma concentration-time curve (AUC $\pm$ SD) was found to be $3,080.7 \pm 190.2$, $2,137.1 \pm 130.5$ and $1,485.2 \pm 80.1$ ng·h/ml in F1, F2 and F3, re-

Fig. 2. AUC ($\pm$ SD) of different vitamin A formulations after single oral dose administration.

Fig. 3. Plasma maximum concentration ($C_{max} \pm$ SD) of different vitamin A formulations after single oral dose administration.

spectively. Again, the difference between the three groups was found to be significant at $p < 0.05$. The results of AUC and C_{max} are presented in figures 2 and 3, respectively. The calculated relative bioavailability of F1 and F2 relative to F3 based on AUC was 207.4 and 143.8% for F1 and F2, respectively.

Discussion

The most important determinant of product performance is the *in vivo* bioavailability evaluation. It has been recognized that SNEDDS enhanced the bioavailability of several preparations in humans and animals with an increase in its *in vitro* drug release characteristics [15, 16]. However, in some reports, the bioavailability has not increased even though the *in vitro* dissolution rate or emulsification rate has increased [17, 18]. Although there are some reports about anaphylactic reactions to Cremophor, these reactions were observed during intravenous administration, but not during oral administration, based on cytotoxicity studies for SNEDDS oral formulations containing Cremophor [19, 20]. Our previous studies [12, 13] indicated that SNEDD preparations of vitamin A in semisolid and solid forms have improved emulsification and *in vitro* drug release characteristics. Therefore, it was of interest to perform *in vivo* bioavailability studies of these formulations and compare the results with those of the oily solution of the drug as control. From the results, it is obvious that there was an increase in the rate and extent of drug absorption

from SNEDD formulations compared to the drug oily solution (table 1, fig. 1). The absorption of vitamin A is crucial when it is governed by factors that determine the absorption of lipids. During digestion, vitamin A ester is hydrolyzed to vitamin A alcohol (retinol) by pancreatic and intestinal enzymes and then solubilized by bile acids. Therefore, any disturbance of gastrointestinal enzymes or pancreatic enzymes will lead to disturbance of vitamin A absorption [21]. SNEDDS consist mainly of an oily solution of the drug mixed with a blend of surfactant and cosurfactant. The system readily emulsifies the oily solution of the drug into nanometer-sized droplets (droplet size for the optimized formulation was 42 nm) when exposed to water or gastrointestinal media. Therefore, it is conceivable that a SNEDD preparation that relies on its surfactant/cosurfactant for emulsification rather than the pancreatic enzymes has a much greater chance of absorption. From the results of bioavailability, it is obvious that the newly developed vitamin A SNEDD formulations improved the bioavailability twofold in case of F1 and 1.4 times in the case of F2. It is interesting to observe that the enhancement of bioavailability was correlated with the *in vitro* release study. The results of *in vitro* dissolution showed that after 30 min, the cumulative percent of drug released from F1, which was 99.4, and from F2, which was 54.4, increased to 92.3 after 90 min. On the other hand we could not perform a dissolution experiment for F3 due to phase separation because of lack of surfactants. This correlation indicates that dissolution is the rate-limiting step in absorption and bioavailability of vitamin A from these SNEDDS formulations. Among

the SNEDD formulations (capsules and tablets), it was clear that the SNEDD capsule showed a higher bioavailability compared to tablet formulations, but the difference was not significant at $p < 0.05$. This may be due to the large amount of Avicel PH105 used to adsorb the oily mixture in F2, associated with the tablet hardness produced from compression. The compact mass of the tablets reduced the dissolution rate of vitamin A in gastrointestinal fluids, which influenced the bioavailability results.

Conclusion

Vitamin A SNEDD-filled capsules and compressed tablets showed a significant increase in the rate and extent of drug absorption and in the bioavailability compared to the capsule filled with an oily solution of vitamin A. Based on this finding, SNEDD drug delivery systems may be one of the promising approaches to enhance the absorption of vitamin A in human volunteers and to overcome the formulation difficulties of lipophilic drugs.

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