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RESEARCH ARTICLE

Nanomemulsion of megestrol acetate for improved oral bioavailability and reduced food effect

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Abstract Megestrol acetate (MGA) belongs to the BCS class II drugs with low solubility and high permeability, and its oral absorption in conventional dosage form MGA microcrystal suspension (MGA MS) is very limited and greatly affected by food. In this study, MGA nanoemulsion (MGA NE) was formulated based on solubility, phase-diagram and release studies. Then oral bioavailability of MGA NE and MGA MS was evaluated. A randomized two-way crossover trial was conducted on six male dogs under fed and fasting conditions. Blood concentrations of MGA were analyzed using LC–MS/MS. MGA NE yielded 5.00-fold higher oral bioavailability in fasting conditions and displayed more stable absorption profiles after food intake compared with MGA MS.

Keywords Megestrol acetate · Nanoemulsion · Food-effect · BCS class II drugs · Oral bioavailability

Yixian Li and Chung Kil Song have equally contributed to this paper.

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Introduction

Megestrol acetate (MGA), a synthetic progestin (17α-acetyloxy-6-metylpregna-4,6-diene-3,20-dione), is used early for palliative treatment of advanced breast cancer or endometriosis. MGA stimulates appetite, induces weight gain and precipitates recovery from side effects caused by anti-neoplastic treatments. Hence, MGA is frequently prescribed for management of anorexia, cachexia, or un-explained substantial weight loss in patients with HIV/AIDS, cancer and other chronic diseases. MGA treatment improves the quality of life in patients who suffer from cancer cachexia (Tomiska et al. 2003). However the recommended dose for cancer-related cachexia is 800 mg per day. Such a large dose has been a problematic issue for the dosage regimen against cancer patients. MGA is belongs to BCS class II drugs with low solubility (2 μg/mL in water at 37 °C) and high permeability (Zhang et al. 2009; Sylvestre et al. 2011). The oral absorption of BCS class II drugs is very susceptible to food-intake (Martinez et al. 2002; Wu and Benet 2005). Foods trigger secretion of bile acid, solubilize drug in the intestinal lumen, and rapidly increase the oral absorption. Incidental increase of bioavailability usually causes unwanted side effects. As like other BCS class II drugs, oral bioavailability of MGA is very low and largely affected by food intake (Farinha et al. 2000). This issues challenges physicians to optimize the dosage form of MGA to get reliable dose–response (Alakhov et al. 2004; Deschamps et al. 2009; Cho et al. 2010).

Various pharmaceutical technologies, i.e., polymer co-precipitation, inclusion complex, nanoparticles and others, have been tested for decades. Among them, nanoemulsion noticeably enhances the oral absorption and reduces the food-effect (Kahan et al. 1995; Yang et al. 2006; Woo et al. 2008). A number of studies have proved that nanoemulsion

improves the bioavailability of BCS class II compounds (Mueller et al. 1994; Gao et al. 2011). Lipid-based delivery system triggers bile acid secretion. The secreted bile acid helps emulsification of oils. The emulsified-oil droplets enzymatically degraded to di-and monoglycerides and free fatty acids, forms micelles, and finally solubilization and absorption of drugs occurs (Patel and Sawant 2009). Nanoemulsion with low surface tension and large surface area facilitates oral absorption of drugs without the help of bile juices (Pouton 2000). The accelerated digestion of lipid not only improves absorption but also reduces food effect. But in any cases, compatibility and stability of drug with oils should be asserted during the formulation stages (Rahman et al. 2013).In this study, we formulated MGA nanoemulsion (MGA NE) which can reduce dosage size by three to four folds, and achieved stable and enhanced oral bioavailability of MGA regardless of food-intake. Droplet size of MGA NE was confirmed via dynamic light scattering (DLS) and TEM imaging analysis. Dissolution of MGA NE was evaluated by USP dissolution method. Then oral bioavailability of MGA NE in fed and fasting states was compared with the conventional formulation (MGA microcrystal suspension, Megace®, BMS, USA) in beagle dogs.

Materials and methods

Materials

Megestrol acetate (MGA), medroxyprogesterone acetate (MPA) and phosphotungstic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Megace® oral suspension was purchased in the market as a reference formulation. Acetonitrile, methanol and ammonium acetate were used as HPLC-grade. All other chemicals were of analytical reagent-grade and were used without further purification.

The preparation of MGA NE

Composition of MGA NE was determined based on the solubility of MGA in vehicles (i.e., oil, surfactant and cosurfactant) and the phase diagram study. Based on the results of the solubility studies (supporting information, Table S1), medium-chain triglyceride (MCT oil) was selected as the oil phase. Cremophor RH40 and propylene carbonate were used as surfactant and cosurfactant. The final compositional ratio of MGA NE was determined 1:2:1 for oil:surfactant:cosurfactant based on our previous study. MGA was dissolved in cosurfactant and then mixed with other components. The mixture was stored at ambient conditions and diluted with water before use.

Droplet size of MGA NE

Droplet size of MGA NE was estimated using a dynamic light scattering spectrophotometer (Zetasizer Nano ZS90, Malvern Ins. Ltd., England) equipped with a 15 mW laser diode and a PMT detector. 1 mL of MGA NE was introduced on the Zetasizer. Scattered light was detected at 90° at room temperature. TEM images of nanoemulsion were acquired on a CM200 scope (Philips, Germany). MGA NE was mixed with 2 % phosphotungstic acid solution. The samples were dropped on a glow-discharged grid, allowed to stand for drying and then introduced to the TEM. MGA microcrystal suspension (MGA MS) was also observed on a SEM (SNE4500 M, SEC CO., Suwon, Korea) after centrifugal separation and routine sample preparation for imaging.

In vitro release of MGA from nanoemulsion

In vitro release study was carried out using a USP 37 dissolution apparatus II (708-DS Dissolution Apparatus, Agilent Technologies, CA, USA) with 900 mL of simulated gastric fluids without pepsin (0.2 % sodium chloride in 0.7 % hydrochloric acid) at 37 ± 0.5 °C at a paddle speed of 50 rpm. 5 mL MGA NE, containing 10 mg of MGA, were put into a floating dialysis tube (Float-A-Lyzer, Spectrum Laboratories Inc., CA, USA) and transferred to dissolution medium. 5 mL of medium was periodically withdrawn, filtered (0.45 μm) and introduced to an HPLC (Agilent 1200 series, Agilent technologies, CA, USA). The same volume of fresh medium was added to dissolution media.

Pharmacokinetic study in beagle dogs

All animal care and experiments were carried out following the protocol (PKDD-I12025, Dec 2012) and the guidance approved by the Animal Care and Use Committee, Inha University. Oral bioavailability studies under fed and fasted states were conducted separately. Each study followed randomized two-way crossover design with a 2-week rest period. A certified canine diet (Orient bio, Korea), 25.2 % protein and 9.5 % fat, was supplied twice a day in the morning and evening. Six male dogs, weighing 12.0–15.0 kg, were randomly divided into two groups. Each group received either MGA NE or MGA MS (Megace®) in the first period. After the 2-week rest period, each group received the other drug during the second period. The oral dose of MGA was 100 mg for each dog. For the fasted state, dogs were fasted for 12 h before dosing and for an additional 4 h after dosing. For the fed state, dosing was started 30 min after the full consumption of regular canine food. The other conditions were exactly the same as those of the fasted state. Both formulations were orally administered via the pharynx.

30 mL of water was administered immediately after dosing using a syringe. 3 mL blood samples were obtained from the cephalic vein into heparin tubes (BD Vacutainer®, BD Diagnosis, NJ, USA) at the predetermined time, centrifuged to obtain plasma, and stored at −20 °C until analysis.

LC–MS/MS analysis of MGA

The concentrations of MGA in the blood samples were analyzed using a LC–MS/MS system (Seo et al. 2013). Briefly, 50 ng/mL MPA (internal standard) was added to 100 μL of dog plasma. The samples were then extracted with 1.9 mL of acetonitrile by vortexing and centrifugation at 10,000 rpm for 10 min. 5 μL of supernatant were injected into the LC–MS/MS system. Agilent 1200 series HPLC system (Agilent technologies, CA, USA) was used for separation using a C18 column (2.8 μm, 50 × 2.0 mm) (pursuit XRs ULTRA C18, Varian) at a flow-rate of 0.3 mL/min with a mobile phase of acetonitrile:5 mM ammonium acetate solution (65:35, v/v). As a detector, API 3200 QTRAP (AB Sciex, MA, USA) equipped with a TurboIonSpray® interface was operated in positive MRM mode, and the transition ions at m/z 385.3 → 267.2 for MGA and transition ions at m/z 387.3 → 327.2 for MPA were selected for detection of the two compounds. The optimized MRM transition conditions were as follows: ionspray voltage 5500 V; source temperature 500 °C; collision energy 22.6 V; curtain gas 20 psi; nebulizer gas 45 psi; auxiliary gas 45 psi. The linear range of concentration for LC–MS/MS analysis was 5–100 ng/mL with the standard curve equation: $y = 0.0306x + 0.00048$ ($r = 0.999$). The specificity of LC–MS/MS analysis was good with quantitation limit 5.0 ng/mL (supporting information, Table S2; Fig. S3).

Statistical analysis of data

The data was expressed in terms of mean ± standard deviation (SD). The pharmacokinetic parameters of each formulation were attained using the WinNonlin® program (Version 3.1, Pharsight Co., Mountainview, CA, USA). Statistical analyses between treatments were performed for AUC_{last}, and C_{max} using paired student *t* test.

Results

Preparation and characterization of MGA NE

MGA-loaded nanoemulsion was prepared by gently mixing with oil, surfactant, and cosurfactant. MGA NE formed transparent o/w emulsion as shown in Fig. S1. The morphology and size of MGA MS and MGA NE was observed using TEM image and zeta sizer (Fig. 1). SEM image showed that the suspension contained large and irregular MGA microcrystals over 500 μm in size. In contrast, MGA NE had homogeneous size distribution with a mean droplet size of 26.6 ± 8.1 nm. Homogeneous size of oil droplets can be observed on TEM images. The observed size on the TEM was around 10–20 nm.

In vitro release of MGA from nanoemulsion

Release of MGA from suspension and nanoemulsion was observed using USP dissolution apparatus II. Figure 2 shows that 13.8 % of MGA was released from the nanoemulsion, while 1.5 % was released from MGA MS. The release of MGA from MGA NE was 9.2 times higher than that from MGA MS. Release study was extended to 24 h to prove that MGA NE is capable of maintaining high solubilizing power in the gastro-intestinal tract without the aid of bile acid. Additional release study was performed following USP dissolution apparatus II without using dialysis membrane. The results displayed in Fig. S2. The simple filtration of dissolution sample for HPLC analysis showed much higher release of MGA up to 80 %.

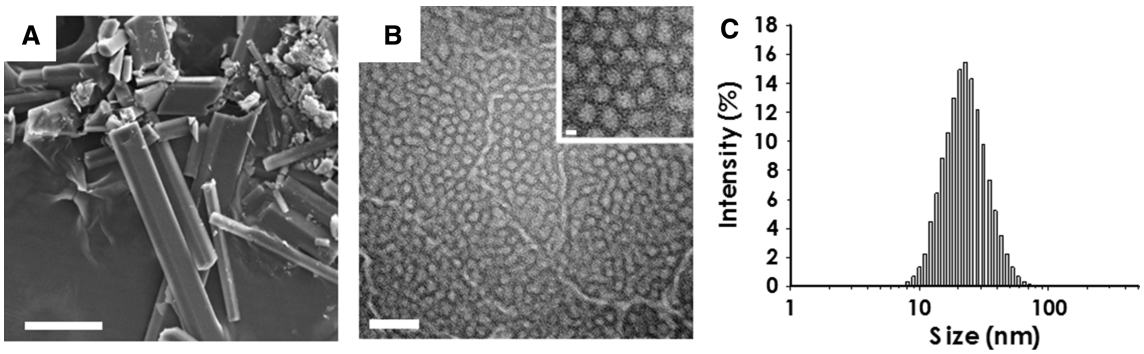

Fig. 1 Scanning electron microscopic image of MGA MS (**a**) and transmission electron microscopic image of MGA NE (**b**), and particle size distribution of MGA NE (**c**). The scale bar of images is (**a**; 500 μm, **b**; 50 nm and figure insert; 10 nm)

Enhanced oral bioavailability of MGA NE

In order to confirm the effect of the bioavailability to nanoemulsion and suspension we conducted in vivo pharmacokinetic experiment in the rat (fasting condition). The pharmacokinetic and plasma concentration profiles after oral administration of MGA NE and MGA MS in the rat was shown in Table S3 and Fig. S4, respectively. AUC_{all} and C_{max} of MGA NE showed 6.46-fold and 9.19-fold increases in comparison with AUC_{all} and C_{max} of MGA MS, respectively. In addition, T_{max} of MGA NE and MGA MS was 52.5 ± 28.7 and 375.0 ± 278.7 min.

Mean plasma concentration profiles after the oral administration of MGA formulations at a 100 mg/dog dose under fed or fasting states are displayed in Figs. 3 and 4. Pharmacokinetic parameters are summarized in Table 1. MGA NE, compared with suspension, exhibited increased AUC and C_{max} in both fed and fasted conditions.

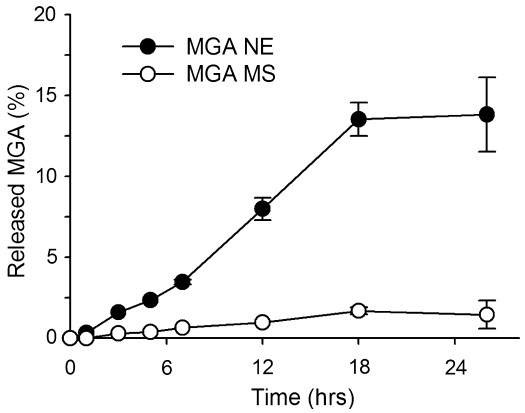

Fig. 2 Release of MGA from MGA MS (*open circle*) and MGA NE (*closed circle*) in pH 1.2 buffer

In fasting condition, $AUC_{0-24\ h}$ of MGA NE and MGA MS was 32,626.9 ± 15,793.3 ng·h/mL and 7899.5 ± 4502.7 ng·h/m, respectively (P = 0.0108). The AUC of MGA NE was 4.13-fold higher than that of MGA MS. The increased AUC from nanoemulsion was also observed in fed conditions. The AUC of MGA NE showed 1.53-fold higher than that of MGA MS (37,265.5 ± 8089.1 vs. 24,316.0 ± 9565.8 ng·h/mL for MGA NE and MGA MS, respectively, P = 0.0304). Along with AUC, C_{max} of MGA NE also increased in comparison with that of MGA MS (3419.7 vs. 721.3 ng/mL in fasting conditions; 4816.7 vs. 1822.7 ng/mL in fed conditions, respectively).

Reduced food effect on oral absorption of MGA

The effect of food on oral absorption of MGA is significantly reduced in the nanoemulsion formulation. As shown in Table 1 and Fig. 3, MGA NE achieves more stable pharmacokinetic profiles irrespective of food intake in beagle dogs. Food increased the individual $AUC_{0-24\ h}$ in MGA MS by 4.25 ± 3.53-fold ($AUC_{fed}/AUC_{fasting}$, P = 0.0065). This result implies the AUC of MGA MS is easily affected by food-intake. Meanwhile, MGA NE displayed a 1.28 ± 0.48-fold increase of $AUC_{0-24\ h}$. No significant statistical difference was observed between AUC of fed and fasting state after administration of MGA NE (P = 0.5411).

MGA NE also showed reduced inter-variation of AUC between dogs (Fig. S5). The reduced inter-variation of AUC achieved by MGA NE may provide therapeutic advantages for clinical application.

As observed in AUC, MGA NE displayed more stabilized C_{max} regardless of food-intake (2.5-fold and 1.4-fold increase of C_{max} for MGA MS and MGA NE, respectively).

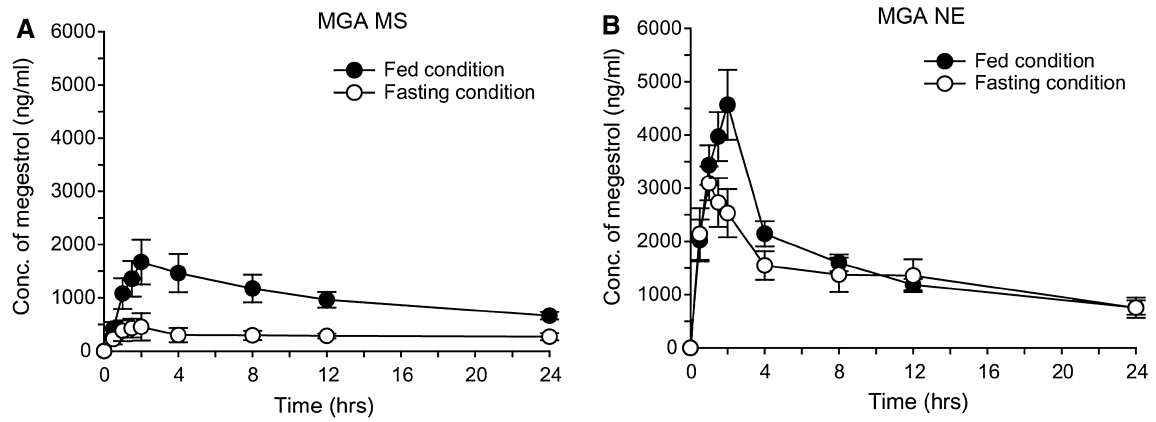

Fig. 3 Mean plasma concentration profiles of MGA after oral administration of MGA MS (**a**) and MGA NE (**b**) at a dose of 100 mg in fed (*filled circle*) and fasting (*open circle*) conditions. The data represent the mean ± SD (n = 6)

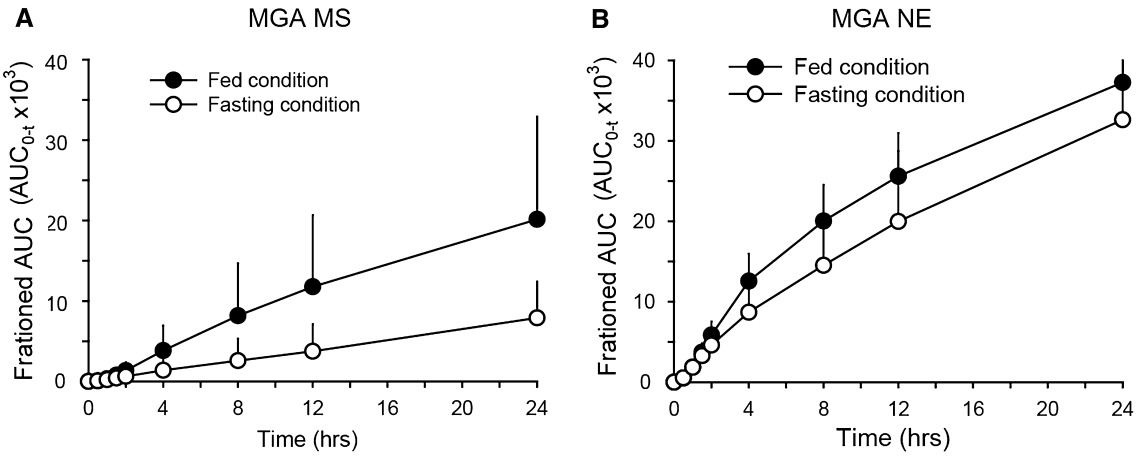

Fig. 4 Time-fractioned oral absorption (AUC_{0-t}) of MGA NE and MGA MS in fasting and fed condition

Table 1 Pharmacokinetic parameters of MGA MS and MGA NE after oral administration of 100 mg of MGA in fed and fasting conditions

Parameters	Fasting condition (A)	Fed condition (B)	*P* values
(A) MGA MS			
AUC_{last} (ng·h/mL)	7899.5 ± 4502.7	24,316.0 ± 9565.8	0.0065
C_{max} (ng/mL)	721.3 ± 571.6	1822.7 ± 830.0	0.0256
T_{max} (h)	12.9 ± 12.1	7.6 ± 8.9	–
$T_{1/2}$ (h)	15.6 ± 6.9	13.2 ± 1.2	–
(B) MGA NE			
AUC_{last} (ng·h/mL)	32,626.9 ± 15,793.3	37,265.5 ± 8089.1	0.5411
C_{max} (ng/mL)	3419.7 ± 911.4	4816.7 ± 1348.0	0.0655
T_{max} (h)	1.3 ± 0.4	1.7 ± 0.4	–
$T_{1/2}$ (h)	21.8 ± 18.9	11.0 ± 3.1	–

The data represent the mean ± SD (n = 6) and statistical analysis was performed using a paired student *t* test

In regard to T_{max}, MGA MS showed varied T_{max} (12.9 ± 12.1 h in fasting conditions and 7.6 ± 8.9 h in fed conditions), but MGA NE displayed more stable T_{max} (1.3 ± 0.4 h in fasting conditions and 1.7 ± 0.4 h in fed conditions) (Table 1).

Discussion

MGA is a progesterone derivative (17-hydroxylated progesterone). Glucocorticoid-related adverse effects such as adrenal insufficiency, osteoporosis and electrolyte imbalances can be observed. Other side effects may include nausea, vomiting, and edema (Chidakel et al. 2006). Stable and predictable pharmacokinetics is clinically very important to obtain reliable therapeutic efficacy and to avoid unwanted toxic events. Various factors such as genetic polymorphisms, transporters, drug interactions, etc. are under investigation to explain the problematic deviation (Nishizato et al. 2003; Yoshida et al. 2013). However, food is the most accountable cause of unstable oral pharmacokinetics. Food affects the oral absorption of drugs in various ways; it (a) neutralizes gastric pH, which changes the solubility of drugs; (b) retards gastric emptying time, which affects the T_{max}; and (c) triggers bile secretion, which facilitates the intestinal dissolution of drug, thus causing a sudden increase of bioavailability (Melander 1978; Courtney et al. 2004; Custodio et al. 2008).

In this study, we formulated MGA NE, and then proved the stable and increased oral bioavailability can be achieved via MGA NE formulation. First of all, MGA NE enhanced oral absorption of MGA. MGA NE showed a 500.1 % enhancement of oral bioavailability compared with conventional suspension. Considering the conventional dose size of MGA is 800 mg per day (20 mL in suspension), MGA NE can optimize the dose size for swallowing, which can be a therapeutic advantage for patients.

MGA NE displayed more stable oral absorption regardless of food-intake. Bioavailability of MGA MS was

increased by 425.4 % after food-intake. Meanwhile MGA NE showed a 128.6 % increase of bioavailability. Figure S5 shows the inter-distribution of AUC and C_{max} of MGA NE and MGA MS with or without food. Even though the experimental size of our study was small ($n = 6$ per group), MGA NE showed a narrower distribution of AUCs than MGA MS in fed conditions.

Especially, T_{max} of MGA MS was so variable in both fed and fasting condition (12.9 ± 12.1 and 7.6 ± 8.9 h for fasting and fed condition, respectively). Interestingly, T_{max} of MGA MS was shortened after food-intake. Usually food delays the gastric emptying time, increases T_{max} of drugs, and causes rapid absorption of drug at the duodenum by triggering secretion of bile juice. But oral suspension of MGA shows extremely low absorption profiles throughout the intestinal tract (Fig. 4). Oral solutions generally show rapid gastric emptying time and are not largely affected by food-intake. But T_{max} of MGA MS seems unrelated to the gastric emptying time. We assume this erratic T_{max} of MGA MS comes from its typical characteristics (i.e., water insolubility and suspension formulation). Meanwhile, more stabilized T_{max} was observed after administration of MGA NE (2.5 ± 1.0 and 1.7 ± 0.4 h in fasting and fed conditions, respectively). As shown in Fig. 4, rapid absorption of MGA was observed after oral administration of MGA NE in both fed and fasting conditions. Those results suggest the absorption of MGA NE is not largely affected by gastric emptying time or bile acid secretion.

Conclusion

In the present study, the oral absorption pharmacokinetics of MGA NE was compared with MGA MS. MGA NE increased oral bioavailability in both fed and fasting conditions and greatly reduced the food effect on the oral bioavailability. Thus we conclude that nanoemulsion of MGA exhibits cardinal benefit, i.e., stable and enhanced bioavailability in respect of food intake, for clinical application.

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Conflict of interest The authors declare that they have no conflicts of interest to disclose.

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