Enhanced absorption of Stachydrine using a self-double-emulsifying drug delivery systems (SDEDDS): in vitro and in vivo studies

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Abstract: Stachydrine, as a quaternary ammonium salt, exhibits low solubility in water, which leads to poor oral bioavailability. Water-in-oil-in-water (w/o/w) double emulsions are potential for enhancing oral bioavailability of drugs with high solubility and low permeability, but their industrial application is limited due to the instability. Herein, we employed a novel formulation, self-double-emulsifying drug delivery systems (SDEDDS) by formulating mixtures of hydrophilic surfactants and water-in-oil (w/o) emulsions, which were easier to be stable through formulations optimization. SDEDDS can spontaneously emulsify to water-in-oilin-water (w/o/w) double emulsions in the mixed aqueous gastrointestinal environment, with drugs encapsulated in the internal water phase of the double emulsions. The current studies have precisely demonstrated the potential utility of SDEDDS for formulating stachydrine with sustained release in vitro and improved oral bioavailability in vivo. The optimal formulation of the stachydrine –SDEDDS was successfully developed. Our study has demonstrated that SDEDDS could be a promising technique for improving the oral absorption of stachydrine with high solubility and low permeability.

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1. Introduction

Stachydrine called proline betaine or N, N-dimethyl proline, is the most simple pyrrole alkaloids. Stachydrine is the main active component of the Leonurus japonicus, mainly for the treatment of dysfunctional uterine bleeding, menorrhagia, dysmenorrhea; As the cardiovascular system, for the treatment of atherosclerosis, heart disease, myocardial infarction, angina pectoris, cerebral arteriosclerosis, cerebral infarction, acute and chronic renal failure, chronic heart failure. It can reduce LDH activity and MDA levels in the blood plasma, thereby increasing cell membrane permeability, reducing the levels of inflammatory factors, reducing lipid peroxidation and antioxidant activity (Wang et al., 2012;3Yin et al., 2010; Liu et al., 2008; Cheng et al.,2010; Ma and Yang 2006). Thus it has a favourable drug research value. Stachydrine, a quaternary ammonium salt, is a amphiphilic substances which can combine as internal salt and may also be a salt with hydrochloric acid. It is dissolved in the ionic state only, absorbing more difficult, and easily lost from the gut. Therefore, Stachydrine has a low bioavailability and high gastrointestinal irritation. Traditional Leonurus japonicus preparation often utilizes high dose or more frequency of administration for patients in ord to achieve therapeutic effect. This is a long-term medication and treatment process (Long et al., 2002). Stachydrine pure hydrochloric acid preparations has not yet appeared on the market after investigation. To increase patient compliance, we made stachydrine SDEDDS self-emulsifying agents.

SDEDDS (self-double-emulsifying drug delivery system) is the homogeneous and transparent solution composed of drugs, oil phase, emulsifiers and co-emulsifier. After oral administration, it is spontaneous emulsificated and rapidly formed W / O / W emulsion but not the O / W emulsion due to the gastric motility. Then, the drugs are wrapped in W / O / W aqueous phase (Qi and Wang 2011). Therefore SDEDDS is more stable than SEDDS (self-emulsifying drug delivery system) and can be filled into hard and soft capsules for benefiting administration and storage, Moreover, it can overcome the shortcomings of unstable to heat, organic solvents and pH changing (Benichou et al., 2004; Su et al., 2006). The rapid spontaneous emulsification and high dispersion in the absorbent placement of the SDEDDS effectively improve the oral absorption of the drug, which has good water-solubility but poor permeability. While avoiding the hydrolysis of water unstable drugs and adverse stimuli on gastrointestinal (Setthacheewakul et al., 2010; Zhao et al., 2010).

This paper presents the design of Stachydrine SEDDS, to increase the oral bioavailability of drugs and provide a theoretical basis for further Stachydrine preparation development. Firstly, we selecte several commonly used self-emulsifyingoil phase, emulsifier, co-emulsifier, through pseudo-ternary phase diagram drawing, and through the establishment of in vitro

HPLC analysis is used to detect the drug in the solubility of various materials and eventually screen SDEDDS prescriptions. Then, particle size, Zeta potential, microscopic morphology, stability, content and other aspects of stachydrine SDEDDS characteristics are also evaluated in vitro. Finally, we utilize rats as animal models for in vivo analysis. For oral pharmacokinetic study, as a control, plasma concentrations of Stachydrine SDEDDS are measured and investigated the relative bioavailability.

2.Materials and Methods

2.1 Reagents and Chemicals

Stachydrine were supplied by the Chengdu Herb purity Company, Ltd (Chengdu, China). Gelatin were purchased from Shanghai Zhaorui Biotech Company, Ltd (Shanghai, China). Tween®80 was obtained from the Wenzhou Qingming Chemical Plant (Wenzhou, China). Span 80, polyglycerol ester of polyricinoleic acid (PGPR), medium chain triglycerides (MCT), oleic acid, soybean oil and olive oil were obtained from Well Chemical Co., Ltd. (Nanjing, China). Methanol of HPLC grade was obtained from Yuwang Chemical Reagents Co. (Shandong, China). Perchloric acid was purchased from Linfeng (Shanghai, China). Isopropanol, sodium dihydrogen phosphate, alcohol, sodium hydroxide and gelatin were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Water was purified by redistillation and filtered through a 0.22_m membrane filter (Zongxing Filter Co., Ltd., China) before use. All other chemicals were of analytical grade.

2.2 Animals

Male Sprague Dawley rats weighing 220g±20g were obtained from the Animal Center of the West China School of Medicine (Chengdu, China) and housed under normal laboratory conditions of temperature, relative humidity, and light. The rats had free access to standard laboratory diet and water before the experiment. Animal experiments were approved by the Animal Experimental Ethical Committee of West China School of Medicine, Chengdu, China.

2.3 Formulation and preparation of Stachydrine -SDEDDS

SDEDDS was prepared through emulsification. Stachydrine hydrochloride (36mg) was dissolved in 0.5% gelatin solution as the inner water phase. The oil phase contained span-80, atolein and tween 80. The inner phase was added to the oil phase with moderate magnetic stirring (400 rpm) at room temperature. The w/o emulsions were then homogenized at 9500 rpm for 5minutes (FJ-200 high-speed dispersion homogenizer; Jiangsujintan Jincheng Instruments Co, Jiangsu, China.) until a clear and transparent formulation was obtained.

2.4 Characterization of SDEDDS formulations

2.4.1 Droplet size analysis and microscopic observation

The morphology and droplet size distribution of the formulation after transformation into w/o/w emulsions were studied under an inverted fluorescence microscope (DM1400B; Leica, Sylvius, Germany) and dynamic light scattering (Nano-S90; Malvern Instruments Ltd, Worcestershire, United Kingdom.) respectively. Three replicate analyses were carried out for each formulation, and data presented as means±SD.

2.4.2 Release of Stachydrine from SDEDDS formulations in vitro

To form the multiple emulsion, 8ml water was added into 4ml the double emulsion, low-speed stirring 5min. Release liquid (40ml) with multiple emulsion (10ml) was mixed at 100 rpm, 37℃. After 0h, 1h, 2h, 4h, 6h and 8h, took 5ml liquid, centrifugal (1000 rpm, 15min),respectively. The supernatant 200ul (reservoir) is added to the 200ul ethanol/acetone mixture (1:1, v/v), 5min shaking, and then centrifuged (13500 rpm, 20min), taking the lower liquid (water layer) contents of stachydrine as measured stachydrine content in emulsion layer; And also took the lower liquid (release liquid layer) 200ul, to determine the content of stachydrine directly.

2.4.3 Pharmacokinetic study of Stachydrine-SDEDDS in vivo

The rats were fasted overnight before the intragastric (ig) administration. Ten rats were divided randomly into two groups and treated (ig) with 80mg/kg of stachydrine solution and stachydrine-SDEDDS formulation respectively. After oral administration, blood samples (0.2 mL) were collected from the eye ground vein at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 400, and 720 minutes. Each sample was immediately transferred to heparinized plastic centrifuge tubes and then centrifuged at 5,000 rpm for 5minutes. Store the 100ul of supernant at -40℃. 100 μL of plasma and 500 μL of acetonitrile were mixed, votexed 10min. After centrifugation (12,000rpm, 10m), 1ul of supernant was measured by LC-MS/MS analysis.

2.5 Statistical analysis

Results are expressed as mean±SD of more than three experiments. Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance

in the differences of the means was determined by Dunnet's methodor Student's t-test.

3. Results

3.1 Characterization of SDEDDS formulations

As shown in Figure 1B, the freshly prepared stachydrine-SDEDDS can formulate fine w/o/w double emulsions after 3-fold dilutions with water under gentle stirring at 37°C for 5 minutes. Stachydrine was encapsulated in the internal water phase of the double emulsions. Dynamic light scattering studies showed that the mean diameter of the transformed w/o/w double emulsions was approximately 19.43 um.

CLSM micrographs showed that the spherical droplets were uniformly distributed in the dispersion medium with narrow particle size distribution(Figure 1A). As shown in Figure 1B, the dispersed oil droplets contained small dispersed aqueous droplets consistent with the characteristics of double emulsions.

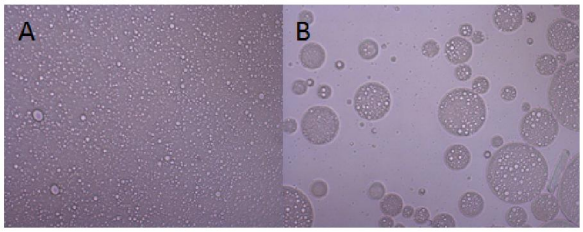

Figure 1 (A) Confocal microscopy images of freshly prepared stachydrine-SDEDDS and (B) formulated fine w/o/w double emulsions after 5-minute dilution with dispersion medium.

3.2 Release of Stachydrine from SDEDDS formulations in vitro

The SDEDDS readily released the lipid phase to form fine water-in-oil-in-water double emulsions. The release profile showed that SDEDDS had significantly decreased the dissolution of stachydrine and provided a sustained release of stachydrine up to 8 h in four releasing samples. Compared with other three samples, SDEDDS for formulating stachydrine had highest released rate continuously and climbed to a top as about 90% at 8 hours in artificial intestinal fluid (Fig. 2).

3.3 Absorption study in vivo

The plasma concentration of stachydrine versus time profiles in rats following oral administration and unformulated stachydrine presented in Figure 3. The pharmacokinetic parameters are summarized in Table 1. Results showed that the AUC (0–12) of stachydrine in SDEDDS increased by 1.8-fold compared to the unformulated stachydrine.T max increased from 0.75 h to 1.70 h. C max (599.9ug/ml) of stachydrine -SDEDDS was not so much different from the Cmax of aqueous solutions (740.4ug/ml).

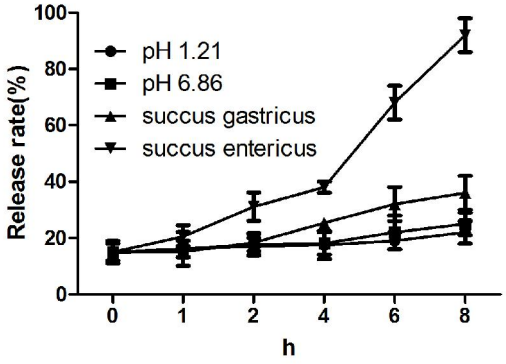

Fig. 2 Release profiles of stachydrine from SDEDDS in four different released sample. Data represent the means±SD (n = 3).

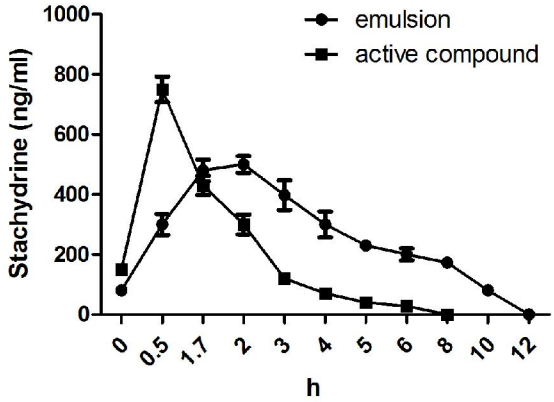

Fig. 3 Plasma concentration versus time profiles after oral administration of stachydrine formulated as SDEDDS, compared with stachydrine pharmacokinetics after dosing aqueous solutions. All values reported are means±SD (n = 10).

Table 1 Pharmacokinetic parameters of after oral administration of – stachydrine SDEDDS and unformulated stachydrine. All values reported are means±SD (n = 6).

	stachydrine	Stachydrine SDEDDS
AUC(0-t)(ug/L*h)	1365.55±479.60	2458.61±928.17
Tmax(h)	0.75±0	1.7±0.27
Cmax(ug/L)	740.40±306.00	599.91±99.28
t1/2z(h)	1.59±0.45	1.946±0.85

4. Discussion

Stachydrine ((2S)-1,1-dimethylpyrrolidinium-2 -carboxylic acid) may be regarded as an essential active principle of the aerial parts of Leonurus japonicus Houtt, which is widely applied in clinics, specially in promoting blood circulation, diuretic swelling and shrinkage in the womb (Kuchta et al.,2013). For cardiovascular system diseases, it is expected to be an excellent treatment. Stachydrine

also could improve blood flow of kidney, and repair renal glomerular and tubular to get the recovery of renal function. It has been recently reported that stachydrine inhibited the activity of breast and prostate cancer cancer cells. They examined the effect of stachydrine on the invasion and metastasis of cancer cells by inhibiting the expression of chemokine receptors (CXCR3 and CXCR4) (Rathee et al.,2012). Therefore, stachydrine has an extremely important research value. However, stachydrine, as a quaternary ammonium salt, exhibits low solubility in water, which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality. How to improve the intestinal absorption rate, for drug compounds, become a big challenge.

In recent years, lipid−based formulations attract extensive attention to improve the oral bioavailability of poor water soluble drug compounds. Of note, in these approaches, self-emulsifying drug delivery systems (SEDDS) are the most popular. It is the incorporation of the drug compound into inert lipid vehicles such as oils (Burcham et al., 1997), surfactant dispersions (Serajuddin et al.,1988; Aungst et al.,1994) and self-emulsifying formulations (Wakerly et al., 1986), emulsions (Toguchi et al.,1990) and liposomes (Schwendener and Schott 1996). SEDDS are a promising approach for the formulation of drug compounds with poor aqueous solubility. The oral delivery of hydrophobic drugs can be made possible by SEDDS, which have been shown to improve oral. However, SEDDS still have some shortcomings to overcome, for instance, unstablility to heat, organic solvents and pH changing.

Herein, we employed a novel formulation system, self-double-emulsifying drug delivery system (SDEDDS), which are the homogeneous and transparent solution composed of drugs, oil phase, emulsifiers and co-emulsifier. Similar to SEDDS, SDEDDS also could spontaneously emulsify in the mixed aqueous gastrointestinal environment. Whereas, the formed emulsions are water-in-oil-in-water (w/o/w) double emulsions not o/w emulsions, and drugs are encapsulated in the internal water phase of the double emulsions(Qi et al.,2011). Compared to conventional thermodynamically unstable double emulsions, SDEDDS are more stable to be filled directly into hard or soft capsules which are easy to administrate and storage. Up to date, in the market stachydrine pure hydrochloric acid preparations has not yet come out. The synthesis and characteristic of stachydrine SDEDDS self-emulsifying agents were a difficult problem.

Our results demonstrated that stachydrine-SDEDDS could enhance the bioavailability of stachydrine with obvious delay in T max. It can be supposed that compared with stachydrine, stachydrine -SDEDDS would disperse and form a w/o/w double emulsions spontaneously in the gastrointestinal fluid, with the active components entrapped in the oil droplets as internal reservoirs. Therefore, the prolonged absorption and elimination of stachydrine may be accounted for the function of the oil coating which can delay the medicine release into the sustained phase, in line with the continuous medicine compound release in vitro (Benichou et al., 2004). At the same time, the absorption-improving outcome of SDEDDS on the intestinal absorption of stachydrine may be in some degree attributed to the phospholipids and medium chain fatty acids contained in the formulation. It was common that phospholipids can act as a penetration enhancer for topically applied drug and could improve the transport of molecules into cells (Kato et al.,1987). For another, there were reports that medium chain fatty acids facilitated the intestinal membrane permeability of hydrophilic compounds bythe paracellular route (Cano-Cebrián et al., 2005).

The current studies have precisely demonstrated the potential utility of SDEDDS for formulating stachydrine with sustained release in vitro and improved oral bioavailability in vivo. The optimal formulation of the stachydrine –SDEDDS was successfully developed. Our study has demonstrated that SDEDDS could be a promising technique for improving the oral absorption of stachydrine with high solubility and low permeability.

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