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15-hydroxystearate micelles for the delivery of aprepitant: Preparation, characterization, pharmacokinetics and tissue distribution in mice

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Abstract: This study developed a novel Aprepitant micells (APPT-Ms) formulation that uses a mixture of 15-hydroxystearate (HS15) as surfactant to solubilize AAPT. This article determines the content of APPT by HPLC. The in vitro test results show that the optimized APPT-Ms has small particle size, excellent stability and long-lasting release. At a test dose of 20mg/kg, the pharmacokinetic study of APPT-Ms showed that it accorded with first-order kinetics in mice, and its AUC value was higher than the pure AAPT about 6 times. The tissue distribution study of mice showed that the APPT-Ms had higher tissue binding ability than pure AAPT. The APPT-Ms could be rapidly distributed to various tissues and it was easier to pass through the blood-brain barrier than APPT. In this study, the APPT-Ms has high antiemetic activity and improves the compliance of patient. The pharmacokinetics and tissue distribution of APPT-Ms after injection administration were studied, which may be of guiding significance for further research.

Keywords: Aprepitant; micelles, pharmacokinetics, tissue, distribution.

INTRODUCTION

Chemotherapy-induced nausea and vomiting (CINV) are the common adverse reactions in cancer treatment (Vanbockstael *et al* 2016; Hosseini and Tirgari *et al*. 2016). Aprepitant (APPT) is the first neurokinin 1 (NK1) receptor antagonist approved by the US FDA in 2003 (Olver, S Shelukar and K Thompson 2007). APPT was categorized as a BCS class IV drug with poor water solubility and permeability.

The micelles constituted by hydrophilic segment and hydrophobic segment is an amphiphilic copolymer. The particle size of micelles is generally less than 100 nm (Kesisoglou and Wu 2008). As a drug carrier, polymeric micelles can not only avoid the metabolism of drugs in the kidney, reduce the phagocytosis of the reticuloendothelial system, but also prolong the circulation time of the drug in the body.

This study developed a novel Aprepitant micells formulation that uses a mixture of 15-hydroxystearate (HS15) as surfactant to solubilize AAPT. HS15 have excellent solubilizing ability, low viscosity and low toxicity. *In vivo* studies of APPT-Ms in mice have shown that the preparation has great pharmaceutical behavior and tissue distribution characteristics. These experimental results provide an important guiding role for the further research of AAPT.

MATERIALS AND METHODS

Materials

HS15 was obtained from BASF (Mumbai, India). APPT was a generous gift sample from 98.5%, Wuhan Ding-hui Chemical Co., Ltd.

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Animals

Sprague-Dawley (SD) male rats (SPF grade, about 250g) and Kunming male mice (SPF grade, about 20g) were obtained from the Traditional Chinese Medicine Experimental Animals Research Institute of Chongqing, China. [No: 0005746]. The animal experiments were performed in accordance with China's guidelines for care and use of laboratory animals and approved by experimental animal ethics committee of School of Pharmaceutical Sciences & School of Chinese Medicine, Southwest University.

Methods

Preparation of Aprepitant-loaded Micelles

Aprepitant is solubilized with surfactant HS15 and co-surfactant ethanol as a mixed surfactant and then added with a pH adjuster, stirred in a 40-50°Cwater bath until APPT completely dissolved.

Characterization of APPT-loaded micelles

The sample size and zeta potential were measured using a Malvern laser particle size analyzer. The polydispersity index (PDI) indicates the degree of dispersion of the micelle solution. The concentration of AAPT in the APPT-Ms solution was determined by HPLC using a high-speed centrifugation method at 25°C.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{weight of APPT in micelles}}{\text{weight of the feeding APPT}} \times 100$$

$$\text{loading capacity (\%)} = \frac{\text{weight of APPT in micelles}}{\text{weight of (APPT in micelles + feeding excipients)}} \times 100\%$$

In vitro release study

APPT-Ms and APPT suspension were loaded into a dialysis membrane bag (MW=1000). The dialysis bag was immersed in fresh PBS solution (pH=7.4, 1% (w/v) Tween 80, 37°C). During a predetermined time, remove 1mL of release medium and replace with an equal volume of the same fresh release medium to maintain a constant volume. The amount of APPT released in the supernatant was determined by HPLC. All assays were performed in triplicate.

Pharmacokinetics and tissue distribution studies

Pharmacokinetic study

Healthy male Kunming mice were injected with APPT-LM and APPT solution at a single dose of 20mg/kg. After that, blood samples (200μL) were collected in EP tubes treated with disodium EDTA at 0.25, 0.5, 1, 2, 4, 6, 12, 24 and 36h. The pharmacokinetic parameters of the analytes were assessed using DAS (Version 3.2.1. Chinese Pharmacological Society, Shanghai, China), were calculated by a non-compartmental method. All data are expressed as the mean ± SD.

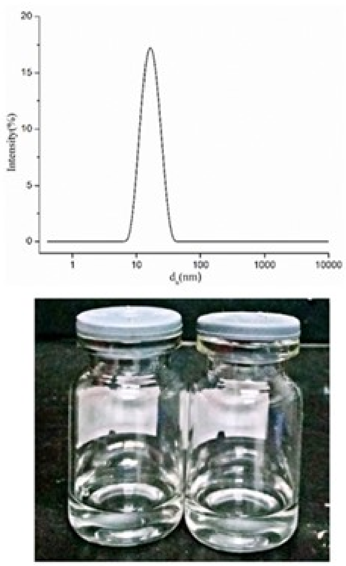

Fig. 1: Size distributions of APPT-Ms (left) and Samples of APPT-Ms (right).

Tissue distribution

The tissue samples were washed with frozen 0.9% saline solution to remove the surface of blood and other content and wiped with filter paper. After that, all tissues were mashed into ten-fold volumes of 0.9% normal saline and then stored at -80°C until further analysis. All data are expressed as the mean ± SD.

HPLC analysis

The analysis of APPT-Ms was carried out by using a Agilent 1260 HPLC system (Agilent, USA). The analyte was separated on a Hypersil BDS-C18 column (250 mm×4.6 mm I.D., 5μm) (Dalian Elite Analytical Instruments Co., Ltd. Dalian, China). The mobile phase consists of methanol, acetonitrile and water (23:51:26, v/v/v) with a flow rate of 1mL/min and diazepam injection (5μg/mL) was used as an internal standard (IS). The injected sample volume was 20μL and detection wavelength 210 nm kept constant throughout the experiment. Column temperature was set at 30°C and the analysis time was 30 min.

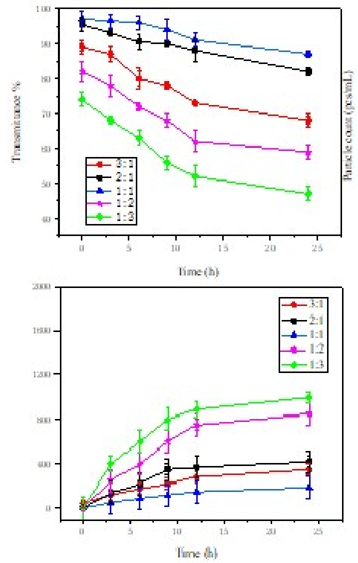

Fig. 2: The left is the transmittance and the right are the number of particles.

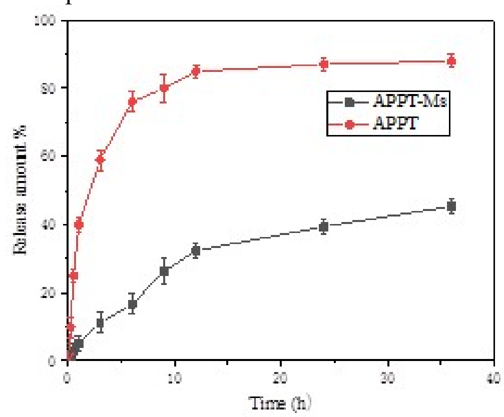

Fig. 3: *in vitro* release from APPT-Ms and APPT solution at 37°C (mean ± SD, n = 3)

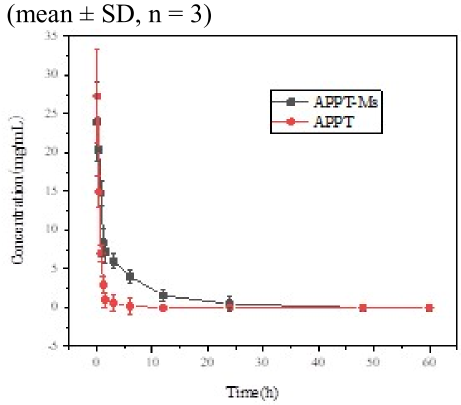

Fig. 4: Mean plasma concentration-time profiles of APPT solution following intravenous administration of APPT-Ms (20mg/kg) to SD male rats (n=5).

Table 1: Characteristics of APPT-loaded micelle system (mean ± SD, n = 3).

HS15: Ethanol	Size (nm)	PDI	Zeta (mV)	EE%	DL%	Clarity
3:1	22.87±0.32	0.113±0.01	-1.8083±0.02	90.30±0.91	6.09±0.13	+
2:1	21.42±0.40	0.101±0.01	-1.7691±0.05	96.01±1.98	6.21±0.25	+ +
1:1	20.91±0.35	0.084±0.00	-1.7103±0.03	98.70±0.52	6.76±0.22	+ + +
1:2	29.59±0.18	0.090±0.01	-1.7068±0.03	82.19±3.47	5.34±0.09	+
1:3	41.68±0.44	0.082±0.06	-1.693±0.02	75.67±5.13	3.91±0.41	+

+ + + means the solution is clear and transparent.

Table 2: Main pharmacokinetic parameters after intravenous administration to SD male rats (20mg/kg) (mean ± SD; n=5).

Parameters	APPT	APPT-Ms
AUC_{0-t} (μg h/L)	14.685±0.023	87.305±10.522
$AUC_{0-\infty}$ (μg h/L)	14.803±0.091	87.391±10.524
$t_{1/2}$ (h)	0.600±0.102	4.073±0.346
Cmax (μg /mL)	28.013±0.301	24.301±5.069
Vz (L/kg)	445.010±21.451	1477.211±188.253
MRT_{0-t} (h)	4.168±2.301	5.733±1.014

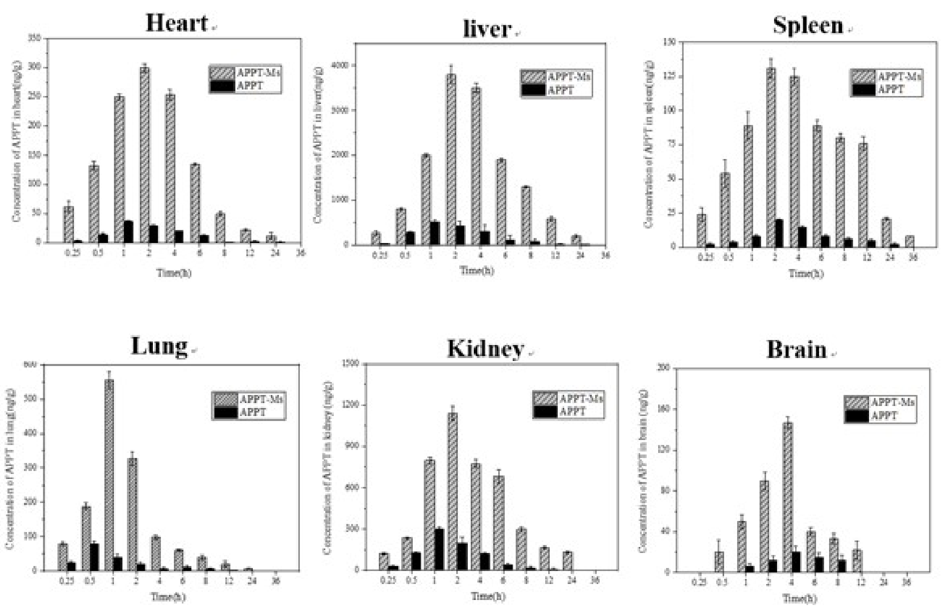

Fig. 5: Tissue distribution of APPT-Ms and pure APPT after intravenous administration for KM mice at dose of 20mg/kg.

DISCUSSION

The results showed that the best ratio of micelles system was 1:1 (HS15: Ethanol). With the concentration of the surfactant increase, the hydrophobic core volume of the micelle increase. As the same, the chain length and the size increase. In general, the ratio of HS15 and Ethanol =1:1 is appropriate.

Characterization: Particle size, Zeta, PDI, EE%, DL%

The average particle size of APPT-Ms was 15.68±0.44 nm and the polydispersity coefficient was 0.082±0.006. The encapsulation efficiency and load of APPT-Ms were 98.70±0.52% and 6.76±0.22%.

Stability

The particle number and transmittance of the solution are positively correlated with the turbidity of the solution. The results of comprehensive particle number and light transmittance indicate that when the ratio of surfactant HS15 to co-surfactant ethanol is 1:1, the stability of APPT-Ms is superior.

In vitro release

The results in fig. 3 indicate that APPT-Ms has a significant sustained release. The release of APPT was 90.3% while the APPT-Ms was 43.5% in 36h.

Pharmacokinetic study

As the table2 shows that the drug reached the highest concentration rapidly and the linear regression analysis (time versus plasma concentration) of APPT and APPT-Ms were performed with the correlation coefficient R^2 of 0.95 and 0.97. Compared with APPT, the $T_{1/2}$ of APPT was 0.600±0.102h, which indicated that APPT-Ms may be eliminated from rat plasma slowly and the action time is longer, thus APPT-Ms can reduce the number of clinical doses and improve patient compliance. The AUC0-t and AUC0-∞ of APPT-Ms were 87.305±10.522 and 87.391±10.524μg h/L. The volume of distribution of APPT-Ms is Vz>100L/kg, which may suggest that its concentration in tissues is higher than in plasma. And because of the poor solubility and outstanding tissue binding ability of APPT, its concentration in brain tissue can be further studied.

Tissue distribution studies

Compared with APPT, the distribution of APPT-Ms increased fourfold in brain tissue. APPT-Ms is rapidly and widely distributed in various tissues and easily penetrates the blood-brain barrier and has antiemetic activity. As shown in the fig. APPT-Ms has a high content in the blood-rich tissues such as heart, spleen, lung, kidney and connective tissue. Since APPT is a CYP3A4 (Milnes *et al*. 2015) inhibitor, it has a high content and is mainly cleared by liver metabolism in the form of inactive metabolites (Bochmanna *et al.* 2016). At the same time, compared with APPT, the concentration in the mice brain of APPT-Ms is higher. That is to say, the amount of APPT passing through the blood-brain barrier depends on its lipophilicity and particle.

CONCLUSION

In this study, a new type of Aprepitant micelles for injection with excellent properties was prepared. The results showed that when the ratio of surfactant HS15 to co-surfactant ethanol was 1:1, the drug-loaded micelle system is the most stable.

Pharmacokinetic studies showed that APPT-Ms met the first-order kinetic characteristics at the experimental dose. Tissue distribution studies showed that APPT-Ms has high permeability, high binding capacity in mice and which was rapidly distributed and widely distributed in various tissues. The distribution of APPT-Ms in brain tissue showed that it is highly permeable to the blood-brain barrier (BBB). In conclusion, this study provides an effective basis and a certain guiding for the industrialized large-scale production and clinical pharmacology and toxicology research of aprepitant.

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