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Preparation of sertraline-loaded chitosan nanoparticles and the pharmacokinetics studies

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Sertraline is a worldwide used antidepressant for perinatal depression. However, the secreting of sertraline into milk arose the concerns for the potentially negative effects on breastfed infants. The present study aims to alter the biodistribution of sertraline by loading the drug into chitosan-nanoparticles. Our results demonstrated an effective way to load the water-soluble sertraline into nanoparticles, and keep the size around 200 nm. The *in vivo* results demonstrated the difference of sertraline concentration in plasma after the intravenous injection through the marginal ear vein in rabbits. Because of the protection of nanoparticles, the drug concentration in plasma increased 5 h post the injection, which may delay the tissue-distribution of sertraline. Thus, our results indicate that the nanoparticles-encapsulation may change the biodistribution of sertraline and offer a safe window for breastfeeding. Nevertheless, the sertraline in breast milk was still detectable after injection of sertraline-loaded nanoparticles. Thus, it still needs caution for lactating women to have antidepressant drugs during breastfeeding.

Key words: Sertraline, chitosan, nanoparticles, pharmacokinetics.

INTRODUCTION

Perinatal depression is an increasingly common psychiatric disease for those women who are in pregnancy and postpartum and antidepressants are often used during these periods, (Cooper and Murray, 1995; Evans et al., 2001; Marcus et al., 2003; Gaynes et al., 2005; Dietz et al., 2007; Patil et al., 2011). Therefore, the administration of antidepressant drugs while breastfeeding is of great concern to both mothers and physicians, because this requires the knowledge of the extent to which drugs are excreted into breast milk. Most antidepressant drugs pass into breast milk to some extent through passive diffusion (Begg, 2006).

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Sertraline (Zoloft) is one of the members belonging to the group of selective serotonin reuptake inhibitors (SSRIs) in brain (Rao et al., 2009). It was introduced firstly in 1988 (Doogan and Caillard, 1988) and noted to be the most prescribed antidepressant with no adverse effects (Whitby and Smith, 2005). Because of some advantages, like an improved tolerability and adverse effect profile and relative safety in over dosage (Doogan and Caillard, 1988; Henry, 1991; Grimsley and, 1992), SSRIs have been one of the first line choices for the treatment of depression (Wen and Walker, 2004; Field, 2008). However, it has been reported that sertraline will be secreted to milk when administrated by breastfeeding mother (Altshuler et al., 1995; Stowe et al., 1997; Kristensen et al., 1998; Wisner et al., 1998), which may cause potentially negative effects of antidepressants on breastfed infants. Stowe et al. (1997) found that sertraline and desmethylsertraline are present in the breast milk of nursing. Detectable concentrations of sertraline are found in three of eleven nursing infants. Moreover, in another case (N=15 women and 182 breast milk samples), the highest concentrations are observed in the hindmilk 8 to 9 h after maternal ingestion of sertraline (Stowe et al., 2003). In addition, the long-term neurobehavioral influence of this antidepressant drug on these infants is more worried. Thus, most postpartum depressed mothers will not breastfeed or reduce the breastfeeding periods of time, as they concern about the potential negative effects of antidepressants on their breast milk and, in turn, on their infants' development (Field et al., 2002; Field, 2008). Therefore, how to avoid or decrease the antidepressants in breast milk has been a challenge for the treatment to perinatal depression.

Nanoparticles are, in general, colloidal particles, less than 1000 nm, that can be used for better drug delivery and prepared either by encapsulating the drug within a vesicle and or by dispersing the drug molecules within a matrix (Saha et al., 2010). Because of the unique physicochemical properties of nanoparticles, nanoparticles based drugs may have improved solubility, pharmacokinetics, and biodistribution as compared to small molecule drugs (Dobrovolskaia et al., 2008). They can alter and improve the biodistribution, pharmacokinetic and pharmacodynamics properties of various types of drug molecules (Vasanthakumar et al., 2010). Furthermore, nanoparticles based drugs can often extend the circulation times in plasma than small molecule drugs, which cause their longer half-lives. Thus, nanoparticulate seems to have the advantages in altering the biodistribution and extending the half-lives of drug molecules.

In the present study, sertraline was used as a model drug to prepare the sertraline-loaded nanoparticles, and compared the biodistribution of sertraline nanoparticles in rabbits' plasma with that of the sertraline solution to investigate whether nanoparticles could change the biodistribution profile of sertraline. Here, chitosan is chosen as the material for drug carrier, because it has great potential as a biomaterial for the construction of nanosized drug, and is generally regarded as non-toxic, biocompatible and biodegradable material (Knapczyk, 1989; Chandy and Sharma, 1990; Hirano et al., 1990; Bersch et al., 1995). The sertraline-loaded chitosan nanoparticles were successfully prepared based on ionic gelation of chitosan with tripolyphosphate (TPP) anions. Many groups have reported the pharmacokinetics of sertraline molecules in breast milk, mother's serum and infant's serum, but the distribution of chitosan nanoparticles encapsulated sertraline have not been reported yet to our knowledge.

Our results demonstrated that chitosan nanoparticles could prolong the residence time of sertraline in circulation when compared with its solution form, and might offer a safe window for breastfeeding. Nevertheless, there was still some sertraline secreted into the breast milk after injection of sertraline-loaded nanoparticles. Thus, it still needs caution for lactating women to have antidepressant drugs during the breastfeeding.

MATERIALS AND METHODS

Reagents and animals

Sertraline hydrochloride was bought from WuHan Weishunda Technology Development Ltd. (China). HPLC-grade acetonitrile and methanol were obtained from Fisher Scientific (USA). 95% n-hexane, acetic acid, potassium dihydrogen phosphate, sodium hydroxide crystals and 1M hydrochloric acid, all of analytical reagent (AR) grade were obtained from Sinopharm Chemical Reagent Co., Ltd, (China). Chitosan was obtained from Yuhuan Ocean Biochemical Co., Ltd, (China). Sodium TPP was bought from Shijiazhuang Shinearly Chemicals Co., Ltd, (China). Milli-Q water was used to prepare the buffers and aqueous solutions.

Pregnant New Zealand White rabbits (Body weight 3.0 to 4.0 kg) were purchased from Zhejiang University of Traditional Chinese Medicine. All procedures involving animals were approved by the Institution Animal Care and Use Committee of this center. Animals were acclimatized in our laboratory 7 days before delivery. During this period, animals were housed separately in cages with controlled light cycle (12/12 h).

Preparation of sertraline-loaded nanoparticles

The sertraline-loaded nanoparticles were prepared by a crosslinking way, which the free amino groups of chitosan would crosslink to the negative ion of TPP to form globular gel. In general, chitosan solution 2.5 mg/ml was prepared by dissolving the polymer in 1% (v/v) acetic acid aqueous solution and stirred for 10 min. Then, 50 mg sertraline was added into the solution and stirred until the sertraline was absolutely dissolved. After that, the pH of the solution was adjusted to 5.0 to 6.0 using 1 mol/L NaOH. The chitosan solution was further stirred for 0.5 h at room temperature. Finally, sodium TPP, the counter ion, was dissolved in pure water to prepare a 1 mg/ml solution, and added to the chitosan solution under mild magnetic stirring to form chitosan nanoparticles. The nanoparticles solution was centrifuged at 18,000 rpm and 4°C for 30 min and the nanoparticles at the bottom were then collected, extensively washed with water to remove the TPP and the acetic acid, and lyophilized at last.

Morphological characterization of nanoparticles

Transmission electron microscopy (TEM) was performed for the morphological examination of nanoparticles. The nanoparticles were stained with 2% (w/v) phosphotungstic acid aqueous solution for 10 s, immobilized on copper grids with formvard and were dried overnight before microscopy.

The particle size and polydispersity index (PDI) of nanoparticles were measured by Nano-S90 laser particles size analyzer (Zetasizer Nano-S90, Malvern, UK) after dilution of nanoparticles suspension with distilled water (30 fold).

Determination of entrapment efficiency of sertraline

The quantity of sertraline entrapped into the nanoparticles was calculated by the ultracentrifugation method. In detail, the nanoparticles were centrifuged at 3000 rpm for 10 min to separate the nanoparticles. The resulting supernatant was transferred to ultracentrifuge tubes (Beckmann Instruments, Fullerton, CA) and further centrifuged at 20000 rpm for 30 min. The sediment obtained was re-suspended in double distilled water (DDW, 10 ml) with the aid of a sonicator and centrifuged at 20000 rpm for 30 min. This process (that is, re-suspension of sediment and centrifugation) was repeated three times and all of the supernatants were collected. The concentrations of sertraline in supernatants were measured by high performance liquid chromatography (HPLC) method.

The entrapment efficiency of the sertraline nanoparticles was calculated as: entrapment efficiency (%) = (total sertraline − free sertraline)/total sertraline.

HPLC analysis

Chromatographic conditions

A modified HPLC–UV method described by Dodd et al. (2000) was used for the quantification of sertraline in various samples. The HPLC system consisted of an Agilent series 1200 Chemstation, Agilent 1200 VWD absorbance detector (Agilent Technologies Singapore (International) Pre. Ltd., USA). Sample separation was performed on a Diamonsil® C18 (150 × 4.6 mm, 5 µm) (Dikma, China) with a guard column (EasyGuard® C18, 10 × 4.0 mm) (Dikma, China). The mobile phase consisting of phosphate solution (15 mM, pH=3.0); Acetonitrile (60:40, v/v) was prepared daily and filtered through a Millipore membrane filter (0.22 µm) and degassed by sonication in an ultrasonic bath before use. The flow rate and the detection wavelength for monitoring the eluents were 1 ml/min and 225 nm, respectively. The analysis was carried out at 30°C. The injection volume was 20 µl. Data collection and processing were performed using Agilent series 1200 Chemstation software.

Preparation of stock and standard solutions

Stock solutions containing 1 mg/ml of individual sertraline were prepared in water. Working standards (100 µg/ml) were prepared by dilution of individual aliquots of stock solution with the same solvent. The solutions were stable at least for 1 month at 4°C. Appropriate dilutions of the individual working solutions of sertraline were made and used for constructing the calibration curves.

Extraction procedure for calibration of plasma specimens

Calibrations for sertraline in plasma were done by adding appropriate volumes of working standard (100 µg/ml) solution of sertraline to 90 µl of plasma in 1.5 ml EP tubes to give a range of sertraline concentrations ranging from 1 to 40 µg/ml. 100 µl of 1M NaOH was added to the specimens and votexed for 15 s. 1 ml of n-hexane and ethyl acetate mixture solution (v/v, 4/1) was then pipetted into each of the specimen tube and votexed for 2 min and shook on an orbiter shaker (QB-600, Kylin-Bell Lab Instruments Co., Ltd. , China) for 15 min. After that, the specimens were centrifuged at 3500 rpm for 15 min. 0.9 ml of the organic layer was then transferred to clean EP tubes and dried using a stream of nitrogen gas at 37°C. At last, the specimens were dissolved in 200 µl of the mobile phase for HPLC analysis.

Extraction procedure for calibration of milk specimens

Calibrations for sertraline in milk were done by adding appropriate volumes of working standard (100 µg/ml) solution of sertraline to 90 µl of milk in 1.5 ml Eppendorf (EP) tubes to give a range of sertraline concentrations ranging from 1 to 20 µg/ml. The followed procedures were similar as mentioned earlier.

Plasma and milk distribution study

Milk and blood sampling was carried out from rabbit during lactating. Sertraline (30 mg/kg) was administered via marginal ear vein. Serial blood samples (0.5 ml) were drawn into heparinized syringe before starting the kinetic study at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after drug administration and were always replaced with an equal volume of saline solution. Serial milk samples were also taken at different time points after drug administration by manual expression into a centrifuge tube connected to a vacuum system. At each time, the gland was emptied as completely as possible. The blood and milk samples were stored frozen at -20°C until analysis. The extraction procedure of plasma and milk specimens were the same as mentioned earlier. Sertraline in various samples was quantified by HPLC-UV method.

Statistical analysis

Statistical evaluation of differences experimental group means was analyzed by multiple Student t-tests. Data were expressed as the mean ± standard deviation (SD). Data points were from at least three independent experiments.

RESULTS

Preparation and characterization of sertraline nanoparticles

In this study, a serial of mass ratios of chitosan (CS)/TPP was set and the particle size, PDI and entrapment efficiency was detected, respectively to investigate the best prescription for sertraline-loaded chitosan nano-particles. Results are shown in Table 1 and Figure 1a. When the mass ratios of CS/TPP were from 8 to 16, the diameter of the prepared nanoparticles was around 200 nm and with the relative low PDI. Although, quite good quality of nanoparticles at mass ratios of CS/TPP was also acquired from 5 to 8; the size of the nanoparticles seems a little large that it may not suit for the intravenous injection. Our former study had reported that the CS/TPP ratio had influence on the stability of the nanoparticles (Li et al., 2010). The stability will be decreased with the

Table 1. Size and size distribution of sertraline nanoparticles with different mass ratios of CS/TPP.

Mass ratio (CS/TPP)	Diameter (nm)	PDI
16.7	240±4	0.077
12.5	217±22	0.230
10	198±29	0.253
8.4	234±6	0.134
6.3	298±28	0.202
5	313±10	0.139

increasing amount of TPP. Here, the nanoparticles, were relative stable below the ratio of 10. In addition, when the mass ratio is at 10, the highest sertraline entrapment efficiency with 15.60% was acquired. Thus, the sertraline-loaded chitosan nanoparticles prepared at mass ratio of 10 were studied in the following experiments. The transmission electron microscopy (TEM) micrographs of the sertraline nanoparticles with the mass ratio of CS/TPP at 10 were demonstrated as shown in Figure 1b. These nanoparticles were generally spherical, and the particle size distribution was in an acceptable range. The mean diameter was 198±29 nm and the PDI was 0.253 (n=3). Figure 1c demonstrated the size distribution of the nanoparticles obtained by the Zetasizer.

HPLC assay of sertraline in plasma and milk

The calibration curves of sertraline in blank plasma and milk were Ap= 20.16 Cp – 32.622 (where Ap represents peak area, Cp represents the concentration of sertraline in plasma, R^2=0.9998) and Am = 34.422 Cm + 50.409 (Am represent peak area, Cm represents the concentration of sertraline in milk, R^2=0.9998), respectively. The retention times of sertraline in plasma and milk were 5.93±0.21 and 6.20 ± 0.18 min, respectively. It was believed from these results that this analysis method was accurate and precise with coefficients of variation with intra-and inter-day relative standard deviation (RSD) below 10% for all the biological samples. The precision and the accuracy of this method were determined by adding known amounts of sertraline to blank plasma and milk and the results conformed to a certain standard within 85.0 to 110.0%. The limit of quantification (LOQ) in plasma was 0.02 µg/ml.

Plasma pharmacokinetics study

After a single intravenous administration of sertraline solution or sertraline nanoparticles in rabbits at a dose of 30 mg/kg, the plasma concentrations of sertraline were assessed. Figure 2 compared the plasma drug concentration-time curves of sertraline solution and sertraline nanoparticles. The main pharmacokinetic parameters were derived by the software of Data Access System 2.0 version (DAS, Mathematical Pharmacology Professional Committee of China, Shanghai, China) and summarized in Table 2. According to the analysis of models and parameters, a two-compartment model presented the best fit to the plasma drug concentration time curves obtained in rabbits. The biological half-lives of sertraline injection and sertraline nanoparticles in the phase of eliminate were 15.269 and 43.565 h, respectively. And the half-lives in the phase of distribution were 0.242 and 43.534 h, respectively. The mean area under the plasma concentration-time curve from zero to infinity ($AUC_{(0-\infty)}$) of sertraline nanoparticles (453.245 µg*h/ml) was about 4 times greater than that of the sertraline solutions (105.126 µg*h/ml). The possible reason for the aforemenntioned results is mainly due to the protection effect of the chitosan nanoparticles. This is because the encapsulated sertraline needs to be released from the nanoparticles in the circulation firstly. And then distributed to the tissues and be eliminated like the sertraline solution. The increased drug concentrations in plasma of sertraline nanoparticles from 4 to 12 h (Figure 2) may be due to the release of sertraline from the nanoparticles.

Concentrations of sertraline nanoparticles in rabbits' milk

The concentrations of sertraline-loaded nanoparticles in lactation rabbits' hindmilk were also investigated to check whether the sertraline was detectable in milk. The samples were taken after a single intravenous administration of sertraline nanoparticles in lactation rabbits. Figure 3 demonstrated the drug concentrations of sertraline nanoparticles in milk. It was believed that the drug concentrations of sertraline-loaded nanoparticles were detectable at 1, 2, and 3 h in rabbits' milk after i.v. administration of sertraline nanoparticles using the analysis method established in this study.

DISCUSSION

Dilemmas about whether or not to contraindicate breast-feeding arise most commonly in relation to postpartum depression as antidepressants took by the nursing

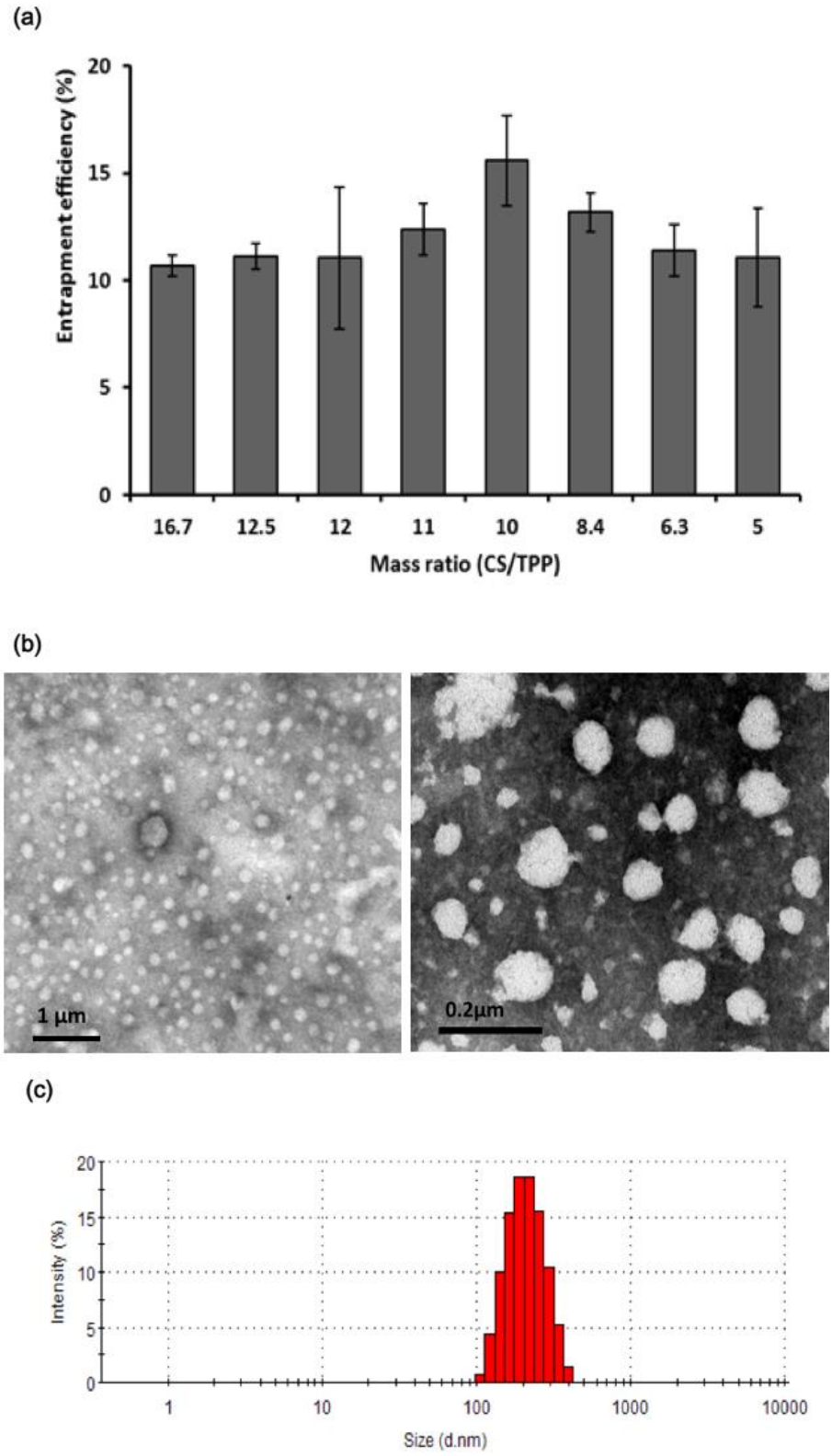

Figure 1. (a) Entrapment efficiency of sertraline-loaded nanoparticles with different mass ratios of CS/TPP. When the mass ratio of CS/TPP equaled to 10, the nanoparticles had the highest entrapment efficiency with 15.6±2.1%. (b) Transmission electron microscopy (TEM) micrograph of chitosan nanoparticles loaded with sertraline (the mass ratio of CS/TPP is 10). (c) The size distributions of the sertraline nanoparticles (the mass ratio of CS/TPP is 10).

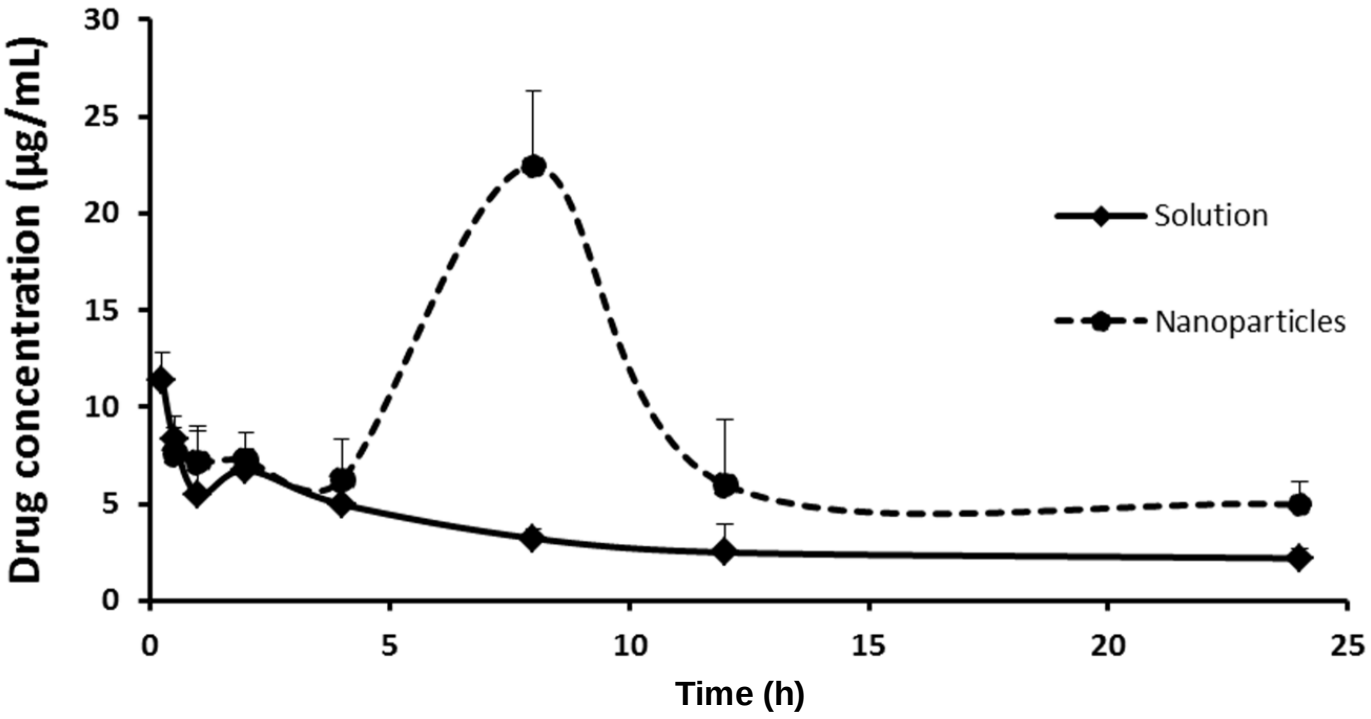

Figure 2. The comparative plasma concentration-time curves after marginal ear intravenous injection at a dose of 30 mg/kg of sertraline and sertraline-loaded nanoparticles injection in rabbits (n = 3).

Table 2. The comparative pharmacokinetic parameters after i.v. administration of sertraline and sertraline-loaded nanoparticles in rabbit (n = 3).

Sample	C_{max} (μg/ml)	$AUC_{(0-\infty)}$ (μg*h/ml)	CL (ml/(kg*h))	V (ml/kg)
Solution	11.382	105.126	0.19	1.112
Nanoparticles	22.434	453.245	0.044	2.674

mother will distribute into breast milk (Stowe et al., 1997; Yoshida et al., 1999). As such, the nursing infants will be exposed to the drugs. Nanoparticles as thought can alter and improve pharmacokinetics and biodistribution of drug molecules contributing to the unique physicochemical properties of nanoparticles. Therefore, we tried to prepare a sertraline-loaded nanoparticle to alter the pharmacokinetics behavior and decrease the diffusion of sertraline to breast milk. Our studies demonstrated that the encapsulation of sertraline into the chitosan nanoparticles prolonged the drug residence time in plasma, as well as the area under curve (AUC). These results indicated that the nanoparticles can maintain more drug in the circulation and prevent the rapid tissue-distribution when compared with the solution. It is believed that by maintaining a high drug concentration in circulation may help decrease the secretion of sertraline into milk. As demonstrated in the plasma drug concentration-time curves, the concentration of sertraline in plasma rapidly decreased during the first 1 h, and then the concentration declined slowly. Whereas, for sertraline-loaded nanoparticles, the concentration in plasma maintained at a lower level during the first 4 h, which is because sertraline was still encapsulated in nano-particles. Then, the concentration reached the peak at 8 h, which may due to the sustained release of sertraline from nanoparticles. And then the drug concentration decreased after 12 h. Thus, it is believed the nanoparticles can create a 4 h window for breastfeeding after the administration of antidepressants. Since most of the sertraline was still encapsulated in the nanoparticles during this period after the drug administration.

Nevertheless, there was still some sertraline released from the nanoparticles in the first 4 h and could be detected in the breast milk. Therefore, it still needs caution for lactating women to have antidepressant drugs during breastfeeding. And more detail studies for the pharmacokinetics in milk are required.

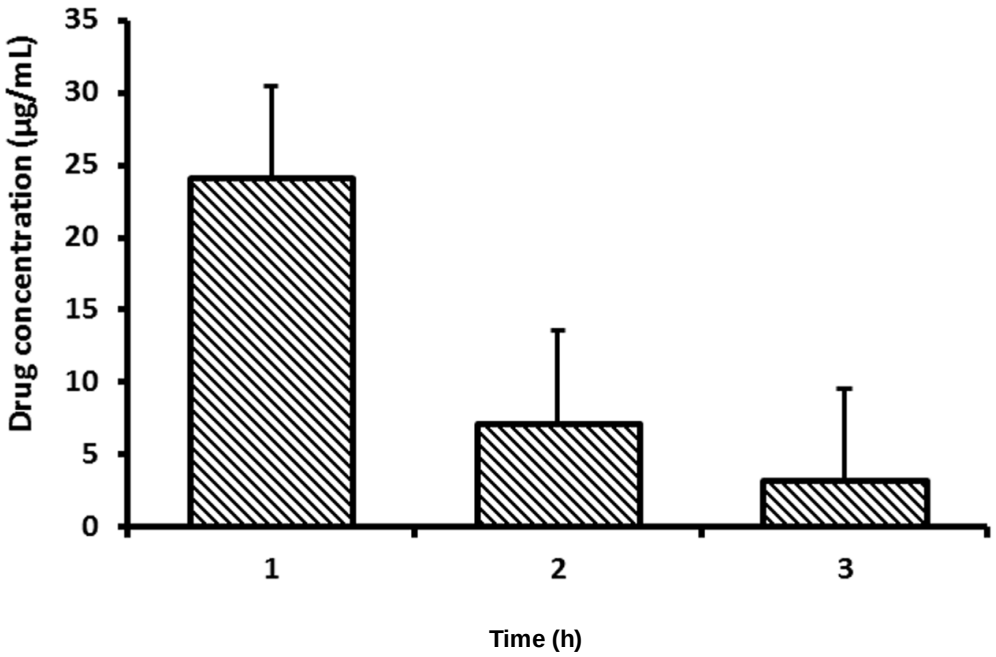

Figure 3. The milk drug concentration after marginal ear intravenous injection of sertraline-loaded nanoparticles in lactation rabbits.

Conclusion

The mass ratio of CS/TPP had an influence on the diameter and entrapment efficiency of the sertraline-loaded nanoparticles. It showed the best size and entrapment efficiency at the ratio of 10. These chitosan nanoparticles carrier could prolong the resistant time of sertraline in plasma and enhance $AUC_{(0-\infty)}$ compared with sertraline solution. This may create a 4 h window for breastfeeding. Nevertheless, the sertraline-loaded nanoparticles should still be used with caution during lactation, as sertraline in milk was still detectable after administrating with the nanoparticles.

Conflict of interest

The authors have not declared any conflict of interest

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