JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 904–910

Received October 20, 2009
Accepted April 11, 2011
DOI
10.1111/j.2042-7158.2011.01295.x
ISSN 0022-3573

Research Paper

Multivesicular liposome (MVL) sustained delivery of a novel synthetic cationic GnRH antagonist for prostate cancer treatment

Tao Wang, Lijun Gao and Dongqin Quan

Beijing Institute of Pharmacology and Toxicology Beijing, China

Abstract

Objectives Multivesicular liposomes (MVLs) are often used as an appropriate carrier for delivering peptides due to high drug loading, relative stability and extended-release behaviour. However, when cationic amphipathic peptides are involved, some challenges may be encountered, including instability of multiple emulsions due to interaction between peptides and lipid membranes (electrostatic and hydrophobic interaction). LXT-101, a cationic amphipathic peptide, is a novel antagonist of gonadotropin-releasing hormone (GnRH) for prostate cancer treatment. The purpose of the current research was to explore simple methods of determining the interaction between peptide and lipid bilayer and to prepare MVLs of LXT-101 (DepoLXT-101) by the modified DepoFoam technique.

Methods The anionic surfactants were added in the process of DepoLXT-101 preparation in order to minimize the effect of instability resulting from cationic peptides.

Key findings DepoLXT-101 was obtained with good efficiency and reproduction. The integrity of encapsulated peptide was maintained as shown by RP-HPLC. DepoLXT-101 particles were characterized by morphology and particle size distribution and in-vitro release was also investigated. The release behaviour *in vitro* in medium of sodium chloride at 37°C showed that 70–90% of LXT-101 was released slowly from MVLparticles over 11 days. According to the fitting results of Ritgar-Pepps model, the in-vitro release of DepoLXT-101 was mainly governed by Fick's diffusion.

Conclusions The data obtained from in-vivo study indicated that a sustained anticancer effect can be achieved over a 7-day period with subcutaneous administration of DepoLXT-101 in rats.

Keywords GnRH antagonist; LXT-101; multivesicular liposome; sustained release

Introduction

More and more biologically active peptides and proteins are being discovered along with the development of genomics and proteomics. Naturally occurring proteins and peptides often possess harmful activity and are not suitable as drugs. Therefore, quite a few synthetic peptides/proteins have been obtained by chemists during past decades.[1–5] Synthetic analogues of gonadotropin-releasing hormone (GnRH),which is a potent drug for sex-hormone-dependent diseases, especially for prostate cancer, have become attractive artificial peptides in recent years.[6–8]

Prostate cancer is the most commonly diagnosed malignancy in men.[9,10] Since the modes of life have changed profoundly such as eating habits, excessive drinking, lacking of motion, the incidence of prostate cancer in China is expected to ascend continuously in the future. It is reported that approximately 80% of human prostatic tumours are androgen dependent and the gold-standard androgen deprivation therapy (ADT) is orchiectomy.[11] As surgical castration is irreversible and has low compliance, a more reversible alternative is chemical castration by a gonadotropin-releasing hormone (GnRH) antagonist or agonist, which reduces the testicular androgen production.[12]

GnRH, also known as luteinizing hormone-releasing hormone (LHRH), is secreted and produced by the hypothalamus, which is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) hormone.[13] Unlike peptide agonists such as leuprorelin (leuprolide), GnRH antagonists act immediately at the receptor, quickly suppressing the release of follicle-stimulating hormone (FSH) and luteotrophic hormone (LH), without a concomitant initial stimulation of hormone ('flare effect'), which tends to initially exacerbate symptoms in patients.

Correspondence: Dongqin Quan, Beijing Institute of Pharmacology and Toxicology Beijing 100850, China.
E-mail: qdqwzb@163.com

Recently, a new compound, LXT-101 (Ac-D-Nal-D-Phe (4-Cl)-D-Pal-Ser- Mop-D-Pal-Leu- Arg-Pro-D-Ala·NH_2), was developed by our institute; it shares a similar structure with GnRH antagonists and might be a potential GnRH antagonist.[14] All our preliminary studies strongly demonstrate that its activity is excellent compared with other GnRH analogues. However, as a peptide, LXT-101 has distinct properties such as easy degradation, instability, low bioavailability and short half-life (4 h in rats). Much attention has been paid to overcoming these limitations and to prolong the biological activity of such peptides in the body. One interesting approach includes using multivesicular liposomes (MVLs) to achieve sustained release of LXT-101.

MVLs are structurally distinct from other types of lipid-based particles. They can encapsulate and deliver both small molecules and macromolecules in a sustained manner from days to several weeks.[15–22] MVLs are characterized by the presence of a continuous outer bilayer with numerous discontinuous aqueous compartments bounded by a continuous, non-concentric network of lipid membrane. Due to their higher aqueous volume-to-lipid ratio and large size compared with unilamellar liposomes (LUVs) and/or multilamellar liposomes (MLVs), these MVLs can act as a high-loading drug depot, providing sustained release of water-soluble drugs delivered from the site of injection to the circulation after subcutaneous administration. They also have a higher mechanical strength and slower clearing rate of tissue macrophages than traditional liposomes.

In this study, the preparation and in-vitro/in-vivo release of MVL-encapsulated LXT-101 were investigated. LXT-101 has four artificial amino acids in the backbone of its molecule and has distinct physicochemical properties, including amphipathicity, low solubility and electropositivity, which lead to its preferentially interacting with lipid film and bring difficulties in the process of the MVLs' preparation, including coalescence of multiple emulsion and drug leakage, which lead to low encapsulation efficiency and poor sustained-release function. Understanding details of the interaction between the drug and lipid bilayer could help us to improve the formulation and preparation technique of MVLs. The effect of interaction between drug and lipid bilayer on the multiple emulsion's stability and MVL preparation has never been published previously. Several simple biophysical methods were applied to determine the interaction between LXT-101 and bilayer in this study and methods to improve the stability of multiple emulsion in the MVL preparation were also investigated. DepoLXT-101 with good reproducibility and high encapsulation efficiency was prepared by a modified Depo-Foam technique. The DepoLXT-101 particles were characterized in terms of size distribution and morphology and release behaviour *in vitro* and *in vivo* was also investigated.

Materials and Methods

Materials

Sodium alginate (MW 48–185 KDa) was purchased from Wenzhou accessory ingredients Co., Ltd (Wenzhou, Zhejiang, P.R. China). Sodium dodecylsulfate (SDS) was obtained from Sigma (Sigma Chemicals, Seezle, Germany). Sodium oleate, Triolein and cholesterol, were kindly gifted from Shanghai Chemical Reagent Co. (Shanghai, P.R., China). L-Lysine was purchased from Beijing Chemical Reagent Co. (Beijing, P.R. China). Polyvinylpyrrolidone (PVP) K-90D and PVPK-30D were supplied by ISP (Frigenstr.4.DE-67065 Ludwigshafen Am Rhein, Germany). Phosphatidylcholine (PC)-rich fraction of egg lecithin with at least 80% PC (EPC, Lipoid E 80) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) were purchased from Lipoid (Ludwigshafen, Germany). LXT-101 was synthesized and isolated by Beijing Institute of Pharmacology and Toxicology (Beijing, P.R. China). Acetonitrile (ACN) was purchased from Fisher Scientific (Fairlawn, USA). Phosphoric acid was from Beijing Chemical Engineering Factory (Beijing, P.R. China). Methanol (MeOH) was supplied by Tianjin Biaoqi advanced science and technology Co. (Tianjin, P.R. China). Acetonitrile and methanol were HPLC grade. All other chemicals were reagent equivalent. All the materials were used without further purification.

Interaction between LXT-101 and lipid membrane

Preparation of small unilamellar liposomes

In this section, blank small unilamellar liposome (SULs) containing the same components as MVLs were produced as a model phospholipid bilayer. The EPC, DPPG, Triolein and cholesterol were dissolved in 10 ml chloroform. After evaporating by a rotary evaporator for 2 h, 20 ml water was added to achieve a suspension of phospholipids. This solution was processed by a probe-type sonicator at 200 W for 30 min, then SULs were obtained.

Zeta potential and particle size

LXT-101 was completely dissolved in water and added stepwise to the SUL solution system. The concentration of LXT-101 was in the range of 0.55–8.88 μmol/l and 20 min equilibration time was allowed before taking measurements. Zeta potential and particle size of SULs were determined by the dynamic light scattering method using a Zetasizer Nano (Malvern, UK) before and after LXT-101 was added to the system. Each measurement was performed in triplicate.

Fluorescence spectrum

The blank SUL solution was added stepwise to LXT-101 solution and the samples, with a ratio of lipid/drug between 53.6 : 1 and 508.6 : 1, were allowed to equilibrate for 60 min in the dark before taking measurements. The fluorescence spectrum (260–440 nm) at an excitation wavelength of 280 nm was carried out on the F-4500 Fluorescence Spectrophotometer (HITACHI, Japan). Each sample was analysed three times.

Fabrication of multivesicular liposome formulation containing LXT-101 (DepoLXT-101)

The MVL particles containing LXT-101 were obtained by a two-step double emulsification method, water-in-oil-in-water (w/o/w), in the presence of emulsifier. Briefly, the first step was to prepare a water-in-oil emulsion. LXT-101 (5 mg) dissolved in the first aqueous solution (1 ml) was emulsified with 1.2 ml chloroform solution containing 15.5 mM DPPC, 20.2 mM cholesterol, 3.1–18.6 mM DPPG and 2.47 mM

triglyceride by ultrasonicator (JY92-11D, Nanjing Xinchen Biotechnology Co. Ltd, Nangjing, Jiangsu, P.R. China). This primary emulsion was then dispersed in a second aqueous solution (5 ml) containing 40–120 mM L-lysine, 5–15% glucose and different emulsifiers, then stirred with vortex mixer (QL-90 vortex; North Tongzheng Biology Development Co., Ltd. Beijing, P.R. China) to produce a w/o/w double emulsion (the second emulsion). Organic solvent was removed by flushing nitrogen over the surface of the mixture at 37–39°C. As an alternative method, chloroform or ethylene chloride was also removed by rotary evaporation under reduced pressure at the same temperature. The resulting DepoLXT-101 particles were harvested by centrifugation at 600*g*, washed and resuspended in isotonic sodium chloride solution. Blank DepoFoam vehicle was also prepared using the same procedure, except the peptide was not included in the first aqueous phase. The DepoLXT-101 particle suspension was stored at 4°C until use.

Characterization of DepoLXT-101 particles

The size distribution of the DepoLXT-101 particles was measured by using coulter counter (Beckman Coulter Multisizer II). The samples were diluted by adding 2 ml of DepoFoam suspension to 50 ml 0.9% sodium chloride and measured the size distribution.

The morphology of the DepoLXT-101suspensions was analysed by a Nicon light microscope (Japan) and the image recorded photographically.

Determination of DepoLXT-101 content

For quantification and characterization of encapsulated LXT-101, the peptide was extracted from MVLs as follows. A 1-ml sample of DepoLXT-101 suspension was centrifuged for 10 min at 600*g*. The supernatant was discarded, the precipitate was dissolved in 100 ml MeOH and assayed by ultraviolet-reverse-phase HPLC equipped with a Zorbax SB-C_{18} column (150 mm × 4.6 μm, 5 μm). Each sample measured in triplicate as described above.

In-vitro release study

For estimation of the release rate of LXT-101 from MVLs *in vitro*, DepoLXT-101 suspension was diluted 10-fold with isotonic sodium chloride preserved with 0.01% sodium azide, and incubated at 37°C under dynamic conditions (gentle rotation at 75 rev/min). At designated times a sample was collected and transferred to a new tube containing fresh sodium chloride. MVL particle pellets and supernatants were separated by centrifugation in an ultracentrifuge at 16 000*g* for 5 min. The amount of peptide remaining in the DepoFoam pellets was then analysed by UV-RP-HPLC after extraction as described above. Resulting data were pooled from 3 independent experiments.

In-vivo study

Pharmacokinetic studies were performed in male Wistar rats to investigate the in-vivo behavior of the MVL-encapsulated LXT-101 (DepoLXT-101) formulation. Rats ($n = 4$) were injected subcutaneously with a single dose of 3.5 mg/kg of either free LXT-101 injection or the DepoLXT-101 formulation in 0.9% NaCl solution. Blood samples (500 μl) were collected from the rats via the eye ground vein at specified time points after administration. Samples were analysed by RP-HPLC to determine the LXT-101 level in plasma. Pharmacokinetic parameters were calculated by Winnonlin software. Our study was approved by the ethical committee of Academy of Military Medical Science of China.

Statistical analysis

Kruskal–Wallis test and Dunn's test in SAS 9.2 were applied to analyse the significance of difference in the formulation study of shelf-life of multiple emulsions in different surfactant group. In-vitro and in-vivo data were analysed by individual models. $P < 0.05$ indicated statistically significant difference.

Results and Discussion

Interaction between LXT-101 and lipid membranes

Zeta potential and particle size

LXT-101 molecules were positively charged (+14.7 mV), while SULs exhibited a high negative charge (−68.8 mV). Zeta potential values of SULs rapidly reduced to −55 mV with increased LXT-101 concentration until a final plateau region was obtained. The concentration range of LXT-101 added was from 0.55 to 8.88 μmol/l. The particle size increased when LXT-101 was added to the SUL solution and the increment 3.15 nm was approximately equal to the transversal length of the LXT-101 molecule. The transversal length of the LXT-101 molecule was calculated as follow: $3 \times \cos\ 25.6° \times R_{C\text{-}C} + 2 \times \cos\ 29.5° \times R_{C\text{-}N} + R_N = 3 \times \cos\ 25.6° \times 154\ \text{pm} + 2 \times \cos\ 29.5° \times 152\ \text{pm} + 150\ \text{pm} = 832.1\ \text{pm}$, where R_N is the Van der Waals radius of the last N atom.

The positive charges of LXT-101 facilitate the electrostatic interaction with the negatively charged head groups of lipids and drug adsorption on the membrane can proceed even at low concentrations. The Gibbs free energy change, ΔG, in this process was determined by the following equation: $\Delta G = \Delta H - T\Delta S$, where ΔH is enthalpy of absorbance, T is the absolute temperature and ΔS is entropy of adsorption. Herein ΔS is given by $\Delta S = \Delta S_H + \Delta S_w$, where ΔS_H is the entropy change of hydrophilic polarity head adsorbed peptide molecules and ΔS_w is the entropy change of water molecules replaced by LXT-101 molecule. The term ΔH is always negative because the electrostatic adsorption process occurs spontaneously. Therefore the sign of ΔG depends on ΔS, the entropy of adsorption, which includes two parts – unfavourable entropic (ΔS_H) contributions and larger favourable entropic (ΔS_w) contributions. A negative value of ΔS_H comes from the lesser degree of freedom due to polarity head connection to the LXT-101 molecules. On the other hand, a greater positive value of ΔS_w is very likely obtained. Therefore, free energy change trends to be negative and electrostatic adsorption between LXT-101 and hydrophilic polarity surface is spontaneous in thermodynamics. Experimental data of zeta potential, as a consequence of electrostatic interaction represent LXT-101 molecules adsorbed on hydrophilic polar head of phospholipid films and the final plateau region may be elucidated by mass balance relating the adsorption.[23,24]

Furthermore, according to the particle size results, a monomolecular adsorption layer was formed on the surface of SUL particles when the adsorption was at equilibrium.

Fluorescence spectrum

The λmax of each sample was constant with the molar ratio of lipid/drug in the range between 53.6 : 1 and 508 : 1 and in accordance with the λmax (340 nm) of free LXT-101 solution. In general, the emission maxima display blue shifts when molecules are in a more hydrophobic environment.[25] If peptide molecules insert into the interior region of the phospholipid bilayer, fluorescence emission maximum will move to a shorter wavelength when liposome is added to peptide solution and the extent of shift becomes stronger along with liposome addition. According to the experimental data, unchanged emission maxima were observed in these spectra, implying that all the adsorbed molecules can not insert into the phospholipid film and the hydrophobic interaction between LXT-101 and phospholipid molecules was not strong. It is worth noting that EPC mixed with cholesterol can stabilize the lipid membrane and make insertion of LXT-101 molecules difficult.[26] Moreover, steric hindrance of LXT-101 molecules may also reduce the hydrophobic interaction. Two main conclusions can be extracted from these tests: (i) it is possible to prepare MVLs containing LXT-101 (DepoLXT-101) because of the absence of a strong hydrophobic interaction between LXT-101 and phospholipid molecules; (ii) LXT-101 molecules will be adsorbed on the o/w interface and affect the surface charge, which may decrease the stability of w/o/w multiple emulsions. To resolve the thermodynamic instability of multiple emulsions, anionic surfactants may be required in the preparation process.

Fabrication of multivesicular liposome formulations containing LXT-101 (DepoLXT-101) formation

The shelf life of multiple emulsions containing different stabilizers in the second aqueous solution was also determined. The shelf life was defined as the time from formation to creaming taking place. The results indicated that anionic surfactants, including SDS, sodium oleate and sodium alginate can be selected as appropriate stabilizers of multiple emulsions to produce longer shelf life (>600 s). The minimum amount of these stabilizers in the formulations was determined by several experiments (data was not shown).The multiple emulsions containing either non-ionic surfactants or polymer surfactants were very unstable, and all the shelf lives were below 60 s when the surfactants were used in high concentration. Compared with other stabilizers, multiple emulsions containing anionic surfactants had the longest shelf life ($P < 0.05$). This phenomenon can be explained by increasing electrostatic repulsion between emulsion particles stabilizing the emulsion. However, Table 1 shows that the stable multiple emulsions can not be obtained until the amounts of electrostatic stabilizer (DPPG) and pH moderator (L-lysine) added to the second aqueous phase were very great. Therefore, the function of anionic surfactants was not only to supply the electrostatic repulsion between emulsion particles, but also steric repulsion. Table 2 depicts the effect of glucose concentration on encapsulation efficient (EE) of DepoLXT-101. The technique of rotary evaporation under reduced pressure was better than flushing nitrogen over multiple emulsions in the DepoLXT-101 preparation.

Table 1 Effect of DPPG and L-lysine on stability of multiple emulsions[a]

DPPG (mmol/l)	Shelf life of multiple emulsion (s)[b]		
	L-Lysine concentration (mmol/l)		
	40	80	120
3.1	<3	<3	<3
6.2	<10	<8	<13
15.5	<14	<13	<12
18.6	<20	<20	<30

[a]No surfactant was added. [b]$n = 3$

Table 2 Effect of glucose concentration and solvent removal technique on encapsulation efficiency[a]

Glucose concentration (%)	%EE of DepoLXT-101[c]	
	Rotary evaporation	Flushing nitrogen the surface
5	32.1* (1.2)[b]	13.1 (1.1)
10	48.9* (1.5)	16.2 (1.8)
12.5	42.7* (1.0)	14.6 (1.1)

[a]SDS was used as emulsifier. [b]Mean and SD. *$P < 0.05$ vs other group, $n = 3$. [c]Drug content was determined by RP-HPLC, mobile phase: 43% acetonitrile /0.2% trifluoroacetic acid /0.2% triethylamine, UV detection: 225 nm, flow rate: 1.0 ml/min, injection volume: 20 µl.

Typically, the double-emulsion method enables entrapping drug into DepoFoam particles. The stable primary emulsion can be prepared by vigorous dispersion conditions. The phospholipid was not only used as membrane material but also an emulsifier in the process.

In a previous report, L-lysine was added to the second aqueous phase as a very important adjuvant,[15,20,22] but the effect of L-lysine in MVL preparation was not studied completely. We found that the effect of L-lysine strongly depended on the pH value of the second aqueous solution. The pH range of 8.5–9.0 was used to prepare multiple emulsion successfully, possibly because L-lysine was negatively charged in the alkali solution and may stabilize the multiple emulsion by increasing the surface charge density of emulsions drops.

Moreover, it was found that multiple emulsion containing the cationic peptide LXT-101 was unstable when prepared by the traditional DepoFoam method and coalescence, even complete phase separation, usually took place within a few seconds. It is possible to assume that both amphiphilicity and charged property of LXT-101 leads to the instability of the multiple emulsion. In such a case, anionic surfactants as stabilizers may be required in the processes of multiple emulsion preparation to form an adsorbed layer at the oil–water interface, this barrier may be both electrostatic and steric in nature. The stabilizing role of the anionic surfactant molecules can be explained by their effect on surface forces (e.g. by modifying

the electrical potential of the drop surface and interface tension or by creating a steric barrier preventing drop–drop coalescence during emulsification). From the results, it was shown that non-ionic surfactants (e.g. Tween-80), electrostatic stabilizers (e.g. DPPG) and steric stabilizer (e.g. PVP) added in the second aqueous solution can not form stable multiple emulsions.

Characterization of DepoLXT-101 particle

Figure 1a shows a light micrograph of a representative DepoFoam formulation containing LXT-101. The picture illustrates the spherical, smooth and multivesicular characteristics of DepoLXT-101 particles. All the DepoLXT-101 formulations studied had similar morphology. Figure 1b shows a volume-weighted size distribution profile of DepoLXT-101 particles calculated by coulter counter. The profile shows that the three formulations of DepoLXT-101 had narrow and monomial distribution of the particles with mean size of 8.3, 8.8 and 12.1 μm, respectively. Over 95% of the DepoLXT-101 particles were in the size range of 5–20 μm and no particles <1 or >100 μm in size were observed.

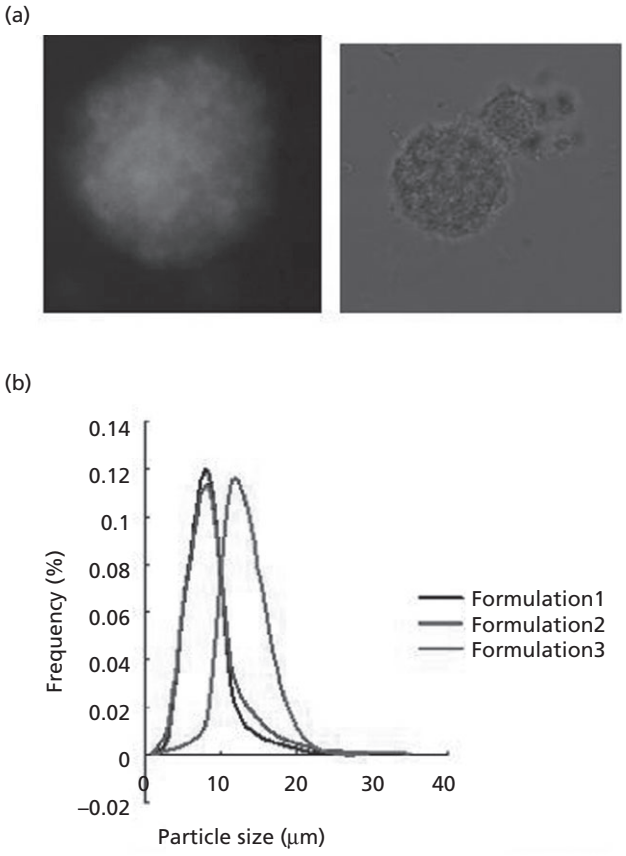

Figure 1 (a) Light micrograph. The right image shows green-fluorescent LXT-101. Surface structure of the particle was observed, illustrating that the particles bounded by LXT-101molecules adsorbed lipid bilayer. This is in good agreement with results observed in the potential and fluorescence spectrum test. (b) Size distribution of DepoLXT-101: formulation 1 (sodium oleate); formulation 2 (SDS); formulation 3 (sodium alginate).

Measurement of LXT-101content in DepoLXT-101

The DepoLXT-101 formulation using a concentration of 5 mg/ml in the first aqueous solution typically had drug loading between 1.66 and 2.58 mg/ml and <1% (w/v) of free peptide present in the DepoFoam suspension.

In-vitro release study

An evaluation of release rate *in vitro* from three DepoLXT-101formulations was performed in saline solution at 37°C (Figure 2) . For these formulations, greater than 70% of encapsulated LXT-101 was released in a sustained manner over 11 days. The in-vitro data showed that the burst release from DepoLXT-101 formulation 1 was lower (~20%); formulations 2 and 3 showed a little faster release. The Ritgar-Pepps model was used to fit the release. The equation for the Ritgar-Pepps model was described as follows: $\ln P = C + k\ln t$, where P is release fraction and t is time. C and k represent the intercept and slope, respectively, of the lnP–lnt straight line; k is used to classify the type of release mechanism. When $k \leq 0.45$, drug release is mainly decided by diffusion and when $k \geq 0.89$, erosion is the primary mechanism of the release process. When the k-value is between 0.45 and 0.89, the release is controlled by two type of mechanism in common. The k-values for three formulations were 0.56, 0.38 and 0.32, and corresponding correlation coefficient, r, were 0.9899, 0.9740 and 0.9902, respectively. Therefore, the mechanism of LXT-101 release from MVLs can be interpreted by Fick's diffusion. Membrane of MVLs was broken at 37°C under dynamic conditions and LXT-101 was released via Fick's diffusion passing bilayer hole until MVL particles were degraded completely.

In-vivo study

Figure 3 shows the pharmacokinetic profile obtained for DepoLXT-101 and LXT-101 solution formulations in rat plasma. The mean residence time (MRT) for two formulations of DepoLXT-101and LXT-101 solution with the same dose was 4.6 h and 3.7 days, respectively; AUC was 17 152.7 and 37 470 h ng/ml and C_{max} was 3799.7 and 427.0 ng/ml, respectively. LXT-101 from MVLs exhibited about 2.2-fold increase in bioavailability and 19.3-fold increase in MRT compared with the normal solution. According to our pharmacokinetic

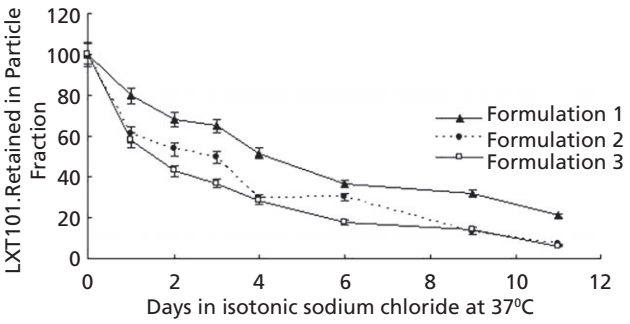

Figure 2 In-vitro release characteristics of DepoLXT-101: formulation 1 (sodium oleate); formulation 2 (SDS); formulation 3 (sodium alginate). The percentage of drug retained in the particle fractions over time was measured by RP-HPLC, and normalized to time 0.

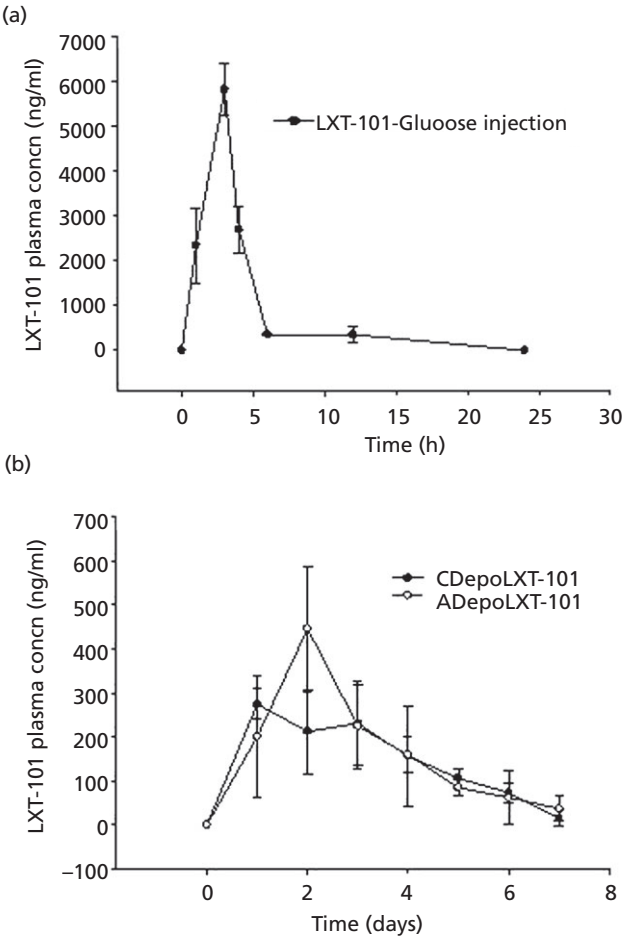

Figure 3 Plasma concentration–time profile of LXT-101 from DepoLXT-101 formulations (a) and LXT-101-glucose solution formulations (b) in rats. Each value represents the mean ± SD ($n = 4$).

study, when the blood concentration of LXT-101 reached 5 ng/ml, it produced a dramatic fall in testosterone to castration level. The data indicated that a sustained suppression of testosterone level in rats was achieved and the anti-cancer effect was maintained over a period of seven days after DepoLXT-101 administration.

Conclusions

Simple methods of using zeta potential and fluorescence spectrum were explored to determine the interaction between peptide and lipid bilayer. DepoLXT-101 by modified method of adding anionic surfactants was obtained with good efficiency and characteristics. The integrity of encapsulated peptide was maintained and 70–90% of LXT-101 was released slowly from DepoLXT-101 over 11 days in medium of sodium chloride at 37°C *in vitro*. According to fitting results of Ritgar-Pepps model, the in-vitro release of DepoLXT-101 was mainly decided by Fick's diffusion. The data obtained from the in-vivo study indicate that a sustained anti-cancer effect can be achieved over a 7-day period with DepoLXT-101.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgements

We acknowledge Fan Fen of Beijing Institute of Biotechnology for his assistance in morphological analysis. We thank Hezhu Liu, Man Ge, Senhao Li and Nan Zhang for helpful discussions and technical assistance. The authors are grateful to Prof Keliang Liu for his kindly supply of LXT-101.

References

1. Toss A *et al*. Amphipathic, alpha-helical antimicrobialpeptides. *Biopolymers* 2000; 55: 4–30.
2. Brogden KA *et al*. Antimicrobial peptides in animals and their role in host defences. *Int J Antimicrob Agents* 2003; 22: 465–478.
3. Bulet P *et al*. Antimicrobial peptides in insects; structure and function. *Dev Comp Immunol* 1999; 23: 329–344.
4. Smet KD, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett* 2005; 27: 1337–1347.
5. Lanlan Y *et al*. Interaction of an artificial antimicrobial peptide with lipid membranes. *Biochim Biophys Acta* 2009; 1788: 333–344.
6. Stangelberger A *et al*. New treatment approaches for prostate cancer based on peptide analogues. *Eur Urol* 2008; 53: 890–900.
7. Schwach G *et al*. Biodegradable microparticles for sustained release of a new GnRH antagonist – part I: screening commercial PLGA and formulation technologies. *Eur J Pharm Biopharm* 2003; 56: 327–336.
8. Padula AM. GnRH analogues – agonists and antagonists. *Anim Reprod Sci* 2005; 88: 115–126.
9. Rittmaster RS *et al*. pharmacological approaches to reducing the risk of prostate cancer. *Eur Urol* 2009; 55: 1064–1074.
10. Jagusch C *et al*. Synthesis, biological evaluation and molecular modelling studies of methyleneimidazole substituted biaryls as inhibitors of human 17a-hydroxylase-17, 20-lyase (CYP17). Part I: heterocyclic modifications of the core structure. *Bioorg Med Chem* 2008; 16: 1992–2010.
11. Poppel HV *et al*. Degarelix: a novel gonadotropin-releasing hormone (GnRH) receptor blocker – results from a 1-yr, multicentre, randomised, phase 2 dosage-finding study in the treatment of prostate cancer. *Eur Urol* 2008; 54: 805–815.
12. Nobes JP *et al*. Metabolic syndrome and prostate cancer: a review. *Clin Oncol* 2009; 21: 183–191.
13. Lanier MC *et al*. Selection, synthesis, and structure–activity relationship of tetrahydropyrido[4,3-d]pyrimidine-2,4-diones as human GnRH receptor antagonists. *Bioorg Med Chem* 2007; 15: 5590–5603.
14. Chi XL *et al*. In vivo characterization of a novel GnRH (gonadotropin-releasing hormone)antagonist, LXT-101, in normal male rat. *Regul Pept* 2006; 136: 122–129.
15. Ye Q *et al*. DepoFoam^{TE} technology: a vehicle for controlled delivery of protein and peptide drugs. *J Controlled Release* 2000; 64: 155–166.

16. Dai CY *et al*. Preparation and characterization of liposomes-in-alginate (LIA) for protein delivery system. *Colloids Surf B* 2006; 47: 205–210.
17. Grayson LS *et al*. Pharmacokinetics of DepoFoam gentamicin delivery system and effect on soft tissue infection. *J Surg Res* 1993; 55: 559–564.
18. Katre NV *et al*. Multivesicular liposome (DepoFoam) technology for the sustained delivery of insulin-like growth factor-I (IGF-I). *J Pharm Sci* 1998; 87: 1341–1346.
19. Ramprasad MP *et al*. The sustained granulopoietic effect of progenipoietin encapsulated in multivesicular liposomes. *Int J Pharm* 2003; 261: 93–103.
20. Ramprasad MP *et al*. Sustained-delivery of an apolipoproteinE-peptidomimetic using multivesicular liposomes lower serum cholesterol levels. *J Controlled Release* 2002; 79: 207–218.
21. Kim S *et al*. Preparation of multivesicular liposomes. *Biochim Biophys Acta* 1983; 728: 339–348.
22. Howell SB. Clinical applications of a novel sustained-release injectable drug delivery system: DepoFoam technology. *Cancer J* 2001; 7: 219–227.
23. Zhao LY *et al*. Investigation of molecular interactions between paclitaxel and DPPC by Langmuir film balance and differential scanningcalorimetry. *J Pharm Sci* 2004; 93: 86–98.
24. Widengren J, Rigler R. Fluorescence correlation spectroscopy as a tool to investigate chemical reactions in solutions and on cell surfaces. *Cell Mol Biol* 1998; 44: 857–879.
25. Jorgensen L *et al*. Preparing and evaluating delivery systems for proteins. *Eur J Pharm Sci* 2006; 29: 174–182.
26. Wydro P, Haüc-Wydro K. Thermodynamic description of the interactions between lipids in ternary Langmuir monolayers: the study of cholesterol distribution in membranes. *J Phys Chem B* 2007; 111: 2495–2502.