Department of Pharmaceutical, China Pharmaceutical University, Nanjing, China

Novel solid lipid nanoparticles as carriers for oral administration of insulin

ZHENHAI ZHANG, HUIXIA LV, JIANPING ZHOU

Received February 11, 2009, accepted March 9, 2009

Prof. Jianping Zhou, Department of Pharmaceutical, China Pharmaceutical University, No. 24 Tongjia Xiang, Nanjing 210009, China
zhoujpcpu@yahoo.cn, lvhuixia@163.com

Pharmazie 64: 574–578 (2009) *doi: 10.1691/ph.2009.9051*

The aim of this study was to propose novel solid lipid nanoparticles (SLNs) loaded with cell-penetrating peptide (CPP), and to evaluate their potential for oral administration of insulin. Octaarginine (R_8) was used as the CPP. SLNs loaded with insulin and R_8 (R_8-Ins-SLNs) were prepared using the spontaneous emulsion solvent diffusion method. The morphological study of R_8-Ins-SLNs showed its spherical shape using transmission electron microscopy (TEM) photography. The mean particle size, zeta potential, encapsulation efficiency (EE %) of insulin and R_8 were 150.8 ± 23.4 nm, 32.65 ± 2.02 mV, 62.29 ± 0.52% and 58.05 ± 0.66%, respectively. The *in vitro* release of insulin or R_8 from R_8-Ins-SLNs was characterized by an initial rapid release and subsequent sustained release in pH 6.8 dissolution media. *In vivo* absorption experiments provided a relative pharmacological bioavailability (PA%) value of R_8-Ins-SLN of 10.39 ± 0.46%. These results suggest that SLNs loaded with CPP could be a promising perioral carrier for insulin.

1. Introduction

Oral delivery of insulin remains an attractive alternative to parenteral delivery and has challenged various attempts at delivery development (Owens et al. 2003). However, successful oral delivery of insulin is difficult to achieve. Due to its high molecular mass, charge and hydrophilic insulin hardly passes the intestinal membranes. Moreover, as a peptide, insulin is susceptible to proteolytic degradation by different gastrointestinal enzymes on the gastric and intestinal mucosa (Morishita and Peppas 2006).

Among the possible strategies of oral delivery of insulin, solid lipid nanoparticles (SLNs) represent a promising approach (Müller and Keck 2004). An obvious advantage of SLNs is that their lipid matrix is composed of physiological compatible lipids, which minimizes the risk of acute and chronic toxicity (Mehnert and Mader 2001). In addition, SLNs can protect encapsulated insulin against enzymatic degradation in the gastrointestinal mucosa (Morishita and Peppas 2006). Furthermore, the carrier itself can be taken up to a certain extent by epithelial cells or the lymphoid tissues in Peyer's patches (Garcia-Fuentes et al. 2002).

Despite the great promise of SLNs as carriers for oral administration of insulin, its ability to penetrate the mucosa is very limited. The mucus and cellular layers represent significant barriers to the absorption of nanoparticles through the gastrointestinal tract (Norris et al. 1998). It has been reported that SLNs containing insulin significantly improved the absorption of insulin, but have not yet achieved adequate blood insulin concentrations (Cui et al. 2006; Zhang et al. 2006). Hence, incorporating new tools into SLNs, which increase its membrane permeability, is essential to attain high oral insulin bioavailability.

In recent years, the development of short peptides, which can cross biological membranes of a variety of cell types, has thrown some promising light on improving nanoparticles' ability to penetrate cell membranes (Zorko and Langel 2005). These peptides are generally known as cell penetrating peptides (CPPs). CPPs may be classified into three groups: basic peptides such as TAT peptide and octaarginine (R_8), basic/amphiphilic peptides such as penetratin, and hydrophobic peptides such as membrane translocating sequence (MTS) (Futaki et al. 2003). The importance of CPPs in drug delivery lies mainly in their ability to efficiently carry various loadings such as macromolecules or liposomes into the cells (Trehin and Merkle 2004; Torchilin 2008). Other advantages include their general inertness, low toxicity, and low immunogenicity (Zorko and Langel 2005; Chung et al. 2008; Torchilin 2008). CPPs are expected to become powerful tools for overcoming the insufficient permeability of macromolecules and particulate carrier systems through epithelial cell membranes.

Up to date, no reports have been concerned with incorporation of cell penetrating peptide (CPP) into SLNs for improving the oral delivery of insulin. In this study, R_8 was used as a CPP. SLNs loaded with insulin and R_8 (R_8-Ins-SLNs) were prepared using the spontaneous emulsion solvent diffusion method. The properties of R_8-Ins-SLNs, including morphology, particle size, zeta potential, encapsulation efficiency (EE %) of both insulin and R_8 and *in vitro* release behavior were determined. SLNs containing insulin without R_8 (Ins-SLNs) were also prepared and investigated using the same method. In comparison with Ins-SLNs, we evaluated whether R_8-Ins-SLNs can improve intestinal absorption of insulin in rats.

2. Investigations, results and discussion

2.1. Preparation of R_8-Ins-SLNs and Ins-SLNs

R_8-Ins-SLNs were prepared using the spontaneous emulsion solvent diffusion method (Siekmann and Westesen 1996; Kawashima et al. 1998; Zhang et al. 2006). We carefully investigated the formulation factors and preparation conditions, and found that the weight ratio of the stearic acid: insulin had significant effect on the EE% of insulin and R_8. An increase in the relative amount of stearic acid to insulin resulted in an increase in the EE% of insulin and R_8. When the ratio reached 20 : 1, the highest EE% of insulin and R_8 had been achieved and even higher ratio would no longer increase them. Soybean phospholipids concentration in the oily phase affected the size distribution of nanoparticles. The appropriate concentration range of soybean phospholipids was 1.6–2.2 mg/ml, because below 1.6 mg/ml the SLNs average diameter was larger than 200 nm, and above 2.2 mg/ml the SLNs polydispersity index (PDI) increased. Furthermore, the amount of R_8 was limited to 4.5 mg in formulation. The higher R_8 amount did not increase its content in the SLNs, inversely lowered its EE%. In addition, because insulin and R_8 were thermolabile, the power and duration of ultrasonication needed to be carefully controlled and 50 W for 15 s were the optimal condition. Meanwhile other influential factors such as the concentration of poloxamer 188, volume of inner aqueous phase and volume of oily phase were investigated. The result showed that they contribute little to the properties of SLNs such as PDI, EE% of insulin and R_8 and particle size.

For comparing with R_8-Ins-SLNs, Ins-SLNs were prepared using the same method as for R_8-Ins-SLNs.

2.2. Physicochemical characterizations of R_8-Ins-SLNs and Ins-SLNs

R8-Ins-SLNs were spherical and uniform in transmission electron microscopy (TEM) photography as shown in Fig. 1. Both R_8-Ins-SLNs and Ins-SLNs exhibited small particle size and narrow size distribution (Table 1). The mean particle size and PDI of R8-Ins-SLNs were 150.8 ± 23.4 nm and 0.206 ± 0.023, respectively. In comparison with the particle size of Ins-SLNs, that of R_8-Ins-SLNs was slightly larger.

Measurement of zeta potential was required to assess the properties of charged particles. In general, nanoparticles could form a stable dispersion when the absolute value of zeta potential was above 30 mV due to the electric repulsion between particles (Komatsu et al. 1995; Müller et al. 2001). As shown in Table 1, the average value of zeta potential of R_8-Ins-SLNs was 32 mV. This demonstrated that the nanoparticles obtained in this study were a dynamic stable system. Because under neutral pH conditions, stearic acid and soybean phospholipids were slightly negatively charged, the value of zeta potential of Ins-SLNs was negative. In contrast, the zeta potential of R_8-Ins-SLNs was positive and the result could be attributed to the dense positive charge of R_8 that was in excess enough to neutralize the negative charge of stearic acid and soybean phospholipids. The positive value of zeta potential of R_8-Ins-SLNs was the evidence that a certain amount of R_8 molecule or cationic group of R_8 molecule were exposed on the SLNs surface.

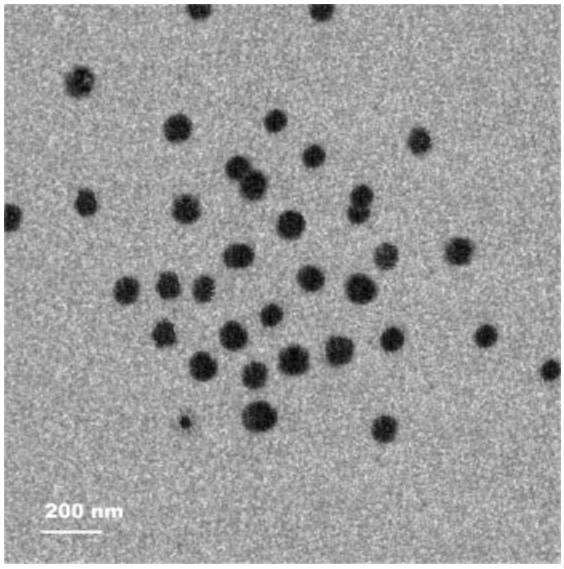

Fig. 1: Transmission electron micrographs of R_8-Ins-SLNs ($\times$ 40,000)

As shown in Table 1, the EE% of insulin in Ins-SLNs was 64% and that in R_8-Ins-SLNs was 62% which was satisfactory. Surprisingly, R_8 EE% in R_8-Ins-SLNs reached 58%. Hydrophilic R_8 was effectively encapsulated into SLNs and the possible explanation for this phenomenon was the electrostatic interactions between densely positively charged R_8 and negatively charged lipid such as stearic acid.

Both R_8-Ins-SLNs and Ins-SLNs exhibited the fine particle size, absolute value above 30 mV of zeta potential and high EE% of insulin and R_8. Therefore, stable and highly dispersible nanometric dispersion could be prepared using the spontaneous emulsion solvent diffusion method.

2.3. In vitro release

The in vitro release profiles of insulin or R_8 from R_8-Ins-SLNs and Ins-SLNs are shown in Fig. 2. For R_8-Ins-SLNs, a biphasic release pattern was observed, which was a rapid release of insulin and R_8 at the initial stage and followed by sustained release. No burst effect was observed for the release of insulin or R_8 at the initial stage. The cumulative release amount of insulin or R_8 from SLNs was no more than 60% at 8 h and no more than 80% at 24 h. The result suggested that the majority of insulin or R_8 was incorporated in the lipid core of SLNs. The insulin release pattern in Ins-SLNs was similar with it in R_8-Ins-SLNs, but the insulin release rate in Ins-SLNs was slightly slower than in R_8-Ins-SLNs.

Table 1: Average diameter, PDI, zeta potential and EE of SLNs (mean $\pm$ S.D., n = 3)

Formulation	Average diameter (nm)	PDI	Zeta potential (mV)	Drug EE (%)	R_8 EE (%)
Ins-SLNs	126.2 ± 20.5	0.173 ± 0.019	-35.41 ± 1.87	64.13 ± 0.30	–
R_8-Ins-SLNs	150.8 ± 23.4	0.206 ± 0.023	32.65 ± 2.02	62.29 ± 0.52	58.05 ± 0.66

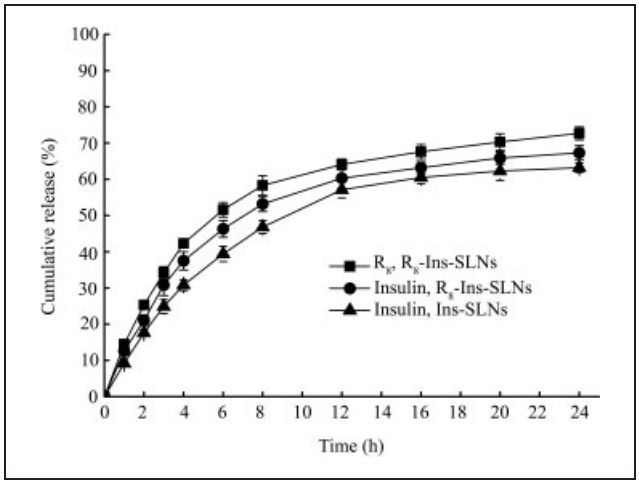

Fig. 2: *In vitro* release profiles of insulin and R_8 from SLNs in the simulated intestinal medium (pH 6.8) (n = 3)

2.4. *In vivo absorption experiments*

In order to confirm the potential use of the incorporation of R_8 into SLNs for oral insulin delivery, the pharmacological effects were evaluated in rats. Figure 3 show the plasma glucose level-time profiles following administration of different insulin formulations to healthy rats. For the control group, after oral administration of insulin solution (20 IU/kg), the blood glucose level decreased only slightly at 12 h, confirming the well-known inefficiency of oral insulin solution. For the R_8-Ins-SLNs group, after oral administration of R_8-Ins-SLNs equivalent to insulin 20 IU/kg, the blood glucose levels decreased to 89% of the initial level at 0.25 h, and reached maximum hypoglycemic effect at 3 h (36 % of the initial level) and continued up to 12 h (90% of the initial level). Comparatively, the blood glucose levels of the Ins-SLNs group decreased to 93.6% of the initial level at 0.25 h, reached the maximum hypoglycemic effect at 4 h (57 % of the initial level) and continued up to 12 h (92.1% of the initial level). The blood glucose levels obtained following oral administration of R_8-Ins-SLNs were significantly lower ($P < 0.05$) than that of obtaining from Ins-SLNs at 0.5–12 h, and those from insulin solution at all the time.

By comparing the area above the hypoglycemic curve versus time profile (AAC) obtained following oral insulin formulations with that of subcutaneous injection of insulin solution, the relative pharmacological bioavailability (PA%) of the oral insulin formulations was determined and the results were shown in Table 2. The PA% obtained following oral administration of insulin solution, Ins-SLNs and R_8-Ins-SLNs was $0.05 \pm 0.02\%$, $5.66 \pm 0.53\%$ and $10.39 \pm 0.46\%$, respectively. The PA% obtained following oral administration of R_8-Ins-SLNs was significantly higher ($P < 0.01$) than that of Ins-SLNs or insulin solution. The PA% of oral insulin solution, and Ins-SLNs was very close to those of other studies published (Zhang et al. 2006). It is clear that compared with Ins-SLNs, R_8-Ins-SLNs shows improvement to the oral delivery of insulin.

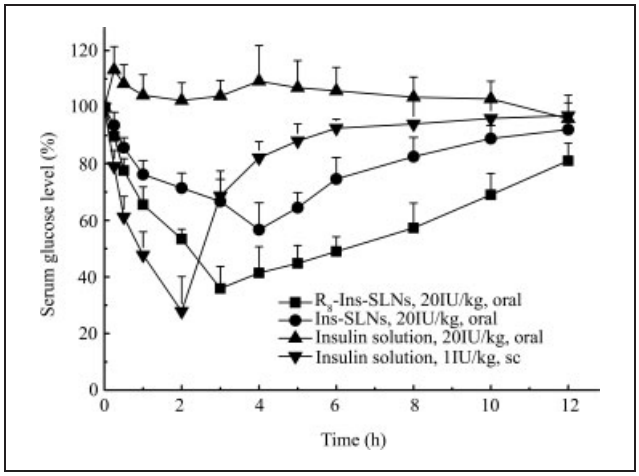

Fig. 3: Serum glucose level versus time profiles after administration of various formulations to rats (mean ± S.D., n = 6)

Table 2: Pharmacokinetic parameters after administration of various formulations to rats (mean ± S.D., n = 6)

Formulation	AAC (% glu. reduc. h)	PA (%)
Insulin solution, sc., 1 IU/kg	238.06 ± 25.60	100
Insulin solution, oral, 20 IU/kg	2.34 ± 1.17	0.05 ± 0.02
Ins-SLNs, oral, 20 IU/kg	269.29 ± 25.62	5.66 ± 0.53
R_8-Ins-SLNs, oral, 20 IU/kg	494.72 ± 22.08	10.39 ± 0.46

During the past decade, many researches have indicated that CPPs could cross cell membranes in a receptor independent manner (Trehin and Merkle 2004; Torchilin 2008). The uptake mechanism of CPPs are still unsettled and controversial, since a range of mechanistic pathways such as conventional endocytosis, involvement of inverted micelles, caveolae, macropinocytosis, formation of ion pair complexes, and others like interactions with cell surface heparin sulfate proteoglycans have been proposed (Zorko and Langel 2005; Chung et al. 2008). Despite the lack of a clear understanding of the uptake mechanisms, CPPs such as TAT peptide, penetratin and octaarginine have been found to be a good enhancer for delivering macromolecular drugs into cells or tissues by covalent linkage to these drugs (Derossi et al. 1994; Fawell et al. 1994; Kosuge et al. 2008; Wender et al. 2000). Although these covalent CPP strategies may be useful, this technology was limited from the chemical point of view, as it was based on a synthetic covalent linkage between CPP and these drugs, which have a big risk of altering the biological activity of the latter (Meade and Dowdy 2007). In order to offer an alternative to the covalent strategies, we have proposed novel SLNs loaded with CPP. In this study, a typical CPP, R_8 was effectively encapsulated into SLNs without covalent linkage to insulin and succeeded in improving oral insulin delivery. In this strategy, the encapsulated insulin and R_8 can be protected against enzymatic degradation in the harsh environment of gut, and the R_8 molecule or cationic groups of R_8 molecules exposed on the surface of SLNs could be responsible to the improvement of the penetration of SLNs through the epithelial mucosa of the gastrointestinal tract. The average value of zeta potential of R_8-Ins-SLNs was positive and the result demonstrated that a certain amount of R_8 molecules or cationic group of R_8 molecules was exposed on the SLNs surface.

As presented here, R_8-Ins-SLNs represent a powerful tool for enhancing oral insulin absorption. The absorption enhancement of R_8-Ins-SLNs may be dependent on the affinity of the R_8 to SLNs, R_8 content in SLNs, and R_8 distribution characters in SLNs such as R_8 concentration on the surface. Hence, further study is needed to clarify the enhancement mechanism.

In our experiment, the PA of R_8-INS-SLNs in rats is still lower than the requirement in clinical application (15%)

(Cui et al. 2006). Some pharmaceutical companies are willing to introduce protein-delivery formulations with bioavailability as low as 10 %. However, for oral insulin delivery, a much higher value might be necessary. Utilization of more effective CPPs might further increase the bioavailability.

3. Experimental

3.1. Materials

Porcine insulin (27.5 IU/mg) was purchased from XuzhouWanbang Biochemical Pharmaceutical Co., Ltd., (China). R_8 (RRRRRRRR, R: L-arginine) was purchased from Beijing SciLight Biotechnology Co., Ltd., (China). Soybean phospholipids were purchased from Shanghai Taiwei Pharmaceutical Co., Ltd., (China). Stearic acid was purchased from Shanghai Chemical Reagent Co., Ltd., (China). Poloxamer 188 was purchased from Nanjing Will Chemical Co., Ltd., (China).All other chemicals were of analytical grade and commercially available.

3.2. Preparation of SLNs

Preparation of SLNs loaded with insulin and R_8 (R_8-Ins-SLNs): The R_8-Ins-SLNs were prepared using the spontaneous emulsion solvent diffusion method. 5 mg (0.86 μmol) insulin and 4.5 mg (3.55 μmol) R_8 were dissolved in 1 ml 0.01 M hydrochloric acid (inner aqueous phase). Then it was added to a 5 ml acetone solution containing 100 mg of stearic acid and 10 mg soybean phospholipids(oily phase). The resulting mixtures were dispersed at 50 W for 15 s with an ultrasonic probe (JY92-II ultrasonic processor, Ningbo Scientz Biotechnology Co., Ltd., China) leading to W/O emulsions, then the emulsions were poured into 50 ml 1.6% poloxamer 188 solution under continuous stirring using (RW20digital agitator, Shanghai Renhe Scientific Instrument Co., Ltd., China) with 800 rpm for 6 h in order to form SLNs. The entire dispersed system was centrifuged at 14,000 rpm for 15 min at 4 °C using (L8−60M, Beckman, USA). The precipitation was washed two times with distilled water to remove free insulin, R_8 and poloxamer 188. Then nanoparticles were dispersed with appropriate distilled water.
Preparation of SLNs containing insulin without R_8 (Ins-SLNs): Ins-SLNs were prepared by the method described above.

3.3. Transmission electron microscopy (TEM)

The morphology of R_8-Ins-SLNs was examined by TEM (H-7650, Hitachi, Japan). Samples were appropriately diluted with distilled water and stained with 2% (w/v) phosphotungstic acid for observation.

3.4. Particle size and zeta potential measurement

The average diameter and polydispersity index (PDI) of particles of R_8-Ins-SLNs and Ins-SLNs were measured by photon correlation spectroscopy (PCS) (Zetasizer 3000HSA, Malvern instruments Ltd., UK). The zeta potential of particles of R_8-Ins-SLNs and Ins-SLNs were measured by Malvern zetasizer Nano ZS90 (Malvern instruments Ltd., UK). Each sample was diluted with distilled water until the appropriate concentration of particles was achieved, and each sample was measured in triplicate.

3.5. Determination of encapsulation efficiency (EE %) of insulin

The R_8-Ins-SLNs or Ins-SLNs were dissolved in 50% ethanol under water bath at 70 °C for 10 min, and then cooled to room temperature to preferentially precipitate the lipid. The insulin content in the supernatant after centrifugation (14,000 rpm for15 min at 4 °C, L8−60M, Beckman, USA) was measured by an HPLC method at 214 nm using an VP-ODS column (4.6 mm × 150 mm, 5 μm, Shimadzu Co., Ltd., Japan) with a mobile phase consisting of 0.2 mol/L sodium sulfate (pH was adjusted to 2.3 with ethanolamine)-acetonitrile (73 : 27) with flow rate 1 ml/min. The EE% of insulin was calculated from the following Eq. (1):

$$\text{EE\% of insulin} = \frac{\text{insulin mass in SLNs}}{\text{insulin mass used in formula}} \times 100 \qquad (1)$$

3.6. Determination of R_8 EE%

The R_8-Ins-SLNs were dissolved in 50% ethanol under water bath at 70 °C for 10 min, and then cooled to room temperature to preferentially precipitate the lipid. The R_8 content in the supernatant after centrifugation (14,000 rpm for 15min at 4 °C, L8-60M, Beckman, USA) was measured by an HPLC method at 210 nm using an VP-ODS column (4.6 mm × 250 mm, 5 μm, Shimadzu Co., Ltd., Japan) with a mobile phase consisting of 2%(v/v) acetonitrile containing 0.05% (v/v) trifluoroacetic acid with flow rate 1ml/min. The R_8 EE% was calculated from the following Eq. (2):

$$\text{EE\% of } R_8 = \frac{R_8 \text{ mass in SLNs}}{R_8 \text{ mass used in formula}} \times 100 \qquad (2)$$

3.7. In vitro release

The *in vitro* release experiment was carried out by suspending a weighed amount of R_8-Ins-SLNs or Ins-SLNs in 50 ml simulated intestinal media without enzymes (Chinese Pharmacopoeia 2005, pH 6.8) containing 1.5% (w/w) glycine as stabilizer. During the experiment (24 h), the sample was shaken at 150 rpm at 37 ± 1 °C in a constant temperature shaker (Wuhan Ruihua instrument Co., Ltd., China). At predetermined time intervals, 0.5 ml aliquots of the buffer medium were taken and 0.5 ml of the simulated intestinal medium was added to maintain a constant volume. The aliquot was centrifuged at 14,000 rpm for 15 min at 4 °C (L8-60M, Beckman, USA) and by the HPLC method described above, the amount of insulin or R_8 in the supernatant was calculated by means of a calibration curve.

3.8. In vivo absorption experiments

Male Sprague-Dawley rats weighing 180 to 220 g were purchased from the Animal Centre of China Pharmaceutical University. Animals were housed in a room with a constant temperature of 22 ± 1 °C, relative humidity of 55 ± 5 % and a standard light/dark cycle, and they had free access to water and food during acclimatization. Animal experiments were performed according to the Guide Principles for the Care and Use of Experiment Animals in China Pharmaceutical University. Male rats were fasted for 16 h prior to the experiment, but received water *ad libitum*. Twenty four rats were separated into four groups. The first group was gavaged with 0.5 ml R_8-Ins-SLNs (insulin 20 IU/kg). The second group was gavaged with 0.5 ml Ins-SLNs (insulin 20 IU/kg).The third group was gavaged with 0.5ml insulin solution (insulin 20 IU/kg) and used as a control for the experiment. Insulin solution was injected subcutaneously to the fourth group (insulin 1 IU/kg). The insulin solution was prepared by dissolving insulin in 0.01 M HCl and phosphate buffered saline (PBS, pH 7.4).
During the experiment, 0.25 ml blood samples were collected from the tail vein immediately before dosing and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after dosing. The plasma was centrifuged at 12,000 rpm for 5 min and blood glucose levels were determined using a glucose meter (ACCU-CHEK Active, Roche Diagnostics Co., Ltd., Mannheim, Germany) and used to represent the biological activity of insulin. Post-dose levels of the serum glucose were expressed as the percentage of the pre-dose level and were plotted versus time. Then the AAC was calculated with the linear trapezoidal method (Liu et al. 2008). The relative pharmacological availability (PA %) of the orally administered insulin could be calculated from the following Eq. (3):

$$\text{PA}(\%) = \frac{\text{AAC}_{\text{oral}} \times \text{DOSE}_{\text{s.c.}} \times 100}{\text{AAC}_{\text{s.c.}} \times \text{DOSE}_{\text{oral}}} \qquad (3)$$

3.9. Statistical analysis

All values are expressed as their mean ±S.D. Statistical tests of significance were performed with SPSS Statistics 17.0(SPSS Inc., USA). A value of $P < 0.05$ was considered statistically significant. For group comparison one-way ANOVA followed by Tukey's multiple comparison tests was applied. Comparison at each time point was made by Independent-Samples T test.

Acknowledgement: We thank the financial support of the Postgraduate Innovation Foundation of China Pharmaceutical University under contract 02704001.

References

Chung SK, Maiti KK, Lee WS (2008) Recent advances in cell-penetrating, non-peptide molecular carriers. Int J Pharm 354: 16–22.
Cui F, Shi K, Zhang L, Tao A, Kawashima Y (2006) Biodegradable nanoparticles loaded with insulin-phospholipid complex for oral delivery: preparation, in vitro characterization and in vivo evaluation. J Control Release114: 242–250.
Derossi D, Joliot AH, Chassaing G, Prochiantz A (1994) The third helix of the antennapedia homeodomain translocates through biological membranes. J Biol Chem 269: 10444–10450.
Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J (1994) Tat-mediated delivery of heterologous proteins into cells. Proc Natl Acad Sci USA 91: 664–668.
Futaki S, Goto S, Suzuki T, Nakase I, Sugiura Y (2003) Structural variety of membrane permeable peptides. Curr Protein Pept Sci. 4 : 87–96.

Garcia-Fuentes M, Torres D, Alonso MJ (2002) Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloids Surf B: Biointerfaces 27: 159–168.

Kawashima Y, Yamamoto H, Takeuchi H, Hino T, Niwa T (1998) Properties of a peptide containing dl-lactide/glycolide copolymer nanospheres prepared by novel emulsion solvent diffusion methods. Eur J Pharm Biopharm 45: 41–48.

Kosuge M, Takeuchi T, Nakase I, Jones AT, Futaki S (2008) Cellular internalization and distribution of arginine-rich peptides as a function of extracellular peptide concentration, serum, and plasma membrane associated proteoglycans. Bioconjug Chem 19: 656–664.

Komatsu H, Kitajima A, Okada S (1995) Pharmaceutical characterization of commercially available intravenous fat emulsions : estimation of average particle size, size distribution and surface potential using photon correlation spectroscopy. Chem Pharm Bull 43: 1412–1415.

Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Q, He Q, Zhang Z (2008) Solid lipid nanoparticles for pulmonary delivery of insulin. Int J Pharm 356: 333–344.

Meade BR, Dowdy SF (2007) Exogenous siRNA delivery using peptide transduction domains/cell penetrating peptides. Adv Drug Deliv Rev 59: 134–140.

Mehnert W, Mader K (2001) Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 47: 165–169.

Morishita M, Peppas NA (2006) Is the oral route possible for peptide and protein drug delivery? Drug Discov Today 11: 905–910.

Müller RH, Jacobs C, Kayser O (2001) Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. Adv Drug Deliv Rev 47: 3–19.

Müller RH, Keck CM (2004) Challenges and solutions for the delivery of biotech drugs – a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 113: 151–170.

Norris DA, Puri N, Sinko PJ (1998) The effect of physical barriers and properties on the oral absorption of particulates. Adv Drug Deliv Rev 34: 135–154.

Owens DR, Zinman B, Bolli G (2003) Alternative routes of insulin delivery. Diabet Med 20 : 886–898.

Siekmann B, Westesen K (1996) Investigation on solid lipid nanoparticles prepared by precipitation in o/w emulsion. Eur J Pharm Biopharm 43: 104–109.

Torchilin VP (2008) Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. Adv Drug Deliv Rev 60: 548–558.

Trehin R, Merkle HP (2004) Chances and pitfalls of cell penetrating peptides for cellular drug delivery. Eur J Pharm Biopharm 58: 209–223.

Wender PA, Mitchell DJ, Pattabiraman K, Pelkey ET, Steinman L, Rothbard JB (2000) The design, synthesis and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters, Proc Natl Acad Sci USA 97: 13003–13008.

Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X (2006) Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm 327: 153–159.

Zorko M, Langel U (2005) Cell-penetrating peptides: mechanism and kinetics of cargo delivery. Adv. Drug Deliv Rev 57: 529–545.