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Preparation and Evaluation of Poly-Butylcyanoacrylate Nanoparticles for Oral Delivery of Thymopentin

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ABSTRACT: Thymopentin (Tp5) was loaded in poly-butylcyanoacrylate (PBCA) nanoparticles (NP) in order to enhance the oral bioavailability of Tp5. PBCA-Tp5-NP was prepared by nanoprecipitation methods. Dialyzing membrane method was employed to examine the *in vitro* release of PBCA-Tp5-NP in PBS, and Tp5 samples in the release medium were detected by HPLC. The cell proliferation test (^{3}H-thymidine) was conducted to verify the PBCA-Tp5-NP bioactivity *in vitro*. The pharmacodynamical studies were performed on preimmunoinhibited rats and in flow cytometer. The size and the entrapment efficiency of PBCA-Tp5-NP were 178 ± 39 nm and $92.21 \pm 1.08\%$, respectively. *In vitro* release data show that less than 60% Tp5 was released from lyophilized PBCA-Tp5-NP while 80% Tp5 was released from the colloidal PBCA-Tp5-NPs in 48 h. The proliferation test showed that PBCA-Tp5-NP had the similar effect as Tp5. The *in vivo* data showed that oral PBCA-Tp5-NPs had similar function as what intravenous Tp5 did. The oral bioavailability of Tp5 could be enhanced by PBCA nanoparticles. PBCA-Tp5-NP had the property of sustained-release and the efficacy of Tp5 was not changed after formulation.
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Keywords: thymopentin; oral drug delivery; bioavailability; PBCA; nanoparticles

INTRODUCTION

The oral delivery of peptide and protein drugs has become an important research area due to a large number of recombinant proteins that are now being investigated for therapeutic applications and the superior convenience of administration for patients. However, the biological and enzymatic barriers *in vivo*, mainly consisting of gastric acid and various enzymes, present scientists a great challenge to develop suitable preparations for the oral delivery of peptide and proteins.

Thymopentin (Tp5), a synthetic pentapeptide, corresponds to the active site of 49 amino acid–human hormone thymopoietin.[1–3] It has the biological activity of thymopoietins and influences the immune system by promoting the differentiation of thymocytes and affecting the function of mature T-cells. Tp5 has been used as an immunomodulator for the treatment of rheumatoid arthritis, acquired immunodeficiency syndrome (AIDS), severe acute respiratory syndrome (SARS), cutaneous T-cell lymphoma/cancer immunodeficiency, and other primary immunodeficiencies.[4–9] However, the half-life of Tp5 is about 30 s and in clinic, it has been used via

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intramuscular administration, which often induces inconvenience in patients. In this sense, to develop a Tp5 oral delivery system is of great scientific and practical significance.

Nanoparticles are a kind of immunogenicity free, stable and less toxic drug delivery system that is easy to prepare and lyophilize. Evidence reveals that nanoparticles between 40 and 200 nm may be absorbed by intestine through the way of either paracellular uptake or endocytosis.[10]

Biodegradable polymers have been widely used in preparing microparticulate drug delivery systems, such as poly-lactic acid (PLA), poly(lactide-*co*-glycolide acid) (PLGA) and chitosan, etc. The fragile drugs can be encapsulated into polymeric nanoparticles and thus go through the GI tract.[11–16] Poly-butylcyanoacrylate (PBCA) is such a fine biodegradable polymer that has been used as surgery glue in clinic.[17–23] In addition, the former experiments done in our lab showed that the method to produce PBCA nanoparticles was much easier than the others.

In this paper, PBCA was selected as the carrier material for Tp5 on account of serial screen tests in our lab, based on the drug entrapment efficiency (EE), compared with PLA and PLGA Tp5-loaded nanoparticles.

According to literature, it is not difficult for water insoluble drugs to be encapsulated in polymers compared with the water soluble ones. However, Tp5 is a highly hydrophilic drug, which presents another challenge for this study. Although there are various methods for the preparation of polymeric nanoparticle,[24–30] nanoprecipitation method was used to prepare PBCA-Tp5-NP in this study. The effective dose of Tp5 is about 1 mg/day with respect to the instruction of Tp5 injection used in clinic. Therefore, it is reasonable to deliver Tp5 in oral dosage forms as long as the amount of Tp5 loaded in the nanoparticle is suitable.

MATERIALS AND METHODS

Materials and Animals

Thymopentin (Tp5) was gift sample from Chengdu Di'ao Group (Chengdu, China). PBCA was purchased from Guangzhou Baiyun Medical Gel Ltd. (Guangzhou, China). Pluronic F68, RPMI1640 and Concanavalin A (Con A) were purchased from Sigma (Chengdu, China). Cyclophosphamide (CTX) was provided by Hualian Pharmaceutical Company (Shanghai, China). Tritiated thymidine (^{3}H-TdR) was ordered from Chinese Academy of Sciences Beijing Institute of Atomic Energy (Beijing, China).

Mouse anti-rat CD_4: RPE, mouse anti-rat CD_3: FITC, and mouse anti-rat CD_8 alpha: RPE-CY5 antibodies were obtained from Serotec Ltd. (Oxford, UK).

Wistar rats and Bal b/C mice were provided by West-China Experimental Animal Center of Sichuan University.

Method of Preparation of PBCA Nanoparticles

An optimized nanoprecipitation method was used to prepare the nanoparticles after the selection tests of different preparation conditions. Tp5 1.0 mg was carefully weighed and dissolved in 10 mL 2.5% Pluronic F68 solution, and then the pH of the solution was adjusted to 2.5 with 0.1 mol/L HCl. Fifty microliters of PBCA was dropped into the water phase by slow injection while stirring. After stirring for 30 min, the pH value of the solution was adjusted to 7.8 with 0.1 mol/L NaOH, and then kept stirring for another 1 h. The colloid was freeze-dried (Savant Instruments Inc, NY, SNL216V) and stored at -20°C before use.

Particle Size and Morphology of Nanoparticles

The size of nanoparticles was determined by Mastersizer 2000 (Malvern, UK), in triplicate. The morphology of nanoparticles was observed by negative staining under the transmission electron microscope (TEM) (Hitachi H-600, Minato-ku, Japan). Nanoparticle colloid was dropped on a grid and dyed with 2% phosphotungstic acid (PTA). The pictures were shown in Figure 1.

Entrapment Efficiency (EE)

The obtained nanoparticle colloid was diluted with distilled water to 10 mL in a volumetric flask. The percentage of drug incorporated into the nanoparticles was determined by centrifuging (Hitachi CP60E, Japan) the drug-loaded nanoparticles at 40000 rpm $\times$ 1 h, with supernatant separated. Free drug in the supernatant was determined by HPLC (Shimadzu LC-10AT, Kyoto, Japan) at 275 nm through RP C_{18} column (4.6 mm $\times$ 250 mm, 5 μm, Vydac 218TP54, USA). Mobile phase was consisted of PBS (0.1 mol/L, pH 7.0): MeOH (1:9), temperature was 30°C, flow rate was 1 mL/min, injection volume was 10 μL. EE was calculated by the difference between the amount of drug entrapped and the total amount of drug.

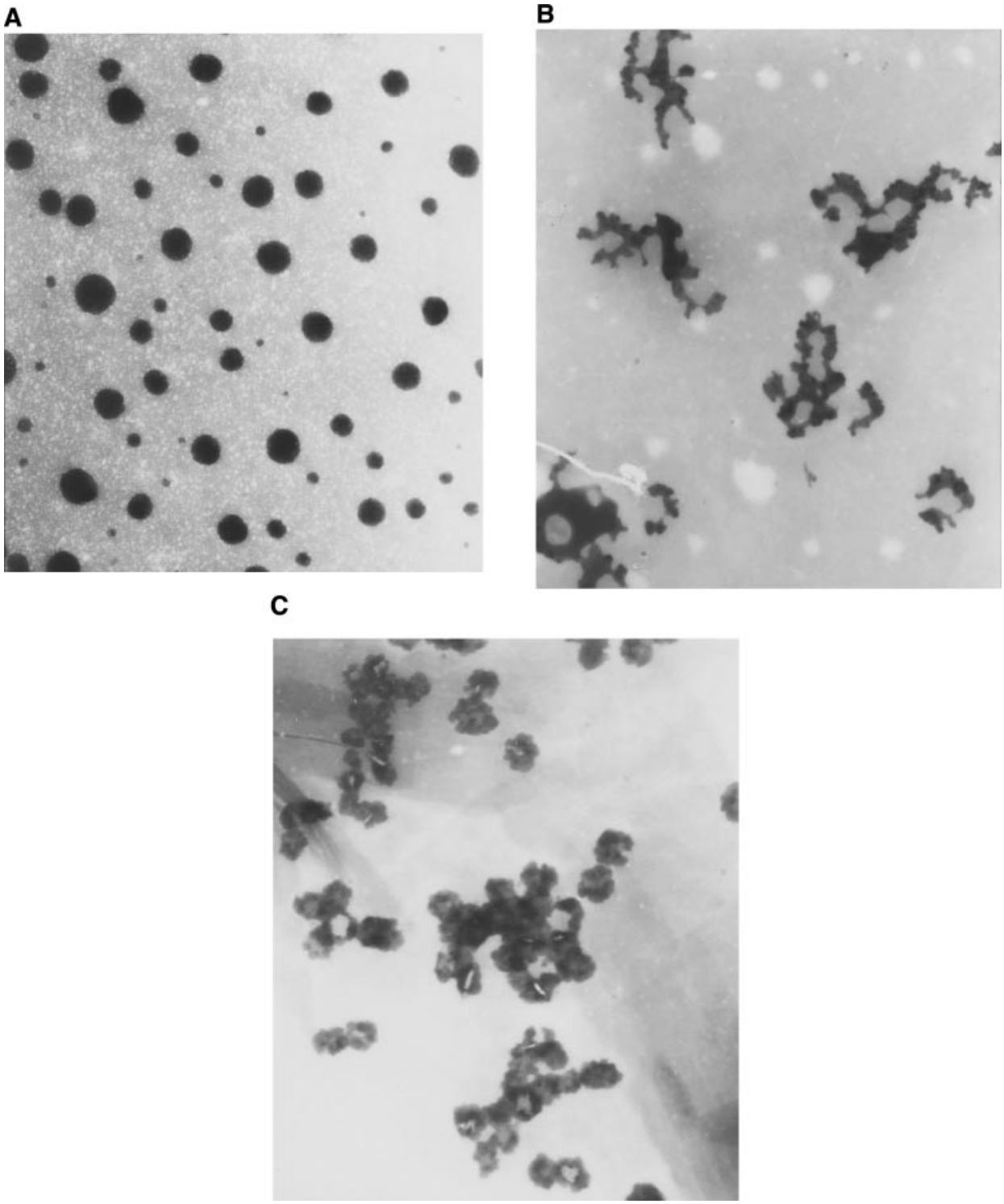

Figure 1. (A) TEM micrograph of lyophilized PBCA-Tp5-NP (30000×), (B) TEM micrograph of PBCA-Tp5-NP colloid stored at room temperature for 3 months (30000×), and (C) TEM micrograph of lyophilized PBCA-Tp5-NP keeping at room temperature for 3 months (20000×).

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In Vitro Release Study

The release of Tp5 from the PBCA-NP was examined as follows. One microliter of colloidal solution, lyophilized nanoparticle solution and Tp5 solution, which containing the same amount of Tp5 (100 μg/mL), were put into dialysis membrane tubing. Twenty microliters pH 7.4 phosphate buffer solution was used as medium for the release test. The tubes were placed in a water bath maintained at 37°C and shook at a speed of 50 rpm. At given time intervals, 1 mL of release medium was withdrawn and the 1 mL fresh PBS was added into the media. The amount of Tp5 in the solution was measured by HPLC analysis. The cumulative release rate of Tp5 from the nanoparticles was calculated. The release curve was shown in Figure 2. The release data were fitted to different pharmacokinetics release models by statistica 5.0 (Fig. 3).

In Vitro Cell Proliferation Assay of PBCA-Tp5-NP

The *in vitro* efficacy evaluation of PBCA-Tp5-NP was studied using cell proliferation assay.[31–33] Thymocytes and spleen cells were collected from mice and suspended in RPMI 1640 at the concentration of 2.0×10^6 cell/mL, respectively. PBCA-Tp5-NP (50 μL/well) were added on 96-well plate with or without 6 μL Concanavalin A (Con A, 300 μg/mL) triplicately. RPMI 1640 containing 5% BSA media was added to each well till 100 μL/well. Then 100 μL of cell suspension above was added into each well and mixed, then kept at 37°C, 5% CO_2 for 72 h. ^{3}H-TdR (1 μCi) was added into each well 14–16 h before the end of culture. Free ^{3}H-TdR was washed out by distilled water for 20–30 times, and the samples were collected onto the glass microfiber filter and detected by liquid scintillation counter system. The results were shown in Figure 4.

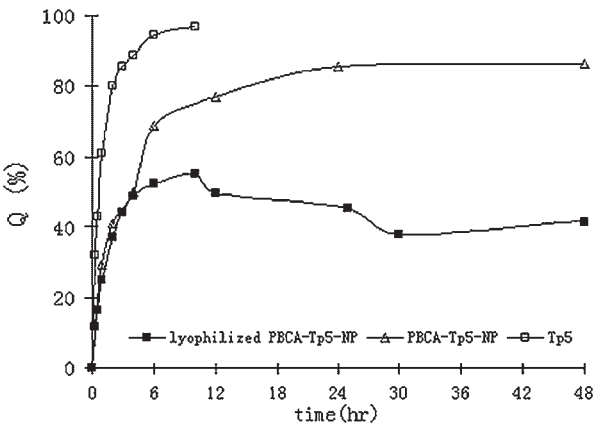

Figure 2. The cumulative release curve of different Tp5 dosage forms *in vitro* at 37°C, 50 rpm in water. (□) The cumulative release curve of uncoated Tp5 was stopped at 10 h as more than 90% of the drug had been released to the outside water by that time, (△) the cumulative release curve of lyophilized PBCA-Tp5-NP, (■) the cumulative release curve of PBCA-Tp5-NP colloid.

In Vivo Efficacy Evaluation of PBCA-Tp5-NP

Twenty-eight Female Wistar rats weighing between 160 and 200 g were divided into seven groups randomly and treated with cyclophosphamide (CTX) intraperitoneally (i.p.) for 3 days continuously, and the immune inhibition model was developed according to the report.[34] The administration was done following Table 1, once per day, 7 days totally. Blood samples (0.5 mL) of each rat were collected into tubes which were precovered by heparin at day 8 and detected by flow cytometry analysis (EPICS ELITE/ESP, Coulter, Fullerton, CA) (Fig. 5).

One hundred microliters of blood sample was added with 20 μL corresponding mono-antibody and was vortexed. After standing for 20 min at RT, 500 μL cell lysis buffer was added and the mixture stood for another 20 min. At last, 500 μL of PBS (0.1 mol/L, pH 7.4) was added and vortexed and stood 20 min before FC analysis. The chart obtained is shown in Figure 4. The ratio of CD_4^+/CD_8^+ in each group was calculated accordingly.

Statistical Analysis

All calculated parameters were shown in the format of mean values ± standard deviations (SD). For comparison between the experimental groups and corresponding controls, Student's *T*-test was employed. Values of $p < 0.05$ were set as a significance limit. If the calculated $p < 0.05$, there existed a significant difference.

RESULTS AND DISCUSSION

Characteristics of PBCA Nanoparticles

The lyophilized powder was clean white and easy to re-suspend in water homogeneously.

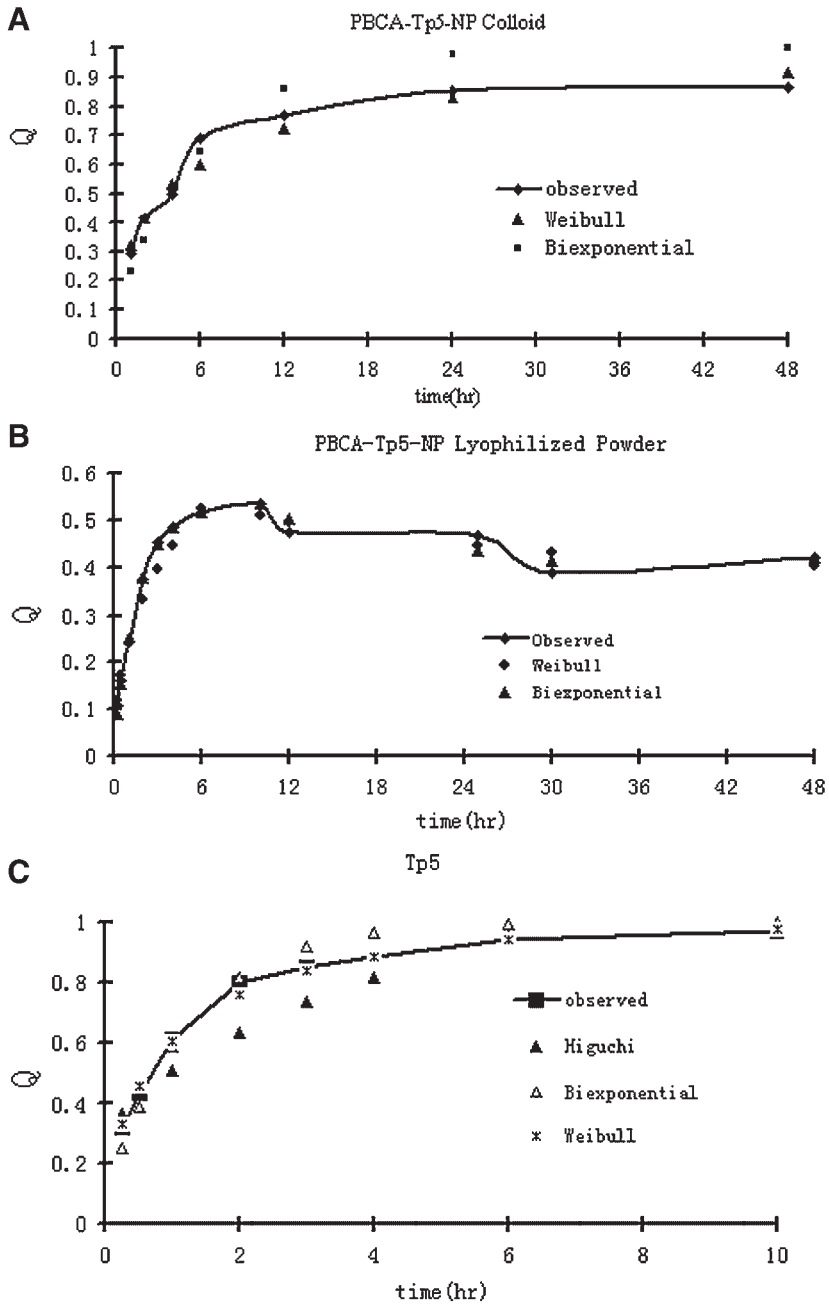

Figure 3. The comparison between observed and calculated value of *in vitro* release data for different preparations. (A) The observed release curve of PBCA-Tp5-NP colloid is compared with the value calculated from Weibull equation ($r = 0.9706$) and Biexponential equation ($r = 0.9505$), respectively. (B) The observed release curve of PBCA-Tp5-NP lyophilized powder is compared with the value calculated from Weibull equation ($r = 0.9787$) and Biexponential equation ($r = 0.9986$), respectively. (C) The observed release curve of free Tp5 within 10 h is compared with the value calculated from Weibull equation ($r = 0.9959$), Biexponential equation ($r = 0.9843$) and Higuchi equation ($r = 0.9169$), respectively.

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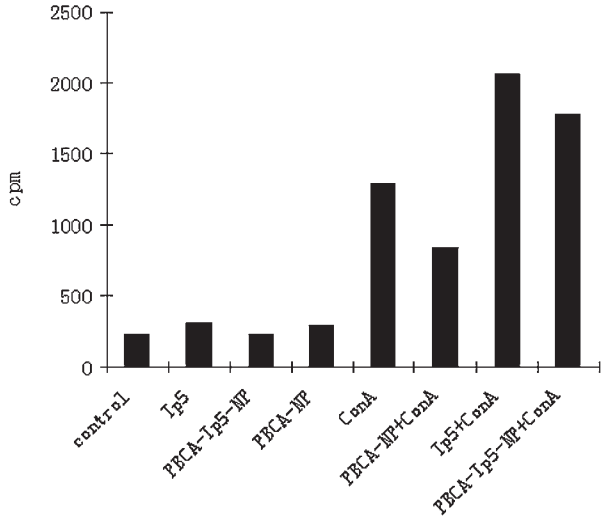

Figure 4. ^{3}H-Thymidine incorporation test of PBCA-Tp5-NP and Tp5 on mice spleen cells *in vitro*. The concentration of Tp5 in the treated groups was equal to 0.1 μg/mL. The count per minute (cpm) value was obtained by liquid scintillation counter system.

Particle Size and Morphology of Nanoparticles

The TEM of nanoparticles with Tp5 were shown in Figure 1A. Most nanoparticles were observed to be of spherical or ellipsoidal shape with smooth surface. TEM of PBCA-Tp5-NP colloid and the lyophilized powder after 3 months in RT were shown in Figure 1B and C, respectively. The results demonstrated that structure of nanoparticles powder was more stable than that of nanoparticles colloid.

The size of PBCA-Tp5-NP was 178 ± 39 nm. Pluronic F68 would increase the size of the nanoparticles when its percentage was more than 3%. Moreover, the pH value was another factor influencing the nanoparticle size. When pH value was higher than 3.0, the particle size would increase significantly.

Entrapment Efficiency (EE)

The EE of PBCA-Tp5-NP was $92.2 \pm 1.08\%$. The data showed that pH 2.5 was helpful for a higher EE at the beginning of nanoparticle formation. Increased F68 increased encapsulation efficiency, but will also increase the particle size. In addition, the increasing ratio of polymer/drug would increase EE.

In Vitro Release Studies

The release profile of PBCA-Tp5-NP colloid, powder, and uncoated Tp5 were shown in Figure 2. All release patterns were noted to be typically biphasic. There was no burst effect

Table 1. The Regression Release Equation of PBCA-Tp5-NP Colloid (I), PBCA-Tp5-NP Lyophilized Powder (II),* and Tp5 (III)

Model	Preparation	Regression Equation	r
Monoexponential	I	$\ln(1-Q) = -0.7791 + 0.0612t$	0.7670
	II	$\ln(1-Q) = -0.1981 - 0.0756t$	0.8614
	III	$\ln(1-Q) = -0.5414 - 0.3419t$	0.9517
Higuchi	I	$Q = 0.2165 + 0.1197t^{1/2}$	0.8859
	II	$Q = -0.0491 + 0.1879t^{1/2}$	0.9508
	III	$Q = 0.2085 + 0.3045t^{1/2}$	0.9169
Niebergull	I	$(1-Q)^{1/2} = 0.7691 - 0.0109t$	0.7848
	II	$(1-Q)^{1/2} = 0.9078 - 0.0305t$	0.8441
	III	$(1-Q)^{1/2} = 0.7507 - 0.0742t$	0.8600
Hixcon–Crowell	I	$(1-Q)^{1/3} = 0.8361 - 0.0087t$	0.8053
	II	$(1-Q)^{1/3} = 0.9370 - 0.0218t$	0.8500
	III	$(1-Q)^{1/3} = -0.8257 + 0.0640t$	0.8936
Weibull	I	$\ln\ln(1/(1-Q)) = -0.9460 + 0.4814\ln t$	0.9706
	II	$\ln\ln(1/(1-Q)) = -1.2967 + 0.5618\ln t$	0.9787
	III	$\ln\ln(1/(1-Q)) = -0.0693 + 0.6191\ln t$	0.9959
Biexponential	I	$1-Q = 0.8996\,e^{-0.1544t}$	0.9505
	II	$1-Q = 0.5108\,e^{-0.6225t} + 0.4747\,e^{-0.0023t}$	0.9986
	III	$1-Q = 0.2294\,e^{-0.8111t}$	0.9843

The critical value of the coefficient $r = 0.834$ when the parameters were chosen ($\upsilon = 6$ and $\alpha = 0.005$).
PBCA-Tp5-NP colloidal (I), lyophilized PBCA-Tp5-NP (II), and Tp5 lyophilized powder (III).
*Data used from 0 to 10 h.

observed. For the uncoated Tp5, more than 95% of it was released after 8 h. At 48 h, the cumulative release of Tp5 from colloidal solution reached 80%; however, cumulative release from lyophilized powder was only 50%. This result indicated that the release of Tp5 was delayed after being lyophilized. On the other hand, Tp5 itself was not stable in water at 37°C after 8 h.[35] The drop down of the release curve in the lyophilized nanoparticle group showed that the released amount of Tp5 was less than that of the degraded Tp5 after 12 h, and the Tp5 could be released from the lyophilized powder for at least 48 h.

The different fitting model results were shown in Table 1. The critical value of the coefficient $r = 0.834$ when the parameters were chosen ($\upsilon = 6$ and $\alpha = 0.005$). PBCA-Tp5-NP colloid release curve was fitted to Higuchi, Weibull, and Biexpotential equations ($p < 0.005$). The release curves of PBCA-Tp5-NP lyophilized powder and Tp5 were fitted to all the equations listed in Table 1, respectively ($p < 0.005$). The release curve of PBCA-Tp5-NP colloid between 10 and 48 h was fitted well to the following equations when the parameters were set ($\upsilon = 2$, $\alpha = 0.10$, $r = 0.800$) ($p < 0.10$):

Weibull equation

$$\ln(1 - Q) = 0.1417 - 0.2077 \ln t, \quad r = 0.8092$$

Biexponential equation

$$1 - Q = 1.2664\, e^{0.2535t} - 0.8479\, e^{0.0308t},$$

$$r = 0.8653$$

The data above showed that the release curves could be described by different equations. Sum should be calculated to select the best equation according to the following formula:

$$\mathrm{SUM} = \sum_{i=1}^{n} (Q_i - \hat{Q}_i)^2$$

The smaller the sum was, the better the equation fitted. Q_i was the observed value, $\hat{Q}_i$ was the value calculated according to different equations. All the values were listed in Table 2. The comparison between observed and calculated value of *in vitro* release data for different preparations were shown in Figure 3. It was indicated that some of the equations could mimic the release curve *in vitro* very well, such as Weibull equation and Biexponential equation.

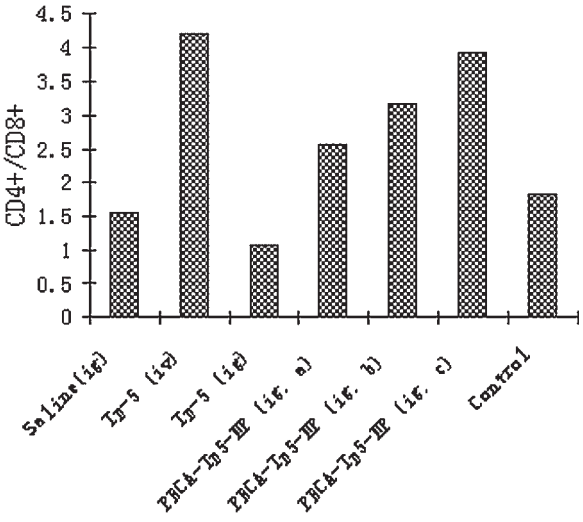

Figure 5. Comparison Chart of $CD4^+/CD8^+$ in different rat groups ($n = 4$). The value of $CD4^+$ and $CD8^+$ was obtained from the flow cytometry system by detecting the signal of corresponding labeled antibody.

In Vitro Cell Proliferation Assay of PBCA-Tp5-NP

The reports[36,37] showed that Con A had the function to induce T cell activation. ^{3}H-Thymidine incorporation is a proliferation assay method which is used widely in the evaluation of the cell proliferation *in vitro*. The Con A has the ability to increase the cell division. Figure 4 showed that the cells in groups without Con A had limited population while the cell amount in groups treated with Con A together had very significant increase. The blank PBCA-NP itself had no function stimulated the proliferation of spleen cells, but it decreased the effect of Con A to induce the proliferation of spleen cells *in vitro* while the PBCA-Tp5-NP could collaborate with Con A increasing this proliferation just as what the free Tp5 did.

In Vivo Efficacy Evaluation of PBCA-Tp5-NP

The concentration of Tp5 in blood cannot be determined because of the extremely short half-life. As an immunomodulator, Tp5 would improve the inhibited immune system. CD factors are

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Table 2. SUM for Good Fitting of the Release Data of PBCA-Tp5-NP Colloid (I), PBCA-Tp5-NP Lyophilized Powder (II),* and Tp5 (III)

	Higuchi	Monoexponential	Niebergull	Hixcon–Crowell	Weibull	Biexponential
I	0.1402	4.9889	0.1400	0.0772	0.1596	0.0628
II	0.0303	0.1725	0.0326	0.0159	0.1550	0.0009
III	0.1417	1.0550	0.1682	0.0897	0.0355	0.0277

*Data used from 0 to 10 h.

suitable to illustrate the efficacy of the administration of the different forms of prepared Tp5. CD_4^+/CD_8^+ was chosen to indicate if the immunization was improved or not. The value of CD_4^+/CD_8^+ in rat blood was decreased after being treated with CTX. Compared to all those treatment groups, Tp5 i.g. group showed no effect on increasing the decreased value of CD_4^+/CD_8^+ in blood, while the PBCA-Tp5-NP groups showed a dosage-depended effect on increasing the CD_4^+/CD_8^+, just like the effect resulted by Tp5 i.v. group (Fig. 5). The results indicated that the PBCA-Tp5-NP administrated orally delivered the Tp5 into the rat blood successfully. Tp5 can be protected by encapsulated in the PBCA nanoparticles so that Tp5 can be effective by oral administration. This is promising in the oral delivery of short half-life drugs.

Student's *T*-tests were performed on the data between group C and groups D–F. The value of CD_4^+/CD_8^+ in control group had no significant difference from the saline group. There was significant difference between group C and groups D–F ($p < 0.05$). The effect/dosage (a pharmaceutical effect) was also calculated and listed in Table 3. The pharmacodynamical effect of group D was the highest compared to the other i.g. groups. It seemed like the most drug dosage into the rats might not give the best result, depending on the adjustment by the immune system with unclear mechanism.

CONCLUSION

In this study, PBCA-Tp5-NP with high entrapment efficiency was prepared by an optimized nanoprecipitation method and showed the property of sustained release in PBS. Tp5 in PBCA-Tp5-NP maintained the biological efficacy and showed positive cooperation with Con A in the proliferation test on lymphocyte transmission. An oral pharmacodynamical study was conducted in immunosuppressed rats by flow cytometry. The results showed that PBCA-Tp5-NP had a significant improvement on the oral absorption of Tp5 than regular uncoated Tp5 solution. The mechanism of Tp5 working on immune system is not clear yet. The suitable dosage for a treatment should be test before coming to a conclusion. Through this study, a feasible and promising oral delivery system using PBCA as the carrier was established to enhance the oral absorption and bioavailability of peptide or protein drugs.

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Table 3. Experiment Arrangement of PBCA-Tp5-NP for the Effect on Rats and the Results *In Vivo* ($n = 4$)

Group	CTX (mg/kg/day)	Dosage (mg/kg)	$(CD4^+/CD8^+)$/Dosage
A: Saline (i.g.)	35	0.00	—
B: Tp-5 (i.v.)	35	0.09	39.09 ± 18.5
C: Tp-5 (i.g.)	35	0.90	1.66 ± 1.1
D: PBCA-Tp5-NP (i.g., a)	35	0.90	2.83 ± 0.9
E: PBCA-Tp5-NP (i.g., b)	35	1.35	2.02 ± 0.8
F: PBCA-Tp5-NP (i.g., c)	35	1.80	2.18 ± 0.9
G: Control[a]	0	0.00	—

$(CD4^+/CD8^+)$/dosage is the adjusted effect factor. The difference between groups D, E, F, and C is significant. $p_{D/C} = 0.012$, $p_{E/C} = 0.021$, $p_{F/C} = 0.003$.

[a]Control means the rats were treated with nothing; all the other rats were immune inhibited.

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REFERENCES

1. Wang J, Lu WL, Liang GW, Wu KC, Zhang CG, Zhang X, Wang JC, Zhang H, Wang XQ, Zhang Q. 2006. Pharmacokinetics, toxicity of nasal cilia and immunomodulating effects in Sprague-Dawley rats following intranasal delivery of thymopentin with or without absorption enhancers. Peptides 27:826–835.
2. Zheng AP, Wang JC, Lu WL, Zhang X, Zhang H, Wang XQ, Zhang Q. 2006. Thymopentin-loaded pH-sensitive chitosan nanoparticles for oral administration: Preparation, characterization, and pharmacodynamics. J Nanosci Nanotechnol 6:2936–2944.
3. Zhang RL, Jiao Q, Wang BG. 2003. Controlled clinical study on 49 patients of SARS treated by integrative Chinese and Western medicine. Zhongguo Zhong Xi Yi Jie He Za Zhi 23:654–657.
4. Giordano N, Biasi G, Taddeo A, Marcolongo R, Bertelletti D. 1989. An immunomodulating drug, thymopentin, in the treatment of progressive systemic sclerosis. Clin Rheumatol 8:293–294.
5. Goldstein G, Audhya TK. 1985. Thymopoietin to thymopentin: Experimental studies. Surv Immunol Res 4:1–10.
6. Greenhalgh D, Gamelli RL. 1987. Immunomodulators and wound healing. J Trauma 27:510–514.
7. Hamilton G, Zoch G, Rath T, Meissl G. 1989. Thymopentin (TP-5) in the treatment of the postburn and postoperative immunodeficiency syndrome. Prog Clin Biol Res 308:995–999.
8. Heavner GA. 1985. Thymopentin structure and relation to thymic factors. Surv Immunol Res 4:11–16.
9. Heavner GA, Audhya T, Goldstein G. 1990. Peptide analogs of thymopentin distinguish distinct thymopoietin receptor specificities on two human T cell lines. Regul Pept 27:257–262.
10. Mathiowitz E, Jacob JS, Jong YS, Carino GP, Chickering DE, Chaturvedi P, Santos CA, Vijayaraghavan K, Montgomery S, Bassett M, Morrell C. 1997. Biologically erodable microspheres as potential oral drug delivery systems. Nature 386:410–414.
11. Gomez-Orellana I. 2005. Strategies to improve oral drug bioavailability. Expert Opin Drug Deliv 2:419–433.
12. Pandey R, Ahmad Z, Sharma S, Khuller GK. 2005. Nano-encapsulation of azole antifungals: Potential applications to improve oral drug delivery. Int J Pharm 301:268–276.
13. Tao SL, Desai TA. 2005. Gastrointestinal patch systems for oral drug delivery. Drug Discov Today 10:909–915.
14. Bruschi ML, de FO. 2005. Oral bioadhesive drug delivery systems. Drug Dev Ind Pharm 31:293–310.
15. Bernkop-Schnurch A, Guggi D, Pinter Y. 2004. Thiolated chitosans: Development and in vitro evaluation of a mucoadhesive, permeation enhancing oral drug delivery system. J Control Release 94: 177–186.
16. Pandey R, Zahoor A, Sharma S, Khuller GK. 2003. Nanoparticle encapsulated antitubercular drugs as a potential oral drug delivery system against murine tuberculosis. Tuberculosis (Edinb) 83:373–378.
17. Li X, Zhu YF, Zhao MJ. 2003. The research on IR and TEM of polybutylcyanoacrylate nanoparticles. Guang Pu Xue Yu Guang Pu Fen Xi 23:266–269.
18. Ramge P, Unger RE, Oltrogge JB, Zenker D, Begley D, Kreuter J, von BH. 2000. Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human and bovine primary brain capillary endothelial cells. Eur J Neurosci 12:1931–1940.
19. Ratcliffe JH, Hunneyball IM, Smith A, Wilson CG, Davis SS. 1984. Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs. J Pharm Pharmacol 36:431–436.
20. Scherer D, Mooren FC, Kinne RK, Kreuter J. 1993. In vitro permeability of PBCA nanoparticles through porcine small intestine. J Drug Target 1: 21–27.
21. Shen LF, Zhang YD, Shen HJ, Zeng S, Wang X, Wang C, Le Y, Shen H. 2006. Liver targeting and the delayed drug release of the nanoparticles of adriamycin polybutylcyanoacrylate in mice. Chin Med J (Engl) 119:1287–1293.
22. Sun X, Wu F, Lu W, Zhang ZR. 2004. Sustained-release hydroxycamptothecin polybutylcyanoacrylate nanoparticles as a liver targeting drug delivery system. Pharmazie 59:791–794.
23. He Q, Zhang Z. 1998. Determination of valaciclovir polybutylcyanoacrylate nanoparticles. Hua Xi Yi Ke Da Xue Xue Bao 29:272–274.
24. Katsikogianni G, Avgoustakis K. 2006. Poly(lactide-co-glycolide)-methoxy-poly(ethylene glycol) nanoparticles: Drug loading and release properties. J Nanosci Nanotechnol 6:3080–3086.
25. McCarthy JR, Perez JM, Bruckner C, Weissleder R. 2005. Polymeric nanoparticle preparation that eradicates tumors. Nano Lett 5:2552–2556.
26. Dong Y, Feng SS. 2005. Poly(D,L-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. Biomaterials 26:6068–6076.
27. Garcia-Fuentes M, Torres D, Martin-Pastor M, Alonso MJ. 2004. Application of NMR spectroscopy to the characterization of PEG-stabilized lipid nanoparticles. Langmuir 20:8839–8845.
28. Santos MC, Mehnert W, Schaller M, Korting HC, Gysler A, Haberland A, Schafer-Korting M. 2002.

Drug targeting by solid lipid nanoparticles for dermal use. J Drug Target 10:489–495.
29. Zambaux MF, Bonneaux F, Gref R, Dellacherie E, Vigneron C. 1999. Preparation and characterization of protein C-loaded PLA nanoparticles. J Control Release 60:179–188.
30. Page-Clisson ME, Pinto-Alphandary H, Ourevitch M, Andremont A, Couvreur P. 1998. Development of ciprofloxacin-loaded nanoparticles: Physicochemical study of the drug carrier. J Control Release 56:23–32.
31. Laszlo A, Petri I, Gyurkovits K. 1987. Spontaneous and stimulated lymphocyte transformation test in homozygous children with cystic fibrosis. Acta Paediatr Hung 28:101–105.
32. Koszinowski U, Volkmann B, Thomssen R. 1975. In-vitro demonstration of cell-mediated immunity to vaccinia virus in man. Z Immunitatsforsch Exp Klin Immunol 148:451–461.
33. Conti B, Panico M, Ventura CA, Giunchedi P, Puglisi G. 1997. Thymopentin loaded microsphere preparation by w/o/w emulsion technique: In vitro/ex vivo evaluation. J Microencapsul 14: 303–310.
34. Li LQ, Wang JX, Song DM, Fan SG, Mei L. 1996. Building up of an animal model of conditioned immunosuppression and analysis of its possible mechanism. Yao Xue Xue Bao 31:477–480.
35. He W, Zhang Z, Jiang X, Nie Y, Wu F. 2003. Stability evaluation of thymopentin in preparation process. Sichuan Da Xue Xue Bao Yi Xue Ban 34: 292–294.
36. Galvin F, Freeman GJ, Razi-Wolf Z, Hall W, Jr., Benacerraf B, Nadler L, Reiser H. 1992. Murine B7 antigen provides a sufficient costimulatory signal for antigen-specific and MHC-restricted T cell activation. J Immunol 149:3802–3808.
37. Galvin F, Freeman GJ, Razi-Wolf Z, Benacerraf B, Nadler L, Reiser H. 1993. Effects of cyclosporin A, FK 506,and mycalamide A on the activation of murine CD4+ T cells by the murine B7 antigen. Eur J Immunol 23:283–286.