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Biodegradable polymeric microspheres for nalbuphine prodrug controlled delivery: in vitro characterization and in vivo pharmacokinetic studies

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Abstract

The objective of this work was to study the in vitro characteristics as well as in vivo pharmacokinetic performance of a series nalbuphine (NA) prodrug-loaded microspheres. An oil-in-water solvent evaporation method was used to incorporate the various NA prodrugs into poly(D,L-lactide-co-glycolide) (PLGA)-based microspheres. The morphology of microspheres under the scanning electron microscopy (SEM) revealed a spherical shape with smooth surface. Drug release rates for the microspheres were found to be a function of prodrug hydrophilicity, with higher drug release rates for microspheres loaded with more hydrophilic prodrugs. The release profiles fit well to the Baker and Lonsdale's spherical matrix model, suggesting the drug release from microspheres was consistent with a diffusion mechanism. The in vivo pharmacokinetic studies after s.c. injection of microspheres into rabbits showed sustained plasma NA-time profiles, with approximately 104.7, 67.2, and 41.0% relative bioavailability for microspheres loaded with nalbuphine propionate (NAP), nalbuphine pivalate (NPI), and nalbuphine decanoate (NDE), respectively. The in vitro release characteristics correlated well with the in vivo pharmacokinetic profiles. The results indicated that the prodrug hydrophilicity had significant effects on the in vitro as well as in vivo drug release kinetics. The present study demonstrates the feasibility of using biodegradable polymeric microspheres for controlled delivery of NA prodrugs.

Keywords: Controlled release; Nalbuphine prodrug; Microsphere; Poly(D,L-lactide-co-glycolide)

1. Introduction

Biodegradable polymeric microspheres have received considerable attention in the design of injectable controlled release formulations to sustain the release of therapeutic agents. Among various biodegradable polymers, the aliphatic polyesters based on lactide/glycolide are most widely used and studied. The major advantage of using biodegradable polymers in injectable delivery system is that it enables the site-specific or systemic administration of pharmaceutical agents without the need for subsequent retrieval of the delivery system. Various therapeutic agents, such as antibiotics, anti-inflammatory drugs, anticancer drugs, narcotics, steroids, peptides, and proteins have been incorporated in the lactide/glycolide-based copolymer systems (Sanders et al., 1986; Ike et al., 1992; Mauduit et al., 1993; Niwa et al., 1993; Zhang et al., 1993; Aso et al., 1994; Lambert and Peck, 1995; Chandrashekar and Udupa, 1996; Sung et al., 1998; Yen et al., 2001; Kim and Burgess, 2002). Since

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the physicochemical and pharmacokinetic behaviors of various therapeutic agents are different, the relationship between in vitro drug release characteristics and in vivo pharmacokinetic performance of each formulation should be well studied and characterized in order to achieve optimal blood concentration and therapeutic effect.

Nalbuphine (NA) is a narcotic drug used effectively in the treatment of both acute and chronic pain. It is a morphine-like drug with partial agonist activity at the κ-opiate receptor and antagonist activity at the μ-opiate receptor (Pugh and Drummond, 1987). It is a potent analgesic with relatively lower incidence of side effects such as respiration depression and withdrawal symptoms (Pugh and Drummond, 1987). The recommended injection dose is 40–80 mg daily for a 70 kg adult. Due to its short elimination half-life and poor oral bioavailability, frequent injection (e.g. every 3–6 h) is often needed. Patient compliance and therapeutic effectiveness in pain management may thus be improved by prolonging blood NA concentration. As a result, a series of NA prodrugs, such as nalbuphine propionate (NAP), nalbuphine pivalate (NPI), and nalbuphine decanoate (NDE) have been synthesized (Wang, 1992). Efforts have also been made to deliver these prodrugs utilizing various dosage forms and delivery routes, including injectable oils, buccal disks, biodegradable implants as well as transdermal delivery systems (Sung et al., 1998, 2000; Han et al., 1999; Yen et al., 2001). The release of NA prodrugs from various dosage forms was significantly prolonged and the release rates were affected by the hydrophilicity of prodrugs as well as the rate-controlling polymers (Sung et al., 1998, 2000; Han et al., 1999; Fang et al., 2001). Although the release rates of NA prodrugs from the various delivery devices may be adequately adjusted by using different formulation variables, several adverse attributes such as the surgical procedure required for implants and local irritation of buccal disks may limit their use. Accordingly, it is desirable to develop an injectable and biodegradable formulation with rate-controlling characteristics for NA prodrugs, which can be easily administered once or twice a week and the patient acceptance may, therefore, be improved.

In the present study, the lactide/glycolide-based microspheres loaded with various NA prodrugs were developed. The in vitro release kinetics and in vivo rabbit pharmacokinetics of the microspheres were characterized. In addition, the relationship between prodrug hydrophilicity and in vitro/in vivo release kinetics was assessed. The obtained in vitro data along with the in vivo pharmacokinetic results can be utilized as a useful reference in developing various NA and its prodrug-based injectable formulations.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA) with lactide/glycolide ratio of 50/50 was purchased from Medisorb Technologies (Cincinnati, OH). The manufacturer reported average molecular weight was in the range 40,000–100,000. NA and its various prodrugs, including NAP, NPI, and NDE were synthesized and supplied by the National Defense Medical Center, Taipei, Taiwan. All the other chemicals were purchased from the Sigma Chemical Company (St. Louis, MO) and used as received.

2.2. Preparation of microspheres

The NA prodrug-loaded PLGA microspheres were prepared by an oil-in-water solvent evaporation method modified from the previous reports (Shiga et al., 1996; Yen et al., 2001). The dispersed phase was obtained by dissolving the prodrug and PLGA in methylene chloride, with final concentration of 2% for the prodrug and 2% for PLGA, respectively. The drug–polymer solution was then slowly added into an aqueous solution containing 7 mM sodium lauryl sulfate and 0.5 M sodium chloride with vigorous stirring. The volume of dispersed phase was approximately 5% of the continuous phase. Stirring was maintained at 35 °C for 1.5 h until methylene chloride was completely evaporated. The solidified microspheres were obtained by filtration through 0.2 μm membrane and washed twice with cold water. The microspheres were then collected and vacuumed dried for further studies.

2.3. Microsphere characterization and determination of drug loading

Microspheres were observed and photographed by scanning electron microscopy (SEM) after ion-

sputtering with gold. The mean diameter and distribution of microspheres were calculated from the results of SEM. The loading percentages of the various microspheres were determined by dissolving 10 mg of the dried microspheres in 10 ml of acetonitrile. The resulting solution was then diluted with HPLC mobile phase and injected into HPLC system to obtain the concentration as well as the amount of drug. The loading percentage of drug was calculated by the following equation:

$$\text{Loading percentage} = \frac{\text{the weight of drug in microspheres}}{\text{the weight of microspheres}} \times 100$$

2.4. In vitro drug release studies

The in vitro drug release studies were performed in stopped Erlenmeyer flasks with 400 ml of phosphate buffer (pH 7.4, 0.025 M) as the release medium. The dissolution was conducted in a shaking bath, with temperature and shaking rate of 37 °C and 40 rpm, respectively. At the sampling times of 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, and 96 h, 500 μl samples were removed. The medium removed from the flask was replaced with fresh buffer after sampling. The samples were then filtered through 5 μm membrane and pending for further HPLC analysis.

2.5. In vivo pharmacokinetic studies

The pharmacokinetic studies of various prodrug-loaded microspheres were performed using male New Zealand white rabbits ($n = 3$, weighing between 2.8 and 3.3 kg). Each rabbit was kept in a metabolic cage in an animal room for at least 1 week before the study. Gentle manipulation was maintained through the study. One hour prior to the experiment, each rabbit was held in a restraining device, the ears were shaved with a razor blade and the auricular artery was cannulated. The pharmacokinetic study of NA was performed by dissolving 50 mg of nalbuphine hydrochloride in 10 ml of normal saline and then injected the resulting solution into ear vein. After injection, 3 ml of blood samples were withdrawn from the artery at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h. For the pharmacokinetic studies of the prodrug-loaded microspheres, appropriate amount of microspheres (containing prodrug equivalent to 50 mg nalbuphine hydrochloride in moles) were dispersed in 4% sodium carboxylmethlycellulose solution. The suspension was then injected subcutaneously into the ventral part of left thigh of rabbit. After injection, 3 ml of blood samples were withdrawn from the artery at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 h after injection. The obtained blood samples were immediately centrifuged and the plasma was frozen at −20 °C subjected for further HPLC analysis.

2.6. Partition coefficient measurements

The partition coefficients of NA prodrugs were determined in *n*-octanol/distilled water at 37 °C. Drug concentrations in aqueous phase before and after partition were determined by HPLC. The partition coefficients were calculated as the ratio of the equilibrium drug concentration in *n*-octanol and the equilibrium drug concentration in distilled water.

2.7. HPLC analysis

To determine drug loading, partition coefficient, and in vitro drug release, the drug concentrations were obtained by direct injection of samples into a HPLC system. Pretreatment of plasma samples was needed for the determination of plasma NA concentration. The HPLC system consisted of a pump (HITACHI 655-A40), an autosampler (HITACHI L6000), a UV detector (HITACHI L4000), and an integrator (HITACHI D2500). A normal phase silica column (μ-porasil, 3.9 mm × 300 mm, 10 μm, Waters) was utilized for drug separation. An acetonitrile-pH 3.5 acetate buffer system (80:20) was used as the mobile phase. The flow rate and UV wavelength were 1.5 ml/min and 210 nm, respectively. The resulting retention times of NA, NAP, NPI, and NDE under the chromatographic condition were approximately 9.8, 8.1, 7.4, and 6.3 min, respectively. The drug concentrations were determined by comparing the peak areas to a standard curve established from a series of standards with known concentrations.

The pretreatment procedure for plasma samples included one step of solvent extraction. One milliliter of plasma sample was mixed with 1 ml of pH 9.25 phosphate buffer (0.5 M). The mixture was extracted with 4 ml of *n*-hexane/isoamyl alcohol (9/1) using

a rotary shaker for 20 min. After centrifugation for 10 min at 1800 rpm, 3 ml of the organic layer was taken and dried with nitrogen. The drug containing residuals were then reconstituted with 200 μl of mobile phase and injected into HPLC. The detailed analytical method can be referred elsewhere (Wang, 1992; Ho et al., 1996).

3. Results and discussion

3.1. Microsphere morphology and loading percentage

Fig. 1A–C show the SEM photographs of the various prodrug-loaded microspheres. The microspheres were spherical with smooth surface. From the SEM photographs, the mean diameter of NAP-, NPI-, and NDE-loaded microspheres were 31.6±8.2, 40.1±3.5, and 39.5 ± 7.1 μm, respectively. The results demonstrate that the size of prepared microspheres was uniform and can be administered to rabbits via s.c. injection.

The loading percentages of NAP, NPI, and NDE in microspheres after determination from three batches were estimated to be 25.0 ± 2.5, 30.1 ± 0.7, and 25.7 ± 0.9%, respectively. The loading percentage indicated the weight of PLGA polymers in microsphere was approximately three times of NA prodrugs. In order to minimize the total volume of injected microspheres and to obtain optimal in vitro/in vivo performance, an increase in loading percentage by adjusting formulation and process variables is needed, such as varying the drug/polymer ratio, incorporating adjuvants in polymeric systems, changing the solvent evaporation time and stirring speed (Ogawa et al., 1988; Brannon-Peppas, 1995; Chandrashekar and Udupa, 1996; Crotts and Park, 1998). Accordingly, more studies are underway to characterize the optimal parameters in order to obtain the desired microsphere characteristics with higher prodrug loading percentages.

3.2. In vitro drug release studies

The in vitro release profiles of various NA prodrugs from PLGA-based microspheres are shown in Fig. 2. A significant higher drug release rate was observed for

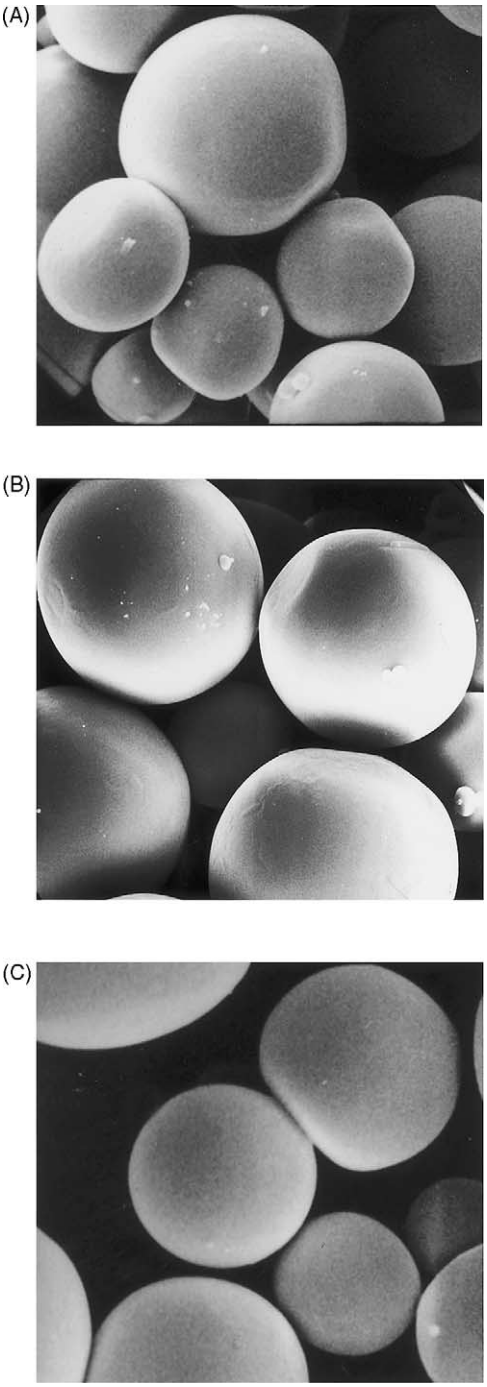

Fig. 1. The SEM photograph of (A) NAP-loaded, (B) NPI-loaded, and (C) NDE-loaded PLGA microspheres.

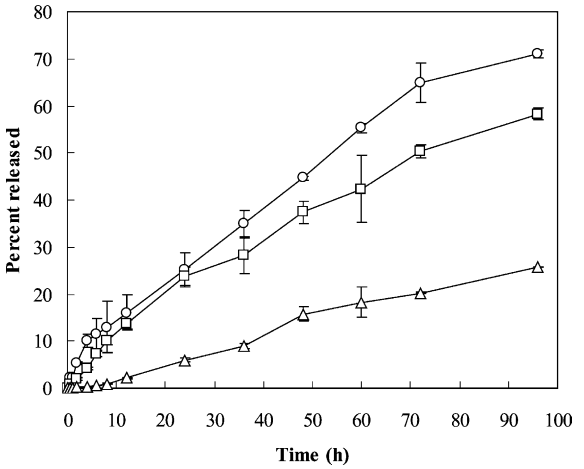

Fig. 2. The percent drug released vs. time profiles for NAP-loaded (○), NPI-loaded (□), and NDE-loaded (△) PLGA microspheres. Mean ± S.E. are presented ($n = 3$).

microspheres loaded with more hydrophilic prodrug, i.e. prodrug with shorter ester side chains. For example, approximately 55.3, 42.3, and 18.3% of drug have released from microspheres loaded with NAP, NPI, and NDE after 60 h. For microspheres loaded with NAP and NPI, faster drug release was observed in the initial 6 h, which may correspond to release of drug on the surface of microspheres. The drug release rates from 8 to 72 h were relatively constant, suggesting that the entrapped prodrug began to release. The release of NDE from microspheres was much slower relative to NAP and NPI. The NDE release was slightly faster after the first 6 h, nevertheless, with only around 25.7% of drug released after 96 h.

For a drug incorporated in PLGA-based microsphere, several possible mechanisms may be involved in the release processes: the drug diffusion from microsphere, matrix erosion resulting from degradation/dissolution of PLGA polymers and the combination of the above two rate processes. Since the initial PLGA degradation is relatively slow and the drug release time (96 h) in this study is much shorter than the degradation lifetime of PLGA (Shih, 1995; Hsu et al., 1996), the degradation/dissolution of PLGA polymer may not be significant. Accordingly, the predominant mechanism controlling the release of NA prodrug was the diffusion mechanism, and the drug release rate may thus be a function of prodrug hydrophilicity/solubility. For a drug incorporated in a spherical matrix, a quantitative model has been developed by Baker and Lonsdale to account for drug release. According to the model, a straight line is expected for the $(3/2)[1-(1-Q)^{2/3}]-Q$ (where Q is the release percentage) versus time plot if drug release from the spherical matrix is based on a diffusion mechanism (Baker and Lonsdale, 1974; Leucuta, 1989). By fitting the data shown in Fig. 2 to the spherical matrix model, the correlation coefficients of 0.96, 0.98, and 0.97 were obtained for the release of NAP, NPI, and NDE, respectively. Both the results of this simple analysis and the above inference suggest that the release of NA prodrugs from these PLGA-based spherical matrices was consistent with a diffusion mechanism.

3.3. *In vivo pharmacokinetic studies*

Fig. 3A and B show the in vivo NA concentration–time profiles after i.v. injection of NA solution as well as s.c. injection of prodrug-loaded microspheres, respectively. Previous studies (Wang, 1992; Yen et al., 2001) have shown that NA prodrugs can be readily hydrolyzed to its parent drug (NA) in the blood stream; accordingly, the plasma NA concentrations are reported in Fig. 3B to demonstrate the pharmacokinetic performance of various prodrug-loaded microspheres. For the pharmacokinetic profile after i.v. injection, the rapid decrease in NA concentration indicated the disposition and elimination of NA was fast. For instance, the concentration of NA 1 h after i.v. injection was approximately 1008 ng/ml and decreased to 23 ng/ml 8 h after injection. The fast decrease in NA concentration suggests frequent injection is needed to maintain plasma NA level. After analyzing the concentration–time profiles by a two compartment model, the elimination rate constant (K) and area under the curve (AUC_∞) after i.v. injection were determined to be $0.52 \pm 0.01\ h^{-1}$ and 4462 ± 354 ng h/ml, respectively.

The pharmacokinetic profiles for all the prodrug-loaded microspheres after s.c. injections showed higher NA concentration in the initial 12 h (Fig. 3B). The concentrations were then gradually decreased and approached zero at 96 h. Prolonged NA concentration–time profiles were observed from 24 to 72 h, suggesting the PLGA-based microspheres were able to sustain the release of NA prodrugs. According to the

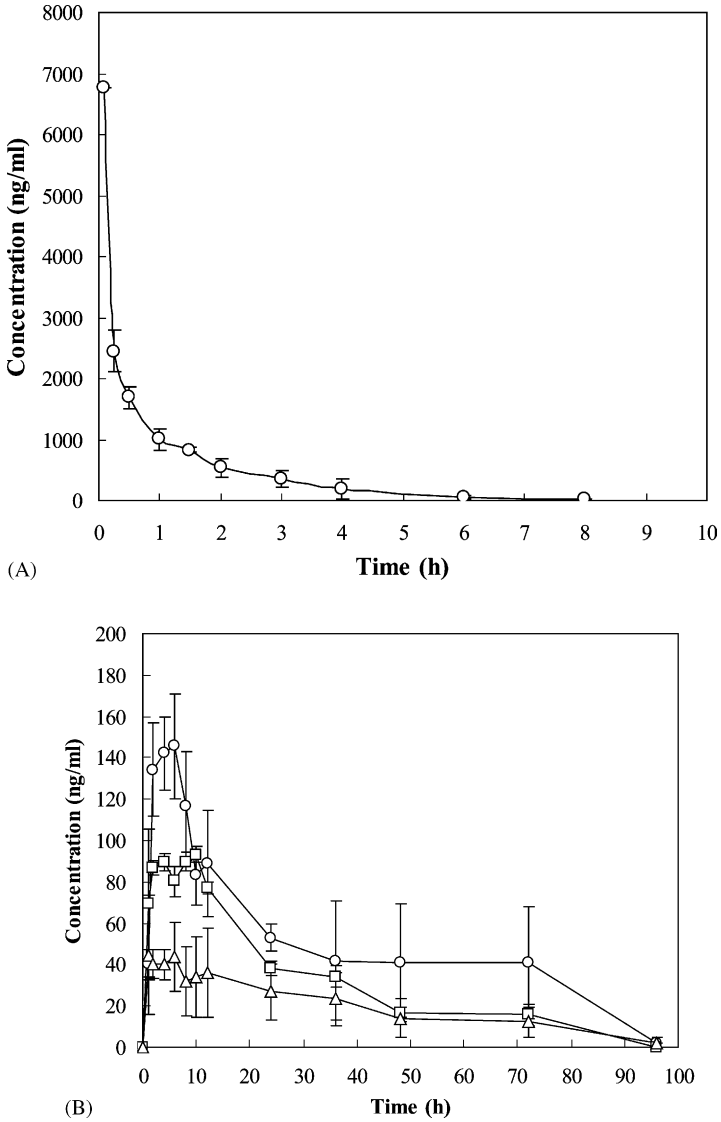

Fig. 3. (A) The in vivo NA concentration vs. time profile after i.v. injection of nalbuphine hydrochloride solution. Mean ± S.E. are presented ($n = 3$). (B) The in vivo NA concentration vs. time profiles after s.c. injection of NAP-loaded (○), NPI-loaded (□), and NDE-loaded (△) PLGA microspheres. Mean ± S.E. are presented ($n = 3$).

previously reported pharmacokinetic/pharmacodynamic relationship of NA in rabbits using the paw pressure model (Yen et al., 2001), the concentration range achieved by NAP- and NPI-loaded microspheres within 24–72 h may produce approximately 10–25% of the full analgesic effect. Although the microspheres had similar characteristics in concentration–time profiles, the magnitudes in NA concentrations for the various microspheres were significantly different. A higher NA concentration–time profile was observed for microspheres loaded with more hydrophilic prodrug. In most cases, the concentrations of NA at each time points were in the order of NAP > NPI > NDE. Table 1 shows the calculated AUC from 0 to 96 h ($AUC_{0–96}$) for the various prodrug-loaded microspheres using the trapezoidal rule. By comparing

Table 1
AUC_{0-96} and relative bioavailability after s.c. injection of various prodrug-loaded microspheres

Drug	AUC_{0-96} (ng h/ml)	Relative bioavailability (%)
NAP-loaded microsphere	4670 ± 1858	104.7
NPI-loaded microsphere	2998 ± 118	67.2
NDE-loaded microsphere	1830 ± 901	41.0

Mean ± S.E. are presented ($n = 3$).

the AUC_{0-96} obtained from s.c. injections of microspheres to the AUC obtained from i.v. injections, the relative bioavailability was calculated (Table 1). The approximate 100% bioavailability for the NAP-loaded microspheres indicates that all the incorporated NAP has released from microspheres 96 h after injection, whereas the 67.2 and 41.0 % relative bioavailability for the NPI- and NDE-loaded microspheres suggests that a substantial amount of drug was still incorporated in the microspheres.

The pharmacokinetic profiles (Fig. 3B) for various prodrug-loaded microspheres correlated well with the in vitro release profiles (Fig. 2). For the release of NAP- and NPI-loaded microspheres. Fig. 3B demonstrates higher NA concentrations in the initial 12 h. The higher initial drug concentrations were due to faster in vivo NAP and NPI release from the injected microspheres, which was in accordance with their faster initial in vitro drug release (Fig. 2). The relative constant plasma NA concentrations from 24 to 72 h shown in Fig. 3B were also consistent with their in vitro drug release profiles shown in Fig. 2, which demonstrated the relative constant in vitro drug release from 24 to 72 h. For the release of NDE-loaded microspheres, the peak phenomenon in the pharmacokinetic profile was not obvious, suggesting the release of drug in vivo was slow in the first several hours. The results also corresponded with the slower initial in vitro release of NDE (Fig. 2). Comparing to the NAP- and NPI-loaded microspheres, its lower plasma NA concentrations at each time point can be reflected well by its slower in vitro release. Accordingly, all the results mentioned-above show that the trend of in vitro release data matched well with the in vivo pharmacokinetic profiles.

Fig. 4 shows the relationship between the AUC_{0-96} of various prodrug-loaded microspheres and the logarithm of partition coefficients of prodrugs. The

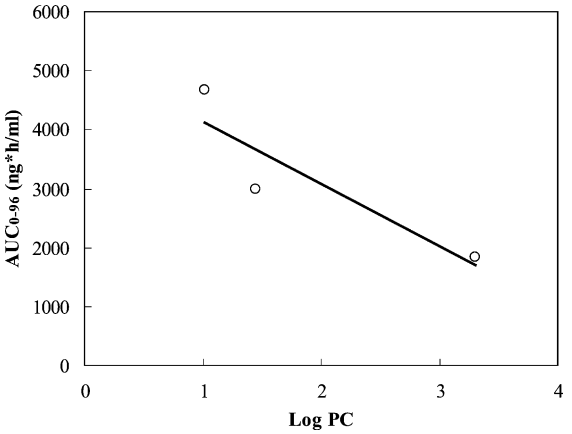

Fig. 4. AUC_{0-96} vs. logarithm of prodrug partition coefficient (log PC) plot for various prodrug-loaded microspheres. The slope and correlation coefficient of the linear regression were −1062 and 0.90, respectively.

negative slope of the linear regression (with correlation coefficient = 0.9) demonstrates the in vivo drug release may not be complete for the more hydrophobic drug. Since the PLGA polymer was hydrophobic and its swelling potential was low, the loaded hydrophobic drug was not able to diffuse easily from the core of microsphere. Thus, the release rate can be very slow after 96 h, resulting in the undetectable plasma concentration and the observed low bioavailability. Due to the relatively hydrophilic nature of NAP, the drug may diffuse more easily from the core of microsphere and thus the bioavailability approached 100%. Nevertheless, the faster initial release and the observed peak suggest higher blood NA fluctuations may be obtained via applying the NAP-loaded macrospheres. Both the lower relative bioavailability for NPI and NDE microspheres as well as higher NA fluctuations for NAP microspheres are untoward properties for the controlled release formulations. As a result, more studies on formulation adjustments as well as studies on choosing an appropriate type of NA prodrug are in progress to obtain the optimal NA pharmacokinetic profiles.

In summary, the in vitro characterization and in vivo pharmacokinetic performance of a series NA prodrug-loaded microspheres were assessed in the present study. The SEM photograph and loading percentage of various microspheres showed the preparation method was adequate and reproducible. Prolonged in vitro drug release profiles from various microspheres were

observed, with faster drug release for microspheres loaded with more hydrophilic prodrugs. The in vitro release data fit well to the Baker and Lonsdales' model, suggesting a diffusional release mechanism. The in vivo concentration–time profiles after s.c. injection of NAP- and NPI-loaded microspheres demonstrated a peak phenomenon in the initial 12 h and showed a sustained NA concentration until 72 h. Higher NA concentrations were obtained for microspheres loaded with more hydrophilic prodrugs. The approximately 100% relative bioavailability indicated most NAP has released from microspheres, whereas the 67.2 and 41.0% relative bioavailability for NPI- and NDE-loaded microspheres suggested that the release of NPI and NDE was not complete after 96 h. The in vitro drug release characteristics were found to correlate well with the in vivo pharmacokinetic profiles. The correlation between the AUC_{0-96} of microspheres and logarithm of partition coefficients indicated the optimal NA pharmacokinetic profile may be obtained by choosing appropriate NA prodrug as well as by adjusting formulation variables. This study demonstrated the feasibility of controlling the release of NA and its prodrugs by using the PLGA-based microspheres.

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