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Controlled release of nalbuphine propionate from biodegradable microspheres: in vitro and in vivo studies

Shu-Yang Yen [a], K.C. Sung [a,*], Jhi-Joung Wang [b], Oliver Yoa-Pu Hu [c]

[a] *Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Jen-Te Hsian, Tainan Hsien, Taiwan 71710, ROC*
[b] *Chi-Mei Medical Center, Tainan Hsien, Taiwan 71710, ROC*
[c] *School of Pharmacy, National Defense Medical Center, Taipei, Taiwan, ROC*

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Abstract

The objective of this work was to assess the in vitro characteristics, in vivo pharmacokinetics and in vivo pharmacodynamics of nalbuphine propionate (NAP)-loaded microspheres. An oil-in-water solvent evaporation method was used to incorporate NAP into poly (d,l-lactide-co-glycolide) (PLGA)-based microspheres. The morphology of the microspheres were evaluated using scanning electron microscopy which showed a spherical shape with smooth surface. A prolonged in vitro drug release profile was observed, with ~71.1% of incorporated drug released in 96 h. The release profile fit well to the Baker and Lonsdale's spherical matrix model, suggesting the release of NAP from microspheres was consistent with a diffusion mechanism. The in vivo pharmacokinetic studies after subcutaneous injection of NAP-loaded microsphere showed a sustained plasma nalbuphine (NA)-time profile, with 100% relative bioavailability comparing to the AUC obtained after intravenous injection. The in vitro release pattern correlated well with the in vivo pharmacokinetic profile. The pharmacodynamic studies evaluated using paw pressure model also showed a prolonged pharmacological response after injection of microspheres. A linear correlation between the percent analgesic effect and the logarithm of plasma NA concentration was obtained, suggesting the pharmacological response can be reflected by plasma drug concentration. This correlation may be utilized for evaluating the pharmacological responses of various NA and its prodrug-based formulations with known plasma NA concentrations.

Keywords: Controlled release; Nalbuphine propionate; Microsphere; Poly (d,l-lactide-co-glycolide)

1. Introduction

Nalbuphine (NA) is a narcotic analgesic used effectively in the treatment of acute as well as 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

* Corresponding author. Tel.: +886-6-2903362; fax: +886-6-2903362.
E-mail address: hkcsung@mail.chna.edu.tw (K.C. Sung).

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(Schmidt and Tam, 1985; Pugh and Drummond, 1987). It is almost as potent as morphine but with relative lower incidence of side effects such as respiration depression and withdrawal symptoms (Schmidt and Tam, 1985; Pugh and Drummond, 1987). Nevertheless, due to its poor oral bioavailability and short elimination half-life, patients always suffer from frequent injections (e.g. every 3–4 h). It is obvious that patient compliance and therapeutic effectiveness in pain management can be improved by maintaining the blood NA concentration. As a result, a series of NA prodrugs, including nalbuphine propionate (NAP), nalbuphine pivalate, nalbuphine enanthate and nalbuphine decanoate were synthesized via esterification of nalbuphine with various carboxylic acids (Wang, 1992). The analgesic effects of those prodrugs in oily formulations have been extended from several hours to several days in animal studies (Wang, 1992). Moreover, various controlled release formulations of nalbuphine prodrugs such as implant and buccal disk have also been developed and evaluated (Sung et al., 1998; Han et al., 1999). The release of nalbuphine prodrugs from biodegradable implant and buccal disk were significantly prolonged and the release rates were influenced by the physicochemical properties of prodrugs as well as the rate-controlling polymers (Sung et al., 1998; Han et al., 1999). Although several formulation variables may be utilized to formulate the implants as well as buccal disks to obtain desired release rates, the surgical procedure in using implants and local irritation of buccal disks may limit their use. Accordingly, it is desirable to develop an injectable and biodegradable controlled release formulation for nalbuphine prodrugs, which can be easily administered and the patient acceptance may therefore be improved.

Biodegradable microspheres have been used and investigated intensively as the injectable controlled release system to prolong the release of therapeutic agents. Among various biodegradable polymers, the aliphatic polyesters based on lactide/glycolide are most widely used and studied. Various therapeutic agents, such as antibiotics, anti-inflammatory drugs, anticancer drugs, steroids, peptides and proteins have been incorporated in the lactide/glycolide 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). Since the physicochemical and pharmacokinetic/pharmacodynamic properties of various therapeutic agents are different, the relationship between in vitro drug release, in vivo pharmacokinetics as well as in vivo pharmacodynamic response of each formulation should be well characterized in order to achieve optimum therapeutic effect.

In the present study, the lactide/glycolide-based microspheres loaded with NAP were developed by an emulsion/solvent evaporation method. The in vitro release kinetics and in vivo rabbit pharmacokinetics of the microsphere were evaluated. Finally, the pharmacological response of rabbits after applying the microspheres was assessed by using paw pressure test and the pharmacokinetic/pharmacodynamic relationship was also established. The obtained in vitro data along with the pharmacokinetic/pharmacodynamic relationship can be utilized as a useful reference in developing various NA and its prodrug-based 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, USA). The manufacturer reported molecular weight range of the polymer was 40 000–100 000 (weight average molecular weight). NA and NAP were synthesized and supplied by National Defense Medical Center, Taipei, Taiwan. All the other chemicals were purchased from the Sigma Chemical Company (St. Louis, MO, USA) and used as received.

2.2. Preparation of microspheres

An oil-in-water solvent evaporation method modified from that previously reported (Shiga et al., 1996) was used to prepare the NAP-loaded

PLGA microspheres. The dispersed phase was obtained by dissolving NAP and PLGA in methylene chloride, with the final concentration of 2% for NAP and 2% for PLGA, respectively. The drug–polymer solution was then added dropwise into an aqueous solution containing 0.5 M sodium chloride and 7 mM sodium lauryl sulfate with vigorous stirring. The volume of dispersed phased was $\sim 5\%$ of the continuous phase. Stirring was continued at 35°C for 1.5 h until methylene chloride was completely evaporated. The solidified microspheres were collected by filtration through a 0.2 μm membrane and washed twice with cold water. The wetted microspheres were then vacuum 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 also obtained from the results of SEM. To determine loading percentage of NAP in the microspheres, 10 mg of the dried microspheres were dissolved in 10 ml of acetonitrile. The resulting solution was then diluted with HPLC mobile phase and injected into HPLC system for determination of the concentration as well as the amount of NAP. The drug loading percentage was calculated as follows:

Loading percentage

= (the weight of drug in microspheres/ the weight of microspheres)*100

2.4. In vitro drug release studies

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

2.5. In vivo pharmacokinetic and pharmacodynamic studies

Male New Zealand white rabbits weighing between 2.8 and 3.3 kg were used in the study. Each rabbit was housed in an individual, well ventilized metabolic cage in an animal room for at lease 1 week before the study. Gentle manipulation was maintained all through the study. One hour prior to the experiment, each rabbit was held in a restraining device individually, the ears were shaved with a razor blade and the auricular artery was cannulated. Blood samples were collected from the artery at various sampling times after injection of drug. For the pharmacokinetic study of NA, 50 mg of nalbuphine hydrochloride was dissolved in 10 ml of normal saline and the resulting solution was injected into ear vein. Blood samples (3 ml) were withdrawn from the artery at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 h after injection. For the pharmacokinetic study of NAP-loaded microsphere, 210 mg of microsphere (containing NAP 52.5 mg; equivalent to nalbuphine hydrochloride 50 mg in moles) were dispersed in normal saline containing 4% sodium carboxylmethlycellulose as the suspending agent. The suspension was then injected subcutaneously into the ventral part of left thigh of rabbit ($n = 3$). Blood samples (3 ml) were withdrawn from the artery at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96 h after injection. The samples obtained from the pharmacokinetic studies were immediately centrifuged and the plasma was frozen to -20°C pending for further HPLC analysis.

The pharmacodynamic studies were performed using a paw pressure model (Hu et al., 1996). In brief, a hand-held pressure algometer (Somedic Sales AB, Sweden) with a pressure sensitive strain gauge at the tip was used to measure the pressure-pain threshold. A pen recorder (No. 805721, Central Denshikeisoku, Japan) was used to monitor the pressure change during testing. For each measurement, the right hind paw of rabbit was gently put on the ground, while pressure was exerted by

a weight between 0 and 1999 kPa on the algometer with a speed of 40 kPa/s. The pressure-pain threshold was taken at the point which the rabbit made a vigorous attempt to remove the paw and the pressure reading was taken from pen recorder. Prior to each set of paw pressure experiment, the control values were obtained at 60, 30, and 15 min before the experiment. The experimental values from various sampling times were then compared with the average of baseline values; each animal served as its own control. A predetermined cut-off pressure of 60% above baseline values was set for each animal (Hu et al., 1996). This cut-off pressure was considered to be the maximum pressure; that is, under this cut-off pressure, the pressure-pain thresholds of rabbits in the paw pressure test were all returned to the pretest values. Accordingly, the pharmacological response (percent analgesic effect) of the applied analgesics was expressed as follows:

Percent analgesic effect

= (the observed paw pressure threshold

− baseline value)

× /(the cut-off pressure − baseline value)*100.

The pharmacological response was measured immediately after withdrawal of blood samples. The sampling times were 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8 h after intravenous injection of NA; 0, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96 h after subcutaneous injection of NAP-loaded microspheres.

2.6. HPLC analysis

For the drug loading test and in vitro release studies, the drug concentrations were obtained by direct injection of samples into a HPLC system; whereas pretreatment procedures were required before injection of the plasma samples into HPLC. The chromatographic 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, while 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. Under these chromatographic conditions, the retention times of NA and NAP were ~9.6 and 7.8, respectively. The drug concentrations were determined by measuring the peak area and compared it with the peak area of known standards.

The pretreatment procedures for analyzing plasma samples included one step of solvent extraction. In brief, 1 ml of blood sample was mixed with 1 ml of pH 9.25 phosphate buffer (0.5 M). The resulting mixture was extracted with 4 ml of *n*-hexane/isoamyl alcohol (9/1) on a rotary shaker for 20 min. After centrifugation under 1800 rpm for 10 min, 3 ml of the organic layer was taken and dried under nitrogen. The residual were reconstituted with 200 μl of mobile phase and injected into HPLC system. More details concerning the analysis method can be referred to elsewhere (Wang, 1992; Ho et al., 1996).

3. Results and discussion

3.1. Microsphere morphology and loading percentage

Fig. 1 shows the SEM photograph of NAP-

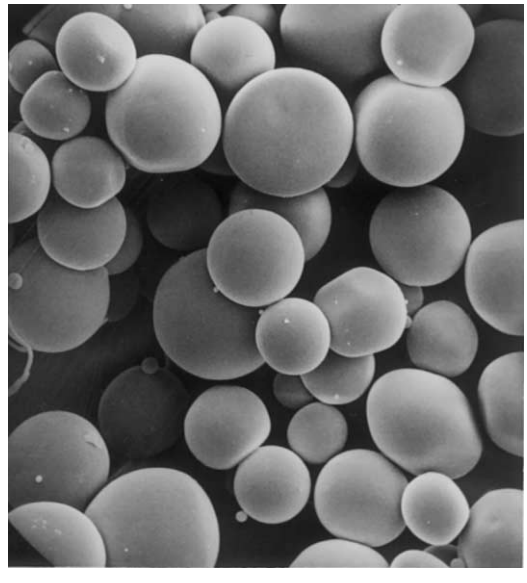

Fig. 1. Scanning electron microscopy photograph of NAP-loaded PLGA microspheres.

loaded microspheres. The microspheres were spherical in shape with a smooth surface. From various SEM photographs, the mean diameter and its distribution of the microsphere were 31.6 and 8.2 μm, respectively. The results demonstrate that the size of the prepared microspheres was uniform and appropriate for administration to rabbit via subcutaneous injection.

The loading percentage of NAP after determination from three batches was estimated to be $25.0 \pm 2.5\%$, indicating that the preparation method was reproducible. Previous reports (Ogawa et al., 1988; Brannon-Peppas, 1995; Chandrashekar and Udupa, 1996) have shown that several formulation as well as process variables may affect the loading percentage, including drug/polymer ratio, incorporation of adjuvants in polymeric systems, solvent evaporation time and stirring speed. An increase in loading percentage and decrease in total volume of microsphere administered would be desirable to apply those microsphere via subcutaneous injection. Accordingly, more studies are underway to characterize the optimum parameters in order to obtain the desired microsphere characteristics with higher NAP loading percentages.

3.2. In vitro drug release studies

Fig. 2 shows the in vitro release profile of NAP from PLGA-based microspheres. Higher release rates were observed in the initial 6 h, which may correspond to release of drug on the surface of microspheres. From 8 to 72 h, the drug release rates were relatively constant, suggesting that the entrapped NAP began to release. The drug release became slower after 72 h and $\sim 71.1\%$ of the loaded drug released at 96 h. The prolonged NAP release from PLGA microsphere may be attributed to the hydrophobic nature of NAP as well as PLGA polymer. Two possible mechanisms may involve in the release of NAP from PLGA microspheres: the dissolution/diffusion of drug from the spherical matrices as well as the matrix erosion resulting from degradation/dissolution of PLGA polymers. Since the bulk degradation of PLGA is trivial during the initial period, moreover, the drug release time (96 h) in this study is considerable

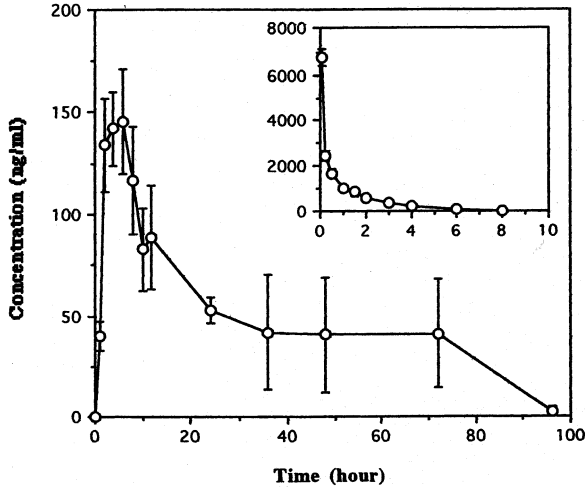

Fig. 2. The percent drug released versus time profiles for NAP-loaded PLGA microspheres. Mean ± standard error are presented ($n = 3$).

shorter than the degradation lifetime of PLGA (Shih, 1995; Hsu et al., 1996), the polymer backbone may retain its integrity without significant degradation/dissolution. As a result, drug release from the microsphere may be attributed to the diffusion mechanism. According to the model developed by Baker and Lonsdale, for a drug incorporated in a spherical matrix, 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 observed data shown in Fig. 2 to the spherical matrix model (up to 60% of total drug release), a correlation coefficient of 0.96 was obtained. This simple analysis of the release data, within the limitation of the model, suggesting that the release of NAP from these PLGA-based spherical matrices was consistent with a diffusion mechanism.

3.3. In vivo pharmacokinetic studies

Fig. 3 shows the in vivo NA concentration–time profile after subcutaneous injection of NAP-loaded microspheres as well as intravenous injection of NA solution (see insert in Fig. 3). Previous studies (Wang, 1992) have shown that

NA prodrugs can be soon converted to its parent drug (NA) in the blood stream; as a result, the plasma NA concentrations are reported here in the pharmacokinetic study of NAP-loaded microsphere. For the pharmacokinetic profile after intravenous injection, the rapid decrease in NA concentration suggests the elimination of nalbuphine was fast. For example, the concentration of NA 1 h after intravenous injection was ~ 1008 ng/ml and it decreased to 23 ng/ml 8 h after injection. The fast elimination of NA indicates the need for frequent injection in pain management. After fitting the concentration–time profiles by a two compartment model, the elimination rate constant (Kel) and area under the curve (AUC8) after intravenous injection were determined to be 0.52 ± 0.01 (h^{-1}) and 4462 ± 354 (ng•h/ml), respectively.

The pharmacokinetic profile of NAP–loaded microsphere after subcutaneous injection showed higher NA concentration in the initial 12 h; with a peak concentration of 145.5 ng/ml at 6 h. The drug concentrations decreased after 6 h and showed a sustained NA concentration from 24 to 72 h. The drug concentration gradually decreased after 72 h and approached zero at 96 h. The calculated AUC from 0 to 96 h for the microspheres using the trapezoidal rule was 4670 ± 1858 (ng•h/ml). By comparing the AUC obtained from subcutaneous injection of NAP-loaded microsphere to the AUC obtained from intravenous injection, a relative bioavailability of 105% was observed. This higher value of bioavailability may be attributed to the variations associated with in vivo studies; nevertheless, the $\sim 100\%$ bioavailability suggests that all the incorporated NAP has released from microsphere 96 h after injection.

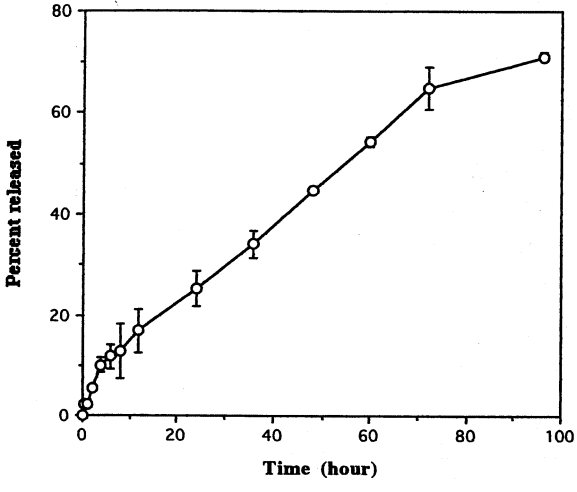

Fig. 3. The in vivo NA concentration versus time profile after subcutaneous injection of NAP-loaded PLGA microsphere. Insert shows the concentration–time profile after intravenous injection of NA solution. Mean ± standard error are presented ($n = 3$).

The pharmacokinetic profile of NAP-loaded microsphere (Fig. 3) correlated well with the in vitro release profile (Fig. 2). Fig. 3 demonstrates higher NA concentrations in the initial 12 h and a peak concentration at 6 h. This higher initial drug concentration was due to faster in vivo drug release from the injectable microspheres, which is consistent with the faster in vitro drug release in the initial 6 h (Fig. 2). The peak concentration phenomenon (Fig. 3) and faster initial release (Fig. 2) were both characteristics of matrix formulation. The constant plasma NA concentration from 24 to 72 h shown in Fig. 3 was also in accordance with the in vitro drug release profile shown in Fig. 2, which demonstrated a relative constant in vitro release from 24 to 72 h. The decrease in plasma NA concentration after 72 h suggests a decrease in vivo release rate of NAP from microspheres. This phenomenon was also reflected in Fig. 2, demonstrating slower in vitro drug release after 72 h. All those above results show that the in vitro release data correlated well with the in vivo pharmacokinetic profile.

3.4. Pharmacodynamic studies and pharmacokinetic/pharmacodynamic relationship

Fig. 4 shows the in vivo pharmacological response (analgesic effect) versus time profile for rabbits after subcutaneous injection of NAP-loaded microsphere as well as intravenous injection of NA solution. Similar to the pharmacokinetic profile, a rapid decrease in pharmacological response was observed via injection

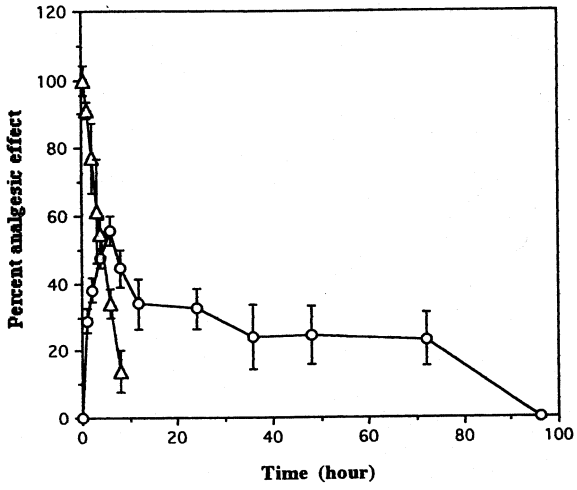

Fig. 4. The percent analgesic effect versus time profile after subcutaneous injection of NAP-loaded PLGA microsphere (○) and after intravenous injection of NA solution (△). Mean ± standard error are presented ($n = 3$).

of NA, demonstrating that the analgesic effect was not able to be maintained after intravenous injection. For the pharmacological response versus time profile after subcutaneous injection of microsphere, the analgesic effect increased initially, with a peak at 6 h and decreased afterwards. The percent analgesic effect was maintained within 20–40% from 12 to 72 h, indicating that the sustained plasma NA concentration may produce a prolonged pharmacological response. The percent analgesic effect decreased with time after 72 h, which may be attributed to the drop in plasma NA concentrations after 72 h. Those pharmacokinetic and pharmacodynamic observations suggest that the plasma NA concentration correlated well with the pharmacological response.

Fig. 5 shows the percent analgesic effect (in the range 20–80% analgesic effect) versus logarithm of plasma NA concentration plot obtained from intravenous injection of NA as well as subcutaneous injection of microsphere. The slope and correlation coefficient of linear regression was 43.0 and 0.97, indicating that the percent analgesic effect positively correlated with logarithm of plasma NA concentrations. The results suggest that the paw pressure model may be used to explore the analgesic effect of various formulations of NA and its prodrugs. The established pharmacokinetic–pharmacodynamic relationship may also be utilized to obtained the pharmacological response of various dosage forms of NA and its prodrug once the plasma NA concentrations are known.

In summary, the in vitro characterization and in vivo pharmacokinetic–pharmacodynamic evaluation of NAP-loaded microsphere were performed in the present study. The SEM photograph and loading percentage (25.0 ± 2.5) showed the preparation method was appropriate and reproducible, nevertheless, the loading percentages can be further increased by adjusting formulation as well as process variables. A prolonged in vitro drug release from microsphere was observed, with a sustained effect for ~ 72 h. The in vitro release data fit well to the Baker and Lonsdales' model, suggesting a diffusional release mechanism. The in vivo concentration–time profile after subcutaneous injection of microsphere demonstrated a peak phenomenon in the initial 6 h and showed a sustained NA concentrations until 72 h. The in vitro drug release data

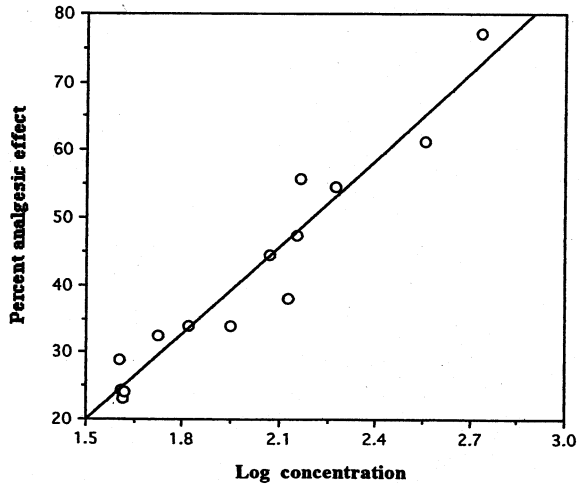

Fig. 5. The percent analgesic effect (in the range 20–80% of analgesic effect) versus logarithm of plasma concentration plot. Data were obtained from the intravenous injection of NA and subcutaneous injection of NAP-loaded microspheres. The slope and correlation coefficient of the linear regression were 43.0 and 0.97, respectively.

correlated well with the in vivo concentration–time profile. The percent analgesic effect observed from paw pressure test also demonstrated a prolonged pharmacological response until 72 h. A correlation was observed between pharmacological response and logarithm of plasma NA concentration, suggesting the analgesic effect was affected the plasma NA concentration. The relationship demonstrated that the present paw pressure model was effective in reflecting the pharmacokinetic profile of NA; it may be also utilized in the future to evaluate the pharmacological response for various NA and its prodrug-based dosage forms from the pharmacokinetic profile. The present work demonstrated the feasibility of controlled delivery of NA and its prodrug utilizing PLGA-based microsphere.

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