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pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A

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Abstract

The purpose of this work was to improve the oral bioavailability of cyclosporine A (CyA) by preparation the CyA-pH sensitive nanoparticles. The CyA-pH sensitive nanoparticles were prepared by using poly(methacrylic acid and methacrylate) copolymer. The characterization and the dispersion state of CyA at the surface or inside the polymeric matrices of the nanoparticles were investigated. The in vitro release studies were conducted by ultracentrifuge method. The bioavailability of CyA from nanoparticles and Neoral microemulsion was assessed in Sprague–Dawley (SD) rats at a dose of 15 mg/kg. The particle size of the nanoparticles was within the range from 37.4 ± 5.6 to 106.7 ± 14.8 nm. The drug entrapped efficiency was very high (from 90.9 to 99.9%) and in all cases the drug was amorphous or molecularly dispersed within the nanoparticles polymeric matrices. In vitro release experiments revealed that the nanoparticles exhibited perfect pH-dependant release profiles. The relative bioavailability of CyA was markedly increased by 32.5% for CyA-S100 nanoparticles ($P < 0.05$), and by 15.2% and 13.6% for CyA-L100-55 and CyA-L100 nanoparticles respectively, while it was decreased by 5.2% from CyA-E100 nanoparticles when compared with the Neoral microemulsion. With these results, the potential of pH-sensitive nanoparticles for the oral delivery of CyA was confirmed.

Keywords: Cyclosporine A; pH-sensitive nanoparticles; Poly(methacrylic acid and methacrylate) copolymer; Neoral microemulsion; In vitro release; Oral bioavailability

1. Introduction

Cyclosporine A (CyA), a highly lipophilic undecapeptide is commonly used as immunosuppressant to prevent allograft rejection in various organ transplantations such as kidney, liver, heart, lung and pancreas (Matzke and Luke, 1988). The drug has also been shown to be effective in the treatment of systemic and local autoimmune disorders (Borel and Gunn, 1986; Richardson and Emery, 1995). However, in spite of the great therapeutic interest of this drug, the

Abbreviations: CyA, cyclosporine A; CyD, cyclosporine D; SD rats, Sprague–Dawley rats; QESD, an adaptation of the quasi-emulsion solvent diffusion technique; CyA-E100 nanoparticles, cyclosporine A Eudragit® E100 nanoparticles; CyA-L100-55 nanoparticles, cyclosporine A Eudragit® L100-55 nanoparticles; CyA-L100 nanoparticles, cyclosporine A Eudragit® L100 nanoparticles; CyA-S100 nanoparticles, cyclosporine A Eudragit® S100 nanoparticles

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bioavailability after oral dosing is low (10–60%) with a higher variability (Lindholm et al., 1988). The incomplete and variable bioavailability of CyA has been attributed to its high molecular weight, high lipophilicity, low intestinal permeability (Ismailos et al., 1991; Tjai et al., 1991), as well as the cytochromes involved in its biotransformation (CYP3A) are present in the liver and the intestinal mucosa. Simultaneously, the presence of the exorption pump P-glycoprotein prevents drug accumulation inside the cells (Wacher et al., 1998).

CyA is dispensed as an oily solution (Sandimmune®) or microemulsion (Sandimmune® Neoral) containing a high concentration of polyoxyethylated castor oil (Cremophor EL®). However, in spite of microemulsion enhances CyA absorption and reduces inter- and intra-subject variability (Kovarik et al., 1994), Cremophor EL® has been reported to be nephrotoxic (Luke et al., 1987) and may cause anaphylactic reactions (Cavanak and Sucker, 1986).

In order to overcome the difficulties as described above, and to improve the therapeutic efficacy of CyA and decrease its side effects, many research efforts have been made. Alternative dosage forms have been suggested including incorporation of the drug into particulate carriers (Ruxandra et al., 2001; Varela et al., 2001; El-Shabouri, 2002). Particulate formulations have previously been proven to be an efficient approach to achieve better pharmacokinetic profiles and even to increase the oral bioavailability of several drugs (McClean et al., 1998). Consensus has not been reached on the mechanisms of particle uptake by intestinal epithelia. Most evidence suggests that the favoured site for uptake is the PP lympho-epithelial M cell (Jani et al., 1992). However, paracellular transport of particles has been favoured by others (Aprahamian et al., 1987) while there is also evidence for particle endocytosis by intestinal enterocytes (Kreuter et al., 1989). Indeed, it was recently reported that particles in the size range 40–120 nm were translocated both transcellularly and paracellularly (Mathiowitz et al., 1997). In addition to the potential for enhancing drug bioavailability via particle uptake mechanisms, particulate oral delivery systems can protect labile macromolecules from stomach acid and from the first-pass metabolism in the gastrointestinal tract. Likewise, particulate formulations also can increase transit times than larger dosage forms and can increase the local concentration gradient across absorptive cells. Thereby enhancing local and systemic delivery or both free and bound drugs across the gut (Kreuter et al., 1989).

Previous studies have described the use of pH-sensitive polymers such as hydroxypropylmethylcellulose phthalate (Klipstein et al., 1983), Eudragit® L100 and Eudragit® S100 (Morishita et al., 1993; Jaeghere et al., 2000) or cellulose acetate phthalate (Lin et al., 1991) to encapsulate antigens or proteins for oral administration. These pH-sensitive particles are matrix-type dispersed systems. Release of the highly dispersed drug at a specific pH within the gastrointestinal tract, as close as possible to the absorption window of the drug, is expected to increase the probability of drug absorption and to minimize the first-pass metabolism of drug.

On the basis of the above mentioned considerations, it was thought plausible to combine the advantages of nanoparticles as oral delivery systems with the benefits of the pH-sensitive property. Thus, the objective of the present study was to develop and to characterize CyA as pH-sensitive nanoparticles as an attempt for improving its gastrointestinal uptake and its overall bioavailability. Eudragit® E100, Eudragit® L100, Eudragit® L100-55 and Eudragit® S100 were selected as pH-sensitive polymers. The bioavailability and pharmacokinetics of CyA from these nanoparticles in comparison with the currently available microemulsion pre-concentrate formulation (Sandimmune® Neoral) were assessed in SD rats.

2. Materials and methods

2.1. Materials

CyA was kindly donated by North China Pharmaceutical Group Co. Ltd. (China). Sandimmune® Neoral capsule was purchased from Novartis (Switzerland). The pH-sensitive poly(methacrylic acid-*co*-methyl methacrylate) copolymers (Eudragit® L100-55, Eudragit® L100, Eudragit® S100) and cationic polymer with a dimethylaminoethyl ammonium group (Eudragit® E100) were gifts from Röhm (Darmstadt, Germany). Poloxamer 188 (PF-68) was purchased from Sigma (USA). All other reagents

were of analytical grade, except those for HPLC assay which were of HPLC grade. Sprague–Dawley (SD) rats were obtained from Animals Center of Peking University Health Science Center. All of the animal experiments adhered to the principles of care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center.

2.2. Nanoparticle preparation

The pH-sensitive nanoparticles with theoretical drug loading of CyA at 20.0% (mg of CyA/100 mg of carrier) were prepared using an adaptation of the quasi-emulsion solvent diffusion technique (QESD) (Kawashima et al., 1989). CyA and the pH-sensitive polymer were co-dissolved at room temperature in anhydrous ethanol.

The solution was quickly injected into the aqueous solution of Poloxamer 188 at room temperature. During injection, the mixture was stirred at 500 rpm. The solution immediately turned into a pseudo-emulsion of the drug and polymer–ethanol solution in the external aqueous phase. The counter-diffusion of water and ethanol in and out of the emulsion micro-droplets, respectively, and the gradual evaporation of the organic solvent determined the in situ precipitation of the polymer and the drug, forming matrix-type nanoparticles. Ethanol residues were left to evaporate with stirring at 500 rpm in a 60 °C water bath.

2.3. Particle size analysis

The nanoparticles were dispersed in ultrapure water and the mean particle size was measured using a 90Plus Goniometer (Brookhaven Instruments, Holtsvile, NY, USA) which works on the principle of dynamic light scattering. Each value resulted from a triplicate determination.

2.4. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) analysis was performed using a JEM-1230 instrument (JEOL Co. Tokyo, Japan). TEM samples were diluted with ultrapure water and stained with a 2% solution of osmium tetraoxide before analysis.

2.5. Evaluation of drug content and entrapment efficiency

An appropriate volume of the suspension of nanoparticles containing 0.04% CyA was filtered through a 0.45 μm filter (HVLP Durapore®, Millipore, Switzerland) to remove non-soluble polymer residues and CyA microcrystals. Then, the filtered suspension was ultracentrifuged (120 000 × *g* for 60 min) and the supernatant was sampled. The concentration of CyA in the filtered suspension and in the supernatant were determined by a reversed-phase HPLC method.

The HPLC system consisted of an isocratic pump (HP1100, HP, USA), UV–vis detector (HP1100, HP, USA) set at 210 nm. The chromatographic column used was a ZORBAX SB-C18 (5 μm in 4.6 mm × 250 mm, Agilent, USA) thermostated at 70 °C. The mobile phase consisted of acetonitrile/methanol/water (58:21:21) and the flow rate was 1.5 ml/min (Malmary et al., 1995).

The yield of nanoparticles was calculated as the percentage of CyA in the filtered suspension relative to the theoretical drug amount added. The drug entrapment efficiency was expressed as percentage of the CyA difference between the filtered suspension and the supernatant relative to the total amount of CyA in the filtered suspension. The drug loading was estimated as the ratio of CyA incorporated to the theoretical carrier amount added.

2.6. X-ray powder diffraction (XRD) studies

X-Ray diffraction experiments were performed in a Rigaku (Japan) X-ray diffractometer using Cu Kα rays with a voltage of 40 kv and a current of 30 mA. Samples were scanned from 2.6 to 40° 2θ.

2.7. Surface analysis

The presence of CyA at or near the surface of the nanoparticles was investigated by X-ray photoelectron spectroscopy (XPS Axis Ultra, Kratos Co. UK). Taking into account that an elemental composition in N (N (%)) of 12.9% corresponds to 100% of CyA at or near the surface. Thus, the percentage of CyA at or near the surface in the nanoparticles (surface % CyA) can be calculated according to following equation:

Surface % CyA = N (%) × 100/12.9 (Ruxandra et al., 2001).

2.8. In vitro release studies by ultracentrifuge

The suspension of nanoparticles or Neoral microemulsion containing 0.25 mg CyA were dispersed in a flask filled with 25 ml of 0.1 M phosphate buffer containing 0.05% SDS at different pH values. Then the suspension was incubated in a shaking incubator thermostated at 37 °C. Thirty minutes after, an appropriate volume of the suspension was sampled from the flask and ultracentrifuged (120 000 × *g* for 60 min) (Ruxandra et al., 2001). The amount of CyA in the supernatant was determined by RP-HPLC.

2.9. Bioavailability study

For this study, 240 male SD rats weighing 250 ± 20 g were selected and randomly divided into five groups (48 animals each). They were maintained in cages for 48 h before the experiments, with a preserved 12:12 h dark/light cycle and free access to standard food and tap water. Before treatment, the animals were fasted overnight and had access to water ad libitum.

Sandimmune® Neoral capsules were dissected and rinsed with distilled water to make Neoral microemulsion adjusted CyA concentration to 1.5 mg/ml. Four kinds of CyA-pH sensitive nanoparticles were prepared under experimental conditions adjusted containing CyA 1.5 mg/ml. A single CyA oral dose (15 mg/kg) was given to each animal by gavage between 09:00 and 10:00 h to avoid chronopharmacokinetic effects (Malmary et al., 1995). Group 1 received Neoral microemulsion and groups 2–5 received four kinds of CyA-pH sensitive nanoparticles respectively. 0.6 ml whole blood samples were withdrawn by venepuncture in the eyepit from ether anaesthetized animals at 0.5, 1, 2, 3, 5, 7, 9, 12, 24, 36, 48 and 72 h post-treatment. Each rats was punctured only twice at an interval of at least 6 h. The samples were collected in heparinized vials, thoroughly mixed and frozen at −20 °C until analysed.

Frozen samples were reequilibrated to room temperature by incubating for 20 min in a 37 °C water bath. 0.5 ml blood samples were taken and submitted to the extraction procedure. Internal standard, cyclosporine D (CyD) (50 μl of 60 μg/ml solution in methanol) and hydrochloric acid (1 ml of 180 mmol/L solution) were added to a 0.5 ml sample of blood. This mixture was vortexed for 1 min, and then diethylether (5 ml) were added. After horizontal shaking for 10 min and centrifugation at 1000 × *g* for 10 min, the ether layer was collected, followed by the addition of sodium metabisulfite (2.5 ml of 1% solution) and sodium hydroxide (1 ml of 95 mmol/L solution). After horizontal shaking and centrifugation, the ether layer was transferred into a clean tube and evaporated to dryness under nitrogen at 40 °C. The residue was redissolved in 120 μl of acetonitrile–water (70:30) solution and 1 ml of *n*-hexane, by vortexing for 2 min. After centrifuged (1000 × g, 8 min), the *n*-hexane layer was discarded and 1 ml of *n*-hexane was added again. To repeat previous procedure, the lower acetonitrile-water (70:30) solution transferred into the inserts. The tubes were then tightly capped and 40 μl aliquots were injected onto the HPLC column for analysis (Malmary et al., 1995).

CyA concentrations in whole blood were determined by a reversed-phase HPLC method which HPLC conditions as mentioned above. CyA (in methanol solution, 1 mg/ml) was added to drug-free pooled whole blood to provide concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 μg/ml, in the presence of internal standard CyD (6 μg/ml). Each blood standard was then taken through the sample preparation procedure described above. Quantitation was done by determination of peak-area ratio of CyA/CyD against the drug concentrations. The concentrations of unknown samples were determined by using the linear regression line (unweighted) of the concentration of the calibration standard versus peak-area ratios. Under these conditions, percentage recovery of CyA in the blood samples was 100.84 ± 3.86% and the within-day and between-day coefficients of variation did not exceed 5% for the same batch of reagents. The recovery obtained for the rest of sample matrices amounted on average to 109.76 ± 4.92% and 100.58 ± 4.74%, respectively for CyA and CyD as compared to drug solutions in the absence of blood. The limit of quantification was 10 ng and no interference of the compounds used in Neoral microemulsion and pH-sensitive nanoparticles were observed.

2.10. Pharmacokinetic analysis

The pharmacokinetic parameters were estimated by noncompartmental methods. The zero-order moment area under the curve (AUC) and the first-order moment mean residence time (MRT) were determined by standard methods applying the linear trapezoidal rule. The differences found between pharmacokinetic parameters in both groups were statistically evaluated by the *t*-test. Differences were considered to be significant at a level of $P < 0.05$.

3. Results and discussion

3.1. Preparation and characterization of the pH sensitive nanoparticles

The main advantage of the QESD technique are the avoidance of toxic organic solvents, commonly used in micro- and nanoparticle solvent evaporation techniques, which increases the possibility of modifying particle morphology by choosing the agitation speed, the polymer concentration in the initial ethanol solution as well as the volume and injection rate of the solvent (Kawashima et al., 1989).

CyA-loaded nanoparticles using different pH-sensitive polymers were prepared. Such variables could influence the nanoparticles characterization (Table 1). The drug entrapped efficiency were approximately 99% and the production yields were over 96% except for CyA-E100 nanoparticles (89.8%). The final CyA loading values were approximately as high as 20%. The high entrapped efficiency and production yield are due to the hydrophobic character of CyA and pH-sensitive polymers. Since CyA is a very poorly water soluble drug, it was preferentially partitioned in the organic phase of the emulsion and consequently, small amount of the drug is lost in the aqueous phase. Furthermore, it has been reported that, CyA is less soluble in Poloxamer 188-water mixtures than in water alone at temperatures between 20 and 37 °C (Molpeceres et al., 1996). Thus, the presence of Poloxamer 188 in the formulation plays an important role not only as a co-surfactant for nanoparticle stability but also in achieving higher CyA entrapped efficiency.

The particle sizes of nanoparticles were listed in Table 1. The narrow size distributions were observed and all of which exhibited standard normal distribution. With various pH-sensitive polymers, the particle sizes of these nanoparticles were different from 37.4 to 106.7 nm.

3.2. Morphological properties of the pH sensitive nanoparticles

The shape and surface characteristics of nanoparticles were shown in Fig. 1. The nanoparticles were non-aggregated solid spherical particles, whereas the surface of those nanoparticles was not smooth. The possible reason for this was the adsorption of CyA molecules on the surface of those nanoparticles, which have been proven by the experiments of surface analysis and in vitro release experiments.

3.3. Physical state of CyA in the pH sensitive nanoparticles

X-ray analysis was performed in order to establish the physical state of both the polymer and drug in

Table 1
Characteristics of CyA-pH sensitive nanoparticles; mean ± S.D. ($n = 3$)

Formulation	Yield (%)±S.D	Drug entrapped efficiency (%) ± S.D	Drug loading (%) ± S.D	Mean particle diameter (nm) ± S.D
CyA-E100 nanoparticles	96.7 ± 0.52	90.9 ± 0.05	18.9 ± 0.22	98.7 ± 13.4
CyA-L100 nanoparticles	89.8 ± 0.03	98.2 ± 0.08	19.9 ± 0.14	106.7 ± 14.8
CyA-L100-55 nanoparticles	96.3 ± 0.20	99.9 ± 0.01	20.6 ± 0.20	60.4 ± 6.0
CyA-S100 nanoparticles	98.8 ± 0.60	99.9 ± 0.05	20.3 ± 0.12	37.4 ± 5.6

Yield (%) $= \frac{C_c \times V}{W_c} \times 100\%$ Drug entrapped efficiency (%) $= \frac{(C_c \times V) - (C_f \times V)}{C_c \times V} \times 100\%$ Drug loading (%) $= \frac{(C_c \times V) - (C_f \times V)}{W_e} \times 100\%$ C_c: the concentration of CyA in the filtered suspension of CyA-pH nanoparticles; V: the volume of the filtered suspension of CyA-pH nanoparticles; C_f: the concentration of CyA in the supernatant of CyA-pH nanoparticles; W_c: the theoretical amount added of CyA; W_e: the theoretical amount added of carrier.

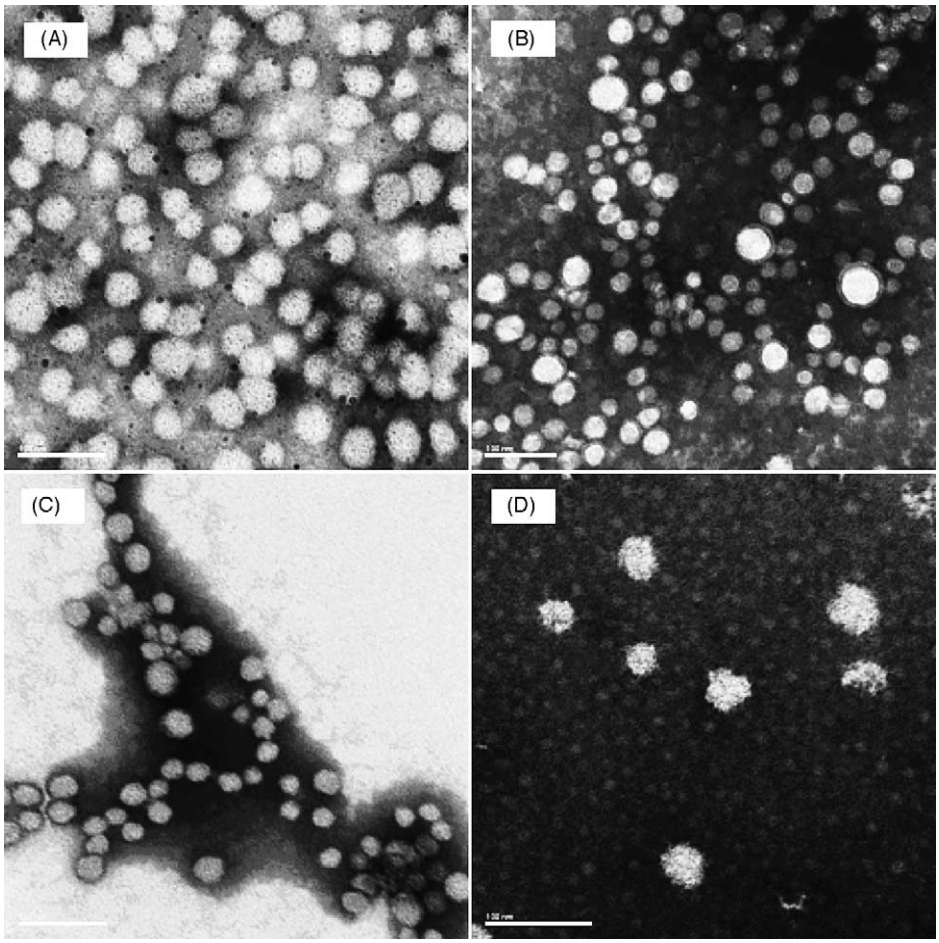

Fig. 1. TEM micrographs of CyA-pH sensitive nanoparticles: (A) CyA-E100 nanoparticles; (B) CyA-L100-55 nanoparticles; (C) CyA-L100 nanoparticles; (D) CyA-S100 nanoparticles.

the nanoparticle matrices. Fig. 2 clearly shows that the original crystal structure of the drug was not found in the nanoparticles, despite the relatively high drug loading of CyA in the nanoparticles (∼20%). The diffraction pattern of the physical mixtures can be clearly explained as superimposition figures of the patterns of the pure components. The absence of crystallinity in the nanoparticles indicated that the drug was amorphous or molecularly dispersed within the polymeric matrices of the nanoparticles, which may be expected to enhance the bioavailability of CyA.

3.4. Surface analysis of the pH-sensitive nanoparticles

In order to determine the presence of CyA molecules at the surface of the pH-sensitive nanoparticles, we used X-ray photoelectron spectroscopy (XPS), which provides quantitative elemental and chemical state information (functional group analysis) on the composition of the material under investigation in the top layers (around 10 nm depth). The atomic composition and the amount of CyA at the surface of the nanoparticles are presented in Table 2. The

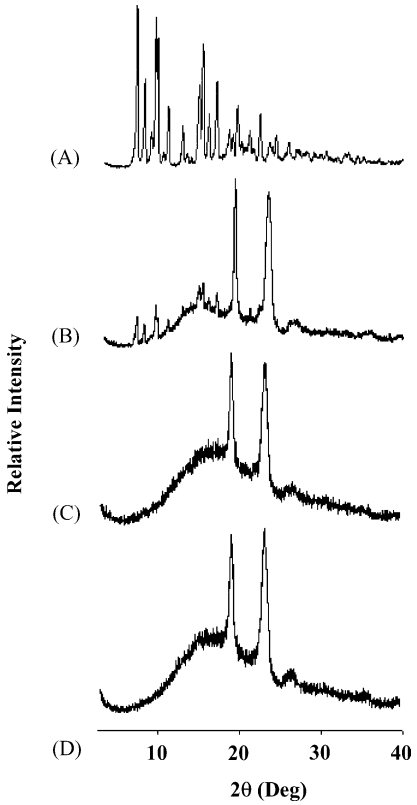

Fig. 2. X-ray diffraction patterns of (A) CyA; (B) physical mixture of CyA, Eudragit S100 and Poloxamer 188; (C) physical mixture of Eudragit S100 and Poloxamer 188; (D) CyA-S100 nanoparticles (other patterns from CyA-E100, CyA-L100-55 and CyA-L100 nanoparticles were not shown).

differences in the percentage of CyA located at the surface of different polymer nanoparticles were in accordance with those differences in drug entrapment efficiency.

3.5. In vitro release experiments of the pH sensitive nanoparticles

An appropriately designed in vitro release study is often difficult to conduct because of a number of technical problems associated with it (Washington, 1996). Sink conditions are rarely achievable for a lipophilic drug during the whole course of a release experiment, since the sensitivity of the analytical assay usually does not allow for sufficient carrier dilution in commonly used aqueous acceptor media. Another problem inherent to all release methods where the free drug is not determined in situ, is possible masking of the actual release profile due to the physical separation of the carrier and released drug (Washington, 1989; Washington, 1990). Because of these interferences the examination of the early-time drug release is often inaccurate, while in extreme cases the data obtained during the whole course of an experiment may be of little relevance (Washington, 1990). A number of improvements in release methods addressing the inaccuracy associated with the separation have been successively reported (Levy and Benita, 1990; Magenheim et al., 1993; Magalhaes et al., 1995), none of which, however, was capable of providing a satisfactory solution for the early-time release distortion. Indeed the suitability of a method has to be critically evaluated for each particular application.

With these limitations in mind, we focused on the development and evaluation of a suitable in vitro release technique for pH-sensitive polymers nanoencapsulated CyA. In the ultracentrifuge release method developed for the purposes of this study the nanoparticles were diluted into the sink medium. The released CyA was assayed after the nanoparticles separation from the assayed medium. Due to the high dispersion

Table 2
Atomic composition of the CyA-pH sensitive nanoparticles and quantification of CyA at or close to their surface, as determined by X-ray photoelectron spectroscopy

Sample	Elemental ratio (%)			CyA at the surface (%)
	C	N	O	
CyA	73.0	12.9	14.1	
CyA-E100 nanoparticles	72.4	3.0	24.7	23.3
CyA-L100 nanoparticles	68.4	1.6	30.1	12.4
CyA-L100-55 nanoparticles	67.6	1.7	30.8	13.2
CyA-S100 nanoparticles	66.3	1.6	32.1	12.4

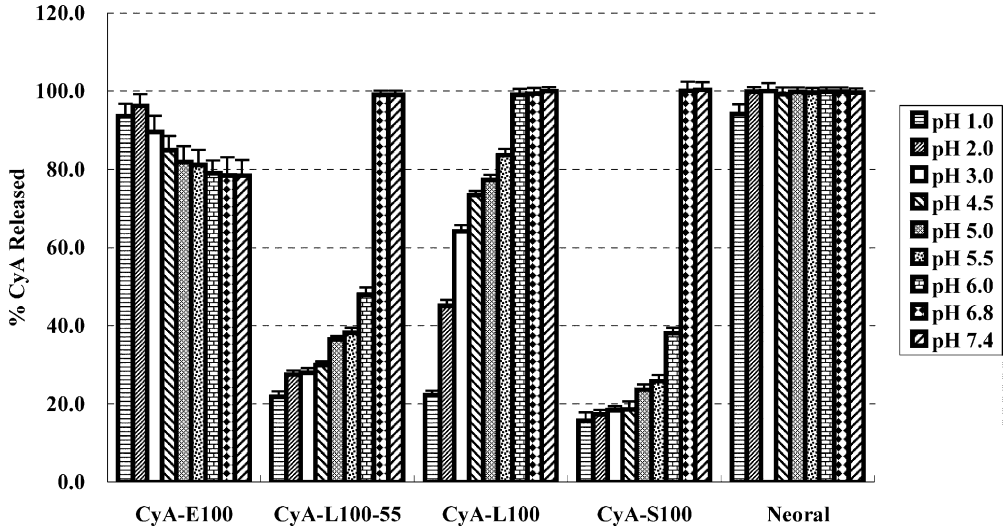

Fig. 3. In vitro drug release profiles from CyA-pH sensitive nanoparticles and the reference Neoral microemulsion by ultracentrifuge method; mean ± S.D. ($n = 6$).

of CyA in the nanoparticles and the pH-sensitivity of the polymer matrices, CyA can release instantly in the mediums at specific pH values. Therefore the release profile produced by this technique is inevitably affected by the separation process. However, the final amount of CyA released from nanoparticles can be assayed accurately.

In the experiments of in vitro release by ultracentrifuge method, the release profiles of Neoral microemulsion and different nanoparticle formulations were evaluated at varying pH values. The in vitro release profiles shown in Fig. 3 indicate the differences between Neoral microemulsion and nanoparticles caused by the polymer specific dissolution characteristic. Acrylic polymers such as Eudragit® E100, Eudragit® L100, Eudragit® L100-55 and Eudragit® S100 are commonly used for coating of tablets and preparation of controlled-release formulations. They are co-polymers of poly(methacrylic acid and methacrylate). These polymers can dissolve rapidly upon deprotonation of carboxylic acid groups at specific pH values. Thereby the release profiles of these nanoparticles exhibit significant pH-sensitivity, which is possible to make CyA mainly released at its specific absorption part of the gastrointestinal tract, decreasing the degradation by gastric acid and the first-pass metabolism by gastrointestinal enzymes, increasing the oral bioavailability of CyA.

3.6. Bioavailability study of the pH-sensitive nanoparticles

The incorporation of CyA into polymeric nanoparticles had been initially thought as a way of increasing its oral bioavailability. The mean blood levels of CyA after oral administration of a single dose of each type of nanoparticles and in comparison with those of the reference Neoral microemulsion are shown in Fig. 4. The relevant pharmacokinetic parameters derived by non-compartmental analysis are listed in Table 3.

The blood concentration–time curve showed a wide variability, especially for those time points describing the absorption phase. In all cases, CyA-S100 nanoparticles showed the highest C_{max} (2243.6 ± 329.3 ng/ml) at T_{max} of 2.50 ± 0.18 h and the $AUC_{0–72}$ was also the highest (35286.1 ± 2859.0 ng/ml h). Furthermore, the $AUC_{0–72}$ from CyA-S100 nanoparticles was increased by 1.32-fold when compared with the reference Neoral microemulsion, while CyA-E100 nanoparticles showed the lowest C_{max} (929.1 ± 85.1 ng/ml) at T_{max} of 4.25 ± 0.34 h and the lowest $AUC_{0–72}$ (25241.9 ± 2551.8 ng/ml h), which was decreased by 5.2% in comparison with Neoral microemulsion. In case of CyA-L100-55 and CyA-L100 nanoparticles, the C_{max} of CyA was found to be 2107.5 ± 253.6 ng/ml at 2.06 ± 0.71 h and 1672.2 ± 430.1 ng/ml at 3.88 ± 0.74 h, respectively. The $AUC_{0–72}$ from CyA-L100-55 or

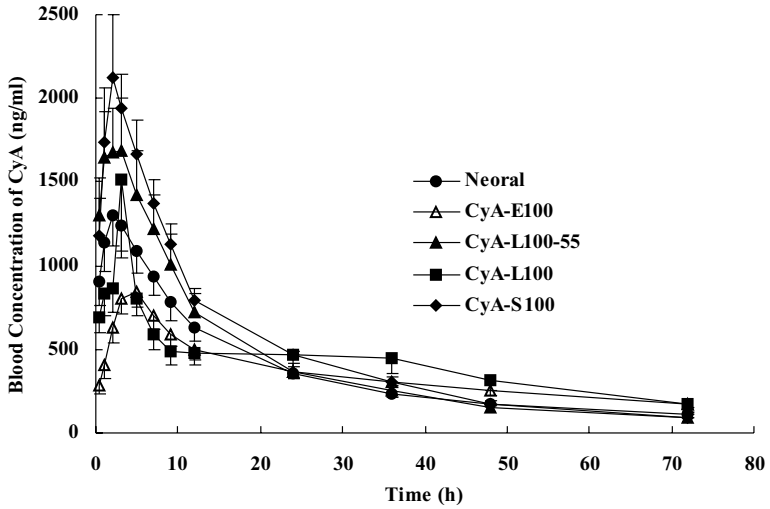

Fig. 4. Blood concentration profiles of CyA after oral administration of loaded Eudragit® E100 nanoparticles (open triangles), Eudragit® L100-55 nanoparticles (filled triangles), Eudragit® L100 nanoparticles (squares), Eudragit® S100 nanoparticles (diamonds) and the reference Neoral microemulsion (circles) into fasted SD rats at a dose of 15 mg/kg; mean ± S.E.M. ($n = 8$).

CyA-L100 nanoparticles was increased by about 1.15-fold when compared with Neoral microemulsion. The MRT from CyA-S100 nanoparticles exhibited significant differences comparing to that of the Neoral microemulsion, while the one from CyA-E100 and CyA-L100 nanoparticles showed highly significant differences.

The relative bioavailability of CyA from CyA-S100 nanoparticles increased by 32.5%, and increased by 15.2 and 13.6% from CyA-L100-55 and CyA-L100 nanoparticles, respectively. While in case of CyA-E100 nanoparticles, it was decreased by 5.2%. The absorption of particles from intestine is a well-known process (McClean et al., 1998) affected by a number of factors among which particle size is prominent. Thus, it was evident from these results that CyA-S100 nanoparticles have the smallest size (37.4 ± 5.6 nm) relative to that of CyA-L100-55 (60.4 ± 6.0 nm), CyA-E100 (98.7 ± 13.4 nm) or CyA-L100 (106.7 ± 14.8 nm) nanoparticles, and gave the highest C_{max} and $AUC_{0–72}$. The statistical analysis revealed that the $AUC_{0–72}$ of CyA-S100 nanoparticles shows significant differences ($P < 0.05$) compared with that of Neoral microemulsion. These results indicated that the particle sizes are the possible factors responsible for improving the oral absorption of CyA. On the other hand, in spite of CyA-E100 nanoparticles have the smaller particle size than CyA-L100 nanoparticles, their C_{max} and $AUC_{0–72}$ were lower. These results could be attributed to the protection effect of the enteric polymers. Eudragit® L100-55, Eudragit® L100 and Eudragit® S100 are pH-dependent anionic polymer solubilizing above pH 5.5, 6.0 and 7.0 for targeting drug delivery in the duodenum, jejunum or ileum respectively. Accordingly, the nanoparticles prepared by these enteric polymers are insoluble in the stomach, which can protect CyA from the degradation of gastric acid or enzymes. The previous studies have proven that CyA is absorbed primarily in the small intestine (Drewe et al., 1992). Therefore, choosing an appropriate enteric acrylic polymer to prepare the CyA nanoparticles can target the drug to the specific site of gastrointestinal tract and create high concentration as close as possible to the absorption window of CyA for improving its oral bioavailability. Furthermore, CyA is known to be metabolized by CYP3A4 and, to a lesser extent, by CYP3A5. Since both CYP3A4 and CYP3A5 have been shown to exist in the intestine as well as the liver, it seems reasonable that the metabolism of CyA would also occur at both sites (Hebert, 1997). Webber et al. (1992) demonstrated that CYP3A can be detected in microsomes made from human duodenum and ileum. In addition, these microsomes were able to metabolize CyA to its three

Table 3
Pharmacokinetic parameters of CyA incorporated pH sensitive nanoparticles and the reference Neoral microemulsion after oral administration to fasted SD rats; mean ± S.E.M. ($n = 8$)

Pharmacokinetic parameters	Neoral	CyA-E100 nanoparticles	CyA-L100-55 nanoparticles	CyA-L100 nanoparticles	CyA-S100 nanoparticles
C_{max} (ng/ml) ± S.E.	1341.1 ± 175.1	929.1 ± 85.1*	2107.5 ± 253.6**	1672.2 ± 430.1	2243.6 ± 329.3*
T_{max} (h) ± S.E.	2.38 ± 0.17	4.25 ± 0.34**	2.06 ± 0.71	3.88 ± 0.74**	2.50 ± 0.18
MRT_{0-72} (h)	21.35 ± 0.89	27.47 ± 0.76**	19.20 ± 1.39	27.14 ± 1.11**	18.49 ± 1.03*
AUC_{0-72} (ng/ml h) ± S.E.	26633.5 ± 2450.6	25241.9 ± 2551.8	30673.5 ± 3403.4	30250.9 ± 3625.1	35286.1 ± 2859.0*
Fr (%) ± S.E.	–	94.8	115.2	113.6	132.5*

* $P < 0.05$.
** $P < 0.01$ vs. Neoral.

primary metabolites (AM1, AM9 and AM4N). The metabolism of CyA is greatest in the duodenum, with the ileum producing less metabolite. Results from the in vitro release studies by ultracentrifuge indicated that the amount of CyA released from CyA-S100 and CyA-L100-55 nanoparticles in the medium above pH 5.5 was less than 25.8% and 38.2%, respectively. While the amount of CyA released from CyA-L100 and CyA-E100 nanoparticles was more than 83.6 and 96.3%, respectively. Therefore, CyA-L100-55 nanoparticles, especially CyA-S100 nanoparticles, can mainly release drug in the ileum. These may be the primary factors responsible for improving the oral absorption and overall bioavailability of CyA. In case of CyA-E100 and CyA-L100 nanoparticles, the metabolism of CyA will be greater than that from CyA-S100 and CyA-L100-55 nanoparticles in the upper parts of intestine. The significant decrease in C_{max} for CyA-E100 nanoparticles and the greater values of T_{max} and MRT for CyA-E100 and CyA-L100 nanoparticles probably could be explained by these.

Increased bile salt secretion and delayed gastric emptying occurring in the postprandial state generally favor the absorption of poorly water soluble drugs. However, small alterations of the pH in the gastrointestinal tract can significantly affect the dissolution pattern of the pH-dependent dissolving particles. Therefore, higher than normal gastric pH in the fed state could have resulted in premature release and precipitation of the drug in the stomach, which would result in the lower bioavailability than that in the fasted state (Charman et al., 1997). In addition, the competition for absorption and/or complexation between the released drug and food elements may also be lead to the lower absorption in the fed state (Welling, 1989). Therefore, the effect of the nutritional state to the oral bioavailability of CyA-pH nanoparticles has been the subject of my further studies.

4. Conclusion

The results presented in this paper indicate that pH-sensitive nanoparticles can be designed as new CyA carriers, showing promising characteristics as compared with present marketed CyA formulations,

which opens up viable possibilities to improve present CyA-based therapies and to widening the possible areas of CyA biomedical application. Furthermore, this formulation approach can be used to improve the oral bioavailabilty of other poorly soluble and poorly absorbable drugs.

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