Nanoparticles made of multi-block copolymer of lactic acid and ethylene glycol containing periodic side-chain carboxyl groups for oral delivery of cyclosporine A

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The purpose of this study was to evaluate the potential of new carboxylated multi-block copolymer of lactic acid and ethylene glycol (EL14) for nanoparticle (NP) formation and their ability to deliver high molecular weight hydrophobic drug—cyclosporine A (CsA). CsA-loaded EL14 NPs were compared with traditional poly(lactide-co-glycolide) (PLGA) NPs, both prepared by emulsion–diffusion–evaporation process. On the one hand, the increase in drug payload from 10 to 30 per cent for EL14 NPs showed no difference in particle size, however the entrapment efficiency tends to decrease from 50 to 43 per cent; on the other hand, the more hydrophobic PLGA showed an increasing trend in entrapment efficiency from 20 to 62 per cent with increasing particle size. Over 90 per cent of CsA was released *in vitro* from both the nanoparticulates; however, the release was much slower in the case of more hydrophobic PLGA. On *in vivo* evaluation in rats, the NPs made of EL14 showed a higher $C_{\max}$, a faster $T_{\max}$ and enhanced tissue levels to that of PLGA that are crucial for CsA's activity and toxicity; however, the overall bioavailability of the nanoparticulates was similar and higher than Neoral. Together these data demonstrate the feasibility of NPs made of low molecular weight, hydrophilic polymer EL14 for efficient delivery of CsA.

Keywords: drug delivery; nanoparticles; pharmacokinetics; polymer

1. INTRODUCTION

The introduction of cyclosporine A (CsA) transformed the field of organ transplantation as it dramatically reduced the frequency of acute graft rejection (Cecka & Terasaki 1991). In addition to graft prevention, CsA found several applications in the treatment of psoriasis, Crohn's disease and rheumatoid arthritis. In spite of its wide applications, CsA is associated with adverse effects of which nephrotoxicity is the most severe (Faulds *et al.* 1993; Rezzani 2004). CsA also causes cardiovascular (Textor *et al.* 1994) and neurological complications (Polson *et al.* 1985) along with other non-nephrotoxic side effects, which include hypertrichosis, tremors and gingival hyperplasia (David-Neto *et al.* 2000). CsA being a critical dose drug with narrow therapeutic window, over- or under-exposure of the drug greatly affects the outcome of the therapy.

Over-exposure to CsA results in toxicity while under-exposure causes graft rejection.

CsA is a highly lipophilic neutral cyclic peptide with a molecular formula of $C_{62}H_{111}N_{11}O_{12}$. CsA exhibits low oral bioavailability owing to its poor biopharmaceutical properties like high molecular weight (1202.64 Da), low solubility and permeability, and high presystemic metabolism (Faulds *et al.* 1993; Italia *et al.* 2006). Several formulations have been developed to overcome the poor biopharmaceutical properties of CsA. Introduction of the micro-emulsion, Neoral, has resulted in significant advancements as it established more dependable and consistent absorption profile (Mueller *et al.* 1994). There still remains a small degree of inter- and intra-patient variability in the absorption of the drug, however less than Sandimmune (Kahan *et al.* 1995), which can affect the therapeutic outcome of CsA treatment (Lindholm & Kahan 1993). Several polymeric nanoparticulate approaches have been investigated for CsA delivery, which includes pH-sensitive nanoparticles (NPs) of methacrylic acid–methacrylate copolymer (Dai *et al.* 2004), poly(isobutyl 2-cyanoacrylate) and polycaprolactone nanoparticulate

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formulations (Varela *et al.* 2001) and poly(lactic acid-*b*-poly(ethylene glycol)) (PLA-PEG) NPs (Gref *et al.* 2001). The developed formulations did improve overall bioavailability however, the efficacy and toxicity still remains a concern. In a recent report by our group, CsA-loaded poly(lactide-co-glycolide) (PLGA) NPs ($C_{\max}$, 448.5 ng ml⁻¹) were found to be less nephrotoxic than commercial formulation Neoral, however the $C_{\max}$ achieved was significantly lower than that of Neoral ($C_{\max}$, 1448.4 ng ml⁻¹; Italia *et al.* 2007) that may fall well below therapeutic levels (Lindholm & Kahan 1993). Besides therapeutic efficacy, $C_{\max}$ is found to be correlated with the occurrence of side effects. Hence, administration of CsA requires a delivery system that decreases the frequency and the severity of side effects, however still maintaining blood concentrations within the therapeutic levels.

Several reports have established the role of polymer matrix in determining the release profile of drugs (Gref *et al.* 2001; Mittal *et al.* 2007). PLGA has been exploited utmost for particulate delivery, because of its desired properties of biocompatibility, degradability and ease of fabrication (Anderson & Shive 1997; Bala *et al.* 2004; Mohamed & Van der Walle 2008). However, in the present case for CsA delivery, the regular approximately 40 kDa PLGA appears to fail in delivering therapeutic levels. Therefore, in an attempt to increase the $C_{\max}$, which is thought to play a crucial role in preventing graft rejection (Grant *et al.* 1999), we synthesized a more hydrophilic multi-block copolymer of lactic acid and ethylene glycol containing periodic side-chain carboxyl groups. The synthesized polymer should release CsA faster owing to decreased hydrophobicity and quicker hydrolytic degradation assisted by periodic side-chain carboxyl groups (Tracy *et al.* 1999) to achieve the desired CsA concentration.

2. MATERIAL AND METHODS

2.1. Materials

CsA (Fluorochem Ltd. Derbyshire, UK), PLGA (Resomer RG 50:50, MW 35 000–40 000, Boehringer Ingelheim, Ingelheim, Germany), *N*, *N*-didodecyl-*N*, *N*-dimethylammonium bromide (DMAB) (Aldrich) and HPLC grade ethanol (Fluka) were used as received. Labrasol was a gift sample from Gattefosse (France). All other chemicals used were of analytical grade.

2.2. Preparation of carboxylated chain-extended (ABA)_n polymer

The polymer EL14 was prepared as described previously (Ankola *et al.* 2009). In brief, a solution of L-lactide, dried polyethylene glycol (PEG, MW 400) and 0.1 per cent (w/w) stannous octoate were heated at reflux in dry toluene for 24 h under dry nitrogen. Chain extension of lactide-ethylene glycol-lactide triblock copolymers was performed by introduction of triethylamine and pyromellitic dianhydride (1:2:1 copolymer:amine:dianhydride molar ratio) into the flask containing the pre-polymer solution under dry nitrogen atmosphere. The mixture was heated at

110°C for 1 h under stirring and then toluene was removed under vacuum. The dried polymer was dissolved in dichloromethane, washed with cold 0.5 N HCl and water, then precipitated from dichloromethane solution into diethyl ether. The precipitate was collected by filtration and dried under vacuum. The polymer was further purified by precipitation from acetone in 5 per cent NaHCO₃, washing with water and dried under vacuum. The synthesized polymer had a MW of 16 300 and its comprehensive characterization was reported earlier (Ankola *et al.* 2009).

2.3. Preparation of NPs

Emulsion-diffusion-evaporation technique was used to prepare the NPs (Italia *et al.* 2007; Ankola *et al.* 2010). In brief, the method used was as follows: 50 mg of EL14 or PLGA were dissolved in 2.5 ml of organic solvent (ethyl acetate for PLGA and 4:1 ethyl acetate: dichloromethane for EL14) at room temperature and stirred for 2 h. The organic phase was then emulsified into an aqueous phase containing DMAB (0.25% w/v) as stabilizer. The resulting o/w emulsion was sonicated (Soniprep 150, MSE) at 10 µm amplitude for 1 min. Water was added to the above emulsion with constant stirring which resulted in nanoprecipitation. CsA-loaded NPs were prepared by dissolving the drug in minimum volume of organic phase and adding it to the polymeric solutions and proceeding as described above. The effect of drug loading on particle characteristics was studied at 10, 20 and 30 wt% of polymer load.

2.4. Size and zeta potential measurements

The mean particle size of the prepared NP suspensions was measured by dynamic light scattering (Nano ZS, Malvern, UK) taking the average of five measurements, which represented the hydrodynamic diameter of the particles. Mean size was derived from Cumulants analysis of the measured correlation curve, wherein a single particle size is assumed and a single exponential fit is applied to the autocorrelation function. In addition to the mean particle size, the instrument reports the Particle Dispersion Index (PDI) between 0 (monodispersed particles) and 1 (polydispersed particles). The zeta potential was estimated on the basis of electrophoretic mobility under an electric field as an average of 30 measurements. The frequency shift of an incident laser beam caused by these moving particles is converted to the zeta potential by the application of the Smoluchowski equation. The temperature was kept constant at 25°C during size and zeta potential measurements.

2.5. Entrapment efficiency

The percentage of CsA incorporated during NP preparation was determined by centrifuging the drug-loaded NPs at 14 000 *ref* for 30 min and separating the supernatant. The pellet obtained was washed twice with water and CsA was extracted from the pellet using acetonitrile, followed by estimation of CsA in triplicate by HPLC. Entrapment efficiency was calculated as the percentage ratio of drug

Table 1. Characteristics of CsA-encapsulated nanoparticles. EE, entrapment efficiency; ZP, zeta potential. All values are represented as mean $\pm$ s.d. ($n = 3$). Zeta potential was measured at pH 4.1 ± 0.5 pH. PDI of particles was in the range of 0.08–0.16.

loading (%)	PLGA				EL14			
	size (nm)	EE (%)	CsA entrapped per 50 mg of PLGA (mg)	ZP (mV)	size (nm)	EE (%)	CsA entrapped per 50 mg of EL14 (mg)	ZP (mV)
10	107 ± 7	20 ± 2	1.0 ± 0.1	78 ± 6	136 ± 7	51 ± 11	2.5 ± 0.6	51 ± 6
20	108 ± 7	46 ± 4	4.6 ± 0.4	73 ± 12	139 ± 7	47 ± 3	4.7 ± 0.3	54 ± 9
30	119 ± 5	62 ± 4	9.3 ± 0.6	70 ± 7	138 ± 4	43 ± 3	6.4 ± 0.5	53 ± 7

incorporated into the NPs to that initially added for loading. Estimation of CsA was carried out using Hypersil GOLD (15 cm $\times$ 4.6 mm, 5 μ m) (C18) column and Hypersil 10 $\times$ 4 mm, 5 μ m drop in guards connected by UNIGAUARD (Thermo Scientific). Elution of CsA was achieved using isocratic mobile phase (ethanol:water) at 210 nm. The HPLC system consisted of ChromQuest acquisition software, Auto-sampler connected with LC Pump and PDA detector (Thermo Finnigan Surveyor System). The analytical method was validated according to the guidelines of the international conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use. Parameters validated included precision (repeatability (intra-day) and intermediate precision (inter-day)) and accuracy. Both the intra- and inter-day relative standard deviations of QC standards were less than 5 per cent over the selected range.

2.6. In vitro drug-release studies

Release of CsA from the nanoparticulates was observed using the dialysis membrane method. CsA-loaded NPs of PLGA and EL14 were centrifuged and redispersed in 1 ml of release medium (5% Labrasol solution in pH 7.4 phosphate buffer) and placed in the dialysis bags (Sigma) with a molecular mass cut-off of 12 000 Da. The bags were suspended in glass vials containing 5 ml of release medium. All the vials were kept in a shaker water-bath maintained at 37°C and 50 r.p.m. The release medium was completely replaced with fresh buffer at every sampling time interval. The sampling points were 6 h followed by sampling at every 24 h for the first week and every 48 h at later stages of release. The CsA content in the release medium was quantified using a validated HPLC method as described above.

2.7. Pharmacokinetic and tissue distribution studies

All animal experiments were carried out under a Project Licence issued under the U.K. Home Office Animals (Scientific Procedures) Act 1986. For the pharmacokinetic and tissue distribution studies, male SD rats weighing 200–250 g were used. Neoral and CsA nanoparticulate formulations of PLGA and EL14 were administered to rats by oral gavage tube at a dose of 15 mg kg⁻¹ body weight. Blood samples were collected from animals by venipuncture of peripheral blood vessel at 0.5, 1, 2, 6, 12, 24, 36, 48 and 72 h in

microcentrifuge tubes containing ethylenediaminetetraacetic acid sodium salt as anticoagulant. Blood samples were analysed for CsA concentration by cyclosporine-specific EIA KIT (Immunotech, Czech Republic). At the end of 72 h, animals were sacrificed and all major organs isolated. CsA concentration in all the organs was determined. In brief, the organs were isolated and weighed, to which water was added at 1:4 ratio and then homogenized to produce a fine homogenate. Two hundred microlitres of homogenate was transferred into a microcentrifuge tube to which 800 μ l of acetonitrile was added to extract CsA from the tissues. The tubes were vortexed for 5 min and centrifuged. The samples were concentrated by collecting 800 μ l of supernatant and evaporating it to dryness under vacuum. The obtained pellet was analysed for CsA content by cyclosporine-specific EIA KIT (Immunotech).

3. RESULTS AND DISCUSSION

3.1. Preparation of NPs

A careful consideration of polymer and drug properties is needed to optimize the formulation of NP-entrapped drug as both the surfactant and the solvent nature can significantly influence the particle characteristics that include particle size and drug encapsulation (Sahana *et al.* 2008). The method previously optimized for PLGA-CsA NPs (Italia *et al.* 2007; Ankola *et al.* 2010) was adapted with appropriate minor modifications for preparation of EL14 NPs. For PLGA NPs ethyl acetate was the oil phase, however for EL14, ethyl acetate:dichloromethane (4:1) was used for the preparation, as small amount of dichloromethane was required for complete polymer dissolution.

The EL14 and PLGA NPs were prepared by loading different concentrations of CsA (10–30% w/w of polymer weight). A slight increase in particle size from 107 to 119 nm was observed by increasing the drug payload from 10 to 30 per cent (table 1), whereas a significant increase in the entrapment efficiency from 20 to 62 per cent was observed for PLGA. On the other hand, EL14 NPs did not show any size increase, whereas CsA entrapment decreased. At 10 per cent loading, EL14 NPs were able to entrap higher amounts of CsA when compared with PLGA. These high entrapments at 10 per cent payload may be owing to several factors including the ionic interactions of CsA with the carboxyl groups present in the side chain of EL14. On the other hand, the commercial PLGA has only

terminal carboxyl end groups therefore, the role of ionic interaction may be negligible when compared with EL14 that contains about 12–16 free carboxyl groups in polymer side chain (Ankola *et al.* 2009). The better orientation of CsA within the EL14 matrix than in PLGA could be another possibility.

By increasing the load to 30 per cent w/w of polymer, the efficiency of CsA entrapment decreased for EL14 NPs, whereas an increase was observed for PLGA NPs. The observed decrease for EL14 could be due to several factors, such as the limitation of the matrix itself owing to its lower molecular weight (16 300) compared with that of PLGA (approx. 40 000). Another reason could be a faster saturation of EL14, more hydrophilic matrix to hold highly hydrophobic CsA molecules in comparison with more hydrophobic PLGA. The entrapment of CsA was also found to depend upon the concentration of DMAB, which can be lowered to increase the CsA entrapment (Italia *et al.* 2007) as low entrapment would result in higher polymer administration at desired CsA dose. The NPs prepared using both the polymers were of low PDI (0.08–0.16). The observed zeta potential for both nanoparticulate formulations was around 75 mV for PLGA and 50 mV for EL14 NPs at pH 4.1 ± 0.5 . The low PDI and high zeta potential indicates the high stability of both the nanoparticulate formulation. In the literature, besides PLGA several other polymers have also been explored for preparing CsA-loaded NPs. Block copolymers of lactic acid and ethylene glycol were used to prepare CsA-loaded NPs (Gref *et al.* 2001). Hyaluronic acid-coated poly(ϵ -caprolactone) particles have been explored for the delivery of CsA to the cornea (Yenice *et al.* 2008). In addition to polymers for specific molecules, several polymers for broader drug delivery and biomedical applications have also been synthesized. Several functional polyesters with pendant hydroxyl groups or tailored side chains have been developed (Cerbai *et al.* 2008). Amphiphilic diblock and triblock block copolymers consisting of alternating poly(ethylene glycol) and poly(ϵ -caprolactone) segments were synthesized (Signori *et al.* 2005). EL14 also represents a new class of polymer that can effectively entrap CsA, in the form of nanoparticulates in the current circumstances. As 20 per cent loading resulted in NPs with similar entrapment of CsA in PLGA and EL14 NPs, it was used for *in vitro* and *in vivo* evaluation.

3.2. In vitro drug-release studies

The *in vitro* release of drugs from polymeric NPs is mediated by diffusion-cum-degradation process and the release profiles for 20 per cent drug-loaded particles recorded were shown in figure 1. A 53 per cent release was recorded at 48 h for EL14 NPs and corresponding to that PLGA NPs recorded 25 per cent. After 48 h, EL14 NPs released approximately 7 per cent of drug daily and about 90 per cent by day 9, whereas PLGA NPs released 86 per cent of drug by day 18 with approximately 4 per cent daily release. Several factors could govern the faster release of CsA from EL14 NPs. The hydrophilic nature and low molecular weight of EL14 could have contributed to faster release

Figure 1. *In vitro* release pattern of CsA from PLGA (open circles) and EL14 (open diamonds) nanoparticles. All values represented as mean $\pm$ s.d. ($n = 3$).

of CsA from EL14 NPs. Other reasons may also include large amounts of surface-bound drug on EL14 NPs and faster degradation of EL14 polymer matrix.

The release profile of CsA from both the nanoparticulate formulations were modelled for different release mechanisms, mainly zero order (Najib & Suleiman 1985), first order (Desai *et al.* 1966), Higuchi's square root plot (Higuchi 1963), Hixson–Crowell cube root plot (Hixson & Crowell 1931) and Peppas–Korsmeyer power plot (Korsmeyer *et al.* 1983; table 2). CsA release from EL14 and PLGA NPs could have followed either Higuchi's model (diffusive) or first-order release based on higher correlation coefficient (R) and lower sum of squared errors (χ^2). No conclusive differences between Higuchi's model (diffusive) or first-order release were found particularly if experimental errors (5–10%) were taken into account. However, if data for less than 85 per cent release are considered, the release of CsA from EL14 NPs fits exceptionally well with the Higuchi model. To further confirm, fitting to Peppas–Korsmeyer gave diffusional coefficient ($n = 0.47$ for EL14 NPs ($n < 0.5$, Fickian diffusion) indicating a diffusive release mechanism. The somewhat larger $n = 0.685$ was observed for PLGA NPs indicating a small deviation from the diffusive model, suggesting that diffusion is accompanied by erosion ($n > 0.5$, non-Fickian diffusion). The difference between the two polymers can be attributed to: hydration of hydrophilic EL14 NPs that facilitates drug diffusion; limited diffusion inside rather hydrophobic PLGA NPs and hence a diffusion–erosion mechanism. The faster but controlled release from EL14 NPs would help in achieving higher CsA blood levels in comparison to PLGA but at a controlled $C_{\max}$, desired for optimal CsA therapy.

3.3. Pharmacokinetic and tissue distribution study

Pharmacokinetics and tissue distribution studies are noteworthy in the assessment of a delivery system, as they provide valuable information about the safety and efficacy of the formulation and also help in

Table 2. *In vitro* release data fit to models.

kinetic model	equation	parameters ^a	PLGA NPs	EL14 NPs ^b
zero order	$Q_t = k_0 t$	R χ^2 k_0	0.972 1590 5.78	0.914 1516 9.72
Higuchi	$Q_t = k_H t^{1/2}$	R χ^2 k_H	0.997 155 21.5	0.988 (0.999) 260 (11.9) 34.5 (37.0)
first order	$\ln Q_t = \ln Q = -k_1 t$	R χ^2 k_1	0.994 105.1 0.0912	0.990 62.1 0.388
Hixon–Crowell	$Q_0^{1/3} - Q_t^{1/3} = k_{HC} t$; $Q_t^{1/3} = Q_0^{1/3} - k_{HC} t$	R χ^2 k_{HC}	0.800 44 565 0.165	0.733 43 164 0.337
Peppas–Korsmeyer	$Q_t/Q_0 = k_{PK} t^n$; $\ln Q_t = \ln Q_0 k_{PK} + n \ln t$	R χ^2 k_{PK} n	0.994 187.8 0.170 0.685	0.993 200.8 0.366 0.470

^a Q_t is the amount of drug released at time t ; Q_0 is the initial amount of the drug in the formulation; n is the diffusional coefficient; k_0 , k_1 , k_H , k_{HC} and k_{PK} are the release rate constants for zero-order, first-order, Higuchi, Hixon–Crowell and Peppas–Korsmeyer rate equations, respectively. n is the diffusional coefficient in Peppas–Korsmeyer rate equations; R is the correlation coefficient and χ^2 is the sum of squared errors.

^bValues in parentheses refers to release values lower than 85 per cent.

estimating clinical doses. Comparison of nanoparticulate formulation with the currently marketed formulation of CsA would provide additional information on therapeutic implications of the new formulations. Considering the above, the pharmacokinetic profile of EL14 and PLGA NPs of CsA were compared with currently marketed CsA formulation Neoral. The blood CsA levels with time for Neoral, EL14 and PLGA NPs on oral administration at 15 mg kg^{-1} are shown in figure 2 and the derived pharmacokinetics parameters in table 3.

Entrapment of CsA into EL14 and PLGA NPs resulted in the sustained release of the drug in comparison with the Neoral which is a micro-emulsion-based formulation. The $T_{\max}$ was 2 h for Neoral and 12 and 24 h for EL14 and PLGA NPs, respectively. The $C_{\max}$ of the formulations varied widely, depending upon the ability of the formulation to sustain the CsA release. $C_{\max}$ was 1328, 1006 and 682 ng ml^{-1} for Neoral, EL14 NPs and PLGA NPs, respectively. Neoral resulted in a quick absorption profile, while PLGA NPs sustained the release of CsA longer than EL14 NPs. By 72 h after administration, EL14 and PLGA NPs had similar blood levels. The relative bioavailability of EL14 and PLGA NPs were 126 and 120 per cent, respectively, in comparison with Neoral and 105 for EL14 NPs in comparison with PLGA NPs.

In literature several studies have demonstrated the correlation of CsA concentrations to the incidence of side effects. The toxicities are found to depend on $C_{\max}$ and the area under curve (AUC) of the drug. In a recent study in paediatrics with stable renal transplant it was found that hypertrichosis was related to AUC, whereas the tremors were more related to $C_{\max}$ of CsA (David-Neto et al. 2000). Similar facts have also been established in acute nephrotoxicity caused

Figure 2. Comparative *in vivo* plasma concentration versus time profiles of CsA administered orally as Neoral (open triangles, 15 mg kg^{-1}), CsA NPs of PLGA (open circles, 20% DL) and EL14 at 15 mg kg^{-1} (open diamonds, 20% DL). All values reported are mean $\pm$ s.e.m. ($n = 3$). DL, drug loading.

by CsA, where decrease in glomerular filtration rate was closely related to the $C_{\max}$ and not AUC (Perico et al. 1992). It was further suggested that hypertrichosis and tremor can be prevented by maintaining the $\text{AUC}_{0-4} \leq 4200 \text{ ng ml}^{-1} \text{ h}^{-1}$ and $C_{\max} \leq 900 \text{ ng ml}^{-1}$, respectively (David-Neto et al. 2000). In circumstances to the above, EL14 NPs could be a preferred formulation for CsA delivery as observed $C_{\max}$ was in the desired range, unlike Neoral that exceeded the limit significantly by approximately 400 ng ml^{-1} .

After 72 h of oral administration, the animals were sacrificed and tissue concentration determined to have an insight on CsA biodistribution (table 4). CsA concentrations in all organs, except brain were lower for

Table 3. Pharmacokinetic parameters. AUC, area under curve.

groups	AUC _{0–72}	C _{max} (ng ml ⁻¹)	T _{max} (h)	relative bioavailability ^a	relative bioavailability ^b
Neoral	29 046 ± 1437	1328 ± 120	2	100	n.a.
PLGA	34 854 ± 2157	682 ± 66	24	120	100
EL14	36 616 ± 2843	1006 ± 119	12	126	105

^aIn relative to Neoral.

^bIn relative to PLGA (all data are represented as mean ± s.e.m., *n* = 3).

Table 4. Tissue concentrations of various CsA formulations at 72 h. All data are represented as mean ± s.e.m. (*n* = 3).

tissue	µg g ⁻¹ of tissue or µg ml ⁻¹ of blood		
	Neoral	PLGA	EL14
blood	0.04 ± 0.01	0.22 ± 0.04	0.17 ± 0.02
brain	3.4 ± 0.7	1.9 ± 0.2	2.7 ± 1.1
heart	5.0 ± 0.8	6.4 ± 1.5	6.8 ± 0.8
lung	5.3 ± 0.7	8.9 ± 3.6	10.7 ± 0.6
kidney	9.9 ± 1.6	14.0 ± 1.3	15.3 ± 1.5
spleen	6.9 ± 1.8	9.3 ± 2.1	10.8 ± 1.2
liver	10.7 ± 1.3	11.7 ± 0.8	16.4 ± 1.1
intestine	6.8 ± 0.8	7.5 ± 1.5	7.7 ± 1.2

Table 5. Tissue distribution coefficient.

tissue	<i>K_p</i>	
	PLGA	EL14
blood	1	1
brain	9	16
heart	29	41
lung	40	64
kidney	64	91
spleen	42	64
liver	53	98
intestine	34	46

Neoral than for PLGA and EL14 NPs. For brain, Neoral had the highest CsA concentration followed in the order by EL14 and PLGA NPs, which may be owing to the fact that free CsA penetrated blood–brain barrier more easily than that entrapped in polymeric particles. When both the nanoparticulate formulations were compared with each other, EL14 NPs produced higher CsA tissue levels at lower blood CsA concentrations than PLGA NPs. This may be owing to the ability of EL14 NPs to distribute more in tissues as observed by higher tissue distribution coefficient (*K_p*, ratio of CsA's tissue concentration to blood concentration) as depicted in table 5. From the observed kinetic and tissue profile, it was clear that the nature of polymer matrix played a key role in determining the performance of given delivery system. EL14 in comparison with PLGA generated a kinetic profile, which is much closer to Neoral. The current commercial formulation of CsA experiences erratic bioavailability owing to its extensive metabolism in the gut, making it difficult to predict trough levels. The better bioavailability of CsA in the form of EL14 NPs could be

because of the protective role of PEG present in the polymer chain, which has been found to have protective effects on proteins and peptides in gastric fluids (Tobio *et al.* 2000). The decreased gut-wall metabolism would allow better therapeutic monitoring of CsA.

4. CONCLUSION

Considering the critical dose drug type nature of CsA, which includes low and variable absorption, formulation-dependent bioavailability and individualization of dosing, nanoparticulate approach would be an ideal strategy to improve its performance and the current study indicates the potential of EL14 NPs for CsA delivery. EL14 NPs were successfully able to deliver CsA via oral route and at a controlled rate, withstanding drastic GI environment and metabolizing enzymes and complex elimination–excretion process of the body. These NPs when compared with PLGA NPs, released CsA faster; resulting in a higher *C_{max}* but similar bioavailability.

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