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Intranasal delivery of zidovudine by PLA and PLA–PEG blend nanoparticles

Rubiana Mara Mainardes[a,*], Najeh Maissar Khalil[a], Maria Palmira Daflon Gremião[b]

[a] Universidade Estadual do Centro-Oeste/UNICENTRO, Departamento de Farmácia, Rua Simeão Camargo Varela de Sá 03, Guarapuava, PR 85040-080, Brazil
[b] Universidade Estadual Paulista Júlio de Mesquita Filho/UNESP, Faculdade de Ciências Farmacêuticas, Departamento de Fármacos e Medicamentos, Rod. Araraquara-Jaú Km 01, Araraquara, SP 14801-902, Brazil

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ABSTRACT

This study describes the preparation and evaluation of biodegradable poly(L-lactide) (PLA) and poly(L-lactide)–poly(ethylene glycol) (PLA–PEG) blend nanoparticles containing zidovudine as model drug. The prepared nanoparticles were characterized in terms of size, zeta potential, morphology and drug entrapment efficiency. The pharmacokinetics of zidovudine following intranasal administration in mice was assessed. The results showed that although PLA and blend nanoparticles had the same morphology, the particle size and zeta potential were changed by the PEG. The drug entrapment efficiency was increased by PEG presence. The pharmacokinetic study showed that all the nanoparticles were able to sustain zidovudine delivery over time, but greater efficiency was obtained with PLA–PEG blend nanoparticles, whose T_{max} was twice that of PLA nanoparticles. The PLA and PLA–PEG nanoparticles formulations increased the zidovudine mean half-life by approximately 5.5 and 7 h, respectively, compared to zidovudine aqueous solution. The relative bioavailability of zidovudine-loaded PLA–PEG blend nanoparticles was 2.7, relative to zidovudine-loaded PLA nanoparticles and 1.3 relative to aqueous solution formulation. Thus, the PLA nanoparticles were unable to increase the zidovudine bioavailability compared to aqueous solution formulation. The results obtained in this study indicate the potential of the PLA–PEG blend nanoparticles as carriers for zidovudine delivery by the intranasal route.

1. Introduction

Zidovudine (AZT) was the first antiretroviral agent developed and, upon approval in 1987, it became a key feature in the therapy of acquired immune deficiency syndrome (AIDS) (Chien and Wearley, 1989; Warnke et al., 2007). AZT is an analog of deoxythymidine and is activated to its mono-, di-, and triphosphates by cellular enzymes in both human immunodeficiency virus (HIV) infected and uninfected cells (Furman et al., 1986). The active metabolite, AZT triphosphate, inhibits HIV-1 reverse transcriptase as well as mitochondrial DNA polymerase-g (König et al., 1989).

The major limitation in the use of AZT is the occurrence of severe side effects (Yarchoan et al., 1989). The most serious and frequent is the hematological toxicity, characterized by bone marrow suppression, which is usually manifested as anemia, neutropenia and thrombocytopenia (Gill et al., 1987; Kennedy et al., 1991). These toxic effects are dose-dependent. The adverse side effects of AZT may necessitate dose reduction or even cessation of therapy.

The pharmacokinetics of AZT have been studied in humans and in animal species, showing that when it is administered orally, AZT is rapidly absorbed from the gastrointestinal tract, but is then rapidly metabolized to the inactive glucoronide with a mean elimination half-life ($t_{1/2}$) of 1 h, resulting in low oral bioavailability (~60–65%) (Blum et al., 1988). An alternative route for AZT administration could be an interesting way to increase it bioavailability. Also, a colloidal carrier used to entrap AZT could deliver it at a continuous rate and reduce the dose-dependent toxicity by minimizing the fluctuations in plasma concentrations.

Colloidal drug carriers are interesting in drug delivery systems because their small size allows them to permeate through biological barriers (Nakada et al., 1996). The novel drug delivery systems for anti-HIV agents include micelles and microemulsions, liposomes, polymeric microparticles and nanoparticles (Ojewole et al., 2008). For nearly three decades, polymeric nanoparticles have been extensively studied because of their unique and valuable physicochemical and biological properties. Indeed, nanoparticles can protect the drug from degradation, enhance its transport and prolong its release; therefore, they may improve the plasma half-life of the drug (Oppenheim, 1981; Allémann et al., 1993). The pharmacokinetic parameters are altered with the nanoparticles and its surface composition plays an important role in drug bioavailability, that can be greater or lower than drug solution/powder depending on the polymer used (Ubrich et al., 2005; Hoffart et al., 2006). Since some nanoparticles' characteristics, such as particle size and surface charge can be modulated by modifying some pro-

* Corresponding author. Tel.: +55 42 36298137; fax: +55 42 36298102.
E-mail addresses: rubianamainardes@pq.cnpq.br, rubianamainardes@hotmail.com (R.M. Mainardes).

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cess parameters on the formulation, they can be used in various applications, involving different routes of administration.

The nasal mucosa is an attractive site for the delivery of drugs and vaccines, because it has a relatively large absorptive surface and low proteolytic activity. Some formulation factors should be considered while designing the drug delivery system for intranasal administration. The formulation should be designed so as to provide rapid transport of the drug across the nasal mucosa and a long residence time in the nasal cavity, to overcome nasal mucociliary clearance (Ugwoke et al., 2001). A critical parameter for transport across the nasal mucosa is the particle size. It has been shown that nanoparticles can play an important part in improving the bioavailability of drugs delivered intranasally (Tobio et al., 1998; Mainardes et al., 2006). Nanoparticles cross the mucosal epithelium better than microspheres since are better taken up by microfold cells overlay the nasal associated lymphoid tissue (NALT) (Huang and Donovan, 1998; Brooking et al., 2001). The polymeric composition of the nanoparticles also influences its transport. The potential of polyethylene glycol (PEG)-corona nanoparticles for nasal protein administration has been explored and had a positive effect in preserving the stability of nanosystems in contact with mucosal components (Alonso, 2004). A slight interaction with the mucus aids the access of the nanosystem to the epithelium, but this interaction should not lead to the aggregation of nanoparticles at the mucus level.

In this study, we show that PLA and PLA–PEG blend nanoparticles prolong the plasma circulating time of AZT after single-dose intranasal administration. The PEG had a crucial role in the formulation since it promoted a great increase in the bioavailability of AZT. The PLA and PLA–PEG nanoparticles represent promising versatile carriers for AZT taken by the intranasal route.

2. Materials and methods

2.1. Materials

The polymers poly(lactic acid) (PLA) (Mw 40–100 kDa) (viscosity 0.15–0.25) and poly(ethylene glycol) (PEG) (Mw 6 kDa) were obtained from Sigma (St. Louis, MO, USA). The surfactant used in the emulsification process was polyvinyl alcohol (PVA) (Mw 18 kDa – 85% hydrolyzed) from Sigma (St. Louis, MO, USA). The organic solvent was methylene chloride (Labsynth Ltd., São Paulo, Brazil). Zidovudine was a gift from FURP (São Paulo, Brazil). Thiopental was purchased from Cellofarm Ltd. (São Paulo, Brazil). Methanol HPLC grade was used (Mallinkrodt, Rio de Janeiro, Brazil). All the reagents used for buffer preparation were of analytical grade.

2.2. Preparation of zidovudine-loaded nanoparticles

The nanoparticles were produced by the double emulsion solvent evaporation technique, as described elsewhere (Zambaux et al., 1998). For the preparation of PLA nanoparticles, approximately 50 mg PLA was dissolved in 2 mL methylene chloride. This solution was poured rapidly on to 200 μL of PVA solution (0.1%, w/v) containing AZT (20 mg) and emulsified by means of sonication for 30 s (55 W) (XL 2002 Sonicator® ultrasonic liquid processor). The resulting water-in-oil (W/O) emulsion was further emulsified with 4 mL of PVA solution (0.7%, w/v) by sonication for 1 min (55 W), giving rise to the water-in-oil-in-water emulsion (W/O/W). Next, the organic solvent was rapidly eliminated by evaporation under vacuum (20 min) at 37 °C. The particles were then recovered by centrifugation (168,000 × *g*, 20 min, 25 °C – Hitachi centrifuge) and washed twice with water to remove the surfactant.

PLA–PEG blend nanoparticles were prepared by mixing 50 g PLA and 12.5 g of PEG in methylene chloride (2 mL), giving a 1:0.25 PLA–PEG blend. Nanoparticles of this blend were made exactly as described above for PLA nanoparticles.

2.3. Physicochemical characterization of nanoparticles

2.3.1. Particle size

Mean particle size and polydispersity index were determined in double distilled water by photon correlation spectroscopy (Brookhaven Instruments Corp.). The analyses were performed at a scattering angle of 90° and a temperature of 25 °C. For each sample, the mean particle diameter, polydispersity and the standard deviation of ten determinations were calculated.

2.3.2. Zeta potential

The zeta potential of the nanoparticles was obtained from their measured electrophoretic mobility (Zeta Plus, Brookhaven Instruments Corp.) The samples were appropriately diluted with KCl 0.1 mM in order to maintain a constant ionic strength, and placed in the electrophoretic cell where a potential of ±150 mV was applied. Three measurements were made for each sample and the zeta potential was calculated.

2.3.3. Morphology

The morphology of nanoparticles was examined by scanning electron microscopy (SEM) (JEOL JSM-T330A), operating at 10 and 20 kV. The nanoparticles were fixed on supports and coated with gold.

2.4. Drug entrapment efficiency

The percentage of drug incorporated during nanoparticle preparation was determined indirectly. Solid nanoparticles were separated from the supernatant containing excess of AZT by ultracentrifugation, as described in Section 2.2. After appropriate dilutions in HPLC mobile phase, 100 μL of the sample was injected into the HPLC system and the drug concentration in the supernatant was obtained by comparison with an analytical curve previously constructed. Before injection, all solutions were previously filtered through a membrane filter (pore size 0.22 μm, Millipore). The amount of AZT entrapped in nanoparticles was obtained by subtracting the quantity of drug in the supernatant from the total amount used for the preparation. The analyses were performed in triplicate with the same HPLC system and conditions described below in Section 2.5.3 (*In vivo* quantitation of AZT), except that the mobile phase consisted of a mixture of methanol:water (1:4, v/v).

2.5. Pharmacokinetic study

2.5.1. Intranasal administration

Male adult Wistar rats with a mean body weight of 190 ± 10 g were fasted overnight, prior to the experiments, with free access to water. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg kg^{-1}. A cannula was used to instill 100 μL of aqueous suspension of AZT-loaded nanoparticles (containing 160 μg mL^{-1} de AZT) into each nostril, in 5 drops of 20 μL at 4-min intervals. This spaced instillation was intended to allow a high dose of particles to be delivered, while minimizing the overflow into the gastrointestinal tract. The AZT aqueous solution also 160 μg mL^{-1} was instilled drop wise into the nostrils in exactly the same way as the suspension, in a control group of rats. In all cases, blood samples (300 μL) were withdrawn from the tail vein of the rats at 0 (pre-dose) and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 24 h after the intranasal administration. The study was previously approved by the research ethics committee of Faculdade de Ciências Farmacêuticas de Araraquara, Brazil.

Table 1
Nanoparticle characteristics.

Polymer	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Encapsulation efficiency (%)
PLA	265.8 ± 15.3	0.311	−20 ± 1.2	42 ± 6
PLA/PEG blend (1:0.25)	328.1 ± 8.6	0.383	−6.5 ± 2.1	52 ± 4

Values are reported as mean ± S.D. ($n = 3$).

2.5.2. Sample preparation

To determine AZT in rat plasma, blood samples were taken from the tail vein and collected in tubes containing sodium heparin. The tubes were centrifuged (850 × *g* for 20 min) at 27 °C, to separate the plasma and it was stored at −20 °C until analysis. In order to ensure the effective separation of AZT from plasma components by HPLC, the plasma was deproteinized. To 100 μL of plasma was added 1 mL of acetonitrile. The mixture was vortexed for 1 min and centrifuged for 15 min at 1600 × *g*. The supernatant was transferred to a tube and evaporated to dryness (approximately 15 min), under a stream of nitrogen. The resulting residue was reconstituted in 200 μL of the HPLC mobile phase.

2.5.3. In vivo quantitation of AZT

A Varian HPLC system (Pro-Star 330) was used to quantitate AZT in plasma, with the UV detection set at 265 nm and with a LiChrospher® 100-RP-18 (250 mm × 4 mm i.d., pore size 5 μm) analytical column. AZT was eluted by isocratic flow at a rate of 1 mL min^{-1}, with a mobile phase comprising a mixture of sodium acetate buffer (55 mM – 7.0 pH) and acetonitrile (91:9, v/v). The HPLC method was validated and the sensitivity was 90 ng mL^{-1} (Mainardes and Gremião, 2009).

2.5.4. Data analysis

The maximum observed plasma concentration (C_{max}) and the time taken to reach it (T_{max}) were obtained from the curve of AZT concentration vs. time. The area under this curve, from $t = 0$ to ∞ ($AUC_{0-\infty}$) was calculated by the trapezoidal rule and the first-order elimination rate constant (K_e) was estimated by least-squares regression of the points describing the terminal log-linear decay phase. The half-life ($t_{1/2}$) was derived from K_e ($t_{1/2} = \ln 2/K_e$).

2.5.5. Statistics

All data are reported as mean ± standard error of mean (S.E.M.) and the difference among groups were compared using one-way analysis of variance (ANOVA) and difference greater at $p < 0.05$ were considered significant.

3. Results

3.1. Preparation and characterization of AZT-loaded PLA and PLA–PEG blend nanoparticles

The choice of a particular method of encapsulation is usually determined by the solubility characteristics of the drug. Owing to the hydrophilic characteristics of AZT, nanoparticles were prepared by the double emulsion–evaporation method, with appropriate modifications.

The nanoparticles obtained were examined by SEM (Figs. 1 and 2). From these micrographs, nanoparticles prepared with either PLA or PLA–PEG blends containing AZT were spherical in shape and did not show aggregation.

The basic physicochemical characteristics of the nanoparticles are presented in Table 1. It can be seen that the presence of PEG in the blend altered several physicochemical characteristics of the particles. The PLA–PEG blend nanoparticles were larger than those prepared from PLA alone and had a higher polydispersity index, indicating a broader size distribution. The zeta potential was calculated to assess whether the PLA/PEG blend might modify the particle surface charge. The most negative zeta potential was displayed by the PLA nanoparticles, probably reflecting the presence of carboxyl end groups on the PLA chains at the surface of the particles. A significant decrease in the zeta value was observed in PLA–PEG (1:0.25) blend nanoparticles, possibly due to PEG chains covering of the PLA terminal carboxyl groups and masking their negative charges. The PLA nanoparticles had a much lower zeta potential than the PLA–PEG blend nanoparticles, indicating the formation of a coat of PEG on the nanoparticle surface. This effect is very relevant to understanding the *in vivo* behavior of the particles.

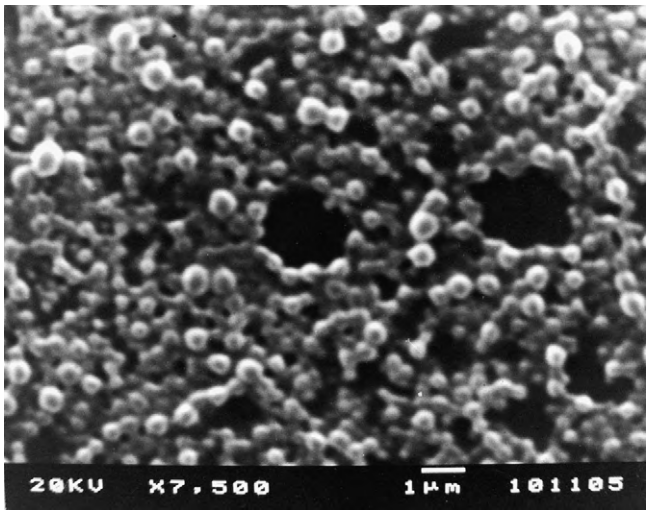

Fig. 1. SEM image of AZT-loaded PLA nanoparticles.

The encapsulation efficiency achieved for AZT was enhanced by the presence of PEG on the PLA chains (Table 1). Thus PLA

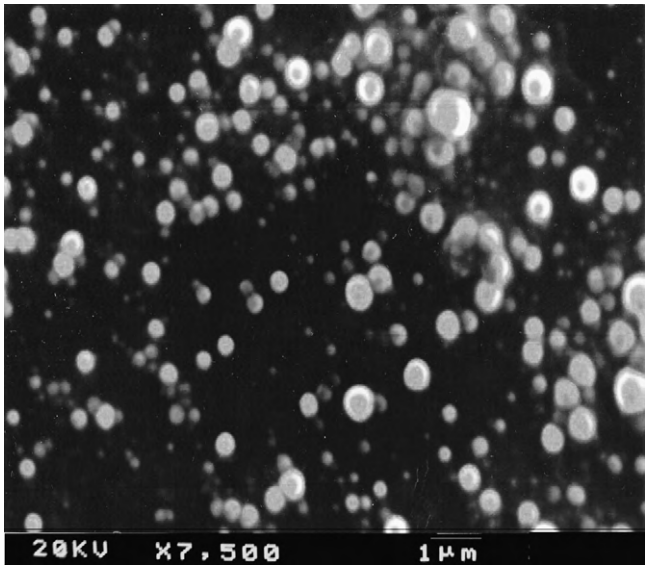

Fig. 2. SEM image of AZT-loaded PLA–PEG blend nanoparticles.

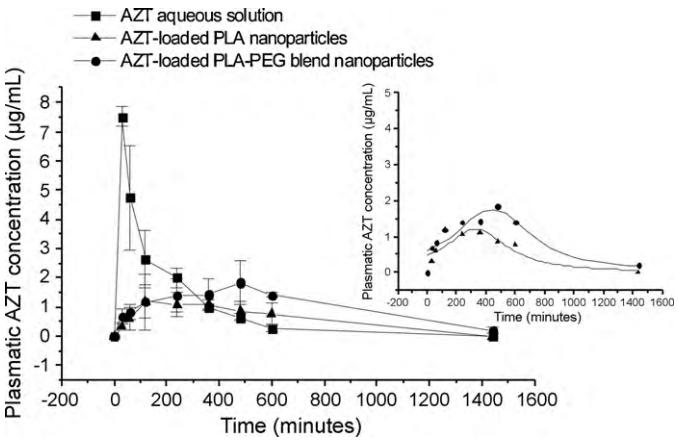

Fig. 3. Comparative *in vivo* plasma concentration vs. time profiles of the different AZT formulations after intranasal administration. All values are reported as mean ± S.D. ($n = 6$).

nanoparticles showed around 42% efficiency, while PLA–PEG blend nanoparticles reached 52% ($p < 0.05$).

3.2. Pharmacokinetic study

AZT-loaded PLA and PLA–PEG blend nanoparticles were designed to improve the drug bioavailability by the intranasal route. Blood levels after intranasal administration of the nanoparticle formulations were compared with those achieved with AZT aqueous solution. Fig. 3 shows the mean AZT plasma concentration–time profiles after intranasal delivery of: AZT aqueous solution, AZT-loaded PLA nanoparticles and AZT-loaded PLA–PEG blend nanoparticles, on AZT dose of 160 μg mL^{-1} in rats. Table 2 lists the relevant pharmacokinetic parameters. The AZT aqueous solution was absorbed quickly after intranasal administration and the maximum plasma concentration of approximately 7.5 μg mL^{-1} was reached in 30 min. Thereafter, the AZT plasma concentration decreases abruptly, as it was rapidly metabolized, resulting in high K_e and short $t_{1/2}$, approximately 1.19 h. This result is consistent with the literature (Blum et al., 1988; Yarchoan et al., 1989). AZT, 10 h after intranasal administration, was still detected (0.27 μg mL^{-1}), but it was not detectable at 24 h. The AZT from nanoparticles showed a different pharmacokinetic profile with a sustained release of AZT over hours. It can be observed that 30 min after intranasal administration of AZT-loaded PLA nanoparticles, the mean plasma concentration was approximately 0.3 μg mL^{-1}, and for AZT-loaded PLA–PEG blend nanoparticles the plasma concentration was approximately 0.7 μg mL^{-1}. The values of C_{max} obtained with the two nanoparticle formulations were not significantly different, but the T_{max} with PLA–PEG nanoparticles was about twice that with PLA nanoparticles and 16-fold the T_{max} for AZT solution, demonstrating an obvious sustained release of AZT from PEGylated nanoparticles. AZT from PLA–PEG nanoparticles, 24 h after intranasal administration, was still detectable in plasma (0.21 ± 0.08 μg mL^{-1}), but AZT from PLA nanoparticles was no longer detected. The relative bioavailability of AZT-loaded PLA–PEG blend nanoparticles was 2.7 with respect to AZT-loaded PLA nanoparticles and 1.3 compared to AZT aqueous solution. Thus, AZT from PLA nanoparticles showed lower intranasal bioavailability than AZT aqueous solution, although the drug had a prolonged release profile and longer $t_{1/2}$. Both nanoparticles formulations increased the $t_{1/2}$ of AZT, but the PLA–PEG blend nanoparticles gave the better result, increasing the AZT $t_{1/2}$ to 7 h (Table 2). The results indicate that PLA–PEG blend nanoparticles improved the intranasal AZT bioavailability and maintained a prolonged drug release profile over 24 h.

4. Discussion

To reach the therapeutic target sites, antiretroviral drugs must cross several biological barriers such as mucous membranes, blood–brain barrier and cell membranes. The physicochemical and metabolic properties of these drugs contribute to reducing the amount of drug in the blood and affected tissues (Aungst, 1999; Li and Chan, 1999). Variability also occurs in the bioavailability profile of antiretroviral drugs and represents a significant factor in the failure of some drug regimens (Csajka et al., 2003; Aarnoutse et al., 2003). The development of delivery systems for antiretroviral drugs has focused on overcoming these barriers to drug delivery in adequate amounts at appropriate rates, thus improving the drug pharmacokinetics (Ojewole et al., 2008; Sosnik et al., 2009). In the present work, PLA and PLA–PEG blend nanoparticles were developed to maintain a prolonged AZT release after intranasal administration. The influence of PEG on the physicochemical characteristics and pharmacokinetic parameters were investigated.

The mean hydrodynamic diameter and polydispersity index of the particles were analyzed and it was found that the presence of PEG lead to an increase in particle size. This was to be expected, since the addition of PEG made the organic phase more viscous, hindering the dispersion of the aqueous phase in the organic phase, during the first emulsion formation. This first emulsion, subsequently, had a low efficiency in reducing the globule diameter during the second emulsion phase.

Analysis of the zeta potential demonstrated that the nanoparticles of PLA–PEG blends differed in their surface charge from PLA nanoparticles. A significant decrease in this potential was observed in PLA/PEG nanoparticles, resulting from the covering of the PLA terminal carboxyl groups by the PEG chains, masking the negative charge on these carboxyl groups. The presence of a hydrophilic PEG steric barrier would shift the "slipping plane" (between particle and surrounding phase) away from the surface of the PLA core and this would also result in a reduced zeta potential (Stolnik et al., 1994; Gref et al., 1995). The PLA–PEG blend is more amphiphilic than PLA alone, and the PEG, due to its hydrophilic character, shows more affinity for the aqueous phase of the emulsion; thus, when the organic solvent is evaporated, the PLA precipitates on the emulsion globule and PEG chains predominate on the particle surface. These results suggest a possible steric protection of the nanoparticles by the PEG in the blend.

The encapsulation efficiency of AZT in PLA and PLA/PEG blend was determined and compared. The results showed that the

Table 2
Pharmacokinetic parameters of AZT from formulations.

AZT formulation	$AUC_{0-\infty}$ (μg min mL^{-1})	C_{max} (μg mL^{-1})	T_{max} (min)	K_e (h^{-1})	$t_{1/2}$ (h)
Aqueous solution	1126.8 ± 129.2	7.5 ± 0.3	30	0.5837	1.19
PLA nanoparticles	552.2 ± 208.6	1.18 ± 0.9	240	0.1224	5.66
PLA/PEG blend nanoparticles (1:0.25)	1482.7 ± 197.7	1.8 ± 0.8	480	0.0988	7.01

Values are reported as mean ± S.D. ($n = 6$). AUC: area under the plasma concentration–time curve from 0 h to ∞; C_{max}: peak concentration; T_{max}: time to reach peak concentration; K_e: constant of elimination; and $t_{1/2}$: mean half-life.

presence of PEG favored an increase in the AZT encapsulation by approximately 10% relative to PLA nanoparticles. Owing to its hydrophilic character, PEG shows more affinity for AZT, a hydrophilic drug, improving the emulsification process and increasing the incorporation of AZT in emulsion globules. The enhancement of the interaction between polymer and drug can reduce the drug loss during the emulsification process (Dong and Feng, 2004).

The determination of physicochemical characteristics of nanoparticles intended for controlled drug release is very important since they can govern the type of application of the system and can be used to predict its behavior in the biological medium. The size and surface charge are important parameters in a nanostructured system since they interfere directly in biological processes, such as transport across biological membranes, recognition by mononuclear and polymorphonuclear cells of the defense system and also the biodistribution (Owens and Peppas, 2006; Beletsi et al., 2005).

A correlation between particle surface charge and opsonization has also been demonstrated *in vitro*, with research showing that neutrally charged particles have a much lower opsonization rate than charged particles (Roser et al., 1998). Since the initial opsonization of particles is so critical to the process of phagocytic recognition and clearance from the bloodstream, researchers have focused on trying to stop or block this step of the process thus increasing the blood circulation half-life and effectiveness of the particle (Carstensen et al., 1992; Norman et al., 1992). Polymers containing long hydrophilic chains adsorbed in particle surface can block the electrostatic and hydrophobic interactions that help opsonins bind to particle. Some examples of polymer systems polysaccharides, polyacrylamide, poly(vinyl alcohol), poly(*N*-vinyl-2-pyrrolidone), PEG, and PEG-containing copolymers such as poloxamers, poloxamines, polysorbates, and PEG copolymers. Of all the polymers tested to date, the most effective and most commonly used are the PEG and PEG-containing copolymers. These polymers are very flexible, highly hydrophilic and present neutral charge, which can help shield even hydrophobic or charged particles from blood proteins and lessens the effect of electrostatic interactions (Owens and Peppas, 2006). In the case of PEG nanoparticles, another important parameter in determining the clearance rate of nanoparticles and its biodistribution, is the PEG layer itself. The characteristics of this layer such as thickness, charge, surface density, functional groups, and conformation impact the way in which the particle interacts with opsonins (Stolnik et al., 1995).

The pharmacokinetic study showed that the nanoparticles were able to sustain the AZT delivery over time. The slow and sustained release of AZT from nanoparticles accounts for the long time (T_{max}) required to attain C_{max}. The greatest efficacy was obtained with PLA–PEG blend nanoparticles, that exhibited AZT sustained release over 24 h. The T_{max} for this formulation was increased twofold compared to AZT from PLA nanoparticles, and 16-fold relative to AZT aqueous solution. AZT plasma levels were detectable up to 10 h after administration in aqueous solution or PLA nanoparticles. The $t_{1/2}$ of AZT also varied among the formulations. The slow elimination rate (K_e) resulted in significantly prolonged $t_{1/2}$ of AZT from PLA and PLA–PEG blend nanoparticles compared to AZT solution. Due to the slow release of AZT from nanoparticles, its metabolic breakdown is made slower, increasing the mean half-life. The significant increase ($p < 0.05$) in the AUC value of the PLA–PEG nanoparticles AZT formulation in comparison to that PLA nanoparticles and AZT aqueous solution distinctly indicates the improved intranasal bioavailability with the blended system.

The great difference between the AUCs of AZT-loaded PLA nanoparticles and PLA–PEG nanoparticles can be explained in two ways: the interaction of the polymers with the nasal epithelium, which influences the transport across the nasal mucosa and the speed of clearance from the blood by mononuclear phagocyte system (MPS). In the first case it has been shown that PEG can play an important role in transport across mucosae (nasal and intestinal). The PEG coating can preserve the stability of nanosystems in contact with mucosal components, preventing the aggregation of nanoparticles at the mucus level and possibly facilitating their transport across the nasal epithelia (Tobio et al., 1998; Vila et al., 2004; Alonso, 2004). PLA nanoparticles aggregate immediately following their incubation with lysozyme, an enzyme that accumulates at mucosal surfaces. This aggregation process may have a negative effect on the further transport of these particles (Vila et al., 2002). The interaction of the polymeric nanoparticles with nasal mucus also is dependent of the surface charge and affects the particle transport across nasal epithelium. This interaction can be explicated in terms of the mucoadhesive properties of the nanoparticles. Mucoadhesion of the particles would improve the drug absorption for it could prolong the intimate contact time of the particle on the nasal mucosa by adhering to the surface of mucus layer. Since the nasal epithelium is negatively charged, excellent mucoadhesion of positive nanoparticles occurs due electrostatic attraction between particle and mucin. Negative nanoparticles cause negative charge repulsion with mucus, and the particle transport is reduced (Wang et al., 2006; Brooking et al., 2001; Morimoto et al., 2001; Schipper et al., 1997). The PLA nanoparticles and PLA–PEG blend nanoparticles reported in this study both presented negative charge, but the presence of PEG on the surface of particle decreased the value of negative charge, almost nearly to neutrality. In this case, the repulsion with mucus could be lower than the PLA nanoparticles and the hydrophilic functional group from PEG could form hydrogen bonds with mucus, thus producing some adhesive force of this polymer, resulting in higher drug absorption. These associated effects can explain the positive effect of the PEG on their ability to overcome the nasal mucosal barrier. Other statement that helps explain the efficacy of PEG nanoparticles in improving the drug bioavailability after intranasal administration is its ability to escape from phagocytosis. After absorption the uncoated nanoparticles in blood are rapidly cleared by the MPS (Fernández-Urrusuno et al., 1996). The rapid particle uptake is due to the opsonization process, which depends on the particle size and surface charge and makes the particle susceptible to recognition by macrophages and circulating monocytes. Thus, opsonized particles exhibit a short blood circulating time and consequently the drug bioavailability falls (Owens and Peppas, 2006). We have recently reported that AZT-loaded PLA nanoparticles were more efficiently delivered into polymorphonuclear cells than PLA–PEG blend ones (Mainardes et al., 2009), illustrating that the presence of PEG influences the particle–cell interaction.

Summarizing, the AZT and other antiretroviral drugs used in current AIDS therapy have some pharmacokinetics limitations, such as: (i) transport to the CNS in ineffective concentrations, and (ii) fast hepatic metabolism leading to the short mean half-life, necessitating therapy with AZT in large and frequent doses, giving rise to hematological toxicity. In light of these considerations it is important to develop systems that improve the pharmacokinetic of AZT. The large and frequent AZT doses could be replaced by a sustained drug release over longer period of time, reducing the fluctuations of the drug in the plasma, and the toxicity. Polymer nanoparticles are able to meet these objectives. The results obtained here should be useful for a future application of AZT-loaded nanoparticles in AIDS treatment. The presence of PEG in the nanoparticle formulation, in the form of a blend, modified the characteristics of the particles and changed their pharmacokinetic profile. The AZT bioavailability after intranasal administration was increased. Our results indicate the potential of the PLA–PEG blend nanoparticles as carriers for AZT delivered by intranasal route.

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References

Aarnoutse, R., Schapiro, J.M., Bouchr, C.A.B., Hkster, Y.A., Burger, D.M., 2003. Therapeutic drug monitoring. Drugs 63, 741–753.
Allémann, E., Gurny, R., Doelker, E., 1993. Drug loaded nanoparticles preparation methods and drug targeting issues. Eur. J. Pharm. Biopharm. 39, 173–191.
Alonso, M.J., 2004. Nanomedicines for overcoming biological barriers. Biomed. Pharmacother. 58, 168–172.
Aungst, B.J., 1999. P-glycoprotein, secretory transport, and other barriers to the oral delivery of anti-HIV drugs. Adv. Drug Deliv. Rev. 39, 105–116.
Beletsi, A., Palazi, Z., Avgoustakis, K., 2005. Biodistribution properties of nanoparticles based on mixtures of PLGA with PLGA–PEG diblock copolymers. Int. J. Pharm. 298, 233–241.
Blum, M.R., Liao, S.H.T., Good, S.S., Miranda, P., 1988. Pharmacokinetics and bioavailability of zidovudine in humans. Am. J. Med. 85, 189–194.
Brooking, J., Davis, S.S., Illum, L., 2001. Transport of nanoparticles across the rat nasal mucosa. J. Drug Target. 9, 267–279.
Carstensen, H., Muller, R.H., Muller, B.W., 1992. Particle-size, surface hydrophobicity and interaction with serum of parenteral fat emulsions and model-drug carriers as parameters related to RES uptake. Clin. Nutr. 11, 289–297.
Chien, Y.W., Wearley, L.L., 1989. AIDS and chemotherapy. Drugs Today 25, 19–25.
Csajka, C., Marzollini, C., Fattinger, K., Décosterd, L.A., Telenti, A., Biollaz, J., Buclin, T., 2003. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. Clin. Pharm. Ther. 73, 20–30.
Dong, Y., Feng, S.-S., 2004. Methoxy poly(ethylene glycol)–poly(lactide) (MPEG–PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials 25, 2843–2849.
Fernández-Urrusuno, R., Fattal, E., Rodrigues Jr., J.M., Féger, J., Bedossa, P., Couvreur, P., 1996. Effect of polymeric nanoparticle administration on the clearance activity of the mononuclear phagocyte system in mice. J. Biomed. Mater. Res. 31, 401–408.
Furman, P.A., Fufe, J.A., St. Claire, M.H., Weinhold, K., Rideout, J.L., Freeman, G.A., Nusinhof-Lehrman, S., Bolognesi, D.P., Broder, S., Mitsuya, H., Barry, D.W., 1986. Phosphorylation of 3%-azido-3%-deoxythymidine and selective interaction of the 5%-triphosphate with human immunodefiency virus reverse transcriptase. Proc. Natl. Acad. Sci. U.S.A. 83, 8333–8337.
Gill, P.S., Rarick, M., Brynes, R.K., Causey, D., Loureiro, C., Levine, A.M., 1987. Azidothymidine associated with bone marrow failure in the acquired immunodeficiency syndrome (AIDS). Ann. Int. Med. 107, 502–505.
Gref, R., Domb, A., Quellec, P., Blunk, T., Muller, R.H., Verbavatz, J.M., Langer, R., 1995. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. Adv. Drug Deliv. Rev. 16, 215–223.
Hoffart, V., Lamprecht, A., Maincent, P., Lecompte, T., Vigneron, C., Ubrich, N., 2006. Oral bioavailability of a low molecular weight heparin using a polymeric delivery system. J. Control. Release 113, 38–44.
Huang, Y., Donovan, M.D., 1998. Large molecule and particulate uptake in the nasal cavity: the effect of size on nasal absorption. Adv. Drug Deliv. Rev. 29, 147–155.
Kennedy, C.A., Griffith, H.S., Mathison, G.E., 1991. Erythrocytosis after zidovudine for AIDS. Ann. Int. Med. 114, 250–251.
König, H., Behr, E., Löwer, J., Kurth, R., 1989. Azidothymidine triphosphate is an inhibitor of both human immunodeficiency virus type 1 reverse transcriptase and DNA polymerase gamma. Antimicrob. Agents Chemother. 33, 2109–2114.
Li, X.L., Chan, W.K., 1999. Transport, metabolism and elimination mechanisms of anti-HIV agents. Adv. Drug Deliv. Rev. 39, 81–103.
Mainardes, R.M., Gremião, M.P., Brunetti, I.L., da Fonseca, L.M., Khalil, N.M., 2009. Zidovudine-loaded PLA and PLA–PEG blend nanoparticles: influence of polymer type on phagocytic uptake by polymorphonuclear cells. J. Pharm. Sci. 98, 257–267.
Mainardes, R.M., Gremião, M.P.D., 2009. Reversed phase HPLC determination of zidovudine in rat plasma and its pharmacokinetics after a single intranasal dose administration. Biol. Res. 42, 357–364.
Mainardes, R.M., Urban, M.C., Cinto, P.O., Chaud, M.V., Evangelista, R.C., Gremião, M.P., 2006. Liposomes and micro/nanoparticles as colloidal carriers for nasal drug delivery. Curr. Drug Deliv. 3, 275–285.
Morimoto, K., Kstsumata, H., Yabuta, T., Iwanaga, K., Kakemi, M., Tabata, Y., Ikada, Y., 2001. Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. Eur. J. Pharm. Sci. 13, 179–185.
Nakada, Y., Fattal, E., Foulquier, M., Couvreur, P., 1996. Pharmacokinetics and biodistribution of oligonucleotide adsorbed onto poly(isobutylcyanoacrylate) nanoparticles after intravenous administration in mice. Pharm. Res. 13, 38–43.
Norman, M.E., Williams, P., Illum, L., 1992. Human serum-albumin as a probe for surface conditioning (opsonization) of block copolymer-coated microspheres. Biomaterials 13, 841–849.
Ojewole, E., Mackraj, I., Naidoo, P., Govender, T., 2008. Exploring the use of novel drug delivery systems for antiretroviral drugs. Eur. J. Pharm. Biopharm. 70, 697–710.
Oppenheim, R.C., 1981. Solid colloidal drug delivery systems: nanoparticles. Int. J. Pharm. 8, 217–234.
Owens, D.E., Peppas, N.A., 2006. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int. J. Pharm. 307, 93–102.
Roser, M., Fischer, D., Kissel, T., 1998. Surface-modified biodegradable albumin nano- and microspheres. II. Effect of surface charges on *in vitro* phagocytosis and biodistribution in rats. Eur. J. Pharm. Biopharm. 46, 255–263.
Schipper, N.G.M., Olson, S., Hoogstraate, J.A., deBoer, A.G., Varum, K.M., Artursson, P., 1997. Chitosan as absorption enhancers for poorly absorbable drugs. 2: Mechanism of absorption enhancement. Pharm. Res. 14, 923–929.
Sosnik, A., Chiappetta, D.A., Carcaboso, A.M., 2009. Drug delivery systems in HIV pharmacotherapy: what has been done and the challenges standing ahead. J. Control. Release 138, 2–15.
Stolnik, S., Dunn, S.E., Garnett, M.C., Davies, M.C., Coombes, A.G., Taylor, D.C., Irving, M.P., Purkiss, S.C., Tadros, T.F., 1994. Surface modification of poly(lactide-co-glycolide) nanospheres by biodegradable poly(lactide)-poly(ethylene glycol) copolymers. Pharm. Res. 11, 1800–1808.
Stolnik, S., Illum, L., Davies, S.S., 1995. Long circulating microparticulate drug carriers. Adv. Drug Deliv. Rev. 16, 195–214.
Tobio, M., Gref, R., Sanchez, A., Langer, R., Alonso, M.J., 1998. Stealth PLA–PEG nanoparticles as protein carriers for nasal administration. Pharm. Res. 15, 270–275.
Ubrich, N., Schmidt, C., Bodmeier, R., Hoffman, M., Maincent, P., 2005. Oral evaluation in rabbits of cyclosporine-loaded Eudragit RS or RL nanoparticles. Int. J. Pharm. 288, 169–175.
Ugwoke, M.I., Verbeke, N., Kinget, R., 2001. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J. Pharm. Pharmacol. 53, 3–21.
Vila, A., Gill, H., McCallion, O., Alonso, M.J., 2004. Transport of PLA–PEG particles across the nasal mucosa: effect of particle size and PEG coating density. J. Control. Release 98, 232–244.
Vila, A., Sanchez, A., Calvo, P., Tobyo, M., Alonso, M.J., 2002. Design of biodegradable particles for protein delivery. J. Control. Release 78, 15–24.
Wang, J., Tabata, Y., Morimoto, K., 2006. Aminated gelatin microspheres as a nasal delivery system for peptide drugs: evaluation of *in vitro* release and in vivo insulin absorption in rats. J. Control. Release 113, 31–37.
Warnke, D., Barreto, J., Temesgen, Z., 2007. Antiretroviral drugs. J. Clin. Pharmacol. 47, 1570–1579.
Yarchoan, R., Mitsuya, H., Myers, C.E., Broder, S., 1989. Clinical pharmacology of 3′-azido-2′,3′-dideoxythymidine (zidovudine) and related dideoxynucleosides. N. Engl. J. Med. 321, 726–738.
Zambaux, M.F., Bonneaux, F., Gref, R., Maincent, P., Dellacherie, E., Alonso, M.J., Labrude, P., Vigneron, C., 1998. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. J. Control. Release 50, 31–40.