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Improved bioavailability of orally administered mifepristone from PLGA nanoparticles

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Abstract

The objective of this study was to prepare an oral dosage formulation of mifepristone that will improve the oral bioavailability of mifepristone and sustain the release of mifepristone for at least 3 days to effectively control reproduction, especially in coyotes. Nanoparticles containing mifepristone were prepared from DL-lactide/glycolide copolymers (PLGA). Encapsulation efficiency of the nanoparticles was determined by HPLC. In vitro release study was done in 30% isopropyl alcohol in water. In vivo bioavailability study was performed in male rats. Mifepristone and drug-loaded 50/50 PLGA, M_W 4.4 kDa, nanoparticles (equivalent to 100 mg/kg mifepristone) were administered orally to rats. The concentration of mifepristone in serum at different time intervals was determined by HPLC. The average sizes of 50/50 PLGA (M_W 4.4 and 13 kDa) nanoparticles containing mifepristone were 516 and 468 nm, respectively. The drug encapsulation efficiency was 75.6% at 20% drug loading in 50/50 PLGA (M_W 4.4 kDa) nanoparticles. In vitro cumulative release of mifepristone from the 50/50 PLGA (M_W 4.4 and 13 kDa) nanoparticles with 20% drug loading was 60% and 48% in 72 h, respectively. In vivo studies in rats demonstrated that PLGA-1A-nanoparticles increase the bioavailability of mifepristone. We are currently using the nanoparticles containing mifepristone for efficacy studies in coyotes.

Keywords: Mifepristone; Poly(DL-lactide-co-glycolide) (DL-PLGA); Nanoparticles; Oral sustained release; Bioavailability

1. Introduction

Mifepristone (RU 486; 11β-[4-dimethylamino]phenyl-17β-hydroxy-17[1-propynyl]estra-4,9-dien-3-one) is a remarkably active antiprogesterone and an abortifacient in many species (Heikinheimo et al., 2003). It is used in the medical termination of first-trimester pregnancy and in emergency contraception in human (Sarkar, 2002; Heikinheimo, 1997; Kekkonen et al., 1996). In addition to its antiprogesterone action, mifepristone can be used for other diseases. Literature reviews indicate that mifepristone can inhibit the growth of ovarian carcinoma cells (Wang et al., 2005) and breast cancer cells (Lanari and Molinolo, 2002; Creinin, 2000). Mifepristone can slow down the progression of cognitive decline in Alzheimer's (Belanoff et al., 2002; Pomara et al., 2002; DeBattista and Belanoff, 2005), the probable reason being related to beta-Amyloid efflux mediated by *p*-glycoprotein (Lam et al., 2001). Mifepristone also causes comparable leiomyoma regression (Steinauer et al., 2004; Eisinger et al., 2003). A sustained oral drug delivery system will be useful for the continued treatment of these diseases.

Our previous studies have shown that mifepristone is an effective agent in controlling reproduction in captive coyotes but only when administered successively for 3 days (Stith, 2004). A single oral dosage of mifepristone was not effective in coyotes. When coyotes were administered 10 mg/kg of mifepristone orally in a bait for three successive days, abortion resulted in all subjects. This dosing regimen seems to be the lowest dose and shortest administration time necessary for efficacy of mifepristone in coyotes. It is not feasible to administer multiple doses of mifepristone in a field situation for wild coyotes. A sustained release formulation to maintain the oral release of mifepristone over 3 days was considered for an effective single dose treatment of mifepristone in coyotes.

Biologically adhesive drug delivery systems may offer important advantages over conventional drug delivery systems in the bioavailability of drugs. It is shown that DL-lactide/glycolide copolymer (PLGA) has bioadhesive properties and binds with the mucosa of the gastro-intestinal tract (Shakweh et al., 2005;

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Satturwar et al., 2003; Fulzele et al., 2003). This may increase the residency time of the sustained release formulation and may enhance the drug absorption due to intimacy of contact with the epithelium cells. Also, biodegradable nanoparticles (Np) and microparticles are of particular interest as they provide protection of fragile molecules against enzymatic and hydrolytic degradation in the gastro-intestinal tract (Fattal et al., 2002). They can naturally be taken up by enterocytes (Damge et al., 2000; Florence, 1997) and/or the lymphoid tissues in Peyer's patches (PPs) (Eldridge et al., 1990; Jani et al., 1990).

Thus, nanospheres of PLGA have great potential as an oral bioadhesive, sustained release drug delivery system for mifepristone. We choose PLGA polymer to encapsulate mifepristone and prepare nanoparticles. The nanoparticles were evaluated for their in vitro and in vivo characteristics.

2. Material and methods

2.1. Chemicals and reagents

Mifepristone was purchased from Sigma–Aldrich (St. Louis, MO, USA). The internal standard (I.S.) is an analog of mifepristone, and was synthesized by the Research Triangle Institute (Research Triangle, NC, USA). The I.S. is 11β-4(*N*, *N*-dimethylaminophenyl)-17α-methoxymethyl-17β-hydroxy-estra-4,9-dien-3-one; RTI-3021-003. Purity of both compounds was reported as 98% and both were supplied in a fine crystalline dry powder. A 50/50 poly(DL-lactide-co-glycolide) (PLGA-1A and -2A, M_W 4.4 and 13 kDa) were kindly donated by Absorbable Polymers International (Pelham, AL, USA). The inherent viscosity of the two polymers were 0.10 and 0.16 dl/g, respectively. Polyvinyl alcohol (PVA) (M_W 30,000–70,000) was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile (ACN), dichloromethane (CH_2Cl_2), isopropyl alcohol (IPA) and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Preparation of mifepristone PLGA nanoparticles

Mifepristone and PLGA polymer were weighed in a glass vial and dissolved in 0.5 ml dichloromethane. To the organic solution, 10 ml of 2% PVA was added and mixed with a Sonifier Cell disruptor W185 (Branson Ultrasonics Corporation, USA) with 50 s burst for four times. The mixture was kept stirring by Corning Stirrer/Hot Plate (VWR, USA) at certain speed to evaporate all the organic solution for 3 h. After that the aqueous colloidal mixture was centrifuged at 11,000 × *g* for 30 min by Allegra™ 64R Centrifuge (Beckman Coulter, USA), and lyophilized by freeze dry system/freezone 4.5 (Labconco, USA) for 24 h to obtain the dry nanoparticle powder.

2.3. Encapsulation efficiency (EE) of mifepristone

A weighed quantity of lyophilized powder was dissolved in dimethyl sulfoxide and the concentration of mifepristone was determined by HPLC with two Shimadzu LC 600 pumps, and a Waters (Milford, MA, USA) 486 tunable absorbance detector. HPLC separation column (symmetry 150 mm × 4.6 mm, C_{18}) and guard column (μBondapak C_{18}) were all purchased from Waters (Milford, MA, USA). System management and hardware interface for data acquisition was done using the Millennium 3.1 computer software package from Waters. From the concentration, we calculated the encapsulation efficiency of mifepristone in the nanoparticles.

$$\mathrm{EE}\,(\%) = \frac{\mathrm{mifepristone}_{\mathrm{in\,Np}}\,(\mathrm{mg})}{\mathrm{mifepristone}_{\mathrm{total}}\,(\mathrm{mg})} \times 100\%$$

2.4. In vitro release study of mifepristone–PLGA-1A and -2A-nanoparticles

A weighed quantity of nanoparticles were put into cellulose dialysis membrane tubing (Spectrum Laboratories Inc., CA) and immersed in a 50 ml 30% isopropyl alcohol solution. Thirty percent isopropyl alcohol in water was used as the dissolution medium because of the poor solubility of the drug. The solution was kept at 37 °C and 50 rpm in shaking incubator 4.0CFT 1570 (VWR International Int., OR). One millilitre solution was withdrawn at different time intervals. The media was replaced with the same volume of fresh solution. The solution was analyzed by HPLC and the cumulative release of mifepristone from the nanospheres was calculated.

2.5. Pharmacokinetic study of mifepristone–PLGA-1A-nanoparticles in rats after oral administration

Male Sprague-Dawley rats (385–480 g) were used for the study. Mifepristone–PLGA-1A (M_W 4.4 kDa) nanoparticle (equivalent to 100 mg/kg mifepristone) was weighed in a 1.5 ml centrifuge tube and suspended in 1.5 ml of water. The nanoparticle suspension was administered by a long blunt animal feeding needle (Popper & Sons, NY). Uncoated mifepristone was weighed into mini-capsules and were given by Torpac® dosing kits for rodents (Fairfield, NJ) followed by 1.5 ml of water. Nanoparticles containing mifepristone was not administered in capsules as the weight of nanoparticles would require five to six of mini-capsules which would be difficult to administer in rats. On the other hand, suspending uncoated mifepristone in water is difficult as the drug is very hydrophobic. Addition of carboxymethylcellulose or hydroxylpropylmethylcellulose as suspending agent may provide mucoadhesive properties to the drug particles and therefore these suspending agents were not used. Approximately 1.5 ml of blood sample from each of these rats was collected for analysis by tail bleeding. Each rat was used for three blood sampling over the 3 days time period. Blood samples were collected into 1.5 ml centrifuge tubes and allowed to clot at 4 °C for approximately 3 h, centrifuged for 7 min at 10,000 × *g* under 4 °C. The serum was harvested and stored at −80 °C until analysis was performed.

A *t*-test was performed on the serum concentrations at each time point for both groups. The *p*-value was set at 0.05. The area under the curve (AUC) was calculated by the trapezoidal method.

Table 1
Particle size of three batches of nanoparticles ($n = 3$) prepared with different PVA concentrations and polymers

	Particle size of three batches (nm)			Mean size (nm)	S.D.
	1	2	3		
2% PVA-2A-Np	334	329	330	331	2.2
5% PVA-2A-Np	333	344	327	335	8.3
2% PVA-2A-DNp	468	465	471	468	3.0
5% PVA-2A-DNp	561	551	549	554	6.6
2% PVA-1A-DNp	515	517	515	516	1.3

Np means nanoparticles; DNp means drug (mifepristone)-loaded nanoparticles. S.D. is standard deviation.

2.6. HPLC determination of mifepristone

The HPLC method described by Stith and Hussain (2003) was used to determine mifepristone in serum. Briefly, the mobile phase was ACN–water (60:40) pumped at 1.0 ml/min. The column temperature was not regulated and ambient temperature for the system ranged from approximately 22 to 24 °C. Absorbance was measured by UV detector set at a wavelength of 305 nm. Data were collected, integrated and analyzed using Waters Millennium software.

Serial dilutions of the 1000 ng/ml standard were made with blank serum to obtain additional standards of 10, 20, 50, 100, 200, 500 and 1000 ng/ml. Standard serum concentrations were analyzed in duplicate.

Except for the blank, serum samples (0.5 ml) were spiked with 25 μl of I.S. working solution (10 μg/ml) and vortexed. All samples (blank, unknowns, and standards) were extracted using disposable solid-phase extraction cartridges (Oasis HLB 1 ml, 30 mg).

2.7. Observation of morphology and determination of size

The size of the mifepristone–PLGA-nanoparticles was determined by suspending the powder in water, and then detected by Zetasizer Nano ZS (Malvern Instruments, UK). The morphological picture was obtained by dropping the same colloidal suspension onto a copper grid, dyed with 2% uranate acid, dried by air for 15 min and then observed under the Hitachi H-7000 Electron Microscope (Hitachi, Japan).

3. Results and discussion

3.1. Size, morphology and encapsulation efficiency of nanoparticles

The determination of the size was performed automatically by the Zetasizer. The following Table 1 shows the particle sizes of the nanoparticles from different conditions. Five percent PVA had a higher viscosity and caused a slightly larger DNp. A representative particle size distribution for mifepristone–PLGA-1A-Np is shown in Fig. 1.

The morphology of mifepristone–PLGA-nanoparticles as determined by the electron microscope are shown in Fig. 2. The TEM study demonstrated that these nanoparticles were round and did not stick to each other. It appeared that the lyophilized powder could be re-dispersed in water easily. This would be helpful for the reconstitution of the dry powder and stability during stocking.

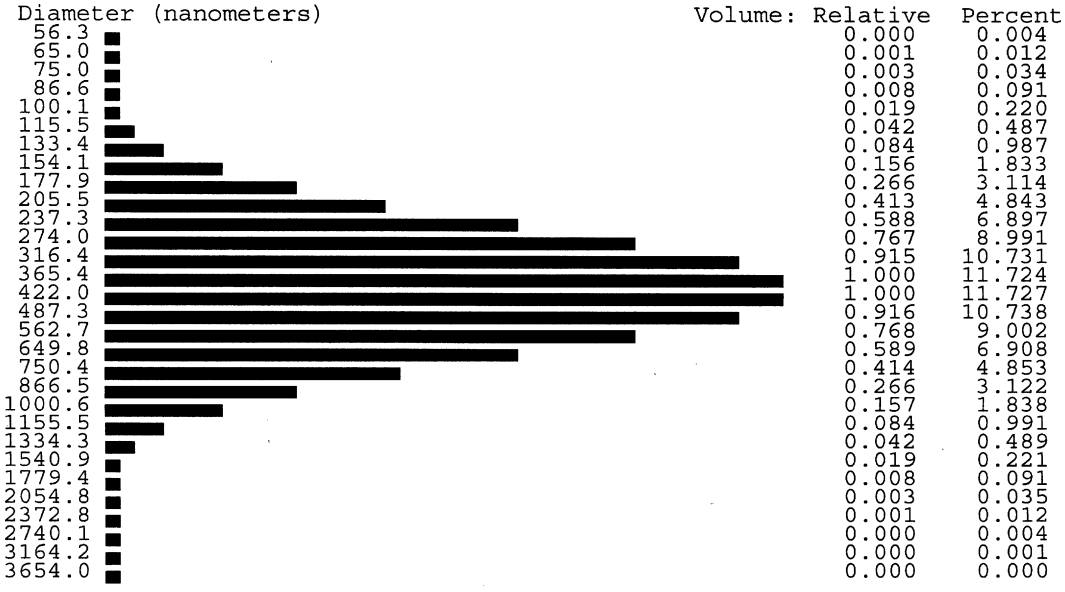

Fig. 1. A representative particle size distribution chart for mifepristone–PLGA-1A-Np obtained from the Zetasizer.

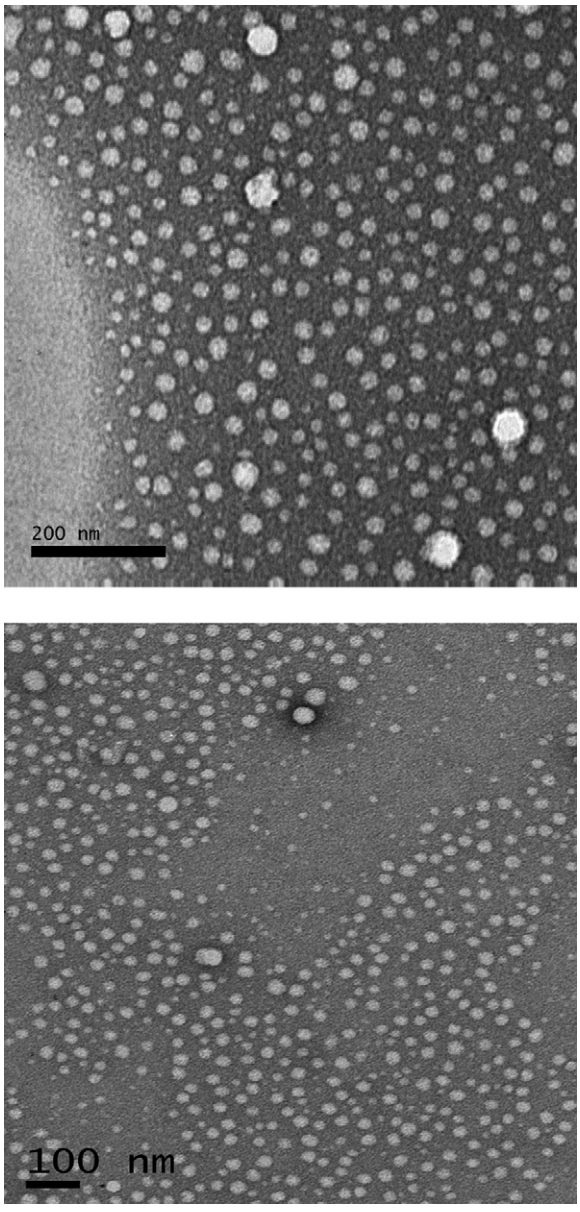

Fig. 2. Transmission electron microscopy (TEM) picture of mifepristone–PLGA-1A-Np (upper picture) and mifepristone–PLGA-2A-Np (lower picture). The drug-loaded nanoparticles were prepared from 2% PVA.

The sizes obtained from the two methods used here for the same nanoparticles were different. TEM pictures show that the sizes of the nanoparticles are much smaller than the size determined by the Zetasizer Nano. First, the operation of each method was different. The sample was in colloidal suspension when the size was determined by Zetasizer Nano while the samples for TEM were dried in the air and then were observed under the microscope. Second, a microscope produces a number distribution and a Zetasizer Nano instrument (a measurement method using photon correlation spectroscopy, PCS) produces an intensity distribution. Samples prepared for electron microscope examination are often harshly treated; this treatment can distort soft materials. PCS in contrast measures the hydrodynamic diameter of dispersed particles in their native environment. When the particles are in solution, the charge may cause the aggregation of the small particles. Also, the Zetasizer tends to give an average dispersion while the TEM tend to give the observation of each particle. These factors may account for the differences in the size measurement by the two methods. We are reporting in Table 1 the size of the nanoparticles by the Zetasizer Nano, which is much bigger than the size determined by TEM. At least 80% of the nanoparticles were below the mean value of the particle size.

Table 2
Encapsulation efficiency of the nanoparticles for mifepristone

V_o/V_w	PVA (%)	Mifepristone/PLGA-Np (w/w, %)	EE (%)
1/4	2	10[a]	26.08
1/4	5	10[a]	24.55
1/10	1	20[a]	36.36
1/10	2	50[a]	57.09
1/20	2	11[a]	55.58
1/20	2	20[b]	75.6

[a] Mifepristone/PLGA-2A-Np (w/w, %).
[b] Mifepristone/PLGA-1A-Np (w/w, %).

The encapsulation efficiency of mifepristone in the PLGA-nanoparticles is presented in Table 2. The data of PLGA-2A-DNp containing mifepristone shows that the concentration of PVA does not significantly affect the EE of the nanoparticles. Increasing 2% of PVA to 5% could not enhance the EE of PLGA-2A-DNp. It was determined that 2% PVA, 20% mifepristone drug loading and 1:20 ratio of organic phase to aqua phase may be used as the optimum conditions to make PLGA-2A-Np containing mifepristone (PLGA-2A-DNp) for good EE. The physicochemical properties of PLGA-2A and PLGA-1A are similar. PLGA-2A has a higher molecular weight and is degraded slower than 1A. The optimized condition for the DNp was applied to PLGA-1A-DNp.

3.2. *In vitro release study*

The cumulative release of mifepristone from different dosages is shown in Fig. 3.

More than 60% of uncoated mifepristone were released into the media within 24 h while just 40% of mifepristone was released from those mifepristone-loaded nanoparticles. Mifepristone–PLGA-1A-nanoparticles released the drug more quickly than mifepristone–PLGA-2A-nanoparticles. The mixture of 1A- and 2A-nanoparticles (2:1) released mifepristone with an intermediate rate between the two types of nanoparticles. As 3–4 days sustained release was sufficient for us, we chose 1A-nanoparticles for further studies.

3.3. *Pharmacokinetic study of mifepristone–PLGA-1A-nanoparticles in rats after oral administration*

The standard curve of mifepristone in rat serum was obtained between the concentrations of 10 and 1000 ng/ml. The regression equation was: $y = 0.0028x + 0.0005$, $R^2 = 0.9993$. The serum mifepristone concentrations in the treated rats were calculated from the regression equation.

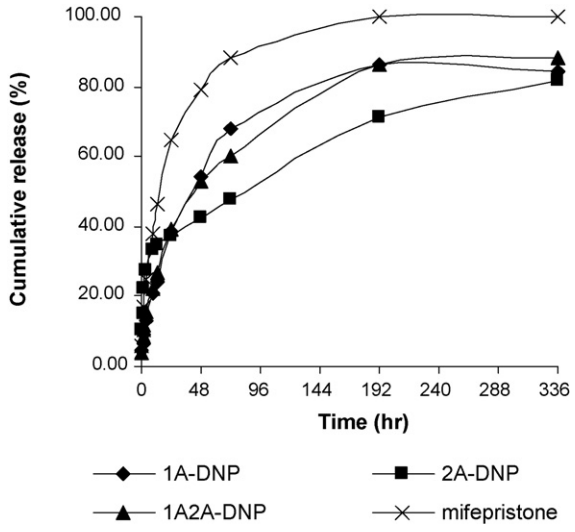

Fig. 3. In vitro cumulate release from different nanoparticles of mifepristone and uncoated mifepristone (DNp means mifepristone-loaded nanoparticles, 1A2A-DNp means mifepristone–PLGA-1A-nanoparticles and mifepristone–PLGA-2A-nanoparticles mixed with the ratio of 2:1).

The serum concentration versus time profile of mifepristone in the rat at different time interval is shown in Fig. 4. The AUC of each curve was calculated and listed in Table 3.

PLGA-1A-DNp released mifepristone quickly at the beginning and then released more smoothly than uncoated mifepristone. On the third day, the concentration of mifepristone was higher in the group of nanoparticles than the mifepristone group. The rate and extent of absorption of mifepristone was higher in the nanoparticle group. Mifepristone was immediately released/absorbed in nanoparticles group (2 and 24 h serum concentrations of mifepristone were significantly higher in the nanoparticle group than the uncoated mifepristone group). Mifepristone may be present at the surface of the nanoparticles

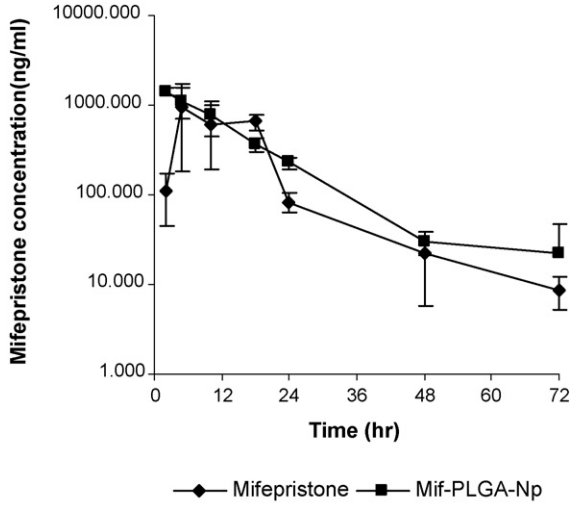

Fig. 4. Mifepristone concentration in rat serum after oral administration of uncoated mifepristone (MIF) and mifepristone–PLGA-1A-Np (MIF–Np). Each data point represents the average of four rats. The error bar represents standard deviation. Two and 24 h serum concentrations of mifepristone were significantly higher ($p = 0.001$ and 0.0004, respectively) in MIF–Np group.

Table 3
The AUC values of the mean serum concentration of mifepristone at different time intervals

Time intervals (h)	AUC (MIF treated group)	AUC (MIF–Np treated group)
0–2	108.3	1442.6
2–5	1587.0	3850.2
5–10	3882.3	4772.1
10–18	5704.5	4593.8
18–24	1867.7	1780.9
24–48	1256.6	3122.0
48–72	368.8	638.8
Total (0–72)	14775.2	20200.3

AUC values were calculated by the trapezoidal rule.

and helped the dissolution of mifepristone in the gastro-intestinal (GI) fluid. The large surface area produced by the nanoparticles allows more drug and GI fluid interactions. The drug may have more dissolution at the surface of the nanoparticles than the drug powders and this may have increased the absorption. The bioadhesive properties of the polymer may have contributed to the increased rate of absorption. Also, the nanoparticle was given in colloidal suspension while uncoated mifepristone was given inside the mini-capsules, immediately followed by the same amount of water (1.5 ml). It was very difficult to suspend mifepristone homogeneously in water and to administer the dose completely without loss. Therefore, the uncoated mifepristone was administered in the mini-capsule. On the other hand, the nanoparticles were easily suspended in the water and the dose could be administered easily without loss. The concentration of mifepristone was maintained at a significantly higher level after 24 h in the nanoparticles group. The concentration of mifepristone in the serum was maintained higher at 48 and 72 h, although the numbers are not statistically significant ($p = 0.205$ and 0.210, respectively). The AUC's are much higher (almost doubled) at these time points in the nanoparticle group. This may be due to released drug in the intestine, bioadhesiveness of the polymer [14–16] and uptake of some of the nanoparticles into the systemic circulation by the enterocytes and PPs (Eldridge et al., 1990; Galindo-Rodriguez et al., 2005; Pandey et al., 2005). In addition to higher bioavailability, there was less inter-subject variability in the absorption of mifepristone after administration of nanoparticles containing the drug than administering the drug itself.

4. Conclusion

Bioadhesive nanoparticles containing mifepristone have been prepared. In vitro studies show that these nanoparticles, especially the PLGA-1A-nanoparticles can improve the oral bioavailability of mifepristone and decreases the inter-subject variability of the oral absorption of mifepristone. It may be possible to sustain the release of the mifepristone from PLGA-nanoparticles for more than 3 days in rats. We are currently evaluating the bioavailability and efficacy of these nanparticles in captive coyotes. Also, studies on the effects of the nanoparticles on ovarian and breast cancer cells are ongoing.

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