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Magnetic poly(D,L-lactide) nanoparticles loaded with aliskiren: A promising tool for hypertension treatment

Iryna Antal [a,*], Martina Kubovcikova [a], Vlasta Zavisova [a], Martina Koneracka [a], Olga Pechanova [b], Andrej Barta [b], Martina Cebova [b], Vitaliy Antal [a], Pavel Diko [a], Martina Zduriencikova [c], Michal Pudlak [a], Peter Kopcansky [a]

[a] Institute of Experimental Physics, SAS, Watsonova 47, 040 01 Kosice, Slovakia
[b] Institute of Normal and Pathological Physiology, SAS, Bratislava, Slovakia
[c] Cancer Research Institute, SAS, Bratislava, Slovakia

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ABSTRACT

In this study anti-hypertensive drug called aliskiren was encapsulated in magnetic poly(D,L-lactide) nanoparticles by the modified nanoprecipitation method. The effect of magnetite and drug concentrations on the size distribution and zeta potential of polymer nanoparticles was investigated. The optimized loadings were as follows: theoretical magnetite loading was 20 mg/100 mg polymer nanoparticles and aliskiren was encapsulated in magnetic poly(D,L-lactide) nanoparticles at theoretical loading 0.6 mg aliskiren/100 mg magnetic polymer nanoparticles. The physicochemical characteristics of nanoparticles were studied, with spherical shape of nanoparticles sized between 58 and 227 nm being one of the observed results. Differential scanning calorimetry and infrared spectroscopy confirmed that aliskiren was successfully identified in the magnetic poly(D,L-lactide) nanoparticles. The *in vivo* experiments indicated that encapsulated aliskiren decreased blood pressure of the studied male spontaneously hypertensive rat even more significantly than common administered drug.

1. Introduction

In the early 21st century, hypertension (high blood pressure) is a global public health issue contributing to the burden of heart disease, stroke and kidney failure and premature death and disability [1]. The renin–angiotensin–aldosteron system (RAAS) is a coordinated hormonal cascade that governs cardiovascular, renal, and adrenal functions by regulating fluid and electrolyte balance as well as arterial blood pressure. Aliskiren is a direct renin inhibitor that works by binding to the active site of renin, the initial step of RAAS, and decreases production of vasoconstrictors in this cascade including Angiotensin II. Thus, blood vessels can normally relax and the heart can pump blood more efficiently. However, the limiting factor in clinical practice is relatively low bioavailability of aliskiren (2–3%) [2]. One of various ways to increase aliskiren bioavailability and maximize the effect of aliskiren on kidney function is nanoencapsulation of aliskiren. Polymer nanoparticles (PLA-NP) created by poly(D,L-lactide) (PLA) were used for drug encapsulation. PLA polymers based on polyesters belong to the class of biodegradable materials extensively used in controlled drug delivery systems. Their main advantage is that they easily undergo degradation due to the hydrolysis of the ester bond and the hydrolysis products are metabolized and removed from the body via normal metabolic pathways [3,4].

The aim of this study was to formulate, characterize and analyze aliskiren entrapped in magnetic poly(D,L-lactide) nanoparticles and to study the effect of encapsulated aliskiren on systolic blood pressure of male spontaneously hypertensive rats.

2. Materials and experimental methods

2.1. Materials

Aliskiren was a generous gift from Novartis Pharma AG, Switzerland. Poly(D,L-lactide) with molecular weight 75000 g/mol, Pluronic F68 were purchased from Sigma company and sodium oleate was obtained from Riedel-de Haën.

2.2. Instrumentations

Prepared samples were observed by Scanning Electron Microscopy (SEM) to obtain information on the morphology and particle size of the polymer nanoparticles. While SEM gives us detailed

* Corresponding author at.
E-mail address: iryna.antal@saske.sk (I. Antal).

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shape and morphological information, Dynamic Light Scattering (DLS) and Differential Centrifugal Sedimentation (DCS) are used for quantitative particle size distribution analysis of samples in the submicron region. The DLS method is based on capturing the light scattered by the nanoparticles undergoing thermal motions. The scattered light fluctuations can be related to the diffusion coefficient of a particle, which in turn can be converted into particle diameter. The particle size distribution measurements by the DLS method were carried out using a Malvern Zetasizer Nano ZS. DCS, a high resolution particle size technique, sizes particles based on their sedimentation velocity through a density gradient under the action of a centrifugal force. The DC24000 UHR disc centrifuge (CPS Instruments, Inc.) was used as a complementary technique to obtain weight-averaged particle size distributions. Particle size was also measured in triplicates using NanoSight NS500 device (Malvern Instruments Ltd., UK). Data were captured and analysed by Nanoparticle Tracking Analysis (NTA) 2.3 software provided by the manufacturer. This method is based on visualising and tracking the light scattered from each particle in solution undergoing Brownian motion.

Fourier transform infrared spectroscopy (FTIR) is an appropriate technique to study the chemical absorption process. Thus, FTIR spectroscopy was carried out using a FTLA2000-100 instrument (from ABB) acquiring 32 scans/specimen at the nominal resolution of 4 cm^{-1}. Baseline corrections were applied for some regions of the spectra.

Thermal properties of the samples were investigated by using of NETZSCH STA F1 *Jupiter* Differential Scanning Calorimeter (DSC). The measurements were performed in a pure argon atmosphere with a dynamic heating rate of 5 K/min from 40 °C up to 520 °C. Aluminium crucibles were used during measurements and the sample masses were in a range of 3–5 mg.

2.3. Synthesis of magnetic poly(D,L-lactide) nanoparticles loaded with drug aliskiren

Magnetite (Fe_3O_4) nanoparticles were synthesized by coprecipitation of iron (II) and iron (III) in alkaline medium and they were coated with sodium oleate to prepare stable water based magnetic fluid. The detailed procedure and full characterization of magnetite nanoparticles has been described previously [5]. A modified nanoprecipitation method [6] was used for entrapment of magnetite nanoparticles and antihypertensive drug aliskiren into polymer nanoparticles. Briefly, 100 mg of PLA and 5 mg of aliskiren were dissolved in 10 ml of acetone to prepare the organic phase. An aqueous colloid was prepared by mixing a solution of Pluronic F68 as a stabilizing agent (25.6 mg in 5 ml) and 0.8 ml magnetic fluid (45 mg Fe_3O_4/ml). Then, the organic phase was added dropwise into the aqueous colloid and stirred vigorously for several hours to allow complete evaporation of the organic solvent at room temperature. A turbid nanoparticle suspension (referred to as aliskiren-MNP from now on) was formed.

2.4. Effect of magnetite and drug concentrations on the size distribution and zeta potential in obtained PLA-NP

To optimize the magnetite loading, a batch of 11 different samples was prepared and examined as a function of the initial amount of magnetite, ranging from 2.5 to 250 mg while the amount of polymer was kept constant (100 mg PLA). Similarly, for the optimization of aliskiren loading, a batch of 13 different samples was prepared and studied as a function of the initial amount of drug ranging from 0.5 to 10 mg while the amount of polymer was fixed (100 mg PLA). The magnetite entrapped into the polymeric matrix (MNP) was measured by detection of free iron (Fe^{3+}) with a UV/visible spectrophotometer (Specord 40), a colorimetric method (λ_{max}=700 nm) that uses the Prussian blue reaction [7]. The amount of aliskiren loaded in PLA-NP (aliskiren-NP) was determined by the spectrophotometric method at 281 nm having taken not-loaded nanoparticles as baseline.

2.5. In vivo experiments

All *in vivo* procedures and experimental protocols were approved by the Ethical Committee of the Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava and conform to the European Convention on Animal Protection and Guidelines on Research.

12-Week-old male spontaneously hypertensive rats were assigned to control group treated with no drug, aliskiren treated group (25 mg/kg per day), group treated with aliskiren-NP (25 mg/kg per day) and group treated with PLA-NP for only 3 weeks by a gavage. Blood pressure was measured by the plethysmographic method on the tail artery of rats. Six animals were used in each group and the same number was used for each of the average systolic blood pressures documented.

3. Results and discussions

To avoid the risk of embolism caused by intravenous administration of nanoparticles, a desirable diameter of particles should be <300 nm, therefore, knowledge of shape and particle size is a key requirement. While SEM confirmed spherical shape of nanoparticles (Fig. 1), DLS, DCS and NTA gave us information about nanoparticle diameter that was in the range from 58 up to 227 nm in dependence of method and amount of encapsulated material (Fig. 2). The DCS method is capable of providing information about the actual diameter of the nanoparticles, while DLS and NTA give us information about the hydrodynamic diameter. This is the

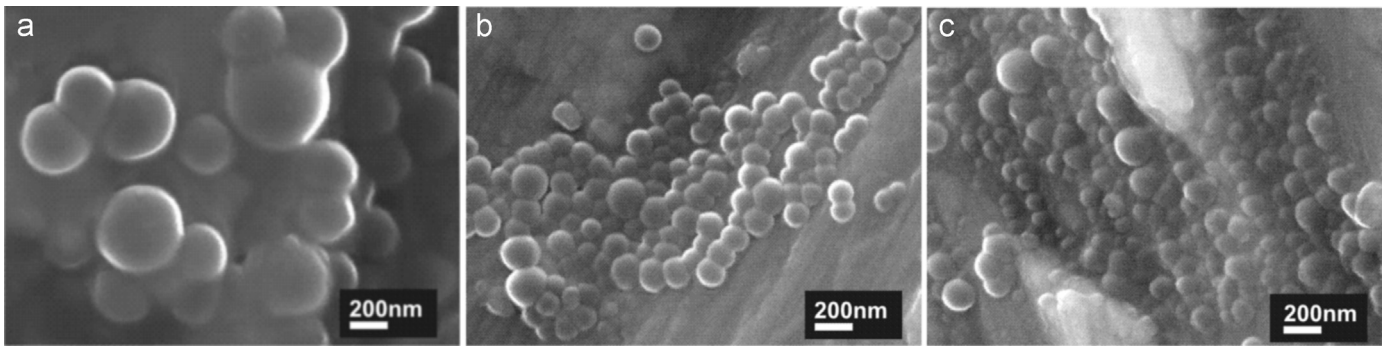

Fig. 1. SEM images of aliskiren-NP (a), MNP (b) and aliskiren-MNP (c).

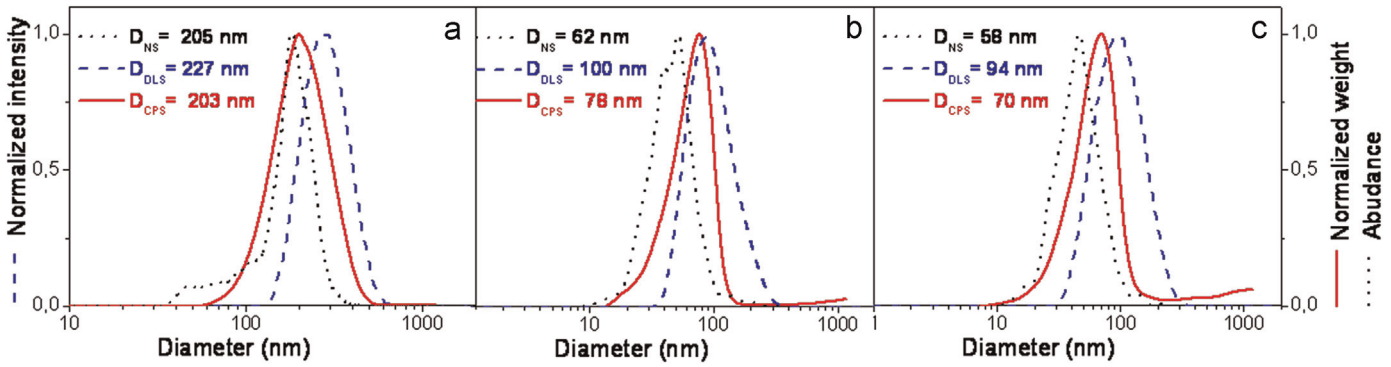

Fig. 2. Particle size distributions of aliskiren-NP (a), MNP (b) and aliskiren-MNP (c) measured by DLS (dashed line), DCS (solid line) and NTA (dot line).

reason why the size obtained by the DLS method was about 20 nm larger than the size obtained by the DCS method. The size distribution of all prepared samples: aliskiren-NP, MNP and aliskiren-MNP measured by DLS showed one narrow peak with low polydispersity index (0.13–0.30), confirming the good quality of formulations.

In comparison to DLS results, NTA distribution is slightly shifted towards smaller value (especially when sample contained magnetite nanoparticles). Nevertheless, NTA is very useful for polydispersed suspension samples; the shift may have been caused by the presence of higher number of free (unbound) smaller sized magnetite nanoparticles.

The concentration of aliskiren was varied to identify the effect on size and drug entrapment, keeping the other parameters constant as in the procedure described above. Fig. 3 shows the effects of various theoretical aliskiren loadings with regard to drug entrapment and drug content. The preparation with 5% w/w theoretical loading of aliskiren, which provided a drug content of 6.3% w/w and drug entrapment of 75%, good morphological features and a relatively high nanoparticle recovery of 78% and hydrodynamic particle size of 227 nm was selected as the optimal starting formulation for *in vivo* studies.

The effect of magnetite loadings on size and zeta potential of prepared magnetic polymer nanoparticles (MNP), whose values varied from 85 to 170 nm and from −25 to −60 mV respectively, is shown in Fig. 4. The most convenient particle size along with maximum zeta potential was found at theoretical weight ratio Fe_3O_4/PLA 0.7 w/w corresponding to 20 mg Fe_3O_4/100 mg PLA-NP. The same impact was investigated in the formulations with various theoretical aliskiren loadings while magnetite and polymer concentrations were constant (Fig. 5). Fitting the experimental data resulting in curve made it possible to set the optimal weight ratio aliskiren/PLA to 0.02 matches up to 0.6 mg aliskiren/100 mg MNP. As a complementary technique, DCS was used for determination of particle size distribution. Displayed in Fig. 6, there are normalized size distributions of the formulations with various theoretical aliskiren loading in magnetic poly(D,L-lactide) nanoparticles. Unlike other procedures, the DCS method results in additional peak of weight distribution in the region of smaller size (about 7 nm) with the aliskiren content in aliskiren-MNP being 5.0 wt%, which is probably related to the presence of free (unbound) aliskiren.

For determination of aliskiren and magnetite entrapment, the amount of drug, as well as magnetite present in the supernatant after centrifugation was determined by a UV spectrophotometer. The obtained results indicated that the optimal Fe_3O_4/PLA weight ratio in MNP was 0.7 (see inset in Fig. 4) and the aliskiren content in aliskiren-MNP was 2 (see inset in Fig. 7). The results are in good agreement with outcomes obtained from DLS (Fig. 5) and DCS (Fig. 6) measurements.

DSC studies of pure aliskiren, magnetite nanoparticles, PLA-NP, aliskiren-NP, aliskiren-MNP and physical mixture of aliskiren, magnetite nanoparticles and PLA-NP, (Fig. 8) were carried out to define the physical state of the drug in this carrier and the possibility of any interaction between the drug and polymer nanoparticles. The endothermic melting peak of pure aliskiren (106 °C) was only observed in the thermogram of the physical mixture

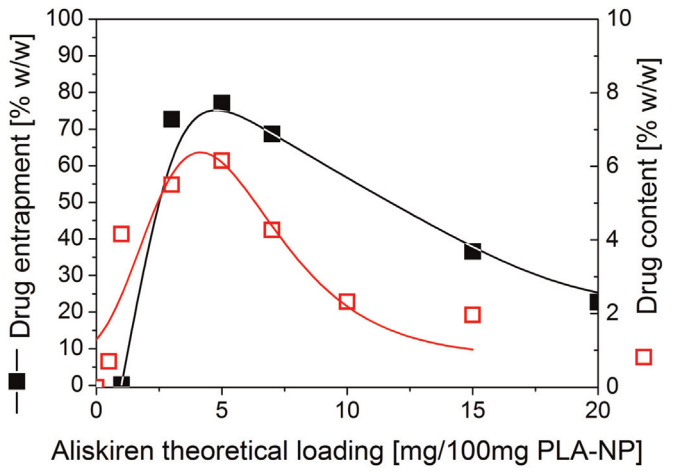

Fig. 3. Effects of various theoretical drug loadings with regard to drug entrapment and drug content.

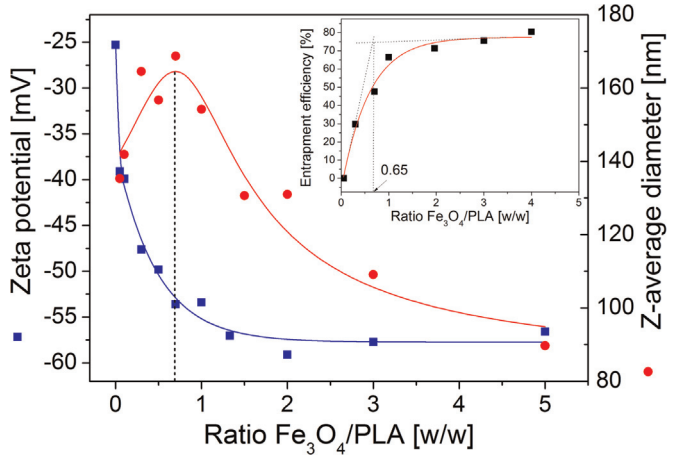

Fig. 4. Effects of various theoretical magnetite loadings with regard to Z-average diameter and zeta potential. (Inset: effect of various Fe_3O_4/PLA weight ratios with regard to magnetite entrapment efficiency).

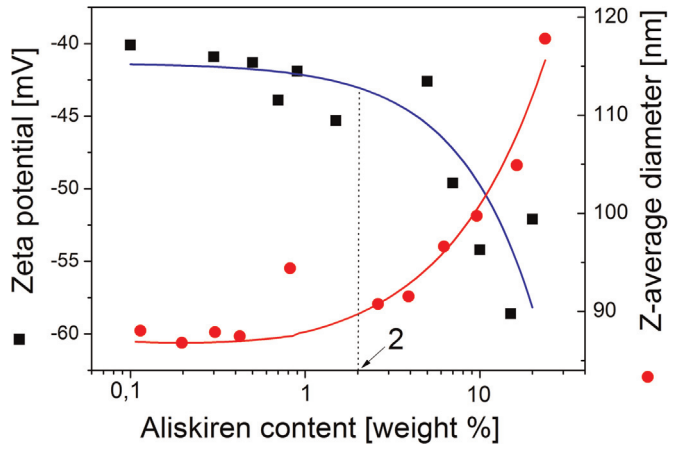

Fig. 5. Effect of various theoretical aliskiren contents with regard to Z-average diameter and zeta potential.

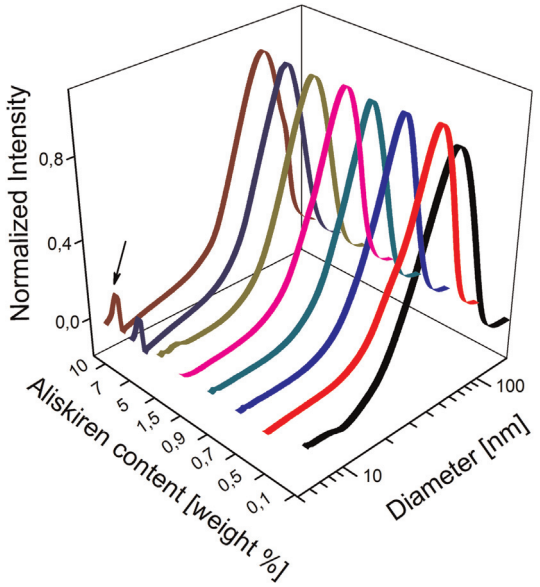

Fig. 6. Particle size distributions of formulations with various aliskiren contents measured by DCS.

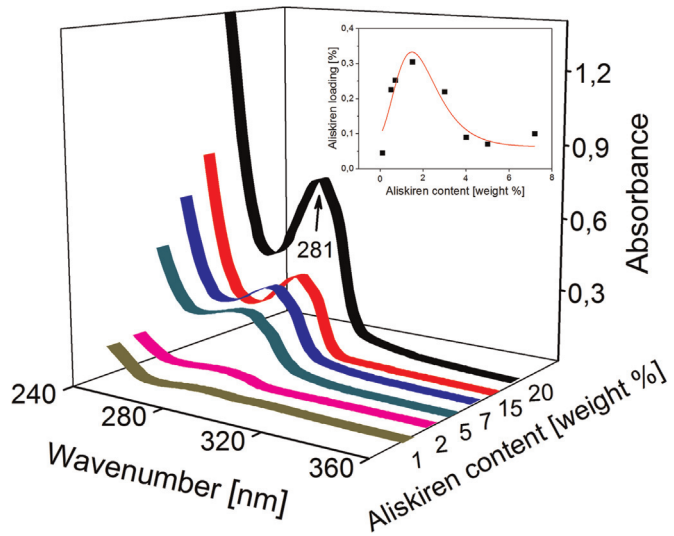

Fig. 7. UV absorption spectra of the supernatants after removing free aliskiren from aliskiren-MNP using a 100-kDa Amicon filter. (Inset: fitting curve of the drug loading dependence of free aliskiren in aliskiren-NP samples.).

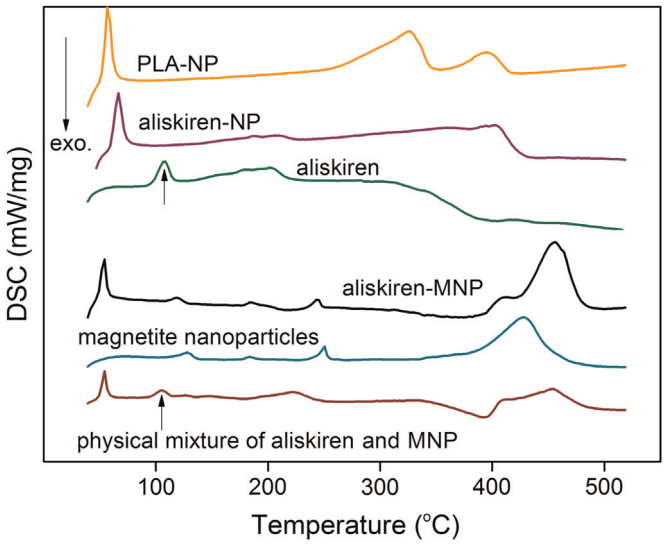

Fig. 8. DSC curves of studied samples.

containing aliskiren, magnetite nanoparticles and PLA-NP (simply mixing aliskiren, magnetite nanoparticles and PLA-NP) and it was slightly shifted to a lower temperature (103 °C), whereas DSC traces of aliskiren-NP and aliskiren-MNP had no such peak, which proved encapsulated aliskiren to be in either amorphous form or disordered crystalline phase of molecular dispersion or a solid solution state in the polymer matrix after the production [8].

The intercalation mode of drugs into prepared matrix can be proved by FTIR analysis, comparing the spectrum of the host matrix with the spectrum of the intercalated with drug loaded matrix, as depicted in Fig. 9. The infrared spectrum of aliskiren presents weak peaks, centered at 3267–3087 cm^{-1} and 2956–2842 cm^{-1} that can be attributed to the ν(N–H) and ν(C–H) vibration of aliskiren anions respectively (Fig. 9a). The two bands also meet at 1608 cm^{-1} and 1660 cm^{-1} being attributed to ν(C=O) vibration. The band at 1539 cm^{-1} is related to the stretching vibrations of the amine group [9,10]. In the spectrum of PLA-NP (Fig. 9c) the C=O stretching at 1749 cm^{-1}, 1712 cm^{-1} and the O–C=O stretching at 1190–1080 cm^{-1} are characteristics of PLA ester bonds [11,12]. The comparison of the spectra of aliskiren-NP (Fig. 9d) and its physical mixture (Fig. 9b) shows the difference between the peak positions or shape changing of each spectrum. The FTIR spectrum of oleate adsorbed on magnetite nanoparticles is shown in Fig. 9f. The CH_2 and CH_3 stretching bands in the spectral region 2800–3000 cm^{-1} and the vinylic =C–H at 3006 cm^{-1} are the most prominent bands about 2000 cm^{-1}. The bands observed about 1500 cm^{-1} are typical bands of carboxylates (1558 and 1445 cm^{-1}) and magnetite (580 cm^{-1}), respectively [13,14]. The strong absorption band of $\nu(Fe^{III}–O)$ is also in the spectrum of aliskiren-MNP (Fig. 9e) which confirmed the presence of magnetite nanoparticles in this sample. The spectrum of aliskiren-MNP is very similar to the spectrum of aliskiren-NP (Fig. 9, lines e and d respectively). The presence of characteristic bands of PLA and aliskiren in IR spectra of synthesized aliskiren-MNP (Fig. 9e) confirmed successful encapsulation of the aliskiren and magnetite nanoparticles into the PLA matrix.

3.1. *In vivo experiment*

At the end of experiment, systolic blood pressure of spontaneously hypertensive rats in aliskiren treated group was significantly lower (178.7 ± 1.8 mmHg) than in the control group

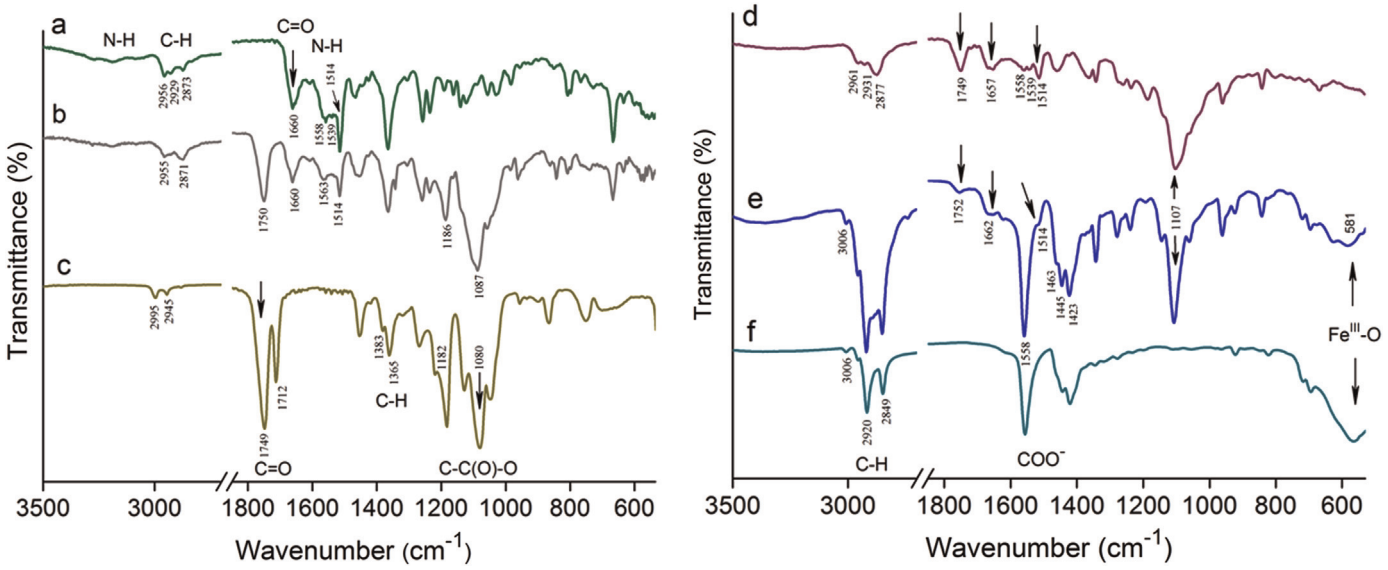

Fig. 9. The FTIR spectra of pure aliskiren (a), physical mixture of aliskiren and PLA-NP (b), PLA pure (c), aliskiren-NP (d), aliskiren-MNP (e) and magnetite nanoparticles (f).

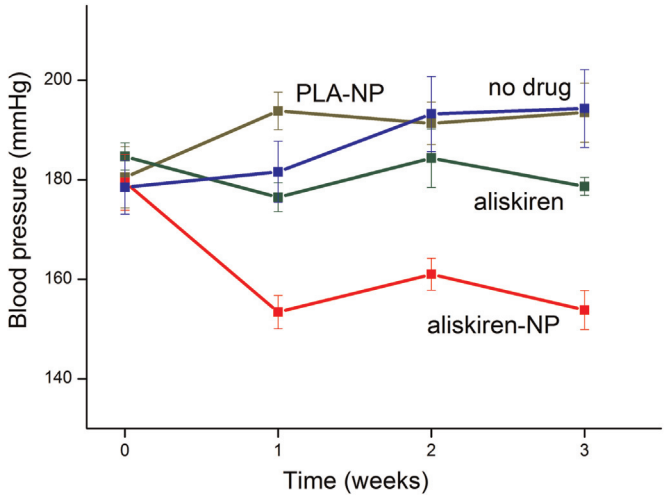

Fig. 10. Blood pressure development of spontaneously hypertensive rats for 3 weeks of aliskiren treatment: control group treated with no drug, aliskiren treated group, group treated with aliskiren-NP and group treated with PLA-NP.

treated with no drug (203.4 ± 4.3 mmHg). In addition, in aliskiren-NP group (aliskiren/PLA-NP at the concentration 5 mg aliskiren/100 mg PLA-NP) blood pressure was decreased to 153.8 ± 3.9 mmHg - the level significantly lower in comparison to both, control group treated with no drug and aliskiren treated group, whereas PLA-NP did not change the value of blood pressure (194.3 ± 2.5 mmHg) (Fig. 10).

In our further study, we are certain to study the effect of aliskiren-MNP on systolic blood pressure of spontaneously hypertensive rats.

4. Conclusion

To conclude what we have dealt with previously, we can say that we have prepared and characterized magnetic poly(D,L-lactide) nanoparticles loaded with aliskiren of spherical shape with mean diameter lower than 300 nm, which is a suitable size for intravenous administration. Differential scanning calorimetry suggested that aliskiren was molecularly dispersed in the polymer matrices. Using infrared spectroscopy, aliskiren was successfully identified in the magnetic poly(D,L-lactide) nanoparticles and the *in vivo* experiments showed that encapsulated aliskiren decreased blood pressure of the studied male spontaneously hypertensive rats even more significantly than common administered drug. Thus, encapsulated aliskiren may represent an effective, novel approach to the treatment of hypertension and hypertension-related disorders. The encapsulation may protect drugs against degradation and increase their bioavailability in organs.

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