Potential of Sustained Release Microparticles of Metformin in Veterinary Medicine: An in Vivo Pharmacokinetic Study of Metformin Microparticles as Oral Sustained Release Formulation in Rabbits.

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Research article

Keywords: Controlled-release, microparticles, pharmacokinetics, poly (lactic acid), In Vitro/In Vivo (IVIVC) Correlation(s)

Posted Date: October 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-80177/v1>

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Abstract

Background: Metformin hydrochloride a biguanide derivative has been widely used in the treatment of type 2 diabetes in humans. In veterinary medicine, metformin has been increasing his potential in different species as equids for insulin dysregulation, dogs and cats with diabetes. It is a highly soluble hydrophilic drug, shows incomplete absorption from the gastrointestinal tract and the absolute bioavailability is 40-60 % with a short biological half-life of 1.5-1.6 h in humans. In this study, to improve its efficacy a sustained-release microparticles of metformin was developed by loading within poly lactic acid (PLA) polymer followed by an *in vivo* pharmacokinetics study in rabbits.

Results: Pharmacokinetic study in rabbits showed the sustained-release characteristic from the prepared microparticles with delayed time to reach maximum concentrations T_{max} , decreased C_{max} , increased Mean Residence Time (MRT) and half-life compared to the pure drug solution. Physicochemical characterization suggested that PLA and metformin hydrochloride interacted within the microparticles via hydrogen bonds and that the drug was transformed to an amorphous state.

Conclusions: The The pharmacokinetics parameters resulted in delayed T_{max} , increased MRT and $t_{1/2}$, decreased C_{max} of metformin from microparticles that show promise for prolonged/sustained release of metformin after oral administration in different animal species affected by insulin disorders. PLA microparticles provided sustained release of the drug, and these systems can be useful as drug carriers for hydrophilic drugs in long term disease treatment such as diabetes.

Background

Diabetes mellitus is a chronic problem in humans characterised by high blood glucose levels induced by; a deficient secretion of insulin, anomalous of insulin action or both. It is a main factor of developing health complications such as cardiovascular disease, neuropathy, nephropathy and eye disease, leading to retinopathy and blindness. Diabetes is classified into three main types; gestational diabetes, type 1-diabetes and type 2-diabetes. The latest one represents 90% of all the cases [1].

In veterinary medicine, diabetes affects to different animal species, it is more frequently reported in dogs and cats [2], and rarely in other animal species like rabbits [3]. In equids, insulin dysregulation is the primary endocrine disorder in equine metabolic syndrome (EMS) affecting also some equids with pituitary pars intermedia dysfunction (10–22% prevalence) [4, 5]. There are several reports about the use of the hypoglycemic drug metformin in clinical cases in different animal species [2, 4]. However, its efficacy is controversial mainly due to its low oral bioavailability [2, 4].

Metformin hydrochloride (metformin, Fig. 1.A) a biguanide derivative of galegine alkaloid, is used as a first line treatment for type 2 diabetes. In the presence of insulin, it acts by lowering the blood glucose concentration mainly through the inhibition of the gastrointestinal glucose absorption and the hepatic glucose production, increasing the glucose uptake and insulin sensitivity in muscle [6]. Metformin a highly soluble hydrophilic drug with low permeability via biological membrane belongs to

Biopharmaceutics Classification System class III drug (BCS). The low permeability of this drug results in poor drug absorption which is the rate limiting step in attaining suitable bioavailability. After oral administration, metformin is incompletely absorbed from the gastrointestinal tract. It has 40–60% absolute bioavailability with a short biological half-life (1.5–1.6 h) [7]. To maintain effective plasma concentration, it should repeatedly be taken at high doses (2.5 g/day) with a consequent of serious gastrointestinal side effects [8]. Furthermore, metformin is required to be taken for a long period by patients.

In such therapeutic treatment, the development of polymeric controlled drug delivery systems (CDDSs) is an important strategy to overcome the above limitations. The purpose of these systems is to reduce side effects, sustain drug release and increase the bioavailability of drugs at a controlled rate for a sustained time (days, weeks or months) [9]. Thereby the drugs concentration is maintained between the therapeutic windows over a specified period of time. In this context, microparticles formulated with polymers, are intensively used in designing CDDSs for a various therapeutic drugs [10, 11].

Poly lactic acid (PLA, Fig. 1.b) is a hydrophobic polymer belongs to the α -hydroxyl acid family. As a biodegradable and biocompatible, this polymer is widely used for many applications in pharmaceutical and medical areas because its decomposition products are not toxic and are easily excreted from human organism [12, 13]. Several methods for the preparation of CDDs microparticles from PLA polymer were reported in the literature.⁸ Because of the easy preparation process, solvent evaporation method (double emulsion) is mostly used to encapsulate water soluble drugs.

The literature does not indicate studies involving microparticles containing metformin, using PLA as polymer. Recently, an optimisation of metformin encapsulation/formulation in PLA microparticles in terms of encapsulation efficiency was investigated by using response surface design [14]. However, *in vivo* bioavailability of the prepared microparticles was not done. It is of important to investigate the *in vivo* performance of the formulated microparticles formulation. For this purpose, this study was conducted to investigate the *in vivo* metformin sustained release and the pharmacokinetics parameters of PLA microparticles after oral administration to rabbits and compared to pure drug solution administered by oral and IV routes.

Results

Encapsulation efficiency and characterization of PLA microparticles

Metformin HCl was encapsulated within PLA microparticles by double emulsion solvent evaporation method. Many batches were prepared; the percentage of encapsulation efficiency of metformin calculated was $76 \pm 2.72\%$.

The obtained microparticles were analyzed by scanning electron microscopy (SEM). SEM micrograph of the PLA encapsulated metformin is shown in Fig. 2. The microparticles had almost spherical shape with smooth surfaces with particles adhered to the surface. In addition to surface morphology characteristic, it is interesting to note that the size of microparticles is variable, it is ranged from ~ 1 to 55 μm . SEM images also suggested that metformin was dissolved in the microparticles, since no individualized drug crystals observed.

Fourier transforms infrared (FTIR) spectroscopy

FTIR spectroscopy was used to investigate the encapsulation process of metformin loaded microparticles. FTIR spectra of pure metformin, blank PLA MPs and metformin loaded microparticles are shown in Fig. 3. The FTIR spectrum of pure metformin (Fig. 3a) shows characteristic peaks at 3390, 1633 and 1045 cm^{-1} corresponds to the stretching of primary N-H, stretching of C = N, and stretching of C-N respectively. For the blank MPs (Fig. 3b) four characteristic peaks were observed at 1764, 2993 – 2943 and 1087 which are attributed to C = O stretching of the ester group, asymmetric and symmetric vibration of the CH₃ group -CH₃ (asym), CH₃ (sym) and C-O ester bond. As shown in the microparticles spectra (Fig. 3c), the intensity of the peak of C = O ester groups of PLA at 1764 cm^{-1} after metformin loading was reduced, this is due to hydrogen bond formation between carbonyl of ester of PLA and hydrogen of amine groups of metformin (Fig. 1). It is reported that the elongation of C = O band to C-O-H band after hydrogen bond formation resulted in shift of absorption band to lower wave number. These results confirm that metformin was successfully encapsulated in PLA microparticles.

X-ray powder diffraction (XRPD)

Figure 4 illustrates the X-ray diffraction (PXRD) diffractogram of pure metformin, blank MPs and metformin loaded microparticles. The PXRD diffractogram of Met revealed the distinct peaks at 2θ : 12.17°, 17.62°, 24.47°, 28.2°, 31.17°, 37.07°; these sharp peaks confirmed the highly crystalline nature of metformin. Blank MPs showed the peaks at 2θ : 16.6°, 18.92° indicating the semi crystalline nature of the PLA [15]. The PXRD diffractogram of MPs shows no peaks. As observed in Figure (4), the peaks of both PLA and metformin are disappeared as compared to Blank MPs and pure metformin. This may be attributed to the incorporation of metformin into PLA matrix leading to a change in the crystallinity of the PLA and metformin. The results showed a good entrapment of Met in PLA with interaction which are aligned with FT-IR analysis.

Differential scanning calorimetry (DSC)

In addition to IRTF and XRD, DSC analysis was performed to obtain more results about the physical state of metformin in PLA microparticles and intermolecular interactions between the drug and the polymer. The DSC curves of samples were recorded during the first heating process Fig. 5. The metformin DSC curve exhibited a very sharp endothermic peak at ~ 232 °C that indicates its crystalline nature. DSC curve of the blank PLA (unloaded) microparticles showed a broad peak at ~ 178 °C, while this peak is shifted to 168 °C in metformin loaded PLA microparticles. No peak of metformin in the loaded microparticles was recorded. The water soluble metformin has interaction with the PLA via the electrostatic force between

NH₃⁺ and COO⁻, consequently changes in the thermal behaviour of both metformin and PLA polymer are occurred [16]. This result indicates that metformin is dispersed or dissolved into the polymer matrix during microencapsulation process.

In vivo Pharmacokinetic studies in rabbits

The *in vivo* study was conducted to assess the pharmacokinetics of metformin loaded PLA microparticles after three routes of administration (intravenous solution, oral solution and oral PLA microparticles). The mean plasma concentration–time profiles after a single administration at the same dose (5 mg / kg) to rabbits of the IV, oral solutions and the oral microparticles of metformin are illustrated in Fig. 6. Quantification of metformin in plasma after 24 h was not detected by HPLC. No adverse events were observed during the study.

After oral administration of pure metformin solution, the drug was absorbed gradually to reach a maximum of absorption at a T_{max} of 110 min with a higher C_{max} of 3.19 ± 0.16 µg/ml followed by a decrease to ~ 1 µg/mL within 300 minutes. In contrast, the metformin concentration in plasma was maintained at low levels within ~ 600 min post administration of metformin-PLA-MPs, showed lower C_{max} (1.36 ± 0.05 µg/ml) and higher T_{max} (138 min) than that of metformin oral solution, this may be attributed to the delayed release of metformin from PLA microparticles.

The pharmacokinetic parameters derived from plasma concentration–time data for the different routes are summarized in Table 1. The results indicated that the Mean Residence Time (MRT) was prolonged with PLA microparticles loaded with drug (369.08 min) as compared to MRT of pure metformin solution given by IV and oral route respectively (156.52 min, 254.59 min). This longer MRT enables to maintain the concentration of metformin for prolonged period in rabbit plasma.

Regards the half-life time (t_{1/2}), it was noted that the t_{1/2} of metformin loaded PLA microparticles following oral administration (223.30 ± 21.30 min) was higher in comparison to the t_{1/2} obtained from pure metformin solution administered by oral (153.78 ± 4.19 min) and iv (139.61 ± 4.39 min) routes, respectively.

This result indicates that incorporation/encapsulation of metformin in the PLA microparticles achieved high half lifetime.

The AUC_{0-∞} after oral administration of metformin PLA microparticles was 617.88 ± 12.92 µg/ml/min, while AUC_{0-∞} of pure metformin solution was 882.85 ± 23.90 µg/ml/ min and 2440.77 ± 68.71 µg/ml/min after oral and IV administration respectively. The oral bioavailability of metformin MPS is calculated to be 25.59 ± 0.46%. Significantly low AUC_{0-∞} of metformin PLA microparticles indicates low release of metformin from the developed microparticles as compared to oral solution. In the case of the PLA microparticles, the decrease in AUC_{0-∞} and the low C_{max} value could be explained by a further decrease in the diffusion of metformin from the PLA microparticles which limit the release of the drug.

It was also observed that the values of other pharmacokinetics parameters such as V_d (2606.33 ± 233.54 mL/Kg), Cl (8.09 ± 0.17 mL/min/kg) and MAT of metformin encapsulated in PLA microparticles (206.95 ± 31.91 min) were higher than the pharmacokinetics parameters observed for pure metformin solution after oral and intravenous administration (Table 1).

Table 1
Pharmacokinetic parameters of metformin loaded PLA microparticles and pure drug suspensions from healthy rabbits given a single dose (after single oral administration of 5 mg/ kg body weight to rabbits. Mean $\pm$ S.D. ($n = 5$).

Parameter	IV solution	Oral solution	Oral microparticle
C_{max} (μ g/ml)	-	$3.19 \pm 0,16$	1.36 ± 0.05
T_{max} (min)	-	110.5 ± 2.37	138.94 ± 4.73
$T_{1/2}$ (min)	$139.61 \pm 4,39$	153.78 ± 4.19	223.30 ± 21.30
$AUC_{0-\infty}$ (μ g/ml/ min)	2440.77 ± 68.71	$882.85 \pm 23,90$	617.88 ± 12.92
V_d (mL/Kg)	412.65 ± 9.63	1257.59 ± 58.98	2606.33 ± 233.54
Cl (mL/min/kg)	2.05 ± 0.06	5.67 ± 0.15	8.09 ± 0.17
MRT(min)	$156.52 \pm 3,81$	254.59 ± 4.54	369.08 ± 18.98
MAT(min)	-	98.05 ± 3.36	206.95 ± 31.91
F (%)	-	36.19 ± 1.27	25.59 ± 0.46

Discussion

The oral route is by far the preferred route of drug administration for many dosages forms. However, many anti-diabetic drugs suffer from low bioavailability following oral administration especially BCS class III like metformin due to its high solubility and low permeability [17]. Therefore, microencapsulation into hydrophobic polymer microparticles systems is an effective strategy to achieve sustained release over time and enhance drug bioavailability in human and veterinary medicine.

In a previous study, we showed the successful preparation of poly (lactic acid) microparticles loaded metformin using the double emulsion (w/o/w) solvent evaporation method. In this method, the effect of microencapsulation parameters such as the amount of metformin, pH of the external aqueous phase, PVA concentration and the stirring rate on the encapsulation efficiency, microparticles size and zeta potential were evaluated and optimized using response surface methodology by Box–Behnken Design [14]. In the present study after microparticles characterization, the potential of poly (lactic acid) microparticles loaded metformin to sustain the *in vivo* release carried out on rabbits under standard fed conditions.

Metformin loaded PLA microparticles, under the conditions reported previously, exhibited high encapsulation efficiency ($76 \pm 2.72\%$) and spherical shape with no porous surfaces. In the FTIR diagrams, some variations in the absorption bands could be observed before and after loading metformin onto the microparticles. These changes could be resulted from the physicochemical interactions between the microparticles and metformin to form metformin-loaded microparticles during the process of microencapsulation [18]. The solid state characteristic is important in controlling drug release. High energy is required in the crystalline states to separate the molecules that led to low aqueous solubility and consequently low physiological bioavailability, while the amorphous state need low energy to separate molecules and drug solubility and bioavailability is superior [10]. SEM images, XRD and DSC results indicate that metformin was dissolved in the microparticles with the presence of intermolecular interactions between the drug and the polymer, and the drug was encapsulated in the microparticles in a non-crystalline state. The crystallinity of metformin and PLA disappeared after microencapsulation because of the fast diffusion of dichloromethane to the outer phase during evaporation step, thus inhibits the formation of crystalline structure [15].

Microencapsulation of metformin in the PLA microparticles caused a change in the pharmacokinetics of the drug in the rabbit model used in this study. C_{max} , T_{max} and Bioavailability (F) reflect the *in vivo* absorption rate of drug. In our study, metformin concentration in plasma was maintained at a low level for 24 h after oral administration of metformin-PLA-MPs, demonstrating slow released metformin which may provide an efficient therapeutic concentration for a prolonged period of time. The peak plasma concentration ($C_{max} = 1.36 \pm 0.05 \mu\text{g/ml}$) for metformin-PLA-MPs is 2.34-fold lower than the peak plasma concentration of oral administration of pure metformin solution. In addition, the time (T_{max}) needed to reach the maximum plasma concentration was significantly delayed in the metformin-PLA-MPs group which may be due to the low absorption of metformin in the cells of the gastrointestinal tract. This type of behaviour is expected to PLA microparticles and it has been found for extended release formulations. The mean value of bioavailability (F) obtained in the oral solution and microencapsulated administration groups were 36.19% and 25.59%, respectively. This value is similar to bioavailability of metformin in diabetic rabbits (36.73%) [19] and rats (34.1%) [20], lower than in cats (48%) [21] and humans (50–60%) [22]. However, significantly higher than in horses (3.9%) [23]. The F value of the metformin-PLA-MPs formulation (25.59%) is promising showing the characteristic sustained-release of metformin of extended release formulations.

Particle size and size distribution could influence the *in vivo* dissolution rate as well as the oral bioavailability of a drug. As shown from the SEM image (Fig. 2), PLA microparticles obtained had a large particle size that leads to slower degradation rate of the polymer matrix. As a result, a delayed of the drug diffusion through PLA microparticles occurs, this may explain the lower C_{max} and the $AUC_{0-\infty}$ of the metformin-PLA-MPs compared to the oral solution [24].

The nature of the drugs to encapsulate is an important factor which influences the release of drug from polymer matrix [25]. The experimental finding by IRTF and DSC suggest the presence of interaction between protonated metformin and carboxylic end group of PLA which affect the drug release and led to

a slow release rate over time. Our results are consistent to those obtained by Proikakis et al. [25]. Besides, the amorphous state of metformin in the microparticles could enhance the release of the drug; however, it is not the case in our study. This could be due to the slow degradation of PLA polymer [26].

Based on these findings, it could be concluded that a promising extended-release PLA microparticles of the highly water soluble drug, metformin, was successfully designed. However, because of the low bioavailability of metformin, future works are needed to improve the bioavailability of the PLA microparticles by evaluating the influence of PLA microparticle's properties on the release of metformin.

Conclusions

Metformin hydrochloride loaded into PLA microparticles was prepared according to their individual optimised formulations developed in our previous study. The obtained microparticles were spherical with heterogeneous size distribution. The pharmacokinetics parameters resulted in delayed T_{max} , increased MRT and $t_{1/2}$, decreased C_{max} of metformin from microparticles that show promise for prolonged/sustained release of metformin after oral administration in different animal species affected by insulin disorders. Low bioavailability is the consequence of slow release of metformin from PLA microparticles. Therefore, further work is needed to investigate the importance of microparticle size distribution on the release from PLA matrix and the release mechanism of these sustained-release microparticles.

Methods

Materials

Metformin HCl Mw 165.6 g/mol. Poly (lactic acid) PLA was purchased from Evonik Industries AG (Germany). Poly (vinyl alcohol) (PVA, 87–89% hydrolyzed, average Mw = 30000–70000) was provided by Sigma Aldrich (Barcelona, Spain). High performance liquid chromatography (HPLC) grade acetonitrile, methanol and water were purchased from sigma Aldrich (Barcelona, Spain), Sodium Dodecyl Sulfate (SDS) ultrapure 288.38 g/mol and ammonium acetate 77.08 g/mol were purchased from (Panreac, Barcelona). All other reagents were of analytical grade.

Animals

Fifteen healthy New Zealand white female rabbits were obtained from the Faculty of Veterinary of the University of Murcia (Murcia, Spain). The rabbits were housed in cage in laboratory animal rooms of the laboratory of pharmacy, pharmacology and therapeutics of Veterinary faculty. The animals were kept under a photoperiod of 12 h light/12 h dark. They received standard laboratory chow diet (Nanta, Madrid, Spain) and tap water. All experiments protocols were carried out in accordance with the requirements of applicable national legislation and approved by the Bioethics Committee of the University of Murcia. After

completion of the study, the animals were donated to be adopted as pets as they all were healthy after physical examination.

Preparation of Metformin HCl-microparticles

Metformin HCl loaded PLA microparticles were prepared by the W/O/W solvent evaporation method described previously [14]. Briefly, 1 mL of aqueous internal phase was emulsified for 5 min in 10 mL of methylene chloride (containing 200 mg of PLA) with an ultrasound bath (Branson 5510, BioBlock Scientific, Spain) at 135 W output. This primary emulsion was gradually added into 40 mL of a 1.5% PVA aqueous solution and homogenized for 30 min at 700 rpm in order to create the W/O/W emulsion. The Solvent evaporation was achieved at room temperature and atmospheric pressure under a 400 rpm agitation (Heidolph-Hei-Tec D91126, Germany). Microparticles were obtained after centrifugation of the colloidal suspension for 30 min at 44000 rpm (Centrifuge 5702, Eppendorf Ag, Germany). Drug free microparticles (Placebo MPs) were prepared with the same procedure.

Determination of the encapsulation efficiency (EE)

The amount of metformin entrapped within polymeric microparticles was determined according to an established High-Performance Liquid Chromatography (HPLC) method by measuring the amount of non-encapsulated metformin in the external aqueous solution, which was recovered after centrifugation of microparticles. The assay was repeated using different samples from independent preparations. The encapsulation efficiency (EE) is defined as the amount of metformin present in the microparticles (m_1) divided by the initial amount added in the inner aqueous phase (m_0) and it is calculated with the following formulas:

$$EE(\%) = \frac{m_1}{m_2} \times 100 \quad (1)$$

Microparticles characterisation

Scanning electron microscopy (SEM)

The morphology of metformin loaded microparticles was investigated by a scanning electron microscopy (SEM) (Zeiss Evo 50, Germany) at a working distance of 9.5 mm and accelerating voltage of 8 KV. The microparticles were prepared by fixing onto carbon adhesive tape and spotter coating with 10 nm Pd/Au layer under vacuum (EVO/LS 15). The microparticles were examined for shape, size and surface characteristic.

Fourier transforms infrared (FTIR) spectroscopy

In order to examine the drug polymer interaction, the FTIR spectra of pure metformin, blank PLA MPs and metformin loaded microparticles were performed from IR Affinity-1 CE spectrophotometer (Shimadzu, Japan). One milligram of samples was finely mixed with 100 mg of purified potassium bromide and pressed in a mechanical die press to form a pellet (90N, 5 min). These pellets were scanned, and spectra were recorded from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} .

X-ray powder diffraction (XRPD)

Pure metformin, blank PLA MPs and metformin loaded microparticles were investigated by wide angle X-ray diffraction (XRPD) technique using X-ray diffractometer (X Per-Pro, PANalytical, Netherland). All the samples were scanned at 40 KV, 30 mA using Cu-K α radiation ($\lambda=1.54059 \text{ \AA}$) at the range of (2θ) from 5 to 80 degree with a scanning speed of 5° min⁻¹.

Differential scanning calorimetry (DSC)

The thermal properties of samples were evaluated by differential scanning calorimetry (DSC-131, Setaram, France). DSC thermograms of pure metformin, blank PLA MPs and metformin loaded MPs were obtained using SETSOFT software. The samples (8-12 mg) were weighted and sealed into aluminium pans; an empty sealed pan was used as a reference. DSC curves were obtained at a heating rate of 10 °C from 30-300 °C.

In vivo Pharmacokinetic study

Fifteen New Zealand healthy white rabbits (n=15) were selected for the pharmacokinetic study. The rabbits were randomly (Excel random numbers tables generator) divided into 3 groups (n=5) in 3 separated individual and marked cages, and were received a single oral dose administration of metformin microparticles suspension (Group A) pure metformin solution (Group B) by gastric intubation using oral feeding needle respectively, and intravenous (IV) of pure metformin solution by injection from the marginal ear vein (Group C). Investigators were not blinded to sample collection or sample analysis at any stage of the study. There is no hypothesis testing involved in this pharmacokinetics study, and therefore a power analysis is not required for estimating sample size as previously described [27]. The microparticles and pure metformin were suspended/dissolved in saline before each administration at a dose of 5mg/kg body weight. At predetermined time points post administration (10, 20, 30, 45, 60, 90, 120 minutes and 4, 6, 8, 10, 24, 34 and 48 hours), blood samples were extracted (0.5 mL) from the marginal ear vein of the rabbits by heparinised syringe and immediately centrifuged (10.000 rpm, 10 min) to separate plasma that was frozen at -50 °C until further analysis.

Plasma analysis

To quantify the drug in plasma rabbits a validated method [28] modified by Carceles-Rodriguez et al. [29] was used. For this, samples were thawed. Then, acetonitrile (200 µL) was added to plasma (200 µL) vortexed for 15 s then shaken in ultrasonic bath for 5 min to allow a complete mixing, followed by centrifugation at 12.000 rpm for 10 minutes to extract metformin. Afterwards, 200 µl of the supernatant was mixed to HPLC mobile phase (ratio of 1:3) transferred into HPLC vials and analyzed. The pharmacokinetic parameters were calculated using a non-compartmental model.

HPLC method

HPLC analysis was performed using a JASCO series HPLC and a JASCO 1575 UV/VIS detector set at a wavelength of 236 nm. The chromatographic separation was achieved by a Kromasil C-18 reverse-phase column (250x4.6 mm of 5µm, (AnálisisVíncicos S.L., C. Real, Spain) at 25 °C. The mobile phase employed was acetonitrile: ammonium acetate (25mM) - dodecyl sulphate sodium (9mM) (pH 7.02) in a volume ratio of 45:55. The flow rate and injection volume of metformin were 1 mL/min and 100 µL, respectively. A standard calibration curve was performed with metformin in plasma. The established linearity range was 100-10000 µg/ L ($r > 0.99$). The duration of the analysis is 10 min and the retention time of metformin is approximately 5 min.

Pharmacokinetics analysis

The plasma metformin time-concentration data were analyzed by non-compartment methods. The Area Under the Concentration-time Curve ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule with extrapolation to infinity. Mean Residence Time was calculated as $MRT = AUMC/AUC$. The systemic clearance as $Cl = Dose/AUC$. The apparent volume of distribution (area method) and apparent volume of distribution at steady state were calculated as $V_z = Dose / (AUC \cdot I_z)$ and $V_{ss} = (Dose \cdot AUMC) / AUC^2$, respectively.

Statistical analysis

Descriptive statistical parameters as mean, standard deviation (SD) and coefficient of variation (CV) were calculated. Harmonic means were calculated for the half-lives of elimination and absorption. Kruskal-Wallis test was used to check for normal distribution of parameters and concentrations ranges between animals. The Wilcoxon Rank Sum and Student's t tests were used to test parameters for significant differences between the different routes of administration. The statistical software used was SPSS Version 19.0 (SPSS Statistic Programme, Chicago, USA). Values of $P < 0.05$ were considered significant.

Abbreviations

PLA: poly lactic acid; $T_{\max}$: time to reach maximum concentrations; $C_{\max}$: maximum concentrations; MRT: Mean Residence Time; BCS: Biopharmaceutics Classification System class III drug; CDDSs: polymeric controlled drug delivery systems; SEM: scanning electron microscopy; FTIR: Fourier transforms infrared spectroscopy; XRPD: X-ray powder diffraction; DSC: Differential scanning calorimetry; HPLC: High Performance Liquid Chromatography; EE: Determination of the encapsulation efficiency; $AUC_{0-\infty}$: Area Under the Concentration-time Curve; SD: Standard deviation; CV: coefficient of variation.

Declarations

Ethics approval and consent to participate

All experiments protocols were carried out in accordance with the requirements of applicable national legislation and approved by the Bioethics Committee of the University of Murcia.

Consent for publication

Not applicable.

Availability of data and material

Research data are available as supplementary files.

Competing interest

The authors declare that they have no conflict of interest.

Funding

This research was funded by the Government of the Region de Murcia (Spain) by the Fundación Séneca (project 20950/PI/18). Fundación Séneca had no role in the design of the study, collection, analysis, interpretation of data and in writing the manuscript.

Authors' contributions

SB: Investigation, writing. AAG: Investigation. CMCR: Investigation, writing, statistical analysis, Pharmacokinetics analysis. FR: Conceptualization, writing. EFV: Funding acquisition, investigation, project administration, writing. All authors have read and approved the manuscript.

Acknowledgements

Not applicable.

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Figures

Figure 1

Chemical structure of metformin (a), PLA (b) and their potent groups for hydrogen bonding formation.

Figure 2

SEM picture of surfaces of the investigated metformin loaded PLA microparticles. Microparticles had almost spherical shape with smooth surfaces with particles adhered to the surface. The size of microparticles is variable, it is ranged from ~1 to 55 μm.

Figure 3

FTIR spectra for pure metformin, blank MPs and metformin loaded microparticles. The FTIR spectrum of pure metformin (blue line) shows characteristic peaks at 3390, 1633 and 1045 cm⁻¹ corresponds to the stretching of primary N-H, stretching of C=N, and stretching of C-N respectively. For the blank MPs (red line) four characteristic peaks were observed at 1764, 2993-2943 and 1087 which are attributed to C=O stretching of the ester group, asymmetric and symmetric vibration of the CH₃ group -CH₃ (asym), CH₃ (sym) and C-O ester bond. The microparticles spectra (green line) shows the intensity of the peak of C=O ester groups of PLA at 1764 cm⁻¹ after metformin loading was reduced.

Figure 4

XRD spectra for pure metformin, blank MPs and metformin loaded microparticles. The PXRD diffractogram of metformine (blue line) revealed the distinct peaks at 2θ : 12.17° , 17.62° , 24.47° , 28.2° , 31.17° , 37.07° ; these sharps peaks confirmed the highly crystalline nature of metformin. Blank MPs (red line) showed the peaks at 2θ : 16.6° , 18.92° indicating the semi crystalline nature of the PLA. The PXRD diffractogram of MPs (green line) shows no peaks.

Figure 5

DSC spectra for pure metformin, blank MPs and metformin loaded microparticles. The DSC curves of samples were recorded during the first heating process. The metformin DSC curve (blue line) exhibited a very sharp endothermic peak at ~ 232 °C that indicates its crystalline nature. DSC curve of the blank PLA (unloaded) microparticles (red line) showed a broad peak at ~178 °C, while this peak is shifted to 168 °C in metformin loaded PLA microparticles (green line). No peak of metformin in the loaded microparticles was recorded.

Figure 6

Mean plasma concentration-times profiles of metformin following a single dose administration of its intravenous, oral solution and oral microparticles in rabbits (n=5). The dose of the drug administered was 5 mg/kg ($\pm$ SD).

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