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Research paper

Novel extended-release formulation of lovastatin by one-step melt granulation: *In vitro* and *in vivo* evaluation

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ABSTRACT

The objective of this study was to apply a one-step melt granulation method to develop an extended-release formulation of lovastatin (LOV-ER). We prepared a formulation using PEG 6000 as binder agent in a laboratory scale high-shear mixer. *In vitro* dissolution studies showed that the release of the drug from the new formulation followed a zero-order kinetic with no differences in the release profile with either the pH media or the agitation rate. The pharmacokinetic of lovastatin and its metabolite lovastatin acid was evaluated after the administration of the new formulation to Beagle dogs in fasted conditions and after a high-fat meal, and compared to the marketed formulation Altoprev®. After the administration of LOV-ER, extended plasma profiles of lovastatin and its active metabolite were achieved in both fasted conditions and after the high-fat meal. Plasma levels of lovastatin and lovastatin acid were always higher when the LOV-ER formulation was administered with the high-fat meal. A high variability in plasma levels and pharmacokinetic parameters was obtained, being this variability higher when the formulation was administered under fasting conditions. Our results suggest that there is an increase in lovastatin bioavailability when the formulation is administered after the high-fat meal. When we compare LOV-ER and Altoprev®, both administered after the high-fat meal, we found significant differences ($p < 0.05$) in C_{max} of lovastatin and in $AUC_{0-\infty}$ and MRT of lovastatin acid. No differences were detected between both formulations in fasting conditions. In this regard, the high-fat meal seems to increase the absorption extent of lovastatin from LOV-ER formulation and to delay the absorption rate of the drug from Altoprev®. In conclusion, we developed a lovastatin formulation that provided extended plasma levels that confirm that one-step melt granulation in high-shear mixer could be an easy and cost-effective technique for extended-release formulation development.

1. Introduction

Extended-release (ER) formulations have been introduced into drug therapy with two main purposes: to reduce the number of single doses per day improving patient compliance of treatments and to decrease the fluctuations of plasma levels, in order to obtain better therapeutic efficacy and lower toxicity. There are many extended-release pharmaceutical systems currently known, ranging from monolithic matrices, membrane reservoirs, erodible polymers, to the more technologically complex and sophisticated pH independent formulations, ion exchange resins, osmotically, and geometrically modified systems. Many of these systems are not produced in a form that is amenable to large-scale manufacturing processes and usually do not exhibit the desirable zero-order-release kinetics. In addition, the cost of formulation development, raw materials, and manufacture technology are among the principal factors in ER delivery systems formulation for oral dosing [1].

An interesting approach to develop ER matrix formulations is based on melt granulation in high-shear mixer, which is a very short and easy one-step technique converting fine powders into granules. The powder agglomeration is promoted by the addition of a low melting point binder, which is solid at room temperature and melts at relatively low temperatures (50–80 °C). The interest in melt granulation has increased due to the advantages of this technique over other ER delivery technologies. Since it is a solvent-free process, the drying phase is eliminated and thus it becomes less consuming in terms of time and energy [2,3]. Moreover, melt granulation is one of the most widely applied processing techniques in the array of pharmaceutical manufacturing operations due to its simplicity and easy scale up [4–6]. In recent years, melt granulation has also been successfully employed to improve the dissolution rate of poorly soluble compounds increasing the bioavailability of these kinds of drugs [7–9], and in the development of ER formulations [10–12].

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Lovastatin is an inactive lactone prodrug that is hydrolyzed rapidly to the corresponding β-hydroxyacid metabolite (lovastatin acid), a potent and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzime A (HMG-CoA) reductase [13,14]. HMG-CoA reductase is an essential enzyme that catalyses an early rate-limiting step (the conversion of HMG-CoA to mevalonate) involved in the biosynthesis of cholesterol [15]. The inhibition of cholesterol formation results in reduced intracellular cholesterol. This effect increases LDL-receptor expression on the cell surface of hepatocytes, which, in turn, increases the clearance of LDL-cholesterol from plasma. The oral bioavailability of lovastatin is approximately 5% and highly variable [16]. Cytochrome P450 3A4 metabolizes the lactone form of lovastatin into hydroxy acid form (active metabolite) [17]. In the Expanded Clinical Evaluation of Lovastatin study (EXCEL), two immediate-release (IR) lovastatin formulations were compared. Lovastatin 20 mg formulation given twice daily produced a significantly greater reduction in LDL-cholesterol than 40 mg lovastatin formulation given once daily [18]. Subsequently, McClelland et al. [19] showed that the efficacy of HMG-CoA reductase inhibitors could be enhanced by the oral administration of a ER dosage form of the β-hydroxyacid simvastatin salt. These findings suggest that compared to a conventional dosage form, an ER dosage form of lovastatin may provide a dose-sparing advantage and improved safety profile.

Taking into account the enhanced efficacy associated with the extended-release form of lovastatin [20–22], and because nowadays there is only one ER formulation of lovastatin in the market that is exclusively commercialized in the United States, the objectives of the present study were (1) to develop a novel hydrophilic matrix extended-release formulation of lovastatin by melt granulation in a high-shear mixer, (2) *in vitro* evaluation of the formulation to investigate the influence of the media pH and agitation rate on the release profile and the release mechanism, and (3) *in vivo* evaluation after oral administration to beagle dogs in fasted and feed conditions. Moreover, a comparison with the marketed extended-release formulation of lovastatin (Altoprev® 60 mg) will be established.

2. Materials and methods

2.1. Materials

Lovastatin and simvastatin (internal standard) were supplied by Sigma–Aldrich Quimica S.A. (Madrid, Spain). Lovastatin acid sodium salt (active metabolite) was obtained from Mikromol Chemical Reference Substances (Luckenwalde, Germany). Hydroxypropylmethylcellulose (HPMC Methocel® K4M Premium) supplied by Colorcon (Kent, UK) and mannitol were used as starting materials and magnesium stearate (Kirsch Pharma, Madrid, Spain) as lubricant. PEG 6000 (Vencaser, S.A., Bilbao, Spain) was used as meltable binder. All other solvents and reagents were of analytical or HPLC grade and used as received.

2.2. Preparation of hydrophilic matrices

Lovastatin extended-release formulation (LOV-ER) was prepared by melt granulation in a laboratory scale one-step high-shear mixer Rotolab® (Zanchetta, Italy) using PEG 6000 as binder agent. Table 1 shows the composition of LOV-ER hydrophilic matrix tablet.

The granulation procedure was standardized on the basis of preliminary trials [23]. The mixture composed of lovastatin, HPMC K4M, PEG 6000, and mannitol was mixed at low impeller speed (250 rpm) for 5–10 min. Afterwards, the impeller speed was increased up to 950 rpm and the mixture was heated up to the melting point of the binder in order to obtain granules. At the end of the granulation process, the granules were cooled at room temperature by decreasing the jacket temperature and tilting the bowl to avoid agglomerate formation.

Table 1
Composition of LOV-ER formulation.

	LOV-ER composition (%)
Lovastatin	20
HPMC K4M	40
PEG 6000	15
Mannitol intragranular	15
Mannitol extragranular	9.5
Magnesium stearate	0.5

The granules obtained were sieved in order to remove lumps, mixed with magnesium stearate and extragranular mannitol, for 15 min using a rotating V-blender and compressed into tablets on a reciprocating single punch tablet machine at a breaking strength of 60 N. The breaking strength was determined using a Pharma-test GmbH durometer. Tablets weighted around 300 mg ± 5% and contained 60 mg of lovastatin.

2.3. In vitro dissolution studies

Drug-release assays were performed using the USP type II (paddle) apparatus at 50 rpm stirring rate and 37 ± 0.5 °C. The tablets ($n = 12$) were placed in 750 mL HCl 0.1 N for 2 h and continued in 0.05 M sodium phosphate buffer pH 6.8 up to 24 h. The change in pH was made by the addition of 250 mL 0.2 M tribasic sodium phosphate. Additionally, three different dissolution media were used to study the influence of pH on the dissolution profile: 0.1 N HCl pH 1.2, 0.02 M acetate buffer pH 4.5, and 0.05 M sodium phosphate buffer pH 6.8. All dissolution media contained 2% of sodium lauryl sulphate. The influence of stirring rate was also evaluated at 100 rpm in phosphate buffer pH 6.8. Samples were extracted at regular time intervals and assayed spectrophotometrically at 238 nm. The UV method was validated in terms of specificity to ensure that excipients and dissolution media do not interfere in the quantification of lovastatin. Moreover, the method was shown to be accurate and precise.

The similarity factor (f_2) was calculated in order to compare the release profile of the formulation in the different dissolution media with that obtained in a pH change medium. In this approach, a value between 50 and 100 indicates similarity between two dissolution profiles.

In order to characterize the kinetics and to define a model of drug release from the matrices, the data were fitted to the zero-order equation (Eq. (1)), the exponential Korsmeyer–Peppas equation (Eq. (2)), and the Weibull function (Eq. (3)).

$$dQ/dt = k \tag{1}$$

$$M_t/M_\infty = kt^n \tag{2}$$

$$M_t/M_\infty = 1 - \exp(-\alpha t^b) \tag{3}$$

where Q is the amount of drug released at time t, M_t/M_∞ is the fractional drug-release percentage at time t, k is the kinetic constant, n is the diffusional exponent that characterizes the drug transport mechanism, and α and b are constants of the Weibull function. The Korsmeyer–Peppas equation (Eq. (2)) is often used to describe the drug-release behavior from polymeric systems when the mechanism is not well known or when more than one type of release phenomena is involved [24]. For cylindrical matrix tablets, if the exponent $n = 0.45$, then the drug-release mechanism is Fickian diffusion, and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous

diffusion. An exponent value $n > 0.89$ is indicative of Case II Transport or typical zero-order release [25]. Experimental data were fitted to equations by using the Winnolin 4.1® Pro (Pharsight, Mountain View, USA) [26].

2.4. In vivo study

The pharmacokinetic of lovastatin hydrophilic matrices was studied in Beagle dogs ($n = 5$) weighing 15 ± 1.5 kg from "Centro de Cirugía de Mínima Invasión-Jesús Usón" (CCMI Cáceres, Spain". Principles in good laboratory animal care were followed and animal experimentation was in compliance with the "Ethical committee of animal experimentation (CEEA) of CCMI".

Each animal received the new formulation LOV-ER 60 mg and the commercial formulation Altoprev® 60 mg in fasted conditions and after a high-fat meal in a crossover study. Besides, the dogs were given 12 mg of lovastatin acid sodium salt intravenously. Lovastatin was not administered intravenously due to the difficulty to obtain a formulation in which it was soluble. A wash-out period of a week was allowed between the different treatments. When the oral formulations were administered to fasted dogs, after the last ordinary meal, food was withheld for 12 h before treatment, but free access to water was allowed. When the formulations were assayed in the dogs receiving a high-fat meal, the animals were given the meal in the morning of the test day (at 7 am) and allowed to eat until they finished their meals (approximately 5–10 min). They had free access to water. The treatments (LOV-ER or Altoprev®) were administered 1 h later (at 8 am). Serial blood samples were collected from cephalic vein immediately before administration and at predetermined time points up to 12 h in the case of intravenous administration and up to 48 h after oral administration. All blood samples were taken in heparinized tubes, and plasma was separated by centrifugation at 4 °C and 3500 rpm and immediately stored at −80 °C until analysis.

2.5. LC/MS/MS determination of lovastatin and lovastatin acid in plasma

Determination of lovastatin and lovastatin acid plasma concentrations was performed by a sensitive and specific liquid chromatography/ion spray tandem mass spectrometry (LC/MS/MS) with a HT Alliance 2795 apparatus coupled to a Micromass Quattro spectrometry detector (Waters™ Corp, Massachusetts, USA). A reversed phase HPLC column was used at room temperature (NovaPack® C18 4 μm, 3.9 × 150 mm). The mobile phase contained a mixture of acetonitrile/formic acid aqueous solution (10 mM) (85/15 v/v) and was delivered at a rate flow of 1 mL/min. The injection volume was 50 μL, and the autosampler was maintained at 4 °C.

The electrospray ionization (ESI) was performed in the positive ion mode, and detection was operated in the multiple-reaction monitoring (MRM) mode. The transition ions m/z 405 → 199, 445 → 343, and 419 → 199 were selected for lovastatin, lovastatin acid $[M+Na]^+$, and simvastatin (used as internal standard), respectively.

The analytical method was previously validated according to FDA and ICH Guidelines [27,28]. The assay for lovastatin and lovastatin acid was linear over plasma concentrations ranging from 0.5 to 50 ng/mL. The intraday and interday coefficients of variation for both analytes ranged from 3.91% to 12.34% at the three concentrations tested (1.5, 7.5, and 40 ng/mL). The bias at these concentrations ranged from 91.3% to 109.89%. The limit of quantification for lovastatin and lovastatin acid was considered the lowest level included in the calibration curve (0.5 ng/mL) and measures of intraday and interday coefficients of variation ranged from 3.04% to 12.15% and bias ranged from 98.0% to 109.5%. Lovastatin and lovastatin acid plasma samples stored at −80 °C were stable for at least 2 months.

2.6. Analytical procedure of plasma samples

Plasma samples were adjusted to pH 5 to avoid the described interconversion between lovastatin and lovastatin acid [29]. Walpole acetate buffer pH 4 (41 mL acetic acid 0.2 M and 9 mL sodium acetate 0.2 M in 100 mL distilled water) was employed to buffer plasma samples.

In order to analyze samples, 50 μL of IS solution (50 ng/mL of simvastatin) and 300 μL of Walpole acetate buffer were added to 300 μL of a plasma aliquot. The sample mixture was then extracted with 5 mL of tert-butylmethylether. After agitation for 10 min and freezing the aqueous solution at −80 °C, the organic phase was transferred to another tube and evaporated to dryness at 37 °C. The residue was reconstituted in 100 μL of ACN/water (75/25) and a 50 μL aliquot was injected.

2.7. Pharmacokinetic analysis

Plasma levels of lovastatin and lovastatin acid in dogs were plotted against time and pharmacokinetic parameters were determined according to a non-compartmental analysis by using the software WinNonlin 4.1 [26]. The area under the plasma concentration vs. time curve up to the last quantifiable time point, AUC_{0-t}, was obtained by the linear and log-linear trapezoidal method. The AUC_{0-t} was extrapolated to infinity ($AUC_{0-\infty}$) by adding the quotient C_{last}/K_{el}, where C_{last} represents the last measured concentration and K_{el} represents the apparent terminal rate constant. K_{el} was calculated by the linear regression of the log-transformed concentrations of the drug in the terminal phase. The half-life of the terminal elimination phase was obtained using the relationship $t_{1/2} = 0.693/K_{el}$. The C_{max} and T_{max} were obtained directly from the data. Oral clearance (Cl/F) was calculated as dose divided by $AUC_{0-\infty}$. The apparent volume of distribution was obtained from the equation $Vdz/F = D/(AUC_{0-\infty} \times K_{el})$. Mean residence time (MRT) was determined by division of AUMC (area under the first moment curve) by $AUC_{0-\infty}$.

2.8. Statistical analysis

Statistical analyses were performed with SPSS 17 software for Windows® (SPSS® Inc., Chicago, USA). The Shapiro–Wilks test was used to verify normality. The significance of the difference between the pharmacokinetic parameters obtained after oral administration was evaluated using a paired sample t-test. Box plot was used to assess the evolution of pharmacokinetic parameters and to compare the behavior of variables in different groups. The Willcoxon test was used to analyze the data of T_{max}. Statistical significance was assessed at $p < 0.05$.

3. Results and discussion

3.1. In vitro dissolution study

In order to test the influence of the dissolution medium pH and the agitation rate on the release of lovastatin from the LOV-ER formulation, dissolution tests were carried out at various pHs and agitation rates. Fig. 1 features the lovastatin-release profile from the new formulation in the different media ($n = 12$).

In vitro dissolution results showed that LOV-ER provided a sustained release of the drug with no differences in the release profile with either the pH or the agitation rate. In fact, the release profiles were almost superimposable at all conditions tested. The similarity

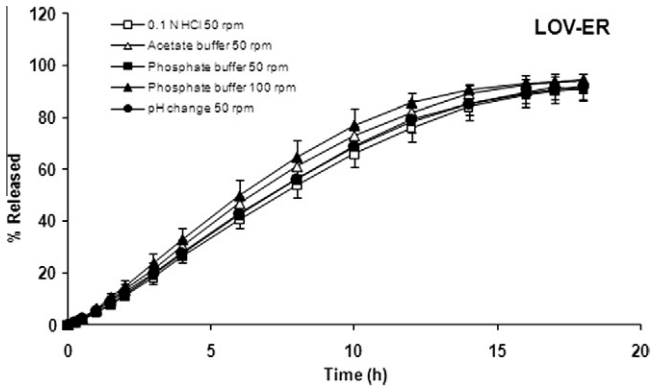

Fig. 1. *In vitro* dissolution profiles for LOV-ER 60 mg in different dissolution media. Bars represent the standard deviation obtained from the individual data (n = 12).

factor (f_2), which was used to compare the dissolution profile in the different conditions with that obtained in pH change medium, reached values of 83.61, 77.63, 96.17, and 64.97 for the HCl pH 1.2, acetate buffer pH 4.5, phosphate buffer pH 6.8 at 50 rpm, and phosphate buffer pH 6.8 at 100 rpm, respectively. These results indicate that lovastatin release from the matrix is independent from the dissolution medium pH and the agitation rate. This is a desirable characteristic for ER formulations, since less intra-inter subject variation in both gastric and gastrointestinal pH influence is then expected. However, differences in the dissolution conditions *in vitro* and *in vivo* make this characteristic less relevant than desired.

In order to describe the release behavior of lovastatin from the formulation, we fitted the data to several kinetic models. For this purpose, the release profile obtained with the pH change medium was used. LOV-ER formulation showed a very good correlation to zero-order kinetics equation (r^2 = 0.992) indicating that the drug release is independent of the drug concentration in the matrix system. The corresponding plot for the power law equation indicated a good linearity (r^2 = 0.995). The release exponent n was 0.86, a value close to 0.89, which is indicative of Case II transport. This suggests that matrix erosion might be the predominant mechanism for drug release from this formulation. These results are in line with the idea that the release of poorly soluble drugs from matrix systems is mainly controlled by erosion of the tablet [30]. The Korsmeyer–Peppas equation is confined to the description of the first 60% of the release curve [31,32]. However, the Weibull function is used to describe the drug-release mechanism of the entire drug-release curve. The fitting of release values to Weibull equation showed a good correlation (r^2 = 0.999). A b > 1 value indicates that a complex mechanism governs the release process, with an initial nonlinear increase up to an inflection point and, thereafter, an asymptotic decrease. These results are in line with previous studies that provide experimental evidence for the successful use of the Weibull function in the entire drug-release studies [33].

A dissolution study was also carried out with the marketed formulation in the same media than the LOV-ER tablets. Fig. 2 features the release profiles of lovastatin from Altoprev®. As can be seen, lovastatin release was dependent on the dissolution pH and agitation rate, pointing out a significant influence of these parameters in the drug-release profile. This influence was expected because this formulation is an osmotic tablet with a pH sensitive polymeric coating that increases its permeability as the pH increases. Therefore, the release of lovastatin takes place through a limited number of pores formed in the weakest points of the coating due to the increasing hydrostatic pressure in the osmotic core [34]. In the pH change medium, the marketed formulation presented a 2-h "lag time" (corresponding to the HCl pH 1.2 phase) and showed a worse correlation to zero-order-release kinetic (r^2 = 0.973) than the new LOV-ER formulation (0.992).

3.2. In vivo study

In vivo studies in dogs were carried out in order to assess whether an extended plasma concentration profile of lovastatin from the new formulation could be achieved. Moreover, the effect of food on drug absorption was also studied, since it is of particular importance with extended-release dosage forms assuring consistent drug delivery when administered with meals. It must be taken into account that drug release from ER tablets may be influenced by the presence of food in the gastrointestinal tract [35,36]. Several studies have reported that a high-fat meal causes decreased absorption of lovastatin from an extended-release formulation [37,38], whereas other studies have found that food increases the absorption of lovastatin [37,39].

Dogs are generally considered to be the best model for such studies, since solid oral dosage forms designed for humans can be administered and canine food composition can be similar to human meals [40]. However, physiological differences between dogs and humans such as those in gastric pH, gastric emptying, and intestinal transit time can result in differences in food effect. These differences may be relevant if dog studies are to be predictive for human [41]. The main goal of this work was to demonstrate the ability of the formulation to provide an extended drug concentration profile and to check the influence of a high-fat meal on the pharmacokinetic and not to predict the behavior of the formulation in humans.

Fig. 3 shows the evolution of the average plasma levels of lovastatin acid after intravenous administration (12 mg). This

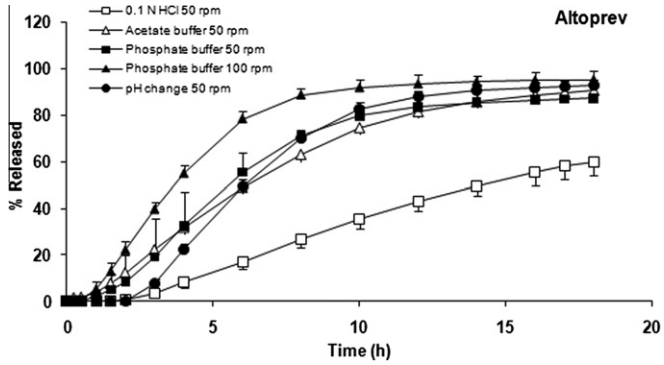

Fig. 2. *In vitro* dissolution profiles for Altprev® 60 mg in different dissolution media. Bars represent the standard deviation obtained from the individual data (n = 12).

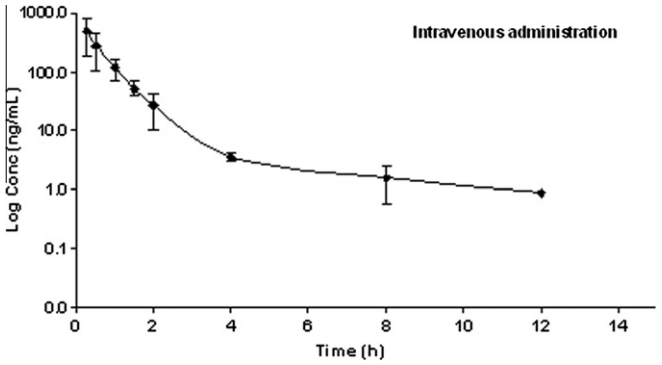

Fig. 3. Plot of mean (±SD) plasma lovastatin acid concentration time curve in dogs (n = 5) after IV administration.

Table 2
Mean pharmacokinetic parameters of lovastatin acid in dogs ($n = 5$) following IV administration.

	IV lovastatin acid Mean (SD)
$t_{1/2}$ (h)	1.75 (1.49)
C_{max} (ng/mL)	486.36 (305.18)
$AUC_{0-\infty}$ (ng h/mL)	492.86 (235.96)
MRT (h)	0.74 (0.17)
Cl (L/h)	0.03 (0.01)
Vz (L)	0.07 (0.07)

Table 3
Mean pharmacokinetic parameters of LOV-ER in dogs ($n = 5$) following oral administration of the formulation.

	Lovastatin		Lovastatin acid	
	Fasted state Mean (SD)	Feed state Mean (SD)	Fasted state Mean (SD)	Feed state Mean (SD)
$t_{1/2}$ (h)	4.25 (1.59)	4.05 (3.50)	2.24 (1.25)	2.96 (2.64)
T_{max} (h)	4.00 (2.00)	4.00 (2.00)	4.00 (0.00)	4.00 (1.41)
C_{max} (ng/mL)	24.06 (29.65)	39.68 (20.17)	22.11 (14.48)	56.77 (37.23)
$AUC_{0-\infty}$ (ng h/mL)	135.09 (129.68)	211.42 (125.75)	142.37 (94.42)	273.49 (102.71)
MRT (h)	8.12 (3.66)	5.30 (1.55)	5.60 (1.50)	5.20 (1.74)
Cl/*F* (L/h)	0.89 (0.81)	0.50 (0.30)	0.70 (0.65)	0.25 (0.09)
Vz/*F* (L)	4.30 (2.46)	3.27 (4.27)	1.72 (0.99)	0.93 (0.69)

evolution has two phases: the first one, with a faster decrease in the plasma levels, is followed by a second phase in which the decrease is slower, reaching a value of 0.87 ng/mL after 12 h of administration. Table 2 lists the main pharmacokinetic parameters.

Fig. 4 shows the evolution of the average plasma levels of lovastatin and lovastatin acid obtained after oral administration of LOV-ER in fasting conditions and after a high-fat meal. As can be seen, extended plasma level profiles of lovastatin and its active metabolite were achieved in both situations. Table 3 summarizes the mean plasma lovastatin and lovastatin acid pharmacokinetic parameters according to a non-compartmental analysis.

Plasma levels of lovastatin were always higher when the LOV-ER formulation was administered with the high-fat meal rather than in fasting conditions. Although no statistical differences were found, a higher lovastatin C_{max} mean value (39.68 ± 20.17 ng/mL vs. 24.06 ± 29.65 ng/mL), higher $AUC_{0-\infty}$ mean value (211.42 ± 125.75 vs. 135.09 ± 129.68 h ng/mL), and lower MRT mean value (5.30 ± 1.55 vs. 8.12 ± 3.66 h) were achieved when the formulation was given with the high-fat meal. However, similar absorption rate and plasma elimination half-lives of lovastatin were obtained in both situations (4.25 ± 1.59 and 4.05 ± 3.50 h for fasted and feed state, respectively). The elimination half-life mean value of lovastatin from this ER formulation was similar to that obtained by Fu et al. [42] after the administration of an immediate-release formulation in beagle dogs; this is indicative of absence of a flip-flop effect.

No significant difference in the elimination half-life of lovastatin acid was found between the intravenous and oral administration, which indicates that lovastatin acid elimination rate was not conditioned by the absorption rate of lovastatin from the formulation. As for lovastatin, no significant differences in pharmacokinetic parameters of lovastatin acid were found when the oral formulation was administered in fasting state or after a high-fat meal, although, as in the case of lovastatin, higher C_{max} and $AUC_{0-\infty}$ mean values were obtained in feed condition.

Lovastatin is a poorly absorbed drug attributed mainly to its poor solubility in gastrointestinal fluids, poor permeability through the mucosal membrane, and/or extensive small intestinal mucosa and hepatic first-pass metabolism [43]. As previously mentioned, increased systemic exposure with food is often seen for lipophilic drugs like lovastatin and it may be attributed to improve solubilization due to higher bile salt and lipid concentration. Moreover, food could accelerate the erosion of the matrix inducing a faster release of the drug in the gastrointestinal tract [36]. Our results suggest an increase in lovastatin bioavailability due to food. The increase in bioavailability of the formulation in presence of food is in line with that described in the package insert for lovastatin IR tablets, in which plasma concentrations of lovastatin and its active metabolite after oral administration under fasting conditions were lower than when the tablet is administered after a standard meal [44]. Due to the lack of statistical significance in the pharmacokinetic parameters, the interpretation of this finding is complicated by large standard errors around the mean for C_{max} and $AUC_{0-\infty}$. As shown in Fig. 4 and Table 3, high variability in plasma levels and pharmacokinetic parameters was obtained. This high variability was also observed in other studies [22,45–47]. An interesting finding is that higher variability in pharmacokinetic parameters was obtained when the formulation was administered under fasting rather than feed conditions. This could be explained by an increase in lovastatin solubility in the presence of high-lipid meal that may have improved the absorption of lovastatin, leading to a decrease in the variability. A reduced pharmacokinetic variability (i.e., more consistent absorption) was also found by other authors when ziprasidone was administered with meals rather than under fasting conditions [48].

Plasma levels of lovastatin and lovastatin acid after the administration of Altoprev® to the dogs under fasting conditions and with a high-fat meal are presented in Fig. 5. As expected, extended levels of lovastatin and its active metabolite were obtained in both situations. Table 4 summarizes the mean pharmacokinetic parameters following oral administration of the marketed formulation.

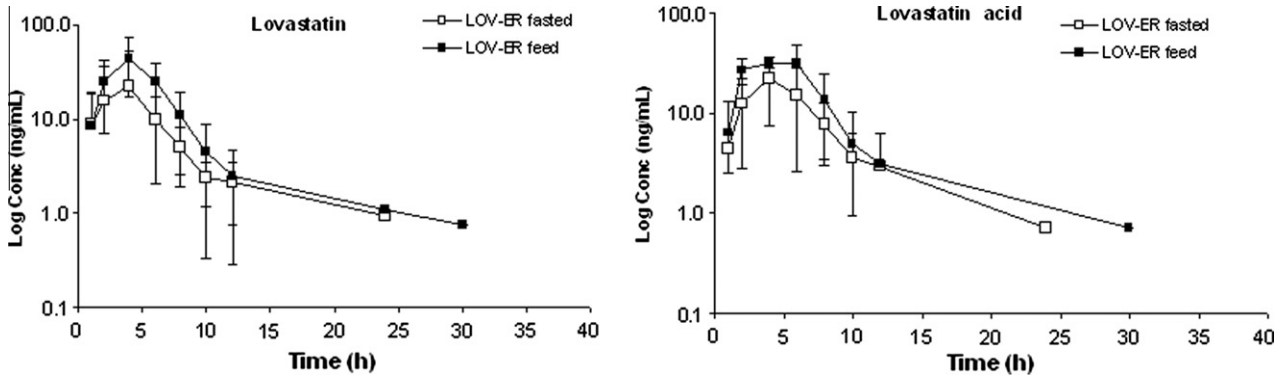

Fig. 4. Plot of mean (±SD) plasma lovastatin and lovastatin acid concentration time curves in dogs ($n = 5$) after oral administration of LOV-ER in both fasted and feed states.

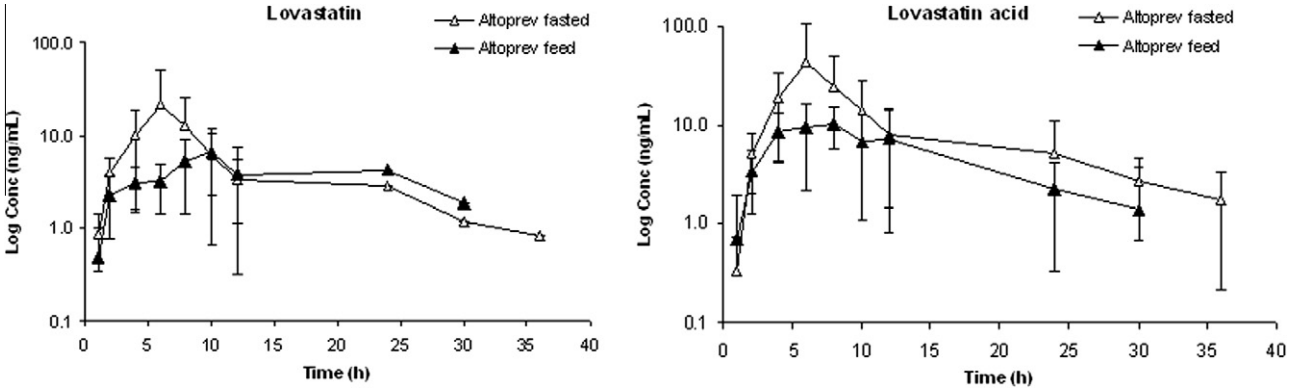

Fig. 5. Plot of mean (±SD) plasma lovastatin and lovastatin acid concentration time curves in dogs ($n = 5$) after oral administration of Altoprev® in both fasted and feed states.

Table 4
Mean pharmacokinetic parameters of Altoprev® in dogs ($n = 5$) following oral administration of the formulation.

	Lovastatin		Lovastatin acid	
	Fasted state Mean (SD)	Feed state Mean (SD)	Fasted state Mean (SD)	Feed state Mean (SD)
$t_{1/2}$ (h)	4.20 (2.54)	3.80 (1.72)	4.21 (1.86)	3.46 (3.75)
T_{max} (h)	4.50 (1.00)	8.50 (3.42)	5.50 (2.52)	8.00 (1.63)
C_{max} (ng/mL)	23.02 (28.63)	8.73 (3.00)	46.47 (62.73)	15.50 (2.37)
$AUC_{0-\infty}$ (ng h/mL)	137.92 (118.74)	76.77 (55.79)	318.06 (281.87)	149.30 (89.54)
MRT (h)	7.32 (2.16)	9.45 (3.43)	10.36 (4.14)	9.12 (3.08)
Cl/F (L/h)	1.06 (1.07)	1.02 (0.45)	0.48 (0.49)	0.55 (0.32)
Vz/F (L)	5.65 (4.73)	5.35 (3.62)	2.65 (2.29)	1.70 (0.99)

When Altoprev® was administered to the dogs after a high-fat meal, lovastatin C_{max} mean value was lower than that obtained when the formulation was administered under fasting conditions (8.73 ± 3.00 vs. 23.02 ± 28.63 ng/ml). Moreover, food produced a reduced $AUC_{0-\infty}$ (76.77 ± 55.79 vs. 137.92 ± 118.74 h ng/mL) and longer T_{max} (8.5 ± 3.42 vs. 4.5 ± 1.00 h) mean values. A similar behavior was found with the active metabolite, lovastatin acid. The variability of the data was also reduced when Altoprev® was administered with meals.

In spite of non-significant differences, the decrease in C_{max} and the increase in T_{max} suggest that the high-fat meal may decrease the absorption rate, justified by a delay in the gastric emptying. Food could make the wetting of the system difficult and, subsequently, the release and the absorption of the drug, providing a lower C_{max} and $AUC_{0-\infty}$. These results are in accordance with those obtained from Sun et al. who showed a reduction in the bioavailability of lovastatin from ER osmotic system when it is administered with food [20].

When we compare the LOV-ER and Altoprev® formulations, both administered after the high-fat meal, we found significant differences ($p < 0.05$) in C_{max} of lovastatin and in $AUC_{0-\infty}$ and MRT of lovastatin acid. No differences were detected between both formulations in fasting conditions. These data reinforce the idea that the meal induced changes in the behavior of the formulations when compared to the administration under fasting conditions, despite the lack of statistical significance. In this regard, the high-fat meal seems to increase the absorption extent of lovastatin from LOV-ER formulation and to delay the absorption rate of the drug from the marketed formulation.

4. Conclusions

We have developed a novel ER lovastatin formulation by melt granulation in high-shear mixer that provided extended plasma levels of lovastatin and its active metabolite after oral administration to dogs. When the new formulation is administered to dogs in fasted conditions, no significant differences in pharmacokinetics parameters were found compared to the marketed formulation Altoprev®. However, significant differences in bioavailability were found among the two formulations when administered with a high-fat meal. Moreover, a lower variability in pharmacokinetics parameters was observed in the presence of meal. The high-fat meal seems to increase the absorption extent of lovastatin from LOV-ER formulation and to delay the absorption rate of the drug from the marketed formulation. Further studies need to be carried out in human volunteers to confirm the results of this trial. In conclusion, one-step melt granulation in high-shear mixer could be an easy and more cost-effective technique for extended-release formulation development.

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