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Pharmacokinetics of Metformin in Combination With Sitagliptin in Adult Horses After Enteral Administration

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ABSTRACT

Insulin dysregulation (ID) is a common metabolic disorder in horses. Recently, incretin hormone release has been suggested to be involved in ID in horses. In human medicine, metformin and sitagliptin are commonly used in combination for metabolic syndrome. This combination could be useful in treating ID in horses. However, no pharmacokinetics data have been reported in this species. The objective of the present study was to establish the plasma concentration–time profile and to derive pharmacokinetics data for a combination of metformin and sitagliptin in horses after enteral administration. Six healthy adult Purebred Spanish horses were used. A metformin (15 mg/kg) plus sitagliptin (1.5 mg/kg) preparation was administered by intragastric route (IG) as an enteral solution. Blood samples were collected from 0 to 48 hours after IG drug administration. Plasma concentrations of metformin and sitagliptin were measured using HPLC methods. The $t_{½\lambda z}$ for metformin was 2.9 hours and for sitagliptin 21 hours. The C_{max} was 442 ± 84 mg/L within 0.9 hours for metformin and 94 ± 14 mg/L within 1.3 hours for sitagliptin. No adverse effects were observed, and the combination of metformin and sitagliptin was well tolerated. Therefore, these results suggest that metformin plus sitagliptin might be a combination to consider in horses with ID. Additional studies are needed to establish the effectiveness and tolerance in equids affected by endocrine disorders.

1. Introduction

Insulin dysregulation (ID) is the primary endocrine disorder in equine metabolic syndrome (EMS) affecting also some equids with pituitary pars intermedia dysfunction (PPID) (10%–22% prevalence) [1–5]. In horses suffering from EMS and some cases of PPID, reducing caloric intake through dietary modification [6,7] and increasing energy expenditure through exercise are the mainstays for reducing obesity and/or improving insulin sensitivity (IS) [8,9]. However, it is important to keep in mind that dietary restriction compliance for some owners can be problematic [10], as well as the fact that some horses with PPID should not be severely calorie restricted (unless obese) due to the risk of exacerbating their catabolic status [6,11]. Until these management changes result in adequate weight loss and improved IS, and in refractory cases in which laminitis may limit ability to increase exercise, pharmacological intervention may also be warranted [1,8,12–14].

Currently, there are no licensed treatments for ID in horses. Consequently, several medications approved for human use such as metformin have been used in an extralabel manner in equids with ID. This antihyperglycemic drug increases tissue IS and is recommended as first-line treatment in type 2 diabetes mellitus in humans [12,15,16]. In equids, although there are several reports about the use of metformin in clinical cases, its efficacy is controversial [17,18] mainly due to its low oral bioavailability [19,20]. In humans, when diet and exercise fail to adequately control glycemia in type 2 diabetes mellitus, a pharmacological intervention within a dual therapy regimen including metformin is usually recommended [21]. Among the possible therapeutic associations, the combination of metformin with sitagliptin is one of the more frequently used. In humans, sitagliptin is a selective and orally administered dipeptidyl peptidase-IV inhibitor that extends the action of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic peptide, following carbohydrate consumption [21]. In equine medicine, the recent evidence of a functional enteroinsular axis in ponies, where alterations in glucose absorption and the secretion of GLP-1 may account for the difference between healthy and dysregulated ponies [22], encourages the trials of other therapies like the metformin-sitagliptin combination, in the attempt to control ID in horses.

Animal welfare/ethical statement: The study was conducted in compliance with applicable national legislation and was reviewed and approved by the Bioethics Committee of the University of Murcia.

Conflict of interest statement: The authors have no financial involvement or financial conflict with the subject matter or materials discussed in the manuscript.

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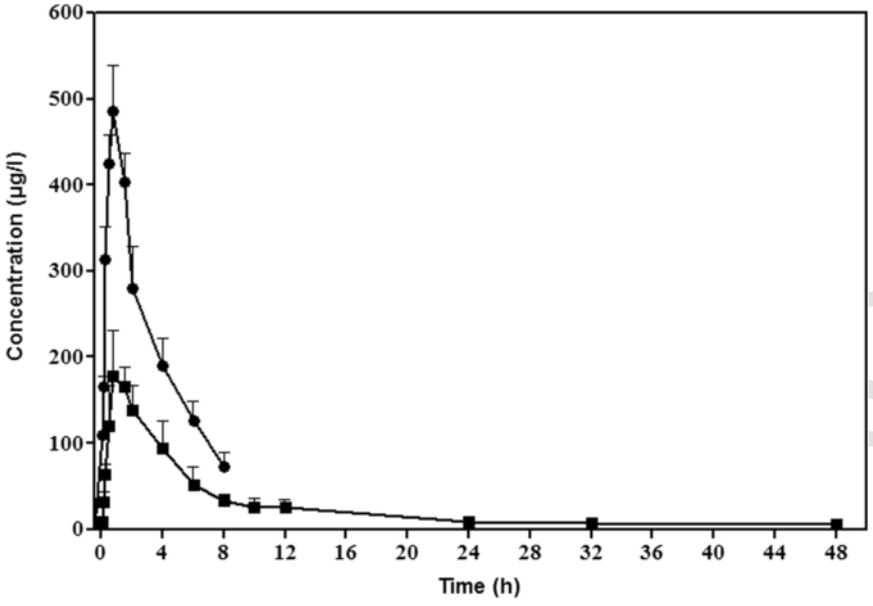

Fig. 1. Plasma concentrations (mean ± SD) of metformin and sitagliptin in horses after intragastric administration at a single dose of 15 mg/kg (metformin, ●) and 1.5 mg/kg (sitagliptin, ■) bodyweight (n = 6).

Table 1
Pharmacokinetic parameters (mean ± SD) of metformin and sitagliptin in horses after intragastric administration at a single dose of 15 mg/kg (metformin) and 1.5 mg/kg (sitagliptin) bodyweight (n = 6).

Parameters	Treatment	
	Metformin	Sitagliptin
λ_z (1/h)	0.2 ± 0.1	0.03 ± 0.01
$t_{½\lambda z}$ [a] (h)	2.9	21
V_{ss} (L/kg)	26.2 ± 9.3	19.3 ± 3.1
V_z (L/kg)	35.3 ± 11.3	34.1 ± 7.1
$AUC_{0-\infty}$ (µg·h/mL)	1822 ± 654	1347 ± 290
MRT (h)	2.7 ± 1.4	19.2 ± 4.8
Cl/F (L/h·kg)	8.3 ± 3.3	1.2 ± 0.3
C_{max} (µg/L)	442 ± 84	93.6 ± 14.3
T_{max}(h)	0.9 ± 0.2	1.3 ± 0.2

Abbreviations: $AUC_{0-\infty}$, area under the serum concentration-time curve from zero to infinity; Cl/F, total body clearance of drug; C_{max}, peak concentration following intragastric administration; $t_{½\lambda z}$, elimination half-life associated with the terminal slope (λ_z) of a semilogarithmic concentration-time curve; T_{max}, time to reach peak concentration following intragastric administration; V_z, apparent volume of distribution calculated by the area method; V_{ss}, apparent volume of distribution at steady state.

[a] Harmonic mean.

The prospect of possible benefits of metformin-sitagliptin combination in horses encourages future clinical studies to evaluate the advantages of this combination. This is supported by the fact that metformin treatment alone is an unappealing option of treatment because of poor bioavailability and questionable efficacy reported in different studies [1,10,17,19]. However, as several of these studies with metformin had shown some benefits, as is the case in human medicine with transition from metformin treatment alone to benefits of metformin-sitagliptin combination treatment, we consider that metformin-sitagliptin combination would be an interesting option to be studied. Therefore, it is important to study the pharmacokinetics of the combination to acquire information for future efficacy and clinical studies. Therefore, the objective of the present study was to establish the plasma concentration–time profile and to derive pharmacokinetics data for a combination of metformin and sitagliptin in horses after oral administration.

2. Material and Methods

2.1. Animals

Six geldings Purebred Spanish adult male horses weighing between 435 and 509 kg were used. All horses were healthy based on physical, hematological, and biochemical examinations. They did not receive any drug treatment before the study. The animals were housed individually in boxes receiving three meals daily of grass hay and were given free access to water. Twelve hours before the start of the treatment, animals were fasted. A complete physical examination was performed including their attitude in the box, vital signs (such as cardiac and respiratory rates, mucous, capillary refill time, motility, and rectal temperature), and findings from palpation of digital pulse and dorsal hoof wall and coronary band temperatures. This clinical examination was carried out before administering the treatment and every 12 hours until 24 hours after finishing the experimental phase of this study.

2.2. Experimental Design

A metformin plus sitagliptin single dose (15 mg/kg of metformin and 1.5 mg/kg sitagliptin) was administered by intragastric route (IG) as an enteral solution previously prepared in 100 mL of water. The metformin (Dianben; Merck, Madrid, Spain) plus sitagliptin (Januvia; Merck) solution was administered through a nasogastric tube (19 × 3000 mm) to each horse followed by 300 mL of deionized water to clean the tube.

Blood samples were collected at 0, 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, and 48 hours after IG drug administration. Blood samples (5 mL) were obtained through a 12-gauge catheter in the left jugular vein. The blood was placed into a 5 mL heparinized tube, and the samples were centrifuged at 1500 g × 15 minutes within 30 minutes of collection. Plasma was immediately

removed, divided into two tubes, and stored at −90°C until being assayed.

2.3. Analytical Method

Plasma concentrations of metformin and sitagliptin were measured using a modified HPLC method for metformin and sitagliptin [23,24].

2.3.1. Metformin

Phenformin (Sigma-Aldrich, Madrid, Spain) was used as the internal standard. After the addition of 20 μL of the internal standard solution to 200 μL plasma, 200 μL acetonitrile was added. Plasma proteins were precipitated by being shaken in an ultrasonic bath followed by centrifugation for 10 minutes at 5000 rpm. The supernatant (300 μL) was transferred to HPLC autosampler vials. The HPLC separation was performed using a reverse-phase column Discovery C_{18}250 × 4 mm of 5 μm particle size (Discovery column; Supelco, Bellefonte, PA) with an injection volume of 100 μL. The mobile phase consisted of acetonitrile (45%) and ammonium acetate (25 mM)–dodecyl sulfate sodium (9 mM) (pH = 7.02) solution (55%), using an isocratic form with a flow rate of 1.0 mL/min. Metformin eluted at approximately 4.8 minutes. The ultraviolet detection was performed at 236 nm.

2.3.2. Sitagliptin

Candesartan (Sigma-Aldrich) was used as the internal standard. After the addition of 20 μL of the internal standard solution to 500 μL plasma, 800 μL of tert-butyl methyl ether was added.

After vortex mixing for 5 minutes, the organic layer was transferred to a glass tube and evaporated to dryness using an evaporator at 40°C under a stream of nitrogen. Then, the dried extract was reconstituted in 250 μL of the mobile phase, and a 100 μL aliquot was injected into the chromatographic system. The HPLC separation was performed using a reverse-phase column Discovery C_{18}250 × 4 mm of 5 μm particle size (Discovery column; Supelco). The mobile phase consisted of acetonitrile (45%) and an ammonium acetate (25 mM)–dodecyl sulfate sodium (9 mM) (pH = 7.02) solution (55%) using an isocratic form with a flow rate of 1.0 mL/min. Sitagliptin eluted at approximately 6.3 minutes. The fluorescence detection was performed at 267 nm (λ_{ex}) and 297 nm (λ_{em}).

2.3.3. Method Validation (Metformin and Sitagliptin)

Quality controls were prepared from a pool of blank horse plasma spiked with seven concentrations of metformin (50–10,000 μg/L) and sitagliptin (5–500 μg/L). Plasma aliquots were stored at −90°C until assay. Aliquots of quality controls were extracted as above and 100 μL was injected into the chromatographic system. Standard curves were obtained by unweighted linear regression of the metformin and sitagliptin/IS ratios. Each point was established from an average of five determinations. Correlation coefficients (r) were >0.99 (metformin and sitagliptin) for calibration curves.

The percentage recovery was determined by comparing the peak areas of blank plasma samples spiked with different amounts of drug and treated in the same way as the other samples, with the peak areas of the same standards prepared in a phosphate buffer. Each point was established from an average of five determinations. The average recovery obtained was 95.1 ± 1.3% (metformin) and 94.1 ± 2.1% (sitagliptin). The assay precision was assessed by expressing the relative standard deviation (RSD) of repeated measurements as a percentage of the mean value. Intraday precision was estimated from six replicates of three standard samples used for calibration curves (RSD < 9% for both drugs). Interday precision was estimated from the analysis of standard samples on three separate days (RSD < 12% for both drugs). The limit of quantification (LOQ) of metformin and sitagliptin in plasma was chosen in the same way as the concentrations used for the lowest concentration level on the calibration curves and for which the RSD was <15% (LOQ metformin: 50 μg/L; LOQ sitagliptin: 5 μg/L).

2.4. Pharmacokinetic Analysis

The plasma metformin and sitagliptin time-concentration data were analyzed by noncompartment methods. The area under the concentration-time curve ($AUC_{0-\infty}$) and area under the first moment curve (AUMC) were calculated using the linear trapezoidal rule with extrapolation to infinity. Mean residence time (MRT) was calculated as MRT = AUMC/AUC, and 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 · λ_z) and V_{ss} = (dose ·AUMC)/AUC^2, respectively.

2.5. Statistical Analysis

Descriptive statistical parameters—mean, standard deviation (SD), and coefficient of variation (CV)—were calculated. Harmonic means were calculated for the half-lives of elimination and absorption. The *Kolmogorov–Smirnov test* was used to check for normal distribution of parameters and concentration ranges between animals. The statistical software used was SPSS, version 19.0 (SPSS Statistic Programme, Chicago, IL). Values of $P < .05$ were considered significant.

3. Results

The mean (±SD) metformin and sitagliptin plasma concentrations following IG administration are shown in Fig. 1, whereas mean (±SD) values for pharmacokinetic parameters are given in Table 1. No concentrations of metformin were found after 8 hours. The terminal half-life ($t_{½\lambda z}$) for metformin and sitagliptin was 2.9 hours and 21 hours, respectively. Clearance value (Cl/F) relative to bioavailability (F), for metformin was 8.2 ± 3.3 L/kg h and for sitagliptin 1.2 ± 0.3 L/kg h. After IG administration, the peak concentration (C_{max}) for metformin was 442 ± 84 mg/L within 0.9 hours and 93.6 ± 14.3 mg/L for sitagliptin within 1.35 hours.

There were no adverse effects or clinical signs that characterize acute laminitis such as bounding digital pulse, hoof heat, and changes in weight bearing during the course of the study. The combination of metformin and sitagliptin was well tolerated by the horses.

4. Discussion

The results of this pharmacokinetics study indicate that metformin plus sitagliptin in horses show a high tolerance and compatible pharmacokinetics profile to be administered together. Marked differences have been found in the pharmacokinetics of metformin in horses compared with human beings [19,20,25]. The bioavailability of metformin in horses after oral administration is reportedly 3.9%–7.1%, depending on the feeding status [19], whereas in humans, it is 40%–60%. In this study, a dose of 15 mg/kg was used, which fell between previously reported doses of 11 mg/kg [19] in horses and 30 mg/kg [20] in ponies. Results of our study demonstrate a relatively low peak concentration after oral administration, similar to what others have found, which supports a low oral bioavailability. Despite this low

bioavailability, metformin may still be effective. It is known that metformin reduces intestinal glucose absorption in rats by altering regulation of the 2 major intestinal glucose transporters and has a pronounced first-pass pharmacodynamic effect [26,27]. There are some findings [14] that indicate that metformin may have similar inhibitory effects on glucose absorption in the equine intestine. Although in the present study the $t_{½\lambda z}$ of metformin after IG administration was higher than the intravenous half-life described (0.4 hours) for metformin alone in horses [19], our results were much shorter (2.9 hours) than the $t_{½\lambda z}$ values reported in humans (9–17 hours) [28] and in insulin-resistant ponies (11.7 hours) [20]. Regarding the ponies study [14], the 8.76-hour difference with this study may be due to different factors like diet-related interactions, as horses of the present study were unfed and ponies were fed at the time of the study. Moreover, the high Cl/F (8.2 L/h kg) (possibly associated with a high rate of absorption), the short $t_{½\lambda z}$ (2.9 hours), C_{max} (0.4 mg/L), T_{max} (0.9 hours), and the last concentrations obtained in the present study were similar to those previously reported [19] and could be explained by this poor bioavailability.

On the other hand, in human medicine, sitagliptin has proven to be equally effective and just as safe as metformin, with an improvement in glycemic control and a lower risk of gastrointestinal side effects and hypoglycemia. Sitagliptin can be administered either together with metformin or alone if metformin is not suitable due to tolerability problems [29,30]. However, in horses, sitagliptin has not been used before, neither alone nor in combination with other drugs, and no pharmacokinetics studies have been reported yet. In our case, the $t_{½\lambda z}$ (21 hours) of sitagliptin was longer than values described in humans [31]. In addition, sitagliptin has also demonstrated a good tolerance, favorable pharmacokinetics profile in this species with extension of plasma concentrations up to 48 hours. The combined use of metformin-sitagliptin tested in the present study was chosen due to different potential actions of these drugs in the control of sugar that the liver produces and the intestine absorbs, and the sensitization of the insulin action that is physiologically produced [29]. It is clear that metformin treatment alone is an unappealing option in the treatment of horses because of its low bioavailability and controversial results of different studies [1,10,17], but we consider that transition from metformin treatment alone to the possible benefits of metformin-sitagliptin combination treatment would be an interesting option to be studied, and consequently, studying the pharmacokinetics of the combination of metformin and sitagliptin is a logical first step toward determining whether this combination would have a useful clinical application. In this context, although hyperglycemia appears to be uncommonly associated with ID, or at least not when horses are analyzed under study conditions, the recent confirmation of the enteroinsular axis influence, in the glycemic response to a meal [24], supports the use of this drug in combination with metformin if no adverse effects are observed.

In conclusion, the results of this study suggest that metformin plus sitagliptin might be a combination to consider in horses with ID due to the high tolerance and favorable pharmacokinetics profile. However, additional studies are needed to establish effectiveness and the tolerance associated when doses are repeatedly administered in equids affected by endocrinopathic disorders.

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