Pharmacokinetics of metformin after enteral administration in insulin-resistant ponies

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Objective—To determine pharmacokinetics and plasma steady-state kinetics of metformin after oral or nasogastric administration in insulin-resistant (IR) ponies.

Animals—8 IR ponies.

Procedures—Metformin (30 mg/kg) was administered to 8 ponies via nasogastric tube. Blood samples were collected at intervals for 24 hours. Plasma concentrations of metformin were measured via liquid chromatography–electrospray tandem mass spectroscopy. Pharmacokinetic variables were determined via noncompartmental analysis. Metformin (15 mg/kg, PO, twice daily [8 AM and 5 PM]) was administered to 4 ponies for an additional 20 days, and blood samples were obtained every 2 days. Plasma concentration at steady state ($C_{ss}$) was determined.

Results—Mean ± SD elimination half-life ($t_{1/2}$) of metformin was 11.7 ± 5.2 hours, maximal plasma concentration was 748 ± 269 ng/mL at 54 ± 32 minutes, mean area under the curve was 355 ± 92 µg•h/mL, and apparent clearance was 90.6 ± 28.1 mL/min/kg. The $C_{ss}$ was 122 ± 22 ng/mL.

Conclusions and Clinical Relevance—Metformin reportedly enhances insulin sensitivity of peripheral tissues without stimulating insulin secretion, but bioavailability in horses is low. The $t_{1/2}$ of metformin in IR ponies was similar to that in humans. Actual clearance of metformin adjusted for bioavailability in IR ponies was similar to that in humans; however, during chronic oral administration at dosages reported in efficacy studies, the $C_{ss}$ of metformin was less than values associated with therapeutic efficacy in humans. The apparent lack of long-term efficacy of metformin in horses is likely attributable to low bioavailability, rather than to rapid clearance. (Am J Vet Res 2010;71:1201–1206)
cine. In humans, metformin enhances insulin sensitivity via enhanced peripheral glucose uptake and decreases blood glucose concentrations via inhibition of hepatic glucose production and of intestinal glucose absorption. Metformin also promotes weight loss and reduces circulating lipid concentrations; adverse effects are rare.12

A small number of reports6,8,9,13,14 have been published that described the effects of metformin use in horses. A single dose of metformin (1.9 mg/kg, PO) administered in combination with an insulin secretagogue13 to a hyperglycemic horse reduced the plasma glucose concentration to a value within the reference interval.9 In a study15 of obese mares, metformin (2.8 mg/kg, PO, q 12 h) administered to horses for 30 days enhanced insulin sensitivity but became ineffective when given for a longer period or at an increased dose. At a dose of 15 mg/kg every 12 hours, metformin administration improved indicators of insulin resistance, insulin sensitivity, and β-cell function for up to 14 days in IR horses and ponies, compared with values from the same animals prior to treatment.16 However, metformin (15 mg/kg, q 12 h) given to a hyperglycemic mare did not improve clinical signs, blood glucose values, or serum insulin concentrations.8 A subsequent study14 of some of the pharmacokinetics of metformin in healthy horses revealed a low bioavailability (ie, fraction of the dose absorbed) of the drug in this species and determined distribution half-life but did not establish the clinically relevant \( t_{1/2} \).

The primary objective of the study reported here was to determine the \( t_{1/2} \) of metformin administered via nasogastric tube in IR ponies. The determination of \( t_{1/2} \) directly influences dosing regimens and has not been reported for metformin in horses and ponies to our knowledge. To extend the time during which plasma concentrations of the drug would be detectable and to characterize \( t_{1/2} \) accurately, the ponies were given a relatively high single dose of metformin (30 mg/kg), and a sensitive LC-MS-MS assay with a lower limit of quantitation of 10 ng/mL was used for detection of the drug. The pharmacokinetics of metformin administered IV and orally to 4 clinically normal horses has been partially described,14 but the study reported here was enhanced by use of a larger sample size and by optimization of the study design to accurately elucidate the clinically relevant \( t_{1/2} \).

The second objective of the study was to determine the \( C_{\text{max}} \) of metformin administered orally at a dose of 15 mg/kg twice daily for 21 days and to investigate the safety of chronic administration of metformin to ponies. We tested the hypothesis that the kinetics of metformin in IR ponies would be comparable with kinetics of the drug reported previously in horses1 and in humans.6–10 If the \( t_{1/2} \) of metformin in IR ponies was similar to that reported in humans and thus enabled oral administration twice daily, we further hypothesized that the \( C_{\text{max}} \) of metformin at a dose of 15 mg/kg would be safe and within the presumed therapeutic range (500 to 1,000 ng/mL)20 for the treatment of insulin resistance.

Materials and Methods

Animals—Eight female Welsh-cross and Shetland-cross ponies (mean ± SD age, 11.0 ± 3.4 years; weight, 209 ± 48 kg) from the Charles Sturt University research herd were included in the study. Ponies had been purchased from local sales and were determined to be IR on the basis of evaluation of neck crest adipose tissue (ie, cresty neck score22) and the results of a combined glucose-insulin test.23 On the basis of results of a 19-hour dexamethasone suppression test,24 all ponies were determined to be free of pituitary pars intermedia dysfunction. Ponies received a physical examination daily and were otherwise healthy. Feeding behavior was monitored at each meal. During the study, ponies were individually stalled, with free access to water. Early cut oat hay in an amount equivalent to 2% of BW was provided twice daily at 8 AM and 5 PM. Stables were cleaned each morning while ponies were allowed free exercise in a communal enclosure. The study protocol was approved by the Charles Sturt University Animal Care and Ethics Committee.

Drug administration and blood collection—The evening before the study began, the area over the right jugular vein was prepared for catheterization. Hair over the area was clipped, and the skin was washed 3 times with 2% chlorhexidine scrub solution alternated with 70% ethanol solution. A local anesthetic was administered (lidocaine [ie, lignocaine] hydrochloride, 60 mg, SC), and a 14-gauge catheter was placed aseptically. Each pony was given a meal of early cut oat hay equivalent to 1% of its BW (ie, half the daily ration). The hay feeding was repeated prior to drug administration on day 1 of the study. The dose of metformin for each pony was prepared by use of a mortar and pestle. The appropriate number of metformin tablets9 was ground to a powder, which was dissolved in 200 mL of tap water. The dose of metformin (30 mg/kg) was then administered via nasogastric tube to ensure accurate and consistent dose delivery and to mimic oral treatment. The solution was delivered through a funnel, and the nasogastric tube was rinsed with 100 mL of tap water to ensure the complete dose was delivered. The nasogastric tube was rinsed thoroughly prior to use for the next pony. Blood samples (10 mL) were obtained immediately before (ie, time 0) and at 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, 480, 600, 720, and 1,440 minutes after metformin administration. The samples were collected into lithium-heparin vacuum tubes and kept on ice; all samples were centrifuged at 4°C within 30 minutes of collection. Plasma was stored at −20°C until assayed for metformin concentration.

A subset of 4 ponies (3 Welsh-cross ponies and 1 Shetland-cross pony) was randomly selected to receive continued treatment with metformin (15 mg/kg, PO, twice daily [8 AM and 5 PM]) on days 2 through 21 (ie, chronic administration). Metformin tablets14 were ground into powder as previously described and dissolved in 100 mL of tap water; this solution was mixed with 100 g of rice bran pellets and fed to the ponies at 8 AM and 5 PM throughout the treatment period. The ponies willingly consumed the ration. For these ponies, 10-mL blood samples were collected into lithium-heparin vacuum tubes via jugular venipuncture for C. analysis of metformin on days 3, 5, 7, 9, 11, 13, 15,17,19, and 21. On days when blood samples were collected,
these were obtained in the morning, prior to the oral administration of metformin. The final dose of metformin was given on day 21.

Instrumentation and operating conditions—The samples were analyzed with liquid chromatography\(^d\) coupled to a tandem mass spectrometer\(^d\) with an electrospray ionization interface (ie, LC-MS-MS); the chromatography column\(^d\) was maintained at approximately 24°C. The mobile-phase program consisted of 4 solutions used in the following sequence: 100% water at 1.2 mL/min for 0.5 minutes; 1% formic acid in methanol at an initial rate of 1.2 mL/min for 0.5 minutes, which was changed to 0.6 mL/min for 0.7 minutes; 100% acetonitrile at 0.6 mL/min for 0.5 minutes; and 100% water. The retention time was 1.06 minutes. The electrospray ionization interface was set in positive mode, and the transitions for metformin and D6 metformin\(^d\) (used in preparation of the internal standard) were 130 to 71 and 136 to 77, respectively. The mass spectroscopy operating conditions included a capillary voltage of 0.8 kV, sample cone voltage of 20 V, and analyzer collision energy of 25 V. The lower limit of quantitation was 10 ng/mL.

Sample preparation—A 7.5-µL aliquot of the control (ie, metformin-free plasma obtained from the same ponies) or each plasma sample was added to 0.5 mL of the internal standard (25 ng/mL of D6 metformin\(^e\) in methanol). After centrifugation (14,000 × g) for 5 minutes at approximately 24°C, 5-µL volumes of each prepared sample were injected into the LC-MS-MS analyzer. Results for each sample were compared to a standard curve from 0 to 3,000 ng/mL that was prepared by use of metformin-containing plasma samples at 800, 1,000, and 2,000 ng/mL concentrations. Samples were analyzed in duplicate. Intraday coefficients of variation at 50 and 3,000 ng/mL were 3.97% and 1.56%, respectively. Interday coefficients of variation at 800 and 1,600 ng/mL were 3.07% and 2.42%, respectively. The concentration-detection relationship was linear over the range of 50 to 3,000 ng/mL and had a correlation coefficient of \(r^2 = 0.998\).

Pharmacokinetic analysis—The \(C_{\text{max}}\) and \(T_{\text{max}}\) were determined directly from examination of data points; \(C_{\text{ss}}\) was determined directly from examination of data point values between days 3 and 21. Other pharmacokinetic variables for samples from each pony were analyzed by use of noncompartmental analysis with a commercial software program.\(^1\) Log-linear regression was used to calculate \(t_{\frac{1}{2}}\), and log-trapezoidal integration was used to calculate the AUC\(_{\text{0-∞}}\). The CI/F (ie, apparent CI after administration via nasogastric tube) was calculated by use of the following formula: CI/F = Dose/AUC\(_{\text{0-∞}}\). The actual CI was subsequently calculated by use of the reported\(^14\) bioavailability value. All results were expressed as mean ± SD.

Results

After a single dose of metformin (30 mg/kg) in water was administered to each of 8 IR ponies via nasogastric tube, the drug was absorbed rapidly (Figure 1). The ponies continued to eat their hay ration. Mean plasma concentrations of metformin were assessed at predetermined intervals over a 24-hour period; the \(t_{\frac{1}{2}}\) was 11.7 ± 5.2 hours, \(C_{\text{max}}\) was 748 ± 269 ng/mL, and the \(T_{\text{max}}\) was 54 ± 32 minutes. The AUC\(_{\text{0-∞}}\) was 355 ± 92 µg·h/mL and CI/F was 90.6 ± 28.1 mL/min/kg.

During the chronic administration period (ie, days 2 through 21) in which 4 of the 8 IR ponies were given metformin (15 mg/kg, PO, twice daily [8 AM and 5 PM])
mixed with water and added to feed, the mean plasma concentrations from samples obtained 15 hours after drug administration and immediately prior to the next scheduled dose (ie, trough values) generally decreased with time (Figure 2). An apparent increase in the mean trough values of metformin on days 13, 15, and 21 were accompanied by large SDs, which were primarily attributed to variation among ponies. The Cl of metformin during the chronic administration period was 122 ± 22 ng/mL (mean ± SD; range, 93 to 157 ng/mL). Throughout the entire study period, the ponies willingly ate their rations and no adverse effects were noticed.

Discussion

Most IR horses and ponies have compensated insulin resistance, in which normoglycemia or mild hyperglycemia is accompanied by hyperinsulinemia. Prolonged hyperinsulinemia increases the risk for laminitis and other diseases. Therefore, the reduction of basal insulin secretion is an important part of the treatment for this condition. Metformin may be a good therapeutic option for IR horses and ponies because it is reported to enhance insulin sensitivity of peripheral tissues but does not stimulate insulin secretion by pancreatic β cells.

Reported doses of metformin in humans range from 500 to 2,550 mg/d (6.25 to 31 mg/kg/d), and this dose range is similarly reported in published studies of metformin use in horses. However, there are marked interspecies differences in pharmacokinetics of various drugs; the adaptation of metformin regimens established in humans for use in horses may not be appropriate. In humans, the T_max of metformin after oral administration is 1 to 3 hours, which approximates the finding of the present study in the IR ponies where the T_max was detected approximately 1 hour after administration, similar to the findings reported by investigators in a study of metformin in healthy horses. The T_1/2 of approximately 12 hours that was determined for ponies after administration of metformin via nasogastric tube in the present study is similar to a T_1/2 of 4 to 12 hours determined after oral administration in humans. To the authors' knowledge, this information has not been reported for horses prior to the present study.

Our calculated Cl/F value of 90.6 ± 28.1 mL/min/kg for a single dose of metformin was very high for horses and difficult to reconcile with the 11.7-hour result of the chronic administration experiment. Because the dosage regimen used in the study reported here was similar to that in another study of metformin in horses, pharmacokinetics were compared. The bioavailability of metformin in horses after oral administration in that study was reportedly 3.9% to 7.1%, depending on feeding status; in contrast, bioavailability in humans is 40% to 60%. Estimates of Cl/F are higher than Cl because bioavailability is the determining variable. By use of the bioavailability values reported for horses, Cl of metformin in the IR ponies in the present study was calculated to be 3.5 to 6.4 mL/min/kg. In studies in humans, Cl was reported to be in the range of 4.5 to 7.25 mL/min/kg following IV administration. When standardized units of Cl were used, the estimated Cl of metformin in IR ponies, obtained by use of data from the single-dose experiment in the present study and the published bioavailability, approximated that of humans and was in accordance with results described in healthy horses.

Investigators reported that at 5,720 ± 524 mL/min, Cl of metformin in horses was exceptionally rapid, more than 10 times the 454 mL/min Cl reported in humans. However, the results of the present study show that the actual Cl rate for IR ponies is similar to that of healthy horses after adjusting for BW, as well as being similar to the value determined in humans. Metformin is a highly polar compound that is largely confined to the vascular space and excreted unchanged by the kidneys in both humans and horses. The clearance of drugs that do not undergo hepatic biotransformation and are excreted unchanged by the kidneys is similar between these 2 species.

In the chronic administration experiment of the present study, the Cl of metformin in plasma was reached after 3 half-lives with no evidence of drug accumulation. The mean trough concentration of metformin in IR ponies from days 3 to 21 was 122 ± 22 ng/mL (range, 93 to 157 ng/mL), a value that is much lower than the concentration of 500 to 1,000 ng/mL considered therapeutic in humans. At these plasma concentrations of metformin, the circulating glucose concentration in fasted humans is reported to decrease within 3 to 5 days after treatment is started and to stabilize within 1 to 2 weeks. Although peak concentrations of metformin were not measured during the chronic administration period in the present study, peak values are expected to be twice the trough values when drugs are given at intervals equal to the T_1/2. The results of the present study suggested that, during chronic treatment with metformin administered orally at 15 mg/kg twice daily, the predicted peak plasma concentration of the drug (240 ng/mL) in IR ponies was also well below the range associated with therapeutic efficacy in humans.

Following a single dose of metformin (15 mg/kg, PO) in horses, the C_max of 300 to 400 ng/mL was suggested to be approximately equivalent to the therapeutic range at C_average reported for humans (300 to 1,000 ng/mL). In the study reported here, the C_max of 748 ± 269 ng/mL also approximates the human therapeutic range at C_average. However, the C_max following a single dose, especially because it is short-lived, is of minor clinical relevance. The C_average obtained with chronic dosing is more relevant and more predictive of therapeutic efficacy. Because the elimination half-life of metformin administered orally was not reported in the study of metformin in healthy horses (and the drug was only administered once in that study), conclusions regarding concentrations associated with therapeutic efficacy cannot be made. The predicted peak plasma concentration of metformin after chronic oral administration in the present study was less than the C_max obtained after a single dose, which suggested a reduced potential for the use of metformin as a long-term treatment for insulin resistance in horses and ponies. The reason for this apparent reduction in plasma concentrations of metformin during repeated administration was not determined.
On the basis of results of the investigations in the present study, it appears that the CI of metformin and its hybrid variable, \(t_{1/2}\), are similar between horses and ponies. At a dose of 15 mg/kg administered orally twice daily, trough concentrations and predicted peak metformin concentrations were lower than values associated with therapeutic efficacy in humans. The bioavailability of metformin in horses is appreciably less than that in humans, and trough values during chronic administration at a dose of 15 mg/kg in the present study were approximately one-fifth of the concentrations considered to be therapeutic in humans. Therefore, we suggest that subtherapeutic concentrations of metformin during chronic administration detected in the present study are attributable to the lower bioavailability of metformin in horses and ponies, instead of enhanced CI.

It is possible that a larger dose than that used in the present study should be investigated. However, increasing the dose may not result in therapeutic circulating concentrations of the drug. Evidence in studies of four horses in humans suggests that the bioavailability of metformin is inversely related to dose and that decreased absorption, rather than increased elimination, prevents increased responses that correspond to increased doses. The possibility that bioavailability may decrease as the drug dose is increased suggests that metformin may not be suitable for treatment of insulin resistance in horses and ponies.

References


Correction: Evaluation of body composition and cartilage biomarkers in large-breed dogs fed two foods designed for growth

In this report, published August 2010 (Am J Vet Res 2010;71:934–939), the unit of measure for linoleic acid, arachidonic acid, α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid should have been reported as µg/dL, not mg/L. The unit of measure for vitamin E should have been reported as mg/dL, not ng/mL. The unit of measure for glutathione peroxidase should have been reported as mU/10⁶ RBCs, not U/mL.