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The effect of oral metformin on insulin sensitivity in insulin-resistant ponies

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ABSTRACT

Metformin may be an effective therapeutic option for insulin-resistant (I-R) horses/ponies because, in humans, it reportedly enhances insulin sensitivity (SI) of peripheral tissues without stimulating insulin secretion. To determine the effect of metformin on insulin and glucose dynamics in I-R ponies, six ponies were studied in a cross-over design by Minimal Model analysis of a frequently-sampled intravenous glucose tolerance test (FSIGT). Metformin was administered at 15 mg/kg bodyweight (BW), orally, twice-daily, for 21 days to the metformin-treated group. The control group received a placebo. A FSIGT was conducted before and after treatment. The Minimal Model of glucose and insulin dynamics rendered indices describing SI, glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and the disposition index (DI). The body condition score (BCS), BW and cresty neck score (CNS) were also assessed.

There was no significant change in SI, Sg, AIRg, DI, BW, BCS or CNS in response to metformin, or over time in the control group. There were no measurable benefits of metformin on SI, consistent with recent work showing that the bioavailability of metformin in horses is poor, and chronic dosing may not achieve therapeutic blood concentrations. Alternatively, metformin may only be effective in obese ponies losing weight or with hyperglycaemia.

Introduction

With increasing understanding of the pathophysiology of insulin resistance (IR) and hyperinsulinaemia in horses/ponies (Hess et al., 2006; Asplin et al., 2007; Geor, 2008; de Laat et al., 2010), the goal of owners and veterinarians is to prevent IR, or to treat it before the consequences become manifest. While correct management of energy intake and exercise levels may be effective (Carter et al., 2010; Frank et al., 2010), there are circumstances where pharmacological intervention may be warranted (Geor and Harris, 2009; Frank et al., 2010).

With no licensed medications available for the treatment of IR in horses/ponies, veterinarians increasingly prescribe the off-label use of medications used for IR in humans (e.g., metformin, levothyroxine sodium, glibenclamide) (Johnson et al., 2005; Frank et al., 2008; Durham et al., 2009). An evaluation of the efficacy of these drugs in horses/ponies is, therefore, necessary. The anti-hyperglycaemic drug metformin (dimethylbiguanide) is in widespread human clinical use since it enhances insulin sensitivity (SI) by increasing peripheral glucose uptake. It also decreases blood glucose concentrations by inhibiting hepatic glucose production and intestinal absorption of glucose (Saenz et al., 2005). Metformin promotes weight loss and reduces lipid levels; adverse effects are rare (Salpeter et al., 2008).

Several reports describe the effects of metformin use in horses/ponies with mixed results (Johnson et al., 2005; Vick et al., 2006; Durham et al., 2008, 2009; Firshman et al., 2009; Hustace et al., 2009). A single dose of metformin (1.9 mg/kg bodyweight (BW), orally) administered with an insulin secretagogue to a hyperglycaemic horse reduced plasma glucose concentrations to values within the reference interval (Johnson et al., 2005). Furthermore, metformin (2.8 mg/kg BW, orally every 12 h) administered to 14 obese mares for 30 days enhanced SI, measured with the hyperinsulinaemic-euglycaemic clamp (HEC) (Vick et al., 2006). However, in the same study, metformin became ineffective when given for longer or at an increased dose (Vick et al., 2006).

In another study, at a dose of 15 mg/kg BW orally every 12 h, metformin improved proxy measures of SI and beta-cell function for up to 14 days in 18 insulin resistant (I-R) horses/ponies, compared with pre-treatment values from the same animals (Durham et al., 2008). However, metformin given to a hyperglycaemic mare at the same dose and frequency did not improve blood glucose or serum insulin concentrations (Durham et al., 2009). In six non I-R horses, metformin therapy (15 mg/kg BW, orally every 8 h for 15 days) showed no effect on SI measured with the HEC (Firshman

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et al., 2009). Pharmacokinetic studies of metformin in horses and I-R ponies indicated low bioavailability of the drug in this species (Hustace et al., 2009), and that chronic dosing may not achieve therapeutic blood concentrations (Tinworth et al., 2010).

The aim of this study was to determine the effect of oral metformin on insulin and glucose dynamics in I-R ponies using Minimal Model analysis of a frequently-sampled intravenous glucose tolerance test (FSIGT) conducted before and after 21 days of metformin administration (15 mg/kg BW, orally every 12 h), using six I-R ponies with no signs of laminitis. The study was conducted as part of the aforementioned work that investigated the pharmacokinetics of metformin (Tinworth et al., 2010).

We hypothesised that metformin treatment of IR in I-R ponies would enhance SI, that glucose effectiveness (Sg) would remain unchanged, that the acute insulin response to glucose (AIRg) would be lowered, and that the disposition index (DI) would increase. We also hypothesised that if SI was enhanced there would be a concurrent reduction in neck adiposity.

Materials and methods

Animals

Six female Welsh-cross and Shetland-cross ponies were used, with a (mean ± SD) age of 12.00 ± 2.88 years, weighing 206 ± 53.5 kg. The ponies, purchased from local sales, were not obese, had a body condition score (BCS) of 6.0 ± 0.9, but did display regional adiposity (Frank et al., 2010) and were deemed to be I-R based on the evaluation of neck crest adipose tissue, a cresty neck score (CNS) of $\geqslant 3$ (Carter et al., 2009a) and the results of a combined glucose-insulin test (CGIT) (Frank et al., 2006). The ponies were otherwise healthy and not suffering from pituitary pars intermedia dysfunction based on the results of a 19 h dexamethasone suppression test (Dybdal et al., 1994), where testing was conducted during November (Southern Hemisphere late Spring) (Donaldson et al., 2005).

The protocol described was reviewed on Wednesday 5 March 2008 by the Charles Sturt University Animal Care and Ethics Committee and was approved with the approval number 08/030. The ponies were owned by Charles Sturt University.

Study design

The study was conducted as a 2 × 2 cross-over trial. The two treatments were control (C) and metformin (M), and the six ponies were randomised to two treatment sequences, MC and CM, with three ponies in each group. The two treatment periods were separated by a 21-day wash-out period with ponies kept in a communal paddock. Treatment periods continued for 21 days with a FSIGT conducted on Days 0 and 22 on all ponies. At these times, the physical characteristics of BW, BCS, CNS, neck circumference and the height and thickness of the crest of the neck were also measured. Bodyweight was measured using electronic scales; BCS was scored out of a total of 9 (Henneke et al., 1983). The CNS was assigned according to Carter et al. (2009a), with the neck circumference, crest height and thickness measured as described by Frank et al. (2006).

During treatment periods, all ponies were stabled individually with unrestricted access to water. Stables were cleaned each morning while the ponies were allowed free exercise in a communal yard. Ponies were fed maintenance rations of early-cut oaten hay at a rate of 1–2% of BW twice daily (NRC, 2007). The ponies also received 100 g rice bran pellets (Cool Conditioner, CopRice) twice daily. Ponies were monitored for feeding behaviour at each meal and received full health checks daily. The C animals received exactly the same management as that of the M animals.

Metformin administration

Metformin was given at 15 mg/kg BW, orally every 12 h with meals at 08.00 and 17.00 h. For each pony, the dose of metformin was prepared by powdering the appropriate number of metformin tablets (Metforbell 500 mg, CiplaGenpharm), using a mortar and pestle, and suspending the powder in 100 mL of tap water. This solution was mixed with 100 g rice bran pellets (Cool Conditioner, CopRice) for the ponies to eat. The entire ration was readily consumed within 2 min.

FSIGT

The evening before the FSIGT, a 14 G catheter was placed in the jugular vein of each pony, after aseptic preparation of the site and the administration of local anaesthetic. On the morning of the study, the ponies were fed oaten hay at a rate of 1–2% of BW. The FSIGT was initiated at 09.00 h with a bolus of glucose (Dextrose solution 50%, Baxter Healthcare) (300 mg/kg BW, intravenously [IV]) administered within 2 min. Twenty minutes later, an insulin bolus (Humulin R, Eli Lilly) was administered at a dose rate of 20 mU/kg BW IV within 30 s, as described by Yang et al. (1987). Basal blood samples (10 mL) were taken at 60, 45, and 0 min before the glucose dose. Blood samples were then drawn at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after the glucose bolus. Blood samples were collected into lithium-heparin Vacutainers (Becton-Dickinson), kept on ice and centrifuged at 4 °C (1000 *g* for 10 min) within 20 min of collection.

Sample analysis

Each plasma sample was divided into two aliquots and stored at −20 °C until assayed for glucose and insulin. Plasma glucose concentrations were measured using an enzymatic assay (Glucose hexokinase, ThermoFisher Scientific) on a bench-top biochemistry analyser (Cobas Mira, Roche). Plasma insulin concentrations were determined using a radioimmunoassay (RIA) (Coat-A-Count Insulin RIA, Siemens Medical Solutions Diagnostics), validated for use in equines (Tinworth et al., 2009). Each sample was assayed in duplicate, and intra-assay coefficients of variation <5% or <10% were required for the acceptance of glucose and insulin assay results, respectively.

Minimal Model analysis

Glucose and insulin curves were interpreted according to the Minimal Model of glucose and insulin dynamics (Bergman, 1989; Boston et al., 2003) using MINMOD Millennium software (Boston et al., 2003). Application of this method in horses has been described previously (Hoffman et al., 2003; Treiber et al., 2005a). All the results were expressed as means ± SD.

Statistical analyses

Values for SI, Sg, AIRg, DI and physical characteristics between C and M groups were compared by calculating the difference in each variable between Day 22 and Day 0 for each pony in each period, then conducting a Wilcoxon matched pairs test comparing ponies in the two treatment sequences. The null-hypothesis was rejected if $P \leqslant 0.05$. Analyses were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software).

Results

Pre-treatment physical characteristics of the six ponies are shown in Table 1. Pre-treatment Minimal Model indices are shown in Table 2. No significant differences were found.

Physical characteristics measured on Day 0 and Day 22, of the C group and the M group at pre-treatment (Day 0) and post-treatment (Day 22) are shown in Fig. 1. The results for SI, Sg, AIRg and DI from Minimal Model analysis of the FSIGT of the C group and the M group at pre-treatment (Day 0) and post-treatment (Day 22) are shown in Fig. 2.

Non-fasting basal insulin concentrations of the C group were 8.67 ± 3.77 (mean ± SD) on Day 0 and 7.80 ± 3.89 on Day 22, and of the M group were 9.43 ± 6.19 on Day 0 and 6.10 ± 0.71 on Day 22. Data from the ponies were compared and no significant treatment effects were observed.

Discussion

The results of this study were disappointing but not surprising in the light of the pharmacokinetic results obtained concurrently.

Table 1
The baseline physical characteristics of the ponies at the frequently-sampled intravenous glucose tolerance test, before the treatment period. Each group comprised a sample size of six. Data are presented as means ± SD.

Parameter (units)	Metformin-treated ponies	Control ponies
Bodyweight (kg)	209.50 ± 49.95	209.8 ± 54.05
Body condition score (scale: 1–9)	6.00 ± 0.89	6.00 ± 0.89
Cresty neck score (scale: 1–5)	3.17 ± 0.41	3.00 ± 0.00
Neck circumference (cm)	84.50 ± 6.03	85.67 ± 5.50
Crest height (cm)	10.53 ± 1.86	10.38 ± 1.29
Crest thickness (cm)	7.37 ± 1.31	7.73 ± 1.69