Effect of parenteral administration of ivermectin and erythromycin on abomasal emptying rate in suckling calves

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Objective—To evaluate the effect of parenteral administration of ivermectin and erythromycin on abomasal emptying rate in suckling calves.

Animals—6 male Holstein-Friesian calves < 15 days old.

Procedures—In a crossover study, calves were administered each of 3 treatments (control treatment, 2 mL of saline [0.9% NaCl] solution, IM; erythromycin, 8.8 mg/kg, IM; and ivermectin, 200 µg/kg, IV). Thirty minutes later, calves were bottle-fed 2 L of fresh cow's milk containing acetaminophen (60 mg/kg). Blood samples were collected from a jugular vein at various periods after suckling of milk. Abomasal emptying rate was assessed by use of the time to pharmacokinetically determined maximal plasma acetaminophen concentration.

Results—Administration of erythromycin and ivermectin caused a significant increase in abomasal emptying rate, compared with results for the control treatment, as determined on the basis of time to maximal plasma acetaminophen concentration.

Conclusions and Clinical Relevance—Parenteral administration of erythromycin and ivermectin increased the abomasal emptying rate. The macrolide erythromycin can be an effective prokinetic agent in calves and other animals. Ivermectin is classified as a macrolide but has a number of structural differences from erythromycin. The clinical importance of a slight increase in abomasal emptying rate after IV administration of ivermectin remains to be determined because ivermectin is only labeled for SC, oral, and topical administration. (Am J Vet Res 2009;70:527-531)

Erythromycin is an effective prokinetic agent in humans and domestic animals, including adult cattle1 and calves.2 Erythromycin exerts its effect to accelerate gastric emptying by acting as a motilin-receptor agonist via binding to motilin receptors in the pyloric antrum and proximal portion of the small intestine.3 Motilin is a peptide consisting of 22 amino acids; it is periodically released from endocrine cells in the duodenojejunal mucosa, thereby initiating the migrating motor complex of the mammalian gastrointestinal tract during the interdigestive period. The migrating motor complex is the so-called housekeeper of the gastrointestinal tract.

The group of nonpeptide motilin-receptor agonists, called the motilin-like macrolides (ie, motilides), that interact with the motilin receptor and promote gastric emptying1 has generated considerable interest. Structure-activity studies1,4 have indicated that motilides have 3 main structural requirements that enable them to interact strongly with the motilin receptor: a ring structure (typically a 14-member lactone [cyclic ester] ring), an amino sugar (desosamine) bound at C-5 of the ring in a glycosidic linkage, and a neutral sugar (such as cladinose) bound at C-3 of the ring in a glycosidic linkage. From this 3-part structure, the potency of the motilide is influenced primarily by modifications to the N-dimethylamino group at the 3’ position of the amino sugar and, to a lesser extent, the configuration of the lactone ring structure (C-6 through C-9) and by the presence of a neutral sugar at C-3 that is parallel to the amino sugar at C-5.5,6 Parenteral administration of erythromycin at the label dosage for cattle (8.8 mg/kg, IM) causes an immediate increase in abomasal motility and emptying rate in milk-fed calves.1,7,8 Parenteral administration of erythromycin (10 mg/kg, IM) immediately before surgical correction of abomasal volvulus or left displacement of the abomasum in lactating dairy cattle increases the postsurgical abomasal emptying rate.9,10 Erythromycin

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AUC&lt;sub&gt;480&lt;/sub&gt;</td>
<td>Area under the plasma glucose concentration-versus-time curve for 480 minutes after suckling</td>
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<tr>
<td>Cmax</td>
<td>Maximal plasma concentration</td>
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<tr>
<td>Tmax</td>
<td>Time of maximal plasma concentration</td>
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has a 14-member lactone ring with a dimethylamino sugar (desosamine) at C-5 and a neutral sugar (cladinose) at C-3 in parallel with desosamine. In another study conducted by our laboratory group, we determined that the semisynthetic macroclide tylosin, which has a 16-member lactone ring with a dimethylamino sugar at C-5, also increases the abomasal emptying rate in milk-fed calves, although tylosin was not as effective a prokinetic agent as was erythromycin. In that study, we also found that the semisynthetic macroclide tilmicosin is a weak prokinetic agent in milk-fed calves. Tilmicosin has a 16-member lactone ring, an amino sugar at C-5, a hydroxyl group at C-3 (instead of a neutral sugar), a dimethylpiperidoneoethyl group at C-6, and a side-chain sugar at C-14.

Macrocyclic lactones are broad-spectrum antiparasitic drugs with activity against endoparasites and ectoparasites. Two chemical families of macrocyclic lactones are commercially available for administration to cattle: avermectins (such as ivermectin, doramectin, and eprinomectin) and milbemycins (such as moxidectin). Avermectins are natural compounds produced by a soil-dwelling actinomycete, Streptomyces avermitilis; in comparison, erythromycin is a natural compound produced by the closely related soil-dwelling actinomycete, Streptomyces rimosus. Ivermectin is a semisynthetic derivative of avermectin; it contains at least 80% 22-23 dihydroavermectin B1, and <20% 22-23 dihydroavermectin B1. Ivermectin is classified as a macroide and has some structural similarities to tylosin and tilmicosin in that ivermectin is a 16-member macro cyclic lactone; however, ivermectin has a fused hexahydrobenzofuran unit from C-2 to C-8 that is not found in tylosin or tilmicosin. Ivermectin also has an \( \alpha \)-oleandrose disaccharide attached at C-9 instead of C-3 or C-5. It is possible that ivermectin could have prokinetic activity in cattle, although the lack of a amino sugar in the region of C-5 of the 16-member ring would suggest that prokinetic activity is likely to be less than that of erythromycin.

Therefore, the purpose of the study reported here was to determine the effect of IV administration of ivermectin on abomasal emptying rate in milk-fed calves and to compare the effect for ivermectin with that of a positive control treatment (ie, erythromycin) and a negative control treatment (ie, saline [0.9% NaCl] solution). Two methods (acetaminophen absorption and glucose absorption) were used to assess abomasal emptying rate.

**Materials and Methods**

**Animals**—Six 2- to 4-day-old Holstein-Friesian calves were obtained from the University of Tehran dairy farm. Body weight of the calves ranged from 33 to 45 kg (mean, 39 kg). Calves were housed unrestrained in separate stalls that were bedded with wood shavings. Calves were bottle-fed fresh cow's milk. Calves had access to fresh water at all times. Approval of the study protocol by an animal care and use committee was not required because commercially available formulations were administered at labeled dose rates and because of the minimally invasive nature of the procedures (IV or IM injections and collection of blood samples) performed in the study.

**Experimental design**—Calves were at least 5 days of age when entered into the study. At least 18 hours before each experiment, calves were sedated by administration of xylazine hydrochloride (0.2 mg/kg, IV), and a catheter was then placed in the right jugular vein of each calf. The hair over the right jugular vein was clipped and the skin aseptically prepared. One milliliter of lidocaine hydrochloride was injected SC over the right jugular vein, and the skin was incised (1 cm in length) with a scalpel blade to assist in catheter placement. A 16- or 18-gauge catheter was inserted in the jugular vein, an extension set was attached to the catheter, and the catheter and extension set were secured to the neck. The catheter was flushed every 12 hours with heparinized saline solution (40 U of heparin/mL).

Calves were administered each of 3 treatments in a crossover study. A minimum of 36 hours was allowed to elapse between subsequent treatments. Treatments were not initiated until at least 12 hours had elapsed since a calf had consumed the preceding feeding.

Each calf was weighed and then administered the assigned treatment. The treatments were 2 mL of saline solution, IM (control treatment); erythromycin* (8.8 mg/kg, IM); and ivermectin* (200 \( \mu \)g/kg, IV, administered during a 2-minute period). The first 2 treatments administered were the control treatment and erythromycin, and they were randomly assigned. Ivermectin was the third treatment for all calves. Ivermectin was administered as the last treatment because it is slowly cleared after IV administration.

Thirty minutes after administration of each treatment, the calves were allowed to suckle 2 L of fresh cow's milk at room temperature (19°C to 22°C). The milk contained a dose of acetaminophen (50 mg/kg), and abomasal emptying rate was measured by use of acetaminophen and glucose absorption techniques. Venous blood samples for determination of plasma acetaminophen and glucose concentrations were obtained at ∼30, 0, 15, 30, 45, 60, 90, 120, 130, 180, 210, 240, 300, 360, 420, and 480 minutes (start of suckling was designated as time 0). These time points for obtaining samples were selected in an attempt to provide at least 6 data points before and after the Tmax of acetaminophen to facilitate nonlinear regression analysis for pharmacokinetic modeling. Blood samples were collected into 6-mL tubes containing sodium fluoride and potassium oxalate and centrifuged at 1,000 \( \times \) g for 15 minutes; 3 mL of plasma was harvested and stored at −20°C until analysis.

Plasma was thawed at 19°C to 22°C and analyzed spectrophotometrically by use of a colorimetric nitration assay, as described elsewhere. Actual Cmax and actual Tmax were derived from a plot of the plasma acetaminophen concentration-versus-time data. The first derivative of the Siegel modified power exponential formula was used to model the acetaminophen time curve. The equation was derived from the fact that the acetaminophen concentration-versus-time curve represented as a cumulative dose curve is an inverse analogue of the scintigraphic curve with the following equation: \( C(t) = m \times k \times B \times e^{-k \times t} \times \left( 1 - e^{-k \times t} \right)^{0.5} \), where \( C(t) \) is the acetaminophen concentration in plasma at a specified time point, \( t \) is time, \( m \) is a constant for the total cumulative recovery of acetaminophen when time is
infinite, $k$ is an estimate of the rate constant for abomasal emptying, $\beta$ is a constant that provides an estimate of the duration of the lag phase before an exponential rate of emptying is reached, and $e$ is the natural logarithm. Nonlinear regression was used to estimate values for $m$, $k$, and $\beta$. Values for model $C_{max}$, model $T_{max}$, $k$, $\beta$, and $m$ were obtained by fitting a nonlinear equation to the cumulative dose curve for acetaminophen.

Plasma glucose concentration was determined by use of an automatic analyzer. The actual $C_{max}$ and actual $T_{max}$ of glucose were obtained from a plot of the plasma glucose concentration-versus-time data. Although a delay in actual $T_{max}$ suggests a slow rate of abomasal emptying, actual $T_{max}$ is an insensitive and nonspecific index of abomasal emptying rate because the plasma glucose concentration-versus-time relationship is dependent on the glucose or lactose concentration in the ingested meal, rate of abomasal emptying, small intestinal transit time, surface area available for absorption, rate of glucose entry into cells (which is dependent on the rate and magnitude of insulin release after glucose absorption), and magnitude of glucose loss in the urine when plasma glucose concentration exceeds the renal threshold of 140 to 160 mg/dL. The $AUC_{140}$ was calculated by use of the trapezoidal rule. The $AUC_{140}$ is related to the glucose and lactose content of the ingested meal; thus, it was expected to be similar for all 3 treatments.

**Statistical analysis**—Data were expressed as mean ± SD or geometric mean and range. A value of $P < 0.05$ was considered significant for all statistical analyses. The primary variables of interest were $T_{max}$ for acetaminophen absorption and $T_{max}$ for glucose absorption. A repeated-measures ANOVA was used to determine the main effects of treatment. Variables that did not have normal distributions were logarithmically transformed or ranked before statistical analysis was performed. Bonferroni-adjusted post hoc tests were conducted to compare erythromycin with the control treatment and ivermectin with the control treatment whenever the value for the $F$ test for treatment was significant, which yielded a value for significance of a post hoc test of $P < 0.025$. A statistical software program was used for all comparisons.

**Results**

All calves remained healthy during the study period. Signs of discomfort were detected in calves 2 to 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Erythromycin</th>
<th>Ivermectin</th>
<th>Saline solution</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{max}$ (µg/mL)</td>
<td>34.6 ± 7.5</td>
<td>27.5 ± 13.2</td>
<td>22.4 ± 5.7</td>
<td>0.110</td>
</tr>
<tr>
<td>$T_{max}$ (min)</td>
<td>104 ± 85</td>
<td>150 ± 60</td>
<td>210 ± 66</td>
<td>0.029</td>
</tr>
<tr>
<td>$AUC_{240}$ (mg × min/mL)</td>
<td>8.5 ± 3.2</td>
<td>7.4 ± 3.0</td>
<td>6.2 ± 2.5</td>
<td>0.460</td>
</tr>
<tr>
<td>Model $C_{max}$ (µg/mL)</td>
<td>29.8 ± 7.1</td>
<td>23.2 ± 7.2</td>
<td>19.0 ± 5.6</td>
<td>0.020</td>
</tr>
<tr>
<td>Model $T_{max}$ (min)</td>
<td>102 ± 51</td>
<td>138 ± 50.4</td>
<td>191 ± 52</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$k$ (per min)</td>
<td>0.006 ± 0.0037</td>
<td>0.0063 ± 0.0033</td>
<td>0.0059 ± 0.0039</td>
<td>0.980</td>
</tr>
<tr>
<td>$m$ (µg/mL)</td>
<td>1.89 (1.37–2.29)</td>
<td>2.24 (1.41–3.61)</td>
<td>3.08 (1.26–6.22)</td>
<td>0.140</td>
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| Glucose absorption       |              |            |                |           |
| $C_{max}$ (µg/mL)        | 158 ± 42    | 148 ± 28   | 128 ± 20       | 0.310     |
| $T_{max}$ (min)          | 48 ± 37     | 43 ± 26    | 130 ± 125      | 0.140     |
| $AUC_{240}$ (µg × min/mL)| 45.4 ± 7.7  | 49.4 ± 3.7 | 45.9 ± 6.3     | 0.590     |

Saline solution and erythromycin were randomly assigned as the first 2 treatments; ivermectin was always the last treatment in each calf. A minimum of 36 hours was allowed to elapse between subsequent treatments. Thirty minutes after treatments were administered, calves were allowed to suckle 2 L of cow's milk containing acetaminophen (50 mg/kg). $\beta$ Actual $C_{max}$ and actual $T_{max}$ were derived from a plot of the plasma acetaminophen concentration-versus-time data. $\beta$ Within a row, values differs significantly ($P < 0.05$) from the value for the saline solution treatment. $\beta$ Models $C_{max}$ and model $T_{max}$ were obtained by fitting a nonlinear equation to the cumulative dose curve for acetaminophen. $\beta$ Data are geometric mean (range).

$k$ = Estimate of the rate constant for abomasal emptying. $m$ = Constant that provides an estimate of the duration of the lag phase before an exponential rate of emptying is reached. $m$ = Constant for the total cumulative recovery of acetaminophen when time is infinite.
minutes after IV administration of ivermectin. Rate and depth of respiration typically increased after ivermectin injection, but calves were clinically normal within 15 minutes. Most calves had a few moist coughs for approximately 30 minutes after injection of ivermectin. Hypersalivation and ocular discharge started 3 to 5 minutes after ivermectin injection in some calves and lasted for 1 hour. Intramuscular administration of erythromycin caused signs of restlessness for a few minutes in most calves, but it did not alter respiratory rate and depth or increase salivation or ocular discharge.

Erythromycin significantly increased the rate of abomasal emptying, as assessed by actual Tmax (P = 0.009) and model Tmax (P < 0.001; Figure 1; Table 1). Ivermectin significantly (P = 0.003) increased the rate of abomasal emptying, as assessed by model Tmax.

Model Tmax for the glucose absorption curve was less, but not significantly so, for erythromycin (P = 0.078) and ivermectin (P = 0.094; Figure 2); model Tmax was not significantly different from that of the control treatment. Actual Cmax was similar for all 3 treatments, whereas model Cmax was significantly (P = 0.006) higher for calves when treated with erythromycin, compared with the value when calves were administered the control treatment.

Discussion

We believe that the study reported here is the first in which the effect of ivermectin on the rate of gastric emptying has been investigated. We determined that IV administration of ivermectin caused a small but significant increase in abomasal emptying rate of suckling calves. We also confirmed the findings of 4 other studies (2-6, 8-10) (ie, that parenteral administration of erythromycin increases the rate of abomasal emptying in calves).

Erythromycin exerts its prokinetic effect by acting as a motilin-receptor agonist. The motilin receptor is a class A G-protein–coupled receptor containing 7 transmembrane segments and 3 extracellular loops. An ionic interaction between the protonated dimethylamino group of desosamine in motilin or motilides and the negatively charged glutamic acid at position 119 of the second extracellular segment appears to play an important role in the activation of the motilin receptor by erythromycin. In particular, the size and shape of the electron cloud around the nitrogen atom on desosamine appear to be more important for receptor activation than does the electrostatic effect of attached alkyl groups. Because ivermectin does not possess a dimethylamino group in the fused hexahydrobenzofuran unit spanning C-2 to C-8, ivermectin probably does not contain a suitable charge structure that interacts with the motilin receptor.

The formulation of ivermectin used for the study reported here was a clear sterile solution containing 1% ivermectin, 40% (vol/vol) glycerol formal, and 60% (vol/vol) propylene glycol. The solution was formulated to be administered SC at a dose of 200 μg/kg (1 mL/50 kg). Ivermectin has been administered by the IV route to cattle14-17 and sheep.18 The pharmacokinetics after IV administration of ivermectin in cattle have been described by use of a 2-compartment open model; the volume of distribution (1.9 to 2.4 L/kg) was large as a result of the lipophilic nature of ivermectin, and the clearance was low (0.59 [mL/min]/kg), with an elimination half-time of 1.9 to 2.8 days.14,15,18 The dosage rate of ivermectin administered in the study reported here reflected current label recommendations for cattle, except that ivermectin was administered IV instead of SC to maximize plasma ivermectin concentrations, which were likely to be >1,000 ng/mL during the period when abomasal emptying was assessed.16,17 For comparison, peak plasma ivermectin concentrations after SC administration of ivermectin at a dosage of 200 μg/kg are typically 20 to 30 ng/mL, with effective plasma concentrations of 1 to 2 ng/mL being required for optimal anti-parasitic activity against some endoparasites.11 Assuming a dose-dependent response to IV administration of ivermectin, it is likely that the weak prokinetic effect of ivermectin detected after IV administration does not result after SC injection.

Intravenous administration of an oral formulation of ivermectin containing polysorbate 80, propylene glycol, a preservative, and buffer salts causes transient hemolysis of blood and mild sedation and salivation in sheep.20 Rapid administration (600 μg/kg, IV) of a formulation of ivermectin similar to that used in the study reported here to 4 healthy 8-month-old Jersey bull calves caused signs of depression, ataxia, profuse salivation, tachycardia, difficulty in breathing, miosis, and diarrhea within 15 to 10 minutes after injection, whereas administration of the solvents (glycerol formal and propylene glycol) had no effect.21 Calves treated with ivermectin (600 μg/kg, IV) were clinically normal within 72 hours after administration.20 Clinical signs of ivermectin toxicosis in calves were associated with an increase in serum pseudocholinesterase activity, which suggested that some of the clinical signs (salivation, difficulty in breathing, miosis, and diarrhea) may have been attributable to γ-aminobutyric acid–mediated cholinergic function.21 As such, it is possible that the weak prokinetic effect of ivermectin detected in the calves in the study reported here was attributable to general parasympathetic stimulation and not to activation of motilin receptors. Alternatively, the weak prokinetic ef-
fect after IV administration of ivermectin (and some or all of the adverse clinical effects after IV injection of ivermectin) may have been attributable to augmentation of smooth and skeletal muscle responsiveness to depolarization because ivermectin directly activates ryanodine receptors in skeletal muscles and decreases calcium uptake into the sarcoplasmic reticulum, thereby augmenting contraction in skeletal muscles in response to sarcolemmal depolarization.

Results of the study reported here should not be construed as promoting the extralabel use of ivermectin as a prokinetic agent. As mentioned previously, the prokinetic effect after IV administration of ivermectin is weak and likely to be even weaker when ivermectin is administered via the labeled routes of administration (SC, oral, or topical). Moreover, extralabel use of ivermectin as a prokinetic agent is not appropriate because it may contribute to the development of ivermectin resistance by some intestinal nematodes.

References