Influence of P-glycoprotein modulation on plasma concentrations and pharmacokinetics of orally administered prednisolone in dogs

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Objective—To evaluate the impact of modulation of the membrane-bound efflux pump P-glycoprotein (P-gp) on plasma concentrations of orally administered prednisolone in dogs.

Animals—7 healthy adult Beagles.

Procedures—Each dog received 3 treatments (control [no treatment], rifampicin [100 mg/d, PO, for 21 days, as an inducer of P-gp], and ketoconazole [100 mg/d, PO, for 21 days, as an inhibitor of P-gp]). A single dose of prednisolone (1 mg/kg, PO) was administered on day 8 of each treatment period. There was a 7-day washout period between subsequent treatments. Plasma concentrations of prednisolone were determined by use of a validated liquid chromatography–tandem mass spectrometry method. Duodenum and colon biopsy specimens were obtained endoscopically from anesthetized dogs and assessed for P-gp protein labeling via immunohistochemical analysis and mRNA quantification via real-time PCR assay. Total fecal collection was performed for evaluation of effects of P-gp modulation on digestion of nutrients.

Results—Rifampicin treatment upregulated duodenal P-gp in dogs and significantly reduced the area under the plasma concentration-time curve of prednisolone. Ketoconazole typically downregulated expression of duodenal P-gp, with a subsequent increase in the area under the plasma concentration-time curve of prednisolone. There was a noticeable interindividual difference in response. Digestion of nutrients was not affected.

Conclusions and Clinical Relevance—Modulation of P-gp expression influenced plasma concentrations of prednisolone after oral administration in dogs. Thus, treatment response to prednisolone may be influenced by coadministration of P-gp–modulating medications or feed ingredients. (*Am J Vet Res* 2012;73:900–907)

Oral administration is the most popular, most convenient, and generally safest route for treatment. However, this route is not necessarily the most efficient. Adequate oral bioavailability is essential to achieve therapeutic plasma concentrations for oral administration of drugs.

During oral absorption, drugs can be transported across the intestinal epithelium by the transcellular pathway, paracellular pathway, or a combination of both. The transcellular pathway is limited by the pres-

Abbreviations

AUC	Area under the plasma concentration-versus-time curve
Cl	Total body clearance
Cmax	Maximal plasma concentration
CYP3A	Cytochrome P450 3A
CYP450	Cytochrome P450
GUSB	β-glucuronidase
HPRT	Hypoxanthine-guanine phosophoribosyltransferase
k_a	Absorption rate constant
P-gp	P-glycoprotein
RT	Reverse transcriptase
Tmax	Time to maximal plasma concentration
Vd	Apparent volume of distribution

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ence of ATP-binding cassette drug transporters, such as P-gp.[1]

P-glycoprotein is a membrane-bound efflux pump that is localized in tissues with a barrier function, such as the intestines, blood-brain barrier, bile canaliculi, and renal tubules. It is the product of the ABCB1 (formerly known as MDR1) gene. Because of its strategic location, P-gp can limit uptake of drugs from the intestinal lumen by transporting them from the enterocytes back into the intestinal lumen.[2,3]

The net amount of drug absorbed into the mesenteric blood circulation is the difference between the amount absorbed by the influx process and the sum of the amount extruded by efflux transport together with the amount metabolized in the intestinal epithelial cells by enzymes such as CYP450. In individuals with increased P-gp expression or function, a decrease in oral bioavailability can be expected.[4] P-glycoprotein decreases the bioavailability of orally administered drugs by limiting intestinal absorption.[5–7] There is a tendency for P-gp to have a greater impact on drug uptake than on drug excretion.[4]

In veterinary medicine, little information is available regarding the effect of P-gp modulation on the pharmacokinetics of orally administered drugs.[8] It is known that oral absorption of docetaxel[9] and cyclosporine[10,11] in dogs can be enhanced by coadministration of a P-gp inhibitor. Oral administration of prednisolone is frequently used for chronic enteropathies[12] and as an anticancer treatment[13–15] in companion animals. Coadministration of prednisolone with P-gp–modulating drugs might have consequences for treatment response.

The objective of the study reported here was to evaluate the influence of P-gp modulation on plasma concentrations and pharmacokinetics of prednisolone in dogs. To our knowledge, the study reported here was the first in which investigators evaluated prednisolone as a P-gp substrate in dogs. Moreover, this was the first study in which investigators evaluated the effect of P-gp modulation on oral administration of prednisolone in dogs. Because P-gp is involved in the transport of a broad range of endogenous and exogenous substrates,[16–18] the effect of P-gp modulation on feces composition and digestion was also evaluated.

Materials and Methods

Animals—Four male and 3 female Beagles (age, 4 to 8 years) that weighed 7.5 to 13.0 kg were included in the study. It was determined by use of DNA from serum samples in a competitive allele-specific PCR assay[a] that they were free from the deletion mutation of the MDR1 gene. Results of physical examinations, CBCs, and biochemical analyses of all dogs were within the respective reference ranges. Dogs were dewormed and vaccinated in accordance with standard guidelines. Dogs were housed individually and walked daily. They had ad libitum access to water; food was provided once a day at 8 AM. Food was withheld for 24 hours before endoscopic collection of samples and withheld for 12 hours before collection of samples for pharmacokinetic analysis. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium.

Inhibitor, inducer, and substrate of P-gp—Ketoconazole[b] was used as a P-gp inhibitor, and rifampicin[c] was used as a P-gp inducer. Tablets were placed into gelatin capsules (100 mg of drug/capsule). Prednisolone[d] was used as the P-gp substrate. Prednisolone tablets were placed into capsules and administered at a rate corresponding to a dose of 1 mg/kg.

Experimental design—All dogs received each of 3 treatments (control, inhibitor, and inducer). First, dogs received the control treatment (no premedication before administration of prednisolone). Dogs then received the inhibitor treatment (ketoconazole; 100 mg/d, PO, for 21 days). Finally, dogs received the inducer treatment (rifampicin; 100 mg/d, PO, for 21 days). Each treatment was followed by a 1-week washout period before the start of the subsequent treatment.

The first day of each treatment period was designated as day 1. On day 6 of each treatment period, each dog received an enema, which was followed by gastroduodenoscopy, colonoscopy, and collection of duodenal and colonic tissue specimens. Specimens for histologic and immunohistochemical evaluation were fixed for 24 to 48 hours in neutral-buffered 10% formalin (phosphate buffer). Specimens for mRNA analysis were immediately incubated in an aqueous tissue storage reagent,[e] stored overnight at 4°C, and then stored at –20°C until analysis. In 1 dog, a duodenal biopsy specimen could not be collected endoscopically after rifampicin administration.

On day 8 of each treatment period, a single dose of prednisolone (1 mg/kg) was administered orally, and blood samples were obtained before and 30 minutes and 1, 1.5, 2, 4, 7, 12, and 24 hours after prednisolone administration. Samples were centrifuged, and plasma was harvested and stored at –20°C until analysis for prednisolone quantification. From days 14 to 21, food intake was measured and daily fecal production recorded. Fecal samples were collected and frozen (–20°C) for subsequent analysis. Each morning, fresh (< 30 minutes after defecation) fecal samples were used to determine the fecal pH; samples were diluted with 10% deionized water, and pH was measured with a glass electrode.

Plasma analysis—Plasma prednisolone concentrations were determined via a validated liquid chromatography–tandem mass spectrometry method. Quantification was determined by use of an internal standard, methylprednisolone, which was added to the homogenized plasma samples immediately before analysis. Quality-control and blank samples were analyzed with each batch of samples to assess the extraction and liquid chromatography–tandem mass spectrometry procedure. All quality-control, blank, and calibration curve samples were prepared in pooled drug-free dog plasma.

A 25-µL aliquot of an internal standard working solution (1 µg/mL) was added to 250 µL of each dog plasma sample, which was followed by the addition of 3 mL of diethyl ether. Samples were shaken for 20 minutes and then centrifuged for 10 minutes at 1,258 × *g*. The diethyl ether phase was transferred into a new extraction tube and evaporated to dryness under a gentle stream of nitrogen at 18°C. The dry residue was dissolved in 100 µL of methanol, mixed via a vortexer for

15 seconds, and transferred into an autosampler vial. An aliquot (10 µL) of the extract then was injected onto the liquid chromatography–tandem mass spectrometry instrument.

The high-performance liquid chromatography system consisted of a separation module.[f] Analysis of the extracts was performed on a 100 × 3-mm, 5-µm, C18 column[g] that was used in combination with a guard column of the same type. Gradient elution with 0.1% acetic acid in water and acetonitrile was performed at ratios of 9:1 from 0 to 1 minute, 2:8 from 1 to 9 minutes, and 9:1 from 9 to 20 minutes. A mass spectrometry instrument[h] equipped with an electrospray ionization source was used in the positive ion tandem mass spectrometry mode. The ion transition (m/z, 361.2 > 343.2) was monitored and used for quantification.

Linearity of the method was assessed by analysis of matrix-matched calibration curves for samples fortified in the range of 1 to 1,000 ng/mL. In total, 8 curves were analyzed on different days; correlation coefficients were at least 0.9962, and the goodness-of-fit coefficients were < 9.8%. The limit of detection of the method was 0.67 ng/mL as calculated on the basis of a signal-to-noise ratio of 3:1. The limit of quantification was defined as the lowest concentration in dog plasma for which the method was validated with an accuracy and precision that were within European Union–recommended ranges and was set at 1 ng/mL (accuracy, –6.1%; precision [relative SD], 15.0%). Plasma concentrations below the limit of quantification were not included in the data analysis. The between-day accuracy and precision were assessed by analysis of quality-control samples included in each batch of study samples. The quality-control samples were fortified at 10 ng/mL (n = 30) and 250 ng/mL (30). The accuracy was –4.5% and –6.8% for 10 and 250 ng/mL, respectively, whereas the precision, calculated as relative SD, was 11.7% and 8.3%, respectively.

Pharmacokinetic analysis—The plasma concentration-versus-time data from each dog were subjected to noncompartmental analysis.[i] As an estimation of the absorption, we measured the AUC of prednisolone after oral administration, with the knowledge that this value is the result of absorption, distribution, and elimination processes.

Total AUC was calculated by use of the linear trapezoidal method and the addition of the estimated terminal portion of the curve. Other pharmacokinetic variables evaluated were the elimination rate constant, elimination half-life, Cmax, Tmax, Vd, Cl, k_a, and absorption half-life. The latter 2 variables were calculated via the method of residuals. Values for Cmax and Tmax were determined directly from the data. Values for Vd and Cl were corrected for bioavailability because the bioavailability was not known.

Fecal analyses—Digestibility variables were calculated via the total fecal collection method. Crude protein was calculated from Kjeldahl nitrogen concentrations (nitrogen concentration × 6.25), and fat content was determined by acid hydrolysis followed by ether extraction.[19] Moisture content of the feces was determined as the difference before and after drying at 103°C. Bacterial nitrogen was estimated as soluble nitrogen according to the method of Mason[20] and adapted for diets rich in animal protein[21] by use of SDS to dissolve bacterial material. Bacterial nitrogen was estimated by calculating the difference with and without SDS.

Immunohistochemical analysis of intestinal biopsy specimens—Formalin-fixed tissues were processed, embedded in paraffin, sectioned at a thickness of 4 µm, and stained with H&E in accordance with standard protocols. For detection of P-gp, 2 monoclonal antibodies (C494[j] and C219[k]) were used at dilutions of 1:200 and 1:10, respectively. Each antibody binds to a different internal epitope of the human P-gp molecule.[22,l]

Immunolabeling was achieved with a high-sensitivity horseradish peroxidase mouse diaminobenzidine kit[m] in an autoimmunostainer.[n] This kit also blocked endogenous peroxidase. An antibody diluent[o] with background-reducing components was used to block hydrophobic interactions.

Real-time quantitative RT-PCR assay of intestinal biopsy specimens—Tissue samples were homogenized by use of stainless steel beads in a homogenizer.[p] Next, total RNA was isolated with a kit[q] used in accordance with the manufacturer's instructions. The respective cDNA syntheses were performed by use of 250 ng of total RNA of each sample and with a reverse transcription kit[r] used in accordance with the manufacturer's protocol.

For relative quantification of the canine MDR1 gene, the GUSB gene and HPRT gene were used as endogenous reference genes. Results for the real-time PCR assay were analyzed via the comparative cycle threshold method. The PCR reactions were performed with a real-time PCR system.[s] Two microliters of each cDNA (corresponding to 25 ng of total RNA) were amplified in a volume of 25 µL by use of a universal PCR mix[t] and gene expression assays[u] for each gene.

All samples were assayed in triplicate; nontemplate control samples and non-RT control reactions were included in each assay. A precedent efficiency analysis of

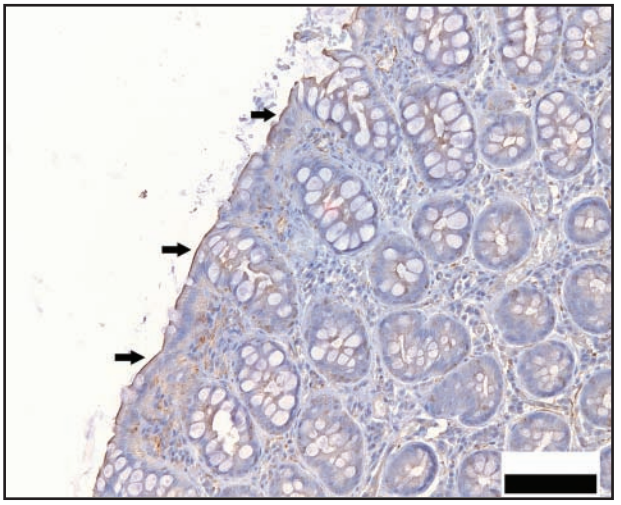

Figure 1—Photomicrograph of a tissue section of colon obtained on day 6 from a Beagle during the control treatment (no medications administered). Notice the localization of P-gp at the brush border of the surface epithelium (arrows). Immunohistochemical stain; bar = 100 µm.

all PCR assays used in the study was performed by application of the same template and dilution steps. The PCR assays for the MDR1, GUSB, and HPRT genes had comparable efficiencies, which ensured an appropriate relative real-time PCR analysis.

Statistical analysis—The AUC data were analyzed by use of a mixed model ANOVA, with treatment as a fixed effect and dog as a random effect ($\alpha = 0.05$). Correctness of the model was confirmed by analyzing the residuals. For all variables, the standardized residuals were normally distributed (confirmed with the Kolmogorov-Smirnov test and Q-Q plot), whereas equality of variances and absence of outliers in the data set were confirmed by means of a scatterplot of the standardized residuals versus the predicted values. To analyze differences between treatments, a post hoc Scheffé test was applied ($\alpha = 0.05$). The Friedman rank test was applied to the results of the RT-PCR assays to compare the relative expression of MDR1 in the duodenum and colon for the control treatment and after premedication with rifampicin or ketoconazole.

Results

Modulation of intestinal P-gp evaluated at the protein level—Immunohistochemical analysis with both antibodies revealed that there was continuous P-gp expression at the brush border of the surface epithelium in the colon for the control treatment in all 7 dogs (**Figure 1**). Expression of P-gp was not immunohistochemically detectable at the apical membrane of duodenal enterocytes (**Figure 2**).

Ketoconazole administration to the 7 dogs resulted in complete loss of immunohistochemically detectable P-gp expression of the surface epithelium in the colon of 2 and 5 dogs for antibodies C494 and C219, respectively (**Figure 3**). Except for that of 1 dog, all biopsy specimens of the duodenum had negative results for P-gp by use of both antibodies.

After rifampicin administration, there was consistent P-gp expression in the colon, similar to the results for the control treatment. However, in 6 dogs, 2 and 5 had strong P-gp expression in the duodenum for antibodies C494 and C219, respectively, at the apical plasma membrane of the villous epithelial cells (**Figure 4**).

Modulation of intestinal P-gp evaluated at the mRNA level—In the duodenum, administration of rifampicin resulted in a significant difference in MDR1 expression relative to the expression of the housekeeping genes GUSB ($P = 0.047$) and HPRT ($P = 0.018$) in 5 of 6 dogs (**Figure 5**). There was a 3- and 4-fold increase in MDR1 expression relative to GUSB and HPRT expression, respectively, after rifampicin premedication.

In the colon, rifampicin administration resulted in a nonsignificant ($P = 0.059$) increase in MDR1 expression relative to expression for the housekeeping gene GUSB. The effect of ketoconazole administration was highly variable, with a decrease of MDR1 expression in the duodenum in 4 of 6 dogs (Figure 5); however, the mean value was not significantly lower, compared with that for the control treatment.

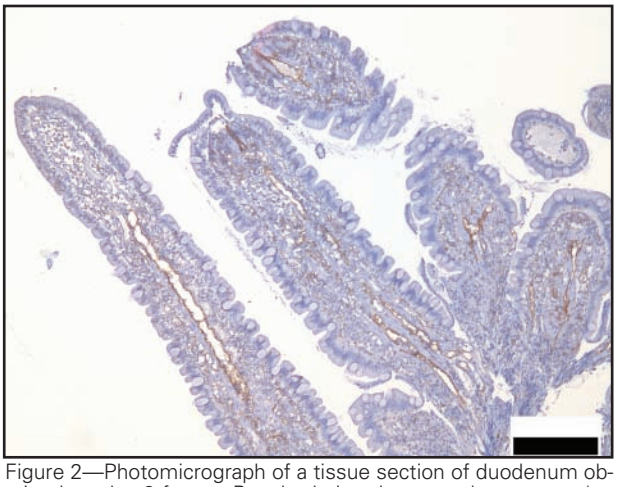

Figure 2—Photomicrograph of a tissue section of duodenum obtained on day 6 from a Beagle during the control treatment (no medications administered). Notice that there is no evidence of P-gp at the apical plasma membrane of the villus tips. Immunohistochemical stain; bar = 200 μm.

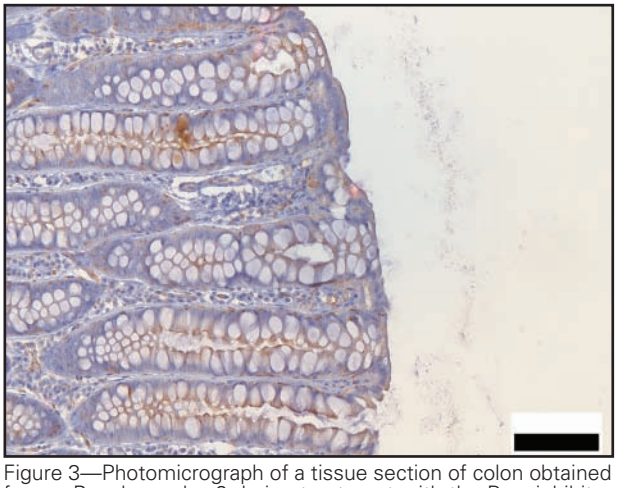

Figure 3—Photomicrograph of a tissue section of colon obtained from a Beagle on day 6 during treatment with the P-gp inhibitor ketoconazole (first day of ketoconazole administration was designated as day 1). Notice the lack of P-gp labeling at the brush border of the surface epithelium. Immunohistochemical stain; bar = 100 μm.

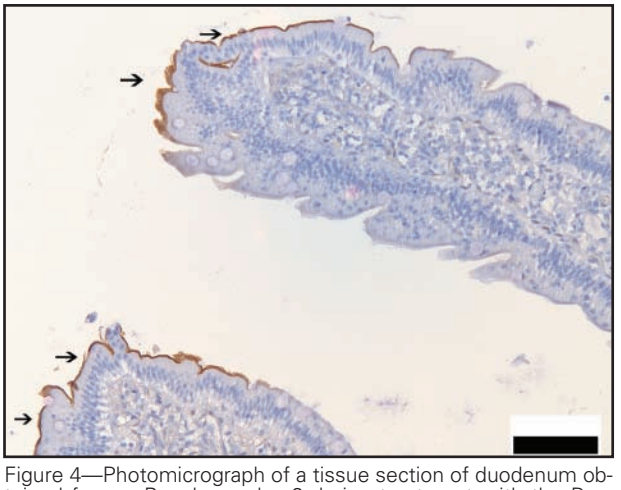

Figure 4—Photomicrograph of a tissue section of duodenum obtained from a Beagle on day 6 during treatment with the P-gp inducer rifampicin (first day of rifampicin administration was designated as day 1). Notice the P-gp expression at the apical plasma membrane at the villus tips (arrows). Immunohistochemical stain; bar = 100 μm.

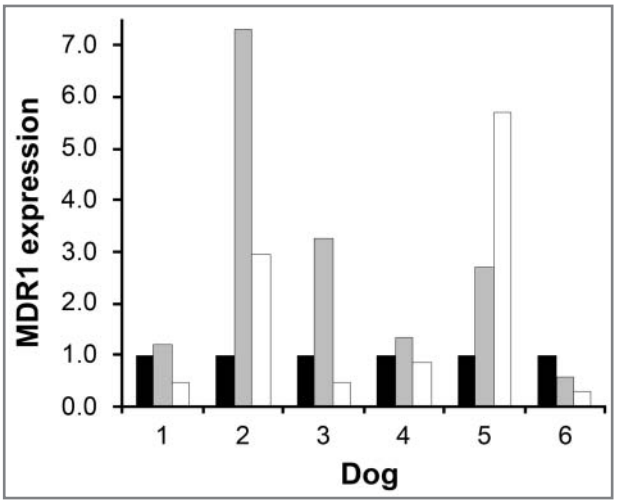

Figure 5—Results of RT-PCR assay for the relative quantification of the MDR1 gene in duodenum tissues obtained from each of 6 Beagles on day 6 of 3 treatment periods. The 3 treatments were control (no medications [black bars]), inhibitor (oral administration of ketoconazole for 21 days [gray bars]), and inducer (oral administration of rifampicin for 21 days [white bars]). Each treatment period was 21 days, with a 7-day washout period between subsequent treatments. Results for the HPRT gene were used as an endogenous reference. For each dog, data for the control treatment are assigned a value of 1 and the data for the ketoconazole and rifampicin treatments are expressed as the fold difference relative to the control treatment.

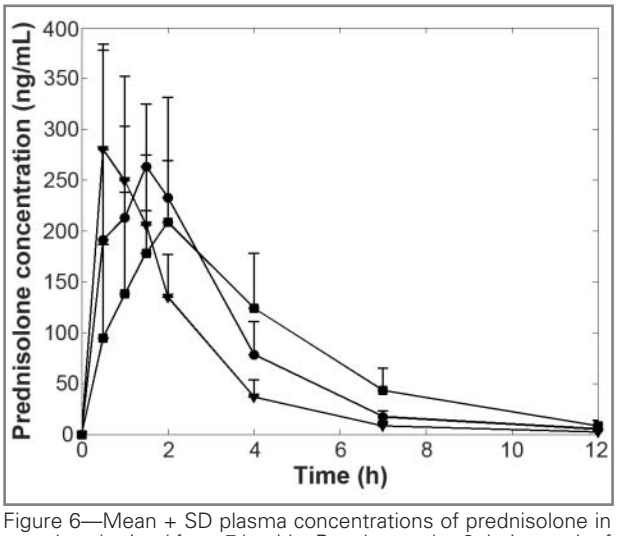

Figure 6—Mean + SD plasma concentrations of prednisolone in samples obtained from 7 healthy Beagles on day 8 during each of 3 treatment periods. The 3 treatments were control (no medications [circles]), inhibitor (ketoconazole, 100 mg/d, PO, for 21 days [squares]), and inducer (rifampicin, 100 mg/d, PO, for 21 days [triangles]). A single dose of prednisolone (1 mg/kg, PO) was administered on day 8 (the first day of each treatment period was designated as day 1). Time of prednisolone administration on day 8 was designated as time 0. There was a 7-day washout period between subsequent treatments.

Influence on plasma prednisolone concentrations and prednisolone pharmacokinetics—Mean plasma concentrations of prednisolone were determined for the 3 treatments (**Figure 6**). The major pharmacokinetic variables were summarized (**Table 1**). In general, the AUCs were significantly ($P = 0.001$) decreased after rifampicin treatment, whereas ketoconazole treatment resulted in a slight, although not significant, increase in AUC. There also was a significantly higher Cl after rifampicin treatment. As a consequence, and because the Vd was not different between the treatment periods, a significantly higher elimination rate constant and shorter elimination half-life were evident for the rifampicin treatment.

Table 1—Mean ± SD results for pharmacokinetic variables in plasma samples obtained from 7 healthy Beagles on day 8 during each of 3 treatment periods.*

Variable	Control	Ketoconazole	Rifampicin
$AUC_{0-\infty}$ (ng•h/mL)	937.09 ± 264.08	1,011.24 ± 237.83	667.83 ± 159.75†
k_{el} (1/h)	0.3135 ± 0.1454	0.3345 ± 0.0850	0.4444 ± 0.1255
$T_{1/2el}$ (h)	2.83 ± 1.67	2.22 ± 0.66	1.67 ± 0.48†
Cmax (ng/mL)	354.00 ± 93.65	230.37 ± 53.39	317.04 ± 61.81
Tmax (h)	1.14 ± 0.56	1.79 ± 1.07	0.86 ± 0.48
Cl/F (L/h•kg)	1.140 ± 0.308	1.038 ± 0.249	1.565 ± 0.332†
Vd/F (L/kg)	4.14 ± 1.41	3.18 ± 0.59	3.69 ± 1.11
k_a (1/h)	2.32 ± 1.85	2.12 ± 1.32	1.01 ± 0.57
Absorption half-life (h)	0.60 ± 0.53	0.47 ± 0.32	0.82 ± 0.46

*The 3 treatments were control (no medications), inhibitor (ketoconazole, 100 mg/d, PO, for 21 days), and inducer (rifampicin, 100 mg/d, PO, for 21 days); there was a 7-day washout period between subsequent treatments. A single dose of prednisolone (1 mg/kg, PO) was administered on day 8 of each treatment (the first day of each treatment period was designated as day 1); time of prednisolone administration on day 8 was designated as time 0. †Value differs significantly ($P < 0.05$) from the value for the control treatment.

$AUC_{0-\infty}$ = Area under the plasma concentration-versus-time curve from 0 to infinity. Cl/F = Total body clearance corrected for bioavailability. k_{el} = Elimination rate constant. $T_{1/2el}$ = Elimination half-life. Vd/F = Apparent volume of distribution corrected for bioavailability.

Fecal analysis—Values for fecal dry-matter content, fecal pH, and apparent digestibility coefficients of crude protein, crude fat, and nitrogen-free extract were not significantly different when the P-gp inhibitor ketoconazole or the P-gp inducer rifampicin were administered, compared with values for the control treatment. Protein intake and the ratio of the fecal bacterial protein to total fecal protein content also were not significantly different among treatments.

Discussion

To our knowledge, the study reported here was the first in which investigators evaluated the effect of P-gp modulation on plasma prednisolone concentrations and prednisolone pharmacokinetics in domestic animals. Prednisolone was chosen as a substrate because of its frequent use in the treatment of chronic enteropathies and neoplasia in dogs. Furthermore, prednisolone is known to be a P-gp substrate in humans.[23,24]

Rifampicin was chosen as a P-gp inducer because in humans, pretreatment with rifampicin results in higher intestinal expression of P-gp and a decrease in the plasma AUC of digoxin[25,26] and ezetimibe.[27] The present study provided evidence of a similar effect in dogs. Because no information on the dose in dogs was available in the literature, the dose used in the present study was adapted from studies in humans. Similar to results in humans, dogs of the present study had increased P-gp expression in enterocytes associated with a reduced AUC of prednisolone. Rifampicin induced an

increase in intestinal expression of P-gp as determined by use of immunohistochemical staining (Figure 4) and as measured in 5 of 6 dogs by use of RT-PCR assays (Figure 5). To our knowledge, the present study is the first in which oral administration of rifampicin has been used for modulation of P-gp expression in dogs.

Ketoconazole is used frequently as an antifungal in human and veterinary medicine and is a P-gp inhibitor in humans and dogs.[28–31] As a P-gp inhibitor, it is often used in combination with other drugs to increase their bioavailability. In humans, coadministration of ketoconazole increases bioavailability of several drugs (eg, mefloquine, quetiapine, everolimus, and cyclosporine).[32–35] In dogs, ketoconazole is used in combination with cyclosporine,[11,36,37] ivermectin,[30] and quinidine.[38] Similar to results for humans,[33] adverse effects of ketoconazole have been described for dogs.[39] In a retrospective study[39] of 632 dogs treated with ketoconazole because of skin disorders, the frequency of adverse effects for ketoconazole treatment was approximately 15%. Adverse effects were more likely to occur when cyclosporine or ivermectin was administered concurrently with ketoconazole. Both inhibition of P-gp and CYP3A by ketoconazole may play a role in the higher prevalence of adverse effects.[39] Therefore, concomitant administration of ketoconazole with a P-gp or CYP450 substrate should be avoided or used with caution.

In the present study, there was an inhibitory effect of ketoconazole on MDR1 expression in enterocytes in 4 of 6 dogs (Figure 5). Because of the low sensitivity of the technique, P-gp expression was not detected at the protein level via immunohistochemical analysis. Therefore, immunohistochemical analysis was not suitable for use in investigating a decrease in P-gp expression in the duodenum. However, RT-PCR assays are extremely sensitive. Use of RT-PCR assays enabled us to detect a decrease in MDR1 expression in the duodenum. This inhibitory effect of ketoconazole may be biologically important. Nevertheless, there was only a limited effect of ketoconazole on pharmacokinetics of orally administered prednisolone, with a slight increase in AUC, which is similar to results in humans.[26]

The reason for the induction of P-gp expression after ketoconazole treatment in duodenal specimens of 2 dogs (results of both immunohistochemical analysis and PCR assay in one dog and results of PCR assay alone in the other dog) is unclear. In humans, ketoconazole can activate or inhibit P-gp activity, depending on the concentration at the target organ.[40] Therefore, it is possible that the 2 dogs responded differently because of a difference in the disposition of ketoconazole. To our knowledge, this phenomenon has not yet been described in domestic animals.

As an efflux pump, P-gp limits uptake of drugs from the intestinal lumen by transporting the drugs from the enterocytes back into the intestinal lumen.[2,3] Moreover, P-gp can affect intestinal drug metabolism by controlling access of the drug to the intracellular metabolizing CYP3A.[41] Through the repetitive process of extrusion and reabsorption, P-gp prolongs the intracellular residence time of drug molecules and increases the probability of exposure to drug-metabolizing enzymes.[42] Consequently, P-gp may enhance intestinal metabolism of drugs. Therefore, modulation of P-gp influences the oral bioavailability of drugs that are a substrate of both P-gp and CYP3A.

Cytochrome P450 3A is considered to be the major phase-1 drug metabolizing enzyme family in mammals.[43,44] There is high expression of CYP3A and P-gp in the villus tip of enterocytes in the gastrointestinal tract, which is the primary site of absorption for orally administered drugs, and CYP3A and P-gp appear to work together to prevent oral absorption of many drugs because substrates of P-gp are often also substrates for CYP3A.

Rifampicin and ketoconazole are also modulators of CYP3A in dogs.[30,45] Given that prednisolone is a substrate of both P-gp and CYP3A in humans,[23,24,46] the effect of P-gp modulation in the present study may be reinforced by the CYP3A activity. In a recent study[47] in humans, the inhibitory effect of ketoconazole on efflux transporters was detected at a concentration one-tenth that of the concentration that caused an inhibitory effect on CYP3A activity. Therefore, it has been suggested that at low concentrations, ketoconazole may increase drug absorption by inhibiting efflux pump–mediated excretion from cells back into the intestinal lumen, whereas at higher concentrations, ketoconazole may increase drug bioavailability by inhibiting the indirect effect on CYP3A-mediated drug metabolism.[47] The dose of ketoconazole used in the present study was adapted from another study[30] on ivermectin in dogs; investigators in that study[30] found that this low dose of ketoconazole does not interfere with the production of ivermectin metabolites, which meant that ketoconazole essentially has an effect on the P-gp efflux pump. Further studies are warranted to examine the dose-dependent effect of ketoconazole on P-gp expression in enterocytes.

It is worthy of mention that the Cl of prednisolone was significantly higher for the rifampicin treatment in the present study. Consequently, and because the Vd was not affected, the elimination rate constant and elimination half-life of prednisolone were significantly increased and decreased, respectively, after rifampicin treatment. This enhanced elimination, with a decrease in AUC, may have been the result of CYP3A induction in the kidneys and liver, given that k_a was not directly affected. Additional studies, including the measurement of possible prednisolone metabolites, would help to clarify this. Also, P-gp function in the liver may be influenced, considering that prednisolone metabolites might also be P-gp substrates. The net effect on prednisolone absorption and elimination probably is the result of inhibition or induction of both CYP3A and P-gp. The extent to which each of them contributes to the net effect is unknown.

As determined via immunohistochemical analysis or PCR assays, oral administration of ketoconazole and rifampicin were able to modulate P-gp expression in the intestines of dogs. Possible modulation of P-gp and CYP450 at nonintestinal sites such as the liver was not investigated. Nevertheless, P-gp has a slightly greater impact on drug uptake than on drug excretion.[4]

Changes in function or expression of ATP-binding cassette transporters that originate from genetic variation, physiologic and pathological conditions, or exog-

enous factors determine individual variability in drug disposition and kinetics and subsequently the individual pharmacological response.[48,49] This means that to design individualized treatment that is more efficacious, knowledge of the individual response to P-gp modulation may be required.

Because transporters such as P-gp are involved in the active transport of phospholipids, peptides, corticosteroids, polysaccharides, amino acids, nucleotides, organic anions, drugs, and toxicants and their conjugates,[16–18] and because many P-gp substrates have not yet been identified, the effect of P-gp modulation on feces composition was also evaluated. Fecal analysis revealed no significant effects of P-gp modulation on fecal pH, bacterial protein, and digestibility of a commercial feed. To our knowledge, this is the first report of an investigation of the effect of P-gp modulation on feed digestibility.

Results of the present study supported the concept of modulation of P-gp expression as a tool to alter pharmacokinetics of orally administered drugs in dogs. This principle may find numerous applications, especially in the field of anticancer treatment, and avoid the need for prolonged hospitalization and the inconveniences of multiple parenteral treatments. Further studies are needed to identify more efficacious inhibitors and inducers of P-gp in dogs.

a. KASPar, Van Haeringen Laboratorium, Wageningen, The Netherlands.
b. Nizoral, 200-mg tablets, Janssen-Cilag, Berchem, Belgium.
c. Rifadin, 150-mg tablets, Sanofi-Aventis, Diegem, Belgium.
d. 5-mg tablets, Kela, Hoogstraten, Belgium.
e. RNAlater solution, Ambion, Lennik, Belgium.
f. Alliance 2695, Waters Corp, Milford, Mass.
g. Nucleosil, Varian, Palo Alto, Calif.
h. Quattro Ultima, Waters Corp, Milford, Mass.
i. WinNonlin, version 5.0.1, Pharsight Corp, Mountain View, Calif.
j. Alexis Biochemicals, Axxora, Zandhoven, Belgium.
k. SA Eurogentec, Ougreé Seraing, Belgium.
l. Georges E, Bradley G, Gariepy J, et al. Mapping of P-glycoprotein monoclonal antibody epitopes: application for immunohistological staining (abstr), in *Proceedings*. 30th Annu Meet Am Assoc Cancer Res 1989;510.
m. Envision DAB+ kit, Dako, Glostrup, Denmark.
n. Cytomation, S/N S38-7410-01, Dako, Glostrup, Denmark.
o. Dako, Glostrup, Denmark.
p. TissueLyser II, Qiagen, Hilden, Germany.
q. RNeasy Plus micro kit, Qiagen Inc, Valencia, Calif.
r. QuantiTect reverse transcription kit, Qiagen Inc, Valencia, Calif.
s. Mastercycler ep Realplex real-time PCR system, Eppendorf AG, Hamburg, Germany.
t. PCR Mastermix, Applied Biosystems, Darmstadt, Germany.
u. TaqMan gene expression assay, Applied Biosystems, Darmstadt, Germany.

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