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AUTHORS: Charlotte A. Johnston DVM; Valerie S. MacDonald Dickinson DVM; Jane Alcorn DVM, PhD; M. Casey Gaunt DVM

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# Pharmacokinetics and oral bioavailability of metformin hydrochloride in healthy mixed-breed dogs

**Charlotte A. Johnston** DVM

**Valerie S. MacDonald Dickinson** DVM

**Jane Alcorn** DVM, PhD

**M. Casey Gaunt** DVM

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From the Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine (Johnston, MacDonald Dickinson, Gaunt), and the College of Pharmacy and Nutrition (Alcorn), University of Saskatchewan, Saskatoon, SK S7N 2Z4, Canada.

Address correspondence to Dr. Johnston (caj311@mail.usask.ca).

**OBJECTIVE**
To investigate the pharmacokinetics of metformin hydrochloride in healthy dogs after IV and oral bolus administrations and determine the oral dose of metformin that yields serum concentrations equivalent to those thought to be effective in humans.

**ANIMALS**
7 healthy adult mixed-breed dogs.

**PROCEDURES**
Each dog was given a single dose of metformin IV (mean ± SD dose, 24.77 ± 0.60 mg/kg) or PO (mean dose, 19.14 ± 2.78 mg/kg) with a 1-week washout period between treatments. For each treatment, blood samples were collected before and at intervals up to 72 hours after metformin administration. Seventy-two hours after the crossover study, each dog was administered metformin (mean dose, 13.57 ± 0.55 mg/kg), PO, twice daily for 7 days. Blood samples were taken before treatment initiation on day 0 and immediately before the morning drug administration on days 2, 4, 6, and 7. Serum metformin concentrations were determined by means of a validated flow injection analysis–tandem mass spectrometry method.

**RESULTS**
After IV or oral administration to the 7 dogs, there was high interindividual variability in mean serum metformin concentrations over time. Mean ± SD half-life of metformin following IV administration was 20.4 ± 4.1 hours. The mean time to maximum serum concentration was 2.5 ± 0.4 hours. Mean systemic clearance and volume of distribution were 24.1 ± 7.8 mL/min/kg and 44.8 ± 23.5 L/kg, respectively. The mean oral bioavailability was 31%.

**CONCLUSIONS AND CLINICAL RELEVANCE**
The study data indicated that the general disposition pattern and bioavailability of metformin in dogs are similar to those reported for cats and humans. (*Am J Vet Res* 2017;78:1193–1199)

Metformin (1,1-dimethylbiguanide) is a biguanide primarily used for the management of non–insulin-dependent diabetes mellitus in humans and is the recommended first-line treatment following diagnosis.[1] Metformin does not appear to affect pancreatic beta cell production of insulin, but to be effective, it requires the presence of circulating insulin.[2,3] Metformin reduces blood glucose concentration by enhancing muscle cell sensitivity to insulin and therefore increases muscle glucose metabolism.[3–7] Metformin also decreases hepatic gluconeogenesis and glycogenolysis. The overall consequence of these actions is to lower blood glucose concentrations without causing hypoglycemia.

**ABBREVIATIONS**

| | |
|---|---|
| AMPK | Adenosine monophosphate–activated protein kinase |
| AUC | Area under the serum concentration–time curve |
| FIA-MS/MS | Flow injection analysis–tandem mass spectrometry |
| GFR | Glomerular filtration rate |
| MDR | Multidrug resistance |
| OCT | Organic cation transporter |
| $T_{max}$ | Time to maximum serum concentration |
| $V_{d\text{-}area}$ | Volume of distribution |

There is clinical evidence that humans receiving metformin for treatment of non–insulin-dependent diabetes mellitus have a reduced lifetime incidence of cancer.[8,9] This finding has led to substantial research to better understand metformin's activity, including attempts to define the precise molecular target of metformin. Metformin's mechanism of action is not understood but appears to target the enzyme AMPK. Increased activity of AMPK enhances glucose uptake from the blood into muscles.[8,10] The upstream regulator of AMPK is a protein kinase known as liver kinase B1, which is a tumor suppressor. Furthermore, results of in vitro investigations[11] suggest that metformin may inhibit the proliferation of MDR cell lines and decrease the expression of the MDR marker, MDR1 (p-glycoprotein), leading to reversal of chemotherapy resistance. This may be related to decreased expression of the drug efflux transporters (eg, MDR1), given that these efflux transporters may be responsible for

reduced effectiveness of chemotherapy agents such as doxorubicin.[11] A further possible explanation of the mechanism is related to the impact of metformin on chronic inflammation. Adenosine monophosphate–activated protein kinase may have the capacity to reduce inflammation, which is an important component of the development and progression of cancer.[10]

A dearth of information exists regarding the ideal therapeutic concentration of metformin in humans. Kajbaf et al[12] performed a systematic review of the human medical literature and found reported therapeutic values ranged widely from 0.129 to 90 mg/L (with boundary values of 0 to 1,800 mg/L). Detailed assessment of the available data suggests that a serum metformin concentration of 2.5 mg/L is the likely upper limit of the therapeutic range, whereas a lower limit could not be determined.[12]

To the authors' knowledge, complete reports of metformin pharmacokinetic or pharmacodynamic data from which to determine an appropriate dose regimen in dogs are lacking. A recent publication[13] reported the development and validation of a high-performance liquid chromatography–tandem spectrometry method for the simultaneous determination of plasma concentrations of metformin and pioglitazone (another orally administered anti-diabetic agent) in 6 Beagles.

The use of metformin in other species has provided basic knowledge of the drug's pharmacokinetics. Metformin is excreted unchanged in urine in humans and cats.[1,4,5,14] As such, normal kidney function is imperative for the excretion of this drug and to prevent its accumulation. In humans, lactic acidosis is a rare life-threatening condition that has been linked to high circulating metformin concentrations and is often associated with renal dysfunction.[4–6] In dogs, the adverse effects of metformin, of which vomiting is most common, have been documented only after accidental oral ingestion of large amounts of the drug; even at high doses, these adverse effects are minimal.[14]

Given the potential benefits of metformin indicated by results of recent human studies,[2,8,9,15] determination of the pharmacokinetics of metformin in dogs will allow for further investigation into its potential use in cancer prevention and treatment for both companion animals and humans. The purpose of the study reported here was to investigate the pharmacokinetics of metformin in healthy dogs after IV and oral bolus administrations and determine the oral dose of metformin that yields serum concentrations equivalent to those thought to be effective in humans.

# Materials and Methods

## Drug treatments

Metformin hydrochloride[a] (500 mg) was dissolved in 10 mL of saline (0.9% NaCl) solution and passed through a filter (pore diameter, 0.2 µm) for IV administration. For oral administration, metformin hydrochloride tablets[b] (500 mg each) were used.

## Dogs

Seven healthy adult mixed-breed dogs (2 sexually intact males, 2 neutered males, 1 sexually intact female, and 2 spayed females) between 2 and 3 years old and weighing between 25.7 and 29.2 kg (mean ± SD weight, 27.96 ± 1.21 kg) were used. Physical examinations and preliminary diagnostic tests including a CBC, serum biochemical analysis, urinalysis, and blood lactate concentration assessment were performed before initiation and at termination of the study. All dogs were considered healthy at the time of the study. The dogs had been well adapted to their environment and were accustomed to being handled. They were housed at the Western College of Veterinary Medicine Animal Care Unit. The study had 2 phases, was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

## Experimental procedures

Phase 1 involved IV and oral administrations of a single dose of metformin in a crossover design study, with 2 experimental periods and an intervening 1-week washout period. All dogs received each treatment, though not in the same order. One day prior to each experimental period, each dog was sedated with butorphanol tartrate (0.4 mg/kg) and dexmedetomidine (2.5 µg/kg) administered IV, and an indwelling jugular sampling catheter[c] was placed aseptically with a modified Seldinger technique. To maintain catheter patency, 2.0 mL of sterile heparin solution (1,000 U/mL) was injected into the catheter every 6 hours. The dogs were housed in the Western College of Veterinary Medicine Animal Care Unit during data collection and received 30 minutes of exercise once daily throughout the experimental and washout periods and the interval between phases 1 and 2.

Food was withheld from each dog for 16 hours before and 6 hours after the IV or oral dose administration. For the IV treatment, each dog was administered 23.4 to 25 mg of metformin/kg (mean ± SD dose, 24.77 ± 0.60 mg) IV over a period of 5 minutes. For the oral treatment, each dog was administered 17.18 to 25.68 mg of metformin/kg (mean dose, 19.14 ± 2.78 mg). Serial blood samples for analysis were obtained by removal of 2 mL of blood from the catheter, after initial removal of 2 mL to clear the catheter of heparin. After sample collection, 2 mL of sterile saline solution was reinstilled to replace blood volume and heparin within the catheter. Following a 1-week washout period, dogs received treatment by the other route of administration. Blood samples (2 mL each) were collected into tubes containing no anticoagulant before (0 hours) and at 0.33, 0.57, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18, 24, 30, 36, 48, and 72 hours after metformin administration for drug

analysis. Portions of the blood samples were used for immediate analysis of blood lactate and glucose concentrations (by use of a bench top lactometer and glucometer, respectively) at the 0-, 6-, 12-, 24-, 48-, and 72-hour time points. Blood samples were then allowed to clot for 45 minutes before centrifugation[d] for 10 minutes. The serum was transferred to 1.5-mL plastic microcentrifuge tubes and stored at −80°C until analysis of metformin concentrations by an FIA-MS/MS method.[16] The indwelling jugular sampling catheter was removed from each dog at the end of each experimental period. During phase 1, the dogs were constantly monitored for the first 3 hours following metformin administration, then hourly until 24 hours, every 6 hours up to 48 hours, and 3 times a day until metformin treatment via the other route of administration or initiation of phase 2. The general demeanor of the dog was evaluated along with signs of nausea, evidence of vomiting or diarrhea, appetite, energy levels, urination habits, and any other behavior deemed to be unusual for the dog. The dog's rectal temperature, heart rate, respiratory rate, and body weight were also assessed on a daily basis.

There was an interval of 72 hours between phases 1 and 2. Phase 2 consisted of twice-daily oral administration of metformin to each dog for 7 days. Each dog was administered 12.89 to 14.24 mg of metformin/kg (mean ± SD dose, 13.57 ± 0.55 mg) orally twice each day. The first dose was administered on day 0, and the last dose was administered on day 6. Blood samples (2 mL each) were collected for analysis of serum drug and blood lactate concentrations before treatment initiation (baseline) and just before the morning drug administration on days 2, 4, and 6; a final blood sample was collected for analysis at the time of the morning drug administration on day 7, but dogs were not treated that day. Portions of the blood samples were used for immediate analysis of blood lactate concentration (by use of a benchtop lactometer). Blood samples were then allowed to clot for 45 minutes before centrifugation for 10 minutes. The serum was transferred to 1.5-mL plastic microcentrifuge tubes and stored at −80°C until analysis of metformin concentrations by an FIA-MS/MS method. During phase 2, the dogs were monitored 3 times a day up to and including day 7. The general demeanor of the dog was evaluated along with signs of nausea, evidence of vomiting or diarrhea, appetite, energy level, urination habits, and any other behavior deemed to be unusual for the dog. The dog's rectal temperature, heart rate, respiratory rate, and body weight were also assessed on a daily basis.

## Assessment of serum metformin concentrations

For each sample from either of the experimental phases, serum metformin concentration was determined by a previously validated FIA-MS/MS method.[16] Briefly, serum samples were thawed to room temperature (approx 21°C) and gently mixed. A 170-µL volume of precipitation solution (acetonitrile containing 0.1% formic acid and 20 ng of metformin-d6 hydrochloride/mL) was added to 50 µL of each serum sample. The resulting mixture was vortexed for 30 seconds and then centrifuged[e] at 4°C for 10 minutes. The supernatant was then transferred to high-performance liquid chromatography vials, and FIA-MS/MS was performed.[16] A 2-µL sample volume was used with a run time of 2 minutes. For 2 quality control concentrations, recovery was found to be 97.3% for 12-ng/mL samples and 94.5% for 117-ng/mL samples. The standard curve was prepared from serial dilution of the stock solution to 7 standard working stock solutions and subsequent addition of 10 µL of the working solution to 40 µL of pooled blank dog serum. A 170-µL volume of precipitation solution was added to each standard sample and processed for FIA-MS/MS as described. The standard curve was constructed from peak-area ratios of metformin to internal standard plotted against the known metformin standard concentration. A linear regression analysis with 1/x weighting gave an $R^2 > 0.9966$ in the range of 5 to 2,340 ng/mL. The intra- and interday accuracy and precision of 3 metformin concentrations at the low end, middle, and high end of the standard curve range (n = 6 assessments/concentration on each of 3 occasions) were within 15% deviation from the known concentrations. Quality control samples representing concentrations on the low end, middle, and high end of the standard curve range were analyzed in duplicate and were used as acceptance criteria for a single analysis run. At a minimum, 4 of 6 quality control samples (allowing for 1 quality control sample within 2 of the 3 quality control concentrations to fail) had to be within 15% of the known concentration; otherwise, the analysis run was rejected.

## Pharmacokinetic analysis

For each dog, pharmacokinetic parameters were estimated from serum metformin concentrations over time (determined in phase 1) by noncompartmental methods,[f] and final pharmacokinetic parameter estimates were expressed as mean ± SD. For noncompartmental analysis, the values of AUC from time 0 extrapolated to infinity were calculated by the linear trapezoidal rule extrapolation method to obtain estimates of systemic clearance and $V_{d\text{-}area}$. The log-linear terminal rate constant, k, was estimated as the terminal slope of the natural logarithmic mean serum metformin concentration-versus-time profile following linear regression analysis; the half-life was estimated as the ratio 0.693/k. The maximum serum concentration and $T_{max}$ were determined from visual inspection of the serum metformin concentration following oral administration-versus-time profiles. Bioavailability (F) was calculated with an equation as follows:

$$F = \frac{AUC_{oral}}{Dose_{oral}} \times \frac{Dose_{iv}}{AUC_{iv}}$$

where $AUC_{oral}$ is the estimated AUC from time 0 ex-

trapolated to infinity following oral bolus administration of metformin, $AUC_{iv}$ is the estimated AUC from time 0 extrapolated to infinity following IV bolus administration of metformin, and $Dose_{iv}$ and $Dose_{oral}$ are the metformin doses administered by the IV and oral routes, respectively.

## Results

All data are reported as mean ± SD. The mean serum metformin concentrations over time after IV and oral administration revealed extensive interindividual variation among the 7 mixed-breed dogs **(Figure 1)**. The terminal linear phase rate constants for the natural logarithmic mean serum metformin concentration-versus-time profiles were similar following IV (0.035 ± 0.007 $hours^{-1}$) and oral (0.057 ± 0.015 $hours^{-1}$) bolus administrations. The IV administration data suggested a possible prolonged redistribution of metformin from peripheral tissue compartments, consistent with the long half-life (20.4 ± 4.1 hours) and large $V_{d\text{-}area}$ (44.8 ± 23.5 L/kg; **Table 1**). The low systemic clearance (24.1 ± 7.8 mL/min/kg) and high $V_{d\text{-}area}$ were also consistent with the long half-life. Following oral administration, metformin had a moderately rapid absorption rate ($T_{max}$, 2.5 ± 0.4 hours), but the extent of absorption was rather low (oral bioavailability, approx 0.31). The mean serum concentration of metformin measured just prior to a dose administration seemed to plateau by 144 hours **(Figure 2)**, consistent with the drug's prolonged half-life.

Two of the 7 dogs developed mild gastrointestinal signs in 1 or both phases of the study. Both dogs had a slight decrease in appetite following administration of the IV bolus in phase 1, and 1 dog had a single episode of vomiting and diarrhea on day 2 of phase 2. No adverse effects were observed in any dog following administration of the single oral bolus. Among the dogs, no episodes of hypoglycemia were identified during phase 1, and no evidence of lactic acidosis was documented during either phase.

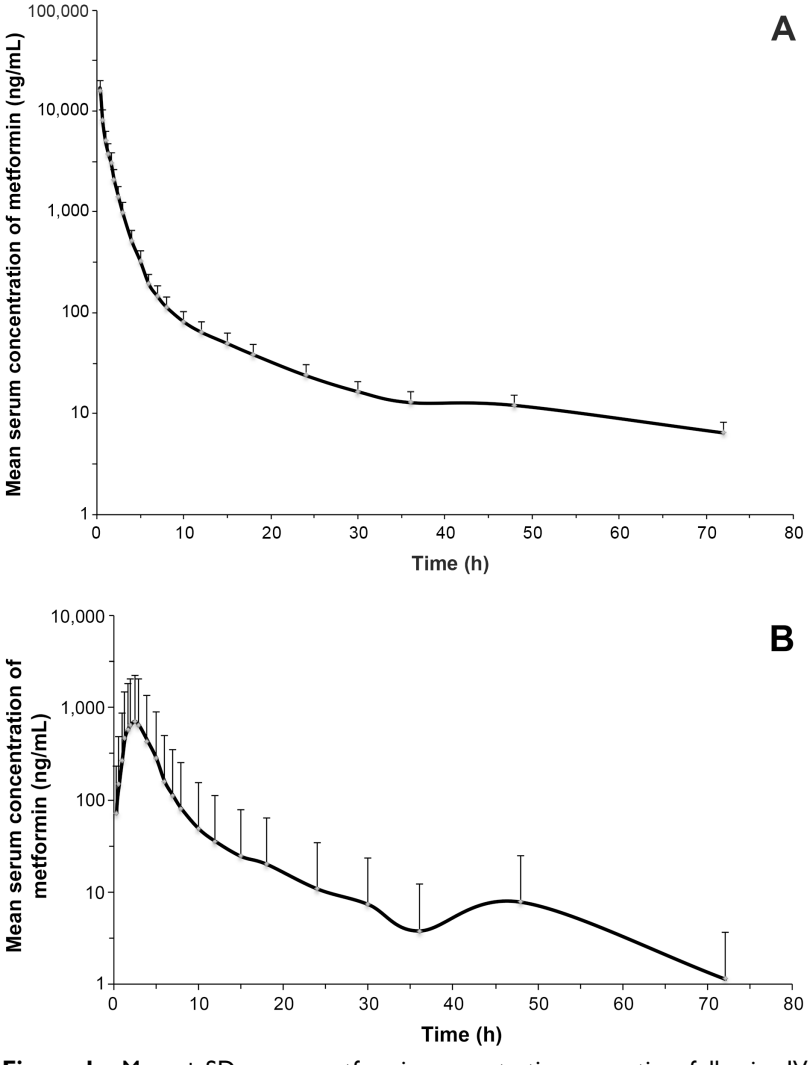


**Figure 1**—Mean + SD serum metformin concentrations over time following IV (A) and oral (B) administration of a bolus dose of metformin hydrochloride to 7 mixed-breed dogs (phase 1) in a crossover experiment (interval of 1 week between treatments). Food was withheld from each dog for 16 hours before and 6 hours after either dose administration. The mean dose of metformin administered IV was 24.77 ± 0.60 mg/kg; the mean dose of metformin administered orally was 19.14 ± 2.78 mg/kg. For each treatment, blood samples were collected into tubes containing no anticoagulant before (0 hours) and at 0.33, 0.57, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18, 24, 30, 36, 48, and 72 hours after metformin administration for drug analysis by FIA-MS/MS.

## Discussion

Metformin is an antihyperglycemic agent used frequently in the management of non–insulin-dependent diabetes mellitus in humans.[1] More recently, it has been evaluated for potential antineoplastic activity on the basis of clinical evidence that patients receiving metformin for treatment of non–insulin-dependent diabetes mellitus have a reduced lifetime incidence of cancer.[8,9] This has led to the questions of whether this finding could be applicable to companion animals and whether data from companion animals treated with metformin could be used to further understand use of this drug in humans. Because information regarding the pharmacokinetics and oral bioavailability of metformin in healthy dogs is lacking, the purpose of the present study was to determine those parameters. The total daily oral dose of metformin currently used in anticancer clinical trials in humans ranges from 1,500 to 2,000 mg.

The pharmacokinetics of metformin after oral administration in dogs in the present study was similar to the pharmacokinetics reported for cats and humans. Peak serum concentration of the drug after oral administra-

**Table 1**—Mean ± SD pharmacokinetic parameter estimates (determined by noncompartmental methods) for metformin following IV and oral administration of a bolus dose of metformin hydrochloride to 7 mixed-breed dogs in a crossover experiment (interval of 1 week between treatments).

| | Route of administration | |
|---|---|---|
| **Parameter** | **IV** | **Oral** |
| $C_{max}$ (ng/mL) | — | 835.8 ± 308.3 |
| $T_{max}$ (h) | — | 2.5 ± 0.4 |
| $Cl_S$ (mL/min/kg) | 24.1 ± 7.8 | — |
| $V_{d\text{-}area}$ (L/kg) | 44.8 ± 23.5 | — |
| F (%) | — | 31 ± 12 |
| $T_{1/2}$ (h) | 20.4 ± 4.1 | 12.6 ± 2.9 |
| K ($h^{-1}$) | 0.035 ± 0.007 | 0.057 ± 0.015 |
| AUC (ng•h/mL) | 18,567 ± 4,892 | 4,687 ± 1,170 |
| $C_0$ (ng/mL) | 13,499 ± 5,401 | — |
| $V_{d\text{-}area}$/F | — | 0.076 ± 0.018 |

Weights of the dogs ranged from 25.7 to 29.2 kg. Food was withheld from each dog for 16 hours before and 6 hours after either dose administration. The mean ± SD dose of metformin administered IV was 24.77 ± 0.60 mg/kg; the mean dose of metformin administered orally was 19.14 ± 2.78 mg/kg. After each treatment, blood samples were collected into tubes containing no anticoagulant before (0 hours) and at 0.33, 0.57, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18, 24, 30, 36, 48, and 72 hours after metformin administration for analysis of serum drug concentrations by FIA-MS/MS.

— = Not applicable. $C_0$ = Serum concentration at 0 hours. $Cl_S$ = Systemic clearance. $C_{max}$ = Maximum serum concentration. F = Oral bioavailability. K = Terminal natural log-linear phase rate constant. $T_{1/2}$ = Half-life.

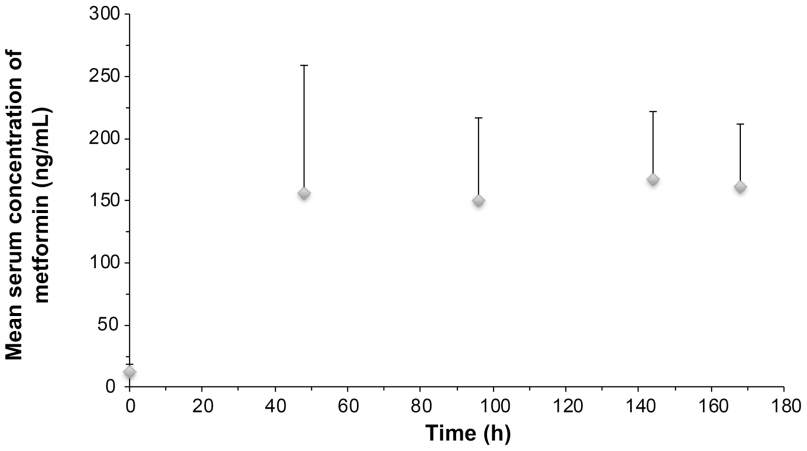


**Figure 2**—Mean + SD serum metformin concentrations over time following twice-daily oral administration of metformin (phase 2) to the 7 dogs in Figure 1. There was an interval of 72 hours between phases 1 and 2. The mean dose of metformin administered was 13.57 ± 0.55 mg/kg. The first dose was administered on day 0, and the last dose was administered on day 6. Blood samples were collected for analysis of serum drug concentrations before treatment initiation (baseline) and just before the morning drug administration on days 2, 4, and 6; a final blood sample was collected for analysis at the time of the morning drug administration on day 7, but dogs were not treated that day.

tion was approximately 2.5 hours, compared with 1 hour in cats[4] and 3 hours in humans.[1] Oral bioavailability of metformin in dogs was 31%, which was similar to 48% reported for cats[4] and 32% to 61% reported for humans.[17] Although oral bioavailability studies are typically conducted in fasted humans or other animals from which food has been withheld, food influences the rate and extent of absorption depending on the formulation of metformin. Use of metformin as adjuvant treatment in cancer patients may warrant further evaluations of the effect of food on absorption kinetics of rapid-release or extended-release formulations of metformin.

It is expected that metformin distribution to peripheral tissue compartments in dogs is similar to the distributions in other animals and humans. Chemically, metformin is a hydrophilic base, which at physiologic pH exists as a cationic species.[1] Therefore, passive diffusion through cell membranes is generally limited. Metformin is transported into cells by membrane transport proteins, such as OCTs and plasma membrane monoamine transporters.[1,2] Hence, the expression of transport proteins on different cell types will affect the bioactivity and the distribution characteristics of metformin. Plasma membrane monoamine transporters are thought to be the major transporters responsible for metformin uptake from the gastrointestinal tract, whereas OCT1 and OCT3 are important transporters of metformin in the liver.[1] In humans, genetic variants of OCT1 and OCT3 exist, which lead to differences in the transport of metformin into cells. Given that transporters are important in the absorption, distribution, and elimination of metformin, genetic variation may lead to a considerable difference in the response to metformin among individuals.[1]

Metformin is extensively excreted into the urine via glomerular filtration and tubular secretion with OCT2 and multidrug and toxin extrusion proteins appearing to have dominant roles.[18–20] A definitive reference range for GFR in dogs is not currently available because of GFR variations associated with age, breed, and concurrent disease. Previously published data obtained from dogs suggest that GFR ranges from 3.05 to 4.85 mL/min/kg depending on the assessment method.[21] The mean systemic clearance determined in the present study (24.1 ± 7.8 mL/min/kg) was considerably higher than the proposed GFR, likely indicating that metformin is principally excreted by active tubular secretion. This was similar to what has been determined for humans, in whom renal clearance of metformin is approximately 5 times the creatinine clearance of metformin.[17] However, in cats, the mean renal clearance of metformin was only 1.4 times the GFR, suggesting active tubular secretion is not as important in the renal excretion of metformin in cats[4] as it is in humans and dogs.

Metformin is rapidly distributed but evidence in humans suggests that drug binding to plasma proteins does not occur. This contributes to a high ap-

parent $V_{d\text{-}area}$. Because metformin is a small, charged molecule at physiologic pH, plasma protein binding of the drug is likely limited in dogs, but this should be confirmed in future evaluations of metformin in dogs. In humans, there is evidence of a slow association of the drug with RBCs.[1] The peak concentration of metformin following administration of a single dose in humans is initially higher in plasma than in erythrocytes. However, the subsequent decrease in concentration in erythrocytes is much slower than that in plasma.[1] This perhaps explains the prolonged elimination half-life of metformin observed in humans (9 to 19 hours) and dogs (20.4 hours), compared with that in cats (11.4 hours).[4,17] Given the metformin half-life in dogs determined in the present study, once-daily dosing would be expected to provide adequate steady-state serum concentrations over time. Once-daily dosing of metformin may also help to limit any gastrointestinal adverse effects.

One objective of the study of this report was to determine the oral dose of metformin that would yield serum concentrations similar to those thought to be effective in humans. One difficulty when trying to achieve this objective was that there is no clear definition of therapeutic concentrations in humans.[12] Many studies have solely used pharmacokinetic data to define therapeutic concentrations. However, such data may predominately evaluate the drug's maximum concentration in the blood after a single dose as opposed to the steady-state concentrations achieved with a long-term dosing regimen.[12] Another concern is that the amount of the drug at its site of action within the tissues may not be reflected by the serum concentration. Through review of the human medical literature, evaluation of trough serum concentrations associated with well-tolerated doses has suggested a likely upper limit of the therapeutic range of 2.5 mg/L.[1,12]

In the present study, metformin appeared to be well tolerated, with development of no adverse effects in most dogs or only mild transient gastrointestinal signs. No episodes of hypoglycemia or evidence of lactic acidosis were documented. However, there have been reports of idiosyncratic, hyperlactemic responses to metformin in humans,[12] and an evaluation of metformin administration in a larger population of dogs is recommended to further assess the safety of the drug's use in this species. Long-term administration of metformin may result in reductions in circulating vitamin B12 and folate concentrations. Clinicians and owners may need to consider supplementation with vitamin B12 and folate in cancer patients receiving long-term metformin treatment.

Results of the present study indicated that a dose of 10 to 15 mg of metformin/kg administered orally twice daily in dogs was well tolerated and resulted in steady-state serum concentrations less than the upper limit of the therapeutic range of metformin in humans (2.5 mg/L). Data from the present study should help inform the selection of dosage regimens and study design of randomized clinical trials to investigate the use of metformin for treatment and prevention of cancer in dogs.

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## Footnotes

a. Metformin-HCl, Sigma Chemical Co, St Louis, Mo.
b. Metformin-HCl tablets, 500 mg, Valeant, Laval, QC, Canada.
c. Indwelling jugular double-lumen sampling catheter, 7F × 20 cm, MILA International Inc, Florence, Ky.
d. Sorvall ST 8 benchtop centrifuge, ThermoFisher Scientific, Burlington, ON, Canada.
e. Allegra 25R centrifuge, Beckman, Indianapolis, Ind.
f. GraphPad Prism 5.0, GraphPad Software Inc, San Diego, Calif.

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