Evaluation of the effect of cephalexin and enrofloxacin on clinical laboratory measurements of urine glucose in dogs

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Objective—To determine the effects of cephalexin and enrofloxacin on results of 4 commercially available urine glucose tests in dogs.

Animals—6 healthy adult female dogs.

Procedure—In a crossover design, cephalexin (22 and 44 mg/kg [10 and 20 mg/lb], PO, q 8 h) or enrofloxacin (5 and 10 mg/kg [2.3 and 4.5 mg/lb], PO, q 12 h) was administered to dogs for 1 day. Urine samples were tested for glucose at 0, 6, and 24 hours after drug administration. In vitro, dextrose was added to pooled glucose-negative canine urine samples containing either no antimicrobial or known concentrations of either antimicrobial; urine samples were then tested for glucose.

Results—In vivo, false-positive results were obtained by use of a tablet test in the presence of both antimicrobials and by use of a strip test in the presence of cephalexin. In vitro, false-positive results were obtained with the tablet test at the highest urine concentration of cephalexin (2,400 µg/mL) and with a strip test at the highest concentration of enrofloxacin (600 µg/mL). Enrofloxacin in urine samples containing dextrose caused the urine glucose tests to underestimate urine glucose concentration.

Conclusions and Clinical Relevance—Cephalexin and enrofloxacin at dosages used in clinical practice may result in false-positive or false-negative urine glucose results, and care should be taken when using urine as a basis for identifying or monitoring diabetic animals. (*J Am Vet Med Assoc* 2004;224:1455–1458)

Cephalexin and enrofloxacin are 2 antimicrobials commonly used to treat canine pyoderma as well as other bacterial infections. Cephalexin and ciprofloxacin, an active metabolite of enrofloxacin, both cause false-positive urine glucose results (pseudoglycosuria) in humans.[1,2] Pseudoglycosuria in humans may reflect either the specific brand of urine glucose test or the possible presence of a drug or its metabolite that interferes with the test result.[3,4] It is likely that pseudoglycosuria also occurs in animals receiving cephalexin.[1] Because ciprofloxacin is structurally similar to, as well as an active metabolite of, enrofloxacin, pseudoglycosuria may occur after administration of enrofloxacin in dogs.

False urine glucose results, whether positive or negative, may lead to incorrect assumptions concerning the health status of the dog. A false-positive urine glucose test result may be interpreted as an indication of diabetes mellitus or abnormal function of the proximal renal tubules.[5] Home monitoring of the diabetic animal may complicate the owner's ability to correctly predict insulin needs. The potential influence of antimicrobials on urine glucose testing may complicate the veterinarian's or owner's ability to monitor urine glucose concentration accurately. An increase in insulin administration as a result of a false-positive glucose test result may lead to life-threatening hypoglycemia. In contrast, false-negative urine glucose test results may lead to uncontrolled diabetes and complications associated with hyperglycemia.

The purpose of the study reported here was to evaluate the effect of cephalexin and enrofloxacin on measurement of glucose in canine urine by use of 4 commercially available tests. In vivo and in vitro conditions were studied to determine the likelihood of effects at clinically relevant urine concentrations of antimicrobials and glucose.

Materials and Methods

Dogs—Six sexually intact female hound-type dogs from 2 to 4 years of age were used in the study. All experimental protocols were reviewed by the University Laboratory Animal Care Committee at Texas A&M University, which assured compliance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Dogs were judged clinically normal on the basis of results of physical examination and clinical laboratory tests (CBC, serum biochemical tests, and urinalysis of free-catch samples) that were performed prior to the beginning of each phase of the study. Food was withheld from each dog overnight prior to starting each part of the in vivo study.

For the in vivo and in vitro study, all urine samples were prepared and tested in a blinded manner and a randomized order. All testing methods involved a color change that correlates with a glucose concentration indicated on a chart specific to each test. If the color that resulted from the reaction did not match any color on the chart, the concentration of glucose was estimated by use of the colors considered the closest match. The range of urine glucose concentration was 100 to ≥ 2,000 mg/dL (strip A[a]), 150 to 500 mg/dL (strip B[b]), 50 to 1,000 mg/dL (strip C[c]), and 250 to ≥ 2,000 mg/dL (tablet[d]).

In vivo study—The in vivo study was performed twice in each dog by use of different dosages of antimicrobials. Dogs were randomly allocated into 2 groups (n = 3/group) to receive either cephalexin[e] at 44 mg/kg (20 mg/lb) every 8 hours for 1 day or enrofloxacin[f] at 10 mg/kg (4.5 mg/lb) every 12 hours orally for 1 day. Following a 2-day wash out period, treatments were crossed over and repeated. After a 3-week wash out period, the dogs were randomly allocated to groups again, and the study was repeated with cephalexin administration at 22 mg/kg (10 mg/lb) and enrofloxacin at 5 mg/kg (2.3 mg/lb).

Urine samples were collected by free catch at 0, 6, and 24 hours after drug administration. Urine samples were evaluated in random order within 15 minutes of collection for

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glucose concentration. Each test was run in triplicate and in accordance with the manufacturer's label recommendations. One person evaluated all tests.

In vitro study—The in vitro study was performed in 3 steps. The first step verified that each of the 4 urine glucose tests accurately detected urine glucose. The second step determined the effects of differing concentrations of cephalexin or enrofloxacin on urine glucose test results. The third step studied the effect of each antimicrobial on the urine glucose test in the presence of various glucose concentrations.

Urine samples collected and frozen at the beginning of the in vivo study (time 0, prior to drug administration) were thawed to room temperature (22°C) and tested for glucose concentration by use of the 4 test methods to confirm negative results. The glucose-free samples were pooled into 1 large sample. For confirmation of accuracy of urine glucose tests, glucose-free urine samples were divided into 4 aliquots of 50 mL each. To 3 of the aliquots, 50% dextrose[g] was added to achieve concentrations of 0.5% (0.5 g/dL), 1%, and 2%. These urine glucose concentrations were within the detectable range for the urine glucose tests and greater than trace glucose concentrations for each of these tests. Urine glucose tests were performed on urine samples of each concentration.

To detect drug-induced, false-positive results, pure cephalexin[h] (3 mg/mL) and enrofloxacin[i] (12 mg/mL) were prepared in stock solutions with a phosphate buffer. Urine collected at the zero time of the in vivo study was aliquoted into 20-mL volumes. A sufficient volume of the stock solution of each antimicrobial was added to the urine such that urine drug concentrations were 1, 10, 30, and 300 times the breakpoint **minimum inhibitory concentration (MIC)** as established in plasma for each drug. For enrofloxacin, concentrations studied were 2 µg/mL, 20 µg/mL, 60 µg/mL, 200 µg/mL, and 600 µg/mL. For cephalexin, concentrations studied were 8 µg/mL, 80 µg/mL, 240 µg/mL, 800 µg/mL, and 2,400 µg/mL. Each urine sample was tested in triplicate by the use of each urine glucose test.

To determine the impact of drugs on known urine glucose, each sample used in the previous step was aliquoted into 3 portions (approx 5 mL) and 50% dextrose solution was added to each to achieve final dextrose concentrations of 0.5%, 1%, and 2%. These dextrose solution concentrations were selected because they were greater than trace concentrations but still within the detectable concentrations for each of the urine glucose tests. The samples were tested in triplicate with each glucose test.

Results

In vivo test results—Each of the triplicate urine glucose tests produced identical results. Neither false-positive nor false-negative test results occurred with strip A or strip B. Both false-positives and false-negatives resulted with the other 2 tests (strip C, tablet; **Table 1**) at various times and drug concentrations. Three of 6 dogs that received the 22 mg/kg dose of cephalexin had false-positive urine glucose results at 6 hours (both tests), but none had false-positives at 24 hours. Six of 6 dogs that received the 44-mg/kg dose had false-positive results at 6 hours (both tests), whereas 2 of 6 dogs (strip C) or 6 of 6 dogs (tablet) had false-positive results at 24 hours. At the 5 mg/kg dose, enrofloxacin resulted in false-positives in 3 of 6 dogs at 6 hours but not at 24 hours, whereas the 10-mg/kg

Table 1—Number of false-positive results for detection of glucose in urine samples by use of 4 commercially available urine glucose tests in 6 dogs that received either low- or high-dose cephalexin or enrofloxacin for 1 day. Samples were obtained before drug administration and 6 and 24 hours after drug administration

Test	Cephalexin 22 mg/kg	Cephalexin 44 mg/kg	Enrofloxacin 5 mg/kg	Enrofloxacin 10 mg/kg
Strip A[a]	0	0	0	0
Strip B[b]	0	0	0	0
Strip C[c]	3*	6* and 2†	0	0
Tablet[d]	3*	6* and 6†	3*	6* and 5†

*Samples obtained 6 hours after drug administration. †Samples obtained 24 hours after drug administration. [a-d]*See* footnotes for identification of tests.

Table 2 —False-positive and false-negative results for detection of glucose in pooled canine urine samples by use of 4 commercially available urine glucose tests. Various concentrations of dextrose and either cephalexin or enrofloxacin were added to the pooled samples

Drug and concentration	Dextrose concentration (%) 0	0.5	1.0	2.0
Cephalexin (µg/mL)				
8	0	0	0	0
80	0	0	0	0
240	0	0	0	0
800	0	0	0	0
2,400	+ (Tablet)	+ (Tablet)	0	0
Enrofloxacin (µg/mL)				
2	0	0	0	– (Strip A)
20	0	– (Strip C)	– (Strip B)	0
60	0	– (Strip C)	– (Strip B and tablet)	– (Strip A and tablet)
200	0	– (Tablet)	– (Strips A, B, and tablet)	– (Strip A and tablet)
600	0	– (Strips B, C, and tablet) + (Strip A)	– (Strips A, B, and tablet)	– (Strip A and tablet)

0 = No false-positive or false-negative results. + = False-positive result. – = False-negative result. *See* Table 1 for key.

dose resulted in false-positives in all dogs at 6 hours and 5 of 6 dogs at 24 hours.

In vitro results—Results of the triplicate urine glucose tests were identical for all of the urine samples analyzed. Enrofloxacin caused each urine glucose test to underestimate urine glucose concentration (**Table 2**). The only exception was that 1 test, strip A, yielded a false-positive test result once (dextrose concentration of 0.5% and enrofloxacin concentration of 600 µg/dL). In contrast, cephalexin resulted in overestimation of glucose concentration, but only in the samples that contained the highest antimicrobial concentration (2,400 µg/mL) and only for the tablet.

Discussion

Urine glucose tests in dogs are used most commonly for the initial diagnosis and subsequent monitoring of the treatment of diabetes mellitus.[6] However, other disorders associated with glycosuria exist including acute renal failure, primary renal glycosuria, hyperadrenocorticism, pancreatitis, pheochromocytoma, hypothalamic lesions, and drug toxicosis (aminoglycoside toxicosis, amphotericin toxicosis).[5,6] A false urine glucose test result after antimicrobial administration may lead to diagnostic testing or erroneous treatment for these medical conditions, contributing to unnecessary cost, stress, and possibly danger to the client and pet.

Limited information is available for urine drug concentrations of cephalexin and enrofloxacin after administration. The typical urine drug concentration for cephalexin in dogs is not known. In humans, urine concentration of cephalexin after a single 500-mg dose is 750 µg/mL, which is within the range of urine cephalexin used in this study.[7] In dogs, more information is available about urine concentrations of enrofloxacin and its active metabolite, ciprofloxacin. In 1 study,[8] the urine drug concentration for enrofloxacin and its metabolite, ciprofloxacin, peaked and were at similar concentrations (approx 173 to 263 µg/mL) at 6 hours after enrofloxacin administration (dosage, 5 mg/kg, daily). In a different study,[9] enrofloxacin and ciprofloxacin urine concentrations 2 hours after a single IV administration of enrofloxacin at a higher dose (20 mg/kg) were 43.9 µg/mL for enrofloxacin and 42.2 µg/mL for ciprofloxacin. Therefore, the ranges of drug concentrations for cephalexin and enrofloxacin added to the urine in our study were within a reasonable range.

Female dogs were selected for this study because female dogs have a greater percentage urine drug concentration than male dogs after cephalexin administration (mean ± SD, 52.0 ± 12.2% in females versus 30.6 ± 4.8% in males).[10] Therefore, female dogs are more ideal for research than male dogs for detecting the potential effects of cephalexin on urine glucose concentration. This study revealed differences in urine glucose test results with not only cephalexin administration but also with enrofloxacin administration. Our study design may have increased our chances of finding these differences. It is not known whether a similar situation would occur in male dogs.

Results of the study reported here indicate that cephalexin and enrofloxacin administration in dogs may alter the accuracy of urine glucose tests. False-positive urine glucose results were caused by strip C and the tablet formulation. Urine glucose dipsticks comprise a double sequential enzyme system with glucose oxidase impregnated on the paper. Oxidation of glucose in the urine results in a color change in the strip. Inappropriate detection and quantification of glucose by strip C may reflect the color-changing agent (chromogen).[6] The color change for this strip is less dramatic, compared with the other 2 strip tests. The color change for strip C is yellow to light green, as opposed to green to brown color change for strip A and yellow to dark green for strip B. In contrast, false-positive results obtained with the tablet formulation in vivo may reflect the different reaction upon which glucose detection is based.[6] The tablet detects glucose by use of a copper reduction test. Any reducing agent, including glucose, can result in the formation of cuprous oxide. It is possible that either the antimicrobial or 1 of its metabolites may have acted as a reducing agent and caused the false-positive urine glucose reaction.[11]

Interestingly, enrofloxacin caused false-positive results in vivo, but false-negative results in vitro. The exact reason for the false-negative test results in vitro was unclear. Several possible explanations exist. Enrofloxacin in its pure form (no metabolites) has the ability to possibly alter glucose concentrations to a lower than normal concentration. When enrofloxacin is administered orally, it normally is metabolized into ciprofloxacin. Ciprofloxacin has been isolated from dogs after enrofloxacin administration and at a similar concentration to that of enrofloxacin in urine.[8,9] Therefore, it may be the parent compound that is causing the disparity in urine glucose test results.

The types of urine glucose tests that yielded false-positive urine glucose results differed between the in vivo and in vitro study results. For the in vivo study, the 2 tests that yielded false-positive urine glucose results were strip C and the tablet. For the in vitro study, the 2 tests that yielded false-positive urine glucose results were strip A and the tablet. This difference may be attributable to the fact that the drug concentrations in the urine samples in vivo were different than drug concentrations used in the in vitro study. The false-positive reactions for the in vitro study occurred at high drug concentrations (2,400 µg/mL for cephalexin and 600 µg/mL for enrofloxacin). Therefore, if high concentrations of drug are present in the urine, the probability of a false-positive urine test result seems to increase.

The in vivo data suggest that, with strip C and the tablet, the probability of getting a false-positive urine glucose result may be the direct result of the drug used, the dosage used, and the period of time after drug administration that the urine sample is collected. At the higher cephalexin dosage (44 mg/kg, q 8 h), false-positive reactions occurred at 6 hours and 24 hours after drug administration with both tests. At the lower cephalexin dosage (22 mg/kg, q 8 h), false-positive reactions only occurred at 6 hours after drug adminis-

tration with both tests. With enrofloxacin, problems with false-positive urine glucose results only occurred with the tablet. For the higher enrofloxacin dosage (10 mg/kg, q 12 h), false-positive reactions may occur at 6 hours and 24 hours after drug administration. For the lower enrofloxacin dosage, false-positive reactions may occur in the 6-hour postadministration samples only.

False-positives occurred more frequently for in vivo tests than did false-negatives for in vitro tests. Several possible explanations exist for this disparity in test results. As previously suggested, the in vivo test results may have been affected by metabolites formed when these antimicrobials are orally ingested. Another possibility is that these drugs induced a transient glycosuria. In addition, the concentrations of these drugs for the 2 tests may have been sufficiently different to cause different reactions. These explanations are all speculative, and other explanations probably exist.

Results of this study suggest that false-positive results for urine glucose may occur in dogs when either cephalexin or enrofloxacin is administered. However, this problem is probably test-dependent, time-of-collection dependent, and dosage-dependent. If the veterinarian is concerned that a false-positive result has occurred, it would be prudent to discontinue administration of the cephalexin or enrofloxacin for several days and repeat the test. The end result would be a more accurate urine test result (no possible drug interference) and more accurate assessment of the status of the dog's health.

[a]Multistix 10 SG, Bayer Corp, Elkhart, Ind.
[b]Urispec 9-way, Henry Schein, Port Washington, NY.
[c]Chemstrips 10 SG, Boehringer Mannaheim, Indianapolis, Ind.
[d]Clinitest, Miles Inc, Elkhart, Ind.
[e]Cephalexin, Teva, Sellersville, Pa.
[f]Baytril, Bayer Corp, Shawnee Mission, Kan.
[g]50% dextrose solution, Abbott Laboratories, North Chicago, Ill.
[h]Cephalexin powder, Sigma Chemical Co, St Louis, Kan.
[i]Baytril powder, Bayer Corp, Shawnee Mission, Kan.

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