Pharmacokinetics of N-acetylcysteine after oral and intravenous administration to healthy cats

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Objective—To describe the pharmacokinetics of N-acetylcysteine (NAC) in healthy cats after oral and IV administration.

Animals—6 healthy cats.

Procedures—In a crossover study, cats received NAC (100 mg/kg) via IV and oral routes of administration; there was a 4-week washout period between treatments. Plasma samples were obtained at 0, 5, 15, 30, and 45 minutes and 1, 2, 4, 8, 12, 24, 36, and 48 hours after administration, and NAC concentrations were quantified by use of a validated high-performance liquid chromatography–mass spectrometry protocol. Data were analyzed via compartmental and noncompartmental pharmacokinetic analysis.

Results—Pharmacokinetics for both routes of administration were best described by a 2-compartment model. Mean ± SD elimination half-life was 0.78 ± 0.16 hours and 1.34 ± 0.24 hours for the IV and oral routes of administration, respectively. Mean bioavailability of NAC after oral administration was 19.3 ± 4.4%.

Conclusions and Clinical Relevance—The pharmacokinetics of NAC for this small population of healthy cats differed from values reported for humans. Assuming there would be similar pharmacokinetics in diseased cats, dose extrapolations from human medicine may result in underdosing of NAC in cats with acute disease. Despite the low bioavailability, plasma concentrations of NAC after oral administration at 100 mg/kg may be effective in the treatment of chronic diseases. (*Am J Vet Res* 2013;74:290–293)

N-acetylcysteine is a sulfhydryl donator and a thiol precursor of L-cysteine and reduced glutathione.[1] N-acetylcysteine is approved for use in humans for the treatment of acute acetaminophen toxicosis and as a mucolytic in respiratory tract disease.[2] Similar uses have proven efficacy in veterinary medicine.[3] The most common use of NAC is for the treatment of acetaminophen toxicosis in cats. In addition, NAC has been used successfully to treat heavy metal toxicosis (as an adjunct treatment to chelation) and to support recovery following poisoning with *Amanita* spp mushrooms. It currently is being evaluated as a precursor of cysteine and glutathione for use in the treatment of nephropathies associated with radiographic contrast material and hepatobiliary disease in canine and feline patients.[4,5] The therapeutic value of NAC is its ability to reduce inflammation, fibrosis, cartilage erosion, and endothelial dysfunction.[1]

Abbreviations

AUC	Area under the plasma concentration–versus-time curve
NAC	N-acetylcysteine

Although both oral and IV dosing regimens of NAC for treatment of acute disease can be found in the literature, there is no information on the pharmacokinetics of NAC in species other than humans.[4] In vitro concentrations of NAC have been identified that yield beneficial responses in cell culture,[1] but to the authors' knowledge, they have not been tested in vivo. Current dosing regimens in veterinary medicine are based on case reports and thus have limited extrapolation to other diseases, other species, and other routes of administration.[3] In general, NAC is considered to be safe in humans; however, vomiting and anorexia have been reported.[6] To the authors' knowledge, there has been no information reported about safety and adverse reactions after NAC administration to cats. Therefore, the purpose of the study reported here was to characterize the pharmacokinetics after IV and oral administration of NAC to healthy cats so that clinicians can expand and refine dosing regimens for the treatment of acute and chronic disease.

Received May 2, 2012.
Accepted July 18, 2012.
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Supported in part by the Western University of Health Sciences Competitive Intramural Grant Program and the Morris Animal Foundation Veterinary Student Scholar Program.
Presented as a poster presentation at the Morris Animal Foundation–Small Companion Animal Symposium, Denver, June 2011.
The authors thank Drs. David Clark and Marc Togneri for technical assistance.
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Materials and Methods

Animals—Six healthy adult cats were used in a crossover study that was approved by the Western University of Health Sciences Institutional Animal Care and Use Committee. Cats were screened for concurrent disease via evaluation of the medical history (free from clinical disease for a minimum of 30 days prior to start of the study); results of physical examination, a CBC, urinalysis, and an expanded serum biochemical analysis (including total thyroxine and triiodothyronine concentrations as well as free thyroxine concentrations measured with equilibrium dialysis); detection of antibodies against FIV (via an ELISA) and feline coronavirus (via an indirect immunofluorescence assay) and antigens of FeLV (via an ELISA); evaluation of a blood smear for detection of feline hemotropic *Mycoplasma* spp; Doppler echocardiography (reviewed by a board-certified veterinary radiologist); ECG; and Doppler blood pressure measurement.

Procedures—Each cat was anesthetized with sevoflurane 24 hours prior to the start of the study to facilitate placement of a double-lumen catheter in a jugular vein. A single dose of meloxicam (0.1 mg/kg, PO) was administered to each cat prior to catheterization. Cats were assigned via a randomization procedure (each cat was assigned a number; numbers were written on pieces of paper, which were then drawn out of a hat) to receive NAC (100 mg/kg) IV (via the internal lumen of the double-lumen catheter) or orally (via syringe, followed by administration of 1 mL of water). Commercial formulations of NAC approved for use in humans for oral[a] or IV[b] administration were used. Blood samples (3 mL/sample) were collected into heparin-containing tubes via the double-lumen catheter at the time of NAC administration (time 0) and 5, 15, 30, and 45 minutes and 1, 2, 4, 8, 12, 24, 36, and 48 hours after administration. After a 4-week washout period, cats received the NAC via the alternate route of administration.

Blood samples were centrifuged immediately after collection. Plasma was harvested and stored at –80°C until analyzed.

Measurement of NAC concentrations—Plasma concentrations of NAC were determined via high-performance liquid chromatography[c] and mass spectrometry.[d] The reference standards for NAC and penicillamine (ie, internal standard) were obtained from the same source.[e] Standard curves for NAC were created by adding known amounts of NAC to untreated feline plasma; these standard curves were linear from 0.2 to 1,000 µg/mL. Standard curves were accepted if the predicted concentrations were within 15% of the actual concentration and the correlation coefficient was 0.99.

Samples for the standard curves and unknown samples obtained from the cats were extracted in an identical manner. Plasma (0.1 mL) was added to 0.1 mL of dithiothreitol (46.3 mg/mL) and 0.1 mL of penicillamine (100 µg/mL). Then, 0.1 mL of 10% trichloroacetic acid was added to precipitate the plasma proteins. The samples were vortexed for 5 seconds and then centrifuged for 10 minutes at 15,000 × *g*. The supernatant was transferred to an injection vial, and the injection volume was 0.05 mL. Mobile phase A consisted of acetonitrile, and mobile phase B consisted of 0.02% trifluoroacetic acid in deionized water. Flow rate for the mobile phase was 0.4 mL/min as follows: 90% mobile phase B from 0 to 1 minute, a gradual decrease to 50% mobile phase B during the next 3 minutes, 50% mobile phase B for 1 minute, a gradual increase to 90% mobile phase B during the next minute, and then 90% mobile phase B for 2 minutes (total run time was 8 minutes). Separation was achieved with a C18 column[f] maintained at 40°C. The qualifying ions for NAC and penicillamine were 164.08 m/z and 149.96 m/z, respectively. The quantifying ions for NAC and penicillamine were 122.00 m/z and 87.10 m/z, respectively. Mean ± SD accuracy of the assay was 99 ± 9%, and the coefficient of variation was 8% (as determined on 5 replicates at each of 3 concentrations [0.2, 2, and 50 µg/mL]). The lower limit of quantification, defined as the lowest concentration on the linear standard curve with predicted concentrations within 15% of the actual concentration, was 0.2 µg/mL, and the upper limit of quantification was 1,000 µg/mL.

Pharmacokinetic analysis—Plasma concentration–versus-time curves were analyzed via compartmental and noncompartmental methods with commercial pharmacokinetic software.[g] Compartmental analysis was evaluated on the basis of the coefficient of determination and the Akaike information criterion for the best-fit model. The AUC was calculated via the trapezoidal method with the last triangle extrapolated to infinity. The elimination half-life was calculated as 0.693 of the mean resonance time. Bioavailability was calculated as the AUC after oral administration divided by the AUC after IV administration.

Results

Six cats received NAC via oral administration, but only 5 cats received NAC via IV administration. One cat did not complete the study because of behavioral complications not related to the medication. Another cat vomited within 1 minute after IV administration of NAC. No cats vomited after oral administration of NAC. Although some cats tried to avoid oral administration after an initial taste of the solution and licked

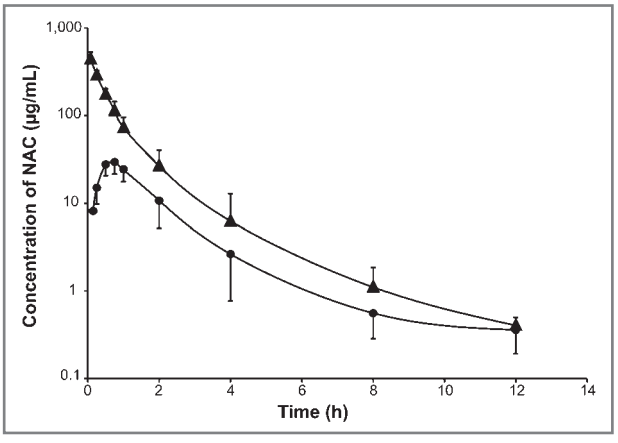

Figure 1—Plasma concentrations of NAC after IV (triangles) and oral (circles) administration of 100 mg/kg to healthy cats (n = 5 and 6 cats, respectively). Error bars represent SD.

Table 1—Values for pharmacokinetic parameters determined via compartmental analysis for IV and oral administration of 100 mg of NAC/kg to healthy cats (n = 5 and 6 cats, respectively).

	Route of administration	
Parameter	**IV**	**Oral**
Tmax (h)	NA	0.85 ± 0.31
Cmax (µg/mL)	394.30 ± 71.44	19.66 ± 6.85
K01 (1/h)	NA	1.58 ± 0.74
K01 half-life (h)	NA	0.53 ± 0.26
K10 (1/h)	1.44 ± 0.20	0.81 ± 0.39
K10 half-life (h)	0.49 ± 0.07	1.49 ± 1.72
K12 (1/h)	0.32 ± 0.51	0.28 ± 0.18
K21 (1/h)	0.40 ± 0.41	0.11 ± 0.11
A (µg/mL)	368.02 ± 33.80	163.93 ± 1,432.06
B (µg/mL)	26.28 ± 49.15	1.39 ± 1.83
α (1/h)	1.91 ± 0.82	1.11 ± 0.24
α half-life (h)	0.41 ± 0.13	0.65 ± 0.16
β (1/h)	0.26 ± 0.14	0.09 ± 0.09
β half-life (h)	3.21 ± 1.39	59.84 ± 113.07
MRT (h)	1.11 ± 0.19	NA
VDss (mL/kg)	408.78 ± 60.11	NA
CL (mL/h/kg)	375.29 ± 85.27	NA
AUC (h•µg/mL)	277.44 ± 61.26	88.87 ± 89.77
F (%)	NA	33.11 ± 25.21

Values are mean ± SD.

A = Zero-time intercept for the rate constant of the distribution phase. α = Rate constant of the distribution phase. B = Zero-time intercept for the rate constant of the elimination phase. β = Rate constant of the elimination phase. CL = Clearance. Cmax = Maximum plasma concentration. F = Bioavailability. K01 = Absorption rate constant. K10 = Elimination rate constant from the central compartment. K12 = Rate constant of transfer from compartment 1 to compartment 2. K21 = Rate constant of transfer from compartment 2 to compartment 1. MRT = Mean residence time. NA = Not applicable. Tmax = Time to maximum plasma concentration. VDss = Volume of distribution at steady state.

Table 2—Values for pharmacokinetic parameters determined via noncompartmental analysis for IV and oral administration of 100 mg of NAC/kg to healthy cats (n = 5 and 6 cats, respectively).

	Route of administration	
Parameter	**IV**	**Oral**
Tmax (h)	NA	0.65 ± 0.31
Cmax (µg/mL)	441.00 ± 87.63	32.05 ± 5.56
VDss (mL/kg)	337.00 ± 78.62	NA
AUC last (h•µg/mL)	317.75 ± 68.19	59.18 ± 16.98
AUC inf (h•µg/mL)	319.89 ± 67.24	60.10 ± 0.18
MRT last (h)	0.92 ± 0.28	1.80 ± 0.29
MRT inf (h)	1.05 ± 0.23	1.99 ± 0.34
Elimination half-life last (h)	0.64 ± 0.19	1.25 ± 0.20
Elimination half-life inf (h)	0.73 ± 0.16	1.38 ± 0.24
CL (mL/h/kg)	324.54 ± 71.53	NA
CL/F (mL/h/kg)	NA	1,756.73 ± 494.51
F (%)	NA	19.30 ± 4.42

Values are mean ± SD.

AUC inf = The AUC calculated on the basis of extrapolation to infinity. AUC last = The AUC calculated on the basis of the last measured time point. Elimination half-life inf = Elimination half-life calculated on the basis of extrapolation to infinity. Elimination half-life last = Elimination half-life calculated on the basis of the last measured time point. MRT inf = MRT calculated on the basis of extrapolation to infinity. MRT last = MRT calculated on the basis of the last measured time point.

See Table 1 for remainder of key.

their lips repeatedly after oral administration, no cats had excessive salivation or other adverse complications associated with oral administration.

Results for both IV and oral administration of NAC were best fit via a 2-compartment model and $1/Y^2$ weighting. Oral administration was characterized by fast absorption with a peak plasma concentration at a mean ± SD of 0.65 ± 0.31 hours. Concentrations of NAC were less than the limit of quantification by 24 hours after administration for both routes of administration.

Concentration-versus-time curves for both routes of administration were plotted (**Figure 1**). The mean ± SD elimination half-life after IV administration was 0.49 ± 0.13 hours and 0.73 ± 0.16 hours via compartmental and noncompartmental analysis, respectively. Mean elimination half-life after oral administration was 1.49 ± 1.72 hours and 1.38 ± 0.24 hours via compartmental and noncompartmental analysis, respectively. Mean bioavailability of NAC after oral administration was 33.11 ± 25.21% and 19.3 ± 4.4% via compartmental and noncompartmental analysis, respectively. Results for both compartmental and noncompartmental analysis were summarized (**Tables 1 and 2**).

Discussion

Pharmacokinetics of NAC in healthy cats is similar to that reported in humans. Bioavailability in humans ranges from 4% to 10%, depending on the study and formulation.[7] Most of the human studies used equivalent formulations (FDA-approved products) but contained large numbers of subjects with variations in sampling times. The low bioavailability in humans is thought to reflect a high first-pass effect from liver metabolism, which could be a mechanism for the similar low bioavailability in cats. Total body clearance in cats of the present study was 324.54 mL/h/kg, which is faster than that reported in humans (207 mL/h/kg).[7] This was reflected in the fact that the elimination half-life (1.38 hours) in the cats of the present study is slightly less than that reported in humans (2.24 hours).[7] Radionucleotide evaluations in humans have confirmed that NAC is metabolized quickly in the liver. Further studies would be needed to confirm a similar metabolism pattern in cats. The volume of distribution (0.34 L/kg) in the cats of the present study was consistent with total body water content, as would be expected for a hydrophilic molecule like NAC. This parallels the volume of distribution reported in humans (0.327 L/kg).[7]

In the present study, 1 cat vomited within a minute after IV administration of NAC. In humans, nausea and vomiting are adverse effects after both oral and IV administration and may become a limiting factor for the administration of NAC.[2] Reported adverse effects in cats also include vomiting.[3] However, this has been reported for clinical patients and may have been biased by the effects of acetaminophen toxicosis, which also include vomiting. The importance of this clinical sign should be interpreted on a case-by-case basis, but clinicians should recognize that vomiting could be caused by the treatment as well as the underlying disease process.

The efficacy of treatment for acute acetaminophen toxicosis in humans is dependent on the AUC and not on peak plasma concentrations. The dosing regimen in humans equates to a constant rate infusion of 23 mg/kg/h.[8] On the basis of the assumption that the pharmacokinetics for a constant rate infusion is equivalent to that after IV administration of a single dose, and given

the slightly shorter half-life in cats than that in humans, a dosing regimen consisting of a constant rate infusion of 23 mg/kg/h would result in steady-state plasma concentrations of approximately 71 µg/mL in cats. This is less than the target plasma concentration of 125 µg/mL in humans. To reach the same AUC as that achieved in humans, a constant rate infusion of 40.5 mg/kg/h would be needed in cats. Again, this is based on the assumption of linear pharmacokinetics and minimal accumulation. Thus, dosing regimens for treatment of acute disease may need to be altered to account for the slight but possibly important differences in pharmacokinetics between cats and humans. Further studies are needed to confirm the predicted plasma concentrations in cats.

In addition to use in the treatment of acute disease, NAC has been used as a treatment for chronic disease processes. Provision of important precursors of metabolism and cellular repair as well as free-radical scavengers are established methods of treatment for both acute and chronic diseases. This strategy has been used successfully for chronic diseases, such as chronic active hepatitis (S-adenosylmethionine) and degenerative joint disease (polysulfated glycosaminoglycan) in feline, canine, and equine patients.[5,9] Past success with provision of metabolic precursors (eg, carnitine in dilated cardiomyopathy) has identified chronic cardiac diseases, such as hypertrophic cardiomyopathy, as possible therapeutic targets.[10]

In a recent study,[11] oral administration of NAC at a dosage of 500 mg/kg/d reduced the pathological changes in transgenic rabbits with established cardiac hypertrophy and fibrosis in β-myosin heavy chains. This dosage was based on target plasma concentrations of 0.8 to 6.5 µg/mL, which have been found to have antifibrotic effects in vitro.[12] Hypertrophic cardiomyopathy is one of the most common chronic cardiac diseases in cats, and there currently is no specific treatment available that is capable of stopping or reducing the cardiac hypertrophy. On the basis of the assumption that administration of multiple doses does not alter the pharmacokinetics of NAC in cats, a target concentration of 4.9 µg/mL and 2.4 µg/mL would be predicted after oral administration of 100 mg of NAC/kg every 12 and 24 hours, respectively. Both of these possible dosing regimens would yield concentrations within the range found to be efficacious in vitro. Therefore, results of the present study provided a possible dosing regimen for oral administration of NAC to cats that should reach target plasma concentrations known to have in vivo effects. Prospective clinical trials for the use of NAC in chronic diseases (eg, hypertrophic cardiomyopathy in cats) should be conducted to establish the efficacy and long-term safety of dosing regimens.

In the present study, the pharmacokinetics of NAC after administration to healthy cats paralleled that reported in humans, but there was a shorter elimination half-life because of faster clearance from the cats. Therefore, current dosing regimens for cats extrapolated from the human medical literature may not provide therapeutic concentrations in plasma and should be evaluated for their efficacy. In humans with acute acetaminophen toxicosis, administration of NAC is initiated with a loading dose, which is followed by administration of maintenance doses every 6 hours. Because dose extrapolation within a species is inherently less risky than extrapolation between species, analysis of our data suggests that administration of maintenance doses of NAC should be performed every 3 hours in cats to account for the faster clearance. Analysis of the pharmacokinetic data indicates that clinicians could use long-term oral administration at a dose of 100 mg/kg if target therapeutic concentrations are < 5 µg/mL. However, these recommendations are based on the assumption of linear pharmacokinetics and little to no accumulation with multiple doses, and additional studies should be performed to establish the efficacy of NAC for the treatment of both acute and chronic diseases.

a. 20% acetylcysteine solution, Hospira Inc, Lake Forest, Ill.
b. Acetadote, Cumberland Pharmaceuticals, Nashville, Tenn.
c. Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, Md.
d. API 2000, Applied Biosystems, Foster City, Calif.
e. Cerilliant Corp, Round Rock, Tex.
f. ACE C18AR, 150 mm × 3.0 mm × 5 µm, MAC-MOD Analytical, Chadds Ford, Pa.
g. WinNonLin, version 5.2, Pharsight Inc, Mountain View, Calif.

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