Faculty of Veterinary Science
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'...improvements in clinical signs in the cats with IC that received NAG treatment suggest that there are clinical benefits of NAG administration in cats with IC...'

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A randomised double-blind, placebo controlled study

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Objective—To determine the effects of once-daily oral administration of N-acetyl-D-glucosamine (NAG) on plasma and urine glycosaminoglycan (GAG) concentrations in cats with idiopathic cystitis (IC).

Animals—19 cats with IC and 10 clinically normal cats.

Procedures—Cats with IC were randomly assigned to receive 250 mg of NAG in capsule form orally once daily for 28 days (n = 12) or a placebo (capsule containing cellulose) orally once daily for the same period (7). In cats with IC, plasma and urine GAG concentrations and urine creatinine concentration were measured on days 0 (immediately before first dose), 7, 14, 21, 28, and 56. For purposes of comparison, those variables were measured in 10 clinically normal cats on day 0.

Results—Mean ± SEM urine GAG-to-creatinine concentration ratios (day 0 data) for cats with IC and clinically normal cats differed significantly (3.11 ± 0.62 µg/mL and 14.23 ± 3.47 µg/mL, respectively). For cats with IC, mean plasma GAG concentration in NAG-treated cats (39.96 ± 5.34 µg/mL) was higher than that in placebo-treated cats (24.20 ± 3.35 µg/mL) on day 21. In the NAG-treated cats, plasma GAG concentration on days 21 (39.96 ± 5.34 µg/mL) and 28 (39.91 ± 6.74 µg/mL) differed significantly from the day 0 concentration (27.46 ± 3.90 µg/mL).

Conclusions and Clinical Relevance—Cats with IC have lower urinary GAG-to-creatinine concentration ratios than did clinically normal cats. Administration of NAG (250 mg, PO, q 24 h) significantly increased plasma GAG concentrations in cats with IC after 21 days of treatment. (*Am J Vet Res* 2011;72:843–850)

Feline lower urinary tract disease is a common disease in cats.[1] In 45% to 70% of cats with lower urinary tract disease, the cause is nonobstructive IC[2–4]; in 29%, the cause is obstructive IC.[5] The clinical signs of IC in cats are dysuria, stranguria, hematuria, pollakiuria, and periuria (urination in inappropriate locations).[6] In women with IC (idiopathic pelvic pain syndrome), similar clinical signs include urinary bladder pain, frequent urination, urination urgency, and nocturia without a diagnosable cause. Clinical signs of IC in cats may result from multiple abnormalities of the urinary bladder, CNS, and hypothalamic-pituitary-adrenal axis.[7] It has been suggested that changes in several factors (eg, changes in environment, reduction in stress, feeding of certain types of diet, increase in daily water consumption, and application of pheromone treatments) can contribute to improvement of clinical signs of IC in cats.[8] Various hypotheses regarding the causes of IC in cats have been proposed and are as follows: the GAGs lining on the transitional epithelium of the urinary bladder becomes compromised, which leads to development of clinical signs[9]; altered gene expression patterns and consequently inhibition of cell proliferation in the urothelium results in clinical signs[10]; or stress from the surrounding indoor environment and patient behaviors induce clinical signs.[11] A specific GAG, called GP-51, is known to be a major component of the surface mucin on the normal bladder urothelium. Glycosami-

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
DMB	1,9–dimethyl-methylene blue
FLUTD	Feline lower urinary tract disease
GAG	Glycosaminoglycan
GP-51	Glycoprotein with a molecular weight of 51 kDa
IC	Idiopathic (or interstitial) cystitis
NAG	N-acetyl-D-glucosamine

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noglycans are believed to prevent bacterial and crystal adhesion and protect the urothelium from noxious or toxic urine substances.[12] Studies[13,14] in women and cats with IC have revealed that the amount of excreted urine GAGs or GP-51 is decreased, compared with findings in individuals without IC.

Glycosaminoglycans are mucopolysaccharide chains composed of long unbranched polysaccharide molecules; when linked to proteins, proteoglycans are formed. There are many GAG components of proteoglycans, such as chondroitin sulfate, dermatan sulfate, heparan sulfate, keratin sulfate, and heparin, which are all different in structure.[14] In animal tissues, GAGs are found mainly in intracellular substance,[15,16] extracellular matrix (mostly chondroitin sulfate and dermatan sulfate), and cell surfaces.[14] Glycosaminoglycan replacement is used to replace the GAG layer of the urothelium. In human patients with IC, there has been some success following treatment with pentosanpolysulphate sodium, an exogenous form of GAG, given via the oral route with or without intravesical administration of heparin.[17] Oral administrations of heparin, hyaluronic acid, and pentosanpolysulphate sodium have been shown to have some efficacy in humans with IC[18,19]; such treatment helps to increase the amount of urothelial GAGs and reduce transitional cell injury.

N-acetyl-D-glucosamine, an exogenous form of GAG, has also been used as a medication administered to cats to increase the amount of GAGs lining the urothelium. However, to our knowledge, clinical studies of the effects of NAG on GAG alteration in the urinary bladder in cats with IC have not been reported. The purpose of the study reported here was to evaluate the effects of oral administration of NAG (250 mg) once daily for 28 days on clinical signs, findings of urinalysis, and plasma and urine concentrations of GAGs in cats with IC. Data were collected for analysis before and during treatment and at 4 weeks after treatment was discontinued. In addition, plasma and urine GAG concentrations and urine creatinine concentration were measured in clinically normal cats on 1 occasion for purposes of comparison.

Materials and Methods

Cats—Clinically normal cats and cats with IC were included in the study. The protocol was approved by the Chulalongkorn University Animal Care and Use Committee.

As part of the study, data were obtained from 10 clinically normal client-owned cats that were brought to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University for vaccination or neutering. Cats were included without sex or breed preferences. The owners of the clinically normal cats were asked to sign written consent statements to allow their cats to participate in the study.

Cats with IC that were brought to the veterinary hospital and had clinical signs of dysuria, stranguria, hematuria, pollakiuria, or urination in inappropriate places were eligible for inclusion in the study. Cats with IC and a history of urolithiasis, urinary tract infection, bladder neoplasia, or congenital deformity of the urinary tract were excluded. Cats were also excluded from the study when signs of urinary tract infection or any other serious condition (eg, severe anemia) were detected via physical examination. The owners of cats with IC were also asked to sign written consent statements to allow their cats to participate in the study. Each owner was allowed to withdraw his or her cat from the study if the cat's condition worsened at any time.

Study design and procedures—A double-blind placebo-controlled study was performed. For the clinically normal cats, history was obtained from the owners; physical examination and blood and urine sample collection (for hematologic and biochemical analyses, urinalysis [including measurement of urine protein and creatinine concentrations], and evaluation of plasma and urine GAG concentrations) were performed on the first day of the study (day 0).

Cats with IC were randomly assigned into 2 groups: a treatment group in which cats received 250 mg of NAG[a,b] orally once daily for 28 days or a placebo group in which cats received a placebo orally once daily for the same period. The NAG was formulated as a capsule. The placebo consisted of a capsule that contained cellulose, a form of organic compound; cellulose is a polysaccharide (as is NAG) that is metabolized to glucose as the only end product. The owners of each cat were also given instruction on changes to the environment and care of their cats.

Each owner of the cats with IC and the clinically normal cats was interviewed by use of a questionnaire to obtain information regarding the cat's type of diet, water intake, lifestyle, and environment. For each cat with IC, an initial physical examination was performed; survey abdominal radiography and double-contrast cystography or ultrasonography (or both) of the lower urinary tract were also performed. A blood sample was collected for a CBC and plasma biochemical analysis (to determine activities of ALT and ALP and concentrations of BUN and creatinine). A urine sample was collected for urinalysis and measurement of urine protein and creatinine concentrations (for calculation of the urine protein-to-creatinine ratio). Analysis of these blood and urine samples provided baseline data (ie, day 0 data). Administration of NAG or placebo was commenced immediately after collection of the day 0 data.

During the study period, NAG- and placebo-treated cats were further evaluated on days 7, 14, 21, 28, and 56. At these time points, evaluations included assessment of clinical signs, a CBC, and plasma biochemical analysis (to determine activities of ALT and ALP and concentrations of BUN and creatinine), urinalysis (including measurement of urine protein and creatinine concentrations for calculation of the urine protein-to-creatinine concentration ratio), and measurement of plasma and urine GAG concentrations. Blood and urine samples were collected immediately before administration of the daily dose of NAG or placebo on each of these evaluation days.

Blood sample collection—At the predetermined time points, a blood sample was collected from each clinically normal cat (ie, day 0) and each cat with IC (ie, days 0, 7, 14, 21, 28, and 56) in a similar manner. Each blood sample (3 mL) was collected from a cephalic or saphenous vein and divided between 2 tubes: 1 tube contained anti-

coagulant (EDTA) and 1 tube contained heparin. A manual blood cell count was performed. Plasma was obtained via centrifugation[c] at 700 × *g* for 10 minutes at 4°C; BUN concentration,[20] creatinine concentration (via the alkaline picrate method),[21] ALT activity,[22] and ALP activity were determined.[23] The remainder of each plasma sample was stored at –80°C for GAG concentration analysis.

Urine sample collection—At the predetermined time points, a urine sample was collected from each clinically normal cat and each cat with IC in a similar manner. The urine sample was obtained via urinary bladder catheterization or during voiding of urine (midstream sample). Analysis was performed by use of a commercial dipstick[d]; data recorded included pH and estimates of concentrations of protein and blood. Urine specific gravity was measured by use of a refractometer. Urine sediment was examined microscopically for presence of casts, RBCs, WBCs, and crystals. Urine protein concentration was measured by use of the Bradford method,[24] and creatinine concentration was measured by use of the alkaline picrate method.[21] Urine samples were inoculated into the transport medium and subjected to microbiological analysis to determine whether bacterial culture was warranted. The remainder of each urine sample was also centrifuged[e] at 700 × *g* for 30 minutes at 4°C, and the supernatant was stored at –20°C for GAG concentration analysis.

Extraction and purification of plasma GAGs—To cleave covalent O-linkages between protein and carbohydrate and to release the GAG chains from proteoglycans or peptidoglycans, 0.1 mL of plasma from each cat was placed in 0.1 mL of 0.5M NaOH at 37°C for 12 hours. The GAG chains were isolated via ion exchange chromatography on a highly cross-linked beaded agarose matrix[f] in chloride form, according to the method reported by Pereira et al.[14]

Extraction and purification of GAGs in urine samples—Each urine sample was diluted with an equal volume of distilled water and adjusted to a pH of 4.0 to 4.5 with 1M HCl. Cetyltrimethylammonium bromide (high-purity grade) solution[g] was added (final concentration, 1 g/L). The solution was incubated at 4°C for 24 hours and centrifuged. The precipitate was washed 2 times with ethanol, dried at 37°C, and dissolved in 0.5 mL of 0.1M NaOH.[25]

Plasma GAG concentration—Sulfated GAGs in plasma were quantified by use of a spectrophotometric method with DMB.[h] The method was modified from that reported by Farndale et al[26]; 2.4 mL of a solution containing 16 µg of DMB/mL (with 3.04 g of glycine, 2.37 g of NaCl, and 95 mL of 0.1M HCl) was mixed with 0.1 mL of plasma. After 5 minutes, the absorbance (at 525 nm) of the mixture was measured by use of a spectrophotometer[i] with a semimicro cuvette.[j] Chondroitin 4-sulphate sodium salt derived from bovine tracheas[k] was used to prepare a standard curve (calibration interval, 0 to 100 mg/L). The results were expressed as plasma GAG concentrations (in µg/mL).

Urine GAG concentration—Sulfated GAGs in urine were quantified by use of a spectrophotometric method with DMB,[h] as reported by Panin et al[27]; 2.5 mL of a solution containing 10.67 µg of DMB/mL (with 5 mL of ethanol, 2 g of sodium formate, and 2 mL of formic acid) was mixed with 0.1 mL of urine and adjusted to a total volume of 3 mL with distilled water. After 5 minutes, the absorbance (at 525 nm) of the mixture was measured by use of a spectrophotometer[i] with a semimicro cuvette.[j] Chondroitin 4-sulphate sodium salt derived from bovine tracheas[k] was used to prepare a standard curve (calibration interval, 0 to 100 mg/L).

The urine GAG concentrations were corrected with regard to the amount of creatinine, as measured by use of the alkaline picrate method. The final results were expressed as both urine GAG concentration (µg/mL) and the GAG-to-creatinine concentration ratio ($\times 10^{-3}$).

Statistical analysis—Data regarding signalment, type of diet, water intake, lifestyle, and environment and urinalysis results were evaluated as descriptive statistics. Results of CBCs and plasma biochemical analyses are reported as mean ± SEM, and values obtained before and after NAG or placebo treatment were compared by use of paired *t* tests. Mean ± SEM values of plasma GAG concentration, urine GAG concentration, urine creatinine concentration, and GAG-to-creatinine concentration ratios in each group were calculated. These values were compared between the placebo and the treatment groups and between the clinically normal cats and cats with IC by use of Student *t* tests. Paired *t* tests were used for within-group comparisons. All analyses were performed by use of statistical computer software.[l] A value of $P < 0.05$ was considered significant.

Results

Cats—Ten clinically normal cats and 19 cats with IC were used in the study. All clinically normal cats were domestic shorthair cats. Mean ± SEM age of these cats was 4.0 ± 0.5 years (range, 1.0 to 6.0 years); mean weight was 3.98 ± 0.51 kg (range, 2.8 to 5.0 kg). There were 6 sexually intact males, 3 sexually intact females, and 1 spayed female. The results of CBCs, plasma biochemical analyses, and urinalysis performed on samples collected from these cats were within reference ranges, confirming their clinically normal status.

Nineteen cats with IC were used in the study. The breeds of the cats with IC were domestic shorthair (n = 15), Siamese (2), and Persian (2). Mean age of the cats with IC was 4.41 ± 3.92 years old (range, 1 to 18 years); mean weight was 4.62 ± 0.97 kg (range, 3.38 to 7.8 kg). There were 11 castrated males, 6 sexually intact males, and 2 spayed females. Of the cats with IC, 10 lived outdoors and 9 lived indoors. Seventeen cats with IC each lived in a household with other cats; 2 cats each lived in a household with no other cats. The types of food consumed by the cats with IC were dry food (n = 10), canned and dry food (7), and homemade diets (2). All cats with IC received water ad libitum.

Of the 19 cats with IC, 7 were assigned to the placebo group and 12 were assigned to the treatment group. Cats in the treatment group received 250 mg of NAG orally once daily for 28 days, and cats in the placebo group received a placebo orally once daily for the same

period; all doses of NAG or placebo were administered successfully to all cats with IC.

Clinical signs in cats with IC—At the initial evaluation, clinical signs among the cats with IC included stranguria, hematuria, periuria, signs of pain during urination, and pollakiuria. Ultrasonography of the lower urinary tract revealed an irregular mucosal surface of the urinary bladder wall in all cats with IC. The double-contrast cystography also demonstrated no uroliths in the urinary bladder. For the clinical signs, owners of NAG-treated cats with IC reported fewer signs of pain at urination on day 28 of the treatment.

Clinicopathologic data from cats with IC—Results of CBCs and plasma biochemical analyses were

Table 1—Results of analyses of urine samples collected from cats with IC before (day 0) and after treatment for 28 days with once-daily oral administration of NAG (250 mg [treatment group]) or placebo (capsule containing cellulose [placebo group]).

	Treatment group (n = 12)			Placebo group (n = 7)		
Variable	**Day 0 (n = 12)**	**Day 28 (n = 12)**	**Day 56 (n = 9)**	**Day 0 (n = 7)**	**Day 28 (n = 7)**	**Day 56 (n = 6)**
Urine protein*	3+ (2) 2+ (1) 1+ (8) None (1)	3+ (1) 2+ (2) 1+ (5) None (4)	3+ (1) 2+ (2) 1+ (4) None (2)	3+ (3) 2+ (2) None (2) —	3+ (1) 2+ (3) 1+ (2) None (1)	2+ (2) 1+ (2) None (2) —
Urine specific gravity	> 1.050 (3) 1.049 (2) 1.045 (1) 1.040 (1) 1.032 (1) 1.026 (2) 1.021 (1) 1.015 (1)	> 1.050 (8) 1.049 (1) 1.039 (1) 1.024 (1) 1.020 (1) — — —	> 1.050 (3) 1.042 (1) 1.040 (1) 1.034 (1) 1.028 (1) 1.024 (1) 1.015 (1) —	> 1.050 (4) 1.040 (1) 1.035 (1) 1.028 (1) — — — —	> 1.050 (6) 1.028 (1) — — — — — —	> 1.050 (4) 1.050 (1) 1.028 (1) — — — — —
pH†	9 (2) 8 (3) 7 (3) 6 (3) 5 (1)	8 (1) 7 (5) 6 (4) 5 (2) —	9 (1) 8 (1) 7 (4) 6 (2) 5 (1)	9 (2) 7 (2) 6 (3) — —	9 (1) 8 (2) 7 (2) 6 (1) 5 (1)	8 (1) 7 (3) 6 (2) — —
RBC count (No. of cells in sediment/hpf)	TNTC (2) 50–100 (1) 20–30 (1) 10–20 (1) 5–10 (1) None (6)	TNTC (1) 3–5 (1) None (10) — — —	100–200 (1) 100 (1) 10–20 (2) 3–5 (1) 2–3 (1) None (3)	TNTC (2) 100–200 (1) 50–100 (1) 10–20 (1) 0–1 (1) None (1)	TNTC (2) None (5) — — — —	100–200 (1) None (5) — — — —
WBC count (No. of cells in sediment/hpf)	TNTC (1) 20–30 (1) 5–10 (1) 3–5 (1) 2–3 (1) None (7)	30–50 (1) 1–2 (2) None (9) — — —	TNTC (1) 10–20 (1) 5–10 (1) 1–2 (2) None (4) —	TNTC (1) 3–5 (3) 1–2 (1) None (2) — —	30–50 (1) 3–5 (1) None (5) — — —	20–30 (1) None (5) — — — —
Amount and type of crystals in sediment	2+ struvite (1) 1+ calcium oxalate (1) Rare struvite (3) None (7) —	2+ struvite (1) Rare struvite (2) Amorphous (1) None (8) —	3+ struvite (1) 1+ struvite (2) None (6) — —	3+ struvite (1) 2+ struvite (1) Rare struvite (2) Rare calcium oxalate (1) None (2)	Rare struvite (2) MAP and calcium oxalate (1) None (4) — —	None (6) — — — — —

Urine samples were collected immediately before the first dose on day 0 and before dose administration on subsequent evaluation days; urine samples were also collected on days 7, 14, and 21 (data not shown). Numbers in parentheses represent the number of cats in each result category; not all urine samples collected for some cats in the 2 groups were available for analysis. The amount of crystals in sediment was scored in increasing order as none, rare, 1+, 2+, or 3+. Urinalysis did not detect glucose and ketone throughout the study in either group of cats.

*Assessed by use of a dipstick. †Assessed by use of a strip test.

— = Not applicable. MAP = Magnesium ammonium phosphate. TNTC = Too numerous to count.

Table 2—Mean ± SEM plasma GAG concentration (µg/mL) in cats with IC before (day 0), during (days 7, 14, 21, and 28), and after (days 28 and 56) treatment for 28 days with once-daily oral administration of NAG (250 mg; treatment group) or placebo (capsule containing cellulose [placebo group]).

	Day					
Group	**0**	**7**	**14**	**21**	**28**	**56**
Treatment (n = 12)	27.46 ± 3.90 (11)	31.89 ± 6.02 (10)	36.22 ± 6.75 (11)	39.96 ± 5.34*† (10)	39.91 ± 6.74† (12)	32.81 ± 3.94 (10)
Placebo (n = 7)	27.01 ± 5.49 (7)	27.46 ± 4.95 (7)	21.94 ± 3.78 (7)	24.20 ± 3.35 (7)	23.44 ± 3.80 (7)	25.66 ± 6.20 (5)

In each group, the number of cats (value in parentheses) varied among time points depending on whether a sufficient volume of plasma was available for analysis.

*At this time point, value is significantly ($P < 0.05$) different from that of the placebo group. †Within a group, value at this time point is significantly different from the day 0 value.

obtained for cats in the treatment and placebo groups on days 0, 7, 21, 28, and 56. For all cats with IC, hematologic and plasma biochemical variables were within reference ranges. With the exception of monocyte counts, there were no significant differences in hematologic variables between the 2 groups of cats with IC at any time point; on day 56, the monocyte count in the treatment group was higher than the value in the placebo group (74 ± 23 cells/µL and 10 ± 10 cells/µL, respectively). There were no significant differences in BUN and creatinine concentrations between the 2 groups from day 0 to 56. Plasma ALT activity differed significantly ($P < 0.05$) between the treatment (167.2 ± 13.6 U/L) and placebo groups (90.5 ± 3.3 U/L) only on day 0. Plasma ALP activities of NAG-treated cats were 77.4 ± 2.7 U/L, 74.8 ± 13.5 U/L, 75.7 ± 1.6 U/L, 79.4 ± 17.9 U/L, and 83.9 ± 22.3 U/L on days 7, 14, 21, 28, and 56, respectively. All values were within reference limits.

Urinalysis data from cats with IC—Results of urinalyses were obtained for most cats in the treatment and placebo groups on days 0, 7, 21, 28, and 56. Hematuria and proteinuria were detected in the cats with IC on day 0 (**Table 1**). At the end of the dosing period (day 28), 2 of the 7 cats in the placebo group had RBCs in the urine sediment; however, only 2 of the 19 cats in the treatment group had RBCs in the urine sediment at that time point. In the treatment group, the amount of RBCs in urine sediment increased in most cats after administration of NAG was discontinued. The urine specific gravity ranged from 1.015 to > 1.050 in the placebo and treatment groups. Urine pH ranged from 5 to 9 in the 2 groups.

White blood cells were found in urine samples from 5 cats on day 0 and in urine samples from only 3 cats on day 28. After NAG treatment was discontinued, WBCs were found in urine samples from 8 cats with IC. Protein concentration (as determined by use of a commercial dipstick[d]) ranged from 0 to 3+ in the treatment and placebo groups.

Magnesium ammonium phosphate crystals were detected in most of the urine samples collected from cats with IC on day 0; calcium oxalate crystals were also detected in 2 cats at that time point. No casts were detected in the cats with IC from day 0 to 56 with the exception of rare waxy casts that were detected in only 1 cat on day 28. Motile rod bacteria, epithelial cells (eg, transitional and squamous cells), RBC clumps, WBC clumps, debris, and fat droplets were detected in the urine of cats with IC on day 0.

Plasma GAG concentrations in cats with IC and clinically normal cats—In the NAG-treated cats with IC, mean plasma GAG concentration was significantly ($P < 0.05$) increased on days 21 and 28, compared with the day 0 value (**Table 2**). Mean plasma GAG concentration in the treatment group was significantly ($P < 0.05$) higher than value in the placebo group on day 21. However, mean plasma GAG concentrations in the clinically normal cats and the cats with IC as a group were not significantly different (**Table 3**).

Urine GAG concentrations in cats with IC and clinically normal cats—Mean urine GAG concentration in the placebo-treated cats with IC was significantly ($P < 0.05$) lower than the value in the NAG-treated cats with IC on day 14 (**Table 4**). In addition, urine creatinine concentration in the placebo group was significantly ($P < 0.05$) higher than the value in the treatment group on day 28. Mean urine GAG concentration (on

Table 3—Mean ± SEM plasma and urine GAG concentrations, urine creatinine concentration, and GAG-to-creatinine concentration ratio in 18 cats* with IC (samples obtained prior to treatment [day 0] with NAG or placebo) and 10 clinically normal cats.

Variable	Cats with IC	Clinically normal cats
Plasma GAG concentration (µg/mL)	27.29 ± 3.10	26.09 ± 2.94
Urine GAG concentration (µg/mL)	7.97 ± 1.20†	44.26 ± 6.16
Urine creatinine concentration (mg/dL)	3.36 ± 0.38	3.89 ± 0.61
Urine protein-to-creatinine concentration ratio	0.58 ± 0.20†	0.09 ± 0.02
GAG-to-creatinine concentration ratio ($\times 10^{-3}$)	3.11 ± 0.62‡	14.23 ± 3.47

*Sufficient volumes of plasma and urine for analysis were not collected from 1 cat on day 0. †For this variable, value in cats with IC is significantly ($P < 0.001$) different from the value in clinically normal cats. ‡For this variable, value in cats with IC is significantly ($P < 0.05$) different from the value in clinically normal cats.

Table 4—Mean ± SEM urine protein, creatinine, and GAG concentrations; urine protein-to-creatinine concentration ratio; and urine GAG-to-creatinine concentration ratio in cats with IC before (day 0), during (days 7, 14, and 21), and after (days 28 and 56) treatment for 28 days with once-daily oral administration of NAG (250 mg [treatment group]; n = 12) or placebo (capsule containing cellulose [placebo group]; 7).

		Day					
Variable	Group	0	7	14	21	28	56
Urine protein concentration (mg/dL)	Treatment	49.39 ± 9.93	ND	ND	ND	62.01 ± 17.8	62.11 ± 22.94
	Placebo	321.1 ± 103.06	ND	ND	ND	146.65 ± 86.7	46.55 ± 11.24
Urine creatinine concentration (mg/dL)	Treatment	2.90 ± 0.47	3.31 ± 0.49	3.16 ± 0.52	3.28 ± 0.51	3.01 ± 0.34	3.57 ± 0.62
	Placebo	4.08 ± 0.60	3.48 ± 0.38	3.34 ± 0.60	4.05 ± 0.65	4.40 ± 0.34*	4.32 ± 0.49
Urine GAG concentration (µg/mL)	Treatment	8.13 ± 1.92	10.80 ± 2.30	9.15 ± 1.39*	9.48 ± 1.87	12.67 ± 2.55	13.65 ± 2.69
	Placebo	7.71 ± 0.92	7.76 ± 1.20	4.89 ± 1.08	6.60 ± 1.78	8.94 ± 4.50	8.13 ± 2.77
Urine protein-to creatinine concentration ratio	Treatment	0.46 ± 0.26	ND	ND	ND	0.2 ± 0.05	0.16 ± 0.04
	Placebo	1.01 ± 0.36	ND	ND	ND	0.34 ± 0.21	0.12 ± 0.05
Urine GAG-to-creatinine concentration ratio ($\times 10^{-3}$)	Treatment	3.71 ± 0.97	3.30 ± 0.57	4.30 ± 1.44	3.07 ± 0.52	5.09 ± 1.25	4.55 ± 1.16
	Placebo	2.17 ± 0.38	2.40 ± 0.47	1.56 ± 0.42	1.60 ± 0.50	2.08 ± 1.12	1.93 ± 0.63

Sufficient volumes of plasma and urine for analysis were not collected from 1 NAG-treated cat on day 0.
ND = Not determined.
See Table 2 for remainder of key.

day 0) in the cats with IC as a group was significantly ($P < 0.001$) greater than the value in the clinically normal cats (Table 3).

Urine GAG-to-creatinine concentration ratio in cats with IC and clinically normal cats—Mean urine GAG-to-creatinine concentration ratio in NAG- and placebo-treated cats with IC fluctuated during the study period (Table 4). In the placebo group, the ratio was slightly increased or decreased from the day 0 value at the subsequent time points; in the treatment group, the magnitude of changes from the day 0 value appeared slightly greater. However, the mean ratios at days 7, 14, 21, 28, and 56 did not differ significantly from the day 0 value in either group. Mean urine GAG-to-creatinine concentration ratio (on day 0) in the cats with IC as a group was significantly ($P < 0.05$) lower than the value in the clinically normal cats (Table 3).

Discussion

The mean age of the cats with IC in the present study was 4.41 ± 3.92 years, which is consistent with reports[28,29] that cats that are 2 to 7 years old have an increased risk of developing FLUTD. Domestic shorthair, the most popular breed of cat in Thailand, was the most common breed among the cats with IC in the present study. There were 2 purebred cats with IC: a Siamese and a Persian. However, specific breed predispositions for development of IC have not been identified in cats,[29] to our knowledge. Most (11/19) of the cats with IC were castrated males, which is consistent with findings of other previous reports.[2,28] In contrast, no sexually intact female cats with IC were included in the study; overall, female cats have a lower risk for development of FLUTD[28] and sexually intact females have lower risk for IC.[28] Although there are many previous reports[28] about the association between neutered male cats and the development of IC, none of those reports explained the exact mechanism of how sex and IC are associated. The cats with IC in the present study were overweight; overweight cats are also at greater risk for development of FLUTD.[28]

The results of the present study also suggested that indoor and outdoor cats have an equal chance of developing IC. In another study,[11] FLUTD developed more commonly in indoor cats. It has been postulated that cats with IC have increased stress response and decreased adrenocortical function.[30,31] Furthermore, it has been suggested that the environment of some indoor cats can be very stressful and that indoor cats may be more stressed than outdoor cats.[11] Conflicts between cats with IC and other cats within the same household has been identified as a source of stress.[7] Seventeen of the 19 cats with IC in the present study lived in a household with > 1 cat; thus, they could have been more stressed than were the clinically normal cats.

In the present study, more cats with IC were fed dry cat food than other types of diet. Dry cat food is a risk factor for the formation of uroliths[8,32] and development of IC.[33,34] Previous studies[33,34] also revealed that cats with IC are typically fed dry food more often than are cats without IC. Decreased water intake is also a risk factor for development of IC. Supersaturation of urine is possible in cats that are fed dry food or that have low water intake; as substances in the urine become more concentrated, irritation of the urinary bladder occurs. All cats with IC in the present study received water ad libitum.[8] It was also recommended to owners to provide an adequate daily water consumption for the purpose of decreasing the concentration of substances in the cats' urine.[8]

Results of CBCs and plasma biochemical analyses were within reference ranges for all cats at all time points in the present study. Because the cats with IC that received NAG had no abnormal clinicopathologic findings, this suggests that administration of NAG to cats with IC causes no adverse effects. A similar lack of adverse effects was also evident in another study[33] of cats with IC treated with NAG for 6 months and in a study[35] in rats treated with NAG for 13 weeks. In the present study, the monocyte counts in the NAG- and placebo-treated cats with IC were significantly different at day 56 (ie, 28 days after administration of the last dose); the monocyte count in cats that had received NAG once daily for 28 days was higher than the value in cats that had received placebo. This difference may be attributable to an effect of NAG on the phagocyte activation of macrophages. One study[36] reported that the number of WBCs in the treatment group was higher than the number of WBCs in the placebo group, which may be a result of a biological effect of NAG on the number of polymorphonuclear cells and their chemotactic activity.

The results of the urinalyses indicated that the cats with IC in the present study had hematuria and proteinuria on day 0. Hematuria and proteinuria without pyuria or bacteriuria in cats with IC has been reported.[2,29] Hematuria is associated with IC more frequently than it is with other urinary tract problems such as urolithiasis or urinary tract infection.[37] After treatment with NAG for 28 days, only 2 of the 12 cats in the treatment group had detectable RBCs in the sediment of urine samples; however, the proportion of cats with RBCs in urine sediment increased after NAG administration was discontinued for 28 days (ie, on day 56). This may be attributable to the action of NAG on the urinary bladder wall and its effect on inflammatory processes because exogenous NAG helps to maintain the amount of GAGs in the urinary bladder wall, thereby preventing development of IC–induced inflammation.[38]

The cats with IC in the present study had concentrated urine, and urine pH was variable. Cats with IC typically have concentrated and acidic urine.[29] The presence of crystals in the sediment of urine samples collected from the cats with IC also varied; however, urine crystals may have no clinical importance in cats because the crystals do not damage healthy urothelium.[2] The sediments of urine samples that have been refrigerated or stored for hours also contain crystals, and this phenomenon is exaggerated in urine that is highly concentrated.[39,40] In a previous study,[28] 31 of 62 (50%) cats with IC had crystalluria.

In the present study, the mean urine GAG concentration and urine GAG-to-creatinine concentration ratio in the clinically normal cats were similar to the values determined in clinically normal cats in a previ-

ous investigation.[13] In that investigation, urine samples were evaluated by use of DMB chloride, which was the same method used in the present study. Comparison of mean urine GAG concentrations in the clinically normal cats and the cats with IC in the present study revealed that the concentration in cats with IC was significantly lower, which is consistent with results of previous studies.[13,14] This difference may be the result of an increase in the permeability of the urinary bladders of cats with IC, compared with bladder permeability in clinically normal cats.[9] Similar differences in urine GAG concentrations between women with and without IC have also been observed.[41,42]

In contrast, mean plasma GAG concentrations of the placebo group were consistently lower than the concentrations in the treatment group, although this difference was significant only on day 21. In the treatment group, mean plasma GAG concentrations on days 21 and 28 were significantly higher than the value on day 0. These findings suggested that the NAG administered orally to the cats with IC in the treatment group was absorbed from the gastrointestinal tract and passed into the circulatory system, resulting in an increase in plasma GAG concentration during the treatment period. In a study by Talent and Gracy,[43] patients receiving NAG orally for treatment of osteoarthritis also had increased plasma concentrations of GAGs, compared with poly-NAG, a sustained-release form of NAG. On the basis of the results of the present study, it appears that it may take up to 21 days to increase plasma GAG concentrations in cats with IC that are receiving oral treatment with NAG (250 mg) once daily.

To our knowledge, no previous studies of the effect of NAG administration on urine GAG concentrations in cats with IC have been reported. Overall, mean urine GAG concentrations in the cats with IC that received NAG orally were higher than the concentrations in the cats with IC that received placebo in the present study; on day 14, this difference was significant. N-acetyl-D-glucosamine is a substrate for GAG biosynthesis, but its half-life is relatively short.[43] Results of a previous study[44] of the metabolism of glucosamine and NAG indicated that NAG was comparatively less completely oxidized in the body, resulting in greater excretion in the urine. N-acetyl-D-glucosamine can also be converted to glucosamine in vivo.[43,45] An investigation of endogenous GAGs for treatment of IC revealed that biotinylated pentosanpolysulfate and heparin had no or weak binding to rabbit and human urinary bladders.[46] However, NAG can bind to lectins in the bladder epithelium.[47] Therefore, endogenous NAG is a more suitable form to bind with the epithelium than other forms of GAGs.

The results of the present study indicated that once-daily oral administration of 250 mg of NAG to cats with IC for 28 days was not associated with adverse effects. Similarly, no adverse effects were detected in rats following oral administration of NAG at a concentration of 5% in their food for 13 weeks.[35] The apparent increase in urine GAG concentration and subjective decrease in the degree of hematuria along with improvements in clinical signs in the cats with IC that received NAG treatment suggest that there are clinical benefits of NAG administration in cats with IC. Cystoscopic investigation of the effect of NAG treatment on the urinary bladder epithelium in cats with IC is needed.

a. Cystaid, VetPlus Ltd, Lytham St Annes, Lancashire, England.
b. Provided by TJ Animal Health (Thailand), Co Ltd, Bangkok, Thailand.
c. Biofuge 22R, Heraeus, Osterode, Germany.
d. Combur test, Roche Diagnostic, Mannheim, Germany.
e. IEC CR-6000, Damon/IEC Division, Needham Heights, Mass.
f. HiTrap Q Sepharose Fast Flow, GE Healthcare Bio-Science Corp, Piscataway, NJ.
g. Cetyltrimethylammonium bromide, Fluka Chemie AG, Buchs, Switzerland.
h. Sigma-Aldrich Chemical Co, St Louis, Mo.
i. Evolution 60 UV-Visible spectrophotometer, Thermo Fisher Scientific Inc, Waltham, Mass.
j. Brand GMBH + CO KG, Wertheim, Germany.
k. Bovine trachea-derived chondroitin 4-sulphate sodium salt, Fluka Chemie AG, Buchs, Switzerland.
l. SPSS, version 17.0, SPSS Co, Chicago, Ill.

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