J Vet Intern Med 2000;14:503–506

Serum Alpha 1–Acid Glycoprotein Concentrations in Healthy and Tumor-Bearing Cats

K.A. Selting, G.K. Ogilvie, S.E. Lana, M.J. Fettman, K.L. Mitchener, R.A. Hansen, K.L. Richardson, J.A. Walton, and M.A. Scherk

The purpose of this study was to evaluate alpha 1–acid glycoprotein (AGP) concentrations in tumor-bearing and healthy cats. The hypothesis of the present study was that AGP concentrations would be significantly increased in tumor-bearing cats. Serum from 51 healthy and 97 tumor-bearing, client-owned cats was harvested at the time of presentation and stored at −80°C until assayed. Cats with measurable, histologically confirmed malignancies, and healthy cats of similar ages were included. Serum was assayed for AGP concentration by using a radial immunodiffusion method. AGP concentrations were significantly ($P = .0051$) higher in tumor-bearing (763 ± 595 μg/mL; mean ± SD) when compared to healthy cats (501 ± 377 μg/mL; mean ± SD). Of the tumor-bearing cats, 35 had carcinomas, 33 had sarcomas, and 26 had discrete, round cell tumors. AGP concentrations were 645 ± 62 μg/mL, 660 ± 540 μg/mL, and 967 ± 860 μg/mL, respectively, and there were no significant differences among the groups.

Key words: Acute phase reactant proteins; Feline; Neoplasia.

Alpha 1–acid glycoprotein (AGP) is an acute phase reactant protein (APRP) found in the seromucoid portion of the perchloric acid–soluble fraction of serum.[1,2] It is produced by hepatocytes[1–7] and lymphocytes[6] in response to cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor alpha (TNF-α).[8,9] It is composed of a single polypeptide chain containing approximately 45% carbohydrate,[6,7] and its conformation resembles beta-adrenergic receptors.[10] There are 2 forms of AGP. The 1st has a molecular weight of approximately 54,000 Da and is produced by lymphocytes. The 2nd form (orosomucoid) has a molecular weight of approximately 40,000 Da and is found in serum, likely as a result of cleavage of sialic acid residues from the 54,000-Da form.[2,6] The role of AGP has not been elucidated, but its serum concentration has been shown to be increased in humans and animals with various types of cancer.[1,3–5,9,11–20] In humans, AGP concentrations have been useful in staging and monitoring malignancies such as lymphoma,[4] colorectal cancer,[5,16,20] cervical cancer,[14] and esophageal cancer,[15] with AGP concentrations being increased with active disease.

Regarding function, one author suggests that AGP (described as an antiprotease) may inhibit tumor-produced enzymes, thereby decreasing invasion to surrounding tissues.[16] AGP also inhibits lymphocyte proliferation, platelet aggregation, and neutrophil function, including phagocytosis, chemotaxis, and superoxide anion generation, and its biological effects may depend on its state of glycosylation.[21] It follows that APRPs may play a role in the defense mechanisms of animals against microbial infection.[22] AGP also has been shown to neutralize the effects of TNF-α in an experimental murine shock model when given preemptively.[8] Lastly, AGP has been investigated for its role in the pharmacokinetics and pharmacodynamics of basic protein-bound drugs. As a saturable or specific binder, it can decrease the amount of free drug in blood.[10,23,24]

Serum AGP concentrations are increased both in patients with cancer and in those with nonmalignant illness. Species in which AGP concentrations have been studied with illnesses other than cancer include humans,[7,9,18] dogs,[3,11] cats,[22,25] pigs,[26] chickens,[21,27,28] and cows.[12] Illness included both viral and inflammatory processes. Clinical studies of veterinary cancer patients are few but include a recent study of dogs with lymphoma in which serum AGP concentrations correlated with remission status. AGP concentrations were markedly higher in dogs with lymphoma than in healthy dogs, and concentrations decreased with remission and increased before clinical detection of recurrent disease.[11] A marked increase in AGP in tumor-bearing dogs is not always present, and one report of dogs with both benign and malignant mammary tumors, as compared to healthy dogs, did not find a statistically significant difference among groups.[29]

To date, few data exist regarding the presence of a similar pattern of AGP concentrations in cats. One report in cats with lymphoma demonstrated significantly increased AGP concentrations in tumor-bearing cats ($n = 9$) when compared to healthy cats ($n = 25$), but a loss of remission was not preceded by an increase in serum AGP concentration.[19] The purpose of this study was to evaluate AGP concentrations in large numbers of tumor-bearing and healthy cats. We hypothesized that serum concentrations of AGP would be markedly increased in cats with malignancies compared to healthy cats.

Materials and Methods

Tumor-Bearing Cats

Ninety-seven client-owned tumor-bearing cats were evaluated from November 1997 to March 1999. Cats did not have a history of prior definitive treatment for their malignancy and ranged in age from 2 to 18 years. All tumor types were accepted, and diagnosis was confirmed histologically. There were 35 carcinomas, 33 sarcomas, and 26 dis-

From the Comparative Oncology Unit, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO (Selting, Ogilvie, Lana, Fettman, Hansen, Richardson, Walton); MedVet Memphis, Memphis, TN (Mitchener); and Cats Only Veterinary Clinic, Vancouver, BC, Canada (Scherk). *Previously presented at the Veterinary Cancer Society meeting, November 13–16, 1999, in Wood's Hole, MA.*

Reprint requests: Kim Selting, Colorado State University Veterinary Teaching Hospital, 300 W Drake, Fort Collins, CO 80523; e-mail: kselting@vth.colostate.edu.

Submitted January 11, 2000; Revised March 31, 2000; Accepted June 1, 2000.

0891-6640/00/1405-0007/$3.00/0

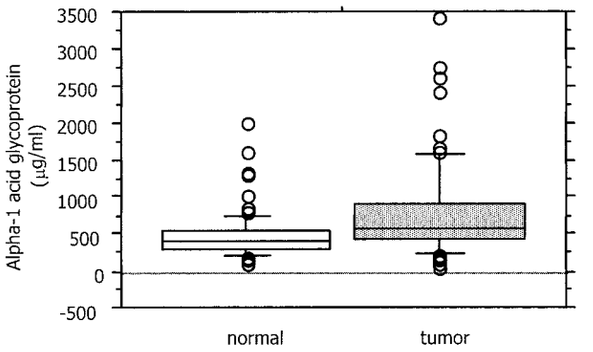

Fig 1. Distribution of alpha 1–acid glycoprotein concentrations in healthy and tumor-bearing cats. The box plot part of the graph demonstrates the 10th, 25th, 50th, 75th, and 90th percentiles with the horizontal lines from bottom to top, where the middle 3 values constitute the enclosed box. Additionally, scatter plots outside the lines represent outlying values. Although these data are normally distributed, this format is used to better demonstrate the data given the overlap of values. Difference between the mean values was significant with $P = .0051$.

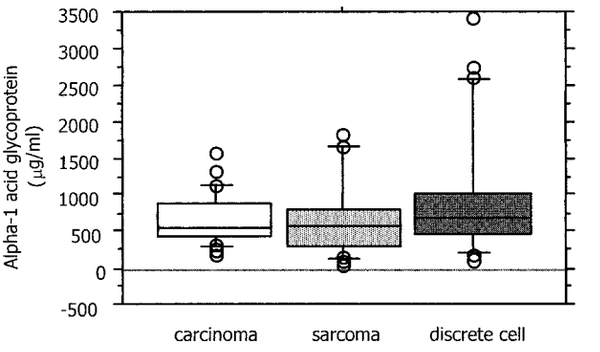

Fig 2. Relative concentrations of alpha 1–acid glycoprotein with respect to tumor type. Differences between any 2 groups were not found to be statistically significant. Data are displayed in the same manner as in Figure 1.

crete, round cell tumors (22 lymphomas, 2 melanomas, and 2 mast cell tumors). Serum was collected at the time of initial diagnostic evaluation and stored at −20°C until it was shipped to a central location, where samples were stored at −80°C until assayed. Duration of storage was not recorded, but previous studies have revealed that freeze-thaw cycles do not affect results.[1]

Healthy Cats

Fifty-one client-owned healthy cats were evaluated. All were presented with no history of illness between February 1998 and April 1999 for routine physical examination. Serum was collected and stored in the same manner as described for tumor-bearing cats.

Analytical Methods

Serum AGP concentration was determined by use of the single radial immunodiffusion (SRID) method of Mancini et al,[30] with minor modifications.[a] Briefly, agarose gel containing anti-feline AGP rabbit sera was prepared on a plastic container, and 2.5-mm-diameter wells were punched out. Serum samples were applied to the wells (5 μg per well). After the gels were incubated for 24 hours at 21°C in a humid chamber, the diameter of the precipitin ring was measured to the nearest 0.1 mm. Serum AGP concentration was determined for each sample by comparing the diameter of its precipitin ring with a standard diameter-concentration curve prepared using standards with known AGP concentrations (500 and 2000 μg/mL) provided in the kit. As described by the company, the SRID test reacts specifically and exclusively with AGP of cats, has a coefficient of variation less than 4%, and is accurate within a range of 100–3000 μg/mL.

Statistical Analysis

Data were evaluated using the Kolmogorov-Smirnov test for normality of distribution. Regression analysis was used to examine the relationship between serum AGP concentration and age. Comparison of serum AGP concentrations between tumor-bearing and healthy cats was done by unpaired *t*-test. A 1-way analysis of variance was used to compare the 3 tumor types. A value of $P < .05$ was considered significant.[31]

Results

Healthy cats had a median age of 10 years (range, 2–18 years) with a mean of 10 ± 4 years (± SD). The tumor-bearing cats had a median age of 12 years (range, 2–18 years) with a mean of 11 ± 4 years (± SD). Serum AGP concentrations (all data) were normally distributed but were not associated with age ($r^2 = 0.023$; $P = .0725$). Serum AGP concentrations for healthy cats were 501 ± 377 μg/mL (mean ± SD), with a median of 400 μg/mL (range = 90–2000 μg/mL). Serum AGP values for tumor bearing cats were 763 ± 595 μg/mL (mean ± SD), with a median of 580 μg/mL (range = 30–3430 μg/mL). Mean concentrations for tumor-bearing cats were significantly higher ($P = .0051$) than for healthy controls. However, the range of serum AGP concentrations for tumor-bearing cats completely included that of the healthy cats (Fig 1).

Comparison of AGP concentrations among tumor types identified differences in results for discrete, round cell tumors, carcinomas, and sarcomas. Carcinomas (n = 35) had a mean concentration of 645 ± 62 μg/mL (range 180–1580 μg/mL), and sarcomas (n = 33) had a mean concentration of 660 ± 540 μg/mL (range 30–1840 μg/mL), whereas discrete, round cell tumors (n = 26) had higher concentrations at 967 ± 860 μg/mL (range 110–3430 μg/mL). Comparing discrete, round cell tumors (primarily lymphoma) to carcinomas ($P = .0533$) and sarcomas ($P = .0953$), and carcinomas to sarcomas ($P = .8201$), there were no significant differences between any 2 groups (Fig 2).

Discussion

This study confirmed that serum concentrations of AGP are significantly increased in cats with cancer. AGP concentrations have been found to be increased in both dogs and humans in a variety of illnesses, including acute and chronic inflammation, pregnancy, and cancer.[1–5,9,11–21,25–28] Although its role in such processes remains unclear, AGP has been shown to be an important marker in monitoring therapy of human cancer patients.[1,4,16,20] A similar pattern may occur in dogs.[11]

Few studies report serum AGP concentrations in cats. One study evaluated different methods of analysis of cat plasma proteins from healthy cats and found that despite species differences, the basic order relationship of plasma proteins in cats is comparable to that found in humans.[32] In

one report AGP was used in the diagnosis of feline infectious peritonitis (FIP), and marked increases in serum AGP concentration were of value in distinguishing naturally occurring FIP from cats without FIP that had similar clinical signs. In the same study, marked increases of serum AGP concentrations were reported in cats with advanced feline immunodeficiency virus infection. As in humans and dogs, the test for AGP concentration in cats is sensitive, but it is not specific with regard to the underlying disease.[24] Also, the report on AGP values in cats with lymphoma demonstrated that AGP did not consistently correlate with disease status in that group of cats.[19]

AGP, as it relates to stage of disease, has been evaluated in some studies. In clinical studies of dogs, one study found no difference in AGP concentrations with respect to stage of lymphoma,[3] and the other study included only 1 stage of patients.[11] Another study of dogs, which investigated the correlation between serum AGP concentration and total sialic acid concentrations in dogs with mammary tumors, found no correlation of AGP concentrations with clinical stage.[29] In humans, data in cervical, esophageal, and colorectal cancer suggest that AGP concentration does correlate with clinical stage of disease.[5,15,16] Stage of disease was not assessed in the present study. Although differences among carcinomas, sarcomas, and discrete cell tumors were not significant in the present study, tumor type may have an influence on AGP concentration. In one study of humans, APRP concentrations were more markedly increased in Hodgkin's than in non-Hodgkin's lymphoma patients.[4] AGP has been shown to be produced by lymphocytes and has an amino acid sequence with marked homology to human immunoglobulin G. Disorders that result in lymphocyte proliferation may therefore contribute to increases in AGP concentration.[6] This observation may in part explain the higher concentrations found in discrete cell tumors, most of which were lymphoma.

A complete overlap in the ranges of serum AGP concentrations between healthy and tumor-bearing cats suggests that this assay may have limitations in monitoring the presence and progression of malignant disease in the cat. Despite wide variations and overlap, statistical analysis demonstrated that the increases in AGP concentration seen in cancer patients are more than expected by chance. The influence of occult chronic inflammatory processes in these cats cannot be ruled out, and the presence of such diseases may account for the variability in AGP concentrations. In one study, AGP concentrations were dramatically increased in cats hospitalized for undisclosed illness, as well as in experimentally and surgically induced inflammation when each group was compared to healthy cats, without overlap between groups.[22] This finding suggests that other factors, such as tumor burden, may account for the variation seen in the present study. Retroviral status was not available in enough cats to evaluate its effect on these results. Despite differences in AGP concentrations among species during the neonatal period, AGP concentration in the mature individual has been reported not to be influenced by age,[15,21,26,28] as observed in this study. Further studies must be performed to evaluate AGP concentrations in nonneoplastic inflammatory processes in cats, as well as to further understand the potential prognostic value of monitoring AGP concentrations in cats with cancer. As in human medicine, AGP may be most useful as one of several components of an APRP profile.[4,16,20]

Footnote

[a] Feline AGP measurement kit. Saikin Kagaku Institute Co, Ltd, Sendai, Japan

Acknowledgment

This study was supported in part by Hill's Pet Nutrition, Topeka, KS.

References

1. Ganz PA, Shell WE, Tokes ZA. Elevation of a radioimmunoassay for alpha 1–acid glycoprotein to monitor therapy of cancer patients. J Natl Cancer Inst 1983;71:25–30.

2. Easton JA, Hardwicke J, Whitehead PH. The estimation of two alpha 1 glycoproteins (orosomucoid and other alpha 1–acid glycoprotein) in health and disease. J Clin Pathol 1962;15:585–590.

3. Ogilvie GK, Walters LM, Greeley SG, et al. Concentration of alpha 1-acid glycoprotein in dogs with malignant neoplasia. J Am Vet Med Assoc 1993;203:1144–1146.

4. Child JA, Cooper EH, Illingworth S, Worthy TS. Biochemical markers in Hodgkin's disease and non-Hodgkin's lymphoma. Recent Results Cancer Res 1978;64:180–189.

5. Avail Lundqvist E, Blad E, Xiao L, et al. Pretreatment serum levels of C-reactive protein, alpha 1-antitrypsin, haptoglobin, alpha 1-acid glycoprotein and tissue polypeptide antigen in cervical carcinoma. Eur J Gynaecol Oncol 1991;12:375–383.

6. Gahmberg CG, Andersson LC. Leukocyte surface origin of human alpha 1-acid glycoprotein (orosomucoid). J Exp Med 1978;148: 507–521.

7. Bennet M, Schmid K. Immunosuppression by human plasma alpha 1-acid glycoprotein: Importance of the carbohydrate moiety. Proc Natl Acad Sci U S A 1980;77:6109–6113.

8. Libert C, Brouckaert P, Fiers W. Protection by alpha 1-acid glycoprotein against tumor necrosis factor-induced lethality. J Exp Med 1994;180:1571–1575.

9. Cooper EH, Stone J. Acute phase reactant proteins in cancer. Adv Cancer Res 1979;30:1–43.

10. Brambilla G, Fiori M, Curiel I, et al. Alpha 1–acid glycoprotein affinity columns for the clean up of adrenergic drugs. Third International Symposium on Hormone and Veterinary Drug Residue Analysis, Bruges, Belgium. Analyst 1998;123:2693–2696.

11. Hahn KA, Freeman KP, Barnhill BS, Stephen EL. Serum alpha 1–acid glycoprotein concentrations before and after relapse in dogs with lymphoma treated with doxorubicin. J Am Vet Med Assoc 1999; 214:1023–1025.

12. Oonaru K, Takahashi K, Kurosawa T, et al. Serum alpha 1-acid glycoprotein in cattle with neoplastic and inflammatory diseases. J Jpn Vet Med Assoc 1990;43:19–23.

13. Bartos H, Lowinger J. Serum mucoids in lymphoma. JAMA 1968;204:1147–1148.

14. Xie JF. Clinical significance of serum ferritin and acute phase reactant protein levels in patients with cervical cancer. Chung Hua Fu Chan Ko Tsa Chih 1991;26:92–94.

15. Saito T, Kuwahara A, Shimoda K, et al. Factors influencing the acute phase protein levels in patients with esophageal cancer. Jpn J Surg 1991;21:402–411.

16. Stamatiadis AP, St Toumanidou M, Vyssoulis GP, et al. Value

of serum acute-phase reactant proteins and carcinoembryonic antigen in the preoperative staging of colorectal cancer. Cancer 1990;65:2055–2057.

17. Durdey P, Cooper JC, Switala S, et al. The role of peptidases in cancer of the rectum and sigmoid colon. Br J Surg 1985;72:378–381.

18. Shetlar MR, Bullock JA, Shetlar CL, Payne RW. Comparison of serum C-reactive protein, glycoprotein and seromucoid in cancer, arthritis, tuberculosis and pregnancy. Proc Soc Exp Biol Med 1955; 88:107–109.

19. Correa SS, Mauldin GN, Mauldin GE, Mooney SC. Alpha 1-acid glycoprotein concentration in cats with lymphoma. VCS Proc 1998:17.

20. Ward AM, Cooper EH, Turner R, et al. Acute-phase reactant protein profiles: An aid to monitoring large bowel cancer by CEA and serum enzymes. Br J Cancer 1977;35:170–178.

21. Inoue M, Satoh W, Murakami H. Plasma alpha 1-acid glycoprotein in chickens infected with infectious bursal disease virus. Avian Dis 1997;41:164–170.

22. Kajikawa T, Furuta A, Onishi T, et al. Changes in concentrations of serum amyloid A protein, alpha 1-acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery. Vet Immunol Immunopathol 1999;68:91–98.

23. Son DS, Hariya S, Shimoda M, Kokue E. Contribution of alpha 1-acid glycoprotein to plasma protein binding of some basic antimicrobials in pigs. J Vet Pharmacol Ther 1996;19(3):176–183.

24. De Rick AF, Belpaire FM, Dello C, Bogaert MG. Influence of enhanced alpha 1-acid glycoprotein concentration on protein binding, pharmacokinetics and antiarrythmic effect of lidocaine in the dog. J Pharmacol Exp Ther 1987;241:289–293.

25. Duthie S, Eckersall PD, Addie DD, et al. Value of alpha 1-acid glycoprotein in the diagnosis of feline infectious peritonitis. Vet Rec 1997;141:299–303.

26. Itoh H, Tamura K, Izumi M, et al. The influence of age and health status on the serum alpha 1–acid glycoprotein level of conventional and specific pathogen-free pigs. Can J Vet Res 1993;57(2):74–78.

27. Takahashi K, Akiba Y, Tamura K. Effect of dietary ascorbic acid on the hepatic microsomal mixed function oxidase system in liver of chicks treated with Escherichia coli lipopolysaccharide. Comp Biochem Physiol 1997;118:301–304.

28. Takahashi K, Naji K, Akiba Y, et al. Plasma alpha 1-acid glycoprotein concentration in broilers: Influence of age, sex and injection of Escherichia coli lipopolysaccharide. Br Poult Sci 1994;35:427–432.

29. Thougaard AV, Hellmen E, Pedersen HD, Jensen AL. Correlation between alpha 1–acid glycoprotein and total sialic acid in serum from dogs with tumours. Zentralbl Veterinarmed A 1999;46:231–237.

30. Mancini G, Carbonara AP, Hermans JF. Immunochemical quantitation of antigen by single radial immunodiffusion. Immunochemistry 1965;2:235–254.

31. Glantz SA. Primer of Biostatistics, 4th ed. New York, NY: McGraw-Hill; 1997:32–89.

32. Furukawa T, Sugiyama F. Analysis of feline plasma proteins by immunoelectrophoresis and polyacrylamide gel electrophoresis. Jpn J Vet Sci 1986;48:643–653.