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Prognostic Factors in Dogs with Urinary Bladder Carcinoma

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Medical records and biopsy specimens were retrospectively reviewed from 25 dogs diagnosed with unresectable urinary bladder carcinoma and treated with chemotherapy. Our intention was to identify clinical, histologic, and immunohistochemical indicators of prognosis. Immunohistochemical stains for P-glycoprotein, glutathione-S-transferase π, and factor VIII–related antigen were applied to archived tissue. There were more spayed female dogs than castrated male dogs (76% versus 24%). Transitional cell carcinoma was the most common tumor (88%, n = 22), followed by undifferentiated carcinoma (8%, n = 2) and squamous cell carcinoma (4%, n = 1). Overall median survival was 251 days. Histologic diagnosis and immunohistochemical characteristics did not correlate with prognosis. Spayed females survived significantly longer than castrated males (358 days versus 145 days, $P = .042$). Dogs that received either doxorubicin or mitoxantrone in addition to a platinum-based chemotherapeutic (either cisplatin or carboplatin) lived significantly longer than those that received only a platinum compound (358 days versus 132 days, $P = .042$).

Key words: Carboplatin; Chemotherapy; Cisplatin; Doxorubicin; Glutathione-S-transferase; Mitoxantrone; P-glycoprotein; Transitional cell carcinoma.

Carcinoma of the urinary bladder is difficult to treat successfully in dogs. The most common treatment modality is chemotherapy with or without piroxicam. Median survival times are 4 to 9 months.[1–4] Factors that adversely influence response to treatment include the following: advanced stage at the time of diagnosis; anatomic location that prevents surgical excision or marked reduction in tumor size; and limited efficacy of chemotherapy with platinum compounds alone and standard radiation therapy protocols.[5–7] Factors that favorably influence prognosis include the following: tumor location within the urinary bladder that allows surgical extirpation,[5] absence of concurrent bladder and urethral involvement,[5] and male gender.[1]

Immunohistochemical examination of malignant urinary bladder cancer in humans is useful in identifying chemotherapy resistance and prognostic factors.[8–10] The detoxification and elimination of drugs and toxins from the body is one of the normal functions of the urinary tract. It has been postulated that as a consequence of this normal function, urinary tract cancer may be less sensitive to chemotherapy than neoplasia in other body systems. Mechanisms of this purported inherent drug resistance may include the cell membrane drug efflux pump, P-glycoprotein, as well as the cytoplasmic detoxification enzyme, glutathione-S-transferase π. Tumor angiogenesis, detectable by various endothelial antigens, including factor VIII–related antigen (factor VIII), is one indicator of poor prognosis in humans with bladder tumors.[11–12]

Given the generally poor response to treatment and paucity of prognostic variables identified for bladder carcinoma in the dog, this study was undertaken to determine if P-glycoprotein, glutathione-S-transferase π, or tumor angiogenesis as measured by factor VIII immunohistochemistry are associated with resistance to chemotherapy. Furthermore, we sought to identify prognostic indicators to aid clinicians in formulating a treatment plan and pet owners in deciding on a course of action for dogs with urinary bladder neoplasia.

Materials and Methods

Treatment records spanning January 1990 to March 1996 of the Donaldson-Atwood Cancer Clinic at the Animal Medical Center were reviewed. Criteria for inclusion in the study were the presence of a nonresectable trigonal mass (with or without urethral involvement, prostatic involvement, or both), availability of the medical record, and a paraffin-embedded tissue specimen suitable for immunohistochemical staining. Data retrieved from each record included the following: age, gender, breed, mass location within the urinary bladder, extent of surgery performed, evidence of metastasis, chemotherapy regimen used, chemotherapy toxicity, and length of survival.

All cases had an incisional biopsy of a nonresectable urinary bladder carcinoma located at the trigone. One of 3 chemotherapy regimens was used according to the clinician's preference: cisplatin[a] only, carboplatin[b] only, or cisplatin or carboplatin alternating with doxorubicin[c] or mitoxantrone.[d] Dosages of each drug used were calculated on the basis of body surface area (BSA) calculated with the following equation: $BSA = [10.1 \times (\text{body weight in grams})^{0.67}]/10^4$. The following dosages were used: cisplatin 60 mg/m^2, by using previously described diuresis and drug administration protocols[13]; carboplatin 150–250 mg/m^2; doxorubicin 25 mg/m^2 for dogs weighing 10 kg or less or 30 mg/m^2 for dogs weighing over 10 kg, given over 5–10 minutes; and mitoxantrone 5.5 mg/m^2. All treatments were given intravenously at 3-week intervals, alternating between the platinum drug and anthracycline in those patients receiving both. Other treatments, such as piroxicam, radiation therapy, and cystostomy catheters were not used.

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Assessment of response to treatment or tumor progression was by history, physical examination, thoracic and abdominal radiography, and abdominal ultrasonography. A standardized assessment of response to treatment was not possible, as this was a retrospective study. Dogs with rapid tumor progression or no detectable response to treatment were included in all analyses.

One of the authors (Patnaik) who was unaware of the treatment or outcome of any case reviewed all biopsy specimens. Tumors were classified according to type, depth of bladder wall involvement (in the specimens with full thickness biopsies), and evidence of lymphatic invasion. The squamous cell carcinoma and undifferentiated carcinomas were not assigned a histologic grade. A grade of 0 to 3 was assigned to each transitional cell carcinoma according to published criteria for human tumors.[14] Grade 0 specimens, which were designated papillomas, were not included in the study. The remaining specimens were designated carcinomas of low (grade 1), intermediate (grade 2), or high (grade 3) grade. The following factors were considered in order to assign a grade: orderliness of growth pattern, papillary or nodular growth, frequency of foci of heterologous differentiation (ie, squamous, glandular, etc), uniformity of cell size, infiltrative growth, cytoplasmic vacuolization, nuclear and nucleolar pleomorphism, chromatin pattern, and mitotic figures.

Immunohistochemical stains for P-glycoprotein C219,[15] glutathione-S-transferase π, and factor VIII were applied to specimens according to the instructions of Signet Laboratories, the manufacturer. Briefly, 0.3-μm-thick sections of formalin-fixed, paraffin-embedded biopsy specimens were placed on glass slides and treated with a proprietary agent[e] to enhance epitope availability in preserved specimens. Specimens were then exposed to the primary antibody against the target substance, followed by conjugation to a secondary antibody, biotinylated anti-immunoglobulin. This was followed by treatment of the slide with the labeling reagent, peroxidase-labeled streptavidin, followed by chromogen and counterstaining with hematoxylin. Positive control tissues were as follows: for P-glycoprotein C219, canine liver; for glutathione-S-transferase π, canine liver; for factor VIII, canine tonsil. Negative control tissues were processed as described, except that phosphate-buffered saline was substituted for the primary antibody. Formal validation of these stains for use in canine specimens has not been undertaken, but canine tissues analogous to the human tissues recommended as positive controls by the manufacturer stained appropriately in each assay. For P-glycoprotein and glutathione-S-transferase π, each specimen was graded either positive (if any staining was seen) or negative. Factor VIII staining was assigned a score equal to the mean number of microvessels counted in 3 high-power fields, selected on the basis of dense activity of factor VIII staining, per specimen.

Survival times were calculated from the date of histologic diagnosis until the death of the dog. The data were explored with Kaplan-Meier survival analysis for the following variables: sex, histologic type and grade, tumor invasiveness, lymphatic invasion, P-glycoprotein, glutathione-S-transferase π, platinum compound, and inclusion of an anthracycline drug in the chemotherapy protocol. The survival functions were then compared by use of the Gehan-Wilcoxon test, with a P value of .05 considered significant. The factors identified as significant by use of the Gehan-Wilcoxon test and the ordinal factors of age and factor VIII score were further evaluated with forward stepwise regression analysis, with a P value of .05 considered significant.[f]

Results

Of 51 cases reviewed for inclusion in the study, 25 met the entrance criteria. Mixed-breed dogs predominated (n = 7). Purebred dogs included the following: Lhasa Apso (n = 3), Shetland Sheepdog (n = 2), Miniature Poodle (n = 2), and one each of Akita, Beagle, Bichon Frise, Dachshund, Doberman Pinscher, Fox Terrier, Puli, Scottish Terrier, Shih Tzu, West Highland White Terrier, and Yorkshire Terrier. The mean age at diagnosis was 11.3 years (range: 8 to 17 years). Spayed females predominated (n = 19, 76%) over castrated males (n = 6, 24%). Of the 6 males in the study population, 3 had prostatic and trigonal involvement at the time of diagnosis, as determined by physical examination, ultrasonography, or exploratory surgery. All 25 dogs died or were euthanized for reasons directly related to their cancers. Overall median survival was 251 days (range: 32 to 1,068). Neither age nor breed correlated with survival. Spayed females survived longer than did castrated males (358 days versus 145 days, P = .042, Fig 1).

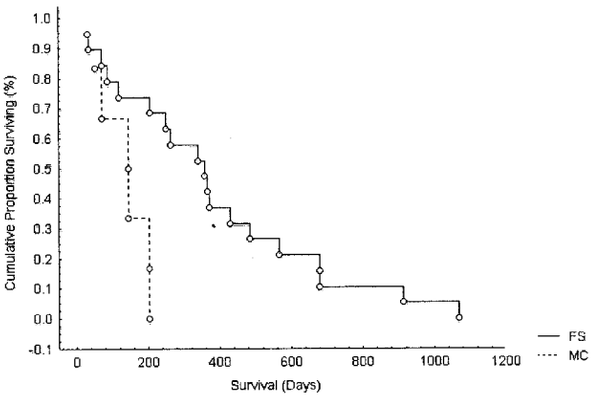

Fig 1. Kaplan-Meier survival curves for dogs with carcinoma of the urinary bladder that were treated with chemotherapy, grouped by gender. Spayed females (solid line, n = 19) lived significantly longer than castrated males (dashed line, n = 6, 358 days versus 145 days, P = .042).

Histologic examination identified 22 cases of transitional cell carcinoma, 2 undifferentiated carcinomas, and 1 squamous cell carcinoma. Of the transitional cell carcinomas, 14 (64%) were classified as the nonpapillary variant (1 solid, 6 microcystic) and 8 (36%) as the papillary variant (4 solid, 4 microcystic). Tumor invasion to the level of the submucosa was identified in 8 cases (44%, median survival: 302 days), to the muscularis in 8 cases (44%, median survival: 305 days), and to the serosa in 2 cases (12%, median survival: 139 days). Small or superficial biopsies prevented determination of exact depth of tumor invasion in 7 cases. Of the transitional cell carcinomas, 8 specimens (36%) were designated grade 2 (median survival: 365 days), and 14 (64%) were designated grade 3 (median survival: 205 days, P = .099). Lymphatic invasion was detected in 9 of 19 specimens (47%), but neither gross nor histologic evidence of metastasis was detected in the study population at the time treatment was initiated. Median survival for dogs with lymphatic invasion was 145 days, and it was 349 days for dogs without lymphatic invasion (P = .178). Positive staining for P-glycoprotein was found in 5 of 18 (28%) specimens evaluated (positive staining median survival: 206 days, negative staining: 263 days, P = .657), and glutathione-S-transferase π in 5 of 16 (31%; positive staining median survival: 87 days; negative staining: 251 days; P = .257). Factor VIII score ranged from 8 to 24 in the 11 specimens evaluated. Limitations of the amount of some biopsy specimens prevented all cases from being evaluated with all immunohistochemical stains. None of these histo-

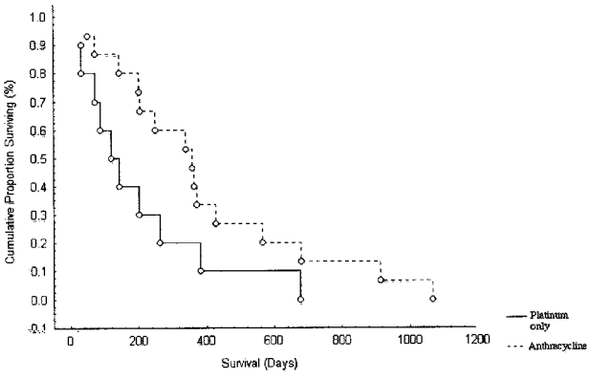

Fig 2. Kaplan-Meier survival curves for dogs with carcinoma of the urinary bladder that were treated with chemotherapy, grouped by chemotherapy protocol. Dogs receiving an anthracycline in addition to a platinum compound (dashed line, n = 15) lived significantly longer than dogs receiving a platinum compound alone (solid line, n = 10, 358 days versus 132 days, $P = .042$).

logic or immunohistochemical variables correlated with prognosis.

A total of 119 chemotherapy treatments were given (range: 1–21, median: 5). Seven dogs received only cisplatin, 3 received only carboplatin, 8 received cisplatin and doxorubicin, 5 received carboplatin and doxorubicin, and 2 received carboplatin and mitoxantrone. Of the 119 chemotherapy treatments, 47 were carboplatin, 37 were cisplatin, 33 were doxorubicin, and 2 were mitoxantrone. There was variation in the dosage of carboplatin among dogs: 150 mg/m^2 was used a total of 37 times in 6 dogs, 200 mg/m^2 was used a total of 8 times in 2 dogs, and 250 mg/m^2 was used a total of 2 times in 2 dogs. Similarly, 4 dogs received doxorubicin a total of 11 times at 25 mg/m^2, 8 dogs received 19 doses at 30 mg/m^2, and 1 dog received both dosages (2 at the lower dose, 1 at the higher dose). Comparison of median survival in those dogs that received cisplatin (203 days) versus carboplatin (365 days) did not reveal a significant difference ($P = .439$). Dogs that received an anthracycline drug in addition to a platinum compound lived longer than dogs receiving a platinum compound alone (358 days versus 132 days, $P = .042$; Fig 2). Both gender and use of an anthracycline drug remained significant under multivariate statistical analysis.

Toxicity attributed to chemotherapy was detected in 10 dogs. Neutropenia and thrombocytopenia followed the administration of doxorubicin in one dog (630 neutrophils/μL; 22,000 platelets/μL). A presumptive diagnosis of neutropenia was made in another dog with a body temperature >106°F (>41.1°C) 9 days after administration of mitoxantrone. Severe vomiting (requiring hospitalization for antiemetic and supportive therapy) occurred in 3 dogs after cisplatin administration. Severe azotemia (creatinine >6.0 mg/dL, blood urea nitrogen >100 mg/dL) occurred in 7 dogs that received cisplatin. It was not possible to determine in all of these cases whether the azotemia was prerenal in origin, renal (ie, cisplatin toxicosis), or postrenal (ie, resulting from tumor obstructing urine outflow). Median survival for these 7 dogs was 203 days (range: 33–483). Chemotherapy was discontinued because of toxicity (cisplatin-induced vomiting) in 3 dogs. Treatment delays and dosage decreases did not occur.

Discussion

In several respects, this study confirms findings in previous studies of bladder carcinoma in the dog. Age at diagnosis and the preponderance of females in this group of dogs were similar to previous reports.[2,4,7,16,17] The overall median survival time of 251 days is comparable to an earlier report of dogs treated with chemotherapy.[4] The small size of the study population does not allow meaningful statements regarding prevalence of bladder carcinoma within specific dog breeds.

The longer survival of female dogs has not been previously reported. At least 2 mechanisms may contribute to this finding. First, the urinary tract of the female is shorter and has fewer anatomic constraints compared with the male (eg, prostate and penis), which may allow extensive tumor progression with minimal change in clinical signs associated with urination. Second, an as-yet-unidentified hormonal factor or factors may affect tumor behavior, progression, or response to chemotherapy. Two of 3 males without prostatic involvement at the time of diagnosis were among the shortest lived dogs (52 and 72 days), suggesting that anatomic constraints may not fully explain the gender-associated difference in survival. Both cases of undifferentiated carcinoma were in males, with survival times of 145 and 203 days. Although one pathologist reviewed all biopsy specimens, the possibility exists that some or all of the 3 dogs with prostatic involvement had a primary prostatic carcinoma, which may respond to chemotherapy differently from urinary bladder carcinoma. Other studies of bladder tumors in dogs treated with chemotherapy have not reported a difference in response or duration of remission correlated with gender, on the basis of sonographic and radiographic measurements.[1,3,7] Assessment of sex hormone receptors in bladder carcinomas would be useful, as would a larger study involving more male dogs.

None of the histologic variables analyzed yielded statistically significant differences in survival. This may be a reflection of sample size, because analysis of the data showed a tendency toward decreased survival for dogs with higher grade tumors ($P = .099$). A previous report of a series of 110 dogs found a statistically significant correlation between tumor grade and survival.[18]

We were unable to identify any prognostic significance of 2 markers of chemotherapy resistance, P-glycoprotein and glutathione-S-transferase π, or the angiogenesis marker, factor VIII. In fact, only a minority of our samples was positive for either of the chemotherapy resistance markers, in contrast to human bladder carcinomas. Potential reasons for this difference include qualitative and quantitative differences in chemotherapy resistance mechanisms of bladder tumors in the dog. Alternatively, inherent chemotherapy resistance may be a less important reason for poor chemotherapy efficacy than other factors. For example, effective delivery of chemotherapy may be hampered by extensive tumor burden in dogs or an insufficiently rigorous chemotherapy dose-time schedule because of concern for toxicity. Also, the statistical power of these data may be a factor.

Statistical power calculations demonstrate low power to detect a difference in prognosis for glutathione-S-transferase π and P-glycoprotein (0.21 and 0.13, respectively). Consequently, a study population of 90 dogs for P-glycoprotein and 40 dogs for glutathione-S-transferase π would be necessary to achieve a statistical power of .80 and allow for more conclusive interpretation of correlation to survival time. Markers of angiogenesis other than factor VIII (eg, CD34) may prove useful. Computer-aided image analysis of immunohistochemically stained specimens would presumably be more accurate than the visual scoring system employed in this study.

Dogs were prescribed a chemotherapy regimen according to the preference of the clinician or owner. It is possible that medical and nonmedical biases may have influenced individual treatment decisions, which in turn may have affected treatment outcome in unknown ways. For instance, an anthracycline-containing protocol may have been opted against in a dog with preexisting cardiac disease. Dog owners may have declined a cisplatin-containing protocol because of the requirement for hospitalization for fluid diuresis. Clinician perception of the threat of toxicosis associated with a particular drug may have resulted in choosing a different drug or dosage when treating a severely compromised patient. Ideally, when comparing different treatment protocols, cases should be randomly assigned to a treatment group to avoid these and similar biases.

In contrast to previous reports on the use of platinum compounds to treat dogs with transitional cell carcinoma, analysis of our data does not show a difference in survival between cisplatin and carboplatin.[7] This is surprising, considering most dogs received carboplatin doses below the current accepted range of 250–300 mg/m^2. This was presumably because of a lack of information at the time of treatment regarding the maximum tolerated dose of carboplatin. The dose discrepancy did not appear to influence efficacy (or lack), as there was no marked difference in survival between the cisplatin and carboplatin groups. The 7 dogs receiving cisplatin that developed severe azotemia had a median survival time identical to all 15 dogs receiving cisplatin, suggesting that cisplatin toxicosis did not adversely affect survival time.

Two dogs (1 castrated male and 1 spayed female) with the shortest survival times (32 and 33 days, respectively) each received only one chemotherapy treatment (carboplatin 250 mg/m^2 and cisplatin 60 mg/m^2, respectively) before death and were assigned to the platinum-only treatment category for statistical analyses. This may have introduced a negative bias into the platinum-only median survival time. Had these cases been excluded from the study, the platinum-only median survival time would increase from 132 days to 174 days. The statistically significant difference in survival time between the 2 treatment groups would thereby be eliminated. Had these 2 dogs originally been prescribed a platinum-anthracycline regimen and consequently been included in that treatment category even though they died before receiving an anthracycline drug, the data are similarly affected. Because these 2 dogs were dead shortly after receiving only a platinum compound, however, this may indicate poor efficacy of these drugs rather than a bias in the data.

On the basis of analysis of our data, more extensive investigation of the anthracycline drugs is warranted. Dogs in our study that were treated with an anthracycline in addition to a platinum compound survived almost 3 times as long as dogs treated with a platinum compound alone. A randomized, prospective study incorporating standardized staging and bladder imaging is needed to confirm the utility of the anthracycline drugs in the treatment of this tumor. If the anthracycline drugs prove to be active agents, further study will be needed to determine if a synergistic relationship exists in dual-agent treatment or if an anthracycline drug alone provides all of the survival benefit.

It is likely that the frequency of chemotherapy toxicosis was underestimated in this study. Routine monitoring for hematologic toxicity occurred at 3-week intervals (ie, when the patient was presented for the next scheduled chemotherapy). Consequently, transient myelosuppression without clinical signs was likely to have gone undetected during the interval between chemotherapies. Similarly, frequency and severity of gastrointestinal signs may have not been apparent when reviewing the medical records.

A limitation of this retrospective study is the lack of a standardized assessment of response to treatment. Although radiographic and sonographic measurements of tumor dimensions taken at prescribed intervals after chemotherapy would have been ideal, they were not available in most cases. Another useful variable to monitor in the treatment of urinary bladder tumors is improvement in clinical signs. Unfortunately, such subjective information is difficult to glean from retrospective analysis of medical records. As a result of the absence of these data, we analyzed another objective figure, patient survival time, for which comparable numbers are available in the literature. Analysis of survival data alone for a tumor whose most common clinical sign is usually non–life threatening (ie, pollakiuria) is problematic. For these reasons, we acknowledge limits to the conclusions that can be drawn in this study. Nevertheless, the marked difference in survival time for both sex and anthracycline use warrant prospective evaluation.

Footnotes

[a] Platinol, Bristol-Meyers Co, Syracuse, NY
[b] Paraplatin, Bristol-Myers Squibb Co, Princeton, NJ
[c] Adriamycin, Pharmacia-Upjohn Co, Kalamazoo, MI
[d] Novantrone, Immunex, Seattle, WA
[e] Target Unmasking Fluid, Signet Laboratories, Dedham, MA.
[f] Statistica for Windows (1997), StatSoft Inc, Tulsa, OK

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