Transitional Cell Carcinomas of the Urinary Tract in a Colony of Beagle Dogs

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Abstract. Gross and light microscopic features of transitional cell carcinomas (TCC) of the urinary tract were examined in Beagle dogs used for the study of the long-term effects of low-dose, whole-body, 60Co gamma radiation. Thirty-eight cases of TCC occurred among 990 dogs that were from 0 to 14 years of age. There was no conclusive evidence of a radiation effect. The 38 TCCs were equally divided between male and female dogs, but there was a significant difference in the sex distribution of urethra-origin TCC. Eleven males had a primary urethral TCC compared to only two females. There was no significant difference between the urethra-origin and bladder-origin TCCs in the number of tumors that caused clinical signs, metastasized, or that contributed to the death of the dog. All cases of urethral TCC in male dogs occurred in the prostatic urethra. The majority of these cases were not recognized to be neoplasms at gross necropsy, but microscopic examination revealed the TCC. Our findings differ from previous reports stating that TCC occurs more frequently in female than male dogs, and they especially differ from reports claiming that urethra-origin TCC is predominately a disease of female dogs.

Primary tumors of the canine urinary bladder are not observed frequently,3,6,8-11,15,21,22,33 and primary neoplasms of the canine urethra are reported even less frequently,13,16,23,27,30-32,34,36 The most common tumor of the canine urinary tract is the transitional cell carcinoma (TCC),15,18,20,22,33 TCCs may originate in the renal pelvis, ureters, bladder, or urethra of the dog. They occur most often in the urinary bladder.12,20,22 It has been suggested that the greater incidence of primary neoplasms in the bladder is associated with retention of urine that allows increased contact time of carcinogenic agents with the bladder epithelium.20

There are conflicting reports in the literature concerning possible breed and sex predilections for canine urinary tract tumors.5,7,12,15,16,18-20,22,24,30,34,36 Human bladder tumors tend to occur three times more often in males than females, but urethral tumors occur five times more frequently in females than males.26,29,35 Suspected causes of human bladder cancer include occupational exposure to carcinogens, tobacco use, chemotherapeutic agents, tryptophan metabolites, bladder infection, and urinary stasis.

In this report, we document the incidence of TCC in a colony of Beagle dogs and call attention to the TCC arising from the prostatic urethra. This urethral tumor in the male dog could be overlooked easily or misdiagnosed. Difficulties associated with recognizing this tumor may account for the misleading hospital prevalence data that suggest that urethral tumors are more common in female than male dogs. The possibility that incidence rates for TCCs differ between Beagles and other dogs is also discussed.

Materials and Methods

Necropsy records of 474 male and 516 female, purebred, laboratory Beagles were reviewed for occurrence of transitional cell carcinoma. The dogs were part of a study of the long-term biological effects of relatively low-dose, whole-body, 60Co gamma radiation using 1,680 male and female Beagles (in equal numbers) conducted at the Collaborative Radiological Health Laboratory (CRHL). The 990 dogs included in this report died between 0 and 14 years of age. The data sets exclude dogs over 14 years of age since necropsy records are not complete for the older dogs. The basic experimental design and dosimetric data for the experiment have been published previously.1,2

The dogs were maintained in outside kennels, were fed a standard dry dog chow, and given water ad libitum. All dogs were observed daily and given a complete physical examination annually. In case of illness, the dogs were given more frequent examinations. Animal care was under the direction of full-time licensed veterinarians. All procedures were conducted in accordance with published federal standards for animal care. Facilities were inspected and approved by the US Department of Agriculture and inspected on a regular basis by the University Animal Care and Use Committee to assure that all animals were kept according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

All dogs that died or were euthanatized when in terminal condition were given a thorough post-mortem examination. A consistent necropsy protocol was followed during the entire study. The CRHL necropsy protocol requires that all lesions be sampled in addition to the standard tissues sampled in every necropsy. These standard tissues examined grossly and then sampled include the following: the entire left and right kidneys (the left kidney was serially sectioned longitudinally.

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and the right transversely); the entire bladder including the trigone; and the pelvic urethra (in male dogs, this tissue was sampled with the serial coronal sections of the prostate gland). The ureters and extra pelvic urethra were examined grossly in all dogs and were sampled if lesions were present. Tissues were fixed by immersion in 10% neutral buffered formalin. Routine samples of various organ systems and lesions were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The routine samples examined microscopically in all dogs included sections of both kidneys (including the cortex, medulla, and pelvis), the urinary bladder, and the pelvic urethra.

Analysis of radiation dose-response relationships for the incidence of transitional cell carcinoma (TCC) was made using a modification of the weighted combination of contingency tables described by Petto et al. According to this method, neoplasms are categorized by the context in which they occur; thus a neoplasm that is responsible for the death of an animal is categorized and analyzed differently from one that is found incidentally at necropsy. Cumulative incidences of TCC were analyzed using Kaplan-Meier procedures. Chi square analyses were used to test for differences in the sex distribution of dogs with TCC, without TCC, with bladder-origin TCC, and with urethra-origin TCC. Chi square analysis was also used to compare the metastatic potential and tendency to contribute to death of bladder- versus urethra-origin TCC.

Results

Transitional cell carcinoma (TCC) of the urinary tract occurred in 38 of the 990 Beagles included in this study. The age at death of the dogs with bladder-origin TCC was 7.5 to 13.9 years, with urethra-origin TCC was 10.8 to 13.9 years, and with multiple-site-origin TCC was 7.6 years to 11.9 years. There were no cases of primary renal TCC. The data for the origin of the TCC, number of dogs affected, sex, number of dogs with tumors causing clinically recognized signs, context of tumor occurrence, number of dogs with tumors that metastasized, and gross diagnosis are summarized in Table 1.

Gross appearance

The bladder-origin TCCs were most often single or multiple, papillary, or nodular mucosal masses, with or without invasion of the propria submucosa and muscle layers (Fig. 1). Only three of the 23 bladder-origin tumors were not recognized as neoplasms during the gross necropsy. The urethra-origin TCCs had a more varied appearance (Figs. 2–4). Two of the three urethra-origin tumors diagnosed as neoplasms at gross necropsy were described as multiple nodules within the prostatic parenchyma, while the third was described as a scirrhouss mass surrounding the prostatic urethra. Of the four urethra-origin tumors diagnosed as inflammation, three occurred in male dogs and resembled suppurative prostatitis, while the fourth occurred in a female and resembled urethritis. Four urethra-origin TCCs were diagnosed grossly as prostatic hyperplasia or hypertrophy. Two dogs (one male and one female) had urethra-origin TCC without lesions being observed at gross necropsy. The two tumors classified as both bladder- and urethra-origin were recognized grossly as neoplasia. One was diagnosed as neoplasia of the bladder neck and urethra, while the other was considered to be a prostatic neoplasm with metastasis to the bladder.

Microscopic appearance

Microscopically, the bladder-origin TCCs were papillary and infiltrating, except one tumor that was non-papillary and noninfiltrating (carcinoma in situ). The papillary and infiltrating tumors consisted of thick papillary fronds of irregularly stratified epithelial cells protruding into the bladder lumen with cords or nests of epithelial cells invading the propria submucosa and muscle layers. Cytomegaly, atypical nuclei, anaplasia, and mitotic figures were frequently present. A scirrhous response was an occasional feature of those tumors that invaded the muscle layers. The following were observed in 11 of 22, 2 of 22, and 9 of 22 infiltrating carcinomas, respectively: invasion of the propria submucosa exclusively; invasion of the propria submucosa, two muscle layers, and vessels; and invasion of all layers including the serosa and vessels. Ten of the dogs with bladder-origin TCC had a single discrete focus of mucosal involvement, seven had seemingly
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Fig. 1. Urinary bladder, dog 14047. Macroscopic view of a papillary bladder-origin transitional cell carcinoma. HE.

Fig. 2. Prostate gland, dog 7367. Macroscopic view of a prostatic urethra-origin transitional cell carcinoma. The neoplasm is confined to the periurethral region and was not detected at gross necropsy. HE. See also Figs. 5 and 6.

Fig. 3. Prostate gland, dog 10155. The distortion of the prostatic urethra and the increased tissue density extending peripherally from the urethra is due to a transitional cell carcinoma.

Fig. 4. Prostate gland, dog 15576. The prostatic glandular tissue has been obliterated by a transitional cell carcinoma. The multiple cystic spaces within the prostate gland are secondary to tumor-associated necrosis and inflammation. HE.

Multiple sites of origin within the bladder, and six had extensive areas of mucosal neoplasia (estimated to be one fourth or greater of the mucosal area) such that a discrete focus of tumor origin was not evident. The most commonly observed sites of metastasis of bladder-origin TCC were the lungs, adrenal glands, and lymph nodes. When lymph nodes contained metastatic foci, the iliac nodes were always involved, but usually multiple nodes were involved including sternal, mediastinal, tracheobronchial, medial retropharyngeal, and hepatic nodes. Other sites of metastasis of bladder origin TCC included the kidneys, ovaries, myocardium, liver, cerebellum, inguinal mammary gland, abdominal mesenteries, dura mater of the lumbar spinal cord, and lumbar vertebrae. One bladder-origin TCC metastasized to the urethra of a female dog, one metastasized to the prostatic urethra of a male dog, and one metastasized to the peripheral portion of the prostate gland.

Microscopic examination of the urethra-origin TCCs revealed two general patterns. In the two female dogs, the tumors had the same papillary and infiltrating pattern as seen in the bladder-origin TCCs. One tumor had invaded the propria submucosa only while the other had invaded through the muscle into the wall of the vagina. Neither of these two tumors had metastasized. The urethra-origin TCCs in male dogs all arose from the prostatic urethra. These neoplasms demonstrated less papillary growth and protrusion into the lumen than the bladder-origin tumors. Typically, the urethra-origin TCCs demonstrated plaque-like thickenings or small papillary growths in the urethral mucosa and a great tendency to invade the prostate gland (Figs. 5, 6). In most cases, most of the architecture of...
The prostate gland was obliterated by the TCC. The degree of scirrhoue response was variable and, when present, tended to occur at the periphery of the gland. The cellular morphology and histologic indicators of cellular anaplasia were indistinguishable in the urethra-origin and the bladder-origin TCCs. Seven of the 11 prostatic urethra-origin TCCs in male dogs had metastasized. In all seven cases, the bladder was one of the sites of metastatic foci. In two of the seven, metastatic foci and vessels containing tumor emboli were observed only in the bladder serosa and muscle layers. In three of the seven, metastatic foci occurred in all layers of the bladder, but the mucosal involvement was minimal compared to the extent of the serosal and muscular foci. In one of the seven, the degree of mucosal involvement was comparable to the serosal and muscular involvement. In one case there was a focal change in the mucosa only. Lymph nodes contained metastatic foci in four of the seven prostatic urethra-origin TCCs that had metastasized. As with the bladder-origin tumors, the iliac lymph nodes were involved in all cases of metastasis to lymph nodes. Other nodes were involved with the same pattern as with the bladder-origin tumors. Other sites of metastasis included the lungs, vertebral venous sinuses, vertebrae, other bones, adrenal glands, and both pelvic and sublumbar muscles.

In two cases, one male and one female, neither gross nor microscopic examination allowed differentiation between bladder- versus urethra-origin of the TCC. In the male dog, there was extensive involvement of the bladder with invasion of all layers and vessels plus there was extensive tumor obliterating the prostatic urethra and prostate gland with tumor present in prostatic vessels. In the female dog, there was transmural involvement of the neck of the bladder and the proximal urethra. In both cases, metastasis to the iliac lymph nodes had occurred.

Results of the Peto-type analysis showed no consistent and convincing evidence of a radiation effect on the incidence of TCC; however, these analyses will be repeated when the data from necropsies for all 1,680 Beagles have been tabulated. For the purposes of the analyses below, the radiation-exposed groups have been combined with the controls.

The incidence of TCC increased with age. The cumulative incidence of TCC in the Beagle dogs that died between 0 and 14 years of age is shown in Fig. 7. The
cumulative incidence curves, representing both sexes, show the probability of finding a TCC at necropsy in Collaborative Radiological Health Laboratory Beagles at any age. The curve may underestimate the actual incidence of this neoplasm, since it is possible that other, living dogs of the older age groups (>14 years) had clinically unrecognized TCC prior to their 14th birthdate.

The chi square analysis showed that TCCs (when considered regardless of origin) are not more common in female than in male dogs in our colony ($P = 0.79$) and that there is no convincing evidence of a difference in the sex distribution of bladder-origin TCC ($P = 0.10$). There is, however, a significant difference in the sex distribution of urethra-origin TCC ($P < 0.01$) reflecting their prostatic origin. There was no significant difference between the bladder-origin and urethra-origin TCCs in the number of tumors that metastasized ($P = 0.26$) or that contributed to the death of the dog ($P = 0.68$).

**Discussion**

Transitional cell carcinomas (TCCs) of the urinary tract occurred with equal frequency in male and female Beagles in our colony. When classified by site of origin, however, it is evident that TCCs of the urethra occur predominately in male dogs. There are conflicting reports in the literature concerning the sex predilection of TCC. Several authors have suggested that TCC of the urinary bladder occurs more frequently in female than male dogs, or that primary urethral tumors (TCCs, squamous cell carcinomas, and adenocarcinomas) occur predominately in female dogs. Others report that there is no sex predilection for urinary bladder TCCs in dogs, and yet another states that carcinomas of the bladder occur more frequently in male than female dogs. One possible explanation for the discrepancy between reports of an increased risk for female dogs for TCC and our results lies in the method of discovering the neoplasms. Other reports rely upon hospital prevalence values that are not incidence statistics. Hospital prevalence values depend upon the owner bringing the animal to medical attention and may vary with the extent of the diagnostic procedures performed and the thoroughness of the necropsies at different institutions. Our report is based upon incidence data derived from necropsy records. All dogs that die in our colony are necropsied, and samples of the kidneys, urinary bladder, urethra and prostate in male dogs are examined microscopically, whether or not a lesion is observed grossly. Several of the neoplasms that we recorded may not have been reported had they occurred in the general canine population, since 23 of the 38 cases of TCC were considered incidental findings at necropsy and since clinical signs referable to the TCC were noted in only 15 of the 38 cases. Differences in tissue sampling between typical hospital necropsy services and our detailed necropsy protocol may affect the reported incidence of urethral TCC. Only three of our 13 cases were suspected as neoplasms at gross necropsy, and the others may not have been evaluated histologically on a routine hospital service. Others have commented on the difficulty sometimes encountered in differentiating prostatic urethral TCC from non-neoplastic causes of prostatomegaly and from primary prostatic neoplasia. Another confounding factor is that some authors list TCC as arising from the prostatic urethra among cases of prostatic rather than urethral neoplasia.

Although both the pelvic and penile urethra of the dog are lined by transitional epithelium, all cases of urethral TCC in our male Beagles occurred in the prostatic urethra. This finding suggests either a sensitivity of this portion of the urethra to carcinogenesis or perhaps the presence of a tumor promoting factor in the prostatic environment. Our findings controvert previous theories that the female urethra is more susceptible than the male to the effects of carcinogens or that the resting prostatic secretion dilutes urine-born carcinogens and spares the male dog from urethral neoplasia.

Urethral TCC in man also arises from the prostatic urethra. In men and dogs, the prostatic ducts close to the urethra are lined by transitional epithelium. It has been suggested that some cases of prostatic urethral TCC in men actually arise from the terminal portions of the prostatic ducts. Although the same may be true in dogs, we have classified all cases of TCC arising in this area as prostatic urethral TCC, as is the convention in man.
Forty-five percent of the TCCs in this study metastasized. This frequency of metastasis is similar to the 51% reported by one group for bladder TCCs,23 but different from an earlier report where only one of 21 bladder TCCs metastasized.11 The most frequent sites of metastasis, regional lymph nodes and lungs, as well as other less frequently affected sites are similar to those reported by others for urinary tract TCC.24 The frequency of synchronous urothelial tumors in men14 and dogs35 has been noted previously. In one study based on hospital prevalence rates, 11 of 31 cases of urethral TCC in dogs had synchronous malignancy in the urinary bladder.30 No effort was made to determine if the urethra or the bladder was the primary site in these cases. In our study, an attempt was made to determine a primary site of origin in cases of synchronous tumors. In two cases it was not possible to choose a site of origin between the bladder and urethra. In eight cases of synchronous neoplasia, the determination of a site of origin was made with confidence based on the greater overall tumor size and extent of transmural involvement at the site of origin coupled with the pattern of growth (from the periphery towards the mucosa) in the presumed secondary site. In one case listed as urethra-origin and one listed as bladder-origin, this determination was made with less certainty. The site listed as the primary contained a large tumor mass with transmural involvement, but only the mucosa was involved in the secondary site. A strong argument could be made for a second independent urothelial tumor in these two cases.

The incidence data in our dogs can be compared with the findings in the general canine population. The best canine data are from the Veterinary Medical Data Program (VMDP) to which many veterinary colleges contribute data on animal neoplasms.28 The data are reported as tumors per animal-years at risk, i.e., whether or not an animal had a tumor diagnosed when seen at a veterinary clinic during a given year of age. The VMDP data cover a total of 523,706 canine-years at risk, of which 511,972 years at risk are for dogs 0 to 14 years of age, 2,740 years at risk are for dogs 15 and over, and 8,994 years at risk are for dogs of unknown age (presumably the majority of these years are for dogs less than 15 years of age). Table 2 compares the VMDP data for TCC cases per 1,000 canine-years at risk with our data. Since the VMDP tumor cases are not reported by year of age, all cases of TCC reported to the VMDP and the total canine-years at risk, including dogs older than 14 years, were used in these calculations. For the Collaborative Radiological Health Laboratory (CRHL) calculations, only data from dogs 0 to 14 years of age were used. Our data show approximately a nine times higher rate of bladder-origin TCC compared to the VMDP data and approximately a 200 times and an eight times higher rate of TCC of the urethra in male and female dogs, respectively.

The differences in TCC rates between our data and the VMDP data would be even greater if the older dogs in the VMDP were excluded. The question arises of whether there is something unique about the CRHL Beagle colony compared to the general canine population or whether the difference is due to a different rate of detection. There are conflicting reports in the literature concerning possible breed predispositions for TCC. Several authors claim that there is no breed predisposition for primary bladder tumors in dogs.10,20,24 According to one report,13 four breeds (Scottish Terrier, Shetland Sheepdog, Beagle, and Collie, in descending order) have a significantly higher relative risk of TCC in the bladder than expected. There is also a report of an increased relative risk of urethral tumors for Beagles,36 but another study shows no breed predisposition for urethral tumors.35 It is interesting that in both reports that suggest an increased relative risk of TCC in the Beagle,15,36 they found that females were at higher risk than males for bladder and urethral TCC. This disparity in sex distribution from our findings implies that dissimilarities in tumor detection cause major discrepancies between our data and hospital prevalence rates, apart from a possibility that the Beagle is predisposed to TCC.

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