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Feasibility of quantitative contrast ultrasound imaging of bladder tumors in dogs

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Abstract — The purpose of this pilot study was to assess the feasibility of Cadence contrast pulse sequencing ultrasound to predict clinical and angiogenic tumor response in dogs undergoing chemotherapy. Contrast ultrasound facilitated visualization of bladder tumors but failed to identify a straightforward relationship between ultrasound measures and clinical outcome.

Résumé — Faisabilité de l'échographie de contraste quantitative des tumeurs des reins chez les chiens. Cette étude pilote avait pour but d'évaluer la faisabilité de l'échographie de contraste par séquençage des pulsations (Cadence™) pour prédire la réponse clinique et angiogénique de la tumeur chez les chiens subissant la chimiothérapie. L'échographie de contraste a facilité la visualisation des tumeurs rénales mais n'a pas réussi à identifier un lien direct entre les mesures de l'échographie et le résultat clinique.

(Traduit par Isabelle Vallières)

Introduction

Increasing evidence indicates that cancer growth and lethality are dependent on and related to angiogenesis (1). Angiogenesis is the process by which tumors recruit new blood vessels to deliver the nutrients and oxygen necessary for growth and metastasis. Microvascular density (MVD) is one of the most widely accepted markers of tumor angiogenesis (2). Elevated MVD is a negative prognostic indicator in a variety of tumor types including hepatocellular carcinoma (2), renal cell carcinoma (3), and bladder carcinoma (4) in humans. Moreover, MVD is known to decrease as angiogenesis is curtailed and vascular normality is restored with anti-angiogenic chemotherapy (5).

While tumor biopsy is routine for diagnosis, repeated biopsy is invasive and carries associated morbidity. Thus, surrogate markers to serially quantify angiogenic response are desirable. Diagnostic imaging is frequently used to evaluate the response of bladder transitional cell carcinoma to treatment. B-mode ultrasound is widely used to assess bladder tumor size because it is non-invasive and the equipment is readily accessible and inexpensive. With the addition of microbubble contrast agents, ultrasound becomes sensitive to capillary-sized vessels and very low flow rates while maintaining the ability to determine tumor size from traditional B-mode imaging. Ultrasound contrast agents are composed of high molecular weight gases encapsulated in an albumin, polymer, or lipid shell (6). A variety of contrast-specific pulse sequences designed to enhance non-linear echoes that are specifically produced by microbubble contrast agents have been developed and employed for the interrogation of regions of myocardial infarction, tumor microvasculature, and responses to therapy in both clinical and research settings (7–9). Cadence contrast pulse sequencing (CPS) is one such sequence which uses a multipulse transmit sequence with precise changes in interpulse amplitude and phase that when received and combined allow for the rejection of linear (tissue) echoes while non-linear contrast echoes are retained (10). Qualitative contrast ultrasound has been used to identify bladder tumors and to evaluate enhancement patterns in humans (11,12). Quantitative estimation of MVD with contrast ultrasound has been reported in a rodent model in which significant correlation was found between MVD and the enhanced area with vessels ranging from 6 to 30 µm (13).

The purpose of this preliminary study was to evaluate the feasibility of using contrast-enhanced CPS ultrasound estimates of MVD to predict clinical and angiogenic response to treatment in dogs with bladder tumors undergoing chemotherapy. We compared results to clinical outcome and urine vascular endothelial growth factor (VEGF) concentration. Angiogenic factors such as VEGF are upregulated by many tumors and evaluation of urine VEGF is thought to be representative of tumor angiogenesis in bladder transitional cell carcinoma (14).

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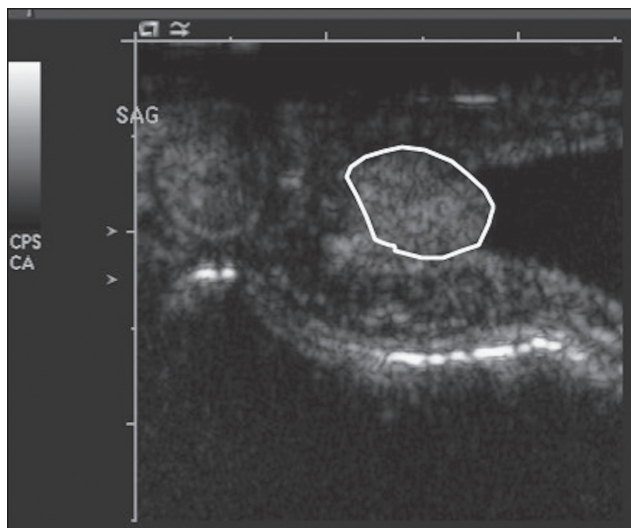
Figure 1. A region of interest (ROI) has been drawn around the tumor arising from the cranioventral bladder wall on this sagittal contrast-enhanced CPS ultrasound image obtained from a dog with transitional cell carcinoma.

We hypothesized that this ultrasound technique would predict which dogs would have clinical and/or angiogenic progression.

Materials and methods

To test this hypothesis, we recruited 6 dogs with 8 lower urinary tract transitional cell carcinomas (6 bladder, 2 prostate) into this pilot study. All procedures were approved by the institutional Clinical Trials Review Board and owner consent for the contrast ultrasound imaging studies was obtained before the procedures were carried out. Dogs were excluded if they had evidence of cardiac disease to eliminate the unlikely possibility of myocardial infarction by microbubbles. Dogs were also excluded if they had a history of anaphylactic reactions to vaccines, contrast agents, or other medications to minimize the likelihood of adverse response to the microbubble contrast agent. Additionally, dogs were not eligible if they had serum creatinine > 265 μmol/L, serum bilirubin > 17 μmol/L, serum ALT > 400 IU/L, hematocrit $< 25\%$, platelet count $< 50\,000/\mu L$ or a urethral stent in place to ensure overall health and reduce risk of adverse response to the microbubble contrast agent. There were 3 spayed females and 3 castrated males. There was 1 each fox terrier, Scottish terrier, West Highland white terrier, pomeranian, Labrador retriever mix, and terrier mix. Mean [$\pm$ SD (standard deviation)] age was 11.5 ± 2.1 y (range: 8 to 14 y). Mean body weight was 9.6 ± 3.7 kg (range: 4.8 to 16.0 kg). All dogs were receiving Piroxicam capsules (Teva Pharmaceuticals Sellersville, Pennsylvania, USA) ($n = 5$) or Meloxicam (Boehringher Ingelheim Vetmedica, St. Joseph, Missouri, USA) ($n = 1$) before and during the study period. Four dogs initially received Mitoxantrone (Teva Parenteral Medicines, Irvine, California, USA), 3 of which eventually either developed progressive disease ($n = 1$) or untoward side effects ($n = 2$) and were switched to Carboplatin (Hospira, Lake Forest, Illinois, USA) ($n = 1$), Vinblastine (APP Pharmaceuticals, Schaumburg, Illinois, USA) ($n = 1$) or Carboplatin, followed

Table 1. The mean ($\pm$ standard deviation) Cadence pulse sequencing (CPS) estimates of microvessel density (MVD) from 6 dogs with 8 bladder tumors undergoing chemotherapy. Dogs are separated into those with partial remission (PR), stable disease (SD), and progressive disease (PD). Also shown is the percent change in MVD and the number of dogs to have increased or decreased estimates of MVD in comparison to the previous examination

	Mean CPS estimate of MVD	% change MVD from previous	Number increased from previous	Number decreased from previous
PR ($n = 6$)	$28.4 \pm 13.8\%$	$39.3 \pm 67.7\%$	4	2
SD ($n = 28$)	$19.9 \pm 8.6\%$	$9.8 \pm 68.6\%$	16	12
PD ($n = 17$)	$22.7 \pm 9.9\%$	$14.7 \pm 81.8\%$	8	9

by Vinblastine ($n = 1$). Two dogs initially received Carboplatin, both of which developed progressive disease and were switched to Mitoxantrone. One of these dogs later received Vinblastine.

Dogs were evaluated with B-mode ultrasound prior to chemotherapeutic induction (t = 0) and at every chemotherapy appointment thereafter (approximately once every 3 wk) until the owners opted to stop therapy or the animal died. B-mode ultrasound of the caudal abdomen was performed using a commercially available ultrasound unit (Acuson Sequoia 512; Siemens Medical Solutions USA, Ultrasound Division, Issaquah, Washington, USA) and a 15L8 MHz linear transducer. Mean tumor size before chemotherapeutic induction was 2.2 ± 2.6 cm^3 calculated as follows: ellipsoid volume = (length $\times$ width $\times$ height) $\times$ $\pi/6$. Considering all 8 tumors, a total of 59 imaging studies were performed, 8 of which were obtained before induction and 51 of which were follow-up evaluations. At follow-up, each tumor's response was categorized as complete resolution of all evidence of tumor (CR) (0/51); partial response (PR), $\geq 50\%$ decrease in tumor volume and no new tumor lesions (6/51); stable disease (SD), $< 50\%$ change in tumor volume and no new tumor lesions (28/51); and progressive disease (PD), $\geq 50\%$ increase in tumor volume or development of new tumor lesions (17/51). Mean survival for 5 dogs was 373 ± 238 d (range: 120 to 755 d). One dog was lost to follow-up at 200 d after chemotherapy induction.

After tumor response was determined, ultrasound contrast material (Definity; Bristol-Myers Squibb, N. Billerica, Massachusetts, USA) was injected intravenously (cephalic or saphenous) as a bolus with dosage based on body weight as previously described (0.1 mL for dogs ≤ 20 kg; 0.2 mL for dogs > 20 kg) (15). Cadence contrast pulse sequencing was engaged and longitudinal video clips were obtained for 20 s starting at the arrival of contrast agent into the imaging plane as previously reported (13). When 2 lesions were present, the primary lesion was imaged first and then the transducer was moved and a second video clip was obtained of the secondary lesion. After the imaging procedure, dogs received their assigned chemotherapy drug. Dogs were monitored for evidence of anaphylaxis (including but not limited to pruritis, head shaking, facial swelling, dermal redness, vomiting) for 1 h following contrast administration.

Image data were recorded digitally and were processed offline (MatLab; The MathWorks, Natick, Massachusetts, USA).

Videoclips were reviewed by a board-certified veterinary radiologist (REP) and 3 frames were selected from the time of maximal contrast enhancement. A user-defined region of interest (ROI) was placed around the entire tumor (Figure 1). Care was taken to exclude large regional blood vessels and adjacent portions of the bladder wall that appeared unaffected. The percentage of pixels with contrast enhancement was determined for each of the 3 frames and the average for the 3 frames was calculated to represent the percentage of enhanced pixels for that imaging session. The percent change in enhanced pixels was calculated for each follow-up imaging session.

Results

No evidence of acute toxicity was observed. All tumors were successfully detected with the contrast ultrasound technique and blood flow was visible at the microvessel level. The mean CPS estimate of MVD prior to chemotherapeutic induction was $24.6 \pm 10.3\%$ indicating that ~25% of the mm scale pixels within the image contained the vascular contrast agent. The data failed to identify a clear trend in CPS estimates of MVD which correlated to clinical assessment of PR, SD, or PD (Table 1) where the mean change in MVD was positive for each group; however, the standard deviation was large.

A total of 25 free catch urine samples were analyzed for VEGF concentration, 5 of which were obtained before induction and 20 were follow-up evaluations. Samples were centrifuged at $1000 \times g$ for 20 min to remove particulate matter and the supernatants were aliquoted for VEGF concentration assessment using an ELISA kit (Canine VEGF DuoSet ELISA Kit, R&D Systems, Minneapolis, Minnesota, USA), following the manufacturer's recommended protocol. Urine VEGF concentrations were normalized to the urine creatinine and expressed as nanograms VEGF/g creatinine. Mean urine VEGF concentration before chemotherapeutic induction was 1117.8 ± 413.3 ng/g creatinine. In dogs with 2 tumors, urine samples were considered to come from SD if both tumors had SD but were considered to come from PR or PD if either tumor was classified as PR or PD and the other was characterized as SD. Data failed to identify a correlation between urine VEGF concentration and CPS estimates of MVD. In addition, there was no clear trend for urine VEGF concentration or percent change urine VEGF concentration with PR, SD, or PD.

Discussion

Three dogs were diagnosed with bacterial cystitis during the study period. There was no clear trend identified between urine VEGF concentration, presence or resolution of cystitis, and percent change in CPS estimates of MVD.

In summary, data collected from this limited number of dogs indicated that contrast enhanced ultrasound of lower urinary tract tumors is feasible in dogs but failed to identify a connection between ultrasound data, urine VEGF concentration, and traditional criteria for tumor response to chemotherapy. Thus, our hypothesis that this ultrasound technique would predict which dogs would have clinical and/or angiogenic progression has been rejected. The disappointing early results have discouraged the continued enrollment of dogs in this study and a new study design with fewer confounding variables (only 1 chemotherapeutic protocol, cystitis as an exclusion criterion) is being considered. Interpretation of results should take into account the limited number of animals in this study.

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