Extracellular pH and P-31 Magnetic Resonance Spectroscopic Variables are Related to Outcome in Canine Soft Tissue Sarcomas Treated with Thermoradiotherapy

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Abstract

Purpose: The objective was to test whether tumor pH and ^{31}P magnetic resonance spectroscopic end points were related to treatment outcome in pet canine patients with spontaneous soft tissue sarcomas treated with thermoradiotherapy.

Experimental Design: Forty-two dogs with evaluable ^{31}P magnetic resonance spectroscopic end points and pH data were included in this study. Tumor variables (grade and volume), extracellular pH (pHe), T_2 relaxation times, intracellular pH, and selected phosphometabolite ratios were examined for correlation with clinical outcome.

Results: From 39 dogs, pHe was a predictor of metastasis-free survival (MFS), with hazard ratio (HR, 0.29; $P = 0.005$) and overall survival (OS) with (HR, 0.36; $P = 0.013$). Tumor volume (>19 cm^3) was related to MFS (HR, 2.14; $P = 0.04$), time to local failure (HR, 3.4; $P = 0.025$), and OS (HR, 2.27; $P = 0.03$). There was no association between T_2 or intracellular pH and clinical outcome. Tumor grade (high versus low/intermediate) and phosphodiester/βATP ratio were identified as significant predictors for MFS, with (HR, 2.66; $P = 0.009$) and (HR, 0.75; $P = 0.027$), respectively, and as predictors of OS with (HR, 2.66; $P = 0.009$) and (HR, 0.76; $P = 0.03$), respectively. The phosphodiester/phosphocreatinine ratio predicted time to local failure (HR, 1.24; $P = 0.017$).

Conclusions: pHe was predictive of metastasis and OS in canine spontaneous sarcomas. To our knowledge, this is the first time that pHe has been shown to be predictive of clinical outcome. The results suggest that additional studies should be considered evaluating the prognostic significance of this variable. Phospholipid resonances, related to membrane metabolism, were related to clinical outcome, confirming recent results reported in human patients with soft tissue sarcomas treated with thermoradiotherapy.

Location, histologic grade, and tumor volume are three well-established predictors of clinical outcome for soft tissue sarcomas (STS) in humans and dogs (1–3). These factors, however, do not adequately identify patients who are at the highest risk for development of metastatic disease. Approximately half of the patients diagnosed with high-grade sarcomas will eventually develop metastases (4), but currently, there is no established test to distinguish which patients in this group are at highest risk. In addition, variation among pathologists leads to disagreement up to 25% of the time when staging sarcomas (4). For these reasons, imaging is being used more frequently to stage tumors in the clinic.

Magnetic resonance imaging (MRI) is used routinely to assess the characteristics and anatomic extent of tumors. Several groups have reported that contrast-enhanced MRI is of prognostic value for survival (5) and local changes in sarcomas in response to therapy (6). Additionally, T_2 relaxation time and phosphorous magnetic resonance spectroscopy (P-31MRS) variables including intracellular pH (pHi) have been associated with the percentage of necrosis and duration of local tumor control following thermoradiotherapy in humans and dogs with STS (7, 8). P-31MRS metabolites reflect the energy status and rates of membrane turnover within the tumor. Previous reports have shown P-31MRS variables to be of prognostic importance in STS (7–11), bone sarcomas (12), head and neck tumors (13, 14), lymphomas (15, 16), and breast cancer (17).

Recently, we reported that phosphomonoester/phosphodiester (PDE), a ratio associated with lipid turnover, was predictive of metastasis in human patients with STS (9). Experimentally, metastasis has also been associated with the PDE/phosphomonoester ratio and extracellular pH (pHe) in a transgenic breast carcinoma model (18). These recent findings

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Received 12/6/05; revised 5/2/06; accepted 6/28/06.

Grant support: NIH grant PO1CA42745 from the Department of Health and Human Services.

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doi:10.1158/1078-0432.CCR-05-2669

suggest phosphorous spectroscopy to be a valuable tool to obtain information related to malignant potential in STS, and more specifically, it indicates a relationship between metastasis and lipid biosynthetic profiles.

Although pHe can also be obtained with phosphorous spectroscopy under experimental conditions using contrast agents (i.e., 3-aminopropylphosphonate), this technique has not yet been introduced into the clinic (19). Needle probes can also be used to monitor pHe (20, 21). Although the probe technique is relatively inexpensive, its invasive nature has limited its introduction into the clinic. Microelectrode probe measurements have been shown to be prognostically important for tumor response and local control in prior thermoradiotherapy trials (21–26), however, to date pHe has not been shown to be related to survival.

Indirect assessment of tumor acid/base status can be obtained by the measurement of lactate concentration. Lactate concentrations >8 μmol/g have been related to metastasis and worse overall survival (OS) in head and neck, colorectal, and cervical adenocarcinomas (27–29). Lactate-lipid/creatinine ratios evaluated with proton (H1)-MRS were also related to survival in a population of 54 glioma patients (30).

In summary, prior studies have delineated the potential relationship between tumor acidity and/or membrane-related phospholipid metabolites and clinical prognosis. This report will focus on the evaluation of the importance of pHe and P-31MRS variables in predicting treatment outcome in pet dogs with spontaneous STS treated with thermoradiotherapy.

Materials and Methods

Patients and protocol. Forty-two dogs with spontaneous STS referred to the North Carolina State University Veterinary Medical Teaching Hospital were accrued onto a phase III clinical trial that included radiation therapy and hyperthermia from 1994 to 2001; the primary objective was to assess the effect of prospective application of controlled thermal dose on local tumor control following treatment. Treatment included 5 weeks of radiation therapy (2.25 Gy/d) using cobalt 60 photons and one hyperthermia treatment per week. Dogs were randomized into one of two thermal dose groups, in which there was a 10-fold difference in thermal dose between the two treatment arms. These animals were a subset of a larger phase III trial, the results of which has been reported elsewhere (31). It is important to note that for the overall trial, a significant difference in local tumor control was observed between the two treatment groups, with the high thermal dose group having a superior outcome. In the same trial, tumor volume and grade were shown to be prognostic for metastasis-free survival (MFS) and OS. This study was approved by the Institutional Animal Care and Use Committees from North Carolina State University and Duke University.

pHe. pHe was determined using interstitial needle electrodes (Microelectrode, Inc., Londonderry, NH; Agulian, Hamden, CT). The probes were precalibrated using standard buffer solutions and then were inserted aseptically into tumors through a small skin incision. pH measurements were obtained at 0.5 to 1 cm intervals along a track, at two to three different tumor locations (depending on the size of the tumor). Measurements were obtained before and after each MR procedure.

MRI/MRS. MRS and MRI studies were acquired before and 24 hours after the first hyperthermia treatment. Animals were anesthetized (isoflurane; Abbott Laboratories, North Chicago, IL) breathing 100% oxygen, and monitored closely during the MR procedure, which typically lasted >90 minutes. Images were acquired using a body coil, allowing the tumor to be in the center of the field of view with various $CuSO_4$ standards for T_2 calculations (40, 150, and 250 ms). Spectra were acquired with a surface dual frequency coil array and triphenyl phosphite standards solutions were placed next to the tumor for pulse calibration. Pulse sequence protocol and data analysis methods have been previously reported in detail (9).

Clinical assessment. All dogs were assessed after treatment for time to local failure, MFS, and OS as clinical end points. The time schedule for treatment and acquisition of data is graphically represented in Fig. 1. Follow-up was done at 3-month intervals for the first 2 years and every 6 months thereafter.

Statistical analysis. Clinical outcome, survival variables (MFS, time to local failure, OS) and their relationship with MRI/MRS variables and pH, were evaluated using Kaplan-Meier graphs, log-rank tests, and Cox proportional hazards analysis. Cut points were determined by using medians (or 0 for a pre-versus post-change variable) of respective continuous variables or by using values from previously reported studies. As a secondary analysis, a multivariable regression analysis was done in order to discern possible interactions among significant variables. To calculate HR for a continuous predictor in a Cox proportional hazards model setting, half of the interquartile range (IQR/2) of the corresponding predictor was used as the increment of the predictor for HR.

Results

Patients. A total of 42 dogs had concurrent pHe and MRS measurements. Of these, three were not included in the analysis because of poor signal to noise from the MRS data, or failure to converge during the fitting algorithm. This left 39 dogs that had data of sufficient quality to be evaluable (93%). From 21 male dogs, 17 were castrated, and from 18 female dogs, 17 were spayed. Most of the dogs were elderly with a median age of 10 years (Table 1).

Tumors. Hemangiopericytoma was the most commonly observed tumor (n = 20) followed by fibrosarcoma (n = 9), myxoma (n = 4), undifferentiated sarcoma (n = 4), and

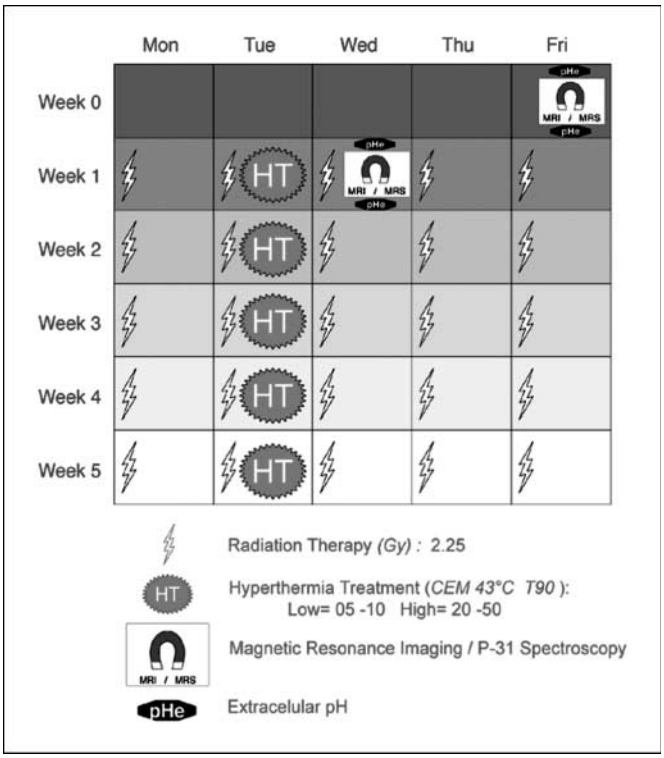

Fig. 1. Graphical representation of treatment and physiologic variables obtained for this trial as a function of time.

Table 1. Patient, endpoints, and treatment characteristics

	Sex	Tumor histology	Tumor grade	Age (y)	Tumor volume (cc)	CEM43°C T90 (HT1)	T90 (HT1)	T_2 (ms)	pHi (pre)	pHi (post)	PDE/ PCr	PDE/ βATP	pHe (pre)	pHe (post)
Low thermal dose														
1	SF	HPC	Low	14	210.6	1.52	41.2	113.71	7.18	—	2.11	1.19	7.06	—
2	SF	HPC	Low	15	13.4	1.01	39.2	100.17	7.23	7.17	0.89	2.68	6.61	6.68
3	SF	MYX	Low	10	11	1.79	40.35	92.98	—	—	—	—	6.47	7.00
4	CM	FSA	Low	8	1.3	0.30	39.9	71.84	—	—	—	—	—	—
5	SF	HPC	Low	10	2.5	0.58	40.2	59.33	—	—	—	—	—	—
6	CM	MYX	Low	7	84.6	2.56	41.8	151.13	6.77		5.20	3.38	6.85	7.00
7	SF	SA	Low	7	3.4	0.62	40.3	3,617.42	—	—	—	—	7.00	
8	SF	NFS	Low	10	15.4	0.75	40.08	1,030.58	7.30	6.44	6.12	9.95	7.01	7.01
9	CM	HPC	Low	9	64.25	0.63	40.1	2,552.45	7.37	7.53	0.57	5.22	7.09	7.07
10	F	HPC	Low	6	4.38	0.64	37.74	—	7.62	7.41	1.02	0.94	7.23	7.26
11	SF	HPC	Low	10	33	0.83	39.6	71.20	7.27	7.77	2.19	1.42	7.28	6.85
12	SF	HPC	Low	13	5.1	1.26	40.3	—	7.07	6.93	0.18	3.92	7.32	7.28
13	CM	HPC	Low	9	257.3	1.12	40.6	—	7.09	—	1.23	2.55	6.95	—
14	M	SA	Low	13	18.8	2.16	39.8	55.21	7.26	—	0.50	2.14	—	—
15	SF	SA	Low	9	38.9	0.67	39.75	80.81	7.18	—	0.25	0.89	7.11	7.16
16	CM	HPC	High	5	125.4	0.96	39.82	92.76	7.19	7.36	0.17	1.03	7.00	6.94
17	CM	FSA	High	10	40.4	0.91	37.9	77.98	—	—	—	—	6.88	6.91
18	CM	FSA	High	11	28.8	0.22	38.82	72.82	7.33	7.42	0.47	1.36	7.12	7.01
19	SF	HPC	High	8	15.7	0.47	39.92	73.53	—	—	—	—	—	—
20	CM	HPC	High	15	13.2	0.64	40	—	7.37	—	0.20	0.64	7.06	7.03
21	CM	SA	High	10	109.03	1.80	40.2	68.71	7.02	7.41	0.53	0.77	—	6.98
High thermal dose														
22	CM	HPC	Low	7	7.3	12.62	40.7	7,360.84	7.04	—	—	4.65	7.05	7.11
23	SF	HPC	Low	14	81.9	12.46	40.9	147.98	7.21	—	15.08	2.19	7.09	—
24	CM	MYX	Low	10	2.7	10.09	40.8	63.83	—	—	—	—	—	7.27
25	CM	FSA	Low	11	12.7	6.81	40.7	108.75	—	—	—	—	6.72	—
26	M	FSA	Low	4	120	2.87	40.53	128.34	7.25	7.09	1.59	2.98	6.89	6.61
27	CM	FSA	Low	6	2.3	8.44	41.66	5.53	7.39		0.20	0.78	—	—
28	SF	HPC	Low	12	122.5	1.55	40.17	61.04	7.58	7.13	5.39	1.22	6.93	6.59
29	CM	MYX	Low	9	15.7	8.04	40.9	4,952.28	7.28	7.20	0.23	1.74	7.32	6.97
30	CM	HPC	Low	11	66.69	12.07	50	79.40	7.28	7.51	3.86	4.11	6.65	7.14
31	CM	NFS	Low	11	13.9	10.97	39.1	—	7.24	—	0.86	3.22	7.25	7.12
32	SF	FSA	Low	8	33.2	0.49	38.29	—	7.69	—	3.19	7.03	7.24	7.06
33	SF	HPC	Intermediate	12	19.1	14.45	40.7	107.45	7.27		0.11	0.27	6.48	6.65
34	SF	FSA	High	14	80.8	8.51	40.3	84.96	7.26	—	2.04	—	—	—
35	CM	HPC	High	11	7.2	7.15	41	53.78	7.31		1.04	3.31	7.21	—
36	M	FSA	High	9	45.3	1.77	40.02	215.48	7.19	7.17	0.21	1.04	6.85	7.08
37	M	HPC	High	11	3.7	5.38	40.9	28.44	7.34		0.28	1.32	—	—
38	SF	HPC	High	13	64.5	9.94	44.12	68.34	—	—	—	—	7.04	6.78
39	SF	HPC	High	13	66.4	7.53	41.7	130.37	7.58	7.45	1.22	3.74	7.23	6.91

Abbreviations: T90, temperature at 10th percentile of the distribution; CEM43°C T90, cumulative equivalent minutes at 43°C for T90; HT1, first heat; T_2, T_2 relaxativity time; M, male; MC, male castrated; F, female; SF, spayed female; HPC, hemangiopericytoma; FSA, fibrosarcoma; MYX, myxoma; SA, undifferenciated sarcoma; NFS, neurofribrosarcoma.

neurofibrosarcoma (n = 2). Most of the tumors were low grade (n = 26). The remaining tumors were intermediate (n = 1) or high grade (n = 12; Table 1). Tumor grade (high versus low/intermediate) was related to MFS and OS with the same hazard ratio (HR) for both end points (HR, 2.66; P = 0.009). Tumor volume (median, 19.1; IQR, 59.39 cm^3) was related to MFS (Fig. 2; HR, 2.14; P = 0.04), OS (HR, 2.27; P = 0.03), and time to local failure (HR, 3.4; P = 0.025; Tables 2 and 3).

Thermal dose. Thermal dose was measured with the CEM43°C T90 (cumulative equivalent minutes at 43°C with temperatures above the 10th percentile of the distribution of temperatures), which is a well-accepted way of measuring heat delivery (32). The low thermal dose group (median, 2.87 CEM43°C T90; IQR, 1.28) was significantly lower than in the high thermal dose group (median, 47.62 CEM43°C T90; IQR, 14.7) $P < 0.0001$ by Wilcoxon-rank sum test. There was a trend toward longer duration of local control in the high thermal dose group, similar to that found to be significant in the whole trial (31).

pHe. The median pHe was 7.0, and this value was used as a cut point for statistical analysis. There was no difference in pHe between the low and high thermal dose groups. pHe was a highly significant predictor of MFS (HR, 0.29; P = 0.005) and OS (HR, 0.36; P = 0.013; Table 2; Fig. 3A). The prognosis was worse overall for tumors with relatively acidic pHe. The change in pHe after the first hyperthermia treatment was also significantly related to MFS (HR, 2.87; P = 0.034). Tumors that acidified (posttreatment) had a better prognosis than tumors that became more alkaline (Fig. 3B). A possible relationship between pHe and tumor volume that was discarded based on multivariable regression analysis; however, was inconclusive when relating pHe to tumor grade (Fig. 4A).

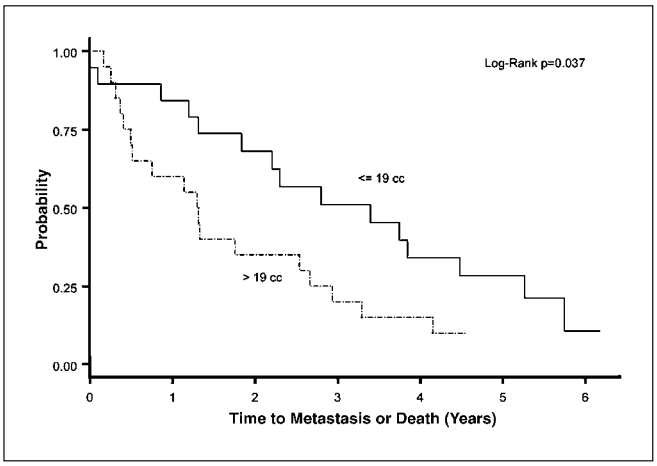

Fig. 2. Kaplan-Meier plot representing MFS for canine STS with tumor volume above and below the median cut point.

MRI/MRS. T_2 measurements of 33 dogs (median, 85 ms; IQR, 61.7 ms) obtained prior to treatment were not related to pHi or pHe, and were not predictive of clinical outcome ($P > 0.05$; data not shown). The remaining six animals were unevaluable for T_2 because of errors in placement of the T_2 standards such that they were not visible in tumor-containing slices.

Figure 5 depicts an example of a P-31 MR spectrum obtained from a fibrosarcoma. Several P-31 MRS variables were examined for their relationship with outcome. Of these, the PDE/βATP ratio was significantly associated with MFS (HR, 0.75; $P = 0.027$) and OS (HR, 0.76; $P = 0.03$). When dichotomized (>2.14 versus <2.14), the PDE/βATP ratio was not significant, but an important median survival difference of 20 months between both arms was found. The PDE/phosphocreatinine (PCr) ratio was also moderately predictive of tumor local control probability (HR, 1.24; $P = 0.017$; Table 2; Fig. 6).

Pretreatment intracellular pH (median, 7.26; IQR, 0.15), its difference posttreatment (post-pre), and the intracellular/extracellular gradient were not related to clinical outcome.

Discussion

Although this trial confirms the importance of tumor volume and tumor grade as factors influencing survival in dogs with STS following thermoradiotherapy, the association of pHe effects and P-31 metabolites with metastasis and survival is provocative. Here, we can see that tumors with acidic extracellular pretreatment pH, based on needle electrode measurements, tend to metastasize more than three times faster than nonacidic tumors. Also, tumors that acidify 24 hours post-first heat, as well as tumors with high PDE/βATP ratios, are less likely to exhibit metastasis. These clinical results show the hypothesized relationship between acidity, lipid metabolism, and tumor metastasis (18).

Relationship between pHe and treatment outcome. Our study shows for the first time the influence of pHe in predicting MFS and OS. STS with pHe <7.0, had a greater probability to metastasize, compared with tumors with pHe >7.0. pHe has been evaluated for its relationship with the likelihood of achieving complete tumor response in two clinical trials involving the use of thermoradiotherapy for recurrent or metastatic superficial tumors in prior studies (22, 23). In a report of 26 patients with heterogeneous histologic types enrolled in a phase II trial, a low average pHe (pHe = 6.88) was observed in patients who achieved complete response, whereas a higher pHe average (pHe = 7.24) was observed in patients who achieved either partial or no response (22). In contrast, the opposite result was observed in 49 patients with predominantly chest wall recurrences of breast cancer. Patients who achieved a complete response had higher average pH (pH 7.36) compared with those who achieved either no or partial response (pH 7.16; ref. 23). Possible explanations for the differences between these trials may be methodologic or may be related to the particular histologic types being evaluated. The measurement of pH by needle electrodes is not technically difficult, but it is subject to error. It is important to wait until the electrode reaches steady state after being inserted into tissue, and calibrations prior to use are highly desirable to verify that the probes are functioning properly. Nevertheless, the microelectrode is a well-accepted instrument to determine pHe *in vivo*.

There is a rationale for hypothesizing that pHe might be involved in tumor progression. Tumor cells exposed to low pHe

Table 2. Cox proportional hazards ratio for physiological parameters on clinical outcome variables

	Type of variable or cut point	*n*	MFS		Time to local failure		OS	
			HR (95% CI)	Wald *P* value	HR (95% CI)	Wald *P* value	HR (95% CI)	Wald *P* value
Tumor volume (cc)	>19 versus ≤19	39	2.14 (1.03-4.42)	0.041	3.4 (1.17-9.9)	0.025	2.27 (1.08-4.8)	0.031
Tumor grade	High versus low/intermediate	39	2.66 (1.27-5.54)	0.009	2.3 (0.87-6.13)	0.095	2.66 (1.28-5.56)	0.009
T_2 (ms)	>100 versus ≤100	33	1.10 (0.51-2.36)	0.812	0.80 (0.28-2.26)	0.675	1.08 (0.50-2.30)	0.850
PDE/PCr	Continuous	29	0.95 (0.81-1.10)	0.478	1.24 (1.04-1.48)	0.017	0.95 (0.82-1.10)	0.505
PDE/βATP	Continuous	29	0.75 (0.59-0.97)	0.027	0.87 (0.65-1.17)	0.347	0.76 (0.59-0.97)	0.030
pHi	>7.26 versus ≤7.26	30	0.54 (0.24-1.25)	0.150	0.49 (0.16-1.46)	0.199	0.58 (0.25-1.33)	0.199
pHi (post-pre)	>−0.06 versus ≤−0.06	15	1.44 (0.45-4.60)	0.538	7.70 (0.89-66.2)	0.063	1.41 (0.44-4.50)	0.565
pHe	>7 versus ≤7	30	0.29 (0.12-0.69)	0.005	0.84 (0.27-2.60)	0.767	0.36 (0.16-0.81)	0.013
pHe (post-pre)	>0 versus ≤0	24	2.87 (1.09-7.58)	0.034	1.59 (0.46-5.46)	0.465	2.54 (0.96-6.74)	0.061
phi/pHe (gradient)	>0.21 versus ≤0.21	25	1.15 (0.47-2.80)	0.757	0.77 (0.23-2.65)	0.681	1.19 (0.49-2.90)	0.697

NOTE: T_2, T_2 relaxation time.

Table 3. Kaplan-Meier and log-rank test results on the significant variables found on this study

Variables	Cut point	MFS				Time to local failure				OS			
		Median	95% CI (lower)	95% CI (upper)	Log rank	Median	95% CI (lower)	95% CI (upper)	Log rank	Median	95% CI (lower)	95% CI (upper)	Log rank
pHe	≤7	1.3	0.31	2.21	0.0029	—	0.26	—	0.7681	1.3	0.54	2.21	0.0102
	>7	3.29	1.31	4.48		2.06	1.02	—		3.29	1.33	4.48	
Change in pHE	≤0	2.15	1.3	4.48	0.0267	1.62	0.72	—	0.4611	2.15	1.3	4.48	0.0525
	>0	0.63	0.25	2.3		—	0.21	—		1.22	0.54	2.3	
PDE/βATP	≤2.14	1.17	0.37	1.31	0.1028	0.59	0.37	—	0.0797	1.25	0.49	1.7	0.1203
	>2.14	2.93	1.83	4.15		—	1.47	—		2.93	1.83	4.15	
Tumor volume	≤19	3.39	1.83	4.48	0.0367	—	1.62	—	0.0179	3.75	1.83	4.62	0.0269
	>19	1.31	0.49	2.66		0.72	0.37	—		1.32	0.57	2.66	
Tumor grade	Low	2.93	1.76	3.84	0.0069	4.08	1.47	—	0.0866	2.93	1.76	4.15	0.0068
	Intermediate/high	1.22	0.37	1.33		0.52	0.35	—		1.22	0.54	1.33	

(6.8) activate proteases (cathepsin B and metalloproteinases) that are involved in extracellular matrix degradation (33). Melanoma cells have been reported to increase the secretion of type IV collagenase at low pHe (34, 35). Mathematical models have predicted that acidification may be a mechanism for tumor cell invasion (36) by activating cysteine proteinases that are important during metastasis (37).

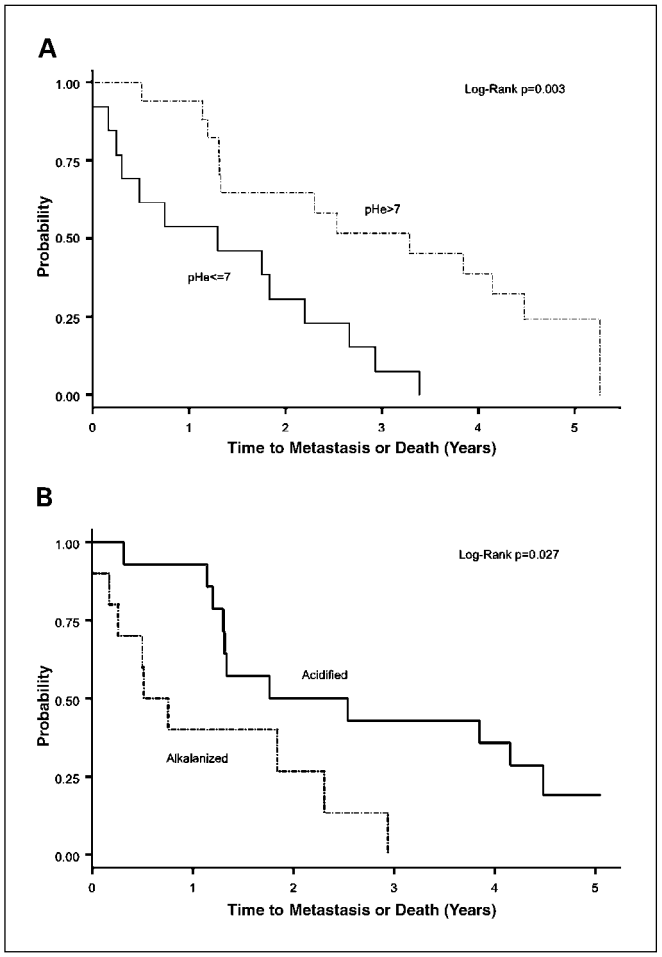

Fig. 3. Kaplan-Meier plot representing MFS for pHe (*A*) and relative change in tumor pHe 24 hours post-hyperthermia treatment (*B*) above and below the median and 0, respectively.

Provocative preclinical data have been published relating to the effect of pHe on metastatic behavior. Bhujwalla et al. examined the differences in extracellular and intracellular pH between two transgenic tumor lines that differed in expression of nm-23H (nonmetastatic protein 23H; ref. 18). Using P-31MRS, they measured both pH values, where pHe was measured using a contrast agent. pHe for highly metastatic tumors was 6.8 compared with 7.13 (average) for wild-type controls. This is indirect evidence for a connection between tumor acidosis and metastatic behavior. Low pH is a characteristic of hypoxic tumors (38, 39), which are radioresistant and typically have poor prognosis (40).

The relationship between tumor metastasis and the increase in pHe, seen 24 hours post-hyperthermia is interesting, but difficult to explain. It is known that high temperatures can acidify the microenvironment up to 24 hours after heating (41). However, we did not observe a significant relationship between the thermal dose achieved and the change in pH (data not shown). In addition, we could see that tumors that were acidic at the beginning became alkaline post-therapy and vice versa (Fig. 4B). Thus, the change in pHe and pretreatment pHe values are probably related and is telling us similar information.

Correlative analysis was done to investigate whether pHe could be an independent predictor of metastasis or OS. We found no relation between pHe (or its gradient) and tumor volume. In previous preclinical rodent studies, tumor pH values decrease whereas tumor volume (or mass) increases (38, 42, 43). For example, large ulcerating mammary carcinomas implanted in rodents are more acidic (0.6-0.8 units) than smaller ones (38) and a decrease of 0.1 on average pHe levels is seen in tumors growing from 1 to 2.5 g and from 4 to 6 g (42). This phenomena is very common in experimental models with fast-growing tumors, but does not seem to be an important relationship in this series of spontaneous canine sarcomas.

Due to the low number of high-grade tumors, the relationship between tumor grade and pHe in the multivariable regression analysis was inconclusive. It is possible that there may be such an association because more aggressive tumors tend to have higher growth rates and may have an increased glycolytic metabolism. Lactate concentration in tumors has been reported to be prognostically important in several small clinical series including head and neck, colorectal, and cervix cancer (27–29). In head and neck cancer, high lactate levels were associated with an increased likelihood for metastases and reduced survival, but not local tumor control (29). None of the

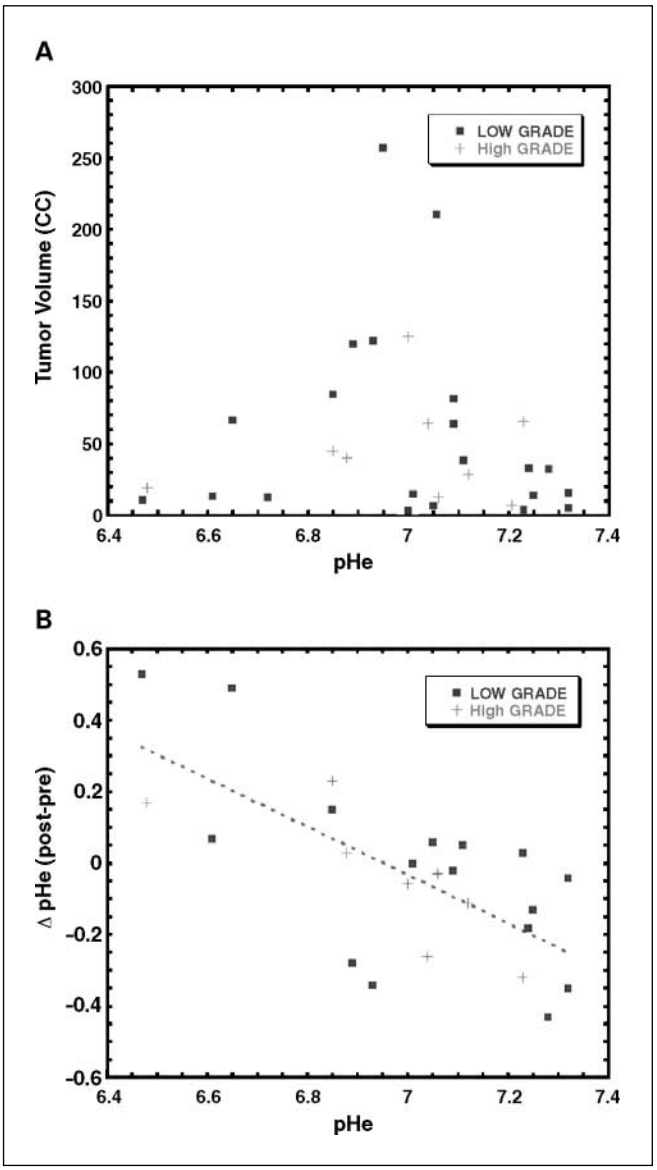

Fig. 4. Relationship between tumor volume (*A*) and relative change in tumor pHe 24 hours posttreatment (*B*) as a function of baseline pHe (pre).

series reported thus far are sufficiently large to perform full multivariate analysis. It will be important to determine whether pH or lactate levels are independently predictive of outcome, compared with other known prognostic factors.

Relationship between pHi and treatment outcome. As previously reported (44), we found that spontaneous canine tumors tend to be alkaline intracellularly (median, 7.26), whereas being acidic extracellularly (median, 7.0). Contrary to pHe, we found that pHi was not significantly related to clinical outcome. In a prior report, we showed that high pHi values obtained before treatment were predictive of pathologic complete response rate in human STS, particularly when this variable was combined with T_2 relaxation times (8). In a recent report of a follow-up trial, neither of these variables were predictive of pathologic complete response (9). There have been significant technologic improvements in P-31MRS between these two reports, with the transition from one-dimensional to three-dimensional chemical

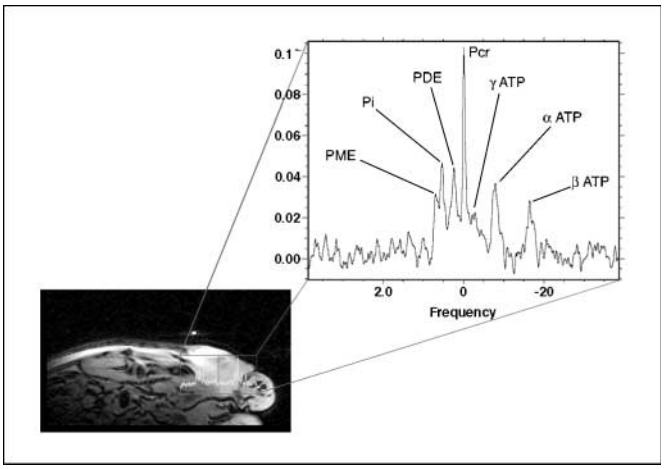

Fig. 5. P-31MRS spectrum from a hemangiopericytoma located above the right elbow. Three-dimensional chemical shift imaging voxels depicted, along with an average spectrum from two tumor-containing voxels.

shift imaging. The practice in the current study of eliminating voxels with significant normal tissue contamination may be particularly important here.

P-31 metabolites and treatment outcome. The PDE/βATP ratio was significant as a continuous variable in predicting MFS and OS. When dichotomized at its median of ratio of 2.14, there is a 21-month difference in median times to metastasis and survival. This canine population was elderly (10 years), and that the average life span for a dog is around 12 years. In this population, therefore, a minimum of 20-month difference in median times to metastasis and survival represents an approximately 20% improvement in life span, which is substantial. If this translates to equivalent differences in survival in humans, it may be a very important prognostic factor for sarcomas. We previously reported changes in the inverse of this ratio to be correlated with pathologic complete response and duration of local control in human and canine patients with STS, respectively (45).

The PDE/PCr ratio was the only ratio that predicted local failure in our study and was also previously reported (in conjunction with the phosphomonoester/PDE ratio) to be related to MFS and OS in 35 human patients with STS treated with thermoradiotherapy (9). An increase in PDEs seems to be

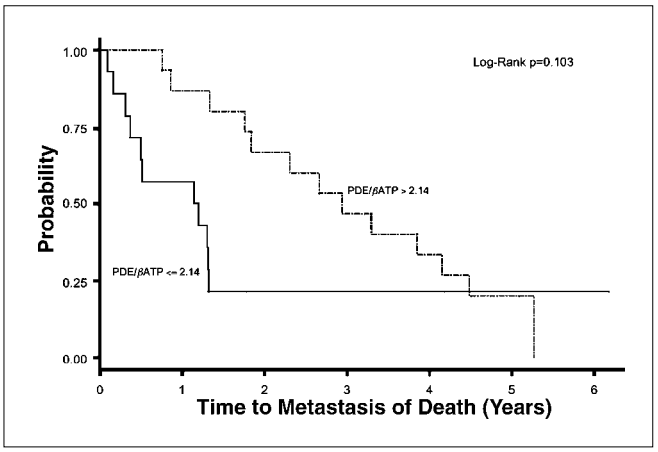

Fig. 6. Kaplan-Meier plot representing MFS for PDE/βATP for canine STS above and below the median cut point.

the main reason for an increase in PDE/PCr (as well as PDE/βATP) because PCr levels are always low in tumor tissues. Elevated values of PDEs are associated with an increase in phospholipid catabolism, elevated cell membrane breakdown, and necrosis in tumors (46, 47). In a prior report, glycerophosphocoline and glycerophosphoethanolamine (PDE) levels were found to be elevated in a low metastatic cell strain suggesting phospholipid signaling mechanisms (18). Although our results are consistent with this prior study, further work will need to be done to clarify the effects of PDE metabolism on tumor progression and invasion.

In summary, we describe the predictive properties of pHe in STS treated with thermoradiotherapy. Higher pretreatment pHe and tumors that did not become alkaline after first hyperthermia treatment are characterized by longer MFS and OS times. Although this concept has been shown in experimental transplantable tumor models, to our knowledge, this is the first time pHe has been identified as a significant prognostic factor in spontaneous tumors. It is still necessary to confirm these findings in a larger population and to evaluate the relationship with tumor grade. The development of noninvasive methods, especially in MRI and spectroscopy, to noninvasively determine pHe in tumors would be extremely helpful in this regard (19, 48–51). The increase in PDE ratios, seen in PDE/βATP and PDE/PCr association with clinical outcome may be explained by elevations in lipid metabolism rates.

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Clin Cancer Res 2006;12:5733-5740.