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Interstitial Hypertension in Human Breast and Colorectal Tumors[1]

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ABSTRACT

The efficacy of present day antineoplastic regimens depends upon the delivery and penetration of therapeutic agents through the tumor vascular and interstitial spaces to the tumor cell target. The distribution of relevant molecules or cells in a solid tumor is often poor and heterogeneous and is believed to be due to a number of pathophysiological factors, including elevated interstitial fluid pressure (IFP). Using the wick-in-needle technique, IFP was measured in primary breast and colorectal carcinomas as well as their respective metastases to the lymph nodes and liver in a total of 17 patients. IFP was also measured in one recurrent renal cell carcinoma, one melanoma metastasis to the lymph nodes, and another melanoma metastasis to the lung. IFP varied from 4 to 50 mm Hg with a mean ± SD of 20 ± 13 mm Hg in the neoplasms (n = 41 measurements; n = 21 tumors), while IFP in normal tissues had a mean of 2 ± 4 mm Hg (n = 11). The mean IFPs for metastatic melanoma, primary breast carcinoma, and liver metastases from a colorectal primary were found to be 33 ± 14, 15 ± 9, and 21 ± 12 mm Hg, respectively. In the renal cell carcinoma, the pressure was 38 mm Hg. These results agree with the findings of our 3 previous studies examining IFP in human superficial melanomas (14.3 ± 12.5 mm Hg, n = 12), cervical carcinomas (15.7 ± 5.7 mm Hg, n = 12), and head and neck tumors (13.2 ± 8.8 mm Hg, n = 19), and indicate that in all types of human tumors studied to date, IFP was significantly elevated above that of normal tissue. This observation may be useful in localizing tumors during needle biopsy.

INTRODUCTION

The delivery of blood-borne therapeutic and diagnostic agents to the tumor cell entails the transport of relevant molecules or cells through the tumor microcirculation, across the microvascular wall, and through the interstitial space (1, 2). Transmural and interstitial transport in tumors are passive processes, occurring predominantly by convection and diffusion. Interstitial hypertension, well documented in experimental tumors since the 1950s (3), would act as a retardant to these transport processes. By reducing the Starling forces responsible for the extravasation of fluid from a blood vessel, extravasation of a solute via convection would be almost eliminated, leaving only the relatively slow process of diffusion. In addition, elevated IFP[5] in a tumor would tend to "wash" the relevant anticancer macromolecules from the tumor periphery to the surrounding normal tissue, where the interstitial pressure is essentially zero (4).

Although interstitial hypertension could have significant implications with regard to the delivery and penetration of anticancer agents in tumors, there is a paucity of data in human tumors. Much of the work in this area has concentrated on theoretical (4) and experimental (5) examinations of interstitial fluid pressure in rodent tumors. Recently, Boucher *et al.* (6) examined IFP in human superficial malignant melanomas using the wick-in-needle method and found that IFP is indeed elevated above that of normal tissue and increases with the size of the lesion. A positive correlation between IFP in human squamous cell carcinoma of the head and neck and tumor volume was also reported by Gutmann *et al.* (7). Roh *et al.* (8) studied IFP in human cervical carcinomas during radiation therapy. For this type of cancer, IFP was found to be elevated above normal values. In addition, a negative correlation was suggested between IFP and tissue oxygenation. Finally, IFP decreased in tumors that showed complete response, suggesting that IFP may have potential as a prognostic indicator for radiation therapy.

A thorough understanding of tumor interstitial milieu would enable a realistic prediction of the efficacy of present day anticancer regimens. Therefore, the objective of this study was to measure the interstitial fluid pressure in a variety of human tumors and also to examine the relationship between IFP and clinicopathological variables such as tumor type, tumor size, TNM stage, histological grade, the degree of vascular invasion, lymph node involvement, and necrosis. Elucidation of the relationship between IFP and these readily available clinicopathological parameters may help define a role for IFP measurements in individualizing therapeutic protocols.

MATERIALS AND METHODS

Patient Selection. The protocol to measure interstitial fluid pressure intraoperatively in human tumors was approved by the Biomedical Institutional Review Board of the University of Pittsburgh, and written informed consent was obtained for all patients who participated in the study. Interstitial fluid pressure was measured in primary breast carcinoma and colorectal carcinoma, liver metastases from colorectal primaries, and nodal metastases from breast cancer. In addition, IFP was measured in one recurrent renal cell carcinoma, and in lymph node and lung metastases from primary melanoma. For each patient, the type of treatment administered (radiation-, chemo-, or immunotherapy), if any, and the time between therapy and the IFP measurement were recorded. The time between the completion of therapy and IFP measurement was quite variable and in almost all cases greater than 2 months. Therefore, an in-depth examination of the possible effect of the anticancer agent on tumor IFP was not attempted. The type of treatment administered, however, is presented for reference. The age of the patients who participated in the study ranged from 22 to 83 years.

Experimental Setup. Interstitial fluid pressure was measured using the wick-in-needle technique originally developed by Fadnes *et al.* (9). Briefly, a 2–3-mm sidehole was drilled 5 mm from the tip of a 23-gauge hypodermic needle. Five, 6–0 ethilon, surgical sutures were threaded through the needle. Following sterilization, the needle was connected to a pressure transducer (model P23XL; Gould, Inc., Cleveland, OH) via

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[5] The abbreviations used are: IFP, interstitial fluid pressure; TNM, tumor-node-metastasis.

Table 1 *IFP measurements for the 19 patients who participated in this study*

Patient	Patient sex/age (yr)	Tumor type	Vol. (cm^3)	IFP (mm Hg)	BP[a] (mm Hg)	Treatment (if any)	Primary tumor stage	Primary tumor grade
A	F33	Renal Cell CA	800	38	90/60	Chemo., immun.	IV	WD
B	F22	Melanoma						
		Nodal met.	133	20, 45, 50	110/60		IV	MD
C	M63	Lung met.	14	22, 27	130/75	Rad., chemo.	IV	MD
D	F61	Breast	13	20	100/58		IIA, T2NOMX	PD
E	F43	Breast	14	5, 8	106/71		IIA, T2NOMX	MD
F	F45	Breast	19	4, 6	120/70		IIB, T2NIMX	[b]
		Nodal met.	14	12	120/70			PD
G	F60	Breast	118	8, 10	95/61	Chemo.	IIIA, T3N2MX	PD
H	F71	Breast	45	30	[b]		IIIB, T4NIMX	PD
I	F83	Breast	23	18, 20	90/60		IIIB, T4N2MX	MD
J	F34	Breast	14	9, 21, 33	[b]		IIIB, T4NIMX	[b]
K	F54	Breast	38	11, 29	[b]	Rad., chemo.	IV, T4NOM1	PD
L	F76	Colorectal	31	25	110/50		T3N2MX, Duke's C	PD
M	M74	Liver met.	4	25	95/55		T3N2MX, Duke's C	MD
		Liver met.	14	27	95/55			
N	F55	Liver met.	14	4, 6, 7	140/60		T3NXM1, Duke's C	MD
O	F59	Liver met.	3	28, 30	130/70		T3NXM1, Duke's D	MD
P	F63	Liver met.	34	5, 5, 10, 29	135/65	Rad., chemo.	T3N1M1, Duke's C	MD
Q	F60	Liver met.	34	37.5, 45	90/50	Chemo.	T3NXM1, Duke's C	WD
R	M62	Liver met.	15	23	110/72		T3N1M1, Duke's C	MD
S	M63	Liver met.	23	14, 18, 32	[b]	Chemo.	T2NOM1, Duke's B	MD

[a] BP, blood pressure; Met, metastasis; Chemo., chemotherapy; Rad., radiation; Immun., immunotherapy.
[b] Data not available.

polyethylene tubing (PE50) filled with sterile, heparinized (70 units/ml) saline. The pressure signal was amplified by a preamplifier (model 134615-50; Gould, Inc.) and recorded on a dual channel chart recorder (model 35-V7202-10; Gould, Inc.).

IFP Measurement. All interstitial fluid pressure measurements were made under general anesthesia with the patient in a supine position. With the patient in this position, the tumor was at approximately heart level, and thus, the contribution of hydrostatic pressure to the interstitial pressure was minimized. The pressure transducer was calibrated, and under sterile conditions the needle was introduced approximately 2 cm into the tumor and left in place without external fixation. In most cases, the interstitial fluid pressure stabilized within a few minutes, after which the fluid communication was checked by slightly compressing and decompressing the polyethylene tubing (6). If the pressures before compression, after compression, and after decompression did not differ by more than 15%, the measurement was considered valid, and a mean pressure was calculated from these three values. In the cases when the interstitial fluid pressure did not stabilize within a few minutes of the needle's introduction into the tumor, the needle was removed, flushed with saline, and reinserted in a new location. Whenever possible, 2–3 measurements were made for each tumor, and for each measurement a new needle was used. If time permitted, a control measurement was made in normal host tissue at the end of the experiment. The systolic and diastolic blood pressures and tumor dimensions were also recorded.

Statistical Analysis. The relationship between parameters was examined using a simple linear regression model. The null hypothesis was rejected if the β_1-coefficient differed from zero at the 0.05 level of significance.

Determination of Clinicopathological Variables. The clinicopathological variables studied were tumor volume, TNM stage, histological grade, and the degree of vascular invasion, lymph node involvement, and necrosis. Tumor volume was determined from the relationship: $V = L_1L_2L_3\pi/6$, where L_1, L_2, and L_3 were measurements of the 3 perpendicular axes of the tumor. For each tumor, at least 3 slides were examined with reference to the histopathological parameters (tumor stage, cellular differentiation, vascular invasion, lymph node involvement, and necrosis). For consistency, all of these evaluations were performed by the same pathologist. Determination of tumor stage was based upon the rules established in TNM classification of malignant tumors (10), while histologically, the tumors were classified as poorly, moderately, or well differentiated. The degree of vascular invasion and necrosis were evaluated on a scale of 0 to 3. Lymph node involvement was assessed as either present or absent. For breast lesions, estrogen and progesterone receptor levels, DNA ploidy and S-phase fractions were also evaluated.

RESULTS

Interstitial fluid pressure was measured in a variety of primary tumors, including breast, renal cell, and colorectal carcinoma as well as in metastatic lesions in a total of 19 patients as shown in Table 1. For all patients, several measurements were made at different locations in the tumor. In some cases, however, individual measurements were discarded due to poor fluid communication. If possible, a control measurement of interstitial fluid pressure was also made in the tumor's host tissue. In normal breast, IFP varied between −0.5 and 3 mm Hg ($n = 8$) and had a mean value of 0 mm Hg, while IFP in normal liver was found to vary between 7 and 10 mm Hg ($n = 3$). When more than one IFP measurement was made for a particular tumor, variations in IFP could be as great as 2–3-fold. For the smaller tumors (volume <15 cm^3), however, there was less variation in the IFP measurements, suggesting a single nodule consistency. In the carcinomas and melanomas examined, interstitial fluid pressure ranged from 4 to 50 mm Hg. The mean interstitial fluid pressures for metastatic melanoma, primary breast carcinoma, and liver metastases from a colorectal primary were 33 ± 14 (SD), 15 ± 9, and 21 ± 12 mm Hg, respectively. It is interesting to note that 2 liver lesions (patients N and P) had IFPs comparable to that observed in normal liver.

Table 1 lists the tumor stage for the primary breast lesions and the stage at initial presentation of the primary colorectal tumors associated with the liver metastases studied. For both the mammary and colorectal lesions, the tumor stage was determined using the TNM classification system, which incorporates the anatomic extent of the disease (T), nodal involvement (N), and distant metastases (M) in its assignment of a tumor stage (10). The relationship between IFP and the extent of primary tumor (T-stage) for primary breast adenocarcinoma is shown in Fig. 1. From this plot, it can be observed that IFP increases as the disease becomes more advanced ($r^2 = 0.41$; $P = 0.01$). IFP was found to be independent of the size of the tumor for both the primary breast carcinomas and the liver metastases from a colorectal primary in the size range examined.

In Table 2, a more complete histological description of various tumors is given. Included in the table is the degree of vascular invasion, lymph node involvement, and tumor

Table 2 *Clinicopathological data for the renal cell carcinoma, melanoma metastases, and breast tumors*

Patient	Tumor type	Vascular invasion (0–3)[a]	Lymph node involvement (+ or −)	Necrosis (0–3)[a]	ER[b]PR[b] (+ or −)	Cytometric data[b]	
						DNA	S-phase (%)
A	Renal cell CA	0	−	1	[c]	[d]	[d]
B	Melanoma, nodal metastasis	0	+	1	[c]	[d]	[d]
C	Melanoma, lung metastasis	2	+	2	[c]	[d]	[d]
D	Breast	2	−	2	−−	1.7	42
E	Breast	0	−	1	−−	1.5	20.6
F	Breast	[d]	+	[d]	+−	2.0	13
	Breast, nodal metastasis	1	[c]	2	[c]	[c]	[c]
G	Breast	2	+	2	++	1.0	1.3
H	Breast	0	+	1	++	1.0	1.7
I	Breast	0	−	1	+−	1.9	9.1
J	Breast	[d]	[d]	[d]	−−	1.1	16.9
K	Breast	2	−	1	+−	1.7	11.7

[a] Vascular invasion and necrosis were evaluated on a scale of 0 to 3, with "3" indicating the greatest degree of that parameter.
[b] Estrogen receptor and progesterone receptor levels, DNA ploidy, and S-phase fractions were evaluated for only breast lesions.
[c] Not applicable.
[d] Data not available.

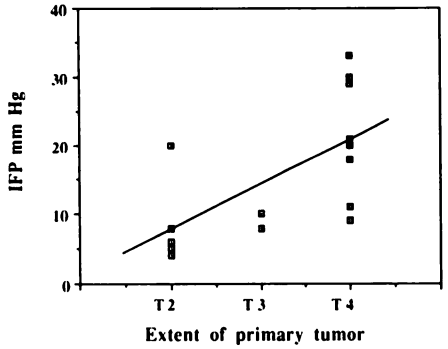

Fig. 1. Individual IFP measurements for primary mammary adenocarcinoma are plotted as a function of the extent of primary tumor ($y = -5.6 + 6.6x$; $r^2 = 0.41$; $P = 0.01$). Tumor stage was determined according to the TNM classification system.

necrosis. The poorly differentiated mammary carcinomas tended to exhibit higher interstitial fluid pressures than the moderately differentiated tumors, although this relationship could not be proven statistically significant. The IFP in mammary carcinoma also did not correlate with hormone receptor levels or cytometric data. Similarly, because all of the liver metastases studied were found to be moderately differentiated, no relationship could be determined between the IFP of the liver metastases and the cellular differentiation of the lesion.

DISCUSSION

The present study demonstrates that interstitial fluid pressure in human tumors, both primary and metastatic, is significantly elevated above that of normal tissue. IFP in the neoplastic lesions ranged from 4 to 50 mm Hg and exhibited much variation not only within tumor types but also between tumor types. Nodal metastases from a melanoma primary exhibited the highest interstitial pressure in the present group of patients. The results of this study agree well with the findings of Boucher *et al.* (6), Gutmann *et al.* (7), and Roh *et al.* (8), in which the IFP of human malignant melanoma (14.3 ± 12.5 mm Hg, $n = 12$), head and neck tumors (13.2 ± 8.8 mm Hg, $n = 19$), and cervical carcinoma (15.7 ± 5.7 mm Hg, $n = 12$), respectively, were examined. IFP measurements for these 3 tumor types ranged from 2 to 48 mm Hg and thus were within the range reported here.

It has been previously demonstrated in rodent tumors (5) and suggested in human tumors (8) that IFP is relatively uniform throughout a tumor growing as a single nodule. In the present study, several mammary tumors exhibited minimal variations in IFP measurements, lending support to the hypothesis that random central IFP measurements may characterize an entire single nodule tumor. For other tumors in this study, however, large variations in IFP for an individual lesion were observed. These large lesions may consist of multiple nodules (6), each nodule having its own biological characteristics. As discussed next, these regional differences may contribute to the observed variations in IFP within a lesion.

In the investigations by Boucher *et al.* (6) and Gutmann *et al.* (7), a positive correlation between IFP and the size of the neoplastic lesion was reported. Roh *et al.* (8) found that IFP was higher in well differentiated cervical carcinoma. In the present study, however, IFP appears to be independent of the size of the neoplastic lesion and is negatively correlated with cellular differentiation for the mammary tumors. This lack of significant correlation could be due to a limited number of tumors and/or limited range of tumor size. Differences in the interstitial fluid pressure among various tumors could also be due to biological factors, *e.g.*, different degrees of invasion of the host tissues (Fig. 1). Previously, interstitial hypertension in tumors has been attributed to 3 mechanisms: (*a*) the absence of functioning lymphatics; (*b*) the high vascular permeability and filtration coefficient of neoplastic blood vessels; and (*c*) the collapse of blood vessels, especially in the relatively low pressure return vessels, due to the proliferation of cells in a confined space (for review, see Refs. 2 and 5). If each of these factors were operating to the same degree in all tumor types for a given stage of development, one might expect to see little variation in IFP among tumor types. Another important factor involved in the maintenance of the interstitial fluid pressure in a given tumor is the hydraulic resistance encountered by the fluid as it moves through the interstitial spaces and across the tumor normal tissue boundary. Finally, Boucher and Jain (11) have recently shown that in conjunction with the above factors the principal driving force for the interstitial hypertension is the pressure in the tumor exchange microvessels. Therefore, it is quite likely that the microvascular pressures differ from one tumor to another depending upon the vascular architecture and viscous resistance offered to blood flow (12–14).

Several studies have suggested that interstitial fluid pressure may have important clinical implications with regard to cancer therapy. Roh *et al.* (8) presented evidence of a possible inverse relationship between tumor IFP and tissue oxygenation and hypothesized that IFP may aid in predicting the efficacy of radiation therapy. In the present study, we examined the correlation between IFP and other prognostic indicators such as

tumor stage and tumor grade. For mammary tumors, IFP tended to increase with the extent of invasion at the primary site and was highest in the less differentiated tumors. However, these trends need to be confirmed with data from a large number of patients. The value of IFP as a predictor of response to radiation therapy, photodynamic therapy, hyperthermia, and chemotherapy should be assessed prospectively. Finally, the knowledge that IFP in tumors is elevated may be used to facilitate tumor localization during needle biopsy.[6]

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