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During the previous year, we completed the animal studies (first phase) of the proposed research using contrast enhanced magnetic resonance imaging for detecting tumor bearing nodes. The R3230AC mammary carcinoma or Freund's adjuvant was injected into the superior aspect of the foot which resulted in metastatic tumor or inflammatory nodes in the politeal area. Studies were done when the nodes were small (4-6 mm) to accurately model the clinical diagnostic question of whether patients have metastatic disease in the axillary nodes. A total of 8 sequences were studied to determine if AMI-227 could enhance discrimination between tumor and inflammatory nodes. The fast spin echo (FSE) sequence resulted in best discrimination between inflammatory and tumor bearing nodes. Statistically significant differences were noted between the two groups but there was significant overlap between the two populations, suggesting that, for small nodes (4-6 mm), the technique may not be diagnostic.

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FOREWORD

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Introduction:

The initial proposed study was to evaluate the use of a contrast agent, AMI-227 (Combidex), to enhance discrimination between inflammatory and tumor bearing nodes, using the R3230AC mammary carcinoma model in rats. The previous report outlined some of the difficulties the project encountered because of the change in animal model requested by the Army (i.e. failure of the lymph nodes to grow, or when they did grow they were small, < 2 mm). Thus the second year of the project focused on the study proposed for the initial year, and permission was obtained from the Army to use to initially proposed model. Attached is an abstract (Appendix 1).

In addition, since the results indicated that AMI-227 would not have high specificity, we began work on a second project, which was agreed to, both before and subsequent to, a site visit. This project has proposed to study two human tumor models, a hormone sensitive tumor (MCF-7), and a hormone resistant tumor (likely to be MDA-MB-468), and its response to a 3 drug combination of metabolic inhibitors, which we found in preliminary work to very effectively enhance the efficacy of radiation. Some preliminary studies from this work are presented.

Body (Results/Discussion):

R3230AC mammary carcinoma and Freund's adjuvant were injected on the superior aspect of the foot as initially proposed. It was decided to try several additional pulse sequences so a total of 8 sequences instead of the original 2 were obtained on all rats studied. The Contrast to Noise Ratio (CNR) relative to muscle was measured pre and post injection of Combidex to determine if the injection of this agent enhanced the discrimination between tumor and adjacent tissue, compared to and inflammatory lesion. Table 1 is a list of pulse sequences studied and Table 2 summarizes the percent tumor for the tumor bearing rats studied, based on histopathologic analyses. Corresponding data is not presented for the inflammatory nodes since they were virtually 100% inflammatory. Tumor nodes were between 4 and 6 mm at the time of study. Imaging parameters included field of view = 80 x 80 mm, 128 x 256 in plane resolution, 2 mm thick slices with a 0.5 mm gap between slices.

Statistically significant changes in CNR were observed for the FSE sequences at all three echo train lengths. None of the other pulse sequences produced significant changes in CNR between tumor and inflammatory nodes. Despite the statistically significant changes in CNR seen with the fast spin echo (FSE) sequences, Fig. 1 demonstrates significant overlap between tumor and inflammatory nodes. The middle column represents samples wherein tumor was not present.

The likely explanation for the unsuccessful outcome lies in the size of the tumor. In a previous study from this laboratory using a prostate tumor model, excellent differentiation was noted between tumor and inflammatory nodes. Those nodes were typically 13-14 mm. In contrast, these experiments utilized a different tumor, but also the size of the nodes were between 4 and 6 mm. These small nodes, with a wide diversity of tumor involvement, often showed evidence of inflammation as part of the metastatic

tumor involvement, often showed evidence of inflammation as part of the metastatic process. It is likely that the presence of both inflammation and tumor has led to the poor results of the study.

Figure 2 shows a preliminary study on an MCF-7 tumor implanted in the mammary fat pad of a mouse. The peaks are identified in the legend of the figure. The spectra were obtained using a 1-dimensional chemical shift imaging sequence (1) which we have used previously (2) for volume localization. Prior to spectral acquisition, an image is obtained for precise determination of the tumor location to ensure that the data analyzed contains only tumors. The tumors are measured by calipers before study and the slice thickness confirmed in the imaging study. A slice thickness for acquisition of spectral data smaller than the tumor thickness in that dimension is selected to ensure minimal contributions from normal adjacent tissue (muscle). Treatment with the three drug combination (N-(phosphonacetyl)-L-aspartate (PALA), methylmercaptopurine riboside (MMPR), 6-aminonicotinamide (6AN)) (PALA (100mg/kg) is administered at t=-17 hours, MMPR (150mg/kg) +6AN (10mg/kg) at t=0 hours) (3) is noted to cause a loss of high energy phosphates (phosphocreatine and nucleoside triphosphates) relative to inorganic phosphate (Pi). In addition, there is the presence of a new peak, assigned to 6-phosphogluconate (based on previous studies (4,5). Quantitation of each of the metabolites will also be done after the data acquisition for the experiment has been completed.

Preliminary data have also been obtained on the effect of 15 Gy of radiation on tumor growth in this model. Table 3 shows the effects to date on a cohort of 10 tumor bearing mice showing that there is approximately a 7 day period without increase in the tumor volume. This study is ongoing, including cohorts receiving saline, PALA + MMPR+6AN, and PALA+MMPR+6AN +radiation. These data have not been quantitated since the data are early.

Conclusions

A. AMI-227 results in an increase in contrast between tumor bearing and inflammatory nodes, particularly using the fast spin echo sequence. However, in this study, wherein small nodes were studied (in contrast to a previous study wherein nodes ~14 mm were studied), there is significant overlap in the contrast-to-noise ratio between tumor and inflammatory nodes. This is likely to be due to the study of smaller nodes, since there is an inflammatory component to the tumor bearing nodes. In the small nodes, this inflammatory component may result in lack of discrimination between tumor bearing and inflammatory nodes.

B. Preliminary studies on the revised proposal of investigating the effect of a three drug combination (PALA, MMPR, 6AN) on the effect of metabolism, demonstrate similar results to that noted previously in a murine tumor study (3-5). Studies are ongoing to

    1. complete the acquisition of NMR spectra of tumor bearing mice treated with this combination and quantitate the results

2. Study the effect of these drugs on radiation sensitivity.

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Table 1

Pulse Sequences	TR/TE
T2 weighted Fast Spin Echo(FSE) Echo train length (ETL) = 8,16,32	4000/105
Proton Density (PD) Fast Spin Echo Echo Train Length = 8, 16,32	4000/15
Gradient Recalled Echo (GRE)	150/20
T2 weighted conventional spin echo (CSE)	2000/20,80

Table 2

Animal #	Per Cent Tumor/Pathologic Evaluation
1	0
2	95
3	5 (very reactive)
4	0 (all reactive)
5	Tumor outside node; no tumor in the node
6	95
7	95 – necrotic
8	100
9	100
10	85
21	100
22	95
23	5
24	95 – necrotic
25	55 –inside tumor; tumor also outside node
26	85
27	100
28	100
31	40
32	95
33	95
34	75

Table 3 Effect of 15 Gy on Tumor Growth (MCF-7)

Animal #	Tumor Volume	
	Day 0	Day 7
1	136	136
2	146	128
3	139	158
4	114	150
5	146	114
6	122	120
7	157	174
8	116	98
9	160	129
10	128	144
Mean	136.4	135.1
Std Dev.	16.2	22.3

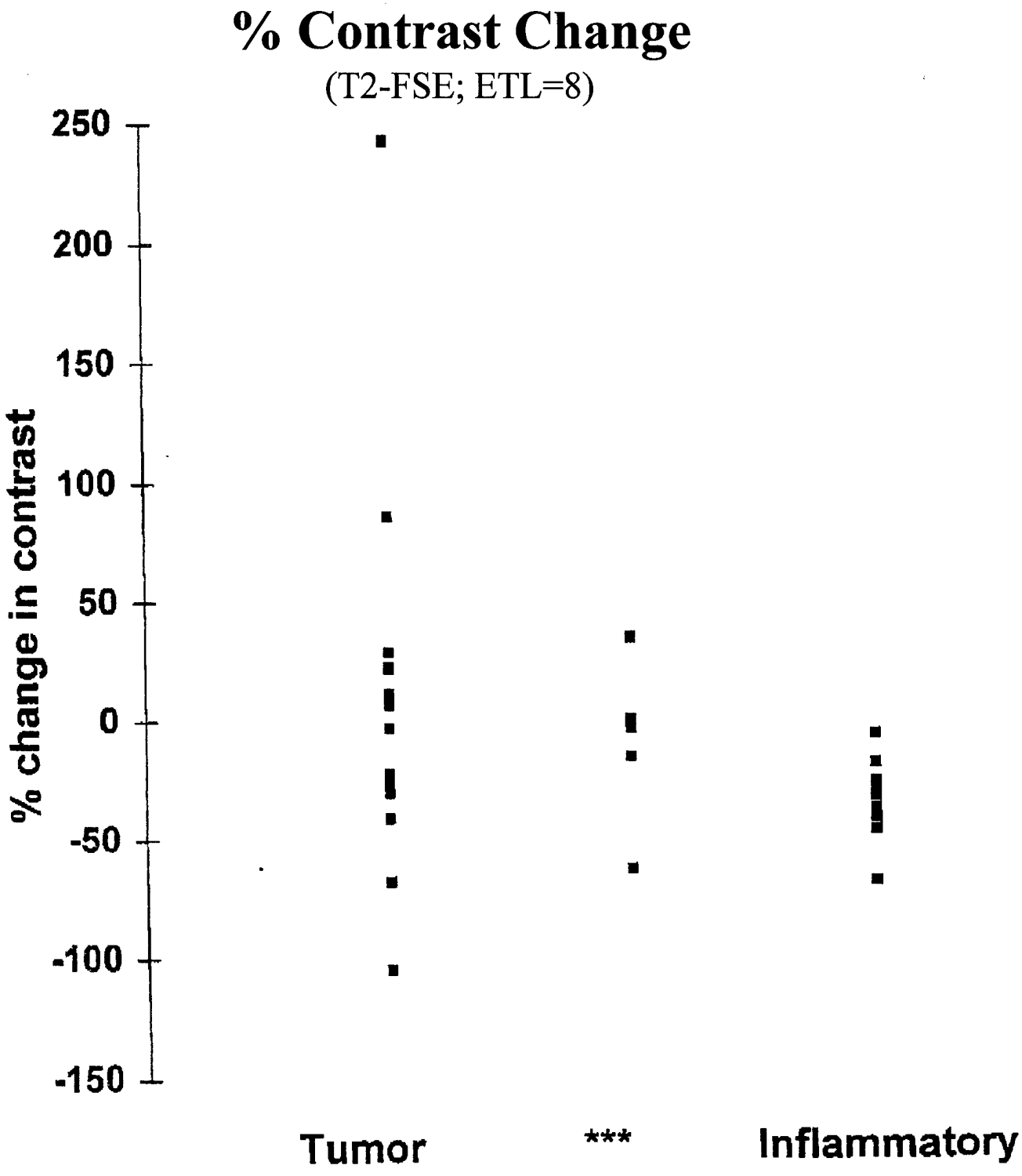

Fig. 1 Per cent change in contrast after injection of AMI-227 (Combidex). The inflammatory nodes show a small decrease in contrast and are all similar. The tumor bearing nodes have great variability in their response to AMI-227. Similar results were obtained with echo train lengths (ETL) of 16 and 32.

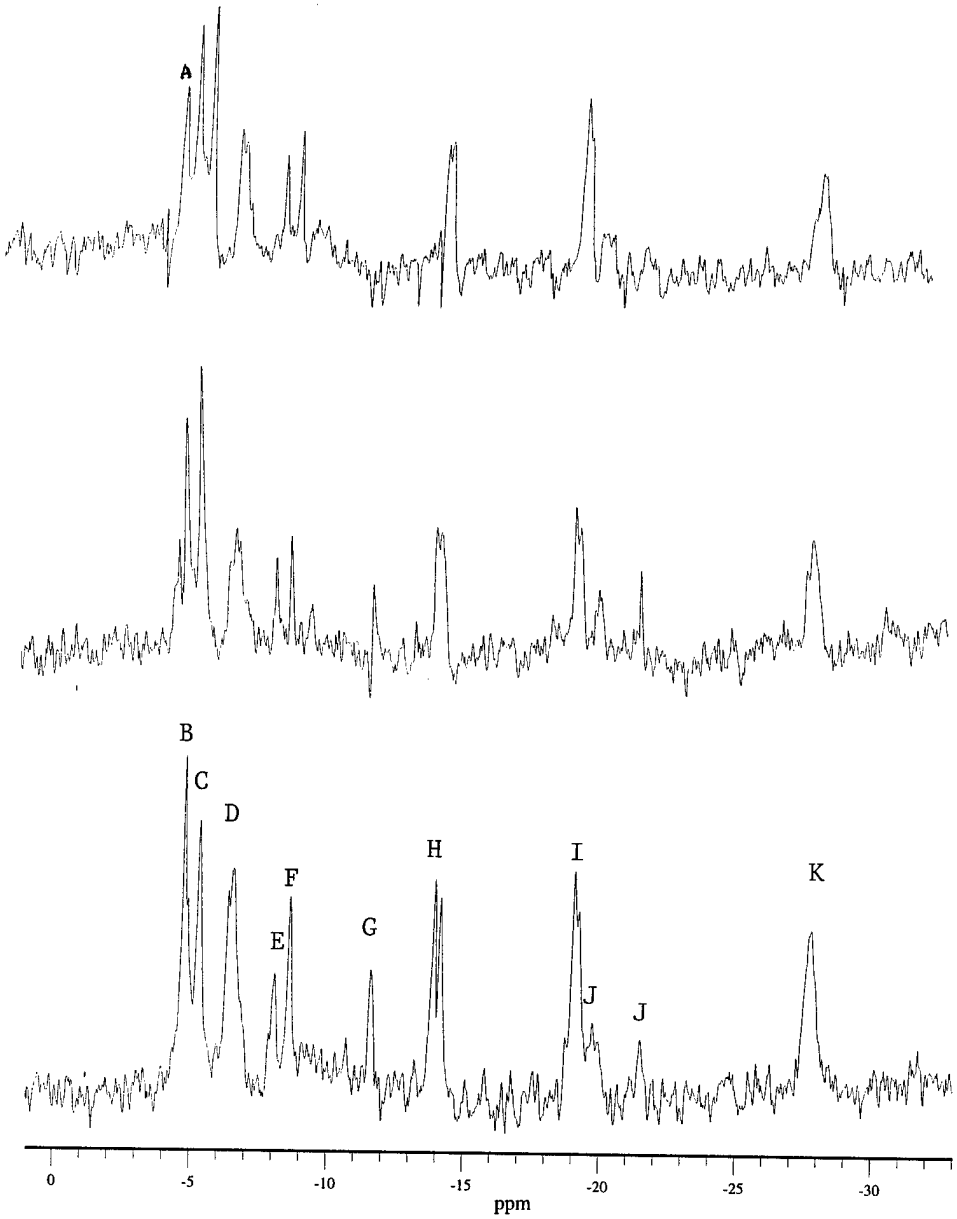

Fig. 2. 31P NMR spectra obtained pre (bottom), 3 (middle) and 10 (top) hours after MMPR + 6AN. Peaks include B= phosphocholine, C=phosphoethanolamine, D=inorganic phosphate, E=glycerophosphocholine, F=glycerophosphoethanolamine, G= phosphocreatine, H=γ nucleoside triphosphate (NTP), I= αNTP, J=diphosphodiesters, K=βNTP. Note the appearance of a peak to the left of peak B which is 6-phosphogluconate, which is present at 3 and 10 hours.

Appendix 1 - to be presented at the Radiological Society of N. AMerica in 11/97

MR Lymphography with Superparamagnetic Iron Oxide

Purpose: To determine if the administration of Combidex (Code 7227) a superparamagnetic iron oxide contrast agent, will discriminate tumor from inflammatory lymphadenopathy in a mammary carcinoma model and to determine the effect on contrast of various pulse sequence parameters.

Materials and Methods: 25 Fischer rat hind paws were treated with a mammary carcinoma tumor model, R3230AC, and were imaged at 1.5T before and after IV Combidex administration. Nine Fischer rat hind paws were injected with Freund's adjuvant to induce an inflammatory response in popliteal lymph nodes and similarly imaged. Conventional spin echo (CSE) (2000/20,80), fast spin echo (FSE-T2 and FSE-PD) (4000/105 and 4000/16 with ETL's of 8,16,32), and gradient echo images were acquired. Contrast to noise ratios (CNR) relative to muscle were calculated for each sequence and histopathologic correlation was obtained for all lymph nodes.

Findings: Histopathologic analysis revealed tumor in 23 of 25 rat lymph nodes treated with the mammary carcinoma model. The mean change in CNR for the inflammatory lymph nodes for the FSE-T2 sequence were -40.1%, -47.3% and -43.2% with ETL's of 8, 16 and 32, respectively, and 3.7%, 6.1% and -9.2% for the tumor-bearing nodes. The mean change in contrast for the inflammatory nodes for the FSE-PD sequence were -59.1%, -55.5%, and -53.85% with ETL's of 8, 16 and 32 respectively and -65.2%, 28.8% and 37.8% for tumor bearing nodes (not significant). The greatest changes in CNR were seen with the gradient echo sequence (79$\pm$ 0.4%), however, no statistically significant differences between inflammatory and tumor- bearing lymph nodes were seen with the CSE and gradient echo sequences. Changes in ETL did not significantly alter the CNR for either the inflammatory or tumor-bearing nodes.

Conclusion: The greatest difference in contrast to noise loss between inflammatory and neoplastic lymph nodes were seen on the fast spin echo pulse sequence. Echo train length did not affect CNR. Both inflammatory and tumor-bearing lymph nodes showed CNR loss with the other pulse sequences.

Take home points:

1. The ability to distinguish inflammatory from neoplastic lymph nodes is aided with Combidex.
2. Pulse sequence paramaters greatly affect the contrast to noise and the ability to discriminate between types of lymphadenopathy.