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TITLE: Tagging of Breast Tumors for Excision and Specimen
Radiography and of Sentinel Nodes for Ultrasound-Guided
Localization Using Novel Particulate Agents

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13. ABSTRACT (Maximum 200 Words) Our initial purpose is to mark the lesion for specimen radiography. This years' experiments have been performed to determine the optimal amount and the persistence of the agent that can be injected without extravasation. The initial study done in 10 rabbits determined the volume from 0.1-1.5ml of ImLN that caused extravasation. Injections were done under ultrasound (US) (n=5) or x-ray guidance (n=5). 1ml was then injected in 10 rabbits and 0.5ml (half the optimal dose) was injected in another 10 rabbits. Lesion and nodes were monitored with US and CT for 6 days. 1ml was the optimum dose as determined from the first 10 rabbits with 1.5-2cm lesions. The second phase of the study showed that the volume that caused extravasation was dependent upon tumor size, injection speed, and needle placement. Local nodes accumulated the agent. Nodal enhancement was greater when extravasation occurred. Although the radiopaque pool in the lesion decreased with time, it was still visible at 6 days. This study shows that we can administer the agent to mark the lesion without extravasation. The variables that increase extravasation, an unwanted event, were defined, and we showed that lesions can be marked for at least 6 days.				
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FOREWORD

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Introduction

Our grant seeks solutions to two related problems in breast tumor imaging: (1) Provide the ability to mark nonpalpable and non-radiopaque lesions to guide surgery and prove their resection on specimen radiography. (2) Mark and localize the sentinel node. The purpose of our first year's study was to prove our first hypothesis:

When ImLN is injected in the lesion it will mark the region in vivo for days and it will mark the lesion on specimen radiography to confirm that the marked lesion is contained in the resected specimen.

We have found that we can administer the agent, ImLN to mark the lesion without extravasation. Furthermore, we were able to identify the variables that increase extravasation, an unwanted event, and we showed that lesions can be marked for at least 6 days.

Body of Progress Report

Our first goal was to determine the volume of agent needed to mark the lesion without extravasation. Since extravasation would mark normal tissues around the lesion, it is important to avoid extravasation.

Study 1:

Ten rabbits were used to determine the optimal dose that could be injected into the lesion without causing leak or significant tissue distortion. Tumor cells (VX2) were injected into each rabbits' calf muscle and allowed to grow between 14-22 days. Half of the tumor lesions were then injected with ImLN (radiopaque fluorocarbon emulsion) using ultrasound guidance and half-using x-ray guidance. The volume injected varied from 0.2-1.4. Following injection rabbits were sacrificed and the calf radiographed on a Faxitron machine to assess whether contrast was located in the lesion, surrounding tissue, or both. Following x-ray, the tumor was resected, its size measured, and the location of the agent assessed. This study showed that that all tumor lesions were marked on specimen radiography. Although tumor size varied from 1 to 2 cm in diameter, the optimal dose was approximately 1 ml. Greater volume caused extravasation and increased backpressure and leakage along the needle tract.

Study 2:

Twenty rabbits were then studied. Ten were injected with 1ml (optimal dose) and 10 with 0.5 ml (half optimal dose). Ultrasound was used to guide needle placement and tumor size measurements. Radiography and ultrasound of the popliteal, iliac, and inguinal lymph nodes were performed on days 1, 3, and 6. CT scans were obtained on days 1, 3, 6 and used to quantitate the amount of ImLN in the lesion, surrounding tissue, and draining lymph nodes. Radiography demonstrated that the 1-ml dose, in comparison to the 0.5 dose, produced greater density, as would be expected, at all time point. At 6 days, the amount of contrast present within the tumor was twice as much after 1ml as 0.5 ml. Although the amount of agent decreased over time by nearly 3 folds, the amount of agent still present at 6 days was sufficient to be visible on specimen radiography.

Despite the smaller dose used in this study than in study 1, all 20 rabbits showed extravasation immediately following injection. There was a general trend in the relationship seen in this study. 1) As the size of the lesion increased, there was more contrast in the lesion and less extravasation; 2) The closer the needle was to the center of the lesion, the less the extravasation; and 3) When the injection was done rapidly, there was greater extravasation.

The lymphatic drainage of the agent from the lesion into draining lymph nodes was clearly shown on CT at both dosages, with more enhancement at the higher dose, and a general trend of greater nodal uptake with greater degree of extravasation.

The following are planned for the coming year. 1) We are testing smaller volumes at significantly slower infusion rates; 2) We are using ultrasound for all measurements of tumor lesion size as well as needle injection guidance following the administration of ultrasound contrast to better define lesion size and location to insure a central injection; 3) The VX2 tumors are allowed to grow for 14-16 days to obtain a more uniform tumor size; 4) Vx2 lesions are being implanted in both calves where one tumor will serve as control for the other; and 5) we are dissecting lesion following the procedure to determine the location of the needle in the tumor and the extent of contrast leakage surrounding tumor.

We are initiating the effort towards the second phase of the study and that is to guide the surgeon to the tumor. We have acquired carbon black to mix with the emulsion to produce a visual guide to the tumor.

We have defined the specification of the non-radiopaque emulsion that will be used to mark the lymphatics and the lymphnodes.

During this year we will be exploring option to color the emulsion. Solutions to this problem will be tested in the 3rd year of the grant.

Key Research Accomplishments

- ⇒ We are able to use ultrasound-guidance to inject contrast and mark the implanted tumor and confirm the area of injection with X-ray and CT Scan.
- ⇒ We are able to quantify contrast enhancement of tumor, leakage, and lymphatic drainage using CT scan ROI's.
- ⇒ We have determined the variables that promoted extravasation
- ⇒ We have demonstrated that we can mark the lesion for several days in-vivo and upon resection, demonstrate the marker within the tumor.
- ⇒ We showed that the presence of the emulsion in the tissues surrounding the tumor promoted lymphnode uptake that was detectable by CT.

Reportable Outcomes

There are no reportable outcomes at this time.

Conclusions

The results of our project indicate that we are able to mark a tumor lesion with contrast radiographically and can visualize and measure contrast drainage into regional lymph nodes via CT scan. We are still seeking the optimal dose of contrast required to mark the lesion but without producing extravasation. We have learned that in addition to volume, tumor size, needle position, and injection speed are key variables to minimize extravasation. We are also determining whether we can add color to the contrast agent to mark the tract and visually guide the resection to the tumor center.