Effect of Hyperthermia on Tumor Uptake of Radiolabeled Anti-Neural Cell Adhesion Molecule Antibody in Small-Cell Lung Cancer Xenografts

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This study was performed to examine the effect of hyperthermia on the intratumor accumulation of a monoclonal antibody (Mab) in an animal model. Mab NE150 (IgG1) recognizes the neural cell adhesion molecule (NCAM) expressed by human small-cell lung cancer (SCLC) cells. **Methods:** Athymic mice inoculated with NCI-H69, an SCLC cell line, received an intravenous injection of ^{125}I- and ^{111}In-NE150 and the serial changes of the biodistribution were determined. Furthermore, athymic mice bearing NCI-H69 were either sham-treated or treated by a single hyperthermia at 42°C or 43°C for 1 hr, with the tumor-bearing leg in a water bath using pentobarbital anesthesia. Immediately after heating, the mice were given an intravenous injection of radiolabeled NE150, and the biodistribution was examined at 24 and 48 hr. **Results:** NE150 localized well in the transplanted tumor when compared with a control Mab. In mice treated at 43°C, there was a 1.34- to 1.67-fold increase in the tumor uptake of ^{125}I- and ^{111}In-NE150 compared to sham-treated mice at both 24 and 48 hr. In addition, a 1.84- to 2.22-fold increase of the tumor-to-blood ratio was demonstrated, since radiolabeled NE150 cleared faster from the circulation in the mice given hyperthermia. A histological study demonstrated the infiltration of neutrophils in the perivascular spaces, indicating an increase of tumor vascular permeability, which might be one of the main reasons for the enhancement of Mab uptake. **Conclusion:** Hyperthermia seems to be a potential method of achieving an increased tumor accumulation of Mab in the radioimmunotargeting of SCLC.

Key Words: hyperthermia; monoclonal antibody; human small cell lung cancer xenograft; neural cell adhesion molecule

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Radiolabeled monoclonal antibodies (Mabs) have been widely used in the diagnosis and therapy for malignancy (*1–8*). A significant problem in Mab-guided tumor targeting is that a sufficient amount of Mabs cannot localize in the targets. Hyperthermia has been studied as a potential enhancer of Mab delivery (*9,10*). Since thermosensitivity is variable in tumor cells and surrounding connective tissues (*11,12*), the effectiveness of hyperthermia as a boost should be examined in each tumor.

Human small-cell lung cancer (SCLC) is one of the most sensitive tumors to radiotherapy and chemotherapy (*13*), but the remission achieved is usually of short duration and the emergence of rapidly growing drug-resistant metastases is frequent. Therefore, radioimmunotherapy has been expected as a potential treatment for SCLC.

In this study, we determined the effect of hyperthermia on the targeting of SCLC using Mab NE150 which recognizes neural cell adhesion molecule (NCAM) (*14–17*). NCAM is expressed by a wide variety of neoplasms, including SCLC, neuroblastoma, glioma, and some other neuroendocrine tumors, as well as by normal tissues such as the brain, peripheral nerves, adrenal gland and natural killer cells (*15–19*).

MATERIALS AND METHODS

Monoclonal Antibodies

NE150 is a murine IgG1 raised in a conventional fashion by immunizing BALB/c mice with primary neuroblastoma cells (*16*). It recognizes human NCAM antigen with an affinity constant of 1.2×10^8 M^{-1} (*17*). An isotype-matched anti-human thyroglobulin Mab, designated 59A, was used as a control antibody.

Mice and Tumors

Female athymic mice used in this study were supplied as 5-wk-old weanlings. The animals were housed in filter-topped cages in a facility controlled for temperature, light, and humidity under near-sterile conditions and were given sterile water and food. A human SCLC cell line NCI-H69 (*20*), which expresses NCAM antigen, was subcutaneously transplanted in the left thigh by the mince-trocar technique. Mice were randomly allocated to groups of five or ten. The mean tumor weight in each group was not significantly different from the others. The mean tumor weight of

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all six groups in the hyperthermia study was 0.33 ± 0.09 g. Prior to studies, the mice were given water containing 0.1% potassium iodine to inhibit the thyroid uptake of radioiodine released from the Mabs.

Radiolabeling of Monoclonal Antibodies

Mabs were radioiodinated using the chloramine-T method (*21*). Purified Mabs (40 μg) in 0.3 *M* phosphate buffer (PB), pH 7.5, and ^{125}I (11.1 MBq) for protein labeling (Amersham International, Buckinghamshire, UK) were mixed with 2.5 μg of chloramine-T (Nakarai Chemicals, Kyoto, Japan) dissolved in 0.3 *M* PB. After 5 min, the radiolabeled Mabs were separated from free radioiodine by Sephadex G-25 gel chromatography (Pharmacia, Uppsala, Sweden). The specific activity of the ^{125}I-labeled Mabs was about 185 MBq/mg.

Mabs were labeled with ^{111}In with diethylenetriaminepentaacetic acid (DTPA) as a bifunctional chelating agent (*22*). In brief, the Mab (2 mg/ml) in 0.1 *M* $NaHCO_3$ was incubated for 1 hr at room temperature with cyclic DTPA anhydride at a DTPA-to-Mab ratio of 3:1 and then unconjugated DTPA was removed by Sephadex G-25 gel chromatography. The DTPA-conjugated Mab in 0.2 *M* citrate buffer was mixed with ^{111}In-chloride (Nihon Mediphysics, Takarazuka, Japan) and was allowed to stand for 1 hr at room temperature. The labeling efficiency was more than 95%.

Biodistribution of Specific and Nonspecific Monoclonal Antibodies Without Hyperthermia

To evaluate the tumor-targeting potential of Mab NE150, serial biodistribution of radiolabeled NE150 and control Mab 59A was determined in mice without hyperthermia. Groups of five mice per time point were injected with a mixture of 37 kBq of ^{125}I-labeled NE150 and 37 kBq of ^{111}In-labeled NE150 via the tail vein (10 μg of Mab per mouse). Another set of NCI-H69-bearing mice were injected with radiolabeled control Mab 59A in the same way. At 6, 24, 48 and 96 hr after injection, the former group of mice were killed, while the latter group of mice were killed at 24, 48 and 96 hr. Tumors and selected organs were removed and the radioactivity was determined with a well-type gamma counter. The results were expressed as the percentage of the injected dose per gram of tissue (%ID/g).

Hyperthermia Protocol and Evaluation of Tumor Uptake

Mice (n = 10 per time point and temperature level) were immobilized in a specially constructed jig by taping the tail and the right leg under 10% pentobarbital anesthesia. The tumor-bearing left leg was completely immersed in a water bath, as confirmed by observation through the side window of the bath. The mice were air-cooled. All temperatures mentioned in this study refer to the water bath temperature. Heating was performed at 42°C or 43°C for 1 hr and then the mice were immediately injected with a mixture of 37 kBq of ^{125}I-labeled NE150 and 37 kBq of ^{111}In-labeled NE150 via the tail vein (10 μg of Mab per mouse). The biodistribution of radiolabeled NE150 was examined at 24 and 48 hr after hyperthermia. Sham-treated mice, which had received anesthesia before the injection of radiolabeled NE150, were examined in the same way. The results were expressed as %ID/g, and the radioactivity in the tumors was compared to that in the normal tissues (tumor-to-organ ratios). Data were subjected to statistical analysis with a computer program including Bonferroni method.

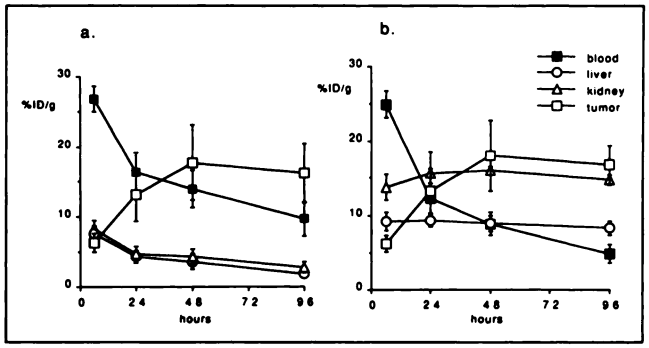

FIGURE 1. Serial biodistribution of (A) ^{125}I-labeled and (B) ^{111}In-labeled NE150 in NCI-H69-bearing athymic mice without hyperthermia. Each point represents the mean ± 1 s.d. of five mice.

Histological Examination

Histological changes of tumors following hyperthermia were evaluated as follows. Hyperthermia was performed as described above, then the mice were killed immediately and at 24 and 48 hr after heating, and tumors were fixed in 10% buffered formalin. Histological sections of 4-μm thickness were prepared for hematoxylin-eosin staining and examined under a light microscope.

RESULTS

Biodistribution of Specific and Nonspecific Monoclonal Antibodies Without Hyperthermia

The maximum tumor uptake of ^{125}I- and ^{111}In-labeled NE150 was seen at 48 hr, reaching a level of 17.8 and 18.8% ID/g, respectively, and the radioactivity in the tumor remained high till 96 hr (Fig. 1). Tumor uptake of ^{125}I- and ^{111}In-labeled 59A control Mab was much lower than that of NE150, reaching 3.8 and 5.5% ID/g at 48 hr (Fig. 2).

Effect of Hyperthermia

Tables 1 and 2 show the mean percentages of radioactivity localized in tumor, blood, liver and other selected organs in the 43°C group, the 42°C group and the unheated control group. At 24 hr after heating, the 42°C group did not demonstrate any change of radioactivity in tumor or normal organ, while 43°C heating produced 34.4% and 36.0% increase of tumor radioactivity compared with the control group for ^{125}I- and ^{111}In-labeled NE150. In addi-

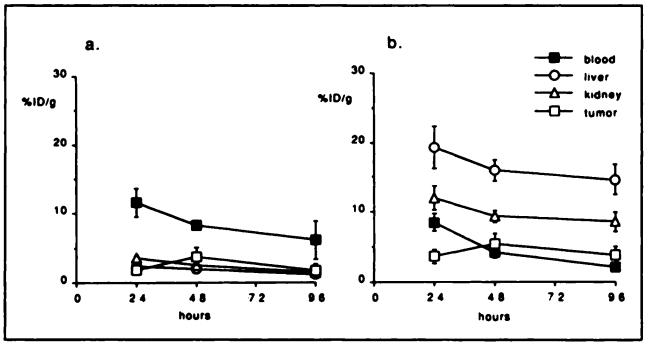

FIGURE 2. Serial biodistribution of (A) ^{125}I-labeled and (B) ^{111}In-labeled 59A in NCI-H69-bearing athymic mice without hyperthermia. Each point represents the mean ± 1 s.d. of five mice.

TABLE 1
Biodistribution (%ID/g) of Iodine-125-labeled NE150 in Athymic Mice Bearing NCI-H69 at 24 and 48 hr After Hyperthermia

Organ	24 hr			48 hr		
	Control	42°C	43°C	Control	42°C	43°C
Blood	16.32 ± 2.42	15.10 ± 1.74	11.31 ± 2.65	13.60 ± 2.05	11.87 ± 1.42	10.76 ± 2.49
Liver	3.87 ± 1.07	3.87 ± 0.87	3.04 ± 0.87	3.11 ± 0.58	2.65 ± 0.44	2.84 ± 1.02
Kidney	5.03 ± 0.74	4.96 ± 0.55	3.79 ± 0.78	4.00 ± 0.49	3.42 ± 0.27	3.31 ± 0.69
Intestine	1.55 ± 0.25	1.57 ± 0.20	1.10 ± 0.23	1.17 ± 0.22	1.08 ± 0.11	1.00 ± 0.22
Stomach	1.59 ± 0.42	2.42 ± 0.82	0.91 ± 0.39	1.27 ± 0.51	1.49 ± 0.66	1.17 ± 0.49
Spleen	2.81 ± 0.30	2.87 ± 0.75	2.41 ± 0.61	2.53 ± 0.56	1.89 ± 0.32	1.87 ± 0.52
Lung	8.36 ± 2.57	7.54 ± 0.90	5.38 ± 0.97	5.56 ± 1.97	5.03 ± 0.78	4.34 ± 1.05
Muscle	1.16 ± 0.10	1.15 ± 0.25	0.81 ± 0.27	0.89 ± 0.14	0.87 ± 0.08	0.79 ± 0.18
Bone	1.62 ± 0.17	1.49 ± 0.26	1.13 ± 0.32	1.37 ± 0.26	1.10 ± 0.15	1.02 ± 0.16
Tumor	12.11 ± 2.70	11.56 ± 2.07*	16.28 ± 3.14†	11.43 ± 4.35	17.77 ± 3.16‡	19.12 ± 4.63§

Mean ± 1 s.d. n = 10.
p values represent a comparison of control and heated groups.
*not significant.
†p = 0.007.
‡p = 0.007.
§p = 0.002.

tion, the radioactivity of the normal organs decreased faster than in the control group (Tables 1 and 2).

At 48 hr, tumor accumulation of NE150 was significantly enhanced in both the 42°C and 43°C groups. The tumor radioactivity of ^{125}I-labeled NE150 increased by 55.5% and 67.5% over that of the control group for 42°C and 43°C groups (Table 1). Similar enhancement was obtained for ^{111}In-labeled NE150 (Table 2). Radioactivity of normal organs cleared faster than that in control group in both the 42°C and 43°C groups.

Figures 3 and 4 show the effect of hyperthermia on the tumor-to-organ ratios. As for ^{125}I-labeled NE150, the increase of tumor uptake and the decrease of normal organs uptake in the 43°C group resulted in 1.96-, 1.75- and 1.81-fold tumor-to-blood, tumor-to-liver, and tumor-to-kidney ratios at 24 hr, respectively (Fig. 3A). At 48 hr, these ratios of the 43°C group increased to 2.22-, 2.01- and 2.10-fold. In the 42°C group, no marked changes were observed at 24 hr, but the tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were enhanced by 1.84-, 1.92-, and 1.84-fold at 48 hr (Fig. 4A). The results obtained with ^{125}I-labeled NE150 were similar to those achieved with ^{111}In-labeled NE150 (Figs. 3B and 4B).

TABLE 2
Biodistribution (%ID/g) of Indium-111-labeled NE150 in Athymic Mice Bearing NCI-H69 at 24 and 48 hr After Hyperthermia

Organ	24 hr			48 hr		
	Control	42°C	43°C	Control	42°C	43°C
Blood	14.22 ± 1.61	12.64 ± 0.95	9.61 ± 1.81	10.19 ± 0.53	8.50 ± 0.78	7.96 ± 1.21
Liver	7.71 ± 0.88	7.62 ± 0.48	6.43 ± 0.69	7.72 ± 0.68	7.61 ± 0.92	7.66 ± 0.86
Kidney	14.51 ± 1.58	15.12 ± 0.95	12.94 ± 1.47	15.14 ± 1.27	14.70 ± 1.29	13.47 ± 2.41
Intestine	2.88 ± 0.36	2.99 ± 0.26	2.01 ± 0.29	2.57 ± 0.29	2.57 ± 0.31	2.22 ± 0.44
Stomach	1.30 ± 0.36	1.77 ± 0.42	0.81 ± 0.40	1.41 ± 0.42	1.41 ± 0.31	0.90 ± 0.30
Spleen	5.69 ± 0.48	5.72 ± 0.74	4.83 ± 0.62	6.24 ± 0.81	5.58 ± 0.72	5.19 ± 0.67
Lung	8.50 ± 2.90	7.46 ± 0.76	5.55 ± 1.15	6.12 ± 0.72	5.18 ± 0.50	4.71 ± 0.66
Muscle	1.35 ± 0.08	1.32 ± 0.23	0.94 ± 0.25	1.13 ± 0.10	1.01 ± 0.04	0.92 ± 0.17
Bone	3.04 ± 0.32	2.86 ± 0.49	2.52 ± 0.52	3.41 ± 0.45	3.02 ± 0.31	2.93 ± 0.45
Tumor	13.88 ± 2.95	13.55 ± 1.85*	18.88 ± 3.50†	14.27 ± 4.67	22.59 ± 4.61‡	23.71 ± 5.76§

Mean ± 1 s.d. n = 10.
p values represent a comparison of control and heated groups.
*not significant.
†p = 0.002.
‡p = 0.004.
§p = 0.001.

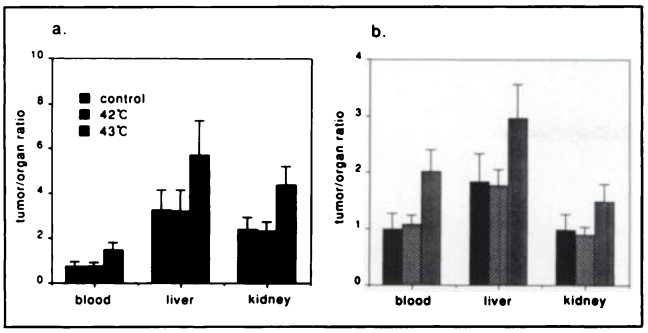

FIGURE 3. Tumor-to-organ ratios of (A) ^{125}I-labeled and (B) ^{111}In-labeled NE150 in NCI-H69-bearing athymic mice at 24 hr after heating. Each point represents the mean ± 1 s.d. of 10 mice.

Histological Findings

In the NCI-H69 xenografts, there were only a few blood vessels and a small amount of connective tissue surrounding the tumor cells (Fig. 5A). Immediately after heating at 43°C, intercellular congestion was observed in the tumor tissue while the blood vessels were dilated in the surrounding connective tissue (Fig. 5B). At 24 hr, many neutrophils had infiltrated in connective tissue, while necrotic areas and obstruction of tumor vessels due to local hyperthermia were not observed (Fig. 5C). No remarkable histological change was found between 24 and 48 hr. In addition, there were no significant differences between the 42°C and 43°C groups (data not shown). Moreover, no vascular occlusion resulting from hyperthermia was observed in the tumors or surrounding tissue at any time.

DISCUSSION

NE150 belongs to the cluster 1 antibodies, as defined by the International Workshop on SCLC antigens (*23*), and NCAM is a target of SCLC cluster 1 antibodies (*19,24*). Both ^{131}I and ^{90}Y have favorable nuclear properties for therapeutic purposes. However, ^{125}I and ^{111}In have been reported to show similar biodistribution with ^{131}I and ^{90}Y (*5*), and we used ^{125}I- and ^{111}In-labeled Mab in the present study. The radiolabeled NE150 localized well in the transplanted SCLC tumors. The maximum tumor radioactivity of radiolabeled NE150 was obtained at 48 hr after injection, and a high tumor uptake persisted at 96 hr. In contrast, tumor uptake of nonspecific Mab 59A was very low, indicating the specific accumulation of NE150 in the xenografts.

Hyperthermia has been studied as a potential enhancer of Mab targeting (*9,10,24*). According to previous reports, hyperthermia augmented absolute tumor uptake and tumor-to-organ ratios in transplanted colon carcinoma and melanoma in Mab-guided tumor targeting. Since properties of tumor cells and structures of surrounding connective tissues are important factors for tumor thermosensitivity (*11,12*), the effect of hyperthermia on tumor uptake of Mab should be investigated in each tumor.

In this study, heating at 43°C enhanced the tumor uptake of radioactivity and decreased accumulation in the normal organs at 24 and 48 hr after injection of both ^{125}I- and ^{111}In-labeled NE150. Heating at 42°C also enhanced tumor uptake and decreased accumulation in normal organs at 48 hr but had little effect at 24 hr.

The tumor-to-normal organ ratios showed no enhancement at 24 hr in the 42°C group, while the tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios at 48 hr of the 42°C group increased by 84.1%, 92.4% and 84.5%, respectively, for ^{125}I-labeled NE150 (Fig. 4A). Similar enhancement was shown at 48 hr for ^{111}In-labeled NE150 (Fig. 4B). In addition, the tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of the 43°C heating group at 48 hr increased by 122.0%, 100.8% and 109.1% for ^{125}I-labeled NE150 (Fig. 4A). Similar enhancement was noted at 24 and 48 hr for ^{111}In-labeled NE150 (Figs. 3B and 4B). No enhancement of normal organ uptake was found in either the 42°C group or the 43°C group. The augmented tumor uptake may be one of the reasons for the diminished accumulation in normal organs.

Histological examination suggested that hyperthermia increased the tumor blood flow immediately after heating, although vascular damage, which largely accounts for the antitumor effect of hyperthermia, was not observed in the tumors and surrounding tissues. But neutrophil infiltration was found at 24 and 48 hr in surrounding connective tissue (Fig. 5), which suggested an acceleration of the permeability of feeding vessels. Increase of Mab supply due to blood flow augmentation immediately after heating and the acceleration of the permeability of feeding vessels after an interval may have contributed to the increase of Mab uptake in the tumor, although these processes do not explain the difference of Mab uptake between the 42°C and 43°C groups.

Nishimura et al. have reported that the intratumor temperature distribution was almost uniform in tumors transplanted in the thigh of mice when the tumors were heated in a water bath more than 5 min (*25*). Moreover, in their mouse model, tumor temperature was lower than water bath temperature only by 0.1–0.2°C. Therefore, the tumor temperature distribution was considered uniform in our model with water bath heating.

Although human SCLC is one of the most sensitive

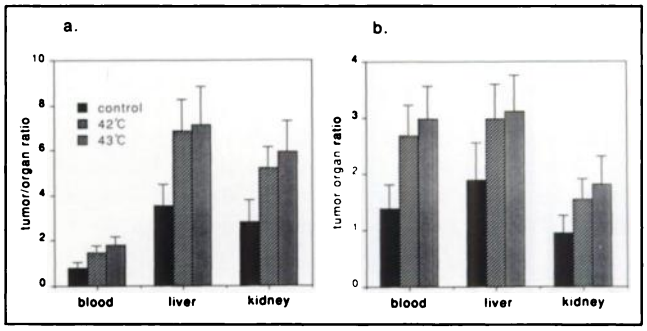

FIGURE 4. Tumor-to-organ ratios of (A) ^{125}I-labeled and (B) ^{111}In-labeled NE150 in NCI-H69-bearing athymic mice at 48 hr after heating. Each point represents mean ± 1 s.d. of 10 mice.

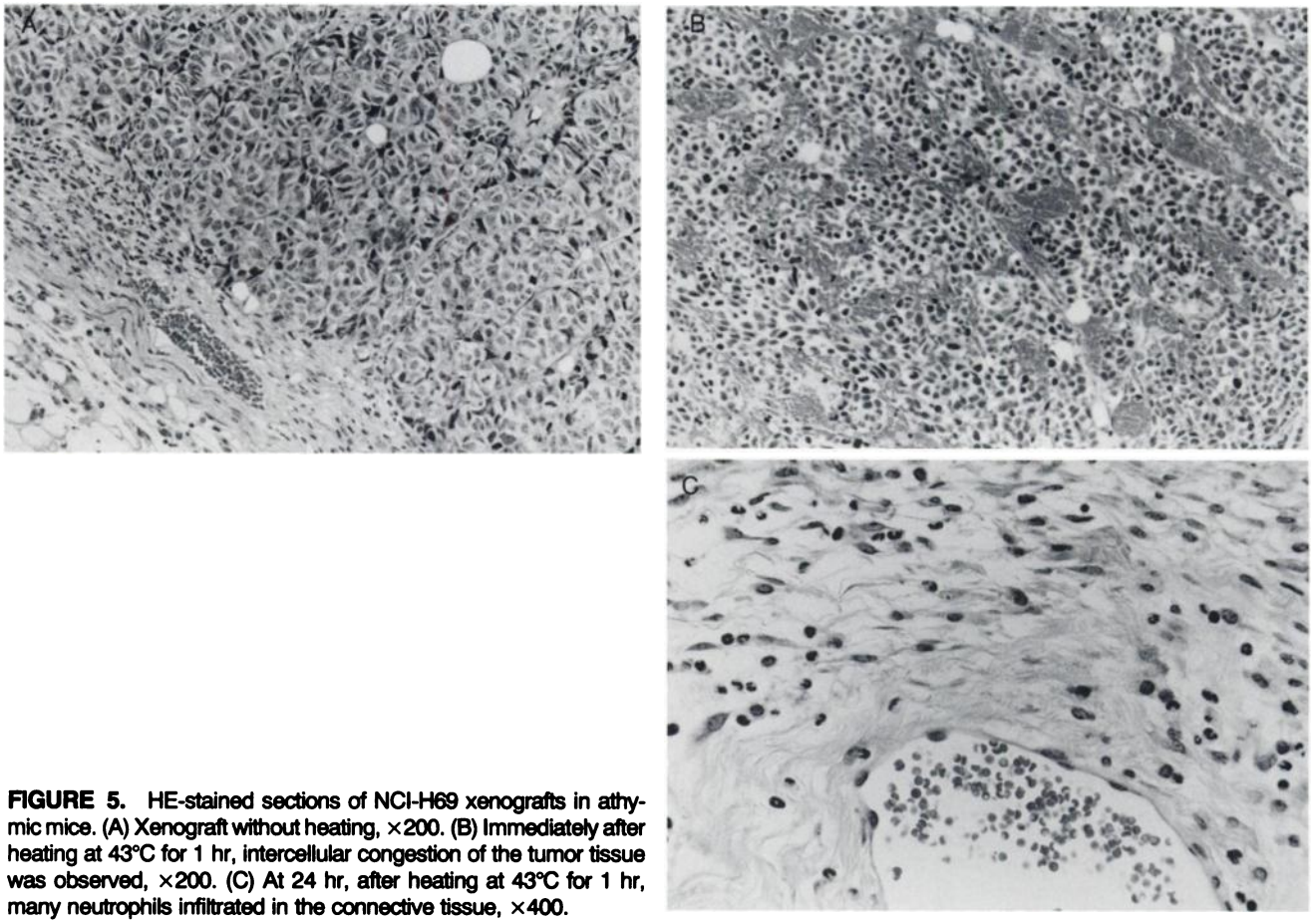

FIGURE 5. HE-stained sections of NCI-H69 xenografts in athymic mice. (A) Xenograft without heating, ×200. (B) Immediately after heating at 43°C for 1 hr, intercellular congestion of the tumor tissue was observed, ×200. (C) At 24 hr, after heating at 43°C for 1 hr, many neutrophils infiltrated in the connective tissue, ×400.

tumors to radiotherapy and chemotherapy, recurrent tumors, often therapy-resistant, develop in most cases (*13*). Therefore, radioimmunotherapy is expected as further strategy in the treatment of SCLC. In patients with SCLC, tumors tend to involve the upper mediastinum and the supraclavicular regions where hyperthermia can be efficiently applied to treat these lesions. As the thermal dose of a single heating at 42°C for 1 hr is clinically achievable (*26*), hyperthermia may deliver higher doses of radiolabeled Mabs to SCLC tumors in patients.

In conclusion, this study suggested that Mab NE150 localized well in SCLC xenografts and that hyperthermia at 42°C or 43°C for 1 hr enhanced the tumor uptake of Mab. Thus, radioimmunotherapy combined with hyperthermia may be helpful in the treatment of SCLC.

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Condensed from
15 Years Ago

Comparison of Xenon-133 Washout and Single-Breath Imaging for the Detection of Ventilation Abnormalities

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Xenon-133 ventilation studies of 115 patients were analyzed to determine the relative abilities of the single-breath and washout phases to detect regional ventilation abnormalities. All ^{133}Xe images were obtained in the posterior projection before six-view perfusion studies with ^{99m}Tc-human albumin microspheres. There were 275 regions with matching V-P abnormalities in the patients. The washout portion of the study detected 258 of these regions (94%) and the single breath detected 175 (64%) ($p < 0.01$). The discrepancies were confined to regions with nonsegmental perfusion defects, where the single breath detected 139 matches and the washout 216. The discrimination ratio between normal areas and areas of obstructive lung disease improved from 2:1 after 1 min of washout to 30:1 after 5 min. The late phases of ^{133}Xe washout are useful in detecting ventilation abnormalities, especially those associated with nonsegmental perfusion defects.

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