Enhanced Delivery of a Monoclonal Antibody F(\text{ab}')_2 Fragment to Subcutaneous Human Glioma Xenografts Using Local Hyperthermia

Darrell A. Cope, Mark W. Dewhirst, Henry S. Friedman, Darell D. Bigner, and Michael R. Zalutsky

ABSTRACT

The purpose of this study was to investigate the effects of tumor-localized hyperthermia at 42°C on the tissue distribution of radiiodinated monoclonal antibody F(\text{ab}')_2 fragments. Paired-label biodistribution measurements were performed in athymic mice bearing D-54 MG human glioma xenografts on one leg. Mice received both the 125I-labeled F(\text{ab}')_2 fragment of Mel-14, reactive with human gliomas and melanomas, and nonspecific 125I-labeled RPC 5 F(\text{ab}')_2. Tumor-bearing legs were placed in a 42°C water bath or a 37°C water bath (control) for 2 or 4 h. In mice sacrificed immediately after 2 h of heating, no hyperthermia-induced differences in the distribution of either fragment were observed. In the 4-h groups, tumor uptake of Mel-14 F(\text{ab}')_2, increased from 7.04 ± 1.59% injected dose (ID)/g at 37°C to 20.65 ± 4.53% ID/g at 42°C (P < 0.0001), and tumor localization of the control fragment rose from 5.23 ± 1.35% ID/g to 14.51 ± 1.37% ID/g (P < 0.0001). In another experiment, F(\text{ab}')_2 fragments were injected, tumors were heated for 4 h, and groups were sacrificed at 4, 8, and 16 h after injection. Statistically significant 2- to 3-fold higher uptake of both fragments in tumor were observed at all time points. Hyperthermic conditions also resulted in higher tumor:tissue ratios for both fragments. These results suggest that it may be possible to use tumor-localized hyperthermia to increase the therapeutic utility of radiolabeled monoclonal antibodies, particularly when labeled with short lived nuclides such as the 7.2-h α-emitter 211At.

INTRODUCTION

The feasibility of using radiolabeled MAbs directed at carcinoma-associated antigens for the external detection of tumors has been demonstrated both in animal models (1-4) and in clinical series (5-10). However, evaluations of tumor biopsies from patients receiving radiolabeled MAbs have shown that the fraction of the injected dose of radionuclide localized in tumor is quite low and generally only on the order of 0.001% of the injected dose per g (5, 6, 10-15). Consequently the delivery of therapeutic levels of radiation to tumor sites, while keeping radiation exposure of normal tissues at acceptable levels, is an exceedingly difficult task and is reflected in the limited efficacy reported for radioimmunotherapy in patients (16-19).

Improving the effectiveness of radioimmunotherapy requires the optimization of multiple variables, some of which are unique for each MAb-antigen combination. These factors include MAb specificity and binding affinity as well as antigen heterogeneity, density, and presence in normal tissues. An additional problem is to minimize the catabolism of radiolabel from the MAb after its administration in vivo. Moreover, delivery of labeled MAbs to tumors also may be impeded by limitations in tumor blood flow, vascularity, and permeability.

Several approaches are being pursued for increasing not only the magnitude but also the rate of MAb delivery to tumor. More rapid uptake of MAb by tumor is essential if promising shorter half-life nuclides such as the 7.2-h α-emitter astatine-211 are to be exploited for therapeutic applications (20). Intra-compartamental delivery of labeled MAbs has been shown to increase the rate and magnitude of tumor uptake in patients with ovarian carcinoma and leptomeningeal malignancies (21, 22). Other investigators have reported that enhanced tumor uptake could be achieved by alterations in tumor vasculature using external beam irradiation (23, 24). A more recent study reports that no statistically significant increase in tumor uptake of labeled MAbs occurs as a result of pre-irradiation (25). A complicating issue is the fact that external beam radiation can decrease tumor mass, an effect which itself increases MAb uptake (26).

Another strategy for increasing uptake of MAbs is to increase tumor blood flow using hyperthermia. Hyperthermia, when applied locally, is known to increase tumor blood flow at temperatures between 40°C and 42°C. However, temperatures above 43°C can create vascular damage and reduction in blood flow (27). Hyperthermia has also been shown to increase vascular permeability in tumors (28). Thus, hyperthermia at ~42°C might be expected to increase MAb uptake in tumors because of an increase in blood flow coupled with increased vascular permeability. This approach might be particularly useful for MAbs labeled with short lived radionuclides, since the hyperthermia could be used to enhance tumor uptake shortly after administration.

Stickney et al. (24) have reported that local hyperthermia increased the uptake of a labeled MAb in s.c. melanoma xenografts in athymic mice by about 200%; however, when tissue specimens were analyzed by direct counting instead of external imaging of intact animals, the enhanced uptake was not considered to be statistically significant.

In the present study, we have evaluated the effects of local hyperthermia at 42°C on the tissue distribution of the radioiodinated F(\text{ab}')_2 fragment of Mel-14, a MAb reactive with the high molecular weight chondroitin sulfate proteoglycan antigen associated with gliomas and melanomas (29, 30). We have shown previously that radioiodinated Mel-14 F(\text{ab}')_2 localizes specifically in s.c. D-54 MG human glioma xenografts in athymic mice (31). In order to obtain information about the specificity of hyperthermia-induced changes, these studies were performed by making direct comparisons to an isotype-matched nonspecific F(\text{ab}')_2 fragment using paired-label analyses (32). Our results indicate that local hyperthermia at 42°C can be used to enhance the delivery of MAb F(\text{ab}')_2 fragments to these s.c. human glioma xenografts over a time period compatible with the use of short-lived nuclides such as 211At.

MATERIALS AND METHODS

Monoclonal Antibodies. The MAb Mel-14, an IgG2a, is reactive with tumor-associated chondroitin sulfate proteoglycan present in melanomas, gliomas, and medulloblastomas (29). RPC 5 (Bionetics, Charleston, SC), which does not bind to any known antigen, was used as the nonspecific IgG2a control MAb. Both MAbs were purified from clari-
fled athymic mouse ascites by Protein A-Sepharose 4B chromatography. F(ab')2 fragments were generated by digestion of MAb's with pepsin, as described previously (31).

Radiiodination. The F(ab')2 fragments of Mel-14 and RPC 5 were labeled with 125I and 131I (Amersham, Chicago), respectively, using a modification of the iodogen method (33). Briefly, 200 ìg of the desired F(ab')2 fragment was reacted for 10 min at room temperature with approximately 500 ìCi of either 125I or 131I in a glass vial coated with 10 ìg of iodogen. Radiiodinated F(ab')2 fragments were separated from unreacted iodide by passage through a Sephadex G-25 column. The specific activities of the labeled proteins were between 1.1 and 1.8 ìCi/ìg. Protein-associated activity, determined by trichloroacetic acid precipitability, ranged from 91 to 97% for these preparations.

Immunoreactivity Assay. The binding characteristics of the 131I-labeled Mel-14 F(ab')2 preparations were evaluated in vitro. Approximately 50 ng of radiiodinated Mel-14 F(ab')2 were incubated in triplicate for 2 h at 37°C with 300 mg of both D-54 MG human glioma tumor homogenates and antigen-negative normal rat liver homogenates in 1 ml of phosphate-buffered saline containing 1% bovine serum albumin. After three washes, the percentage of 131I bound to the homogenates was determined. Specific binding was calculated as the percentage of Mel-14 F(ab')2 binding to D-54 MG minus the percentage bound to liver. The binding of one batch of 131I-labeled Mel-14 F(ab')2 was also determined after a 2-h incubation at 42°C.

Animal Model. Athymic nude mice (BALB/c, nu/nu) were obtained from a closed colony maintained at the Cancer Center Isolation Facility at Duke University. Seven-week-old male mice weighing between 20 and 25 g were used. D-54 MG, the Duke University subline of the human anaplastic astrocytoma line A-172, was used as the tumor model (34, 35). Tumor homogenates were prepared by passing xenograft fragments through a bilayered 20-mesh screen in a tissue press. The resulting homogenate was then passed through successively smaller needles (16, 19, and 20 gauge). Subcutaneous D-54 MG tumors were grown in the mice by injecting 50 ìg of tumor homogenate in the right leg. The foot was suspended, unsnared, so that blood flow to the foot itself would not be impeded. The weight was just sufficient to keep the leg of a fully anesthetized mouse extended.

Temperature Monitoring. Mice were anesthetized by i.p. injection of sodium pentobarbital and then received thermometry probes consisting of calibrated copper/constantan thermistors passed transdermally into the tumor, as well as rectal probes for the measurement of core temperatures. A 3.9-g weight was then attached to the plantar surface of the tumor-bearing leg. The foot was suspended, unsnared, so that blood flow to the foot itself would not be impeded. The weight was just sufficient to keep the leg of a fully anesthetized mouse extended. Animals were then placed on a styrofoam sheet containing holes, which was placed over a 42°C water bath and exposed to room air. Mice were maintained under anesthesia by additional injections of pentobarbital as needed and the tumor and core temperatures were recorded for 120 min. Water bath heating was chosen because it is a convenient method for heating several groups of animals at once. Temperature uniformity is generally within ±0.3°C with this technique (36) and it is more reproducible from mouse to mouse than ultrasound or microwaves.

Biodistribution Measurements. Twenty-four mice with D-54 MG xenografts were randomized with respect to tumor size and then given injections in the tail vein of both 131I-labeled Mel-14 F(ab')2 (4 ìg, 6 ìCi) and 131I-labeled RPC 5 F(ab')2 (4 ìg, 7 ìCi). Immediately following MAB administration, mice were anesthetized by an i.p. injection of pentobarbital, and weights were attached to the tumor-bearing leg as described above. Mice were then positioned over either a 42°C (hyperthermia) or 37°C (control) water bath and maintained under anesthesia with additional injections of pentobarbital, as needed. After both 2 and 4 h, one group each of the hyperthermia and the control animals were sacrificed by halothane overdose. Tissues of interest were removed, weighed, and assayed for both 131I and 125I activity, using a dual-channel automated gamma-counter. A correction was applied for the crossover of 1804

RESULTS

Temperature Monitoring. A preliminary study was performed to examine the intratumoral and rectal temperatures of mice undergoing tumor-localized hyperthermia. Immediately after initial anesthesia and placement of the temperature probes, both mouse core and tumor temperatures decreased to 31.5°C. As shown in Fig. 1, 1 min after submerging the tumor-bearing leg, the tumor temperature equilibrated rapidly to the 37°C temperature of the bath. During the 6 min required to raise the water bath to 42°C, the tumor temperature rose concomitantly with the increasing water temperature and then remained stable between 41.7°C and 42.0°C for as long as the tumor-bearing leg remained in the bath. Interstitial measurements were not made during actual experimental procedures in order to avoid vascular damage which could artifically affect MAB uptake. Mouse core temperatures in these and subsequent experiments never exceeded 39°C with this experimental design, which was well tolerated by these mice. In order to alleviate the problem of hypothermia observed in the brief interval (about 5–10 min) between initial anesthesia and placement in the water baths, the mice were kept under a heat lamp during this period.

Immunoreactivity Assessment. In vitro binding assays were used to determine the specific binding of the 131I-labeled Mel-14 F(ab')2 preparations to D-54 MG human glioma homogenates. After subtracting the nonspecific binding as measured by incubation with rat liver homogenates, the specific binding of the radiolabeled Mel-14 F(ab')2 fragment was 47.3 ± 2.9% in the first experiment and 44.2 ± 5.6% in the second experiment. These values are comparable to those observed in our previous

INVASIVE TUMOR TEMPS

Fig. 1. Tumor and rectal temperatures in athymic mice bearing D-54 MG glioma xenografts on the leg. The tumor-bearing leg was placed in a water bath at 37°C; 3 min later, the bath temperature was raised to 42°C.
studies with this F(ab')2 fragment (31). In order to determine whether hyperthermic conditions influenced specific MAb binding, a homogenate assay was also run at 42°C. No significant difference was observed between the specific binding after a 2-h incubation at 37°C and 42°C. The trichloroacetic acid precipitability of 125I activity in the homogenate supernatant was also determined, in order to see if there was temperature-dependent loss of label from the MAb fragment in vitro. The trichloroacetic acid precipitability of 125I-labeled Mel-14 F(ab')2 was 97%, irrespective of both incubation temperature and type of homogenate.

Effect of 2-h versus 4-h Heating. The purpose of the initial biodistribution study was to compare the effects of 2 h and 4 h of local hyperthermia at 42°C on the tissue distribution of coadministered 125I-labeled Mel-14 F(ab')2 and nonspecific control 125I-labeled RPC 5 F(ab')2. Fig. 2 compares the localization of the two labeled F(ab')2 fragments measured immediately after 2-h exposure to 37°C or 42°C for D-54 MG tumor, blood, liver, muscle, from the heated leg, and muscle from the contralateral leg. Mean tumor weights were 98 ± 70 mg in the control group and 86 ± 32 mg in the hyperthermic group. In tumor, uptake of Mel-14 F(ab')2 increased from 7.6 ± 1.5% ID/g to 9.0 ± 3.3% ID/g and uptake of RPC 5 F(ab')2 increased from 6.2 ± 1.1% ID/g to 8.6 ± 3.0% ID/g as a result of heating; however, neither these differences nor those observed in other tissues were determined to be statistically significant by Student's t test (95% confidence interval).

In Fig. 3 and Table 1, biodistribution data for the mice killed after 4 h of local hyperthermia are presented. Hyperthermic conditions increased the tumor localization of both 125I-labeled Mel-14 F(ab')2 and 123I-labeled RPC 5 F(ab')2 by approximately 3-fold. Tumor uptake of the specific fragment increased from 7.04 ± 1.59% ID/g at 37°C to 20.65 ± 4.53% ID/g at 42°C (P < 0.0001), while the tumor concentration of RPC 5 F(ab')2 rose from 5.23 ± 1.35% ID/g to 14.51 ± 1.37% ID/g (P < 0.0001). Statistically significant 3-fold increases were also observed in heated muscle for both MAb fragments. In general, heating the tumor-bearing leg had no effect on the distribution of either F(ab')2 fragment in other tissues with the exception of stomach, where an approximate 2-fold increase in uptake of radioiodine activity was seen in the hyperthermic animals.

The tissue distribution of both F(ab')2 fragments after 4 h of heating was measured at 4, 8, and 16 h after injection (0, 4, and 8 h after cessation of heating). Mean tumor weights at necropsy were 175 ± 78 mg for the hyperthermic group and 166 ± 96 mg for the control group. The tumor uptake of both Mel-14 F(ab')2 and RPC 5 F(ab')2 observed in the hyperthermia groups was significantly higher (P < 0.0001) than the control groups at all time points (Fig. 4). With all MAb-temperature combinations, maximal tumor localization occurred at 8 h after injection. Hyperthermia-induced enhancement of Mel-14 F(ab')2 tumor uptake was highest (2.6-fold) immediately after heating and decreased to a factor of 1.9 12 h later. For RPC F(ab')2, the ratio of tumor uptake at 42°C versus 37°C was 2.1 at all time points.

Tumor to normal tissue ratios were calculated in order to compare the selectivity of tumor localization under control and hyperthermic conditions. As shown in Fig. 5, exposure of D-54 MG s.c. tumors to 4 h of heating at 42°C increased tumor to normal tissue ratios for both the specific and control F(ab')2 fragments, particularly at earlier time points. At 4 h, tumor to tissue ratios for Mel-14 F(ab')2 at 42°C were 2 to 3 times higher than at the control temperature, indicating that higher tumor uptake could be achieved without compromising tumor selectivity. However, heating also increased tumor to tissue ratios at 4 h for RPC 5 F(ab')2 by almost the same degree, suggesting that the changes caused by local hyperthermia are due to nonspecific processes.

Use of a paired-label format permitted evaluation of the specificity of localization at the two temperatures via calculation of the localization index, the ratio of specific to nonspecific fragment in tissue, normalized to simultaneous blood levels. For all normal tissues and heated muscle, localization indices of about unity were calculated, indicating similar uptake of specific and nonspecific fragments in these tissues (Table 2). In contrast, localization indices in tumor for both control and hyperthermia groups were greater than 1 at all time points, indicating specific tumor uptake was achieved. However, localization indices in tumor were significantly lower (P < 0.001, 8 h; P < 0.05, 16 h) in animals subjected to local hyperthermia, suggesting a nonspecific component to tumor uptake at the higher temperature.

DISCUSSION

Utilization of monoclonal antibodies directed against tumor-associated antigens to deliver radionuclides selectively to malignancies is a conceptually appealing approach to cancer therapy. Unfortunately, particularly when MAbs are administered i.v., the amount of radioactivity localized and retained by tumor is insufficient to achieve adequate therapeutic effects without excessive radiation dose to normal tissues. An additional problem is that nearly all radioimmunotherapeutic clinical trials use MAbs labeled with 131I, an 8-day half-life β-emitter. While this nuclide may be useful for some radiotherapeutic applications, radiation dose rate effects with low linear energy transfer radiation (38, 39) and, assuming similar stabilities in vivo, the loss of nuclide from the MAb over time may make it disadvantageous to utilize shorter half-life β-emitters such as 16.9-h 188Re, 2.6-day 47Cu, and 13.4-h 109Pd. For other therapeutic situations,
exploiting the shorter range and greater biological effectiveness of α-particles might be preferable. However, since the half-lives of the most promising α-particle-emitting nuclides are quite short (212Bi, 61 min; 211At, 7.2 h), strategies must be devised to exploit the shorter range and greater biological effectiveness of α-particles. Recent studies have shown that local hyperthermia at 42°C can enhance the delivery of MAb F(ab')2 to tumors, presumably reflecting their faster blood clearance and greater blood-to-tissue transfer constant compared to intact immunoglobulin (31). Because of the potential for using hyperthermia in tandem with MAb-labeled radiotherapy, biodistribution time points were selected during the first 16 h after MAb injection, the period during which tumor activity were significantly higher in the animals exposed to tumor-localized hyperthermia.

In the current study, animals were exposed to 2 or 4 h of local hyperthermia at 42°C immediately after administration of the radioiodinated MAb labeled with the 7.2-h α-emitter 211At for radiotherapy. biodistribution time points were selected during the first 16 h after injection, the period during which nearly 80% of 211At decays would occur.

Tumor uptake, tumor:organ ratios, and localization indices for Mel-14 F(ab')2 in the control animals are in excellent agreement with the results which we have reported previously for this radioiodinated F(ab')2 fragment (31). The most important aspect of the current study is the demonstration that local hyperthermia at 42°C can enhance the delivery of MAb F(ab')2 fragments to s.c. human tumor xenografts. Statistically significant increases in tumor uptake of up to 1.5-fold were observed in two of the models for both the specific and control MAbs. Similarly, in athymic mice given injections of 131I-labeled anti-p97 MAb 3 h after receiving a dose of 10 Gy, enhanced uptake of activity was observed in s.c. melanoma xenografts 24 and 48 h after MAb injection (24). However, the results of two recent reports indicate no enhancement effect of pre-irradiation on the uptake of radioactivity in mammary carcinoma xenografts exposed to local hyperthermia at 42°C (32), although some enhancements have been observed in other tumor systems (33, 34).

**Table 1. Effect of local hyperthermia on tissue distribution of radioiodinated Mel-14 and control F(ab')2 fragments in athymic mice bearing D-54 MG xenografts.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mel-14 F(ab')2 Control</th>
<th>Hyperthermia</th>
<th>RPC 5 F(ab')2 Control</th>
<th>Hyperthermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>7.04 ± 1.59</td>
<td>20.65 ± 4.53*</td>
<td>5.23 ± 1.35</td>
<td>14.51 ± 1.37*</td>
</tr>
<tr>
<td>Liver</td>
<td>3.29 ± 0.58</td>
<td>3.74 ± 0.40</td>
<td>5.13 ± 1.15</td>
<td>5.82 ± 0.70</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.69 ± 0.60</td>
<td>3.46 ± 0.37*</td>
<td>3.95 ± 0.83</td>
<td>4.69 ± 0.52</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.41 ± 1.06</td>
<td>6.58 ± 2.23</td>
<td>7.35 ± 1.46</td>
<td>10.03 ± 2.83</td>
</tr>
<tr>
<td>Kidneys</td>
<td>28.17 ± 2.43</td>
<td>30.93 ± 7.24</td>
<td>17.12 ± 1.63</td>
<td>18.71 ± 3.93</td>
</tr>
<tr>
<td>Stomach</td>
<td>14.64 ± 6.57</td>
<td>28.25 ± 12.26*</td>
<td>11.21 ± 4.94</td>
<td>21.20 ± 8.78*</td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.21 ± 1.04</td>
<td>2.86 ± 0.64</td>
<td>2.41 ± 0.61</td>
<td>3.24 ± 0.58*</td>
</tr>
<tr>
<td>Large intestine</td>
<td>1.47 ± 0.20</td>
<td>1.48 ± 0.24</td>
<td>1.74 ± 0.29</td>
<td>1.75 ± 0.39</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1.17 ± 0.59</td>
<td>1.73 ± 0.72</td>
<td>1.16 ± 0.65</td>
<td>1.42 ± 0.53</td>
</tr>
<tr>
<td>Unheated muscle</td>
<td>0.97 ± 0.37</td>
<td>1.31 ± 0.36</td>
<td>1.21 ± 0.28</td>
<td>1.70 ± 0.46</td>
</tr>
<tr>
<td>Heatved muscle</td>
<td>1.23 ± 0.42</td>
<td>3.54 ± 1.97*</td>
<td>1.59 ± 0.48</td>
<td>5.91 ± 2.05*</td>
</tr>
<tr>
<td>Blood</td>
<td>9.53 ± 1.76</td>
<td>8.55 ± 1.69</td>
<td>16.75 ± 5.13</td>
<td>14.84 ± 3.43</td>
</tr>
<tr>
<td>Brain</td>
<td>0.28 ± 0.06</td>
<td>0.29 ± 0.09</td>
<td>0.45 ± 0.11</td>
<td>0.42 ± 0.09</td>
</tr>
</tbody>
</table>

* P < 0.0001 compared to control.
* P < 0.05 compared to control.
* Percentage per organ.
* P = 0.01 compared to control.

Fig. 4. Athymic mice with s.c. D-54 MG xenografts given injections of 131I-labeled Mel-14 F(ab')2 and 125I-labeled RPC 5 F(ab')2, and the tumor-bearing leg was exposed to a water bath at 37°C or 42°C for 4 h. Tumor uptake of radioiodine at 4, 8, and 16 h after MAb F(ab')2 administration is shown.

![Graph showing tumor uptake over time](image-url)
with the exception of stomach, normal tissue activity levels did not increase, tumor:tissue ratios also increased by a factor of 2-3, suggesting that local hyperthermia might be useful in enhancing tumor to normal tissue radiation dose ratios.

Although many radioiodinated compounds including MAb fragments are relatively inert to dehalogenation \textit{in vitro}, most undergo deiodination \textit{in vivo}, resulting in significant accumulation of activity in the stomach and thyroid. It is generally believed that the increased loss of label \textit{in vivo} is due to the action of deiodinases (41). Our observation of higher activity in the stomach and, to some degree, the thyroid in animals receiving hyperthermia may reflect increased deiodinase activity at elevated temperature. Future hyperthermia studies will be performed with MAbs radioiodinated using the N-succinimidyl 3-(tri-n-butylstannyl)benzoate reagent, a method which has been shown to render MAbs inert to dehalogenation \textit{in vivo} (42).

The observations that the nonspecific RPC 5 F(ab')2 fragment tumor uptake was increased, that heated muscle uptake of both fragments increased, and that localization indices in tumor decreased at 42°C all suggest that the enhanced tumor uptake caused by hyperthermia is due to nonspecific processes. In all cases, however, tumor uptake and tumor:normal tissue ratios for the specific Mel-14 F(ab')2 fragment were higher than those for the nonspecific control fragment.

The nonspecific nature of increased F(ab')2 uptake with hyperthermia should not be considered as a negative aspect of this method for targeting activity to tumor. With most hyperthermic techniques except total body, tumors tend to get hotter than surrounding normal tissues, which probably reflects the more efficient perfusion in the latter (27). Thus, specific enhanced deliver of MAb should be increased by heating. Recent studies with total body hyperthermia indicate that it may be possible to “target” certain tissue beds such as lungs and liver because of changes in tissue perfusion (43), suggesting that even whole body hyperthermia could play a role in the use of MAbs for the treatment of systemic or metastatic disease.

The increased uptake of MAb fragments in tumor after
hyperthermia treatment is likely due, in part, to changes in blood flow, blood volume, and/or permeability. Hyperthermic treatment at 42°C for 60–100 min previously has been reported to increase tumor blood flow and vascular permeability, at least during the heating session (27, 28). Generally, blood flow drops to baseline or below shortly after heating is stopped. Reductions in blood flow, which are related to vascular damage, occur at various temperature-time combinations but generally require higher than 42°C heating in most tumor models studied. Blood flow, vascular volume, and permeability were not measured directly in this study. Thus, the observed increases in MAB fragment uptake by tumor could be the result of any or all of these processes.

It is hoped that changes in permeability play a role, since this effect may improve the delivery of MABs to the target tissue. Additional studies are now underway using quantitative autoradiography to assess the intratumoral distribution of both intact MABs and F(ab′)2 fragments. Using this technique, it will be possible to ascertain whether MABs are leaving the vascular space and entering the tissue parenchyma and whether the effect extends from the edge into the tumor center.

With MABs, other factors must also be considered. For example, elevated temperatures might be expected to increase the rate of MAB binding to antigen. Although a detailed kinetic study was not performed, the specific binding of 125I-labeled Mel-14 F(ab′)2 after a 2-h incubation in vitro was identical at 37°C and 42°C. Because hyperthermic conditions are known to inhibit protein synthesis, it also is possible that changes in tumor antigen expression could be induced by elevated temperatures. Indeed, heating melanoma cells to 43.5°C or more has been shown to decrease antigen expression during the first day after heating (44, 45). However the magnitude of this effect was much lower at 42°C, the temperature used in our study.

In conclusion, we have demonstrated that local hyperthermia at 42°C applied immediately after administration of a MAB F(ab′)2 fragment results in significantly higher localization in s.c. human tumor xenografts. The applicability of this approach to other tumor models with different permeability and blood flow characteristics remains to be ascertained.

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