Potential of Palladium-109-Labeled Antimelanoma Monoclonal Antibody for Tumor Therapy

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Palladium-109, a beta-emitting radionuclide, was chelated to the monoclonal antibody 225.28S to the high molecular weight antigen associated with human melanoma. The radiolabeled antibody maintained its specific in vitro reactivity with cultured human melanoma cells. Injection of the radiolabeled monoclonal antibody into nude mice bearing human melanoma resulted in significant accumulation of the radiolabel in the tumors: 19% injected dose/g; 38:1 and 61:1 tumor-to-blood ratios at 24 and 48 hr, respectively. The localization of the radiolabeled antibody in liver and kidney also was high, but appreciably lower than that achieved in tumor. These results suggest that Pd-109-labeled monoclonal antibody to tumor-associated antigens may have potential applications in tumor immunotherapy.

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Hybridoma technology has been used to develop monoclonal antibodies to a variety of human tumor-associated antigens (*1–10*). The high degree of specificity of these reagents has rekindled interest in the application of immunotherapy to malignant diseases. In vitro studies and animal model systems have shown that monoclonal antibodies are, in general, ineffective in mediating lysis of tumor cells. Therefore, the emphasis of current studies has focused on the use of antibodies as carriers to deliver toxins, chemotherapeutic agents, and/or radionuclides to tumors. Preliminary studies (*11,12*) have evaluated the use of iodine-131-labeled antibodies for tumor therapy in patients with melanoma. Although the results have been encouraging, labeling of antibodies with I-131 has several limitations, such as in vivo deiodination and possible inactivation of the antibody by attachment of the iodine at or near the antigen-binding site. These limitations have stimulated interest in the utilization of other radionuclides for therapy of malignant diseases.

Monoclonal antibodies to a human melanoma-associated antigen of high molecular weight (HMW-MAA) have been developed recently (*13*), and they have sufficient accumulation and specificity to offer an appropriate carrier for immunotherapy in patients with melanoma. This investigation was designed to determine the feasibility and potential utility of labeling such an antimelanoma monoclonal antibody with palladium-109 (Pd-109), a predominantly beta-emitting radionuclide (E_{max} = 1MeV; T1/2 = 13.4 hr), available in curie quantities and theoretically obtainable in carrier-free form for radiotherapy.

MATERIALS AND METHODS

Monoclonal antibody. The anti-HMW-MAA monoclonal antibody (MoAb) 225.28S, an IgG 2a immunoglobulin, was prepared and characterized as previously described (*13*). It was purified from ascites fluid by the

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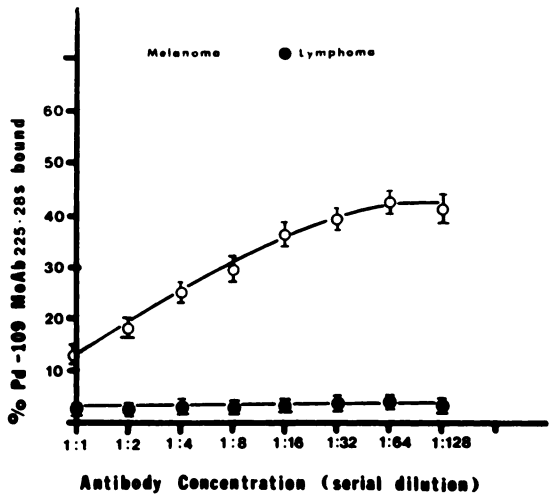

FIG. 1. In vitro binding of Pd-109-labeled anti-HMW-MAA MoAb 225.28S to human Colo 38 melanoma cells (O) and to human B lymphoid cells LG-2 (●). Binding to melanoma is significantly higher ($p < 0.001$) at all antibody concentrations. Error bars represent standard deviation.

caprylic acid method (unpublished data, Russo C, Callegaro L, Lanza E, Ferrone S).

Preparation of $^{109}PdCl_2$. Neutron bombardment of enriched $^{108}PdCl_2 \cdot 2H_2O$ (2.03 mg) in a sealed quartz ampule for 16 hr in the Brookhaven High Flux Reactor (5.5×10^{14} n/cm^2-sec) produced 1.82 Ci of Pd-109. The material was dissolved in 1.6 ml DMSO by gently heating and stirring the solution. An aliquot of this solution containing 45 mCi of Pd-109 was transferred into a 30-ml multi-injection bottle containing 5 ml of a 0.1 *M* acetate buffer, pH 3.95.

Radiometallic labeling. Coupling of DTPA to antibody was accomplished by the cyclic anhydride method (*14*). The anhydride-to-protein ratio was 1:1. The reaction was carried out in HEPES-HCl buffer (pH 7.0). Complexing of the antibody-DTPA conjugate (200 μg) to $^{109}PdCl_2$ (1.8 mCi, 2.5 μg) was accomplished at pH 5-6. The radiometallic antibody was purified on a G-150 Sephadex column and eluted with HEPES-HCl buffer (pH 7.0). Characterization of the radiolabeled antibody was accomplished using TCA precipitation and polyacrylamide gel electrophoresis. The radiolabeling yield was 10%, the low yield due probably to the low specific activity of Pd-109 used in these experiments. The specific activity of the radiolabeled antibody at the time of experimentation was 1.4 μCi Pd-109 per μg antibody.

In vitro binding assay. Cultured human melanoma cells Colo 38, and cultured human B lymphoid cells LG-2 (100,000 each) were incubated for 1 hr at room temperature with sequential 50% dilutions of the radiolabeled antibody preparation in triplicate. The cells then were washed three times and the binding of antibody to cells was measured in a conventional well counter.

Tumor cell lines. The human melanoma cell line Colo 38 and the human B lymphoid cell line LG2 were cultured in RPMI-1640 medium with L-glutamine. Solid tumors were produced in athymic nude mice (Swiss/Webster, nu/nu)* by injection of 10 million melanoma cells in the flank. Tumors were allowed to reach ~5 mm in diameter before experimentation.

Experimental designs and results. *In vitro binding of Pd-109 MoAb 225.28S to melanoma.* Figure 1 demonstrates that Pd-109 MoAb 225.28S achieved significantly higher ($p < 0.001$) in vitro binding (41.3% in the plateau region) to cultured human melanoma cells Colo 38 than to control lymphoid cells LG-2 (3.1%).

In vivo binding of Pd-109 MoAb 225.28S to melanoma tumors. Three groups of athymic nude mice bearing Colo 38 human melanoma tumor (n = 3-6/group) were injected with 10 μCi of Pd-109 MoAb 225.28S and killed 13,24, and 48 hours later for determination of radioctivity (% ID/g). As shown in Table 1, the concentration of Pd-109 MoAb 225.28S in melanoma at 13 hr after tracer administration (19.99%/g) was significantly higher ($p < 0.001$) than that achieved in control studies using melanoma-bearing animals in-

TABLE 1. BIODISTRIBUTION OF Pd-109 MoAb COMPARED WITH Pd-109 CITRATE CONTROL*

Tissue	Pd-109 MoAb 225.28S		Pd-109 Citrate	
	Mean % ID/g	Range	Mean % ID/g	Range
Blood	0.50	0.43–0.58	0.37	0.36–0.37
Tumor	19.99	18.99–21.26	1.73	1.69–1.77
Lung	1.46	1.30–1.59	1.98	1.94–2.02
Liver	5.04	4.29–6.00	4.36	3.06–5.81
Spleen	2.69	2.48–2.89	2.72	2.12–2.61
Kidney	13.67	12.72–14.36	24.82	20.70–28.40
Muscle	0.51	0.42–0.60	0.77	0.75–0.79
Bone	1.35	1.19–1.45	1.06	0.93–1.23

* Results given as percent injected dose per gram (% ID/g) 13 hr after injection.

TABLE 2. CHANGE IN BIODISTRIBUTION OF Pd-109 MoAb OVER TIME*

	Hours after injection					
	13 hr		24 hr		48 hr	
Tissue	Mean % ID/g	Range	Mean % ID/g	Range	Mean % ID/g	Range
Blood	0.50	0.43–0.58	0.51	0.44–0.59	0.30	0.21–0.41
Tumor	19.99	18.99–21.26	19.51	16.66–20.13	18.51	14.79–23.49
Lung	1.46	1.30–1.59	0.94	0.67–1.17	0.64	0.51–0.77
Liver	5.04	4.28–6.00	4.54	3.60–5.47	4.26	3.90–4.66
Spleen	2.69	2.48–2.89	3.15	2.28–3.89	3.01	2.89–3.26
Kidney	13.67	12.72–14.36	11.15	8.34–14.61	10.05	7.66–12.60
Muscle	0.51	0.42–0.60	0.55	0.41–0.79	0.32	0.26–0.39
Bone	1.35	1.19–1.45	1.48	1.25–1.92	1.31	0.99–1.64

* Results given as percent injected dose per gram (% ID/g).

jected with Pd-109 citrate (1.73%/g). Table 2 demonstrates that the concentration of Pd-109 MoAb 225.28S in melanoma at 13, 24, and 48 hr after administration of radioactivity was uniformly higher ($p < 0.001$) than that achieved in other tissues. Tumor-to-blood ratios were very high (38:1 to 61:1 at 13 and 48 hr, respectively), but there also was moderate accumulation of the radiolabel in liver and kidney.

DISCUSSION

Tumor therapy with I-131-labeled antibodies has been reported (*11,12*). However, the affinity of some antibodies for tumor may be diminished or lost with radioiodination, presumably due to the attachment of the label at or near a critical antigen-binding site. The ability to use bifunctional chelation to conjugate monoclonal antibodies with a variety of radioactive metals, including beta emitters, obviates the need to rely solely on radiolabeling with I-131 for tumor therapy. We found relatively high uptake of Pd-109 anti-HMW-MAA MoAb 225.28S in human melanoma implanted in athymic nude mice. Thus Pd-109—a predominantly beta-emitting nuclide with a relatively short half-life—may be a useful agent for treatment of melanoma when bound to anti-MAA monoclonal antibody. Although the concentrations of Pd-109-labeled anti-MAA monoclonal antibody in kidney and liver also were high, these tissues are relatively radioresistant and can withstand much greater radiation doses than the more radiosensitive tumor.

The finding that 60% of the radiolabeled antibody preparation failed to bind to melanoma cells in vitro may reflect the co-purification of IgG without antibody activity from ascites fluid, and/or inactivation of antibody during purification, storage, or radiolabeling. The low binding efficiency of Pd-109 to antibody may be due to presence of carrier Pd-108 in the Pd-109 preparation. Thus, the potential of Pd-109-labeled anti-HMW-MAA MoAb 225.28s for tumor therapy might be substantially enhanced by improving the method of labeling and/or purification of antibody in order to decrease renal and hepatic activity, and by using carrier-free Pd-109 for labeling in order to increase the binding efficiency of Pd-109 to antibody. The results of this study suggest further investigation of the potential of tumor therapy using antibody labeled with radiometallic beta emitters.

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