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Radioimmunotherapy for Pancreatic Carcinoma Using ^{131}I-Labeled Monoclonal Antibody Nd2 in Xenografted Nude Mice

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We investigated the biodistribution, radiolocalization, and radioimmunotherapeutic potential of ^{131}I-labeled Nd2 in athymic nude mice bearing human pancreatic carcinoma xenografts. ^{131}I-Nd2 was accumulated at high levels in the tumor, in contrast to blood, liver, spleen, and other normal organs. The tumor was clearly delineated in scintigraphs. The volumes of tumors of mice injected with 7.4 MBq of ^{131}I-Nd2 were 80% less than those of tumors before injection of radiolabeled Nd2. Fibrous or vacuolar degeneration was seen in histological sections of tumors of 7-week-treated mice. The growth of tumors in mice treated with misonidazole, a hypoxic cell radiosensitizer, and then injected twice with 3.7 MBq of ^{131}I-Nd2 was suppressed over 7 weeks. Neither leucocytopenia nor thrombocytopenia was severe after injection of radiolabeled Nd2. Thus ^{131}I-labeled Nd2 may have clinical application in the radioimmunotherapy of pancreatic cancer.

Key words: Monoclonal antibody Nd2 — Pancreatic carcinoma — Radioimmunotherapy

Pancreatic cancer is still a difficult disease to diagnose early and to treat successfully. In large measure this is due to the advanced stage of tumor growth at the time of diagnosis. By the time the disease is detected, combination therapies such as operation, chemotherapy and irradiation are required. Use of radiolabeled monoclonal antibody against tumor associated antigen for therapy is under active investigation by many groups. Radioimmunotherapy in animals has given successful results.[1-13] Some monoclonal antibodies labeled with various isotopes have been used in clinical trials of hepatocellular-carcinoma,[14] ovarian cancer,[15,16] melanoma,[17,18] and colon carcinoma.[19-22] However, there are only a few reports of radioimmunotherapy for pancreatic cancer. Alisauskus *et al.*[23] and Kamigaki *et al.*[24] have reported positive results using ^{131}I-labeled monoclonal antibodies.

The monoclonal antibody (mAb) Nd2 is a mouse IgG1 which was produced against purified mucin of human pancreatic cancer and previously shown to be reactive with 83% of pancreatic cancers.[25,26] It is not reactive with normal pancreas, chronic pancreatitis, or other organs. In the present study, we investigated the use of ^{131}I-labeled mAb Nd2 in the treatment of pancreatic cancer, and the effect of the radiosensitizer misonidazole on radioimmunotherapy.

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MATERIALS AND METHODS

Tumor models Human pancreatic carcinoma cells SW1990 were cultured in Dulbecco's modified Eagle's medium supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), L-glutamine (2 m*M*), and 10% fetal bovine serum previously inactivated at 56°C for 1 h. Cultures were maintained in 75-cm^2 flasks or 50-cm^2 dishes incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. These cells were used to develop a xenograft model in 5-week-old female athymic nude mice by subcutaneous inoculation. Xenografts were used for study when they were 5–10 mm in diameter, usually 3–4 weeks after inoculation.

mAb Nd2, a mouse IgG1 mAb against human pancreatic carcinoma,[26] was labeled with 131-iodide by the Iodogen method.[27] Labeling was done in 1 ml of 0.1 *M* borate buffer at room temperature for 10 min in a 20-ml vial previously coated with iodogen. The vial was coated as follows: 2 ml of iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril) in chloroform was added to the vial and the chloroform was allowed to evaporate overnight at room temperature. The reaction mixture was purified using a PD-10 column. Analyses of radioactivity and protein for every fraction were done by using a well-counter and the BCA protein assay reagent. The specific activity was 66.6 MBq/mg protein.

Biodistribution study and immunoscintigraphy Tumor-bearing nude mice were injected intravenously with 0.37 MBq of ^{131}I-labeled Nd2. The biodistribution data were

obtained after killing and dissecting animals under ether anesthesia at 12 h, 1, 3, and 7 days after injection. Portions of tumor, blood, urine, liver, kidney, spleen, pancreas, heart, lung, stomach, small intestine, large intestine, muscle, and bone were removed and weighed. The radioactivity in the tissue was measured in a gamma-scintillation counter and expressed as percent of injected dose per gram of tissue (%ID/g) and tissue-to-blood ratio (T/B ratio). The mean and standard deviation were calculated for every time point.

Scintigraphic images were made of mice 7 days after an intravenous injection of 3.7 MBq of ^{131}I-labeled MAb-Nd2. A gamma camera was used to make a posterior view.

Radioimmunotherapy study Mice bearing human pancreatic carcinoma xenografts were divided into three groups of 4–6 animals as follows: (1) mice given a single injection of 3.7 MBq, 7.4 MBq of ^{131}I-labeled Nd2 or ^{131}I-non-specific mouse IgG1; (2) mice injected twice at an interval of three weeks with 3.7 MBq of labeled Nd2; and (3) mice treated with radiosensitizer and injected with 3.7 MBq of labeled Nd2. All antibodies were administered via the tail vein. Tumors were measured in 3 dimensions (length, width and height) on the day of antibody injection (day 0), and on every 3rd or 4th day. Tumor volumes were estimated as $L \times W \times H$, and expressed as ratios to those of day 0. In the 3rd group, we investigated the effect of the radiosensitizer misonidazole on radioimmunotherapy. Misonidazole was made up in saline, and given intraperitoneally (500 mg/kg) twice a day for 5 days, starting 1 h after ^{131}I-labeled Nd2 injection. Mice were observed for 7 weeks. Thyroid uptake of iodide was blocked by adding 0.1% potassium iodide to the drinking water beginning 3 days before the injection of radioiodinated Nd2 and throughout the experiment.

Histologic examination Approximately 7 weeks after the injection of radioiodinated Nd2, mice were killed under anesthesia. The tumors were removed and embedded in paraffin. Histologic sections were stained with hematoxylin and eosin.

Side effects of radioiodinated Nd2 To evaluate the influence of radioiodinated antibody on the bone marrow of test animals, blood was taken from the retrobulbar venous plexus of the group injected with 7.4 MBq of ^{131}I-Nd2 for leukocyte (WBC), hemoglobin (Hb), hematocrit (Ht), and platelet (PLT) counts at different times after injection. In the same group, mice were weighed at three points during the experiments to investigate the effects on the whole body growth.

Statistical analysis Analysis of proportional data in the various experiments at different times post treatment was done by use of the *t* test.

RESULTS

Biodistribution The biodistributions at different times after injection of 0.37 MBq ^{131}I-labeled Nd2 are shown in Tables I and II. The %ID/g of the tumor gradually rose to 49.5±7.3% on day 3, and a high concentration was maintained until day 7. The high concentration in the blood just after injection decreased to 12.3±0.4% on day 7. The %ID/g's of the liver, lung, and spleen, were higher than those of other organs. The values were lower on day 7. Some organs such as the small intestine, large intestine, muscle, and bone showed the highest uptake at

Table I. Biodistribution of 0.37 MBq of ^{131}I-Nd2 (n=4) (expressed as %injected dose/g tissue)

	Time			
	12 h	24 h	3 days	7 days
Tumor	16.5±2.8	35.8±13.4	49.5±7.3	43.2±3.4
Urine	11.9±3.5	6.9±0.7	3.7±0.7	2.9±1.0
Blood	29.7±1.5	27.2±3.1	20.6±1.4	12.3±2.6
Liver	11.7±2.2	6.6±0.1	4.1±1.2	2.2±0.4
Spleen	9.1±2.0	7.6±0.8	4.0±0.6	2.3±0.8
Kidney	8.4±1.0	7.2±0.9	4.8±0.2	3.0±0.4
Pancreas	4.7±1.0	4.2±1.0	3.8±0.4	1.8±0.4
Heart	7.3±0.1	5.3±0.5	3.6±0.1	2.0±0.4
Lung	14.3±4.1	12.2±2.4	7.5±0.7	4.9±1.4
Stomach	3.2±0.7	3.2±0.5	2.4±0.3	1.2±0.2
S. Intes.	3.7±0.5	3.8±0.5	2.5±0.4	1.5±0.3
L. Intes.	4.0±0.8	4.3±1.0	2.7±0.5	1.6±0.2
Muscle	2.3±0.2	2.7±0.4	1.9±0.3	1.8±0.5
Bone	2.5±0.3	5.2±1.8	3.5±0.2	2.0±0.4

mean±SD

Table II. Biodistribution of 0.37 MBq of ^{131}I-Nd2 (n=4) (expressed as tissue/blood ratio)

	Time			
	12 h	24 h	3 days	7 days
Tumor	0.55±0.10	1.31±0.48	2.40±0.34	3.66±0.64
Urine	0.40±0.11	0.25±0.03	0.17±0.04	0.27±0.15
Blood	1	1	1	1
Liver	0.39±0.09	0.24±0.02	0.20±0.06	0.18±0.01
Spleen	0.31±0.08	0.28±0.01	0.19±0.02	0.18±0.03
Kidney	0.28±0.03	0.26±0.00	0.23±0.00	0.25±0.02
Pancreas	0.15±0.02	0.15±0.03	0.18±0.01	0.14±0.01
Heart	0.24±0.01	0.19±0.00	0.17±0.01	0.16±0.00
Lung	0.48±0.15	0.44±0.05	0.36±0.02	0.39±0.03
Stomach	0.10±0.01	0.11±0.01	0.11±0.01	0.10±0.01
S. Intes.	0.12±0.01	0.14±0.01	0.12±0.02	0.12±0.00
L. Intes.	0.13±0.02	0.15±0.02	0.13±0.02	0.13±0.01
Muscle	0.07±0.00	0.09±0.01	0.09±0.00	0.15±0.02
Bone	0.08±0.01	0.18±0.04	0.16±0.00	0.16±0.01

mean±SD

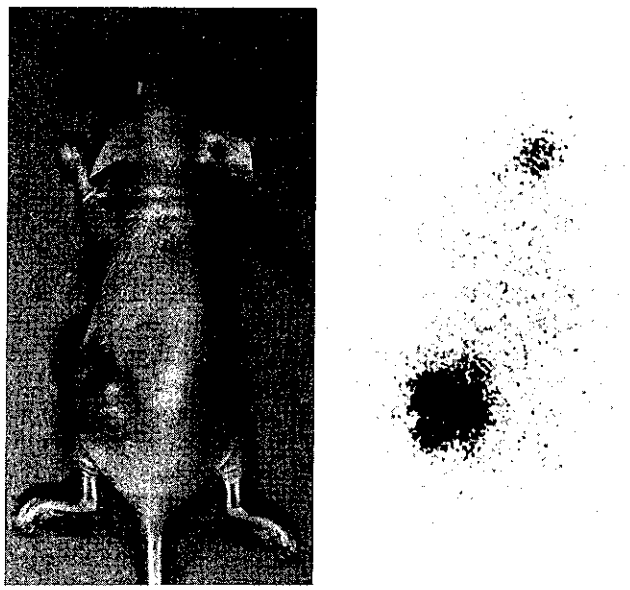

Fig. 1. Radioimmunoscintigraphic study. Tumor-bearing nude mice were injected with 0.37 MBq of ^{131}I-Nd2 and scintigraphic images were made 7 days later with a gamma-camera in posterior view. High levels of accumulation were seen in the tumor and the thyroid. The latter is not unexpected given the normal uptake of iodine by that organ.

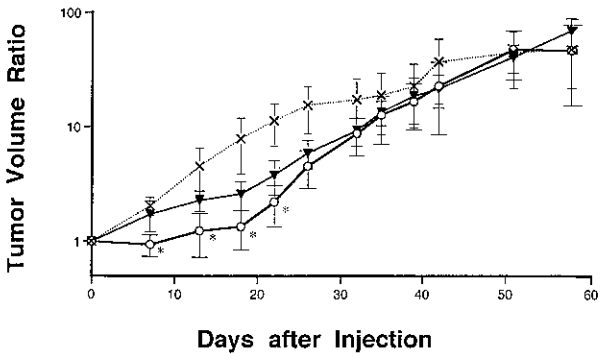

Fig. 2. Tumor growth curves after RAIT. Tumor-bearing mice were administered ^{131}I-labeled Nd2 (○), non-specific IgG1 (▼), or normal saline (×). * $P<0.05$.

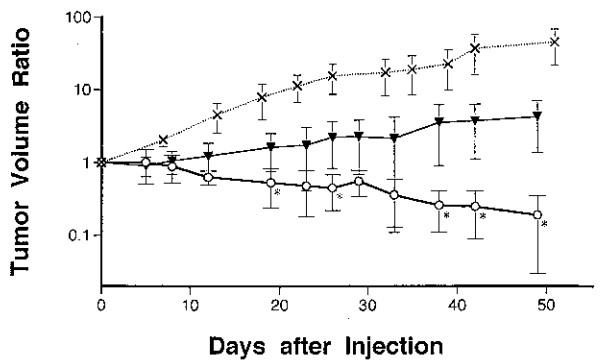

Fig. 3. Tumor growth curves after injections of 7.4 MBq of ^{131}I-Nd2 (○), ^{131}I-non-specific mouse IgG1 (▼) and normal saline (×). * $P<0.05$.

24 h post injection (less than 6.0%). Others had the highest concentration at 12 h (less than 15.0%, Table I). Thus, no organ except the tumor showed a specific uptake of ^{131}I-labeled Nd2. The T/B ratio of the tumor increased from 0.55±0.10 at 12 h to 3.66±0.64 at day 7 post injection. The T/B ratios of liver, lung, and spleen were less than 0.5, and those of the other organs were less than 0.3 throughout the experiment (Table II).

Radioimmunoscintigraphy A scintigraphic image obtained on day 7 from a nude mouse injected with ^{131}I-labeled Nd2 is shown in Fig. 1. This image demonstrated the specific localization of the antibody in the tumor. Organs such as liver and spleen did not take up the radioactivity, so the tumor was definitively identified. High accumulation was seen in the thyroid due to the function of the organ in iodine uptake. Therefore, mice were pretreated with potassium iodide to block thyroid accumulation in subsequent experiments.

Radioimmunotherapy with ^{131}I-labeled Nd2

Single injection: In the first experiment, tumor-bearing mice received a single i.v. injection of 3.7 MBq of ^{131}I-labeled Nd2 or 3.7 MBq of ^{131}I-labeled nonspecific IgG1 or normal saline. As shown in Fig. 2, an anti-tumor effect was seen in the initial three weeks after injection in the group of mice receiving ^{131}I-Nd2. This difference was significant ($P<0.05$) compared to the group of mice receiving ^{131}I-labeled nonspecific IgG1. Tumor volumes showed no marked fluctuation over 2 weeks, but gradually increased thereafter. In the group of mice receiving ^{131}I-IgG1, tumors continued to increase in size after injection, but the growth was not as rapid as that of the group given normal saline. After the 3rd week post injection, tumors in both the Nd2 and IgG1 groups showed progression at the same rate. Tumors had increased 10-fold in volume 5 weeks after injection.

In the second experiment, mice received 7.4 MBq of ^{131}I-Nd2 or 7.4 MBq of non-specific IgG1. In the group of mice injected with ^{131}I-Nd2, a substantial and long-lasting anti-tumor effect occurred (Fig. 3). Tumor reduction was seen about 1 week after injection. Approximately 80% reduction of the original tumor volumes occurred 7 weeks later ($P<0.025$ in comparison with the normal saline group). On the other hand, in the group of mice receiving 7.4 MBq of ^{131}I-IgG1, tumor volumes

gradually increased just after injection, but more slowly than those of mice receiving normal saline.

Two injections of 3.7 MBq of ^{131}I-Nd2: In the group of mice receiving two injections of 3.7 MBq of ^{131}I-Nd2, compared with mice receiving a single injection, tumor progression was slower after the second injection, but the difference was not significant. The tumor volumes were approximately 8-fold larger than the original values 5 weeks after injection, as compared to more than 10-fold in the group receiving a single injection. But no tumor reduction was seen in this group.

The effect of radiosensitizer: The effect of the radiosensitizer misonidazole on radioimmunotherapy is shown in Figs. 4 and 5. Tumor progression was delayed in the mice treated with misonidazole, in comparison with untreated mice ($P<0.05$), especially after the 5th week post injection. However, tumors gradually progressed throughout the experiment (Fig. 4). In the group of mice treated with misonidazole and receiving two injections of 3.7 MBq of Nd2 at an interval of three weeks, tumor growth was continuously inhibited, especially from the 4th week after the second administration (Fig. 5).

Histological examination In the groups of mice receiving 7.4 MBq of ^{131}I-labeled Nd2 and IgG1, histological sections at the 7th week after injection were examined. In the mice injected with ^{131}I-IgG1, tumor cells were seen all around the tumor (Fig. 6a). Lymphocyte-like cell infiltration was seen, but degenerative changes were not obvious. On the other hand, in the mice administered 7.4 MBq of ^{131}I-labeled Nd2, only a small number of tumor

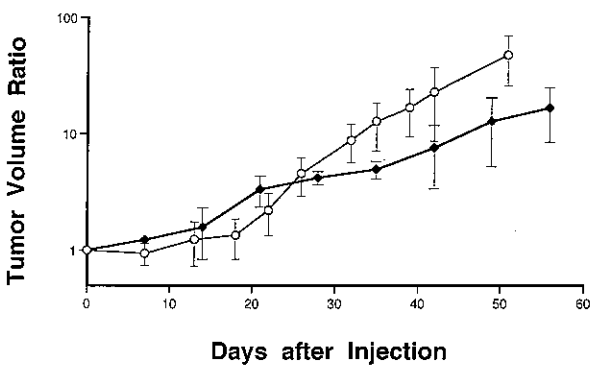

Fig. 4. Tumor growth curves after radiosensitizers. Mice were injected with 3.7 MBq of ^{131}I-Nd2 with misonidazole (◆), and ^{131}I-Nd2 (○).

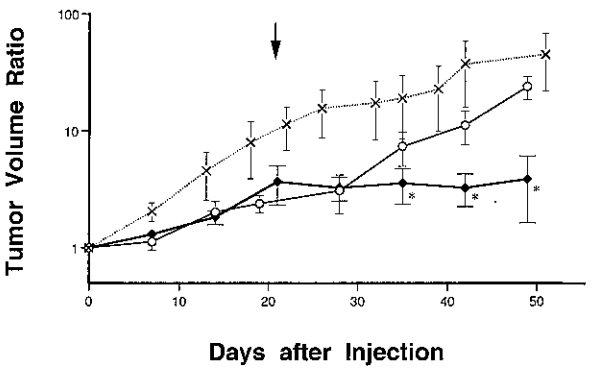

Fig. 5. Tumor growth curves after misonidazole and two injections of ^{131}I-Nd2. Mice were administered 3.7 MBq of ^{131}I-Nd2 twice at an interval of three weeks with (◆), without (○) misonidazole and normal saline (×). The arrow shows the 2nd injection. * $P<0.05$.

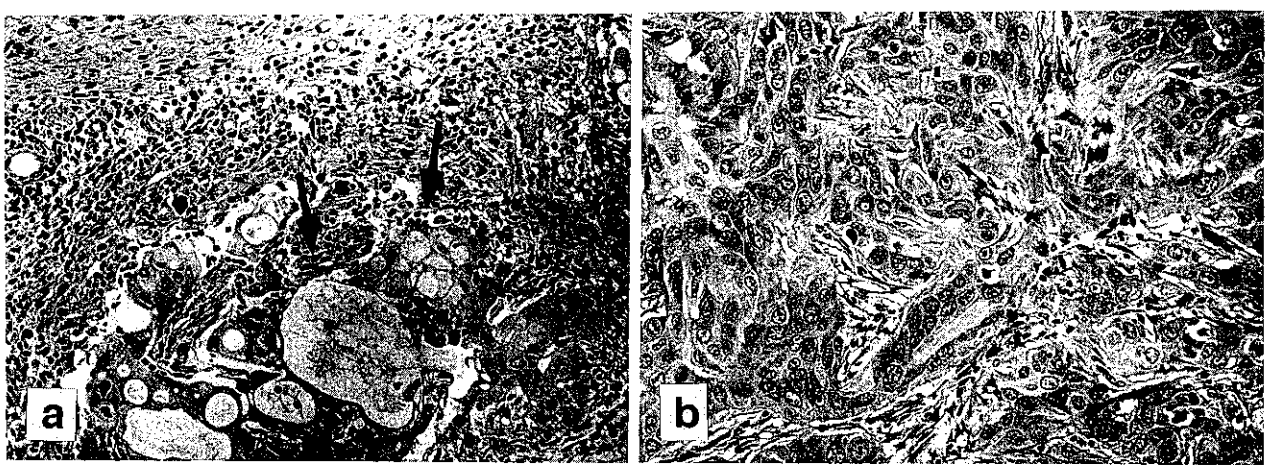

Fig. 6. HE staining of histological sections of tumors after 7 weeks. Tumor-bearing mice were injected with 7.4 MBq of ^{131}I-labeled Nd2 (a) and non-specific IgG1 (b). Arrows show degenerative changes in tumor cells.

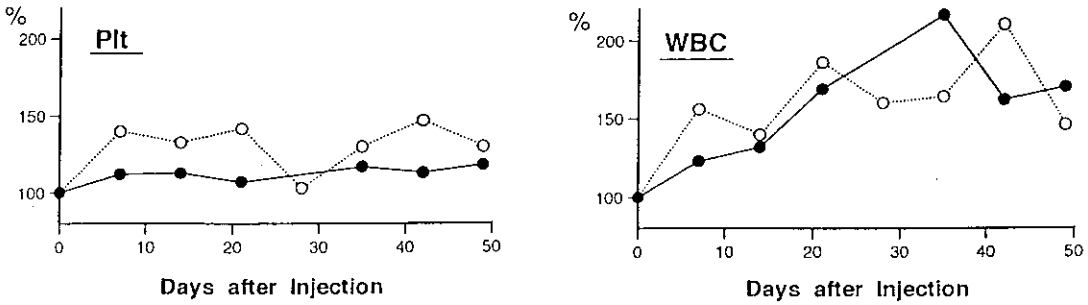

Fig. 7. Blood cell counts after injection of 7.4 MBq of ^{131}I-Nd2 (●), and 3.7 MBq twice with misonidazole (○). Blood was taken from the retrobulbar venous plexus every week after injection of the labeled antibody. The counts of platelet (Plt) and WBC on day 0 were $154.3\times10^4\pm43.2\times10^4$ and 4300 ± 1900.

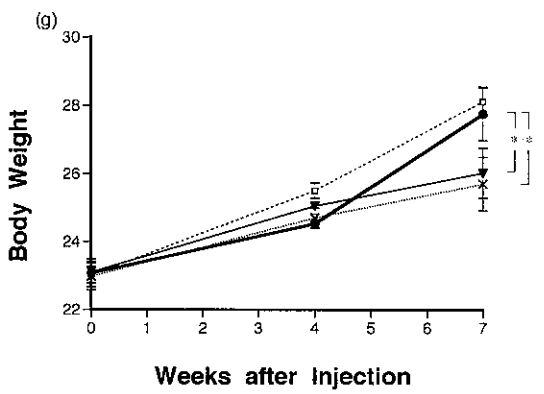

Fig. 8. Body weight changes after injection of labeled antibody. 7.4 MBq of ^{131}I-Nd2 (○), IgG1 (▼), normal saline (×) and mice without tumor (□). * $P<0.05$.

cells were scattered like islets (Fig. 6b). Vacuolar and fibrous degeneration was seen throughout the histological section, with extensive infiltration of lymphocyte-like cells.

Side effects study In the groups of mice receiving 7.4 MBq of ^{131}I-labeled Nd2 or 3.7 MBq of ^{131}I-labeled Nd2 with misonidazole, PLT counts showed no decrease in comparison with the original counts before injection (Fig. 7). WBC counts did not show a marked decrease after injection, but rather increased gradually. In each group, Hb and Ht counts showed no marked change (not shown in figures) at any experimental period. Thus, in all groups, severe pancytopenia was not seen.

Mice in the groups receiving 7.4 MBq of ^{131}I-labeled Nd2 and non-specific IgG1 were weighed at three points throughout the experiment. Weights in each group increased gradually after antibody injection (Fig. 8). Four weeks after injection, there was no significant change in any group. Seven weeks after antibody injection, there was no significant difference between the groups of mice receiving labeled non-specific IgG1 and not treated. However in the group of mice given 7.4 MBq of ^{131}I-Nd2, body weights increased significantly compared with the other two groups ($P<0.05$).

DISCUSSION

The maximum %ID/g of ^{131}I-labeled MAb-Nd2 of the tumors was 49.5 ± 7.3% on day 3 post injection, while the %ID/g of normal tissue was less than 5% (except blood, 12.3 ± 0.4%). Thus, ^{131}I-Nd2 was washed out of all normal tissues. Several investigators[28–30] have reported a maximum tumor uptake of 12–48%. Our results are as good as or better than these values. This is due to the high specificity of Nd2, which rarely reacts with normal organs.[25,26] Furthermore, the antigen recognized by Nd2 is not present in the circulation.[26] The presence of circulating tumor antigens that bind with antibody in the blood could adversely affect tumor therapy.[31] Such circulating antigens would interfere with the targeting of the antibody to tumor cells. In the presence of antigens such as carcinoembryonic antigen, some patients show a transient decrease in antigen level after infusion of antibody. This, taken together with the high specificity of Nd2 for malignancies, makes Nd2 an ideal mAb for radioimmunoscintigraphy and radioimmunotherapy. Nd2 gives clear images in radioimmunoscintigrams, as shown in the present study.

^{131}I-Nd2 suppressed tumor growth compared to ^{131}I-IgG1, and this was confirmed by histological examinations. However, there was only an initial inhibition in the mice injected with 3.7 MBq of ^{131}I-Nd2, whereas in mice injected with 7.4 MBq of ^{131}I-Nd2 there was actual reduction in the size of the tumor. Considering that the tumor volume gradually increased in the mice injected with the same dose of ^{131}I-IgG1, tumor reduction was considered to have been caused by the specificity of Nd2.

Riklund *et al.*[32] used mAb-H7 against human HeLa tumor-bearing mice. They reported that 15 MBq of ^{131}I-H7 suppressed tumor growth at 20 days, but tumor reduction was not seen. Yoneda *et al.*[33] reported tumor reduction with 6.8–17 MBq of ^{131}I-TFS-4 in small cell lung cancer. In these reports, investigators injected up to 17 MBq of radionuclides. In our studies, reduction in the size of tumors was seen in the group injected with only 7.4 MBq of ^{131}I-Nd2. Therefore, it is expected that higher doses of ^{131}I-Nd2 may have even greater effects.

We also wanted to determine if radiosensitizers would enhance the radioimmunotherapeutic effect of ^{131}I-Nd2. Pedley *et al.*[34] reported that when the radiosensitizer misonidazole was given to human colonic xenografted mice, the therapeutic effect was enhanced in comparison with the mice administered radio-antibody only. In the present study, the combined use of misonidazole with ^{131}I-Nd2 enhanced the effect of radioimmunotherapy in each group, especially in the group of mice given two injections of 3.7 MBq of ^{131}I-Nd2. In this group, tumor growth was inhibited more than in the group injected with ^{131}I-Nd2 only. Misonidazole shows electron affinity[35, 36] and mimicks oxygen to radiosensitize the resistant hypoxic cells that occur in tumors. It can also act as a selective cytotoxic drug for hypoxic cells. Hypoxic cells, a major problem in cancer treatment, exist in both animal and human tumors, even in tumors less than 1 mm in diameter. Although hypoxic cells may be quiescent or have prolonged cycling times, rendering many chemotherapy drugs useless, they are still capable of proliferating and causing tumor recurrence.

Apparently ^{131}I-Nd2 at the doses used in this study has few side effects. No severe myelosuppression was seen throughout the experiments. The increased counts of WBC after injection may be due to reaction to injections of antibody. We considered that the mild myelosuppression may reflect the relatively low accumulation of isotope in bone marrow. The body weights of mice injected with ^{131}I-Nd2 increased, but those of mice receiving no treatment or administered ^{131}I-IgG1 increased more slowly. The greater increase in body weight of mice injected with ^{131}I-Nd2 is due to the therapeutic effect of Nd2 in reducing tumor size. The lack of severe side effects in all the experimental groups strongly suggests that clinical application of ^{131}I-Nd2 may be feasible.

Intact mouse IgG1 may cause the production of human anti-murine antibody.[37, 38] Recently a mouse/human chimeric antibody Nd2 has been produced that has the same affinity and specificity as mouse Nd2.[39] We are now studying the use of chimeric Nd2 in a clinical environment.

In conclusion, ^{131}I-Nd2 is accumulated in high levels in tumors, but not in normal tissues. Moreover ^{131}I-Nd2 reduced tumor growth. The radiosensitizer misonidazole enhanced the effect of ^{131}I-Nd2. The side effects of radiolabeled Nd2 were mild. These results indicate that ^{131}I-Nd2 may be useful in radioimmunotherapy for pancreatic cancer.

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REFERENCES

1) Buchegger, F., Vacca, A., Carrel, S., Schreyer, M. and Mach, J.-P. Radioimmunotherapy of human colon carcinoma by ^{131}I-labeled monoclonal anti-CEA antibodies in a nude mice model. *Int. J. Cancer,* **41,** 127–134 (1988).
2) Etoh, T., Takahashi, H., Maie, M., Ohnuma, N. and Tanabe, M. Tumor imaging by antineuroblastoma monoclonal antibody and its application to treatment. *Cancer,* **62,** 1282–1286 (1988).
3) Langmuir, V. K., McGann, J. K., Buchegger, F. and Sutherland, R. M. ^{131}I-Anticarcinoembryonic antigen therapy of LS174T human colon adenocarcinoma spheroids. *Cancer Res.,* **49,** 3401–3406 (1989).
4) Senekowitsch, R., Reidel, G., Möllenstät, S., Kriegel, H. and Pabst, H.-W. Curative radioimmunotherapy of human mammary carcinoma xenografts with iodine-131-labeled monoclonal antibodies. *J. Nucl. Med.,* **30,** 531–537 (1989).
5) Buchegger, F., Pelegrin, A., Delaloye, B., Bischof-Delaloye, A. and Mach, J.-P. Iodine-131-labeled Mab $F(ab')_2$ fragments are more efficient and less toxic than intact anti-CEA antibodies in radioimmunotherapy of large human colon carcinoma grafted in nude mice. *J. Nucl. Med.,* **31,** 1035–1044 (1990).
6) Roeske, J. C., Chen, G. T. Y., Atcher, R. W., Pelizzari, C. A., Rotmensch, J., Haraf, D., Montag, A. and Weichselbaum, R. R. Modeling of dose to tumor and normal tissue from intraperitoneal radioimmunotherapy with alpha and beta emitters. *Int. J. Radiat. Oncol. Biol. Phys.,* **19,** 1539–1548 (1990).
7) Schlom, J., Molinolo, A., Simpson, J. F., Siler, K., Roselli, M., Hinkle, G., Houchens, D. P. and Colcher, D. Advantage of dose fractionation in monoclonal antibody-targeted radioimmunotherapy. *J. Natl. Cancer Inst.,* **82,** 763–771 (1990).
8) Sharkey, R. M., Gold, D. V., Aninipot, R., Vagg, R., Ballance, C., Newman, E. S., Ostella, F., Hansen, H. J.

and Goldenberg, D. M. Comparison of tumor targeting in nude mice by murine monoclonal antibodies directed against different human colorectal cancer antigens. *Cancer Res.*, **50** (Suppl.), 828s–834s (1990).

9) Sharkey, R. M., Weadock, K. S., Natare, A., Haywood, L., Aninipot, R., Blumenthal, R. D. and Goldenberg, D. M. Successful radioimmunotherapy for lung metastasis of human colonic cancer in nude mice. *J. Natl. Cancer Inst.*, **83**, 627–632 (1991).
10) Rowlimson-Burza, G., Bamias, A., Krausz, T. and Epenetos, A. A. Uptake and distribution of specific and control monoclonal antibodies in subcutaneous xenografts following intratumor injection. *Cancer Res.*, **51**, 3251–3256 (1991).
11) Griffiths, G. L., Goldenberg, D. M., Knapp, F. F., Jr., Callahan, A. P., Chang, C. H. and Hansen, H. J. Direct radiolabeling of monoclonal antibodies with generator-produced rhenium-188 for radioimmunotherapy: labeling and animal biodistribution studies. *Cancer Res.*, **51**, 4594–4602 (1991).
12) Schlom, J., Eggensperger, D., Colcher, D., Molinolo, A., Houchens, D., Miller, L. S., Hinkle, G. and Siler, K. Therapeutic advantage of high-affinity anticarcinoma radioimmunoconjugates. *Cancer Res.*, **52**, 1067–1072 (1992).
13) Buchsbaum, D. J., Wahl, R. L., Normolle, D. P. and Kaminski, M. S. Therapy with unlabeled and ^{131}I-labeled pan-B-cell monoclonal antibodies in nude mice bearing Raji Burkitt's lymphoma xenografts. *Cancer Res.*, **52**, 6476–6481 (1992).
14) Order, S. E., Stillwagon, G. B., Klein, J. L., Leichner, P. K., Siegelman, S. S., Fishman, E. K., Ettinger, D. S., Haulk, T., Kopher, K., Finney, K., Surdyke, M., Self, S. and Leibel, S. Iodine 131 antiferritin, a new treatment modality in hepatoma: a Radiation Oncology Group study. *J. Clin. Oncol.*, **3**, 1573–1582 (1985).
15) Epenetos, A. A., Hooker, G., Krausz, T., Snook, D., Bodmer, W. F., Papadimitriou, J. T. Antibody-guided irradiation of malignant ascites in ovarian cancer: a new therapeutic method possessing specificity against cancer cells. *Obstet. Gynecol.*, **68** (Suppl.), 71S–74S (1986).
16) Baldwin, R. W. and Byers, V. S. Monoclonal antibodies in cancer treatment. *Lancet*, **i**, 603–605 (1986).
17) Larson, S. M., Carrasquillo, J. A., Krohn, K. A., Brown, J. P., McGuffin, R. W., Ferens, J. M., Graham, M. M., Hill, L. D. and Beaumier, P. L. Localization of ^{131}I-labeled p97-specific Fab fragments in human melanoma as a basis for radiotherapy. *J. Clin. Invest.*, **72**, 2101–2114 (1983).
18) Larson, S. M. Radiolabeled monoclonal anti-tumor antibodies in diagnosis and therapy. *J. Nucl. Med.*, **26**, 538–545 (1985).
19) Esteban, J. M., Colcher, D., Sugarbaker, P., Carrasquillo, J. A., Bryant, G., Thor, A., Reynolds, J. C., Larson, S. M. and Schlom, J. Quantitative and qualitative aspects of radiolocalization in colon cancer patients of intravenously administered MAb B72.3. *Int. J. Cancer*, **39**, 50–59 (1987).
20) Renda, A., Salvatore, M., Sava, M., Landi, R., Lastoria, S., Coppola, L., Schlom, J., Colcher, D. and Zannini, G. Immunoscintigraphy in the follow-up of patients operated on for carcinoma of the sigmoid and rectum. Preliminary report with a new monoclonal antibody: B72.3. *Dis. Colon Rectum*, **30**, 683–686 (1987).
21) Colcher, D., Esteban, J. M., Carrasquillo, J. A., Sugarbaker, P., Reynolds, J. C., Bryant, G., Larson, S. M. and Schlom, J. Quantitative analyses of selective radiolabeled monoclonal antibody localization in metastatic lesions of colorectal cancer patients. *Cancer Res.*, **47**, 1185–1189 (1987).
22) Carrasquillo, J. A., Sugarbaker, P., Colcher, D., Reynolds, J. C., Esteban, J., Bryant, G., Perentesis, P., Yokoyama, K., Rotman, M., Schlom, J. and Larson, S. M. Peritoneal carcinomatosis: imaging with intraperitoneal injection of I-131-labeled B72.3 monoclonal antibody. *Radiology*, **167**, 35–40 (1988).
23) Alisauskus, R., Wong, G. Y. C. and Gold, D. V. Initial studies of monoclonal antibody PAM-4 targeting to xenografted orthotopic pancreatic cancer. *Cancer Res.*, **55** (Suppl.), 5743s–5748s (1995).
24) Kamigaki, T., Yamamoto, M., Ohyanagi, H., Ohya, M., Shimazoe, T., Kono, A., Ohtani, W., Narita, Y., Ohkubo, M. and Saitoh, Y. Therapy and imaging of pancreatic carcinoma xenografts with radioiodine-labeled chimeric monoclonal antibody A10 and its Fab fragment. Jpn. J. *Cancer Res.*, **86**, 1216–1223 (1995).
25) Sawada, T., Chung, Y. S., Kondo, Y., Sowa, M., Umeyama, K., Ochi, H., Ho, J. J. L., Kim, Y. S. Radioimmunodetection of human pancreatic cancer using ^{111}In-labeled monoclonal antibody Nd2. *Antibody Immunoconjugat. Radiopharm.*, **4**, 493–499 (1991).
26) Ho, J. J. L., Bi, N., Yan, P., Yuan, M., Norton, K. A. and Kim, Y. S. Characterization of new pancreatic cancer-reactive monoclonal antibodies directed against purified mucin. *Cancer Res.*, **51**, 372–380 (1991).
27) Haisma, H. J., Hilgers, J., Zurawski, Jr., V. R. Iodination of monoclonal antibodies for diagnosis and radiotherapy using a convenient one vial method. *J. Nucl. Med.*, **27**, 1890–1895 (1986).
28) Worlock, A. J., Zalutsky, M. R., Metzgar, R. S. Radiolocalization of human pancreatic tumors in athymic mice by monoclonal antibody DU-PAN 1. *Cancer Res.*, **50**, 7246–7251 (1990).
29) Camera, L., Kinuya, S., Pai, L. H., Garmestani, K., Brechbiel, M. W., Gansow, O. A., Paik, C. H., Pastan, I. and Carrasquillo, J. A. Preclinical evaluation of ^{111}In-labeled B3 monoclonal antibody: biodistribution and imaging studies in nude mice bearing human epidermoid carcinoma xenografts. *Cancer Res.*, **53**, 2834–2839 (1993).
30) Gold, D. V., Alisauskas, R. and Sharkey, R. M. Targeting of xenografted pancreatic cancer with a new monoclonal antibody, PAM4. *Cancer Res.*, **55**, 1105–1110 (1995).
31) Bloomer, W. D., Lipsztein, R. and Dalton, J. F. Anti-

body-mediated radiotherapy. *Cancer,* **55,** 2229–2233 (1985).
32) Riklund, K. E., Makiya, R. A., Sundstrom, B. E., Thornell, L. E., and Stigbrand, T. I. Experimental radioimmunotherapy of HeLa tumours in nude mice with ^{131}I-labeled monoclonal antibodies. *Anticancer Res.,* **10,** 379–384 (1990).
33) Yoneda, S., Fujisawa, M., Watanabe, J., Okabe, T., Takaku, F., Homma, T. and Yoshida, K. Radioimmunotherapy of transplanted small cell lung cancer with ^{131}I-labeled monoclonal antibody. *Br. J. Cancer,* **58,** 292–295 (1988).
34) Pedley, R. B., Begent, R. H. J., Boden, J. A., Boden, R., Adam, T. and Bagshawe, K. D. The effect of radiosensitizer on radioimmunotherapy, using ^{131}I-labeled anti-CEA antibodies in a human colonic xenograft model. *Int. J. Cancer,* **47,** 597–602 (1991).
35) Sheldon, P. W. and Hill, S. A. Further investigations of the effects of the hypoxic-cell radiosensitizer, Ro-07-0582, on local control of a mouse tumour. *Br. J. Cancer,* **36,** 198–205 (1977).
36) Hill, R. S. and Bush, R. S. The effect of misonidazole in combination with radiation dose fractionation. *Br. J. Cancer,* **37,** 255–258 (1978).
37) Schroff, R. W., Foon, K. A., Beatty, S. M., Oldham, R. K. and Morgan, A. C., Jr. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res.,* **45,** 879–885 (1985).
38) Shawler, D. L., Bartholomew, R. M., Smith, L. M. and Dillman, R. O. Human immune response to multiple injections of murine monoclonal IgG. *J. Immunol.,* **135,** 1530–1535 (1985).
39) Hirayama, K., Chung, Y .S., Sawada, T., Kim, Y. S. and Sowa, M. Characterization and biodistribution of a mouse/human chimeric antibody directed against pancreatic cancer mucin. *Cancer,* **75** (Suppl.), 1545–1553 (1995).