Enhanced Antitumor Activity of Combined Pretargeted Radioimmunotherapy and Paclitaxel in Medullary Thyroid Cancer Xenograft

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Abstract

A significant antitumor effect associated with moderate toxicity was obtained previously with anticarcinoembryonic antigen × antidiethylene-triaminepentaacetic acid (DTPA)-indium F6–734 bispecific antibody and iodine-131-labeled DTPA-indium bivalent hapten in an animal model of medullary thyroid cancer (MTC). The purpose of this study was to determine whether the cytotoxic agents doxorubicin and paclitaxel, also known as radiosensitizers, improve efficacy of pretargeted radioimmunotherapy (RIT) in experimental MTC. Nude mice bearing TT MTC xenograft were treated with F6–734 and iodine-131-labeled DTPA-indium bivalent hapten injected 48 h apart with or without doxorubicin or paclitaxel. The maximum tolerated dose (MTD) of RIT was 92.5 MBq (as determined previously) and that of doxorubicin and paclitaxel 200 and 1000 μg, respectively. A control group received no treatment. Animal weight, hematotoxicity, tumor volume, and serum calcitonin were monitored for 5 months. Tumor growth inhibition induced by drugs alone, RIT alone, or combined therapy was characterized by measuring relative tumor volume 20, 40, and 60 days after treatment to detect additivity or synergism. Mean tumor volume doubling time (MTVDT) was 13 ± 4 days in the control group, 15 ± 8 days in the group treated with the MTD of doxorubicin, and 32 ± 13 days in the group treated with the MTD of paclitaxel. After RIT alone at 92.5 MBq, MTVDT was 86 ± 22 days. After RIT at 74 MBq (80% of MTD), MTVDT was 56 ± 10 days. MTVDT was not significantly different from this value after RIT plus doxorubicin, 60 ± 16 days (65 and 100% of the respective single-agent MTDs). Combination of RIT with paclitaxel (65 and 100% of the respective single-agent MTDs) prolonged the suppression of tumor growth. One complete response was observed, and MTVDT was 114 ± 44 days. This value was significantly longer than the value obtained with RIT alone at 74 MBq ($P < 0.05$) or with RIT combined with doxorubicin ($P < 0.02$). The change in serum calcitonin levels paralleled those in tumor volume. Analysis of dose-response curves at days 20 and 40 showed additivity between RIT and paclitaxel, and analysis at day 60 suggested a synergistic effect. In conclusion, addition of doxorubicin did not improve RIT efficacy, whereas paclitaxel improved RIT efficacy significantly without increasing toxicity.

Introduction

MTC[2], a neoplasm of parafollicular cells, represents ~10% of all thyroid cancers. As MTC tumor expresses and secretes CEA, it constitutes a potential application for RIT with anti-CEA antibodies (1, 2). RIT efficacy in the treatment of MTC has been demonstrated in preclinical studies performed in mice grafted with human MTC cell lines and in Phase I/II clinical trials performed in patients with recurrences of MTC (2–5). Tumor and/or biological responses showing decreased TCT levels were observed, particularly in small lesions and after repeated courses of RIT.

The AES, a pretargeting technique using a BsMAb and a bivalent hapten, increases tumor:normal tissue ratios by reducing activity levels in normal tissues 3–5-fold as compared with directly labeled MAb fragments (6). Preclinical studies showed that the toxicity of AES RIT using a ^{131}I-di-DTPA-In was significantly lower than with directly labeled MAb fragments and that repeated injections of AES reagents did not increase toxicity (3, 7). Thus, AES is a promising RIT modality, and its moderate myelotoxicity is favorable for combinations with myelotoxic chemotherapeutic drugs.

The purpose of the present study was to assess the toxicity and efficacy of combined chemotherapy and AES RIT using an anti-CEA × anti-DTPA-indium BsMAb and ^{131}I-di-DTPA-In in nude mice grafted s.c. with a human MTC line. In particular, a combination of RIT (65% of the MTD) with the MTD of paclitaxel was studied and compared with the MTD of RIT, 80% of the MTD of RIT, the MTD of paclitaxel, and 65% of the MTD of RIT plus the MTD of doxorubicin.

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[2] The abbreviations used are: MTC, medullary thyroid carcinoma; CEA, carcinoembryonic antigen; RIT, radioimmunotherapy; TCT, calcitonin; AES, affinity-enhancement system; MAb, monoclonal antibody; BsMAb, bispecific antibody; DTPA, diethylenetriaminepentaacetic acid; ^{131}I-di-diethylenetriaminepentaacetic acid-In, bivalent diethylenetriaminepentaacetic acid-indium hapten labeled with iodine-131; MTD, maximum tolerated dose; di-DTPA, Nα-(diethylenetriamine-*N,N,N′,N′′*-tetraacetic acid-*N′′*-acetyl)-tyrosyl-Nϵ-(diethylenetriamine-*N,N,N′,N′′*-tetraacetic acid-*N′′*-acetyl)-lysine.

Materials and Methods

Cell Line. The TT human MTC line obtained from the American Type Culture Collection (Rockville, MD) expresses CEA on cell membrane and secretes TCT. It was grown in adherent-cell monolayers in RPMI medium (Life Technologies, Inc., Cergy-Pontoise, France) supplemented with 10% FCS (Life Technologies, Inc.), 1% glutamine (200 mM L-glutamine; Life Technologies, Inc.), and 1% antibiotic (100 units/ml penicillin and streptomycin; Life Technologies, Inc.).

Animal Model. Nude mice $>$ 10 weeks of age were grafted s.c. in the right flank with 10^6 TT cells in 0.3 ml of sterile physiological serum. The animals were housed under aseptic conditions and used once tumors were ~200 mm^3 ~6 weeks after injection. Lugol's solution 0.1% was added to drinking water (1/100 ml) the week before and then 2 weeks after injection of ^{131}I-labeled hapten.

Antibody, Hapten, and Radiolabeling. F6–734 BsMAb, obtained by chemical coupling of the Fab′ fragment of F6 antibody (anti-CEA IgG1) to the Fab′ fragment of 734 antibody (anti-DTPA-indium IgG1), was kindly provided by Immunotech (Marseille, France), together with the bivalent DTPA hapten di-DTPA.

Di-DTPA-In was provided as a solution in 10 mM citrate and 100 mM acetate buffer (pH 5). The following were added sequentially in a sterile 2-ml plastic tube: 25 μl of di-DTPA-In (25 nmol), 25 μl of 0.3 M phosphate buffer (pH 6), 50 μl of chloramine-T [1 mg/ml in 0.3 M phosphate buffer (pH 6)], and 100 μl of a ^{131}I solution at 14–18 GBq/ml in 0.1 M sodium bicarbonate (pH 8) [^{131}I-S3B; CIS Bio International, Gif sur Yvette, France].

After 10 min of incubation at room temperature, the reaction was stopped by the addition of 50 μl of sodium disulfite [1 mg/ml in 0.3 M phosphate buffer (pH 6)]. The pH of the solution was brought to between 5 and 6 by the addition of 750 μl of *N*(2-hydroxyethyl) piperazine-*N*′ 2-ethane sulfonic acid (1 M).

The resulting solution was purified on a C18-grafted silica cartridge (Sepack-C18; Millipore). Free iodine was eluted with 5 ml of 0.1 M phosphate buffer (pH 7) and radiolabeled hapten with 5 ml of a 0.1 M phosphate buffer (pH 7)-ethanol mixture (3–2).

Specific activity was measured in an ionization chamber. To determine the radiochemical purity of ^{131}I-di-DTPA-In, 10 μl of ^{131}I-di-DTPA-In solution diluted 1:1000 were deposited in tubes coated with 734 antibody (kindly provided by Immunotech) containing 250 μl of 0.1 M phosphate buffer (pH 7) supplemented with 0.5% BSA. Total activity was measured after 1 h of incubation at room temperature with slow stirring. The tubes were washed three times with 0.1 M phosphate buffer (pH 7) and 0.01% Tween 20, and the bound activity was measured.

Chemotherapeutic Agents. Doxorubicin (Adriblastine; Pharmacia & Upjohn S.A., St. Quentin, Yvelines, France), a product of the anthracycline group, is a topoisomerase II inhibitor and an intercalating agent. Doxorubicin was administered i.p. in a 1000-μl sterile isotonic NaCl solution. Paclitaxel (Taxol; Bristol-Myers Squibb Co., Princeton, NJ), a natural product from the taxane group, has novel antimicrotubule properties. Paclitaxel was administered i.p. in 1000 μl of sterile physiological serum.

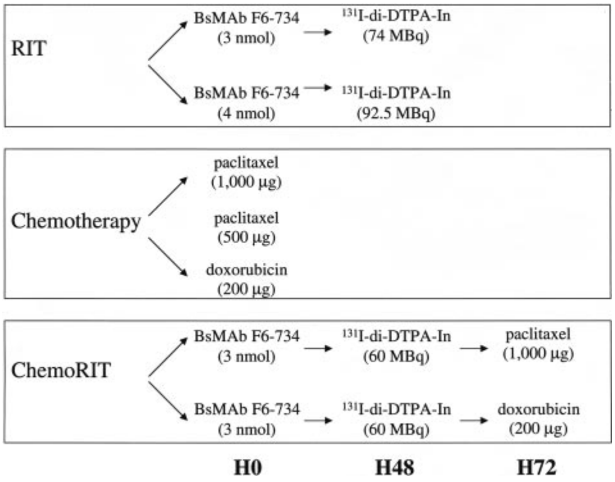

Fig. 1. Description of the treatment procedures. For RIT alone, mice were injected with 3 or 4 nmol of BsMAb F6–734 and 48 h later with 74 and 92.5 MBq of ^{131}I-di-DTPA-In hapten, respectively. The activity of 92.5 MBq was the MTD. For chemotherapy alone, mice were injected with the MTD of paclitaxel (1000 μg), the MTD/2 of paclitaxel (500 μg), or the MTD of doxorubicin (200 μg). For chemotherapy combined with RIT (*ChemoRIT*), mice were injected with 3 nmol of BsMAb and 60 MBq of ^{131}I-di-DTPA-TL 48 h after the BsMAb and the MTD of paclitaxel or doxorubicin 72 h after the BsMAb.

Experimental RIT and Chemotherapy. Eight groups of 6–12 mice each were studied. Initial tumor volumes were not significantly different between the groups. The different treatment procedures are summarized in Fig. 1. BsMAb and hapten diluted in 0.2 ml of sterile physiological serum were injected i.v. into the lateral tail vein. Two groups were injected, respectively, with 3 and 4 nmol of BsMAb F6–734 and then 48 h later with 74 and 92.5 MBq of ^{131}I-di-DTPA-In. Preliminary studies determined that 4 nmol of BsMAb represent an amount less than that required to saturate tumor[3] and that a hapten:BsMAb ratio of 0.5 at injection was most favorable for targeting purposes (8). A preliminary study also determined that the MTD of RIT performed with this scheme was 92.5 MBq.[3] The MTD levels of a single dose of doxorubicin and paclitaxel were established in nude mice, as described previously (9). Three groups were injected i.p. with the MTD of paclitaxel (1000 μg), the MTD/2 of paclitaxel (500 μg), or the MTD of doxorubicin (200 μg). Two groups were treated with RIT (3 nmol of BsMAb and 60 MBq of ^{131}I-di-DTPA-TL hapten injected 48 h apart) in association with the MTD of paclitaxel or doxorubicin delivered 24 h after hapten. Finally, a control group of 12 mice received no injection.

The length (L), width (W), and thickness (T) of tumors were measured with a sliding caliper twice weekly for 150 days. Tumor volume (V) was calculated according to the formula: $V = \pi/6 \times L \times W \times T$. All animals were weighed on the day of injection and then twice a week for 90 days. Biological monitoring was performed on a blood sample drawn from the

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[3] Unpublished data.

Table 1 Toxicity and tumor efficacy of treatments

Groups	Minimal relative tumor volume (%)	Relative tumor volume doubling time (days)	Maximal weight loss (%)	No. of dead mice (day of death)
No treatment	100 ± 0[a]	13 ± 04	3 ± 4	0
RIT (74 MBq)	69 ± 29	56 ± 10	7 ± 6	0
RIT (92.5 MBq)	42 ± 18	86 ± 22	5 ± 4	2 (D52 and D75)
Doxorubicin (200 μg)	100 ± 0[a]	15 ± 08	7 ± 3	0
Paclitaxel (500 μg)	100 ± 0[a]	12 ± 08	2 ± 3	0
Paclitaxel (1000 μg)	87 ± 23	32 ± 13	2 ± 3	2 (D62 and D80)
RIT (60 MBq) + doxorubicin (200 μg)	66 ± 25	60 ± 16	11 ± 4	3 (D68, D78, and D78)
RIT (60 MBq) + paclitaxel (1000 μg)	32 ± 18	114 ± 44	7 ± 6	1 (D78)

[a] No tumor shrinkage occurred in these mice.

inner border of the eye. The parameters used to evaluate the toxicity of each type of treatment were maximal weight loss and variation in the number of leukocytes and platelets measured on days 0, 15, 30, and 60. The parameters used to evaluate the efficacy of each type of treatment were relative tumor volume, mean tumor volume doubling time, and the variation in serum TCT concentration measured by RIA on days 0, 15, 30, 45, 60, and 90 (calcitonin immunoradiometric assay, CIS Bio International).

Analysis of Antitumor Efficacy. The Loewe additivity model was used to determine whether the combination of RIT and paclitaxel had an additive or supra-additive (synergistic) antitumor effect (10, 11). Five groups of mice were used for this study: control group, groups treated with 74 and 92.5 MBq of ^{131}I-di-DTPA-In, and groups treated with 500 and 1000 μg of paclitaxel. Mean relative tumor volumes 20, 40, and 60 days after treatment were used as end points. The dose-response relationships of both RIT and chemotherapy were considered to be exponential and were plotted with a logarithmic scale as relative tumor volume *versus* activities of radiolabeled hapten or doses of paclitaxel (11, 12). The intercepts of the dose-efficacy curves of the single-treatment modalities with the mean relative volumes of tumors treated with combination therapy were determined. These values represented the activities of hapten or the doses of chemotherapy alone that could be expected to be isoeffective, as compared with combination therapy. Combination therapy was considered to be synergistic if $d_{RIT}D_{RIT}^{-1} + d_{chemo}D_{chemo}^{-1} < 1$, additive if $d_{RIT}D_{RIT}^{-1} + d_{chemo}D_{chemo}^{-1} \approx 1$, or sub-additive if $d_{RIT}D_{RIT}^{-1} + d_{chemo}D_{chemo}^{-1} > 1$; d_{RIT} and d_{chemo} were the activities of radiolabeled hapten and doses of chemotherapy actually given in the association, and D_{RIT} and D_{chemo} were the calculated activities and doses expected to be isoeffective when given alone.

Statistical Analysis. Because of the limited number of animals, the means of the quantitative variables of the different groups were compared using nonparametric tests. The Mann-Whitney rank-sum test was used for comparison of two groups. For comparison of more than two groups, the Kruskall-Wallis one-way ANOVA test was used; when P was <0.05 in the first step, multiple comparisons were computed as described for the Mann-Whitney test, with correction in accordance with the number of compared groups (13). Ps ≤ 0.05 were considered significant. BMDP Statistical Software (Cork, Ireland), version 7.0, was used for the analysis.

Results

Radiolabeled Hapten Controls. Specific activity, measured in an ionization chamber, was 59.2–70.3 MBq/nmol. The radiochemical purity of ^{131}I-di-DTPA-In, determined in tubes coated with 734 antibody (ratio of bound activity in tubes after washing to total activity), was >90%.

Toxicity. Maximal weight losses observed after the different treatments are summarized in Table 1. The values were not significantly different after RIT alone, paclitaxel, and doxorubicin, and weight losses were not significantly higher after RIT + doxorubicin or paclitaxel than after RIT alone.

In untreated controls, the mean leukocyte concentration was 2700/mm^3 (range 800-7000) and that of platelets 1.4 10^6/mm^3 (range 0.57–2.7 10^6). Mean leukocyte and platelet concentrations measured after the different treatments are summarized in Table 2. Toxicity on leukocytes and platelets was expressed as the percentage of the variation between the nadir and the basal value at day 0. Leukocyte variations were, respectively, −69 ± 24%, −55 ± 22%, −39 ± 29%, and −30 ± 10% after injections of 74 and 92.5 MBq of ^{131}I-di-DTPA-In, 1000 μg of paclitaxel, and 200 μg of doxorubicin. Leukocyte variation was not significantly higher after RIT + paclitaxel (−45 ± 25%) or RIT + doxorubicin (−34 ± 27%) than after RIT alone (at 74 and 92.5 MBq levels). For platelet, variations were, respectively, −44 ± 28%, −63 ± 13%, −22 ± 16%, and −36 ± 10% after injections of 74 and 92.5 MBq of ^{131}I-di-DTPA-In, 1000 μg of paclitaxel, and 200 μg of doxorubicin. Platelet variation was not higher after RIT + paclitaxel (−54 ± 15%) than after RIT alone (at 74 and 92.5 MBq levels) and was lower after RIT + doxorubicin (−4 ± 6%) than after RIT alone (at 74 and 92.5 MBq levels; $P = 0.006$). After reaching the nadir, hematopoiesis was restored spontaneously in all groups of mice.

Eight animal deaths recorded during the monitoring period (Table 1) occurred long after therapy and were not related to treatment.

Table 2 Blood cells at different days (D) after therapeutic injection

	D0	D15	D30	D60
Leukocytes (/mm^3)				
RIT 74 MBq	2300 ± 900	2200 ± 1200	3850 ± 1450	5100 ± 4200
RIT 92.5 MBq	2200 ± 535	1560 ± 1160	1580 ± 1100	2450 ± 900
Doxorubicin (200 μg)	2160 ± 826	3040 ± 1817	4475 ± 2865	4250 ± 4596
Paclitaxel (1000 μg)	5150 ± 2921	4883 ± 1568	3216 ± 1188	4266 ± 884
RIT + doxorubicin	3500 ± 1266	3357 ± 1367	6214 ± 1786	3400 ± 1964
RIT + paclitaxel	4325 ± 2322	3425 ± 1581	2825 ± 727	4400 ± 1581
Platelets (/mm^3)				
RIT 74 MBq	1.12 ± 0.26	0.93 ± 0.20	1.10 ± 0.42	1.01 ± 0.44
RIT 92.5 MBq	1.12 ± 0.58	0.73 ± 0.38	ND[a]	1.80 ± 0.50
Doxorubicin (200 μg)	1.58 ± 0.85	0.89 ± 0.28	0.70 ± 0.21	1.10 ± 0.71
Paclitaxel (1000 μg)	0.93 ± 0.37	1.15 ± 0.18	0.82 ± 0.32	1.09 ± 0.46
RIT + doxorubicin	0.55 ± 0.15	0.87 ± 0.26	0.99 ± 0.16	0.87 ± 0.27
RIT + paclitaxel	1.10 ± 0.60	0.59 ± 0.18	0.78 ± 0.28	0.55 ± 0.06

[a] ND, not done.

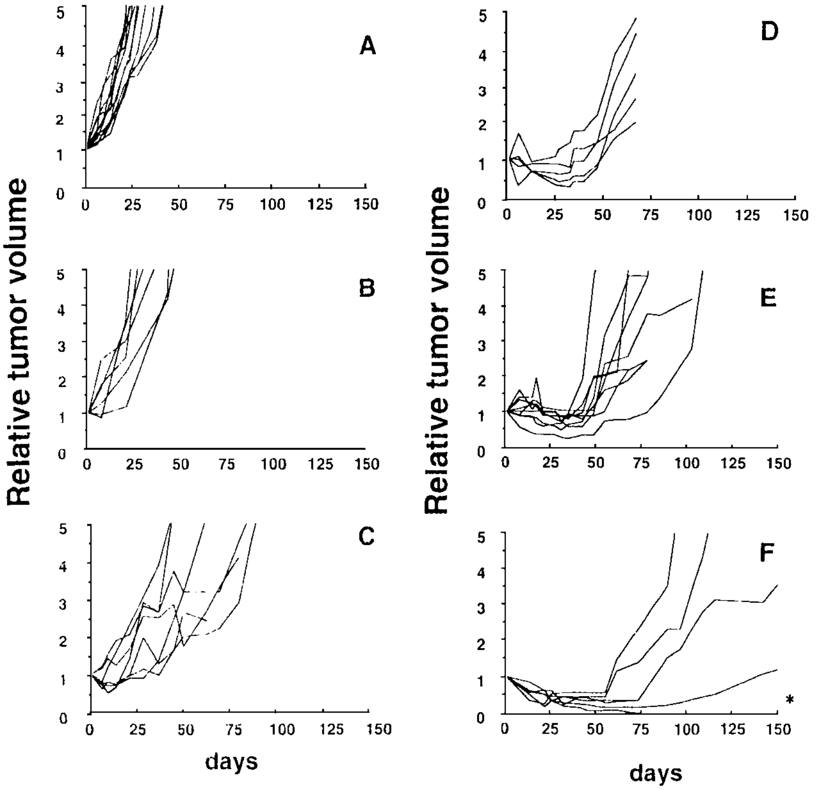

Fig. 2. Variation of TT tumor size in treated mice and controls. Tumor-bearing mice were untreated (*A*, $n = 12$) and given a single injection of 200 μg of doxorubicin (*B*, $n = 6$), 1000 μg of paclitaxel (*C*, $n = 7$), 74 MBq of F6–734/^{131}I-di-DTPA-In (*D*, $n = 5$), 200 μg of doxorubicin 24 h after 60 MBq of F6–734/^{131}I-di-DTPA-In (*E*, $n = 9$), or 1000 μg of paclitaxel 24 h after 60 MBq of F6–734/^{131}I-di-DTPA-In (*F*, $n = 6$). Graphs show relative tumor volume (ratio of tumor volume to initial size before treatment) as a function of time. *, complete response.

Efficacy of the Combination of Doxorubicin and F6–734 BsMAb/^{131}I-di-DTPA-In. Doxorubicin and F6–734 BsMAb/^{131}I-di-DTPA-In were evaluated as single modality therapeutic agents and in combination. Fig. 2 shows the growth curves of TT tumors after various treatment regimens and in the control group. Minimal relative tumor volumes and tumor volume doubling times are summarized in Table 1. In the control group, mean doubling time was 13 ± 4 days. After RIT, all tumors decreased in size, and tumor volume doubling times (respectively, 56 ± 10 and 86 ± 22 days with 74 and 92.5 MBq) were significantly longer than in the control group ($P < 0.005$). After treatment with doxorubicin at the MTD (200 μg), mean doubling time was not significantly different (15 ± 8 days) from that of the control group. The combination of RIT (60 MBq) with doxorubicin (200 μg) did not appear to improve efficacy as compared with RIT alone, giving a tumor volume doubling time of 60 ± 16 days. Changes in TCT concentrations were parallel to those in tumor volume (Fig. 3).

Efficacy of the Combination of Paclitaxel and F6–734 BsMAb/^{131}I-di-DTPA-In. The MTD of paclitaxel was evaluated as a single modality therapeutic agent and in combination with F6–734 BsMAb/^{131}I-di-DTPA-In. Fig. 2 shows the growth curves of TT tumors after the treatment regimens. Tumor volume doubling time was significantly longer after

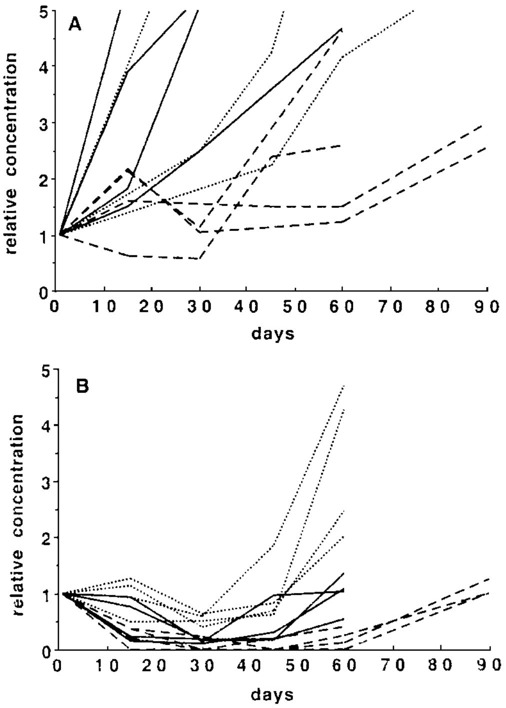

Fig. 3. Variation of calcitonin concentration (pg/ml) in treated mice and controls. Tumor-bearing mice were untreated (*A,* ———; $n = 4$) and given a single injection of 200 μg of doxorubicin (*A,* ····; $n = 3$), 1000 μg of paclitaxel (*A,* ----; $n = 4$), 74 MBq of F6–734/^{131}I-di-DTPA-In (*B,* ———; $n = 4$), 200 μg of doxorubicin 24 h after 60 MBq of F6–734/^{131}I-di-DTPA-In (*B,* ····; $n = 4$), or 1000 μg of paclitaxel 24 h after 60 MBq of F6–734/^{131}I-di-DTPA-In (*B,* ----; $n = 4$). Graphs show the relative calcitonin concentration (ratio of calcitonin concentration to the initial concentration before treatment) as a function of time.

paclitaxel alone (1000 μg; 32 ± 13 days) than in the control group (13 ± 4 days; $P < 0.025$; Table 1). The combination of RIT (60 MBq) with paclitaxel (1000 μg) improved the antitumor effect. One complete response was observed, and tumor volume doubling time was 114 ± 44 days. This value was significantly longer than that obtained with RIT at the 74 MBq level ($P < 0.05$) or with RIT (60 MBq) + doxorubicin (200 μg; $P < 0.02$). The response was also longer than that obtained with 92.5 MBq of RIT, although the difference was not statistically significant at the 95% confidence level, probably because of the small size of the samples. The changes in TCT concentrations were parallel to those in tumor volume (Fig. 3).

Synergy between RIT and Paclitaxel. The dose-response curves of both F6–734 BsMAb/^{131}I-di-DTPA-In and paclitaxel alone showed an exponential pattern (Fig. 4). Dose-response curves based on relative tumor volumes measured at day 20, 40, or 60 were used to calculate the hypothetical doses of the respective single agent treatments (D_{RIT} and D_{chemo}) expected to be isoeffective with the combination. The additivity factor $d_{RIT}D_{RIT}^{-1} + d_{chemo}D_{chemo}^{-1}$ was then calculated according to Loewe (Table 3). At days 20 and 40, factors were close to 1, showing simple additivity, whereas at day 60, the factor was 0.80, suggesting a synergistic effect between RIT and paclitaxel.

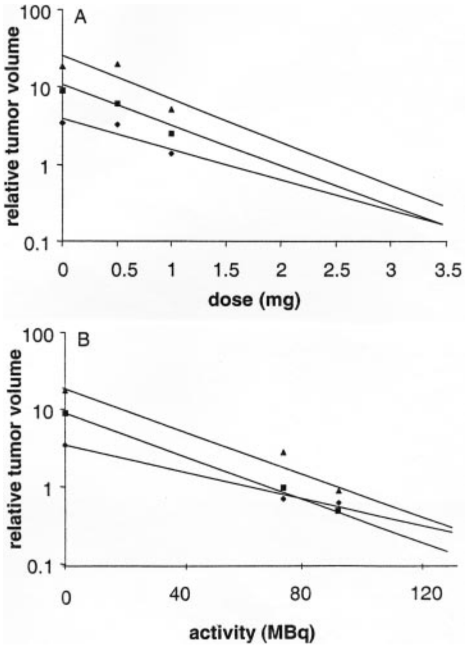

Fig. 4. Dose-response curves of paclitaxel (*A*) and F6–734/^{131}I-di-DTPA-In (*B*) in TT MTC xenografts. Response was evaluated at days 20 (♦), 40 (■), and 60 (▲).

Table 3 Characterization of the antitumor effect of F6-734 BsMAb/^{131}I-di-DTPA-In + paclitaxel

d_{RIT} and d_{chemo} represent the activities of radiolabeled hapten and the chemotherapeutic doses actually given in the association, and D_{RIT} and D_{chemo} represent the calculated activities and doses expected to be isoeffective when given alone.

Days after treatment	d_{RIT} (MBq)	d_{chemo} (μg)	D_{RIT} (MBq)	D_{chemo} (μg)	$D_{RIT}D_{RIT}^{-1}$ + $d_{chemo}D_{chemo}^{-1}$
20	60	1000	103	2400	1.00
40	60	1000	96	2420	1.03
60	60	1000	124	3100	0.80

Discussion

The interest of combination of chemotherapy with external radiotherapy has been documented in many different studies, and concomitant chemoradiotherapy is now a routine treatment for various tumors (14, 15). The combination of chemotherapy to a low-dose rate radiotherapy modality, such as RIT, also appears justified. Additivity is expected when there is no interaction between the treatments. This is the case for a combination of two treatments with spatial cooperation or toxicity independence, allowing each treatment to be used at nearly full doses without increasing damage to normal tissue. Chemotherapy and RIT show different toxicity profiles, except for myelotoxicity. RIT myelotoxicity is significantly decreased with AES as compared with one-step targeting systems (3, 7). Combination therapy may also be designed to capitalize on interactions between the different treatments that produce synergistic effects, *e.g.,* when a radiosensitizer is administered before external beam radiotherapy.

MTC is characterized by good vascularization and strong membrane expression of CEA, allowing RIT to deliver significant radiation doses to tumor (3, 5, 16). However, studies using different targeting systems and radionuclides in animal xenograft models of MTC have shown that isolated courses of RIT induce prolonged tumor responses but that late relapses always occur (3, 4). Heterogeneous perfusion and the presence of hypoxic cells or of cells in nonresponsive cell cycle phases can limit the efficacy of RIT in solid tumors. Autoradiography of TT xenografts has shown a heterogeneous distribution of anti-CEA MAb (17). A histological proliferation study of TT xenografts, using an antibody (MiB1) directed against a nuclear protein expressed in all active cell cycle phases, but absent in G_0 and early G_1, showed that the tumor nodules remaining after RIT were essentially composed of cells in G_0 and early G_1 (18). Moreover, RIT delivers an irradiation dose that decreases with the distance from blood vessels so that radioresistant hypoxic tumors are less irradiated. Chemotherapy, because of a very different biodistribution of the active drug and a different mode of action, could be used as a means of killing these radioresistant cells.

The present study tested two drugs in association with RIT: (*a*) doxorubicin, which has induced the best clinical responses in metastatic MTC as a single agent and enhanced radiation effects *in vivo* (19, 20); (*b*) and paclitaxel, which has shown radiosensitizing effects *in vitro* (21) and given promising results in combination with RIT in human breast carcinoma and MTC xenograft models (22–24).

It is clear that toxicity and antitumor activity depend on the timing between RIT and chemotherapy (23, 24). Doxorubicin, a vasoactive agent that reduces tumor blood flow, may decrease tumor uptake of a subsequently injected antibody (25). Paclitaxel is rapidly distributed to tumor and normal tissues and clears quickly (26). Thus, maximum tissue concentrations are reached shortly after administration. If chemotherapy is to act as a radiosensitizer, tumor cells must be irradiated when the chemotherapeutic agent is administered. A study involving a breast cancer animal model showed that the cure rate was much better when paclitaxel was delivered 6–24 h after the antimembrane glycoprotein ^{90}Y-ChL6 MAb rather than 24–72 h before (23). Another study evaluating anti-CEA ^{90}Y-MN-14 MAb in an MTC animal model showed that administration of doxorubicin 24 h after radiolabeled MAb achieved greater antitumor efficacy than when doxorubicin was given 48 h after RIT (24). A biodistribution study performed with AES reagents (F6–734/^{125}I-di-DTPA) in nude mice bearing MTC tumor showed that tumor uptake was maximal 5 h after hapten injection and then remained high at 24 h, with elevated tumor:nontumor tissue contrast ratios, before decreasing at 48 h (17). These results, as well as the relatively long half-life of iodine 131, led us to administer chemotherapy 24 h after injection of radiolabeled hapten to maximize possible synergistic effects on tumor cells.

Under these conditions, hematotoxicity was not increased when RIT was combined with doxorubicin or paclitaxel as compared with RIT alone. The ^{131}I-di-DTPA-In hapten cleared rapidly from the bloodstream, and only a small fraction of the radiation dose was delivered to marrow after injection of the chemotherapeutic agent. Similarly, in a preclinical study evaluating paclitaxel combined with ^{90}Y-ChL6 MAb, hematotoxicity was not increased when the drug was given after RIT, whereas the combination was more toxic when administered before (22, 23). A study evaluating ^{90}Y-MN-14 combined with different doses of doxorubicin or paclitaxel injected 1 or 2 days after radiolabeled antibody also showed that ~75% of the MTD of the chemotherapeutic agent combined with the MTD of RIT was equitoxic to the MTD of RIT alone (24). It is noteworthy that hematotoxicity with this one-step RIT approach (70–80% reduction of leukocytes) was markedly greater than in our study.

Doxorubicin is an intercalating agent that stabilizes the formation of complexes between topoisomerase II and DNA by altering the three-dimensional structure of DNA and inhibiting enzyme repair of radiation-induced single- and double-strand breaks (20). Thus, doxorubicin may act as a radiosensitizer. This would be especially important because RIT delivers irradiation with a low-dose rate, potentially allowing recovery of radiation-induced damage of DNA. Moreover, doxorubicin improves tumor oxygenation, which is an important factor for radiosensitivity. However, in the present study, doxorubicin alone showed no significant antitumor effect and did not improve the antitumor effect of RIT on MTC xenografts. This is clearly inconsistent with the results obtained by Behr *et al.* (12) in the same MTC xenograft model. These authors found that doxorubicin administered at its MTD (200 μg) induced a significant delay in tumor growth as compared with untreated controls (though with considerable variability in response) and that a combination of doxorubicin with ^{131}I-MN-14 IgG produced a synergistic antitumor effect. However, the i.v. route used in this study for chemotherapeutic injections probably allowed greater delivery of the agent to the entire organism, as well as to the tumor, as suggested by the effects of the high toxicity involved (20–30% weight loss and gastrointestinal side effects). It is also possible that i.p. administration led to slower distribution of the drug to tumor, precluding the expected radiosensitizing effect. Finally, unlike external radiotherapy, RIT delivers low-dose rate irradiation, allowing potential recovery of radiation-induced damage to DNA. The main radiosensitizing effect of doxorubicin appears to be the inhibition of enzyme repair of radiation-induced single- and double-strand breaks. After a single injection, the agent is briefly present in tumor during irradiation, so that damage repair is inhibited for only a short period. Thus, it might be useful to repeat injections, using suitable doses and time intervals.

Paclitaxel is a microtubule stabilizer with significant activity against a variety of solid tumors when used alone or in combination with other drugs (21, 27). In the present study, paclitaxel showed a significant antitumor effect against MTC xenografts and a significant increase in the antitumor efficacy of RIT. The latter was of the same order as that observed by Stein *et al.* (24), who used a different therapeutic scheme (the MTD of ^{90}Y-MN-14 combined with 78% of the MTD of paclitaxel). The moderate toxicity found in our study suggests that a higher hapten activity could be administered or that the interval between BsMAb and hapten administration could be shorter, which would probably provide greater efficacy for chemoradiotherapy with AES reagents and paclitaxel (8, 18). Analysis of the response curves showed that this combination was additive and possibly synergistic when the effect was measured over a longer time interval. This is consistent with the fact that RIT produces long-term effects that have not yet been fully elucidated. In the animal, control of tumor growth was very long after a single administration of AES RIT, even when all tumor cells were not killed (8). In a Phase I clinical study of F6–734 BsMAb/^{131}I-di-DTPA-In in relapsing MTC patients, tumor and biological responses occurred 3–6 months after RIT (5), and this is not an isolated observation. With respect to MTC, this slow response is probably related in part to the slow proliferation rate. The high antitumor potential of the RIT/paclitaxel combination may be because of several properties of the respective antitumor activities of these agents. In mammary or cervical tumor cell lines, paclitaxel has induced arrest in G_2-M, the most radiosensitive phase of the cell cycle (28). In the MTC model of slowly proliferating tumor cells, histopathology has shown that a majority of cells are in G_0 and early G_1 phases of low radiosensitivity (18). Thus, paclitaxel may recruit more tumor cells in the radiosensitive phases of the cell cycle. Moreover, paclitaxel induced apoptosis in an animal model of mammary carcinoma, and low-dose rate irradiation also triggered apoptosis (28, 29). Potentiation could be expected if the pathways of apoptosis induction are different for radiation and the drug. Finally, paclitaxel may increase tumor oxygen pressure and enhance the effect of radioactivity (30).

In summary, this study shows that paclitaxel increased the effect of RIT in inhibiting tumor growth, without producing any significant increase of toxicity. Evaluation of the antitumor effect over a longer time period suggested that the combination is synergistic. It is noteworthy that chemoradiotherapy using AES reagents appeared to be less toxic (for the same efficacy) than that using directly radiolabeled antibodies. Other administration schedules, as well as repeated courses of RIT and paclitaxel, may achieve much better antitumor effects and with limited toxicity. Clinical trials, sponsored by IBC Pharmaceuticals (Morris Plains, NJ), are in progress to optimize the dose and administration schedule of a new AES RIT product. This study clearly suggests that combinations of AES RIT and paclitaxel or other antitumor taxanes should be tested in clinical conditions as soon as possible.

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