Enhancement of the Antitumor Effect of γ -Ray Irradiation in Combination with Camptothecin against Human Colorectal Adenocarcinoma

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The effect of γ -ray irradiation and camptothecin (CAM) on human colorectal adenocarcinoma (LS 174T) growth was examined. On inhibition of DNA synthesis of LS 174T cells, almost all combined treatments presented a synergistic effect, excepting that the additive effects were shown at the lowest or the highest doses of CAM prior to the γ -ray (^{137}Cs) irradiation. The *in vivo* antitumor activity of ^{131}I -NCC-CO-411 monoclonal antibody against carcinoembryonic antigen in combination with CAM was assessed by a tumor growth rate in LS 174T tumor-bearing nude mice. The tumor growth of the combined group was supra-additively suppressed in comparison with that of the individual group. The results suggest that the efficacy of radioimmunotherapy may be enhanced by combination treatment with CAM. A clinical protocol involving a combination treatment with CAM is underway.

Key words combination-radioimmunotherapy; NCC-CO-411 MoAb; camptothecin; human colorectal adenocarcinoma

The prognosis for post-operative recovery from a resection of colorectal cancer is poor. Usually, radiotherapy and chemotherapy are requested instead of the operation. Carcinoembryonic antigen (CEA), a highly glycosylated glycoprotein with a molecular mass of 180 kDa, is the most useful tumor marker of colorectal cancer. Since the development of the hybridoma technique, many monoclonal antibodies (MoAbs) against CEA have been produced by immunization with purified CEA or human cancer cells.^{1–4)} Recently, considerable attention has been focused on imaging and immunotherapy of colorectal cancer by using radiolabelled anti-CEA MoAbs. NCC-CO-411, an anti-CEA MoAb, was produced by a unique immune method.⁵⁾ It was found that the concentration of CEA in feces was significantly higher in patients with colon cancer and other gastrointestinal disorders than that observed in normal adults using a sandwich radioimmunoassay with NCC-CO-411 MoAb.⁶⁾ It was previously shown that CEA in the feces involved amphiphilic molecules (presumably derived from a phosphatidyl-inositol-linked membrane-bound), while serum CEA was hydrophilic. Of these membrane-bound CEA has generally been proposed as a screening marker for gastrointestinal cancer, in particular for colorectal cancer. Earlier studies demonstrated that NCC-CO-411 MoAb was preferentially concentrated in solid LS 174T human colorectal adenocarcinoma, and gave a much clearer tumor image compared to conventional anti-CEA MoAb.⁷⁾

Radioimmunotherapy of cancer using radiolabelled MoAb has been extensively examined in animals and humans, but success has been limited because of the low concentration of radiolabelled antibody in tumor portions.⁸⁾ A variety of methods, such as increasing the specificity of MoAb, the use of antibody fragments,⁹⁾ targeting with recombinant single-chain antibodies,¹⁰⁾ administration of agents to increase tumor blood flow or antigen expression,^{11,12)} and the use of two-step delivery methods^{13,14)} have been investigated to improve the tumor targeting of radioimmunoconjugates. An alternative approach is to effectively elicit the therapeutic action of

radioimmunoconjugates at the tumor site, leading to an elevation of the potency of ionizing radiation and also decreasing the radiation doses which are related with side effects to other normal organs.

Several anticancer drugs such as adriamycin, bleomycin, taxol, *etc.* have been examined for their enhancement of the cytotoxicity of radiation.^{15–17)} Camptothecin (CAM), a natural plant alkaloid isolated from *Camptotheca acuminata*, is the first known drug which specifically inhibits topoisomerase I (topo I).¹⁸⁾

In the present study, preliminary studies on the enhancement of radiotherapy using NCC-CO-411 MoAb highly specific for human colorectal adenocarcinoma by CAM were investigated in *in vitro* cultured LS 174T tumor cells and *in vivo* on human colorectal adenocarcinoma xenografts in nude mice.

MATERIALS AND METHODS

Reagents and Cells CAM was purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). [Methyl- ^3H]thymidine and [^{131}I]NaI were purchased from Du Pont NEN (Wilmington, DE, U.S.A.). Lipiodol ultrafluide (a mixture of the ethyl esters of the iodized fatty acids of poppy-seed oil) was obtained from Laboratoires Guerbet (Bois Cedex, France). CAM stock solution (4.0 mg/ml) was made in sterilized dimethyl sulfoxide (DMSO), and stored at -20°C before use. The LS 174T human colorectal adenocarcinoma cell line was obtained from ATCC (Rockville, MD, U.S.A.). The cells were maintained in modified Eagle's medium (MEM) (Nissui Co., Tokyo, Japan) supplemented with 10% fetal calf serum and 1% 10 mM MEM non-essential amino acids solution (Gibco, Grand Island, NY, U.S.A.) at 37°C in a 5% CO_2 /95% air atmosphere.

MoAbs NCC-CO-411 MoAb (a gift from Dr. S. Hirohashi, National Cancer Institute of Japan, Tokyo) was obtained by hybridizing mouse myeloma cells (P3-X63-Ag8-U1) with spleen cells from nude mice (BALB/c, nu/nu) that had rejected transplanted human

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colorectal adenocarcinoma Co-4 following the intraperitoneal injection of spleen cells from immunocompetent mice (BALB/c). It belongs to the Ig G1 kappa classes.⁵⁾ Anti-human chorionic gonadotropin (anti-hCG) MoAb was a gift from Fuji Film Co. (Tokyo).

In Vitro Cytotoxicity of CAM against LS 174T Cell

The cytotoxicity of CAM against human colorectal adenocarcinoma cells was assessed by measuring cellular DNA synthesis. LS 174T cells were planted into 6-well plates at a density of 6.0×10^5 cells/2.0 ml/well and incubated overnight at 37°C in a 5% CO₂/95% air atmosphere. Ten μ l of a diluted solution of stock CAM was added into each well to a final respective concentration. After the cells were exposed to CAM for 0.5, 2.0 or 8.0 h, the medium was removed by aspiration, and the cells were washed twice with phosphate buffered saline (PBS). Then fresh media were added to each well and further incubated in a CO₂ incubator. Thirty-seven kBq of ³H-thymidine was added to each well at 46 h after adding CAM, and the cells were continuously incubated for 2 h. The cells were detached with 0.1% trypsin in PBS containing 0.02% EDTA and collected into a test tube after washing twice with PBS, then trichloroacetic acid (TCA) solution (4°C) was added into each tube to a final concentration of 5%. After the TCA-containing cell suspensions were kept on an ice bath for 1 h, the TCA-precipitants were filtered through a glass fiber filter (GC/F, Whatman International, Ltd., Maidstone, UK), and washed twice with ice-cold 5% TCA, ethanol-ether (1:1) solution, and ether. The radioactivity of ³H-thymidine incorporated into cellular DNA was measured with a liquid scintillation counter (LSC 9000, Aloka Co., Ltd., Tokyo, Japan). Results are expressed as the mean $\pm$ S.D. of relative DNA synthesis ratio (%) calculated from 3 wells according to the following formula:

$$\text{DNA synthesis (\%)} = \frac{\text{sample dpm} - \text{background dpm}}{\text{control dpm} - \text{background dpm}} \times 100$$

In Vitro Cytotoxicity of γ -Ray Irradiation LS 174T cells were planted into 6-well plates at the same density as described above. After the cells were incubated overnight, they were irradiated by γ -ray with a ¹³⁷Cs source, at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 Gy. Incorporation of ³H-thymidine into cellular DNA was measured using the procedure described above.

Combination Effect of γ -Ray Irradiation and CAM on the *in Vitro* LS 174T Cell Toxicity Two protocols were followed for understanding the combination effect of γ -ray irradiation and CAM on LS 174T cell toxicity. First, the cells were exposed to various concentrations of CAM for 0.5 h before γ -ray irradiation. After washing the cells twice with PBS and changing the fresh medium, the cells were immediately irradiated by γ -ray at dose of 1.0 or 2.0 Gy. Secondly, the cells were exposed to various concentrations of CAM for 0.5 h after γ -ray irradiation at the same doses. In both cases, the incorporation of ³H-thymidine into cellular DNA was measured at 48 h after the beginning of the first treatment of CAM or γ -ray irradiation.

The combined effect of γ -ray irradiation and CAM on the cytotoxicity was evaluated by comparing the actual

value of inhibition of DNA synthesis ratio ($A.I._{\text{DNA}}$) with the expected value of inhibition of DNA synthesis ratio ($E.I._{\text{DNA}}$). If the $A.I._{\text{DNA}}$ was significantly higher or lower (t test, $p < 0.05$) than the $E.I._{\text{DNA}}$, we judged the combined effect to be synergistic or antagonistic, respectively. If the difference was not significant (t test, $p > 0.05$), we judged the combined effect to be additive.¹⁹⁾

The parameters of $A.I._{\text{DNA}}$ and $E.I._{\text{DNA}}$ were calculated by the following formulas:

$$A.I._{\text{DNA}} (\%) = (1 - \text{DNA synthesis ratio}) \times 100$$

$$E.I._{\text{DNA}} (\%) = (1 - \text{DNA synthesis ratio of CAM alone} \times \text{DNA synthesis ratio of } \gamma\text{-ray alone}) \times 100$$

In Vivo Enhancement of Antitumor Activity of ¹³¹I-Labelled NCC-CO-411 MoAb in Combination with CAM

The MoAbs were labelled with [¹³¹I]NaI to a specific activity of about 100 MBq/mg by the chloramine-T method using limited amounts of chloramine-T.²⁰⁾ Seventy female athymic nude mice (BALB/c/nu-nu), 5 weeks old, were obtained from Clea Japan, Inc., (Tokyo). LS 174T cells (1.5×10^7) suspended in 0.2 ml sterilized PBS were inoculated subcutaneously (s.c.) into the back of the right side of the nude mice. On day 6 after the inoculation of tumor cells, the mice were randomly divided into 7 groups. Each group consisted of 10 mice with an average tumor volume about 180 mm³. Animals were s.c. given CAM dissolved in lipiodol at doses of 0, 2.0 or 5.0 mg/kg into the left hind leg,²¹⁾ and 24 h later were injected *via* the tail vein with 1.11 MBq (30 μ Ci) of ¹³¹I-NCC-CO-411. Also, ¹³¹I-hCG MoAb was examined as an irrelevant MoAb. When CAM is administered as a lipiodol suspension, it allows for the sustained release of the drug during localization of radiolabelled antibody at the tumor site. Both treatments were carried out again in the same manner two weeks after the first. The solid tumor size was measured with a caliper twice a week until day 34, and the volume (V) was calculated from the following equation²²⁾:

$$V(\text{mm}^3) = \text{length (mm)} \times [\text{width (mm)}]^2 \times 0.5$$

On the 34th day post-transplantation of tumor cells, the mice were sacrificed, and tumor tissues were removed to measure the weight. All values are expressed as the mean $\pm$ S.D. of 7 mice, because 3 mice died in the control group during the experimental period. The expected value of the inhibition ratio of tumor weight was also calculated using the method described above to judge the combined effect.

RESULTS

In Vitro Cytotoxicity of γ -Ray Irradiation and CAM Alone A dose response effect of cytotoxicity was induced by γ -ray irradiation in LS 174T cells. A low dose of the irradiation (0.5 Gy) seemed to somewhat increase the ³H-thymidine incorporation into the cellular DNA. The 50% inhibitory concentration (IC₅₀) is approximately 1.5 Gy (Fig. 1).

The cytotoxicity of CAM to the cells at varying exposure times and doses were also investigated in the same tumor

Fig. 1. Effect of γ -Ray Irradiation on DNA Synthesis of LS 174T Human Colorectal Adenocarcinoma Cell

Incorporation of [methyl- ^3H]thymidine into cellular DNA was measured at 48 h after γ -ray irradiation by a ^{137}Cs source. Point and bar represent the mean and S.D. of 3 wells, respectively.

Fig. 2. Effect of Camptothecin on DNA Synthesis of LS 174T Human Colorectal Adenocarcinoma Cell

Incorporation of [methyl- ^3H]thymidine into cellular DNA was measured at 48 h after treatment with camptothecin. Point and bar represent the mean and S.D. of 3 wells, respectively.

cells. As shown in Fig. 2, CAM caused cytotoxicity to the cells in both a dose- and time-dependent manner. The values of IC_{50} for different exposure times of 0.5, 2.0, and 8.0 h were 32.0, 4.3, and 2.4 nM, respectively.

Combination Effect of γ -Ray Irradiation and CAM on the *in Vitro* DNA Synthesis in LS 174T Cell The inhibition of DNA synthesis induced in LS 174T cells by 1.0 Gy of γ -ray irradiation was enhanced in a synergistic effect ($p < 0.01$) by post-treatment with CAM at all doses. The synergistic effect was also observed by pre-treatment of CAM at medium doses, while an additive effect was observed at 10^{-1} , 10^0 and 10^4 nM of CAM (Fig. 3). Figure 4 depicts the effect of CAM on the inhibition of DNA synthesis induced by a higher dose of γ -ray irradiation of 2.0 Gy. All combination treatments also showed a synergistic effect ($p < 0.01$), excepting the pre-treatment with CAM at the highest concentration of 10^4 nM. The inhibition values of post-treatment with CAM were higher

Fig. 3. Effect of Combination Treatment of Camptothecin and γ -Ray Irradiation at 1.0 Gy on Inhibition of DNA Synthesis of LS 174T Human Colorectal Adenocarcinoma Cell

■, Expected value of additive effect on inhibition of DNA synthesis in combination treatment with camptothecin for 0.5 h and γ -ray irradiation at 1.0 Gy; ▨, actual value of pre-treatment with camptothecin for 0.5 h; ▩, actual value of post-treatment with camptothecin for 0.5 h. Each column and the bar represent the mean and S.D. of 3 experiments, respectively. ** Indicates synergistic effect ($p < 0.01$); * indicates additive effect ($p > 0.05$), which were compared with the expected value of the additive effect at each concentration of camptothecin using a t test.

Fig. 4. Effect of Combination Treatment of Camptothecin and γ -Ray Irradiation at 2.0 Gy on Inhibition of DNA Synthesis of LS 174T Human Colorectal Adenocarcinoma Cell

■, Expected value of additive effect on inhibition of DNA synthesis in combination treatment with camptothecin for 0.5 h and γ -ray irradiation at 2.0 Gy; ▨, actual value of pre-treatment with camptothecin for 0.5 h; ▩, Actual value of post-treatment with camptothecin for 0.5 h. Each column and the bar represent the mean and S.D. of 3 experiments, respectively. ** Indicates synergistic effect ($p < 0.01$); * indicates additive effect ($p > 0.05$), which were compared with the expected value of the additive effect at each concentration of camptothecin using a t test.

than those of pre-treatment at the same dose of CAM in combination with both 1.0 and 2.0 Gy of γ -ray irradiation.

***In Vivo* Enhancement of Antitumor Activity of ^{131}I -Labelled NCC-CO-411 MoAb in Combination with CAM** The antitumor experiment proceeded in combination treatment with ^{131}I -labelled NCC-CO-411 MoAb and CAM. As shown in Fig. 5A, the tumor volume of the control group increased steeply, reaching $2812.5 \pm 623.4 \text{ mm}^3$ on day 34 after the implantation of LS 174T cells. In contrast, the tumor volumes of ^{131}I -labelled NCC-CO-411 MoAb plus 2.0 mg/kg of the CAM group showed no changes from day 6 to day 34. The volumes of 5.0 mg/kg of CAM, ^{131}I -labelled hCG MoAb plus 5.0 mg/kg of CAM

Fig. 5. *In Vivo* Effect of ^{131}I -NCC-CO-411 MoAb on Tumor Volume of Solid LS 174T Human Colorectal Adenocarcinoma Bearing Nude Mice in Combination Treatment with Camptothecin

A: —○—, solvent control; —●—, ^{131}I -NCC-CO-411 MoAb; —□—, 2.0 mg/kg camptothecin; —■—, ^{131}I -NCC-CO-411 MoAb plus 2.0 mg/kg camptothecin. B: —○—, solvent control; —●—, ^{131}I -NCC-CO-411 MoAb; —△—, 5.0 mg/kg camptothecin; —×—, ^{131}I -hCG MoAb plus 5.0 mg/kg camptothecin; —▲—, ^{131}I -NCC-CO-411 MoAb plus 5.0 mg/kg camptothecin. Point and bar represent the mean and S.D. of 7 mice, respectively. The experimental protocol was described in materials and methods.

Table 1. Antitumor Activity of ^{131}I -NCC-CO-411 MoAb in Combination with Camptothecin on LS 174T Human Colorectal Adenocarcinoma Bearing Nude Mice

Group	Tumor weight Mean $\pm$ S.D. (mg)	Inhibition ratio (%)	<i>p</i> Value (Fisher–Behrens test)					
			Control	2 mg CAM	5 mg CAM	^{131}I -411	^{131}I -411 + 2 mg CAM	^{131}I -411 + 5 mg CAM
Control	1693.1 $\pm$ 361.6	0.0	—	—	—	—	—	—
2 mg CAM	603.3 $\pm$ 167.0	64.4	<0.01	—	—	—	—	—
5 mg CAM	187.1 $\pm$ 42.3	88.9	<0.01	<0.01	—	—	—	—
^{131}I -411	933.7 $\pm$ 208.9	44.9	<0.01	<0.05	<0.01	—	—	—
^{131}I -411 + 2 mg CAM	183.7 $\pm$ 37.6	89.2	<0.01	<0.01	>0.05	<0.01	—	—
		80.4 ^{a)}						
^{131}I -411 + 5 mg CAM	68.0 $\pm$ 14.8	96.0	<0.01	<0.01	<0.01	<0.01	<0.01	—
		93.9 ^{a)}						
^{131}I -hCG + 5 mg CAM	197.3 $\pm$ 28.4	88.3	<0.01	<0.01	>0.05	<0.01	>0.05	<0.01

Group: control, solvent control; 2 mg CAM, 2.0 mg/kg camptothecin; 5 mg CAM, 5.0 mg/kg camptothecin; ^{131}I -411, ^{131}I -NCC-CO-411 MoAb; ^{131}I -411 + 2 mg CAM, ^{131}I -NCC-CO-411 MoAb plus 2.0 mg/kg camptothecin; ^{131}I -411 + 5 mg CAM, ^{131}I -NCC-CO-411 MoAb plus 5.0 mg/kg camptothecin; ^{131}I -hCG + 5 mg CAM, ^{131}I -hCG MoAb plus 5.0 mg/kg camptothecin. *a)* Expected value of additive effect in combination treatment with camptothecin and ^{131}I -NCC-CO-411 MoAb. The actual value of the combination treatment group is higher than the expected value of the same group, indicating a supra-additive effect.

groups also showed no changes, in almost the same manner. The curve of the ^{131}I -labelled NCC-CO-411 MoAb plus 5.0 mg/kg of CAM group came down on the last day of the experiment (Fig. 5B). ^{131}I -Labelled NCC-CO-411 MoAb and the 2.0 mg/kg CAM alone-treated group showed moderately increasing curves (Fig. 5A).

The tumor weights on day 34, at the end point of the experiment, are summarized in Table 1. The control group showed a mean tumor weight of 1693.1 $\pm$ 361.6 mg. The value is significantly higher than those of others ($p < 0.01$). The tumor weights for ^{131}I -labelled NCC-CO-411 MoAb and 2.0 mg/kg of the CAM alone-treated groups were 933.7 $\pm$ 208.9 and 603.3 $\pm$ 167.0 mg, respectively. In contrast, the weight of the combined group with ^{131}I -labelled NCC-CO-411 MoAb and 2.0 mg/kg of CAM showed a significantly low value of 183.7 $\pm$ 37.6 mg, compared with that of the individual groups ($p < 0.01$). Supra-additive effects were obtained in the two combined

groups on the inhibition of colorectal adenocarcinoma growth in nude mice. However, the tumor weight was not significantly different between the irrelevant radio-immunoconjugate (^{131}I -labelled hCG MoAb) plus 5.0 mg/kg CAM group and the 5.0 mg/kg of CAM alone group ($p > 0.05$).

DISCUSSION

Radioimmunotherapy using conjugates of antibodies and radioisotopes is considered to be significantly different from other treatments. Briefly, in radioimmunotherapy, the target cells can be easily killed by radiation, even if radioactive sources are not always taken into the cells, but are just close to them. In other methods, the cytotoxicity is expected to be effective only when the conjugates are internalized by the target cells where the toxins or anticancer drugs are hydrolyzed to a free form.

We had previously indicated that NCC-CO-411 MoAb

preferentially concentrated in LS 174T human colorectal adenocarcinoma portions, and gave a much clearer tumor image than a conventional anti-human CEA MoAb. However, this MoAb scarcely binds with LS 174T cells in an *in vitro* cell binding assay.⁷⁾ It has been found that the *in vivo* accumulation of NCC-CO-411 MoAb in the tumor portions is due to the recognition of CEA released from the tumor cells (unpublished data). Based on this mechanism of NCC-CO-411 MoAb accumulation in tumor portions, we conducted radioimmunotherapy by using conjugates of a radioisotope (¹³¹I) and NCC-CO-411 MoAb, which is thought to be the most suitable way to apply this antibody. We simultaneously examined a combination therapy with radiolabelled MoAb and CAM, which is a potential way of lowering the radiation exposure.

First, the sensitivity of human colorectal adenocarcinoma cells to γ -ray irradiation was examined. As shown in Fig. 1, the tumor cells were relatively sensitive to γ -ray irradiation ($IC_{50}=1.5$ Gy) in comparison with human stomach cancer cells (HGC-27, $IC_{50}=2.3$ Gy, data not shown). The IC_{50} value of LS 174T cells in DNA synthesis induced by γ -ray irradiation was similar to that reported by other investigators.²³⁾ Tumor cell exposure to γ -ray at a low dose of 0.5 Gy seemed to enhance DNA synthesis somewhat. This might be due to the phenomenon of radiation hormesis. In human or animal, it appears as immunoenhancement, obtaining an adaptive response alleviating chromosome damage caused by subsequent larger doses of radiation, but the detailed mechanism is still not clear.²⁴⁾

CAM, a highly specific inhibitor of DNA topo I, also markedly inhibited the DNA synthesis of LS 174T tumor cells (Fig. 2). This drug traps covalent enzyme-DNA intermediates, as a so-called cleavable complex.²⁵⁾ This complex is a reversible topo I-CAM-DNA ternary complex. CAM acts as a stabilizer in this complex, resulting in the inhibition of DNA replication and repair.^{26,27)} So far, many analogues of CAM have been tested against various human cancer xenograft lines carried by immunodeficient mice. Their strong antitumor activities were observed for colon, lung, breast, stomach and ovary carcinomas, particularly for colon cancer.^{21,28)} The higher antitumor activity against colon cancer has been thought to be due to the higher level of topo I in the advanced stage and metastatic stage of human colon cancer tissue than in normal colonic mucosa. The LS 174T cell line was also sensitive to CAM. The higher level of topo I expression in this cell line would be mostly anticipated.

In the *in vitro* combination experiments, almost all combined treatments presented a synergistic effect, while additive effects were shown in the pre-treatment with CAM at 10^{-1} , 10^0 and 10^4 nM for 1.0 Gy of γ -ray, and in pre-treatment with CAM at 10^4 nM for 2.0 Gy of γ -ray. It has already been reported that CAM causes the dramatic radiosensitization of an extremely resistant human melanoma (U1-Mel), and the potency is effectively induced either during or immediately after X-ray irradiation, but ineffective prior to the irradiation.²⁹⁾ However, our data presented here clearly demonstrated that CAM caused radiosensitization in the human colorectal adenocarci-

noma cell line (LS 174T) not only after, but also before the treatment of γ -ray irradiation. The potency is considered to be irreversible, leading to a synergistic effect of this method of treatment as well. Compared with the degree of synergistic effect of CAM between post- and pre-treatment, it has been clearly found that the efficacy post-treatment is significantly greater than that pre-treatment. This may be due to the radiosensitizing effect of CAM being caused to a greater extent after the radiation with γ -ray than before. Detailed molecular analysis of the interaction between CAM and radiation is needed to examine the mechanism of the synergistic potency.

In the *in vivo* combination treatment with ¹³¹I-labelled NCC-CO-411 MoAb and CAM, a supra-additive effect was also observed. It was indicated that ¹³¹I-labelled NCC-CO-411 MoAb markedly inhibited tumor growth at lower doses of radiation (1.11 MBq/head) in combination with 2.0 mg/kg of CAM than conventional doses (Table 1). It was notable that apparent side effects such as body weight loss induced by the toxicity of radiation or CAM were not observed in this trial, while the higher dose treatment of CAM (5.0 mg/kg) alone showed serious cytotoxicity with drastic body weight loss (data not shown). It has been also reported that topo I inhibitors and external beam irradiation enhance the inhibition of tumor growth in the radioimmunotherapy of cancer.³⁰⁻³²⁾ However, it has been a serious problem that these inhibitors were too toxic, and also that high doses of beam irradiation induced skin or bone marrow damage in this experiment.³²⁾ Our preliminary *in vivo* data suggest the possibility of using NCC-CO-411 MoAb with low radiation exposure on radioimmunotherapy in combination with antitumor drugs. It is still too early to decide whether this approach will lead to a potential clinical therapeutic procedure for the treatment of cancer. Detailed *in vivo* trials of this combination treatment are now underway.

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