

Pharmacologic Intervention with Angiotensin II and Kininase Inhibitor Enhanced Efficacy of Radioimmunotherapy in Human Colon Cancer Xenografts

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Induced hypertension and kininase inhibition can enhance tumor targeting of radiolabeled monoclonal antibody (MAb) by altering tumor circulation. This study investigated the effect of this manipulation on the antitumor efficacy of radioimmunotherapy (RIT). **Methods:** Mice bearing human colon cancer xenografts were administered 2.0 µg/kg/min of angiotensin II (AT-II) for 1 h and 30 µg of a kininase inhibitor, enalapril maleate, before the administration of 3.7 MBq ¹³¹I-A7, an IgG1 against 45-kDa glycoprotein on colorectal cancer, and tumor growth was observed thereafter. The mechanism of the manipulation effect was investigated by estimation of the tissue absorbed dose and radioluminography of tumors. **Results:** The pharmacologic manipulation with AT-II and enalapril improved the tumor quadrupling time (T_q) of 3.7 MBq RIT from 24.3 ± 2.75 d to 33.1 ± 2.83 d (*P* < 0.05). Addition of this manipulation made 3.7 MBq RIT as effective as 9.25 MBq RIT alone (T_q, 37.2 ± 2.97 d). Dose estimation showed that the manipulation increased the tumor absorbed dose 1.55-fold without affecting the doses to normal tissues. Uniform intratumoral distribution in the manipulated tumors was shown by radioluminography. **Conclusion:** Larger and more uniform tumor radiation produced by this pharmacologic manipulation can benefit RIT with ¹³¹I-MAb.

Key Words: radioimmunotherapy; tumor circulation; induced hypertension; kininase inhibitor

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Radioimmunotherapy (RIT) can be a good choice for treatment of radiosensitive tumors such as lymphoma (1,2). However, other types of solid tumors, including colon cancer, do not respond well to RIT (3–5). One major reason for the insufficient results of RIT may be the limited and heterogeneous targeting of radiolabeled monoclonal antibody (MAb) to tumors (6). Tumor circulation might be an

important factor affecting the targeting of MAb. Blood supply to solid tumors is usually heterogeneous, which not only makes it difficult for MAbs to reach tumor cells but also increases radioresistant hypoxic cells.

Our previous observations showed that the manipulation of tumor circulation by induced hypertension with angiotensin II (AT-II) and kinin degradation inhibition with a kininase inhibitor, enalapril maleate, improved tumor targeting and intratumoral distribution of radiolabeled MAb (7–9). The rationale of this strategy is that induced hypertension may distend tumor vessels and increase tumor blood flow and vascular area (10–12), which improve delivery of MAbs, and a kininase inhibitor may increase the concentration of bradykinin in tumors by blocking the kininase cascade, which enhances the extravasation of MAbs in tumors (13,14). An increase of tumor blood flow may also better oxygenate tumor cells, which is of benefit in enhancing the radiation effect of RIT. The purpose of this study was to investigate whether this manipulation would improve the antitumor effect of RIT with ¹³¹I-MAb in a mouse model xenografted with human colon cancer.

MATERIALS AND METHODS

MAb, Animal Model, and Pharmacologic Manipulation

A7, an IgG1 murine MAb that recognizes the 45-kDa tumor-associated glycoprotein of colorectal cancer (15), was radiolabeled by the chloramine-T method with either ¹³¹I or ¹²⁵I. The specific activity and immunoreactivity under antigen excess conditions of ¹³¹I-A7 and ¹²⁵I-A7 purified by PD10 columns (Pharmacia LKB Biotechnology, Uppsala, Sweden) were 32.6 MBq/mg and 78.3% and 21.7 MBq/mg and 63.2%, respectively. The MAbs were sterilized by filtration (Millex-GV, 0.22 µm; Millipore, Bedford, MA) before injection into mice.

Animal studies were performed in compliance with the regulations of our institution. BALB/c *nu/nu* mice (females, 20 g) were xenografted subcutaneously with 6 × 10⁶ LS180 human colon cancer cells in the thigh and used for experiments 2 wk later. The number of binding sites on this cell line was determined to be

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$7.90 \times 10^4/\text{cell}$ by Scatchard analysis with ^{125}I -A7. Microosmotic pumps (Alzet model 1003D; Alza, Palo Alto, CA) were implanted subcutaneously on the animal's back under anesthesia with inhalation of diethyl ether. After the start-up period of 4 h, the pumps infuse a solution at a constant flow rate of $1.0 \mu\text{L}/\text{h}$ for 72 h at 37°C . Absorption of the compound by local capillaries results in systemic administration. For the hypertensive manipulation, implanted pumps were filled with $[\text{Asn}^1, \text{Ile}^5]\text{-AT-II}$ (Peptide Institute, Inc., Osaka, Japan) dissolved in saline. AT-II increased the blood pressure of mice dose dependently in the dose range of $0.5\text{--}10 \mu\text{g}/\text{kg}/\text{min}$, and the pressure returned to the baseline pressure soon after removal of the pump (8). In this study, an infusion rate of $2 \mu\text{g}/\text{kg}/\text{min}$ for 1 h was used as determined previously (8,9). Thirty micrograms of a kininase II inhibitor, enalapril maleate (Sigma Chemical Co., St. Louis, MO), dissolved in $100 \mu\text{L}$ distilled water was administered orally as determined previously (9). Mice in the control group were implanted with pumps with saline and orally administered water.

RIT with ^{131}I -A7

The antitumor efficacy of RIT with $3.7 \text{ MBq } ^{131}\text{I}$ -A7 was observed in mice with the manipulation of AT-II and enalapril and compared with that in the control groups, including nontreated mice injected with phosphate-buffered saline and nonmanipulated mice with RIT of 3.7 MBq . This result was also compared with the efficacy of nonmanipulated RIT of 9.25 MBq , which was almost the maximum tolerated dose of this model (16,17). Tumors were measured by a digimatic caliper, and tumor volume was calculated as $\text{length} \times \text{width}^2 \times 0.5$ and expressed as a ratio to the volume on the day of ^{131}I -A7 injection (day 0). Tumor quadrupling time (Tq) was obtained for each tumor. Tumor burden on day 0 was $1.19 \pm 0.10 \text{ cm}^3$, and there was no statistical difference among the experimental groups. Although smaller tumors respond to RIT better, we used tumors of this size because such a model would mimic a current clinical study better than would small tumors (18).

Mechanism of Manipulation Effect on RIT

The effect of AT-II and enalapril on the biodistribution of $74 \text{ kBq } ^{125}\text{I}$ -A7 was observed in mice 6 h through 10 d after the intravenous injection. For the dosimetric assessment, cumulative radioactivity ($37 \text{ kBq} \times \text{h}/\text{g}$) of tissues was obtained by the trapezoid integration method using the biodistribution data. To determine the whole-body dose, the data of whole-body radioactivity up to 10 d were fitted exponentially and integrated. For the tumor dose, trapezoid integration was used for the biodistribution data up to 3 d, and the data of the elimination phase after that time point were fitted and integrated. A homogeneous tissue distribution of radioactivity was assumed, and the absorbed dose was estimated by a formula: $D_{\beta} (\text{cGy}) = 2.13 \times 37 \text{ kBq}/\text{g} \times \text{h} \times E_{\beta}$, where E_{β} of $^{131}\text{I} = 0.19 \text{ g}/(37 \text{ kBq} \times \text{h})$ (18). In this estimation, the contribution of γ emission was neglected.

Radioluminography (autoradiography) of tumors was obtained to assess the effect of manipulation on the intratumoral distribution of MABs. Tumors were excised from mice injected with $740 \text{ kBq } ^{125}\text{I}$ -A7 6 h and 3 d after injection. Frozen sections ($20 \mu\text{m}$) were placed on imaging plates (BAS-SR2025; Fuji Film, Tokyo, Japan) for 13 h, and the radioactivity data were acquired with an imaging analyzer (BAS-5000; Fuji Film) as photo-stimulated luminescence (PSL). For quantitative assessment, PSL/mm^2 was obtained for each section and compared with the biodistribution data. Profiles of sections were delineated to assess the homogeneity of the intratumoral distribution of MAB.

Statistical Analysis

Statistical analysis was performed with a 1-way or repeated ANOVA with Fisher's protected least significant difference to compare the tumor growth. For comparison of the biodistribution data, an unpaired t test was used. In the analyses, the level of significance was set at 5%.

RESULTS

All treated groups showed tumor growth delay compared with the nontreated control ($P < 0.05$ by repeated ANOVA) (Fig. 1). Manipulation with AT-II and enalapril improved antitumor efficacy of RIT ($P < 0.05$ by repeated ANOVA): Tq in the manipulated RIT of 3.7 MBq was $33.1 \pm 2.83 \text{ d}$, whereas Tq was $24.3 \pm 2.75 \text{ d}$ in nonmanipulated RIT of 3.7 MBq and $18.4 \pm 0.68 \text{ d}$ in nontreated control ($P < 0.05$ by 1-way ANOVA). The efficacy of manipulated RIT of 3.7 MBq was close to that of the nonmanipulated RIT of 9.25 MBq , which had a Tq of $37.2 \pm 2.97 \text{ d}$ ($P = 0.23$).

Tumor accumulation of ^{125}I -A7 increased significantly with the manipulation, whereas distribution in normal tissues did not change during the observation up to 10 d (Fig. 2). On day 3, uptake (percentage injected dose/gram [%ID/g]) in the manipulated tumor was 30.0 \%ID/g , whereas

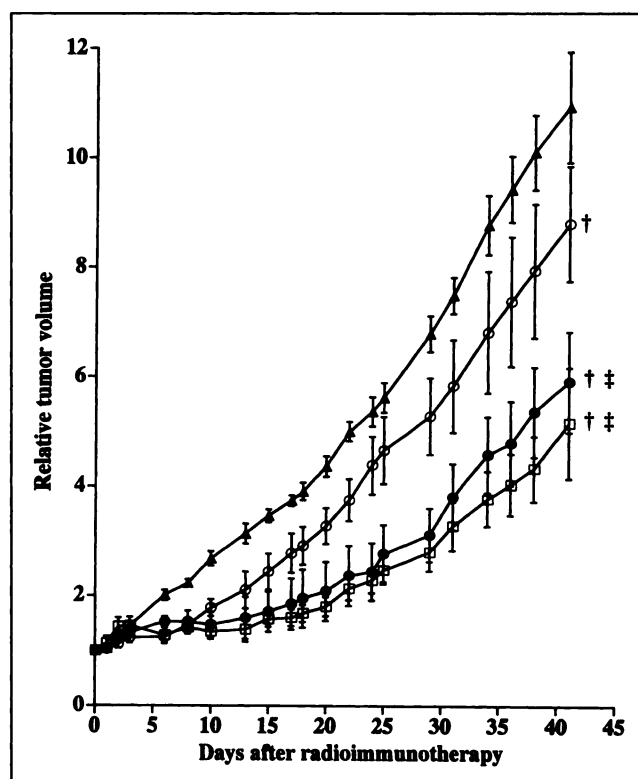


FIGURE 1. Growth of LS180 colon cancer xenografts after RIT with ^{131}I -A7 ($n = 5$). Tumor volume is expressed as ratio to volume on day 0 (mean \pm SE). \blacktriangle , Nontreated control injected with phosphate-buffered saline; \circ , nonmanipulated RIT of 3.7 MBq ; \square , nonmanipulated RIT of 9.25 MBq ; \bullet , RIT of 3.7 MBq with manipulation of AT-II and enalapril. \dagger , Significant versus nontreated control; \ddagger , significant versus nonmanipulated RIT of 3.7 MBq by repeated ANOVA with Fisher's protected least significant difference ($P < 0.05$).

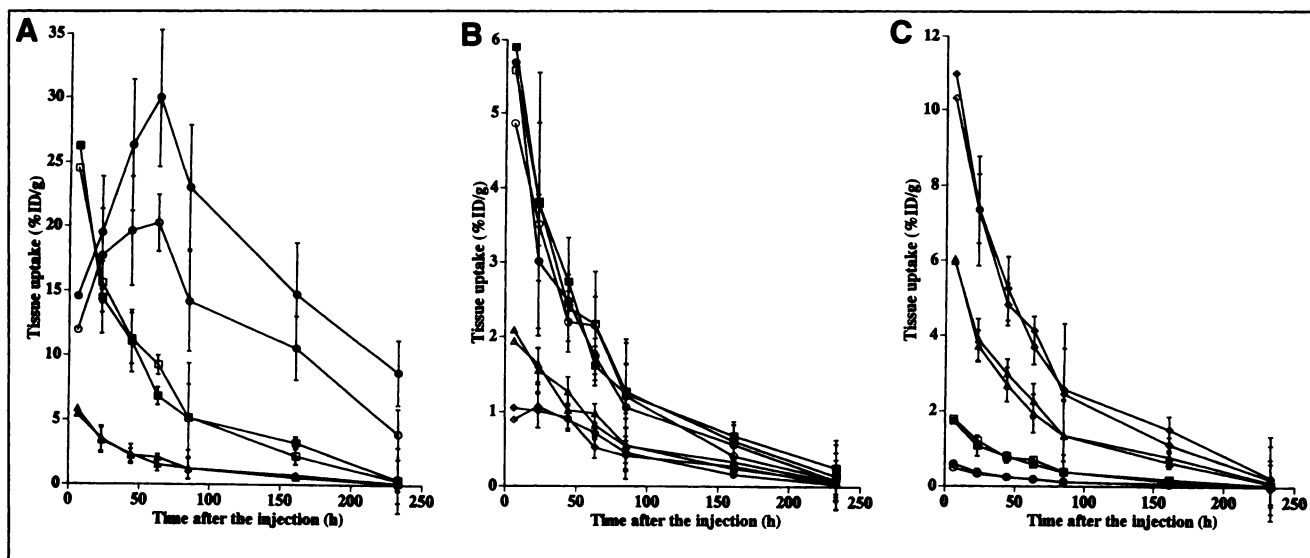


FIGURE 2. Effect of manipulation with AT-II and enalapril on biodistribution of ^{125}I -A7 in mice bearing colon cancer xenografts ($n = 4$). Open symbols, nonmanipulated group; solid symbols, manipulated group. (A) \circ , Tumor; \square , blood; \triangle , liver. (B) \circ , Kidney; \square , spleen; \triangle , bone; \diamond , muscle. (C) \circ , Brain; \square , intestine; \triangle , heart; \diamond , lung. \dagger , Significant versus nonmanipulated group by unpaired t test ($P < 0.05$).

uptake was 20.3 %ID/g in the nonmanipulated tumor, resulting in a 1.48-fold enhancement ($P < 0.001$ by unpaired t test). The manipulation did not affect whole-body clearance of A7 (Fig. 3).

Estimated tissue absorbed doses are summarized in Figure 4. Exponential curves fit well to the experimental data of whole-body radioactivity and the elimination phase of tumors for both the nonmanipulated and the manipulated groups ($r^2 > 0.96$). The manipulation increased tumor dose 1.55-fold from 8.94 to 13.8 cGy/37 kBq, whereas it did not affect doses to normal tissues. Therefore, the therapeutic ratios were improved approximately 50% for all normal tissues and for the whole body. With 3.7 MBq ^{131}I -A7, the tumor absorbed doses were estimated to be 8.94 and 13.8 Gy in the nonmanipulated RIT and manipulated RIT, respectively. The tumor dose in the nonmanipulated RIT of 9.25 MBq was calculated to be 22.4 Gy.

The intratumoral ^{125}I -A7 distribution is shown in Figure 5. Mean intensities in the sections of the nonmanipulated tumors and the manipulated tumors were 110 and 165 PSL/mm² at 6 h and 243 and 353 PSL/mm² on day 3, respectively, showing approximately 50% enhancement by the manipulation; these findings agreed well with the biodistribution data shown in Figure 2. Furthermore, the intratumoral distribution of radioactivity became more homogeneous by the manipulation as observed by both the visual assessment and the profile analysis.

DISCUSSION

Induced hypertension and kininase inhibition, which increase tumor perfusion and vascular permeability (10–14), would produce favorable effects on tumor vasculature for the MAb distribution. The increase in tumor targeting of A7

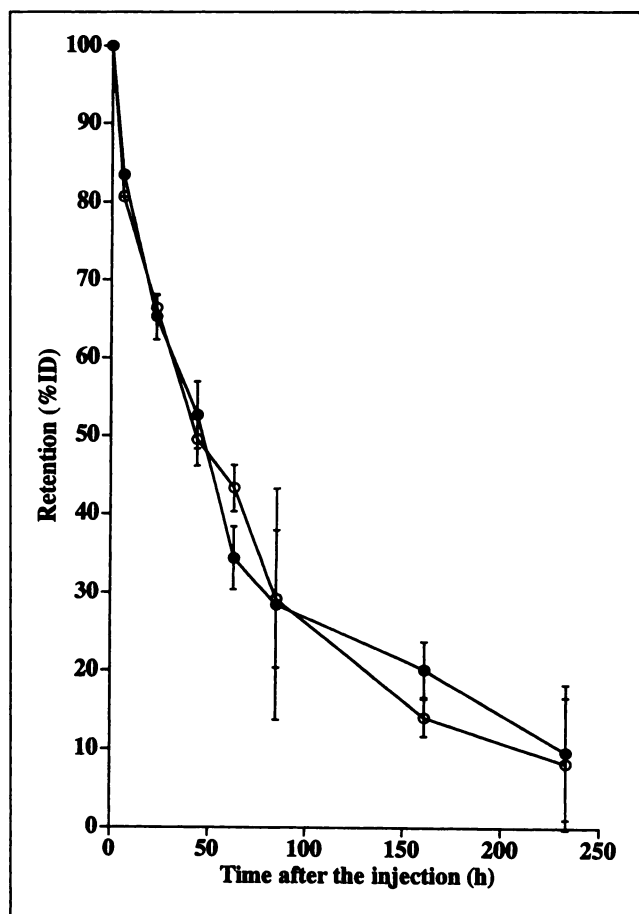


FIGURE 3. Whole-body clearance of ^{125}I -A7 in mice bearing colon cancer xenografts ($n = 4$). \circ , nonmanipulated group; \bullet , manipulated group.

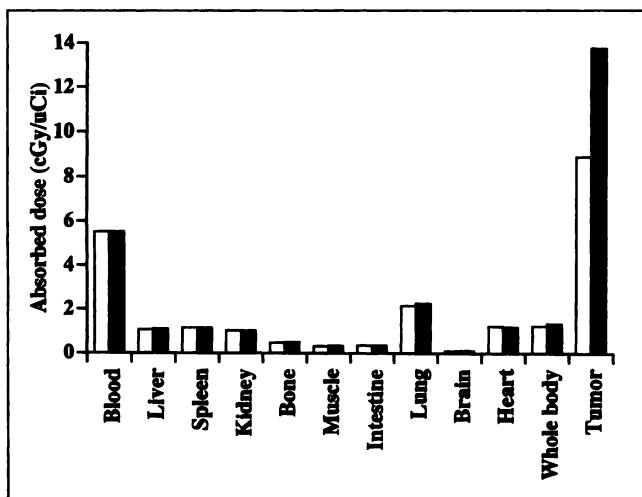


FIGURE 4. Tissue absorbed dose (cGy/μCi [cGy/37 kBq]) of ^{131}I -A7 estimated by biodistribution data of ^{125}I -A7.

MAB by this manipulation was proven by our previous observations (7–9), and in this study it was shown that this actually improved the efficacy of RIT in colon cancer xenografts. The manipulation enhanced the tumor absorbed dose 1.55-fold with ^{131}I -A7. However, the estimated tumor dose of the manipulated RIT of 3.7 MBq was much lower than that of the nonmanipulated RIT of 9.25 MBq, although these treatments showed similar antitumor efficacy. Therefore, factors other than total tumor dose should also be considered to explain the mechanism of the enhanced

antitumor effect obtained with the manipulation. One factor may be the homogeneity of the intratumoral distribution of MAB in the manipulated tumors found by the autoradiographic analyses. Although the contribution of radioactivity inside the distended vascular components to the autoradiographic results could not be excluded completely, artificial improvement by vascular radioactivity would be unlikely because the homogeneity of the intratumoral distribution became more apparent at a later time point when most radioactivity had already been cleared from circulation. Another possible factor for the enhancement of RIT may be the oxygenation status of tumor cells: The increase of tumor blood supply by the manipulation can oxygenate cells better and reduce the fraction of radioresistant hypoxic cells. We postulate that these effects produced by the manipulation worked together to improve the results of RIT, which were greater than anticipated solely from the tumor absorbed dose.

An adverse effect of induced hypertension on the delivery of macromolecules to tumor has been reported by other investigators (19–21), suggesting that the increase in blood pressure might increase not only tumor blood flow but also interstitial fluid pressure (IFP) of the tumor, which is an obstacle for the convective transport of macromolecules such as MABs. A model simulation by Netti et al. (20) predicted that hypertension induced by the administration of AT-II could not increase the tumor uptake of macromolecules because of the increase in IFP. On the contrary, our observations showed the enhancement of tumor targeting of

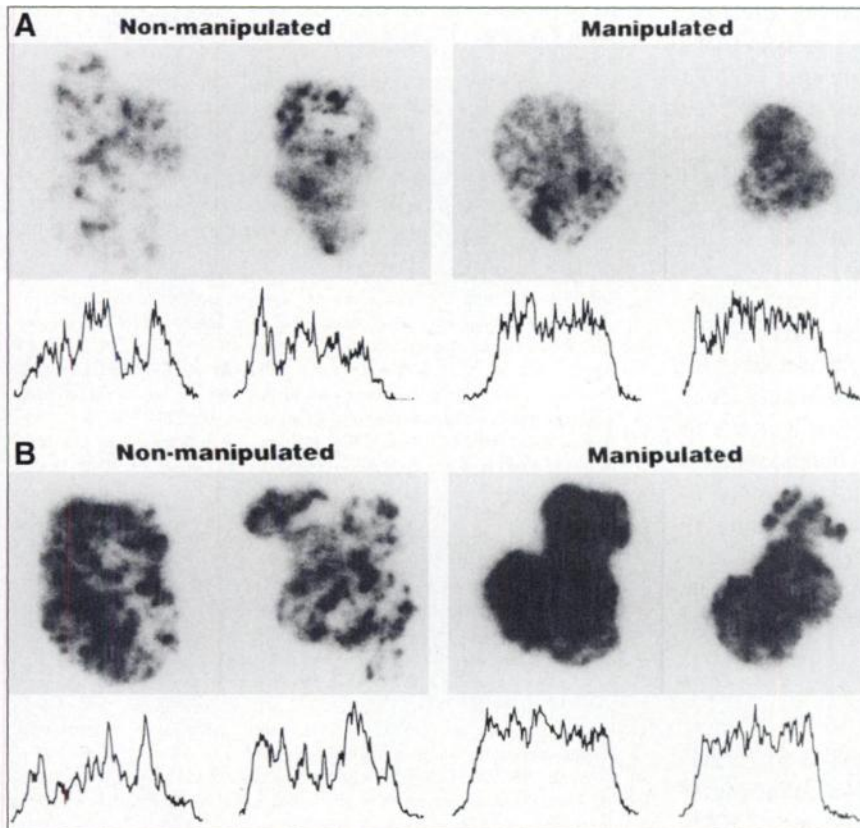


FIGURE 5. Radioluminographs of tumors obtained 6 h (A) and 3 d (B) after injection of ^{125}I -A7. Representative sections from 2 different tumors of each group are shown. Profiles of radioactivity in tumors are shown below images.

MAB and the improvement of RIT outcome by the manipulation. A hysteresis-like response of tumor vessels (12) must be a major cause of the improvement of MAB distribution, which means that the mechanical distension of tumor vessels produced by the induced hypertension persists even after the withdrawal of AT-II infusion. We showed previously that vascular structures were actually more prominent in the manipulated tumors than in the control tumors even 2 d after the manipulation (9). In such a situation, high tumor blood flow can be maintained in the normotensive state when the increase of IFP disappears. However, this phenomenon was not considered in studies that showed the negative effect of induced hypertension (19–21). Recruitment of tumor vessels reported by Hori et al. (11) must be another important factor, indicating that tumor vessels in the deep part of tumors compressed by IFP can be reperfused by the increase in blood pressure. We believe that these multiple positive effects of the short-period AT-II infusion on tumor vessels contributed to the enhanced tumor targeting of A7 MAB that persisted for several days after injection. In addition, the enhancement of vascular permeability caused by the administration of enalapril should have played a role in improving the efficacy of RIT. Matsumura et al. (13) reported that administration of kininase inhibitor increased extravasation of macromolecules by blocking the kinin cascade of the tumor. We reported previously that administration of this agent produced additional enhancement of tumor targeting of A7 in mice with induced hypertension (9). Therefore, the cooperative action of these 2 agents must have been involved in the enhancement of RIT efficacy shown in this study.

Manipulation with AT-II and enalapril did not affect the absorbed dose to normal tissues, indicating that better RIT outcome can be achieved by this manipulation without increasing its toxicity. This phenomenon must be associated with the autoregulation of normal tissues that maintains constant tissue flow by the contraction of their vessels against change in blood pressure (12). Li et al. (22) showed that manipulation did not increase the toxicity of the macromolecular drug styrene-maleic acid copolymer-conjugated neocarzinostatin in a rat model. Furthermore, AT-II-induced hypertension was performed safely in the clinical situation, and a better result was found with hypertensive chemotherapy than with chemotherapy alone (23). Another study also showed the selective increase of tumor perfusion by induced hypertension in human subjects (24). These reports indicate that human vasculature in tumors can be distended by induced hypertension similarly to murine vasculature in xenografts, and the autoregulation mechanism of human normal vasculature prevents the damage of normal organs as found in the murine model of this study.

CONCLUSION

Manipulation of tumor circulation with AT-II and enalapril improved the antitumor effect of RIT in colon cancer xenografts. The increase in absolute tumor uptake of MAB,

homogeneous intratumoral distribution of MAB, and possible better oxygenation of tumor cells may have accounted for this improvement. This manipulation may not affect the normal tissue dose in RIT, so that a better tumor response to RIT can be expected with this manipulation without increasing its toxicity.

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