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Enhanced Tumor Localization of Monoclonal Antibody by Treatment with Kininase II Inhibitor and Angiotensin II

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The effect of kininase II inhibitor, enalapril, on the delivery of monoclonal antibody A7 to the targeted tumor was investigated using athymic mice bearing human colon cancer, SW1116. Enalapril alone, which enhances tumor vascular permeability through the kinin-generating cascade, did not increase the uptake of ^{125}I-labeled A7 (^{125}I-A7) in SW1116 due to the systemic hypotension induced by its inhibitory effect on angiotensin converting enzyme. However, with combined angiotensin II (AT-II) and enalapril treatment, a 2-fold increase in the accumulation of ^{125}I-A7 was seen when compared to A7 alone. This marked increase was presumably due to increased tumor vascular permeability induced by enalapril combined with the absence of hypotension due to the actions of AT-II. This approach might be useful in radioimmunodetection and immunotargeting chemotherapy using monoclonal antibody.

Key words: Monoclonal antibody A7 — Vascular permeability — Kinin generating cascade — Kininase II inhibitor — Angiotensin II

The systemic administration of immunoconjugates of anticancer agents, toxins, and radioisotopes has been investigated for the treatment and diagnosis of cancer,[1,2] but this therapy is limited by the small absolute amount of agent reaching the target. In fact, the binding of monoclonal antibody (MAb)[4] to solid tumor cells is restricted by many barriers such as tumor vasculature, blood flow, vascular permeability, and interstitial tumor pressure.[3,4] Therefore, to improve tumor uptake of MAb, it is important to take into consideration these factors in addition to the immunological factors, such as antibody affinity and antigen density.

Matsumura and coworkers reported that the kinin-generating cascade is present in the human tumor compartments.[5,6] Bradykinin generated by the cascade is one of the most potent permeability-enhancing factors known, and is undoubtedly responsible for the increased vascular permeability commonly observed in various solid tumor tissues. Upon treatment with the kininase II inhibitor, enalapril, a higher kinin level is expected in the tumor, since the degradation of bradykinin is suppressed (see Fig. 1).[5] The kinin-induced increase in vascular permeability may provoke macromolecular leakage from the tumor vessel to the interstitial tumor space, leading to a higher accumulation of MAb in the tumor tissue. The purpose of this study was to improve the tumor uptake of MAb by enhancing vascular permeability using enalapril.

Enalapril maleate, (N-(*S*)-1-ethoxycarbonyl-3-phenylpropyl)-L-proline maleate (kininase II inhibitor) was a gift from Banyu Co., Ltd., Tokyo. The MAb A7, produced against human colon cancer cell line,[7] was labeled with ^{125}I (New England Nuclear, Boston, MA) by a chloramine T method. The specific activity of ^{125}I-labeled A7 (^{125}I-A7) was 2.8×10^5 cpm/μg. The human colon cancer xenograft initiated from the *in vitro* cell line SW1116, to which MAb A7 reacted specifically,[8] was subsequently passed subcutaneously to Balb/c (*nu/nu*) female athymic mice. Seven days after tumor inoculation, the tissue distribution study was started. First, athymic mice bearing SW1116 were divided into two groups; control and enalapril groups. In the control group, mice were injected with ^{125}I-A7 alone. In the enalapril group, the kininase II inhibitor, enalapril, was given orally at a dose of 10 mg/kg 4 h before ^{125}I-A7 injection. In both groups of mice, ^{125}I-A7 was injected into the tail vein at a dose of 5×10^6 cpm in 0.2 ml of PBS. At 3 days after MAb injection, blood was collected from the vena cava and the mice were killed. Organs and tumor were excised and weighed, and their radioactivities were measured in a gamma scintillation counter. The effects of enalapril treatment on the tissue distribution of ^{125}I-A7 radioactivity 3 days after iv injection of

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[4] Abbreviations: MAb, monoclonal antibody; AT-II, angiotensin II; PBS, phosphate-buffered saline; ^{125}I-A7, ^{125}I-labeled A7.

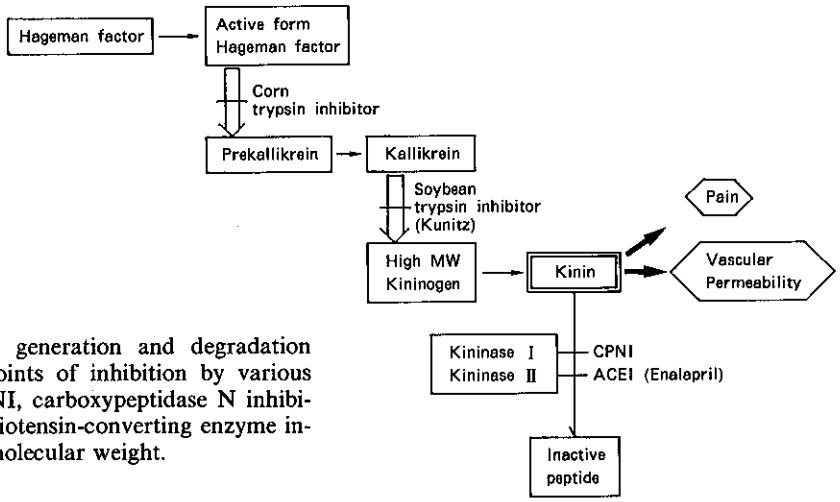

Fig. 1. Kinin generation and degradation cascade and points of inhibition by various inhibitors. CPNI, carboxypeptidase N inhibitor; ACEI, angiotensin-converting enzyme inhibitor; MW, molecular weight.

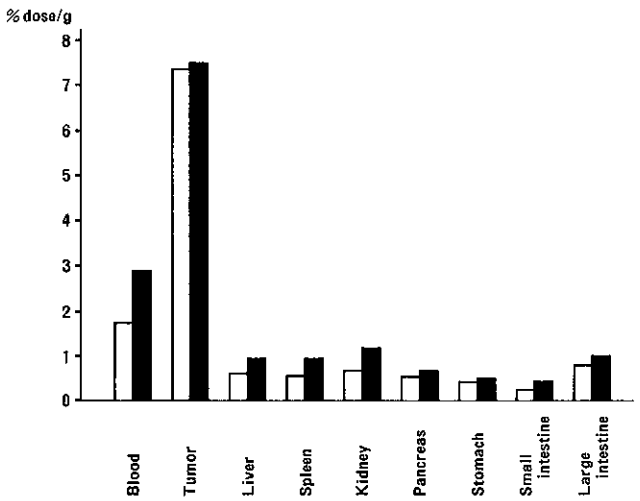

Fig. 2. Tissue distribution of ^{125}I-A7 3 days after iv injection in untreated (□) and enalapril-treated (■) SW1116-bearing nude mice (n=4).

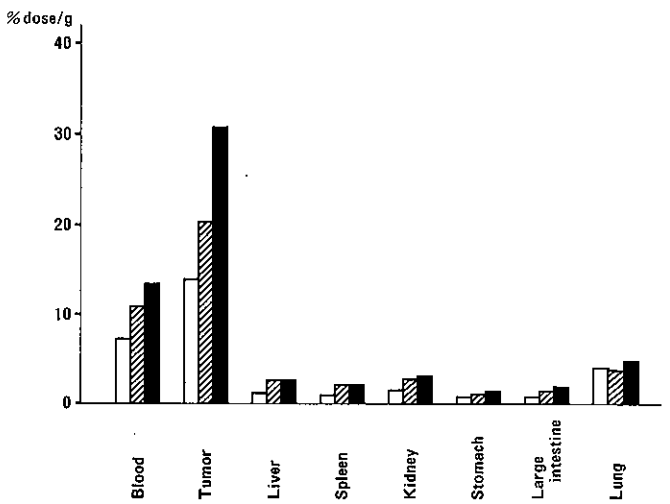

Fig. 3. Tissue distribution of ^{125}I-A7 3 days after iv injection in SW1116-bearing nude mice (n=4) treated with AT-II alone (▨), AT-II plus enalapril (■), or no drug treatment (□).

the MAb into athymic mice bearing SW1116 is shown in Fig. 2. Radioactivity accumulations in the tumors of both groups of mice were nearly identical, i.e., 7.35± 0.93 (mean±SE) %dose/g for the control group and 7.47±1.75 %dose/g for the enalapril group. This result shows that enalapril alone did not increase the tumor uptake of MAb A7. Although the arterial pressure of the mice was not monitored, it is possible that blood flow to the tumor was reduced consequent to a reduced systemic blood pressure, since enalapril inhibits the generation of angiotensin II (AT-II) by angiotensin converting enzyme. It may be presumed that enalapril alone does not enhance vascular permeability since both tumor blood pressure and blood flow are reduced following enalapril treatment. An appropriate tumor blood flow and blood pressure would appear to be essential in promoting increased vascular permeability. With this point in mind, we utilized AT-II to counteract the hypotensive effect of enalapril in the following experiment.

Athymic mice bearing SW1116 were divided into three groups; the control group, AT-II group, and AT-II and enalapril group. In the control group, ^{125}I-A7 was ad-

ministered via the tail vein without additional drugs. With the other groups AT-II (human angiotensin II, Toa Eiyou Co., Ltd.) at a dose of 20 μg/kg in 0.6 ml of PBS, was infused into the tail vein with ^{125}I-A7 by means of an infusion pump over 10 min. In the AT-II and enalapril group, enalapril was given orally at a dose of 10 mg/kg 4 h before AT-II and MAb A7 infusion. The procedure of the tissue distribution study, including the dose of ^{125}I-A7, was the same as described above. Figure 3 shows the tissue distribution of ^{125}I-A7, 3 days after iv injection of MAb into athymic mice bearing SW1116, for untreated mice as well as mice treated with AT-II alone, or the combination of AT-II plus enalapril. The accumulation of ^{125}I-A7 in the tumor tissue was about 1.5-fold greater in AT-II-treated mice (20.21 ± 5.19 %dose/g) than in mice receiving only the MAb (13.91 ± 3.61 %dose/g). This increase is probably due to the increased blood flow in the tumor vessels. Furthermore, an approximately 2-fold-greater accumulation of MAb (30.64 ± 4.82 %dose/g) was seen in mice treated with the AT-II and enalapril combination when compared with mice which only received MAb. This enhanced tumor localization of MAb might be ascribed to enalapril-induced enhancement of vascular permeability and the concomitant maintenance of blood pressure by AT-II.

Suzuki and coworkers found that blood flow increased several-fold in a tumor following AT-II-induced hypertension, whereas blood flow did not increase in any normal tissues.[9] Moreover, Abe *et al.* recently reported that the delivery of intravenously injected fluorescein isothiocyanate-labeled neocarzinostatin to an AH109A (rat ascites hepatoma) tumor implanted into Donryu rats was enhanced about 2-fold under AT-II-induced hypertension.[10] In the present study, as shown in Fig. 2, AT-II itself enhanced the tumor uptake of MAb A7. According to Baba and Taniguchi,[11] using the method employed in this study, the blood pressure of tumor-bearing mice was elevated from 100 to 150 mmHg immediately upon infusion of AT-II and it was maintained near this level with the continuous infusion of AT-II. The enhanced tumor uptake of MAb A7 by treatment with AT-II was supposed to result from the selective increase in tumor blood flow under AT-II-induced hypertension. Furthermore, our results clearly show a greater accumulation of MAb in the tumors of mice treated with both AT-II and enalapril when compared with those treated with AT-II alone. This additional accumulation presumably resulted from a combination of enhanced permeability promoted by the actions of enalapril and increased tumor blood flow induced by AT-II. Thus, it was clarified that tumor localization of MAb A7 could be enhanced by combining kininase II inhibitor and AT-II treatment.

Recently, several approaches have been pursued to increase the magnitude and the rate of MAb delivery to tumors. Success in increasing tumor uptake of MAb has been reported with irradiation,[12] local hyperthermia,[13, 14] vasoactive drugs,[15] and biological response modifiers such as tumor necrosis factor[16] and interleukin 2.[17] Our approach of using kininase II inhibitor and AT-II is unique in that it utilizes the kinin-generating cascade resident in solid tumors to enhance vascular permeability. Although more detailed investigations on the vascular properties and permeability-enhancing factors of solid tumors are needed, the approach described here might provide a useful way to improve the efficiency of MAb for radioimmunodetection and immunotargeting chemotherapy.

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