PREPARATION OF [ARG-GLY-ASP]-[AMINO-POLY(ETHYLENE GLYCOL)] HYBRIDS AND THEIR INHIBITORY EFFECT ON EXPERIMENTAL METASTASIS[1)]

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Hybrids of a fibronectin-related tripeptide (Arg-Gly-Asp) and amino-poly(ethylene glycol) were prepared and their inhibitory effect on experimental metastasis in mice was examined. The hybrids exhibited a potent inhibitory effect on the metastasis of B16 melanoma BL6.

KEYWORDS RGD; poly(ethylene glycol); peptide-poly(ethylene glycol) hybrid; drug-carrier; antitumor; fibronectin

The tripeptide Arg-Gly-Asp(RGD) was first found in the cell attachment domain of fibronectin[2)] and then in other adhesive proteins, such as vitronectin[3)] and collagens.[4)] RGD in fibronectin plays as crucial a role in cell attachment as Tyr-Ile-Gly-Ser-Arg (YIGSR) does in laminin.[5)] RGD- or YIGSR-containing peptides [Gly-Arg-Gly-Asp-Ser(GRGDS),[6)] YIGSR, Cys-Asp-Pro-Gly-Tyr-Ile-Gly-Ser-Arg (CDPG-YIGSR),[5)] poly(RGD) and poly(YIGSR),[7)] etc.] have been reported to be inhibitors in experimental metastasis.

Since poly(ethylene glycol) is stable, low toxic, bioinert and low immunogenic, it will be a promising polymer for a drug-carrier. Recently, we found that the hybrid of YIGSRG and amino-poly(ethylene glycol) (APEG) was a potent inhibitor in experimental metastasis of B16 melanoma BL6.[8)] This paper describes the preparation of RGD-APEG hybrids and their inhibitory effect on experimental metastasis in mice.

Two different types of PEG, #4000(4K;MW,3000-3700) and #6000(6K;MW,7300-9000), were converted into APEG according to the procedure reported by Pillai and Mutter.[9)] APEGs 4K and 6K were purified by carboxymethyl cellurose column chromatography using ammonium acetate buffer as an eluent. The amino contents of APEGs(4K and 6K) were 0.51 meq/g and 0.27 meq/g respectively.

The protected RGD tripeptide, Boc-Arg(Tos)-Gly-Asp(OBzl)-OH, was prepared by the solution method as shown in Fig.1. $[\alpha]_D^{27}$+8.2°(c=1.0,dioxane). Amino acid ratios in an acid hydrolysate(6N HCl, 24h):Arg 0.99; Gly 1.00; Asp 0.97(average recovery 86%). The tripeptide was coupled with APEG by dicyclohexylcarbodiimide(DCC)/1-hydroxybenzotriazole(HOBt) method[10)] and the product was treated with hydrogen fluoride(HF) to remove the protecting groups. The resulting RGD-APEG hybrid was purified by sephadex G-25 column chromatography followed by high performance liquid chromatography (HPLC, YMC ODS-AQ column was used). Amino acid ratios in an acid hydrolysate (6N HCl,48h) of RGD-APEG(4K) and RGD-APEG(6K) were [Arg 0.91; Gly 1.00; Asp 0.94] and [Arg 0.87; Gly 1.00; Asp 0.84] respectively. RGD contents of the hybrid 4K and 6K were 278 μmol/g and 115 μmol/g respectively.

The inhibitory effect of the synthetic hybrids on experimental metastasis of B16 melanoma BL6 were examined in mice according to the method reported in the preceding paper.[8)] In advance, PEG and APEG were independently examined for inhibitory effect on experimental metastasis and were found to have no effect. As shown in Fig.2, the hybrid 4K and 6K inhibit metastasis. The inhibitory effect of the hybrid 6K was nearly equal to that of RGD, but not distinctive. Therefore, the effect of the hybrid 6K was further examined in its diluted concentrations. As shown in Fig.3, diluted hybrid 6K inhibited metastasis dose-

dependently. The inhibitory effect of 0.1 mg of the hybrid 6K nearly equaled that of 1 mg of RGD. Thus it can be said that the inhibitory effect of the hybrid 6K is 10 times and 230 times as potent as that of RGD in terms of weight and molar ratios respectively.

The effect of the mixture of RGD (25 μg) and APEG 6K (300 μg) was examined in the same procedure, and it was less active than that of 300 μg of RGD-APEG 6k hybrid in metastasis inhibition. 300 μg of RGD-APEG 6K hybrid contain 13 μg of RGD. This indicates that the presence of the covalent bond between the tripeptide and APEG is necessary for potentiation of the inhibitory effect. Thus the high inhibitory effect of the hybrid can be roughly explained by the hypothesis that the bulky APEG moiety might prevent enzymatic hydrolysis of RGD and stabilize the binding between RGD and its receptor. Recently Koyama reported that subcutaneously injected PEG in mice was distributed in tumor cells in high concentrations.[11] This fact also suggests a high inhibitory effect of the hybrids.

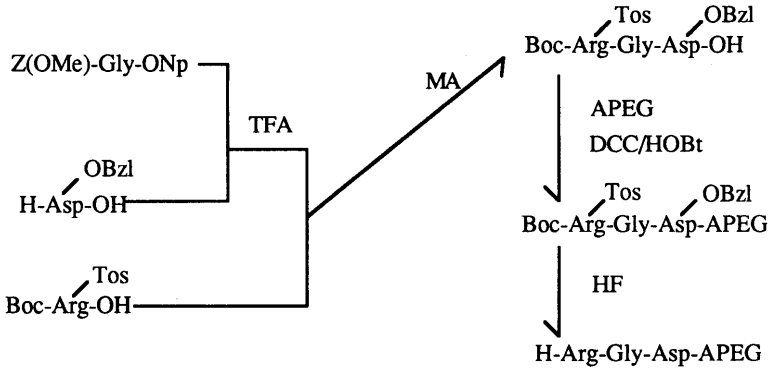

Fig. 1. Synthetic Scheme for RGD-APEG Hybrid
MA:Mixed anhydride method.
DCC:Dicyclohexylcarbodiimide.
HOBt:1-Hydroxybenzotriazole.

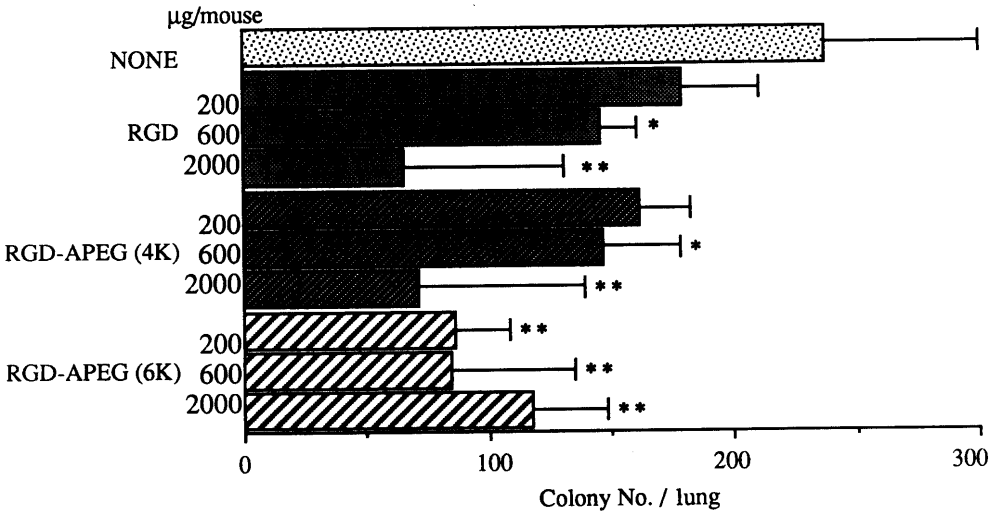

Fig. 2. Inhibitory Effect of RGD-APEG Conjugates on the Formation of Lung Metastasis
B16-BL6 cells ($1x10^5$/0.2ml) were injected i.v. with or without admixing with various concentrations of APEG-RGD into five mice per group. Lung tumor colonies were examined 21 days later. Values were the mean ± SD.
*;$p<0.05$, **;$p<0.01$ compared with untreated control by Student's t-test.

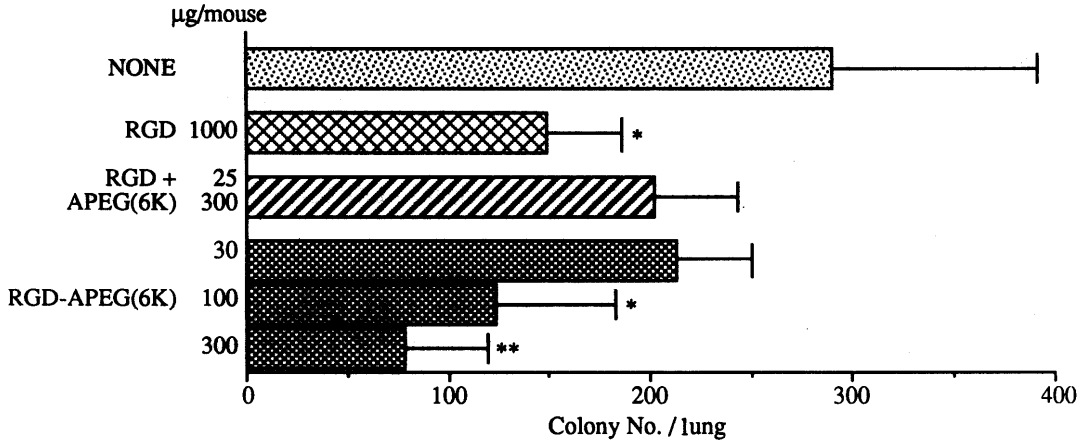

Fig. 3. Inhibition of Lung Colonization by RGD-APEG
The tumor colonization assay was carried out as described in Fig. 2 . Values were the mean ± SD.
*;$p<0.05$, **;$p<0.005$ compared with untreated control by Student's t-test.

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