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AMINO ACIDS AND PEPTIDES.XIV. LAMININ RELATED PEPTIDES AND THEIR INHIBITORY EFFECT ON EXPERIMENTAL METASTASIS FORMATION

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SUMMARY : Inhibitory effects of synthetic laminin related peptides on experimental metastasis formation in mice were examined. Of the synthetic peptides, YIGSRG-[amino-poly(ethylene glycol)] hybrid exhibited the most potent inhibitory effect on the metastasis of B16 melanoma BL6.

Laminin, a basement membrane glycoprotein, consists of three peptide chains: A, B1, and B2. It has interesting biological activities including those to promote the attachment, growth and differentiation of epithelical cells (1). Iwamoto et al. reported that H-Cys-Asp-Pro-Gly-Tyr-Ile-Gly-Ser-Arg-NH_2(CDPGYIGSR) and H-Tyr-Ile-Gly-Ser-Arg-NH_2(YIGSR), corresponding to partial sequences of the B1 chain, inhibited experimental metastasis (2). Later, two YIGSR analogs which have more potent inhibitory effect on experimental metastasis were reported: cyclic YIGSR(3) and poly(YIGSR)(4). These two peptides have specifically cyclic and polymeric forms. These findings suggest a feasible development of a new type of metastasis-inhibiting agents. Various YIGSR analogs were synthesized and examined for their inhibitory effect on experimental metastasis.

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The abbreviations used are : APEG, amino-poly(ethylene glycol); MEM, minimal essential medium.

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MATERIALS AND METHODS

Peptides : YIGSR analogs were synthesized by the solid-phase method. The side chains of amino acids were protected as follows : Cys,p-methylbenzyl group ; Arg, mesitylenesulfonyl or nitro group ; Asp, cyclohexyl group ; Tyr, benzyl group. Alpha amino groups were protected with the t-butoxycarbonyl or p-methoxybenzyloxycarbonyl group which was removed by trifluoroacetic acid. Methylbenzhydrylamine resin(0.64 meq/g) was used for a solid support and the final deprotection was done by the HF procedure. The crude products were purified by high performance liquid chromatography(HPLC). The following peptides were synthesized, all having carboxamide at the C-terminal: YIGSE (I), YIGSR(II, R is dextro), YCGSR(III), $(YCGSR)_2$(IV, dimer through a disulfide bond), $(YIGSR)_2K$(V), YCGSRC(VI, cyclic compound through a disulfide bond), YIGSRG(VII), $(YIGSRG)_2$APEG(VIII). Amino-poly(ethylene glycol) (APEG) was prepared from poly(ethylene glycol)(molecular weight, 3000 - 3700) according to the procedure reported by Pillai and Mutter.(5) VIII was prepared from APEG and Fmoc-YIGSR-OH (Fmoc=fluorenylmethyloxycarbonyl) by the diphenylphosphoryl azide method (6) followed by piperidine treatment. YIGSR and CDPGYIGSR were also prepared by the solid-phase method for standard samples on assay. Details for the synthesis will be reported elsewhere. All peptides were assayed as their hydrochlorides.

Cells and Culture : Highly metastatic B16-BL6 cells, obtained by in vitro selection for invasion(7), were kindly provided by Dr.M.Sano, school of pharmacetical sciences, University of Shizuoka, Japan. They were maintained in an Eagle's minimal essential medium(MEM) supplemented with 10% fetal calf serum(FCS) and L-glutamine.

Experimental Metastasis : Cells were washed once in Ca^{2+} and Mg^{2+}-free phosphate bufferd saline, detached with 1mM EDTA, and sedimented by low-speed centrifugation. The pellet was resuspended gently to $1x10^6$ cells/ml in serum-free MEM and neutralized. The peptides were then mixed with cells in serum-free MEM and single-cell suspensions of $1x10^5$ cells were injected into the lateral tail vein of syngeneic C57BL/6 male mice at 6 weeks of age. Three weeks later the animals were killed , and then lungs were excised and fixed in 10% formaldehyde. The number of surface melanoma colonies were counted macroscopically.

RESULTS AND DISCUSSION

As shown in Fig. 1, I and II did not inhibit experimental metastasis, thus L-Arg in YIGSR might be essential for the activity. This is inconsistent with the finding by Pierschbacher and Ruoslahti(8), who reported that replacement of L-Arg in Gly-Arg-Gly-Asp-Ser-Pro(GRGDSP, fibronectin related peptide) with D-Arg showed no difference in the inhibiting the attachment of rat kidney cells to a fibronectin substrate. The inhibitory effect of III was comparable to that of YIGSR, so Ile can be replaced with Cys or was not essential. III is the first substituted

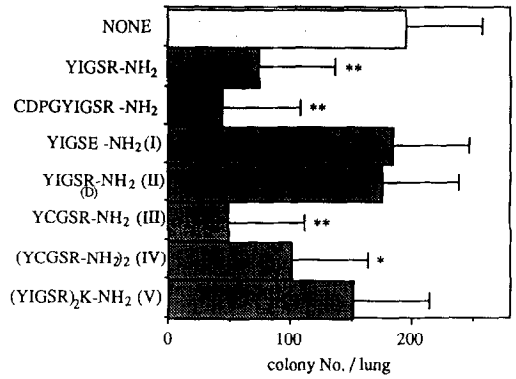

FIG. 1. Effect of various peptides on lung tumor colonizion. B16-BL6 cells($1x10^5$/0.2ml) were injected i.v. with or without admixing with 1mg of peptides into five mice per group. Lung tumor colonies were examined 21 days later. values were the mean ± SD. *;$p<0.05$, **;$p<0.01$ compared with untreated control(MEM) by student's t-test.

analog of which the effect is nearly equal to that of YIGSR. But IV, the dimer of III through a disulfide bond, was less effective than III. V, Dimer through a Lys moiety, was also less active than YIGSR.

Next, cyclic and polymer-bound YIGSR analogs were prepared. YCGSRC was synthesized and oxidized to form a cyclic peptide through an intramolecular disulfide bond. The cyclic peptide, VI, was less active than expected. Poly(ethylene glycol)(PEG) is a polymer which has an outstanding solubility in aqueous and organic solvents. The hybrid of peptide and PEG is a very interesting compound which may has the possibity of increasing the solubility

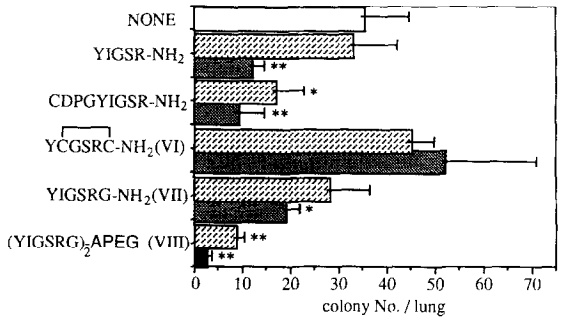

FIG. 2. Inhibitory effect of synthetic peptides on the formation of lung metastasis. B16-BL6 cells($1x10^5$/0.2ml) were injected i.v. with or without admixing with 0.3mg(▨), 1.0mg(▩), 2.0mg(■) of peptides into five mice per group. Lung tumor colonies were examined 21 days later. Values were the mean ± SD. *;$p<0.05$, **;$p<0.01$ compared with untreated control(MEM) by student's t-test.

and activity. For preparation of PEG hybrid, amino-PEG (APEG) was prepared according to the known procedure (5) and coupled with Fmoc-YIGSRG-OH. The resulting protected peptide-APEG hybrid was treated with piperadine to give YIGSRG-APEG (VIII). C-Terminal Gly of YIGSRG was introduced as a spacer. PEG and APEG did not show any inhibitory effect on experimental metastasis and all synthetic peptides did not show any toxic effect to B16-BL6 cells and animals(data is not shown).

As shown in Fig. 2, the inhibitory effect of YIGSRG (VII) was lower than that of YIGSR, but YIGSR-APEG hybrid (VIII) was a potent inhibitor for experimental metastasis, presumably because enzymatic degradation of YIGSRG was prevented by poly(ethylene glycol) (YIGSRG was easily hydrolyzed but the hybrid was hydrolyzed very slow by aminopeptidase M. Data is not shown) and because bulky PEG moiety stabilized the binding between YIGSRG and a receptor.

In this study, APEG with a molecular weight of 3000 - 3700 was used. The effect of the molecular weight of APEG in APEG-peptide hybrid is being studied.

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