POWERFUL ANTIANGIOGENIC ACTIVITY OF HERBIMYCIN A (NAMED ANGIOSTATIC ANTIBIOTIC)

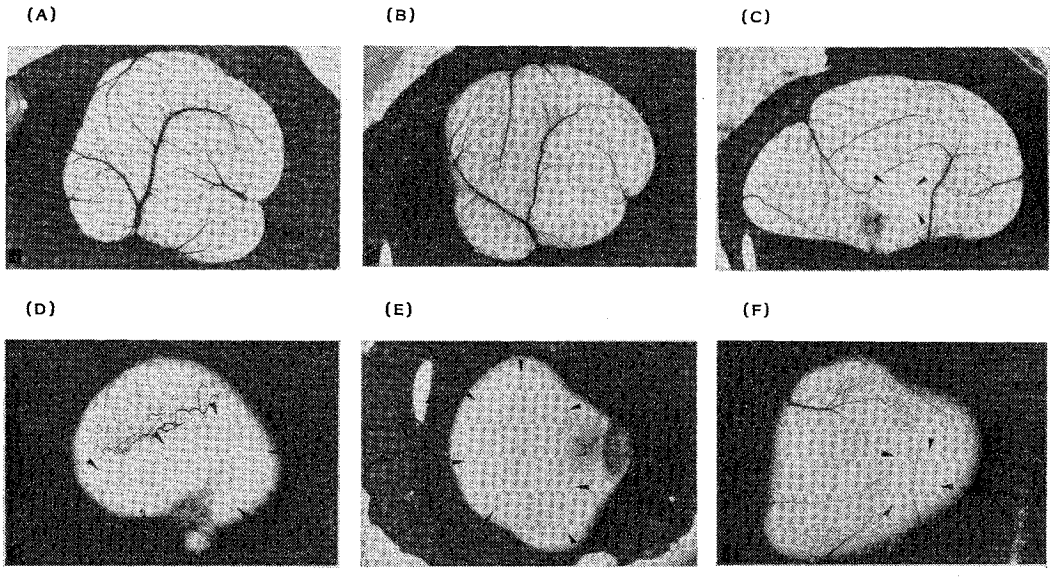
Sir:

Angiogenesis is the process of generating new capillary blood vessels and leads, therefore, to neovascularization[1]. This biological response is observed in a variety of physiological and pathological circumstances, including during embryonic development and progressive growth of solid tumors. Several growth factors and chemical substances have been found to induce angiogenic activity[1,2]. Acidic fibroblast growth factor, one of the putative angiogenic factors, has been reported to stimulate the phosphorylation of plasma membrane protein with MW of 135 kDa, related to the growth factor receptor, on tyrosine residues[3]. Also, protein kinase C activators have been shown to suppress the stimulation of capillary endothelial cell growth by angiogenic endothelial mitogens[4]. This information appears to support the possibility that certain protein kinase is involved in angiogenesis. UEHARA *et al.* have reported that herbimycin A has the ability to decrease the phosphotyrosine content of the total cellular proteins and the phosphorylation level of 36 kDa protein, considered to be the common target substrates for various tyrosine kinase oncogene products[5]. In the present study, we have examined the effect of herbimycin A on angiogenesis in chick embryo chorioallantoic membrane (CAM), the inhibitory activity of the antibiotic being compared with that of medroxyprogesterone acetate (MPA) which significantly inhibited tumor angiogenesis in rabbit corneas as described previously[6], and have found the antibiotic to have powerful antiangiogenic activity. Thus, we wish to name herbimycin A angiostatic antibiotic since angiogenesis-inhibiting steroids are named angiostatic steroids[7]. This is the first paper to report on the antiangiogenic activity of herbimycin A, one of the antibiotics, in CAMs.

Antiangiogenic activity was assayed by the method of TANAKA *et al.*[8] with a slight modification. Samples incorporated in ethylene-vinyl acetate copolymer (EV 40; Mitsui-DuPont Co., Tokyo, Japan) were prepared as described previously[6] and the EV pellets were placed on the 4.5-day CAMs. After the eggs were incubated in a humidified incubator at 37°C for 2 days, appropriate volume of 10% fat emulsion was injected into the 6.5-day chorioallantois. The

Fig. 1. Effect of herbimycin A on embryonic angiogenesis.

EV pellets containing herbimycin A ((A) 0 μg, (B) 0.01 μg, (C) 0.1 μg, (D) 1 μg, (E) 10 μg) and MPA ((F) 100 μg) were implanted on the CAMs of 4.5-day-old eggs.



At 2 days post-implantation, the antiangiogenic activity was determined; original magnification, ×2.5.

Table 1. Inhibitory effect of herbimycin A on angiogenesis in CAM.

Reagents	Dose (μg/egg)	Number of CAM assayed	Number(%) of CAM showing avascular zone	P value[a]
Herbimycin A	0	24	0 (0)	—
	0.01	10	0 (0)	NS
	0.1	10	3 (30)	<0.05
	1	10	10 (100)	<0.001
	10	12	12 (100)	<0.001
MPA	100	10	5 (50)	<0.002

[a] Data as to the incidence of antiangiogenic activity were analyzed by FISHER's exact probability test.
NS: Not significant. The antiangiogenic activity was evaluated on the 2nd day after implantation of the indicated dose of reagent on the 4.5-day CAM.

antiangiogenic response was evaluated by measuring an avascular zone in the CAM beneath the pellet, followed by taking photographs of the CAMs. The response was scored to be effective when the CAM tested had an avascular zone of 3 mm or more diameter according to the method of CRUM *et al.*[7] with a minor modification.

The effects of herbimycin A (0.01 ~ 10 μg per egg) and MPA (100 μg per egg) on embryonic angiogenesis are shown in Fig. 1. Herbimycin A displayed significant antiangiogenic activity in this assay system with as little as 100 ng per egg (Fig. 1C), as compared with the empty pellet without the antibiotic (Fig. 1A). The antibiotic (1 μg per egg) produced the avascular zone in the 100% CAM (Fig. 1D). This dose of the antibiotic apparently caused no damage to the growth of embryos. The highest dose of the antibiotic (10 μg per egg) completely inhibited embryonic angiogenesis, where the treated CAMs showed little or no large and small blood vessels and some toxic effect was observed in half of embryos used (Fig. 1E). On the other hand, the treatment of MPA (100 μg per egg) elicited the avascular zone in the 50% CAM (Fig. 1F). The results are summarized in Table 1. Herbimycin A showed dose-dependent inhibitory activity toward embryonic angiogenesis. Similarly, UEHARA *et al.* demonstrated that herbimycin A inhibited the intracellular $p60^{src}$ kinase activity in temperature-sensitive Rous sarcoma virus-infected rat cells in a concentration-dependent manner[9]. The antibiotic also suppressed angiogenesis induced by crude extract of human cancer in rabbit corneas (T. YAMASHITA *et al.*; personal communication). In addition, the antiangiogenic activity of herbimycin A toward embryonic angiogenesis amounted to more than 200 times that of MPA. Considering the findings by UEHARA *et al.*[5,9] mentioned above, it might be possible that herbimycin A might induce antiangiogenic activity by selectively reducing the activity of certain tyrosine kinase, related to tyrosine kinase oncogene product. In this regard, further investigations are necessary to elucidate the underlying mechanism by which herbimycin A elicits antiangiogenic action.

Microorganisms have been reported to produce various bioactive substances and some of them have been developed as therapeutic drugs for human disease including cancer. Research reagents such as enzyme inhibitors leupeptin, antipain and others have also been discovered. Taking into account these findings and the present findings obtained with herbimycin A, it is highly likely that microorganisms produce a variety of known and unknown substances showing antiangiogenic activity. In this regard, the study for searching novel angiostatic antibiotic is in progress.

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