A NEW ACTIVITY OF HERBIMYCIN A: INHIBITION OF ANGIOGENESIS

Sir:

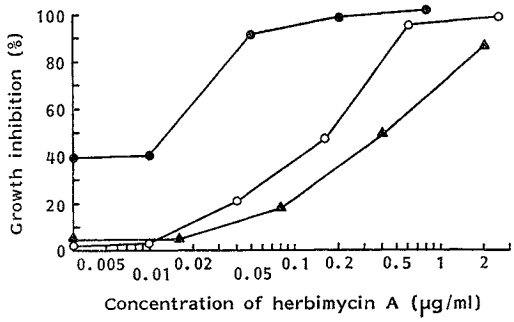
Herbimycin A, a herbicidal antibiotic isolated from the culture broth of *Streptomyces hygroscopicus* AM-3672[1], shows anti-tobacco mosaic virus and antitumor activities[2]. More recently, MURAKAMI *et al.* reported that herbimycin A specifically acts on cells expressing the *src* oncogene and reverses various transformed characteristics to normal[3]. During studies on the inhibition of tumor angiogenesis, we found that herbimycin A also has anti-angiogenesis activity. In this communication, we report the effects of herbimycin A on the proliferation of cultured capillary endothelial cells, the angiogenesis in chick chorioallantoic membrane (CAM), and angiogenesis induced by crude tumor angiogenesis factor (TAF) in rabbit cornea.

The effects of herbimycin A on the proliferation of cultured capillary endothelial cells, fibroblasts, and HeLa S_3 cells were examined. Capillary endothelial cells, and fibroblasts were isolated and cloned from the bovine adrenal cortex and rabbit cornea, respectively. Capillary endothelial cells at a density of 4×10^4 cells per well, fibroblasts and HeLa S_3 cells at a density of 1×10^4 cells per well were seeded into 12-well dishes. These cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum at 37°C under 5% CO_2 in air. After 24 hours, the medium was exchanged with fresh medium containing different amounts of herbimycin A for a further 72-hour incubation. Cell numbers were counted with a Coulter Counter model ZB1. The inhibitory effect of herbimycin A on the proliferation of the three cultured cell lines is shown in Fig. 1. The concentrations of herbimycin A required to inhibit cell growth by 50% for capillary endothelial cells, fibroblasts, and HeLa S_3 cells were 0.013, 0.20, and 0.43 μg/ml, respectively. The activity of herbimycin A was most prominent in capillary endothelial cells.

The inhibition of angiogenesis in chick CAM by herbimycin A was investigated using the method of SCHER *et al.*[4]. A 1-cm^2 window was made in the shells of 7-day-old chick embryos. Next day, five different doses of herbimycin A were soaked into sterile, 6 mm disks of glass fiber filter and implanted onto the CAMs. The angiogenic response was scored qualitatively according to the density of new capillaries developed around the disk 3 days after the implantation. In each group there were 10 embryos. Capillaries grew rapidly in control embryos implanted with solvent disks (Table 1). Herbimycin A at 0.1 μg/disk or more locally inhibited capillary growth around the disk and produced an avascular zone in the CAM in a dose dependent manner.

The inhibitory effect of herbimycin A on angiogenesis induced by crude TAF was examined in the rabbit cornea assay according to the method of GIMBRONE *et al.*[5]. Cell-free crude TAF extracted from the human choriocarcinoma cell line, BeWo, was prepared according to the method of KLAGSBRUN *et al.*[6]. Sterile polymer pellets, 1 mm diameter were prepared by adding 200 μg of crude TAF or different doses of herbimycin

Fig. 1. Inhibitory effects of herbimycin A on the proliferation of capillary endothelial cells (●), fibroblasts (○), and HeLa S_3 cells (▲).



Percent cell number relative to that of saline treated control is indicated. Each value represents the mean of three independent experiments.

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

Dose (μg/disk)	No. of eggs assayed	No. of eggs with following capillary density[a]		
		Normal	Lower	Avascular
0.00	10	10	0	0
0.01	8	8	0	0
0.10	10	3	5	2
1.00	10	1	5	4
10.00	10	0	2	8

[a] Density of capillaries developed around the disk.

Table 2. Inhibitory effect of herbimycin A on capillary growth induced by crude TAF rabbit cornea.

Dose (μg/pellet)	Days after implantation		
	5	7	9
0.000	1.67	1.83	2.67
0.001	1.50	2.08	2.33
0.010	0.50	0.82	1.42
0.100	0.50	0.50	1.08
1.000	0.25	0.75	1.38
10.000	0.00	0.00	0.50
100.000	0.00	0.00	0.75

Values are expressed in mm, represent mean of lengths of each longest capillary induced in 3 eyes.

A to a copolymer mix composed of vinyl acetate and ethylene. A pellet containing herbimycin A was implanted into a cornea pocket in the rabbit which ended 1.5 mm from the limbal vascular plexus. A pellet with crude TAF was simultaneously implanted distal to the herbimycin A pellet, 2.5 mm from the limbus. Three eyes were examined in each group. Corneas were examined and photographed 5, 7, and 9 days after pellet implantation, and the length of the longest capillary extending towards the pellet with crude TAF was measured from the projected picture. Five days after implantation, corneas were penetrated by new capillary sprouts which grew rapidly from the adjacent limbal blood vessels in the untreated eyes (Table 2). In the presence of herbimycin A at a dose of 0.01 μg/pellet or more, capillary growth was suppressed. When herbimycin A was increased to 10 μg/pellet or more, angiogenesis was completely prevented until 7 days after the implantation.

The progressive growth of a tumor is dependent upon the continuous induction of angiogenesis induced by TAF from a tumor[7]. FOLKMAN *et al.* has proposed that the prevention of angiogenesis into a tumor may provide a therapeutic approach[7]. Angiogenesis inhibitors such as protamine[8], combinations of heparin and cortisone[9], tumor necrosis factor α[10], transforming growth factor β[11], and cartilage extract[12] have been shown to inhibit the growth of cultured vessel endothelial cells, and to prevent angiogenesis in CAM or in the rabbit cornea. All of these inhibitors are endogenous polypeptide or steroid. Herbimycin A is the first nonendogenous angiogenesis inhibitor. The cytotoxic activity of herbimycin A against various tumor cells is well known[2]. The results presented here demonstrate that herbimycin A can inhibit the new capillary sprouts elicited by TAF and it is possible that this effect plays a role in its antitumor activity. We have observed hemorrhagic necrosis of tumor tissue in herbimycin A treated mice (data not shown).

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