

INHIBITION OF ANGIOGENESIS BY 15-DEOXYSPERGUALIN

Sir:

Angiogenesis, the formation of new blood vessels, plays an important role in several physiological and pathological conditions, including embryonic development, tumor development, diabetic retinopathy and psoriasis. Thus, treatment with angiogenesis inhibitors might be a novel strategy for managing diseases accompanied by uncontrolled angiogenesis. Previous studies have shown that medroxyprogesterone acetate inhibits the angiogenic response triggered by a rat mammary tumor induced by 7,12-dimethylbenz[*a*]anthracene, probably resulting in the growth inhibition of this tumor¹. We have also found that an antibiotic, herbimycin A, exhibits angiogenesis-inhibitory activity, as assessed with the chick embryo chorioallantoic membrane (CAM) assay system, and have proposed that microorganisms have the ability to produce various angiogenesis inhibitors². 15-Deoxyspergualin, an analogue of the antibiotic spergualin³, has been reported to exhibit antitumor activity and immunosuppressive effects^{4,5}. In this study, we have examined the effect of 15-deoxyspergualin on angiogenesis in CAM of the chick embryo and have found that the antibiotic is capable of inhibiting embryonic angiogenesis in a dose-dependent manner.

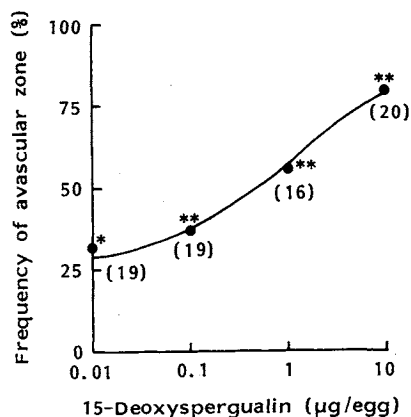
Antiangiogenic activity was evaluated as described previously, except that 1% methylcellulose (20~30 cps; Tokyo Kasei Co., Ltd., Tokyo) was used in place of ethylene-vinyl acetate copolymer^{2,6}. 15-Deoxyspergualin was dissolved in saline at a concentration of 2 mg/ml, sterilized by passing through a 0.2- μ m filter and stored at -20°C until use. Fertilized eggs were incubated in a humidified egg incubator at 37°C for 4.5 days. The stock solution of 15-deoxyspergualin was sequentially diluted with sterile saline immediately before use and resulting dilute solutions were mixed with an equal volume of 2% methylcellulose, previously dissolved in saline and sterilized. Aliquots (10 μ l) of 1% methylcellulose containing various doses of 15-deoxyspergualin were placed within a sterilized silicon ring (5-mm outer diameter, 3-mm inner diameter) put on the 4.5-day CAMs. After incubation at 37°C for additional 2 days, an appropriate volume of a 10% fat emulsion was injected into the 6.5-day chorioallantois. The antiangiogenic effect was assessed by measuring the

avascular zone in the CAM. When the test sample produced an avascular zone of 3-mm or over in diameter, the antiangiogenic response was scored to be effective according to our method^{2,5}. Data as to the incidence of antiangiogenic activity were analyzed by FISHER's exact probability test, with $P < 0.05$ as the level of significance.

The effect of 15-deoxyspergualin on embryonic angiogenesis was examined by placing 1% methylcellulose containing the required doses of the antibiotic on 4.5-day CAM. The dose-response relationship for inhibition of embryonic angiogenesis is shown in Fig. 1. 15-Deoxyspergualin inhibited angiogenesis in a dose-dependent manner. In comparison with the effect of 1% methylcellulose alone in 32 control CAMs examined, the minimum dose required for the induction of significant antiangiogenic activity (*i.e.*, a minimum effective dose) was 10 ng (20 pmol) per egg. The dose required for half-maximal inhibition (ID_{50}) was 480 ng (960 pmol) per egg. Examples of these experiments are shown in Fig. 2. 15-Deoxyspergualin (Fig. 2B) produced significant avascular zones, whereas 1% methylcellulose alone did not affect vascular organization in any of 32 control CAM used (Fig. 2A).

Previous studies have shown that spergualin, an

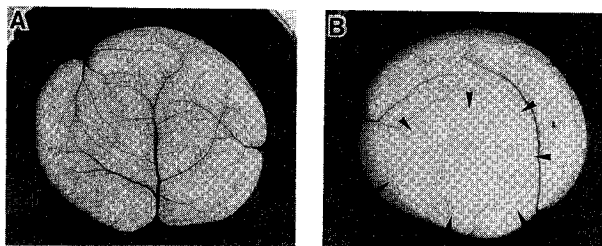
Fig. 1. Inhibitory effect of 15-deoxyspergualin on embryonic angiogenesis.



The 4.5-day CAMs were treated with the indicated doses of 15-deoxyspergualin for 2 days, at which their antiangiogenic effects were assayed by measuring the avascular zone. The number of CAMs studied were shown in parentheses.

* $P < 0.002$, ** $P < 0.001$ compared to control CAMs treated with 1% methylcellulose alone ($n = 32$), which did not cause an avascular zone.

Fig. 2. Effect of 15-deoxyspergualin on angiogenesis in CAMs 2 days after sample implantation.



Samples of 1% methylcellulose alone (A) or containing 15-deoxyspergualin (B, 10 $\mu\text{g}/\text{egg}$) were implanted on the 4.5-day CAMs. An appropriate volume of a fat emulsion was injected into the 6.5-day chorioallantois to show the vascular network better. Note the presence of an avascular zone (surrounded with arrows) in CAM implanted with 1% methylcellulose containing the antibiotic. Control CAMs treated with 1% methylcellulose alone show no disturbance of angiogenesis; original magnification, $\times 2.3$.

antitumor antibiotic isolated from the culture filtrate of *Bacillus laterosporus*, is a unique substance which involves both spermidine and guanidine moieties and that 15-deoxyspergualin is most effective in suppressing tumor growth among various derivatives of spergualin^{3,4}). In this study we have demonstrated that 15-deoxyspergualin dose-dependently caused inhibition of embryonic angiogenesis and that it is active as low as 20 pmol/egg, with an ID_{50} value of 960 pmol/egg. As reported previously²), herbimycin A, an antibiotic produced by a strain of *Streptomyces*, inhibits embryonic angiogenesis, with a minimum effective dose of 170 pmol/egg and an ID_{50} value of 260 pmol. In addition, we have found that retinoids, including retinol (a minimum effective dose of 3,500 pmol/egg; ID_{50} of 4,200 pmol/egg), retinoic acid (a minimum effective dose of 170 pmol/egg; ID_{50} of 330 pmol/egg) and a synthetic retinoid Ch 55 (a minimum effective dose of 2.7 pmol/egg; ID_{50} of 22 pmol/egg), as well as $1\alpha,25$ -dihydroxyvitamin D_3 (a minimum effective dose of 24 pmol/egg; ID_{50} of 340 pmol/egg) and 22 -oxa- $1\alpha,25$ -dihydroxyvitamin D_3 (a minimum effective dose of 2.4 pmol/egg; ID_{50} of 96 pmol/egg) exhibit antiangiogenic activity in the CAM assay system^{6,7}). Taken together, 15-deoxyspergualin seems to have a moderate angiogenesis-inhibitory effect. Additionally, a remarkable feature of 15-deoxyspergualin is that it has the inhibitory effect at a wide range of doses. Similarly, the antibiotic has been found to exhibit antitumor activity at a wide range of doses⁴). Considering these findings, it might be possible that the antitumor activity of 15-deoxyspergualin was, in part, due to the antiangiogenic activity of the antibiotic. Further experiments are in progress to determine whether

15-deoxyspergualin inhibits tumor angiogenesis and whether the antibiotic influences migration or proliferation of blood vessel endothelial cells.

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