COMMUNICATIONS TO THE EDITOR

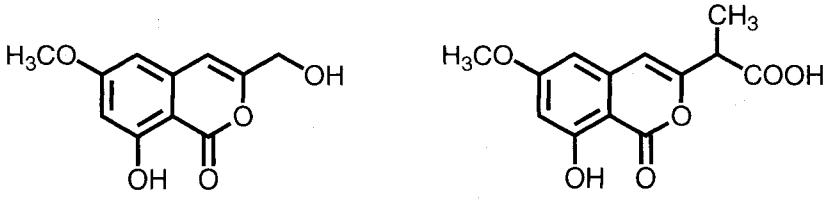
Inhibition of Angiogenesis by a New Isocoumarin, NM-3

Sir:

Angiogenesis, growth and proliferation of new blood vessels, has been reported to be associated with various pathological conditions such as rheumatoid arthritis, psoriasis, diabetic retinopathy and solid tumors[1]. Accordingly, anti-angiogenesis therapy is expected to be a potentially promising approach for the treatment of these diseases. During our studies on the screening program for new antitumor drugs, we found a novel microbial product isolated from a culture filtrate of *Streptoverticillium eurocidicum*, cytogenin (8-hydroxy-3-hydroxymethyl-6-methoxyisocoumarin, Fig. 1), which exhibited *in vivo* antitumor activity, but had only weak cytotoxicity on murine and human tumor cells *in vitro*[2]. Cytogenin was also demonstrated to have efficacy against animal models for human rheumatoid arthritis, including type II collagen-induced arthritis in mice and adjuvant arthritis in rats[3]. As angiogenesis is important in both tumor growth and rheumatoid arthritis, we intended to examine the antiangiogenic effects of cytogenin using mouse dorsal air sac assay system. Consequently, it was revealed that cytogenin is capable of suppressing angiogensis induced by malignant tumor cells (Sarcoma-180, S-180)[4]. We also synthesized cytogenin derivatives and its related compounds, and found that among these compounds NM-3, of which the structure is shown in Fig. 1, has an excellent antiarthritic activity against the animal models, good physico-chemical stabilities and pharmacodynamic properties (unpublished data). In this paper, we describe the antiangiogenic effects of NM-3 in mouse dorsal air sac assay system.

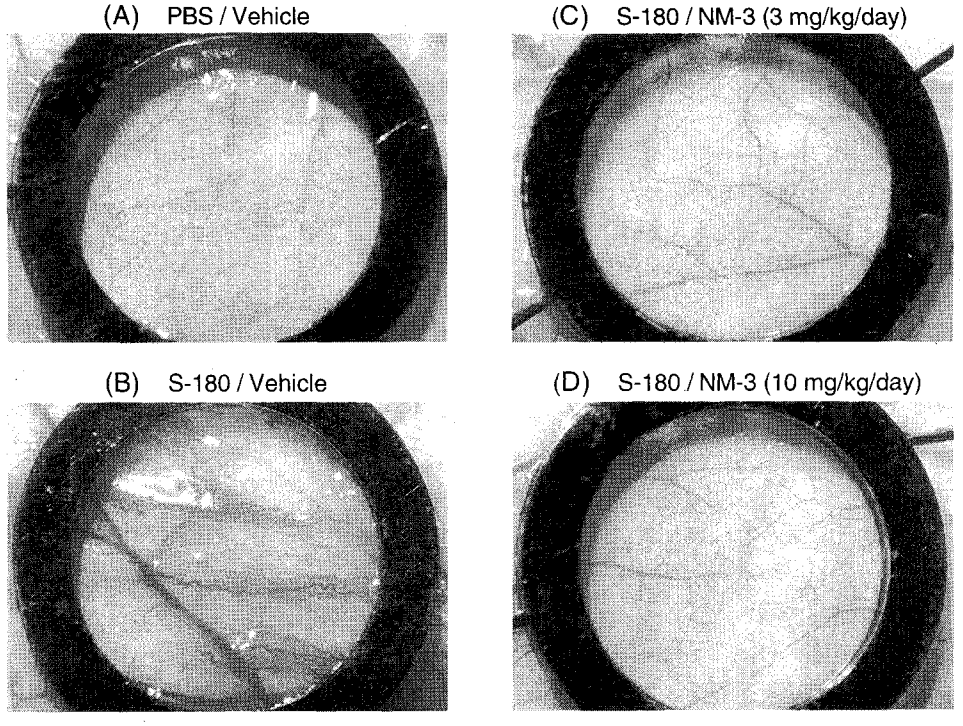
A mouse dorsal air sac assay was performed as described previously[4]. Eight- to ten-week old female ICR mice, purchaced from Charles River Japan (Atsugi, Japan), were used. Both sides of a Millipore ring were covered with Millipore filters of 0.45-mm pore size, and the resulting Millipore chamber was filled with S-180 tumor cells (2×10^7 cells) in 0.15 ml of Mg^{2+} and Ca^{2+} free phosphate-buffered saline (PBS). The S-180 tumor cell-containing chamber was implanted into an air sac formed previously in dorsum of mouse by injection of an appropriate volume of air. NM-3, synthesized at the laboratories of Mercian Corp. (Fujisawa, Japan), was suspended in 0.5% sodium carboxymethylcellulose and administered orally once daily for 5 consecutive days in a volume of 0.1 ml per 10 g body weight from the day of implantation of the chamber containing S-180 tumor cells or PBS. Normal group given the PBS-containing chamber was administered with the vehicle alone. Five days later, the implanted chambers were removed from the subcutaneous fascia of the treated animals, after which a black ring with the same inner diameter as the Millipore ring was placed on the same site. The angiogenic response was assessed under a dissecting microscope by determining the number of newly formed blood vessels of above 3 mm in length within the area encircled by the black ring, and graded into 4 ranks as follows: angiogenesis indices 0, 1, 2 and 3 represented that the number of newly formed blood vessels were 0, 1, 2 and more, respectively. The blood vessels newly formed by

Fig. 1. Structures of cytogenin and NM-3.



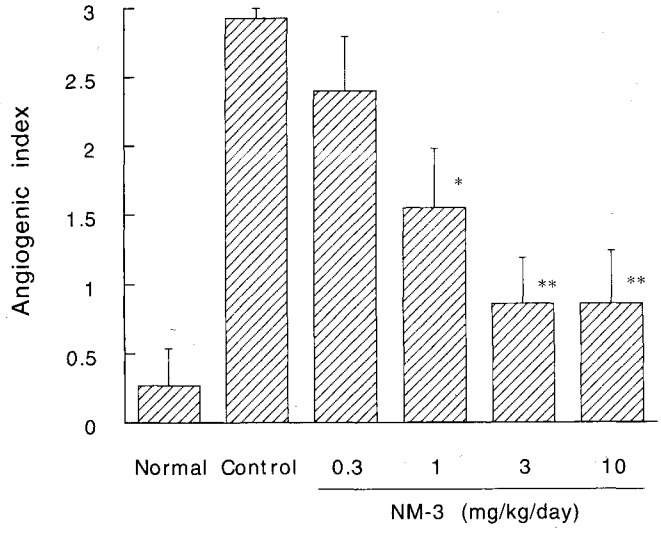
Cytogenin NM-3

Fig. 2. Effects of NM-3 on angiogenesis induced by S-180 tumor cells.



Mice were given chambers containing PBS (Normal, panel A) and S-180 tumor cells (panel B~D), and then administered orally with NM-3 for 5 consecutive days at a dose of 0 (vehicle control, panel B), 3 (panel C) or 10 mg/kg (panel D). Note that in panel B the S-180 tumor cell-containing chamber produced new vasculatures characterized by zigzagging lines. Magnification ($\times 3.8$).

Fig. 3. Inhibitory effect of NM-3 on S-180 tumor cell-induced angiogenesis.



NM-3 or the vehicle (control) was administered orally once daily for 5 consecutive days from the day of implantation of the S-180 tumor cell-containing chamber. Normal mice were given PBS- instead of S-180 tumor cell-containing chamber. Data are the mean $\pm$ S.E.M. of 10 to 14 animals. *,** Significanly different from the control group at $P<0.05$ and $P<0.01$, respectively.

malignant tumor cells were morphologically distinguishable from the preexisting background vessels by their zigzagging character.

Oral administration of NM-3 (0.3~10 mg/kg/day) to mice produced dose-dependent suppression of angiogenesis induced by S-180 tumor cells (Fig. 2 and 3). Angiogenic indices of the groups given NM-3 at doses of 1, 3 and 10 mg/kg/day were significantly reduced to 1.55 ± 0.43, 0.86 ± 0.33 and 0.86 ± 0.38, respectively, when compared with that of control group (2.93 ± 0.07). In addition, no toxicological sign was observed in any mice treated with NM-3 during the experiment period, suggesting that NM-3 is an orally active antiangiogenic agent with low toxicity.

Angiogenesis *in vivo* includes several distinct steps such as proliferation and migration of endothelial cells, vascular cord formation, canalization of the cord, and maturation of the basement membrane surrounding the newly formed vessel walls[1]. In a preliminary experiment, we observed that NM-3, like cytogenin[4], had weak inhibitory activity (about 50% inhibition) against the proliferation of human umbilical vein endothelial cells even at a concentration of 100 μg/ml (unpublished data). Therefore, it is likely that the mode of action of NM-3 is different from those of fumagillin and its analogues, which are also angiogenesis inhibitors of microbial origin and known to selectively inhibit proliferation of endothelial cells *in vitro*[5~7]. Although the precise mechanisms underlying the antiangiogenic action of NM-3 remain to be elucidated, NM-3 may be promising as a novel antiangiogenic agent for the treatment of angiogenesis-associated diseases such as solid tumor and rheumatoid arthritis.

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