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Inhibition of Angiogenesis by Suramin[1]

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ABSTRACT

In this study, we have determined the ability of suramin to inhibit angiogenesis in the chick chorioallantoic membrane. Suramin alone showed significant angiostatic activity in a dose-related manner. Suramin also potentiated the activity of the angiostatic steroids, cortisol-21-phosphate, 17α-hydroxyprogesterone, tetrahydrocortisol, and tetrahydrocortexolone. The presence of heparin decreased the angiostatic activity of suramin. These results suggest that suramin may decrease tumor growth by inhibiting angiogenesis. These novel findings indicate that suramin, perhaps in combination with angiostatic steroids, may be the basis for important new therapeutic approaches for diseases of neovascularization.

INTRODUCTION

Suramin, a polysulfonated naphthylurea compound, was originally developed as a trypanocidal agent (1). This compound has been shown to block the specific cell surface binding of various growth factors, such as PDGF,[3] EGF, TGFβ, IGF-1, basic FGF, and vasculotropin/vascular endothelial cell factor (2, 3). Encouraging results have recently been published indicating that suramin is a useful antitumorigenic agent in human malignancies (1). The mechanisms underlying these antitumorigenic effects of suramin are poorly understood. It has been postulated that the displacement of growth factors from their receptors may affect tumor growth by interfering with a ligand-receptor-mediated mitogen pathway *in vivo*.

The ability of solid tumors to grow to a clinically significant size is dependent on angiogenesis (4). Inhibitors of angiogenesis have become an important potential approach for cancer therapy. Stimulation by basic FGF and vasculotropin/endothelial cell factor is a critical event in angiogenesis (5). Both of these factors are displaced from their receptors by suramin (3).

In this study, we have demonstrated that suramin inhibited angiogenesis in a dose-related manner. In addition, the interaction of suramin with heparin and certain angiostatic steroids is described.

MATERIALS AND METHODS

Suramin FBA (Germany) was obtained from Mobay Chemical Co., New York, NY. Heparin sodium U.S.P. (PM-20487; activity, 171 μ/mg) was a gift from Dr. Judah Folkman, Boston, MA. The steroids were obtained from Sigma Chemical Co., St. Louis, MO, or Steraloids, Inc., Wilton, NH.

The ability of these compounds to inhibit angiogenesis was determined using a modification of the chick CAM assay described by Crum *et al.* (6). The chicken embryos, obtained from Sunrise Farms, Catskill, NY, were cracked and then placed in Petri dishes in a humidified CO_2 incubator (3% CO_2/air) at 37°C on Day 3. Each compound or combination of compounds was dissolved in methylcellulose (0.45%). The solution (10 μl) was air dried on a Teflon-coated metal tray (2-mm diameter) and implanted on the outer third of a 6-day chorioallantoic membrane where capillaries were still growing. The zone around the methylcellulose disk was examined 48 h after implantation. An inhibition of angiogenesis was indicated by an avascular zone of ≥4-mm diameter around the methylcellulose disk. At least 20 embryos were measured for each compound or combination of compounds, and the results were expressed as the percentage of embryos which showed inhibition. The methyl cellulose disk only and cortisol-21-phosphate (100 μg) plus 80 μg of heparin were used as negative and positive controls, respectively.

RESULTS

The effects of increasing amounts of suramin and heparin alone and in combination on the inhibition of angiogenesis are shown in Table 1 and Fig. 1. Suramin alone clearly inhibited angiogenesis when 25 μg were added to the disk (23% inhibition). The inhibition increased in a dose-related manner to 79% inhibition when 200 μg of suramin were added. On the other hand, heparin alone did not inhibit angiogenesis, even at 200 μg. The angiostatic activity by suramin was reduced in the presence of increasing amounts of heparin. This inhibition was overcome by increasing the amount of suramin (Table 1; Fig. 1).

Table 2 and Fig. 2 show the percentage of inhibition of angiogenesis by several steroids alone and in the presence of heparin or suramin. None of the steroids tested showed significant inhibitory activity alone. Medroxyprogesterone acetate did not show angiostatic activity in the presence of 50 or 100 μg of heparin, but it showed 15% and 45% inhibition in the presence of 25 μg and 100 μg of suramin. However, these values were not significantly different from the inhibition by suramin alone (Fig. 1). All of the other steroids tested, cortisol-21-phosphate, 17α-hydroxyprogesterone, tetrahydrocortisol, and tetrahydrocortexolone, showed significant inhibition of angiogenesis in the presence of 100 μg of heparin. Furthermore, each of these steroids showed significant angiostatic activity in the presence of 25, 50, or 100 μg of suramin.

DISCUSSION

Suramin is a polyanionic compound that has been used for several decades as a trypanocidal agent. Recently, it was shown to have antineoplastic activity (1). Its capacity to interfere with the autocrine factors that promote growth in many human tumors suggests great potential in cancer therapy. The results of this study show that suramin can inhibit angiogenesis in the chick chorioallantoic membrane. The inhibition of angiogenesis is dose dependent, increasing from 23% inhibition at 25 μg of suramin to 79% at 200 μg of suramin. The presence of heparin was not required for the angiostatic activity to be expressed.

An important finding of this study is the antagonism between suramin and heparin. Increasing the concentration of heparin clearly inhibited the angiostatic effect of suramin (see Table 1).

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[3] The abbreviations used are: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; TGFβ, transforming growth factor β; IGF-1, insulin-like growth factor 1; basic FGF, basic fibroblast growth factor; CAM, chorioallantoic membrane.

Table 1 *Percentage of inhibition of angiogenesis for different amounts of heparin and suramin alone and in combination*

Compound	Embryos evaluated (positive/total)	% of inhibition
Disk only	0/23	0
25 μg suramin	5/22	23
50 μg suramin	11/24	46
100 μg suramin	15/26	58
200 μg suramin	19/24	79
50 μg heparin	0/22	0
100 μg heparin	0/26	0
200 μg heparin	0/28	0
μg suramin		
25 + 40 μg heparin	0/22	0
50 + 40 μg heparin	3/21	14
100 + 40 μg heparin	8/21	38
200 + 40 μg heparin	18/22	82
400 + 40 μg heparin	23/25	92
μg suramin		
25 + 80 μg heparin	0/24	0
50 + 80 μg heparin	0/22	0
100 + 80 μg heparin	7/23	30
200 + 80 μg heparin	14/20	70
400 + 80 μg heparin	20/24	83

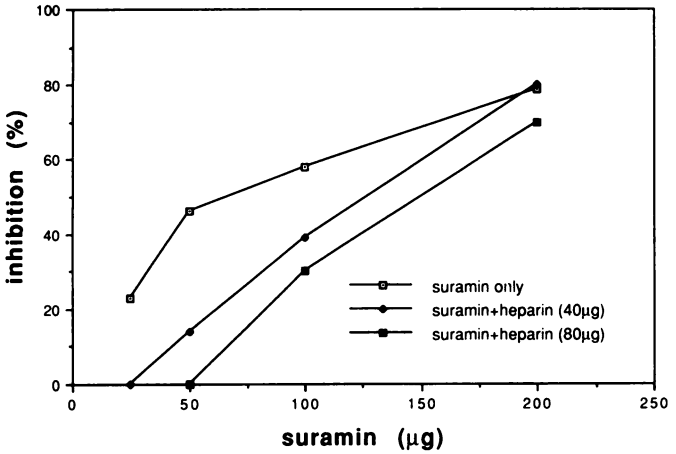

Fig. 1. Percentage of inhibition of increasing amounts of suramin alone and in combination with increasing amounts of heparin.

These results support the hypothesis that suramin may interfere with the effects of growth factors. Suramin has been shown to interfere with the interaction of growth factors, PDGF, basic FGF, and vasculotropin, with their receptors (2, 3).

Folkman *et al.* (7) showed that heparin and fragments of heparin that lack anticoagulant activity inhibited angiogenesis in the presence of cortisone and cortisol. Other members of the pregnane steroids which have no known physiological function, such as 17α-hydroxyprogesterone and tetrahydrocortisol, also showed angiostatic activity in the presence of heparin and heparin fragments (6, 8). Our results indicate that suramin may effectively replace heparin as an activator of angiostatic steroids (see Table 2). These steroids alone had no significant angiostatic activity in the CAM assay. However, when suramin was added with the angiostatic steroids (17α-hydroxyprogesterone, cortisol-21-phosphate, tetrahydrocortisol, and tetrahydrocortexolone), the angiostatic activity was enhanced to levels higher than would be expected in the presence of heparin. Medroxyprogesterone acetate did not show significant angiostatic activity alone or in the presence of either heparin or suramin.

While these studies were being carried out, Wilks *et al.* (9) reported that suramin showed angiostatic activity in the presence of angiostatic steroids (17α-hydroxyprogesterone, cortisone acetate, cortisol) using a modified CAM assay to induce a strong angiogenic response. Their results also suggested that medroxyprogesterone acetate was angiostatic in the presence of suramin, but not heparin. We were unable to demonstrate any angiostatic activity by medroxyprogesterone acetate alone or in the presence of heparin or suramin. Furthermore, Wilks *et al.* (9) did not describe the ability of suramin alone to show angiostatic activity, although their data suggested that this was the case. The differences reported in our study and those by Wilks *et al.* (9) may reflect the angiogenesis-induced model used by Wilks *et al.* This model, which used glass fiber filters on the CAM, involves an inflammatory response in addition to the angiogenic response (10). This type of vascular reaction was not considered characteristic of the tumor angiogenesis factor.

The novel finding reported here that suramin inhibited angiogenesis alone and potentiated the activity of angiostatic steroids suggests an interesting new therapeutic approach for diseases of neovascularization. Further studies are necessary to

Table 2 *Percentage of inhibition of angiogenesis for steroids alone and in the presence of heparin or suramin*

Steroid	Embryos (positive/total)	% of Inhibition
100 μg cortisol-21-phosphate only	8/29	26
+ 100 μg heparin	27/41	66
+ 25 μg suramin	16/27	59
+ 100 μg suramin	18/22	86
100 μg 17α-hydroxyprogesterone only	0/54	0
+ 100 μg heparin	12/23	57
+ 25 μg suramin	11/23	48
+ 100 μg suramin	16/23	70
100 μg medroxyprogesterone acetate only	0/59	0
+ 100 μg heparin	0/24	0
+ 25 μg suramin	3/20	15
+ 100 μg suramin	13/25	45
Tetrahydrocortisol only	0/53	0
+ 100 μg heparin	17/38	45
+ 25 μg suramin	8/23	35
+ 100 μg suramin	18/24	75
100 μg tetrahydrocortexolone only	0/39	0
+ 100 μg heparin	26/58	45
+ 25 μg suramin	14/23	61
+ 100 μg suramin	19/23	83

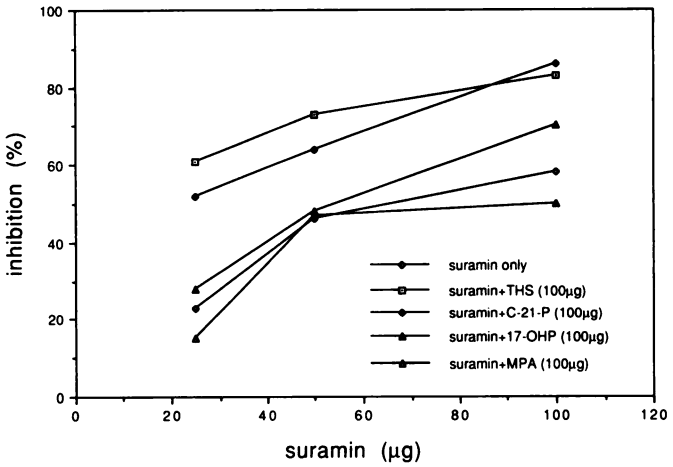

Fig. 2. Percentage of inhibition of increasing amounts of suramin alone and in combination with 100 μg of cortisol-21-phosphate (*C-21-P*), 17α-hydroxyprogesterone (*17-OHP*), medroxyprogesterone acetate (*MPA*), and tetrahydrocortexolone (*THS*).

characterize the interaction between suramin and steroids in inhibiting angiogenesis.

REFERENCES

1. Zaniboni, A. Suramin: the discovery of an old anticancer drug. Med. Oncol. Tumor Pharmacother., *7:* 287–290, 1990.
2. Coffey, R. J., Jr., Leof, E. B., Shipley, G. D., and Moses, H. L. Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2B cells. J. Cell. Physiol., *132:* 143–148, 1987.
3. Bikfalvi, A., Sauzeau, C., Moukadiri, H., Maclouf, J., Busso, N., Bryckaert, M., Plouet, J., and Tobelem, G. J. Cell. Physiol., *149:* 50–59, 1991.
4. Folkman, J., and Klagsbrun, M. Angiogenic factors. Science (Washington DC), *235:* 442–447, 1987.
5. Maione, T. E., and Sharpe, R. J. Development of angiogenesis inhibitors for clinical applications. Trends Pharmacol. Sci., *11:* 457–461, 1990.
6. Crum, R., Szabo, S., and Folkman, J. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science (Washington DC), *230:* 1375–1378, 1985.
7. Folkman, J., Langer, R., Linhardt, R. J., Haudenschild, C., and Taylor, S. Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. Science (Washington DC), *221:* 719–725, 1983.
8. Ingber, D. E., Madri, J. A., and Folkman, J. A possible mechanism for inhibition of angiogenesis by angiostatic steroids: induction of capillary basement membrane dissolution. Endocrinology, *119:* 1768–1775, 1986.
9. Wilks, J. W., Scott, P. S., Vrba, L. K., and Cocuzza, J. M. Inhibition of angiogenesis with combination treatments of angiostatic steroids and suramin. Int. J. Radiat. Biol., *60:* 73–77, 1991.
10. Jakob, W., Jentzsch, K. D., Mauersberger, B., and Heder, G. The chick embryo chorioallantoic membrane as a bioassay for angiogenesis factors: reactions induced by carrier materials. Exp. Pathol. Bd., *15:* S.241–249, 1978.