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Suramin Blockade of Insulinlike Growth Factor I-Stimulated Proliferation of Human Osteosarcoma Cells

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The polyanionic compound suramin is currently being evaluated for antineoplastic activity. On the basis of previous in vitro studies, it has been suggested that the mechanism of action of suramin may be related to its ability to attenuate the mitogenic effects of peptide growth factors, such as platelet-derived growth factor and epidermal growth factor. We recently reported that MG-63 human osteosarcoma cells are mitogenically responsive to insulinlike growth factor I (IGF-I). We now demonstrate for the first time that suramin interferes with the interaction between IGF-I and its receptor and abolishes in vitro IGF-I-stimulated proliferation of these osteosarcoma cells. The fact that cell proliferation resumes when suramin is removed indicates that this is not a cytotoxic effect. We conclude that IGF-I should be added to the list of growth factors whose bioactivity can be attenuated by suramin and that clinical studies of suramin and its analogues are indicated in IGF-I–receptor-positive malignancies such as osteogenic sarcoma. [J Natl Cancer Inst 82:1349–1352, 1990]

Suramin is a polysulfonated naphthylurea that has been used for more than 50 years in the treatment of trypanosomiasis and recently has been found to have antineoplastic activity in humans at concentrations near 300 μg/mL (*1*). Its mechanism of action is poorly understood. Among the plethora of biologic effects of suramin, the compound has been shown to interfere with the action of platelet-derived growth factor (*2,3*) and epidermal growth factor (*4*) in vitro. The mechanism underlying this effect is poorly characterized, but on structural grounds it is highly unlikely that the drug functions as a classic competitive antagonist. It has been proposed that suramin also has in vivo growth factor antagonist properties and that these properties are related to its antineoplastic activity.

Insulinlike growth factor I (IGF-I) is a 76-amino acid growth factor (*5*). Its mitogenic effect follows binding to a specific cell-surface receptor of the tyrosine kinase class (*6*). In a recent report (*7*), we documented that IGF-I is a potent mitogen for osteogenic sarcoma cells in vitro, and we proposed that sarcomas may exhibit dependence on this mitogen for proliferation in vivo. Additional studies (*8–13*) have shown that other neoplasms are IGF-I–receptor positive and may also be IGF-I responsive.

We (*14*) previously proposed that it may be possible to therapeutically exploit the dependence of neoplasms on IGF-I by the use of pharmacologic treatments that lower IGF-I levels. We undertook the present study to determine whether suramin can directly antagonize the mitogenic effect of IGF-I on osteosarcoma cells.

Materials and Methods

Cells, growth factors, and suramin. MG-63 human osteogenic sarcoma cells (*15*) were from the American Type Culture

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Collection (Rockville, Md). Recombinant human IGF-I was from the Amersham Corp. (Arlington Heights, Ill). Suramin was from the Mobay Corp. (New York, NY).

Tissue culture and membrane preparation. MG-63 cells were routinely cultured in RPMI-1640 medium supplemented with 10% fetal calf serum. A plasma membrane-enriched subcellular fraction was prepared from 10^9 cultured cells as previously described (*13*). For determination of growth curves, cells were plated in quadruplicate 2.5-cm^2 dishes in medium with 10% fetal calf serum, which was changed after 24 hours to serum-free medium with various additions as noted in the figure legends. After either 3 or 6 days, the cells were trypsinized and counted with a hemacytometer.

Binding studies. Aliquots of the plasma membrane-enriched subcellular fractions were incubated at room temperature for 1 hour with labeled IGF-I and other substances as noted in the figure legends. Binding studies on whole cells were done as previously described in similar experiments with epidermal growth factor (*16*).

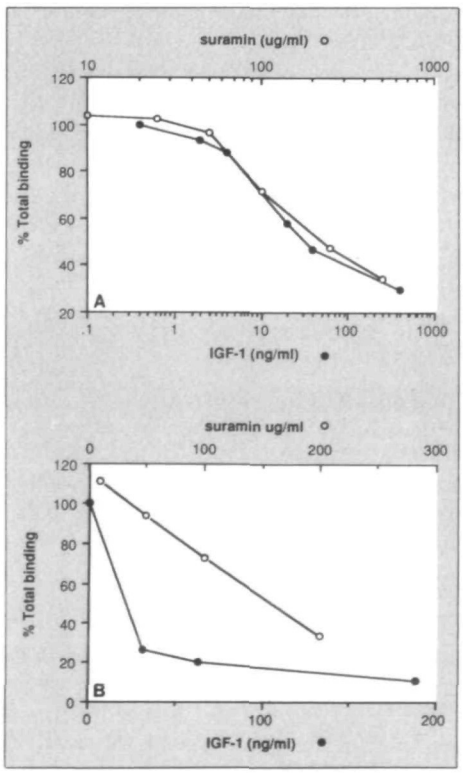

Figure 1. Binding of ^{125}I-labeled IGF-I to MG-63 cells (A) and an MG-63 plasma membrane fraction (B), in the presence of increasing concentrations of suramin or unlabeled IGF-I.

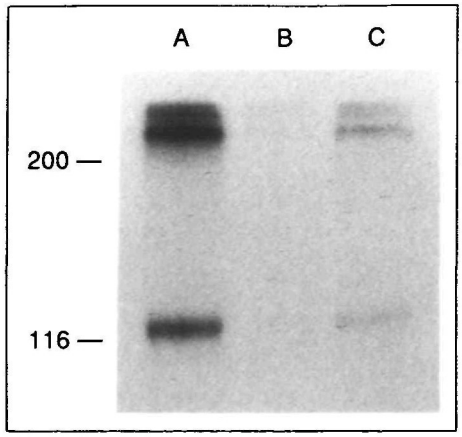

Figure 2. Effect of suramin on affinity labeling of IGF-I receptors of human osteosarcoma cells. Membrane fraction was incubated in the presence of labeled IGF-I either alone (lane A), with excess unlabeled IGF-I (lane B), or with suramin (200 μg/mL) (lane C).

Affinity labeling. Plasma membrane-enriched subcellular fractions were prepared as described (*13*) from cultured osteosarcoma cells. They were incubated for 1 hour at room temperature with radioiodinated IGF-I (20,000 cpm) in the presence or absence of excess unlabeled IGF-I and suramin. Electrophoresis was performed on a 7.5% polyacrylamide gradient gel after the plasma membrane fractions were cross-linked with disuccinimidyl suberate, solubilized with sodium dodecyl sulfate, and reduced in 100 m*M* 1,4-dithiothreitol.

Results

Figure 1 demonstrates the attenuation of binding of labeled IGF-I in the presence of increasing concentrations of suramin in the range of 50–300 μg/mL (3.5×10^{-5}–2.1×10^{-4} *M*). This binding curve was compared with control competition curves obtained with unlabeled IGF-I in the concentration range of 2.5×10^{-11}–5×10^{-9} *M*. Similar curves were obtained when binding experiments were carried out with viable cells in tissue culture or with purified plasma-membrane subcellular fractions. This result suggests that the effect is unrelated to receptor internalization.

The reduction of IGF-I binding to osteosarcoma membranes is confirmed by the affinity labeling experiment shown in figure 2. Here, the band indicating labeled IGF-I–receptor complexes is clearly attenuated in the presence of suramin.

Figure 3A describes experiments that document that suramin at concentrations of 10–200 μg/mL (7×10^{-6}–1.4×10^{-4} *M*) abolishes the in vitro mitogenic effect of IGF-I on osteosarcoma cells. Suramin had minimal effect on slowly proliferating control osteosarcoma cell cultures, but it completely inhibited IGF-I-stimulated proliferation. Serum-stimulated growth was similarly inhibited, and thymidine-incorporation experiments gave results similar to those obtained from the cell proliferation assays (data not shown). Data confirming the noncytotoxic nature of the effect of suramin in this concentration range are shown in figure 3B, where IGF-I is demonstrated to stimulate proliferation of osteosarcoma cells previously exposed to suramin.

Table 1 gives the results of experiments that demonstrate the attenuation of the inhibitory effect of suramin by the pres-

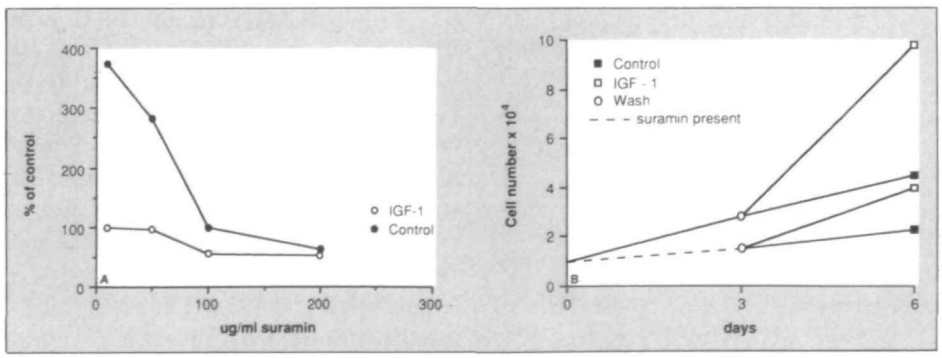

Figure 3. (A) Dose–response curves describing effect of suramin on IGF-I-stimulated proliferation of human osteosarcoma cells. Results are expressed as percentage of cell number observed after proliferation for 6 days in the absence of suramin and IGF-I. (B) Growth curves indicating cytostatic nature of antiproliferative effect of suramin. After attachment of the cells, replicate dishes of osteosarcoma cells were allowed to proliferate in the presence or absence of suramin (200 μg/mL) for 3 days. Cells were then washed and allowed to proliferate for a further 3 days in the absence of suramin, with or without IGF-I.

Table 1. Effect of albumin on antiproliferative effect of suramin

Condition	Percentage of control*
Basal medium (BM)	100 ± 10
BM + albumin (40 mg/mL)	114 ± 17
BM + IGF-I (5×10^{-9} *M*)	221 ± 4
BM + IGF-I (5×10^{-9} *M*) + albumin (40 mg/mL)	279 ± 21
BM + IGF-I (5×10^{-9} *M*) + suramin (200 μg/mL)	96 ± 7
BM + IGF-I (5×10^{-9} *M*) + suramin (200 μg/mL) + albumin (40 mg/mL)	271 ± 14

*Results are expressed as percentages (±SE) of cell number after 3 days in the absence of IGF-I, suramin, and albumin.

ence of a physiologic concentration of albumin in the tissue culture system. Note that the presence of albumin did not attenuate the stimulatory effect of IGF-I: this effect was slightly increased, possibly because the albumin present saturated various proteolytic enzymes in the conditioned medium. However, the inhibitory effect of suramin was eliminated by the presence of albumin in the medium. Dose–response studies indicated that attenuation of the suramin-related inhibition occurred at albumin concentrations as low as 5 mg/mL, considerably lower than the physiologic concentrations used in the experiment described in table 1. Interestingly, this concentration of albumin is, in molar terms, close to the concentration of suramin used in these experiments. This finding is consistent with the possibility of specific bonding interactions between albumin and suramin molecules at a ratio close to 1:1, as suggested by the studies of Müller and Wollert (*17*).

Discussion

Our data indicate that suramin abolishes the potent in vitro mitogenic effect of IGF-I on osteosarcoma cells. No effect of suramin on IGF-I physiology has previously been described. The mechanism underlying the antiproliferative effect is unclear, but we speculate that it is related to the reduction of binding of IGF-I to its receptors observed in the presence of suramin.

Under our experimental conditions, suramin may bind with IGF-I in solution, thereby reducing the effective concentration of free growth factor available for receptor binding. It is also possible that suramin interacts with membrane-associated IGF-I receptors in an undefined manner that results in a reduction of affinity. These mechanisms are not mutually exclusive. In a separate study using placental IGF-I receptors (Pollak M, Polychronakos C, Richard M: manuscript submitted for publication), we have been able to demonstrate that membrane-associated IGF-I receptors presaturated with IGF-I tend to release the mitogen in the presence of increasing concentrations of suramin. The fact that in other in vitro systems suramin interferes with the binding of growth factors having little structural resemblance to IGF-I suggests that the drug does not act as a classic competitive antagonist.

The reversibility of the antiproliferative effect of suramin indicates that any cellular alterations induced by suramin are not permanent and that the agent is cytostatic rather than cytotoxic at the concentrations used in our study. The concentrations active in our in vitro system are comparable with those achievable clinically (*1*). However, the attenuation of the antiproliferative effect by the presence of albumin may be significant in this regard. Suramin is known to bind to albumin (*17*), and little suramin is present in the free state in vivo (*1*). Thus, while the in vitro antiproliferative effect occurs at total suramin concentrations similar to those measured in patients, the concentration of free drug may be considerably lower in vivo.

The nature of the interactions between IGF-I and albumin and those between suramin and albumin is clearly very different. In vivo, circulating IGF-I interacts strongly with specific binding proteins that modulate the bioactivity of this growth factor (*18*), but the interactions with albumin are weak and appear to be physiologically less significant. In contrast, both strong, specific binding and nonspecific interactions have been documented between suramin and human serum albumin and bovine serum albumin (*17*). This observation suggests that despite the technical challenges involved, careful pharmacokinetic studies to address the issue of free versus bound circulating suramin concentrations should be incorporated in future clinical trials of the drug.

Although it is clear that a variety of primary human tumors are IGF-I–receptor positive (*7–13*), further research is required to determine the proportion of sarcomas and other neoplasms that are truly IGF-I responsive. Our data suggest that clinical evaluation of suramin in IGF-I–receptor-positive neoplasms such as osteosarcoma is warranted.

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