

Suramin Interferes with Interleukin-6 Receptor Binding In Vitro and Inhibits Colon-26-mediated Experimental Cancer Cachexia In Vivo

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Abstract

Neoplastic diseases are frequently associated with metabolic changes collectively known as cancer cachexia. The presence of cachexia complicates therapeutic intervention and is an important cause of death in cancer patients. At present there is no effective treatment for cachexia. Recently, the involvement of interleukin-6 (IL-6) in the wasting of colon-26 adenocarcinoma-bearing mice was demonstrated. The research presented here establishes an anticachectic role for the experimental drug suramin, since it partially blocks (up to 60%) the catabolic effects associated with the growth of this tumor in vivo. Suramin prevents the binding of IL-6 to its cell surface receptor subunits, as demonstrated by radioreceptor binding assay and affinity crosslinking experiments. Furthermore, the uptake of radioactive IL-6 by the liver is significantly reduced in suramin-treated mice. On the other hand, the drug is ~ 10-fold less potent in inhibiting the binding of tumor necrosis factor- α to indicator cell line in vitro and fails to block liver uptake of this cytokine in vivo. Collectively, these results suggest that suramin inhibits cancer-associated wasting, in part by interfering with the binding of IL-6 to its receptor. Whether suramin inhibits the action of other factors/cytokines that may also participate in colon-26-mediated cachexia is not yet known. (*J. Clin. Invest.* 1993. 92:2152–2159.) Key words: cancer cachexia • IL-6 • IL-6 receptor • suramin • colon-26 tumor

Introduction

Weight loss is a common manifestation of many chronic illnesses, including cancer, and bacterial, viral, and parasitic infections (1). In humans, neoplastic diseases are frequently associated with a constellation of metabolic changes collectively known as "cancer cachexia" (2, 3). Among these changes are progressive wasting of both muscle and fat tissues, anemia, anorexia, and asthenia. Cachexia long has been recognized as an important cause of death in cancer patients (4), and patients who exhibit cachexia have a reduced response to chemotherapy (5). Wasting is common in cancer patients. Up to 50% of all patients have lost weight by the time of diagnosis, and nearly all patients who die from cancer exhibit wasting (6). Recent attempts to compensate, through total parenteral nutrition, for the negative caloric balance in patients failed to alter

wasting (7). The mitigation of wasting by pharmacological intervention, therefore, is important not only because anticachexia therapy could improve the survival and quality of life of the patient, but also because it could give way to a more effective anticancer therapy.

Cachectin/TNF has been suggested as an important mediator of cancer cachexia because it suppresses key metabolic enzymes and induces anorexia and weight loss in animals (1, 8–10). Recently, however, an experimental cachexia model has been identified that appears to involve another cytokine. The model uses a cell line derived from colon-26 adenocarcinoma (C-26),¹ which retains the transplantability of the original tumor in syngeneic mice and fulfills the criteria of early-onset wasting without apparent anorexia (11). In at least this model, IL-6 appears to have a more significant role than TNF in mediating the myriad parameters of cachexia (11). The cellular source of IL-6 in this model is believed to be derived from the tumor cells, in response to IL-1 provided by tumor-infiltrating mononuclear phagocytes (12).

The experimental drug suramin (a polysulfated naphthylurea) was originally developed as an antitrypanosomal and antifilarial agent (13). Recently suramin was found to inhibit the growth of several tumor cell lines in culture (14, 15), and to inhibit the enzyme reverse transcriptase (16). Previous work suggested that suramin blocks the activity of IL-6 as an autocrine/paracrine growth factor in human multiple myeloma cell lines (17, 18). Suramin can also induce cell differentiation in several systems (19), suppress tumor cell invasion (20), and inhibit the nuclear enzyme DNA topoisomerase II (21). Therefore, suramin is currently being investigated in the clinic for the treatment of AIDS (22) and several inoperable cancers (23, 24). In culture, suramin is known to block binding of various growth factors (among them EGF, IGF-1, TGF- β , PDGF, and basic fibroblast growth factor) to their corresponding cell surface receptors (25–29). In this report, we demonstrate that suramin interferes with the binding of IL-6 to its cell surface receptor, and significantly inhibits the catabolism of C-26-bearing hosts.

Methods

Cell culture. Cell culture was performed in complete medium, which consisted of either RPMI 1640 or DME (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS (Whittaker Bioproducts, Walkersville, MD) and antibiotics, at 37°C in a humidified atmosphere of 5% CO₂. U-266, a human myeloma cell line, and MCF-7, a human breast tumor line, were obtained from the American Type Culture Collection (Rockville, MD). The IL-6-dependent murine cell line B-9 was grown in complete RPMI supplemented with 50 μ M 2-mercaptoethanol and recombinant IL-6.

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1. Abbreviation used in this paper: C-26, colon-26 adenocarcinoma.

Mice. Virus-free, male BALB/c × DBA/2 (CD)F₁ mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). Mice were housed under conventional conditions and were used at 8–14 wk of age.

Radioactive IL-6. Human ¹²⁵I-IL-6 (sp act, 2,000 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL) or was labeled by us. Briefly, IL-6 (R & D Systems, Minneapolis, MN) was labeled using the diiodo ¹²⁵I-Bolton-Hunter reagent (2,200 Ci/mmol; DuPont/NEN, Boston, MA) as previously described (30). The specific activity of the ¹²⁵I-IL-6 was calculated to be 2–4 × 10¹⁵ cpm/mmol. More than 99% of the radioactivity corresponded to a single band of IL-6 when analyzed under both reducing and nonreducing SDS-PAGE, and autoradiography. Human ¹²⁵I-TNFα (sp act, 520 Ci/mmol) was purchased from New England Nuclear (Boston, MA). The biologic activity of the radioactive IL-6 was found to be essentially unchanged as measured using the IL-6-dependent B-9 cell line.

Affinity crosslinking of ¹²⁵I-IL-6 to its receptor. Affinity crosslinking was performed using a modification of a previously published procedure (31). Briefly, cells were washed twice and resuspended at 5 × 10⁷ cells/ml in cold binding medium (RPMI 1640, 0.05% sodium azide). Binding was allowed to proceed on ice for the indicated time with the indicated amount of ¹²⁵I-IL-6. After binding, the cells were washed with cold RPMI 1640/0.05% sodium azide to remove the unbound ¹²⁵I-IL-6 and resuspended in 1 ml of PBS, 1 mM MgCl₂, pH 8.3. Crosslinking was initiated by the addition of 300 μg/ml disuccinimidyl suberate (Pierce Chemical Co., Rockford, IL), as indicated, and allowed to proceed at 4°C for 15 min. The crosslinking reaction was stopped by centrifugation and by immediate lysis of the cells with 50 mM Tris-HCl, 300 mM NaCl, 1% NP-40, 1 mM PMSF, 10 mM leupeptin, and 10 mM pepstatin, pH 7.5 (lysis buffer), for 30 min on ice. The lysate was centrifuged for 10 min at 15,000 g and the supernatant collected for analysis by SDS-PAGE and autoradiography.

Binding assays. This assay was performed as described previously (32). U-266 cells were grown to confluence, medium was removed, and the cells were washed in binding buffer (RPMI 1640 supplemented with 0.1 mg/ml BSA and 25 mM Hepes, pH 7.2), and 0.05-ml aliquots of 10 × 10⁶ cells/ml were dispensed into tubes on ice. Increasing amounts of suramin and 0.8 ng of ¹²⁵I-IL-6 were added simultaneously at the indicated concentrations. The cells were incubated for 90 min at 4°C with gentle agitation. To separate cells bound from free ¹²⁵I-IL-6, 0.2 ml of an oil mixture was injected into the bottom of the tubes, which were centrifuged for 1 min. The fluid was aspirated, the tube tips were cut, and cell-bound radioactivity was determined in a gamma counter. Binding of ¹²⁵I-TNFα (1.0 ng) was performed on confluent monolayers of MCF-7 cells with the same binding buffer. Cells were then washed three times with cold binding buffer, trypsinized, and cell-bound radioactivity was measured.

IL-6 bioassay and ELISA. This assay was previously described (32). Briefly, B-9 cells were grown in human IL-6. To measure IL-6

activity in serum or inhibition by suramin, IL-6-dependent B-9 cells (5 × 10³/well) were cultured at a final volume of 0.2 ml with or without diluted samples in flat-bottomed 96-well plates (Falcon Labware, Oxnard, CA). After incubation for 72 h, cells were pulsed for the final 4 h with 1 μCi/well of [³H]thymidine. Incorporation of radioactivity was determined by standard liquid scintillation counting procedures after collection of cells on glass fiber paper. Results are expressed in units where 1 U was defined as the reciprocal dilution required for half-maximal stimulation of the cells. IL-6 was also quantified by a murine-specific IL-6 ELISA (Endogen, Boston, MA).

Measurement of cachexia markers. Mice were inoculated with 0.5 × 10⁶ C-26.IVX cells subcutaneously to the right flank as described (11, 12). Treatments were performed as indicated in the tables. Mice were weighed between 9 and 11 a.m. several times per week. The length and width of their tumors were measured using an engineering caliper, and estimation of tumor weight was calculated, as previously described, for the same tumor (33). Significant weight loss in C-26-bearing mice occurred between 12 and 14 d after tumor inoculation. Host weight was calculated by subtracting tumor weight (obtained by resection) from total weight. Blood was obtained by cardiac puncture (~0.8 ml), and serum was harvested after the clotting of blood at room temperature for 1 h. Serum was kept frozen (-45°C) until analysis. Measurements of serum glucose were performed using an Ektachem DT-60 analyzer (Eastman Kodak Co., Rochester, NY). Dry weight was determined (after removal of the tumor, blood, and right epididymal fat pad) by oven drying for 3 d at 85°C. The neutralizing mAb against murine IL-6 20F3 was the gift of Dr. C. O. Jacob (Syntex Research, Palo Alto, CA) (11).

Statistical analysis. Results throughout the paper are presented as mean ± SD. Differences in cachexia markers were calculated using computerized analysis of variance (ANOVA).

Results

Inhibition of IL-6 activities by suramin in vitro. The addition of suramin to the B-9 murine myeloma line inhibits the proliferation of these cells in response to IL-6. Half-maximal inhibition of thymidine incorporation was seen at ~30 μM of the drug when various doses (3–30 pg/ml) of recombinant human (rh) IL-6 were added to the culture (Table I). In contrast, 10-fold more drug was required to achieve the same extent of inhibition when B-9 cells were incubated with 1 ng/ml of IL-6 (not shown). The inhibition was not specific to IL-6, since the drug also inhibited B-9 proliferation in response to increasing concentrations of IL-4. When B-9 cells were pretreated with suramin, up to 1 mM for 1 h at room temperature, followed by

Table I. Suramin Inhibits B-9 Cell Proliferation in Response to IL-6 and IL-4

| Cytokine | IL-6 (pg/ml) | | | IL-4 (U/ml) | | |
|--------------|--------------|---------|--------|-------------|---------|--------|
| | 30 | 10 | 3 | 100 | 30 | 10 |
| No compound | 252,895 | 144,526 | 29,728 | 175,269 | 138,423 | 65,445 |
| Suramin (μM) | | | | | | |
| 300 | 5,654 | 1,811 | 1,105 | 3,448 | 2,288 | 1,106 |
| 100 | 59,444 | 9,339 | 2,389 | 23,422 | 6,363 | 1,536 |
| 30 | 229,221 | 81,766 | 11,989 | 103,774 | 70,344 | 23,354 |
| 10 | 265,263 | 139,000 | 32,726 | 162,384 | 150,385 | 74,314 |
| 3 | 233,260 | 156,001 | 32,537 | 159,604 | 139,569 | 81,819 |

Results are expressed as cpm of [³H]thymidine incorporation to B-9 cells. Standard deviation did not exceed 10%. Background proliferation in the assay was 2,301 cpm. The experiment was repeated five times with similar results.

washing of the cells, no inhibition of cell proliferation occurred (data not shown).

Because suramin is known to prevent binding of various growth factors to their cell surface receptors (25–28), it was of interest to determine whether the inhibition of B-9 cell growth was possibly due to the prevention of IL-6 binding to its receptor. To this end, the U-266 human myeloma line, which expresses a relatively high number of IL-6 receptors, was used in standard IL-6 receptor binding assays. At 4°C, suramin inhibited the binding of ¹²⁵I-IL-6 to U-266 cells (Fig. 1). In this representative experiment, the extent of inhibition of cell-associated radioactivity reached almost 100%. The determination of background binding involved the addition of 625-fold excess of cold IL-6. This experiment was highly reproducible, since more than eight experiments in two separate laboratories with different batches of radioactive IL-6 showed similar results. Half-maximal inhibition in these experiments was achieved at ~ 30 μM of the drug. On the other hand, suramin was not as effective in inhibiting the binding of ¹²⁵I-TNFα to the MCF-7 cell line. Half-maximal inhibition of TNFα binding was achieved at only ~ 300 μM of the drug (Fig. 1). To analyze still further the inhibitory effect of suramin on IL-6 binding, affinity crosslinking experiments were performed. Crosslinking membrane-bound ¹²⁵I-IL-6 to U-266 cells generates three IL-6-containing crosslinked complexes with molecular masses of 100, 120, and 150 kD. As shown in Fig. 2, suramin concentrations as low as 25 μM display inhibition of complex formation whereas concentrations ≥ 100 μM completely block the formation of all three IL-6/IL-6 receptor complexes. To better understand this effect, we asked if suramin could dissociate bound IL-6 from the receptor complex. Fig. 3 demonstrates that concentrations of suramin that inhibit the formation of IL-6/IL-6 receptor complexes are unable to dissociate bound IL-6 from the receptor complex. In addition, radioreceptor binding assays confirmed the results presented in Fig. 3. At 4°C, suramin did not displace prebound radioactive IL-6 on U-266 cells (not shown).

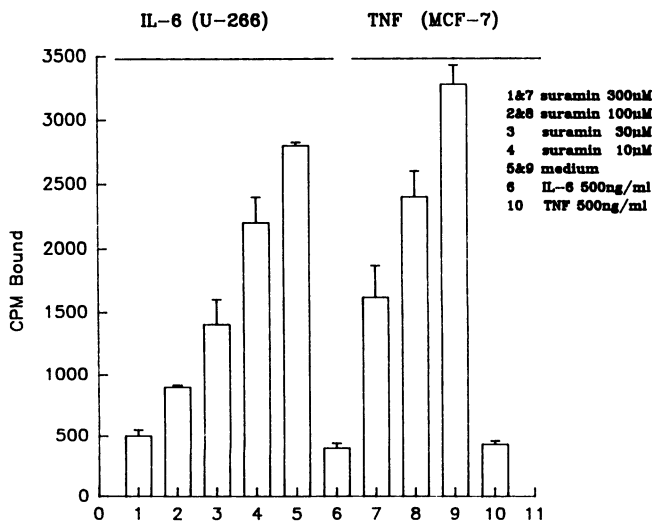


Figure 1. Inhibition of binding of IL-6 and TNFα to indicator cells by suramin. 5×10^5 U-266 or MCF-7 cells were incubated with a saturating amount of ¹²⁵I-IL-6 and ¹²⁵I-TNFα, respectively, and increasing amounts of suramin (right) for 90 min at 4°C. Cell-associated radioactivity was determined as described in Methods.

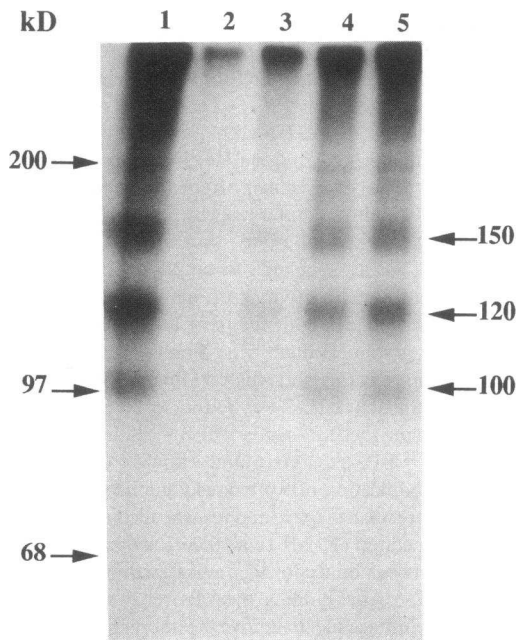


Figure 2. Effect of suramin on the formation of IL-6/IL-6 receptor complexes. 4×10^6 U-266 cells were incubated at 4°C for 2 h with 5 nM ¹²⁵I-IL-6 plus various concentrations of suramin as described in Methods. After removal of suramin and unbound ligand, the cells were crosslinked with disuccinimidyl suberate for 15 min at 4°C, lysed, and analyzed by reducing SDS-PAGE and autoradiography. Lane 1, no suramin; lane 2, 200 μM suramin; lane 3, 100 μM suramin; lane 4, 50 μM suramin; lane 5, 25 μM suramin.

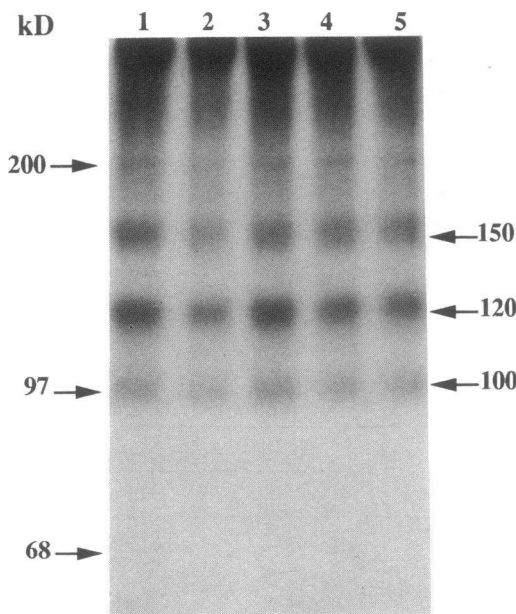


Figure 3. Effect of suramin on prebound IL-6/IL-6 receptor complexes. 4×10^6 U-266 cells were incubated at 4°C for 1 h with 5 nM ¹²⁵I-IL-6. After removal of unbound ligand, the cells were incubated with various concentrations of suramin for 1 h at 4°C. After removal of suramin, the cells were crosslinked and processed as described in Fig. 2. Lane 1, no suramin; lane 2, 200 μM suramin; lane 3, 100 μM suramin; lane 4, 50 μM suramin; lane 5, 25 μM suramin.

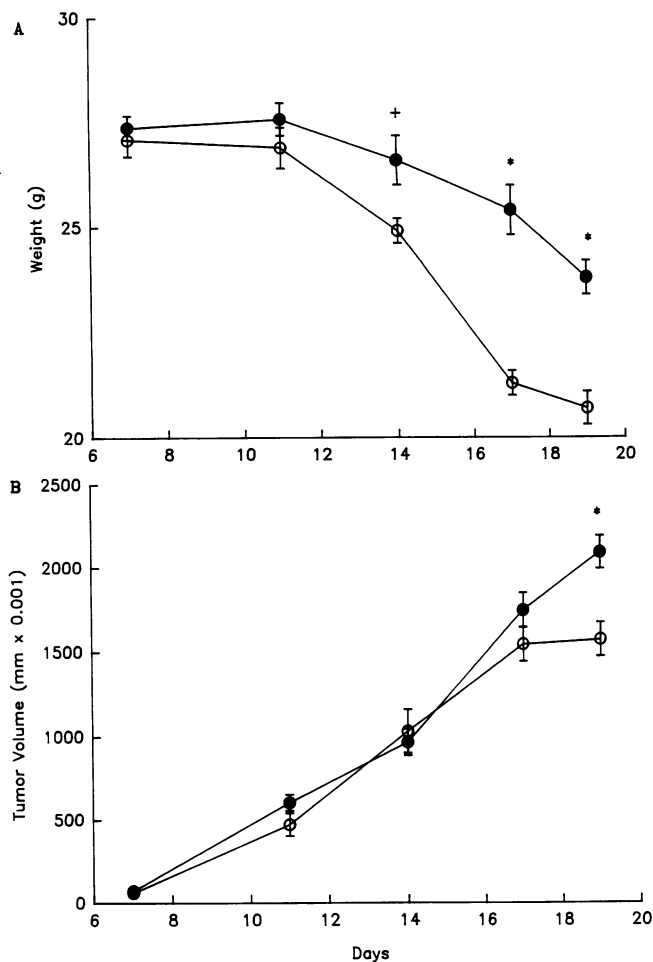


Figure 4. Inhibition of C-26-mediated wasting by suramin. Mice were inoculated with 5×10^5 C-26.IVX cells on day 0. The weight (A) and tumor size (B) were determined as described in Methods. Filled circles represent eight mice receiving 100 mg/kg i.p. of suramin on days 7 and 12, while open circles represent eight mice injected with PBS on the same days. * $P < 0.002$; + $P < 0.002$.

Suramin inhibits C-26-mediated wasting. The administration of suramin to C-26.IVX-bearing mice on days 7 and 12 significantly improved the total weight (host and tumor) of these animals as compared with PBS-injected controls (Fig. 4 A). The measurements of tumor volume in the experiment revealed that, on days 14 and 17 of posttumor inoculation, the suramin-treated hosts were significantly protected from wasting, since they exhibited an identical tumor burden to control-treated hosts (Fig. 4 B). On day 19, suramin-treated animals showed a statistically larger tumor burden. However, for unknown reasons, this augmentation in tumor burden did not occur in all the experiments (for example, see Table II). Of note, non-tumor-bearing mice treated with the drug exhibited weights that were indistinguishable from that of PBS-treated age-matched normal animals (not shown). The beneficial effect of suramin in inhibiting C-26-mediated weight loss required a dose of ~ 75 mg/kg, administered twice on days 7 and 12 posttumor inoculation (Fig. 5). Body compositional analyses of C-26-bearing mice confirmed the protective effect of suramin. Table II depicts three separate experiments where different protocols of suramin treatment and termination days were used. Significant improvements in cachexia markers, including host weight, dry weight, heart weight, epididymal fat weight, and hypoglycemia, could be seen in suramin-treated mice. Of note, irrespective of the tumor burden, serum IL-6 levels in suramin-treated mice tended to be higher than in PBS-treated C-26-bearing mice. Quantification of serum samples using a murine ELISA showed a close correlation with the results obtained in the B-9 assay (data not shown). Thus, the data appear to suggest that the increase in IL-6 in the serum of suramin-treated mice might be due to interference in the binding of IL-6 in vivo.

Modulation of IL-6 but not TNF α sequestration by suramin in vivo. It has recently been shown that IL-6 in the serum is complexed with several other proteins that can camouflage its immunoreactivity and bioactivity (34). Because the interpretation of the findings that suramin increased IL-6 levels in vivo is difficult, we attempted to determine whether suramin would

Table II. Improvement of Cachectic Parameters in Suramin-treated C-26-bearing Mice

| Tumor bearing treatment mice/group parameter | Exp. 1 | | | Exp. 2 | | | | Exp. 3 | | | | |
|--|-----------------|-----------------|--------|-----------------|-----------------|-----------------|----------------|---------|----------------|----------------|----------------|-------|
| | + PBS 5 | + Suramin 6 | NS* | + PBS 6 | + Suramin (1) 6 | + Suramin (2) 6 | - PBS 5 | + PBS 6 | + Suramin 6 | - PBS 6 | | |
| Initial wt. (g) | 29.3 \pm 1.8 | 27.5 \pm 0.8 | NS* | 26.1 \pm 0.4 | 26.4 \pm 0.4 | 26.1 \pm 0.3 | 26.8 \pm 0.6 | NS* | 25.6 \pm 1.1 | 26.1 \pm 1 | 26.1 \pm 0.5 | NS* |
| Final wt. (g) | 21.7 \pm 0.7 | 25.9 \pm 0.8 | 0.0001 | 21.3 \pm 0.8 | 24.5 \pm 0.7 | 25.1 \pm 1.2 | 28.7 \pm 0.9 | 0.0001 | 20.9 \pm 2.4 | 24.9 \pm 1.7 | 27.1 \pm 0.5 | 0.008 |
| Tumor wt. (g) | 1.65 \pm 0.13 | 1.82 \pm 0.49 | NS | 1.46 \pm 0.12 | 1.5 \pm 0.1 | 1.9 \pm 0.4 | | | 1.1 \pm 0.1 | 1.2 \pm 0.1 | | NS |
| Host's wt. (g) | 20.2 \pm 0.8 | 24.2 \pm 1.1 | 0.0001 | 19.6 \pm 0.8 | 23.0 \pm 0.6 | 23.1 \pm 1.2 | 28.7 \pm 0.9 | 0.004 | 19.8 \pm 2.5 | 23.7 \pm 1.6 | 27.1 \pm 0.5 | 0.008 |
| Dry wt. (g) | 5.8 \pm 0.4 | 6.9 \pm 0.5 | 0.02 | 5.9 \pm 0.2 | 7.1 \pm 0.3 | 7.0 \pm 0.4 | 8.8 \pm 0.2 | 0.001 | 6.7 \pm 1.1 | 8.7 \pm 1.2 | 10.6 \pm 0.7 | 0.05 |
| Epididymal fat (mg) | 35 \pm 30 | 118 \pm 61 | 0.02 | 30 \pm 6 | 94 \pm 36 | 95 \pm 30 | 268 \pm 40 | 0.01 | 97 \pm 56 | 197 \pm 43 | 329 \pm 24 | 0.006 |
| Heart wt. (mg) | NT | NT | NT | 102 \pm 7 | 119 \pm 5 | 118 \pm 11 | 136 \pm 7 | 0.01 | NT | NT | NT | NT |
| Serum IL-6 (U/ml) | 158 \pm 32 | 384 \pm 99 | 0.02 | 138 \pm 41 | 290 \pm 146 | 412 \pm 256 | 6 \pm 2 | 0.02 | 115 \pm 28 | 176 \pm 26 | 7 \pm 3 | 0.02 |
| Serum glucose (mg/dl) | 44 \pm 21 | 113 \pm 9 | 0.001 | NT | NT | NT | NT | NT | 40 \pm 18 | 78 \pm 26 | 188 \pm 18 | 0.02 |

CD F₁ male mice were inoculated with C-26.IVX cells on day 0. In exp. 1, mice received PBS (0.5 ml) or suramin (200 mg/kg i.p.) on days 7 and 13, and the experiment was terminated on day 17. In exp. 2, the group designated suramin (1) received a total of 6 mg/mouse, divided over 5 d (1.2 mg/d) between days 7 and 11. The group designated suramin (2) received two injections of suramin on days 7 and 11 (3 mg/injection per mouse) and were killed on day 17. In exp. 3, mice received an identical treatment to that in exp. 2, suramin (1), and were killed on day 15. Results are expressed as mean \pm SD. NT, not tested. * P values represents the difference between suramin-treated and PBS-treated C-26-bearing mice.

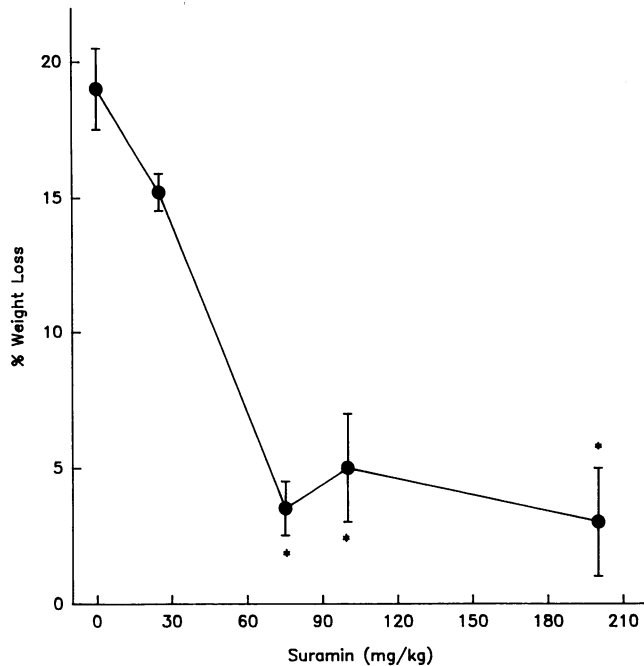


Figure 5. Dose-dependent inhibition of wasting by suramin. Increasing amounts of suramin were injected on days 7 and 12 after tumor inoculation. Shown is percent weight loss on day 17. There were five to six mice per group. * $P < 0.002$.

interfere with IL-6 binding in vivo. Table III illustrates the ability of suramin to significantly reduce the sequestration of ^{125}I -IL-6 to the livers of normal CDF₁ mice. In contrast, suramin increased the radioactivity associated with the kidney (Table III), but did not change the level of radioactivity in the blood (not shown). Under the same conditions in a subsequent experiment, suramin inhibited ^{125}I -IL-6 binding to the liver by 48% and significantly increased radioactivity present in the urine ($111,833 \pm 3,590$ cpm in 0.1 ml urine from suramin-injected mice vs. $45,251 \pm 10,568$ cpm in 0.1 ml urine from PBS-injected mice, measured 1 h after radioactive IL-6 injection). This increase in urine radioactivity suggests that suramin may accelerate the clearance of IL-6 in vivo. Of note, the administration of 15 μg of unlabeled IL-6, together with 1.2 ng of ^{125}I -IL-6, failed to reduce the liver-associated radioactivity (not shown). Therefore, the background binding of ^{125}I -IL-6 in the assay shown in Table III is unknown.

Suramin was ~10-fold less potent in inhibiting receptor binding of TNF α than IL-6 to indicator cells in culture (Fig.

Table III. Suramine Modulates ^{125}I -IL-6 Sequestration In Vivo

| | 30 min | | 60 min | | 120 min | |
|--------|------------------|---------------------|-------------------|---------------------|-----------------|--------------------|
| | PBS | Suramin | PBS | Suramin | PBS | Suramin |
| | <i>cpm±SD</i> | | | | | |
| Liver | 15,792±963 (5.3) | 8,831±1,361 (2.9) | 8,244±467 (2.9) | 4,893±781 (1.6) | 7,135±110 (2.4) | 4,030±441 (1.3) |
| Kidney | 19,618±580 (6.5) | 63,719±7,783 (21.2) | 8,772±1,455 (2.9) | 37,432±1,773 (12.4) | 2,565±218 (0.8) | 17,031±1,506 (5.7) |

Normal CDF₁ mice were injected intravenously with PBS (0.2 ml) or with suramin (5 mg), 20 min before intravenous administrations of ^{125}I -IL-6 (300,000 cpm; ~1.2 ng). At the indicated time points (relative to the injection of ^{125}I -IL-6), mice were killed and organs were removed and counted. Results are expressed as cpm±SD of four mice. Liver radioactivity is expressed as cpm/g tissue. The numbers in parentheses indicate percent of injected cpm.

Table IV. Suramin Does Not Inhibit TNF α Uptake by the Liver

| | TNF α (2.8 ng/mouse) | | IL-6 (2.4 ng/mouse) | |
|--------|--------------------------------|--------------|------------------------|--------------|
| | PBS | Suramin | PBS | Suramin |
| | <i>cpm±SD</i> | | | |
| Liver | 52,684±3,439 | 55,339±9,091 | 42,295±2,068 | 22,378±2,801 |
| Kidney | 10,151±1,443 | 13,774±1,365 | 21,319±796 | 51,123±6,840 |
| Spleen | 3,236±158 | 3,011±475 | 1,625±371 | 1,684±270 |

The experiment was performed and the results are expressed as indicated in Table III. There were three mice per group. Mice were killed 30 min after intravenous administration of radioactive cytokine.

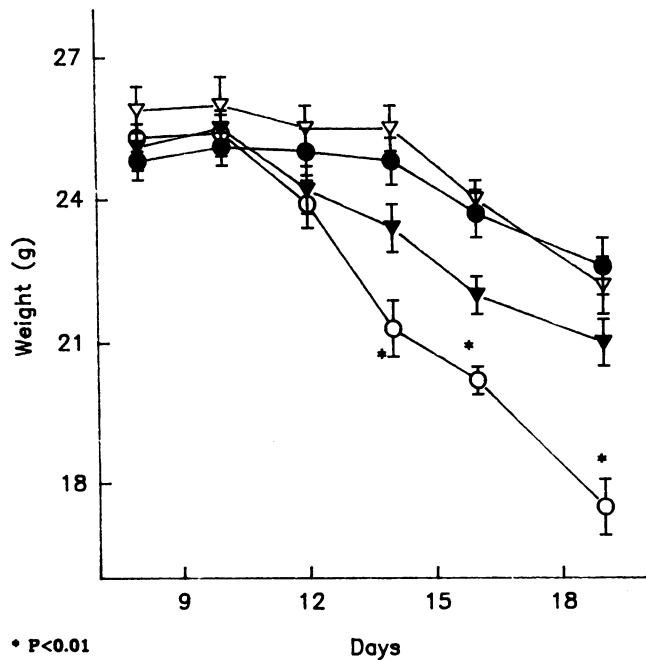
1). Therefore, it was of interest to determine whether suramin would inhibit TNF α uptake by the liver in vivo. Table IV clearly demonstrates that whereas suramin inhibited liver-associated IL-6 radioactivity, the drug failed to decrease liver-associated TNF radioactivity.

Lack of additivity between suramin and anti-IL-6 antibody in vivo. Previous results demonstrated the ability of the rat anti-murine IL-6 mAb 20F3 to inhibit C-26-mediated cachexia (11). The administration of this antibody and of suramin significantly inhibited ($P < 0.01$ from control-treated mice) C-26-mediated wasting (Fig. 6). However, the coadministration of both the 20F3 antibody and suramin did not add to the protection against wasting provided by suramin alone. One possible interpretation for this lack of additivity is that suramin inhibits wasting by interfering with the action of IL-6 in vivo.

Discussion

Previous observations in myeloma cells that suramin inhibits IL-6-stimulated growth and immunoglobulin secretion (17, 18) suggested that suramin could also interfere with experimental cancer cachexia where IL-6 was shown to be involved (11). We demonstrate here for the first time the capacity of a chemical (i.e., suramin) to interfere with the binding of IL-6 to its cell surface receptor and to have a significant beneficial effect on reducing cancer-associated weight loss.

In a dose-dependent fashion, suramin inhibits the proliferation of B-9 cells (a standard IL-6 bioassay) in response to IL-6. The ability of the drug to inhibit IL-6-dependent growth is not restricted to this cytokine, since suramin inhibits the proliferation of the same indicator cells to IL-4 (Table I). The findings



• P < 0.01

Days

Figure 6. Anti IL-6 treatment does not influence suramin inhibition of weight loss. Mice were inoculated with 5×10^5 C-26 IVX cells on day 0. On days 6 and 11, one group of mice was injected intraperitoneally with PBS (open circles). Other groups received the 20F3 mAb (1 mg; filled triangles) or suramin (2 mg; open triangles). The group represented by the filled circles received the same amount of both suramin and of the 20F3 mAb on the same days. There were five mice per group.

that the extent of inhibition had an inverse relation to the amount of IL-6 and, second, that the inhibitory effect of the compound could be removed in pretreatment experiments (up to 1 mM, for 1 h; data not shown), suggest that the effect of suramin on B-9 cells is reversible. Radioreceptor assays conducted at 4°C showed that suramin inhibited the binding of radioactive IL-6 to U-266 human myeloma in a dose-dependent manner (Fig. 1). It is interesting to note that suramin was found to be 10-fold less potent in inhibiting the binding of TNF α to the MCF-7 human breast cancer line. More direct evidence for the interference of IL-6 receptor binding by suramin comes from crosslinking studies. As previously reported, crosslinking membrane-bound 125 I-IL-6 to U-266 cells generates three IL-6-containing crosslinked complexes with molecular masses of 100, 120, and 150 kD (35). The 100- and 120-kD complexes represent one and two molecules, respectively, of IL-6 (20 kD) crosslinked to an 80-kD membrane protein, whereas the 150-kD complex consists of IL-6 crosslinked to a 130-kD membrane protein. These molecular masses exactly correspond with those of two membrane glycoproteins, gp80 and gp130, that participate in the IL-6 receptor system (36). It is possible that suramin exerts its effect by interfering with the initial interaction of IL-6 with the gp80 receptor molecule. The compound was unable to dissociate IL-6 once bound by the receptor complex, as shown by crosslinking experiments (Fig. 3) and radioreceptor assays (data not shown), suggesting that suramin directly interacts with and blocks binding site domains rather than altering the conformation of the ligand or receptor molecules. Whether suramin binds directly to IL-6, to

the receptor subunits, or indirectly to a cell surface component in close proximity to the receptor remains to be established. However, under the conditions used here (4°C), suramin does not appear to act by inducing IL-6 receptor internalization or shedding. It also is possible that suramin exerts its inhibitory action by binding directly to the IL-6 molecule. However, this offers only a partial explanation, since the stoichiometry between IL-6 and suramin requires a vast excess of the drug. Previous studies have shown that suramin inhibits the binding of a variety of other growth factors to their corresponding cell surface receptors (25–29). These results, together with our data, do not support a specific antagonistic role for suramin in preventing/interfering with the binding of IL-6. This differs, for example, from the case of IL-1 receptor antagonist, where the inhibition of binding is highly specific to IL-1 proteins (37). Alternatively, since suramin is a heavily negatively charged molecule, its mechanism of action may involve indiscriminate binding to positively charged regions within the ligand binding site of many receptor types. These possibilities are currently under investigation.

Coincident with the inhibitory action of suramin on IL-6 binding to indicator cells in culture, suramin exhibits a positive effect on cachexia in vivo. The administration of the drug to C-26-bearing hosts reduced the deleterious effects of the tumor in a time- and dose-dependent manner (Figs. 4 and 5). Specifically, two administrations of suramin at doses of ≥ 75 mg/kg prevented, by $\sim 60\%$, the loss of precachectic weight of C-26-bearing hosts. Body compositional analysis revealed that suramin inhibited the wasting of both muscle and fat tissues, and reduced the extent of hypoglycemia (Table II). Because suramin did not prevent C-26-mediated weight loss completely, it is possible that IL-6 is but one of several factors influencing host catabolism in the C-26 cachexia model. These still undetermined factors might be suramin insensitive. One such suramin-insensitive factor may be the competition between progressing C-26 tumors and the host for essential nutrients, a phenomenon known to occur in experimental cachexia (38). The beneficial effect of suramin on weight loss is not due to added nutritional value, since the drug is known to be poorly metabolized (22).

Suramin treatment did not result in a decrease of tumor burden at the same time as it protected weight loss. This suggests that the drug affects the host directly. In some experiments, a small but significant augmentation of tumor size was seen in suramin-treated mice. However, this phenomenon occurred at a time when suramin-treated mice were protected from weight loss, while control-treated animals exhibited significant wasting. In addition, when a given dose of the drug was administered over a 5-d period, rather than injected twice, the increase in tumor burden disappeared (Table II). Moreover, suramin neither inhibited nor augmented the growth of C-26.IVX (data not shown), in contrast to its ability to prevent the proliferation of several other cancer lines in culture (14, 15). Taken together, this information establishes a role for suramin as an anticachectic agent, in addition to its known anti-neoplastic effect. It remains to be established whether suramin prevents wasting associated with the growth of other tumors in vivo.

Suramin inhibits IL-6 activities in vitro and suppresses catabolism of C-26-bearing mice, where IL-6 plays a role in mediating cachexia (11), in vivo. An attempt, therefore, was

made to link these two observations. Experiments with trace amount of radioactive IL-6 showed that suramin modulated the sequestration of the cytokine *in vivo*. Whereas the drug significantly lowered (up to 50%) the radioactivity associated with the liver, it elevated the radioactivity present in the kidney (Table III) and in the urine (now shown). Similar to the situation with suramin, the administration of a mAb against murine IL-6 receptor significantly lowered (50%) the amount of radioactivity associated with the liver and increased the radioactivity associated with the kidney (not shown). Further experimentation is required to establish the effect of the drug on specific binding of this cytokine to the liver. Interestingly, suramin did not influence the uptake of radioactive TNF α by the liver (Table IV). While these results do not support a specific antagonistic role of suramin in preventing IL-6 binding, the data demonstrate that the drug exhibits a certain level of selectivity in its action *in vivo*. The collective implication of the findings presented here support the hypothesis that suramin inhibits C-26-mediated cachexia, in part, by preventing the binding of IL-6 to its receptor *in vivo*. Further support for this hypothesis may be found in the experiment where anti-IL-6 mAb treatment did not increase the protection against cachexia provided by suramin administration *in vivo* (Fig. 6). In addition to IL-6, suramin also prevents the binding of IL-1 to type I and II IL-1 receptors (G. Strassmann, unpublished results). IL-1 has been shown to upregulate IL-6 production by the C-26 tumor *in vitro* (12), and intratumoral, but not systemic, administration of IL-1 receptor antagonist inhibits C-26-mediated wasting (39). Since suramin treatment increases IL-6 levels in the circulation of C-26-bearing mice, it is not likely that inhibition of cachexia *in vivo* is due to tumor IL-1 receptor blockade. It is still possible, however, that suramin inhibits the action of other factors that, together with IL-6, may influence C-26-mediated cachexia.

IL-6 is a multifunctional cytokine. In addition to its involvement in cancer cachexia, it has been implicated in the pathophysiology of rheumatoid arthritis, Kaposi sarcoma, B cell malignancies, Castleman's disease, cardiac myxoma (39), and Alzheimer syndrome (40). Ultimately, then, the identification of suramin as an inhibitor of IL-6 may lead to the discovery of other chemicals capable of exerting even more potent and specific antagonism to this pleiotropic cytokine.

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