Journal of Cellular Biochemistry 29:265–273 (1985)

Suramin Binds to Platelet-Derived Growth Factor and Inhibits Its Biological Activity

Markus Hosang

Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., 4002 Basel, Switzerland

The polyanion suramin was recently found to inhibit binding of ^{125}I-PDGF (platelet-derived growth factor) to Balb/c 3T3 cell membranes. Cultured Swiss 3T3 cells were used to investigate the mode of action of suramin and to monitor its effect on the biological activity of PDGF. Evidence is presented that suramin inhibits cellular binding of PDGF by binding to PDGF itself, thereby preventing it from binding to its cell surface receptor: First, while suramin inhibited ^{125}I-PDGF binding with a half maximum inhibition concentration of ~60 μM or 90 μg/ml in a simultaneous competition assay, it was inactive in a sequential radioreceptor assay, in which an inhibitor is expected to be active if it interacts with the receptor (even with relatively low affinity) but to be inactive if it interacts with PDGF. Second, suramin prevented immunoprecipitation of ^{125}I-PDGF in a dose-dependent manner, with a half maximum effective concentration of ~50 μM. Furthermore, suramin efficiently dissociated ^{125}I-PDGF bound to its cell surface receptor, whereas unlabeled PDGF even in large excess was virtually inactive. This is also in line with the proposed direct interaction between PDGF and suramin, since such an interaction can be envisaged to induce a conformational change in the PDGF-receptor complex, resulting in an increased off-rate of the complex. Reduced ^{125}I-PDGF binding in the presence of suramin correlated directly with a suramin dose-dependent inhibition of PDGF-induced incorporation of ^{3}H-thymidine into quiescent Swiss 3T3 cells and of the proliferation of these cells.

These observations suggest that suramin, by complexing with PDGF, renders this mitogen unable to bind to its physiological receptor and, thereby, prevents PDGF-initiated biological activities.

Key words: platelet-derived growth factor, suramin, protamine sulfate, mitogenic activity, growth inhibition, Swiss mouse 3T3 cells

Platelet-derived growth factor (PDGF) is one of the principal mitogens present in whole blood serum [1–4] in a number of cells of mesenchymal origin *in vitro. In*

Abbreviations used: BSA, bovine serum albumin; PBS, phosphate-buffered saline; pI, isoelectric point; PDGF, platelet-derived growth factor; IC_{50}, inhibitor concentration required for half maximum inhibitory response.

Received April 30, 1985; accepted August 5, 1985.

© **1985 Alan R. Liss, Inc.**

vivo it appears to play an important role in wound healing and arteriosclerosis [5,6]. PDGF binds to high affinity receptors on the surface of its target cells [7,8]. This interaction activates a chain of intracellular events, including tyrosine kinase activation [9–11] and receptor internalization [12], that ultimately lead to increased DNA synthesis. Agents that interfere at any point along this chain of events will help investigators to gain a better understanding of the mode of action of this mitogen. In the current study I have analyzed in greater detail the mode of action of suramin, a polyanionic compound which was recently shown to inhibit ^{125}I-PDGF binding to Balb/c 3T3 cell membranes and to dissociate efficiently and reversibly ^{125}I-PDGF bound to these membranes [13]. It is now demonstrated that suramin inhibits cellular binding of PDGF by binding to PDGF itself, thereby inactivating it as a ligand. Furthermore, it is shown that suramin inhibits PDGF-induced synthesis of DNA in quiescent Swiss 3T3 cells as well as proliferation of these cells.

MATERIALS AND METHODS

Materials

PDGF, purified as previously described [14], and ^{125}I-PDGF, radiolabeled according to Bowen-Pope and Ross [8], were obtained from Dr. R. Ross, University of Washington Medical School (Seattle, WA). Calf serum depleted of PDGF by incubation with carboxymethyl-Sephadex (designated calf CMS-I) was prepared as described by Vogel et al [15]. Suramin (Germanine®, Bayer 205) was purchased from Bayer (Leverkusen, FRG) and protamine sulfate from Sigma (St. Louis, MO). Methyl-^{3}H-thymidine (NET-027Z, 76 Ci/mmol) was from New England Nuclear (Boston, MA). Goat anti-PDGF IgG coupled to Sepharose were obtained from E.W. Raines, University of Washington Medical School. The IgG were isolated from a monospecific antiserum to purified PDGF [E.W. Raines, personal communication] by sodium sulfate precipitation and DEAE Sephacel chromatography.

Cell Culture

Swiss 3T3 cells (obtained from Dr. R. Pollack, State University of New York at Stony Brook) were maintained at 37°C in a humidified atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented to give the final concentrations of the following: 10% calf serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 1 mM each of sodium pyruvate, L-glutamine, and D-glucose (all from Gibco, Paisley, Scotland). For experimentation the cells were plated in 24-well trays (16-mm well diameter, from Costar, Mullingar, Ireland) at a density of approximately 3×10^4 cells/per well in 1.0 ml of the above medium. When cells reached the desired density, the medium was removed and replaced with 1.0 ml of RPMI-1640 medium containing 2% CMS-I.

Assays for Growth-Promoting Activity

Confluent, quiescent cultures of Swiss 3T3 cells (approximately 10^5 cells per well) were preincubated for at least 2 days with RPMI-1640 medium containing 2% CMS-I before they were used for assaying ^{3}H-thymidine incorporation into DNA. Test samples were added directly to the wells in 100 μl of 10 mM acetic acid. After a 20-hr incubation at 37°C the medium was removed and replaced with 0.5 ml RPMI-1640 medium containing 2 μCi/ml ^{3}H-thymidine and 5% calf serum. After an

additional 2-hr incubation period incorporation of radioactivity into trichloroacetic acid-insoluble material was determined following a published procedure [14]. To monitor growth, about 4×10^3 cells in RPMI-1640 medium containing 0.5% calf serum were seeded in each well of a 24-well tray. Following an overnight incubation to allow the cells to attach, the medium was removed and replaced with fresh medium containing 2.5% calf serum and suramin at the concentrations indicated. Cells were incubated at 37°C until they were harvested by trypsinization and enumerated in an electronic particle counter (Coulter Electronics, Ltd., Harpenden, UK).

Determination of Cell-Associated ^{125}I-PDGF

Cultures of Swiss 3T3 cells ($5–10 \times 10^4$ cells per well) used for ^{125}I-PDGF binding experiments were kept for at least 1 day in RPMI-1640 medium containing 2% CMS-I. The medium was aspirated and the cultures rinsed once with 1.0 ml ice-cold binding rinse (PBS containing 1 mM $CaCl_2$ and 1 mg/ml BSA). In the simultaneous competition assay, cultures were incubated for 4 hr at 4°C on an oscillatory platform with 1.0 ml binding medium (Ham's F12 medium containing 2.5 mg/ml BSA and 25 mM Hepes, pH 7.4) containing both suramin and ^{125}I-PDGF at the concentrations desired. Cells were then washed four times with binding rinse and cell-associated ^{125}I-PDGF determined following a published procedure [8]. In the sequential (radioreceptor) assay [16] cells were incubated first for 3 hr at 4°C with binding medium containing inhibitor only, then washed once with binding rinse, and finally incubated for another hour with binding medium containing ^{125}I-PDGF (0.5 ng/ml). In both assays specific ^{125}I-PDGF binding was obtained by subtracting nonspecifically bound ^{125}I-PDGF (determined in parallel cultures by including 20 ng/ml purified, unlabeled PDGF in the binding medium) from total cell-bound ^{125}I-PDGF.

Immunoprecipitation of ^{125}I-PDGF

^{125}I-PDGF (1.0 ng/ml) in PBS containing 1 mg/ml BSA and 0.5% deoxycholate was incubated with goat anti-PDGF IgG coupled to Sepharose (50 μl packed resin/ml incubation medium) for 12 hr at 4°C under end-over mixing. The resin was then washed four times by centrifugation (10,000g, 1 min) and resuspended in the same medium. The radioactivity associated with the final pellet was determined in a gamma counter.

RESULTS

Effect of Suramin on ^{125}I-PDGF Binding to Swiss 3T3 Cells

Suramin inhibited specific binding of ^{125}I-PDGF to Swiss 3T3 cells, with an IC_{50} of approximately 90 μg/ml ($\sim$60 μM) when both ligands were added simultaneously (Fig. 1). Protamine sulfate, a known competitive inhibitor of PDGF binding [17], under the same conditions inhibited specific ^{125}I-PDGF binding, with an IC_{50} of approximately 15 μg/ml (Fig. 1), in agreement with published values [17].

In a sequential radioreceptor assay [16], however, in which cells are first incubated with the test sample alone, washed, and only then incubated with ^{125}I-PDGF, suramin was inactive up to 1.4 mg/ml or 1 mM (Fig. 1). Protamine sulfate was still inhibitory, with an IC_{50} of approximately 35 μg/ml (Fig. 1).

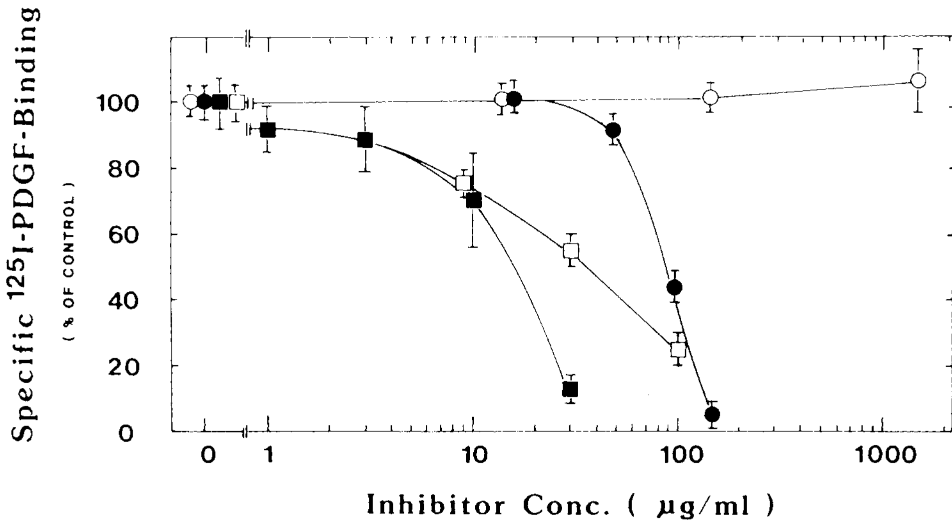

Fig. 1. Effect of suramin and protamine sulfate on the binding of ^{125}I-PDGF to Swiss 3T3 cells. Cells were incubated with ^{125}I-PDGF (0.5 ng/ml) plus the indicated doses of suramin (●,○) or protamine sulfate (■,□), either simultaneously ("coincubation assay";●,■) or sequentially, ie, incubating first with the test substance alone, washing, and only then adding ^{125}I-PDGF ("sequential assay";○,□). Following incubation with ^{125}I-PDGF (3 hr in the coincubation assay and 1 hr in the sequential assay) total and specific ^{125}I-PDGF binding were determined as described in Materials and Methods. Given are the means ± SD of triplicate determinations.

Suramin efficiently dissociated ^{125}I-PDGF bound to Swiss 3T3 cells ($T_{½}$ ~ 5 min), whereas unlabeled PDGF was virtually ineffective (Fig. 2), in agreement with what has already been described for 3T3 cell membranes [13].

Effect of Suramin on Immunoprecipitation of ^{125}I-PDGF

Suramin inhibited the precipitation of ^{125}I-PDGF by anti-PDGF antiserum coupled to Sepharose (Fig. 3). The maximum inhibition of immunoprecipitation achieved was approximately 40%, and a half maximal effect was observed at about 50 µM suramin.

Effect of Suramin on PDGF-induced ^{3}H-Thymidine Incorporation Into Swiss 3T3 Cells

Suramin inhibited PDGF-induced ^{3}H-thymidine incorporation into trichloroacetic acid-insoluble material of quiescent Swiss 3T3 cells (a measure for the mitogenic activity of PDGF *in vitro* [14]) in a concentration-dependent manner, the IC_{50} being approximately 80 µM (Fig. 4). At higher concentrations (> 100 µM) suramin also inhibited basal (no PDGF present) incorporation of ^{3}H-thymidine (data not shown).

Effect of Suramin on the Proliferation of Swiss 3T3 Cells

When cells were grown in the presence of suramin, dose-dependent inhibition of cell proliferation was observed, the IC_{50} for suramin being in the range of 50 µM

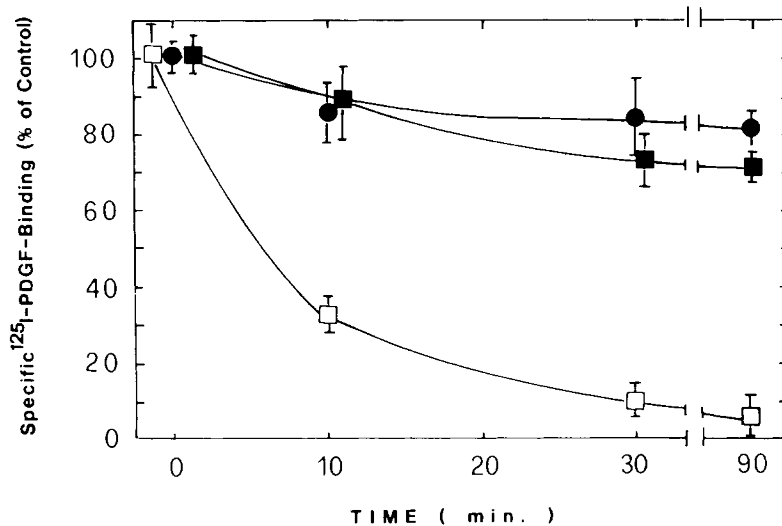

Fig. 2. Dissociation of ^{125}I-PDGF bound to Swiss 3T3 cells by suramin and unlabeled PDGF. Cells were incubated with 0.5 ng/ml ^{125}I-PDGF for 3 hr at 4°C. Following washing, fresh medium containing no further addition (●), 20 ng/ml PDGF (■), or 1 mM suramin (□) was added and incubation at 4°C continued for the time periods indicated. Specific ^{125}I-PDGF binding was determined as described in Materials and Methods.

(Fig. 5). At elevated concentrations (> 100 μM) suramin was cytotoxic, as evidenced by the time-dependent decrease in cell number (Fig. 5).

DISCUSSION

Suramin, a polyanionic compound used therapeutically as an antitrypanosomal and antifilarial drug (see Olenick [18] for review), inhibited binding of ^{125}I-PDGF to intact Swiss 3T3 cells with an IC_{50} of approximately 60 μM when both agents were added simultaneously (simultaneous competition assay). This result confirms a recent report by Williams et al [13] showing that suramin inhibits binding of ^{125}I-PDGF to Balb/c 3T3 cell membranes with an IC_{50} in the range of 50–100 μM. However, this result does not give any information on the mode of action of suramin. In particular, it does not allow one to distinguish between inhibition resulting from an interaction between suramin and the PDGF receptor and inhibition resulting from an interaction between suramin and PDGF. The radioreceptor assay developed by Bowen-Pope and Ross [16] was used to distinguish between these two possibilities. Since in this assay the cells are first incubated with test substance alone, washed, and only then exposed to ^{125}I-PDGF, only compounds interacting with the receptor but not those interacting with PDGF (such as the PDGF binding protein present in serum [19,20]) can be inhibitory. Thus, the finding that suramin in this assay was inactive up to 1 mM (Fig. 1) suggests that suramin inhibits not by interacting with the PDGF receptor, but by interacting with PDGF. However, the radioreceptor assay relies on the high affinity of the test substance for the receptor (the K_d of PDGF that is frequently used as the

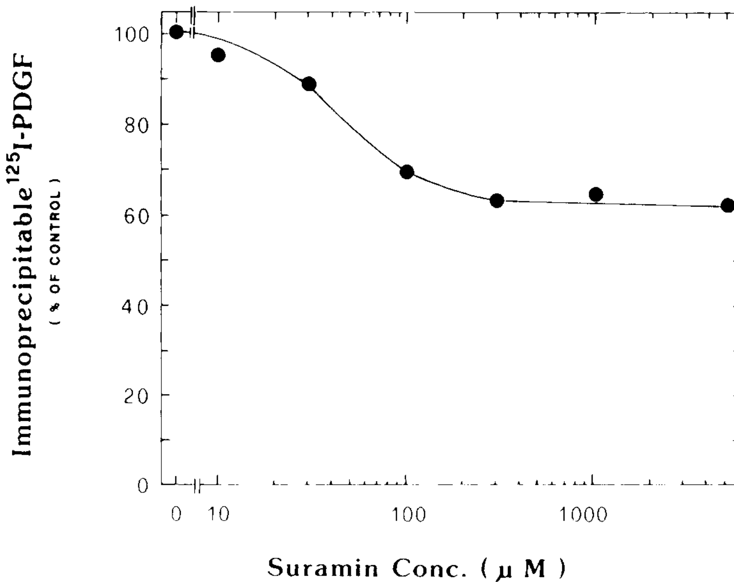

Fig. 3. Effect of suramin on immunoprecipitation of ^{125}I-PDGF. ^{125}I-PDGF (1.0 ng/ml) in the presence of the indicated amounts of suramin was immunoprecipitated with goat anti-PDGF IgG coupled to Sepharose as described in Materials and Methods. Results are expressed as percentages of controls receiving no suramin.

test substance is approximately 10^{-11} M [8]). Therefore, it cannot be ruled out a priori that suramin does bind to the receptor during the initial incubation period of this assay, but is lost during subsequent treatments as a result of its relatively weak apparent affinity. This possibility is rendered unlikely, however, by the finding that protamine sulfate, a known competitive inhibitor of PDGF binding [17] whose inhibitory potency in the simultaneous competition assay was comparable to that of suramin (IC_{50} approximately 15 μg/ml; see Fig. 1 and Bowen-Pope and Ross [16]), was only slightly less active in the sequential assay (IC_{50} approximately 35 μg/ml).

More direct evidence for an interaction between suramin and PDGF comes from immunoprecipitation experiments (Fig. 3) in which suramin inhibited precipitation of ^{125}I-PDGF by goat anti-PDGF antibodies coupled to Sepharose. The concentration of suramin at which a half maximal effect was obtained, approximately 50 μM, is in good agreement with the IC_{50} in the binding assay. The finding that the maximum inhibition achieved is only about 40% may indicate that some antibodies present in this antiserum recognize epitopes on PDGF with which suramin cannot interact.

The finding that suramin inhibited PDGF induced DNA synthesis with an IC_{50} similar to that on PDGF binding (Fig. 4) is in agreement with the current model that PDGF has to bind to its receptor to trigger mitosis.

Suramin is a polyanion and PDGF a polycation (pI about 10 [21]). Therefore, the proposed interaction between these two compounds is likely to involve electrostatic forces. The fact that the basic, arginine-rich protein protamine sulfate is a competitive inhibitor of PDGF binding was taken to suggest that electrostatic inter-

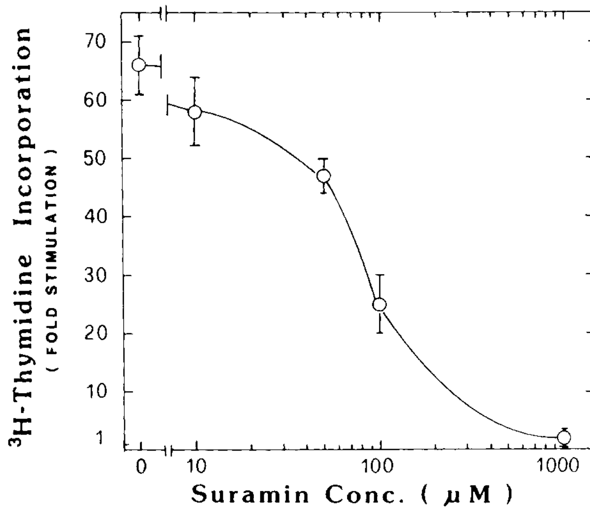

Fig. 4. Inhibition of (PDGF-induced) ^{3}H-thymidine incorporation in quiescent Swiss 3T3 cells. Confluent, quiescent cells were incubated with PDGF (0.5 ng/ml) or with buffer only for 20 hr at 37°C in the presence of the indicated amounts of suramin. Following removal of the medium cells were incubated for another 2 hr with fresh medium containing ^{3}H-thymidine. Incorporation of radioactivity into trichloroacetic acid-insoluble material was then determined as described in Materials and Methods. Results are expressed as fold stimulation (in the presence of 0.5 ng/ml PDGF) over basal incorporation (in the presence of buffer only).

actions play a role also in the binding of PDGF to its cell surface receptor [17]. Thus, suramin may inhibit PDGF binding by interacting with those positively charged amino acid residues with which PDGF interacts with the receptor. Alternatively, suramin may act allosterically, ie, bind to another domain(s) of PDGF, but induce a conformational change in PDGF and thereby render it unable to bind. The latter possibility is supported by the finding that suramin efficiently promoted dissociation of preformed ^{125}I-PDGF receptor complexes, whereas PDGF itself, which is presumably the most potent competitive inhibitor of ^{125}I-PDGF binding, had no effect (Fig. 3). The experiments reported here do not allow one to draw conclusions with regard to the specificity of the interaction between suramin and PDGF. Although preliminary experiments suggest that suramin at low concentrations does not interfere with the mitogenic activity of the anionic mitogen epidermal growth factor (data not shown), the reported binding of suramin to human serum proteins, including albumin [22], suggests that suramin may be a relatively unspecific binder of positively charged proteins in general. Nevertheless, the finding that suramin's inhibition of cell proliferation correlates well with its inhibition of PDGF-induced DNA synthesis and ^{125}I-PDGF binding (IC_{50} values in the range of 50–80 μM) suggests that also in a more complex medium, such as serum-containing medium, neutralization of PDGF is the main antiproliferative effect of suramin at low concentrations. In addition, this finding stresses the role of PDGF as a principle mitogen in whole blood serum for Swiss 3T3 cells and other cells of mesenchymal origin *in vitro*.

It is interesting to note that protamine sulfate has recently been reported to stimulate rather than to inhibit PDGF-induced DNA synthesis [23]. The explanation

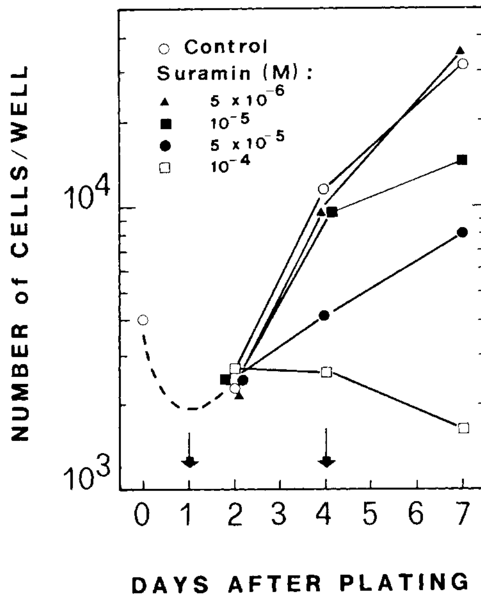

Fig. 5. Suramin inhibits proliferation of Swiss 3T3 cells. Cells were seeded and cultivated in RPMI-1640 medium containing 2.5% calf serum and suramin at the concentrations indicated until they were harvested and enumerated as described in Materials and Methods. On the days indicated by arrows medium was replaced by fresh, suramin-containing medium.

offered by the authors was that protamine sulfate, by partially blocking binding of PDGF to its receptor, may reduce internalization-dependent degradation of PDGF and thereby help to maintain a higher free concentration of PDGF. If this was a more general property of low affinity, site-directed inhibitors of PDGF, our finding that suramin inhibited rather than stimulated PDGF-induced DNA synthesis would also be compatible with an effect of suramin different from site-directed inhibition. It has to be mentioned, however, that this stimulatory effect of protamine sulfate is not firmly established yet: according to a recent report [24] and our own preliminary data (not shown) protamine sulfate can also inhibit PDGF-induced DNA synthesis.

The inhibition of proliferation of Swiss 3T3 cells by suramin suggests that this agent has some potential as inhibitor of the action of PDGF *in vivo* and thus as a therapeutic agent in conditions in which PDGF is believed to play a pathological role, such as arteriosclerosis [6]. However, this potential of suramin is rendered questionable by the known toxicity of this compound in humans [25,26]. Cytotoxicity of suramin at elevated concentrations ($\geqslant 100\ \mu M$) *in vitro* is also evident from the decrease in cell number (Fig. 5) and from an inhibition of basal (no PDGF present) incorporation of ^{3}H-thymidine at these concentrations. Thus, suramin may be mainly of use as a research tool, eg, to unmask occupied receptors or to reverse downregulation of receptors as it has been demonstrated with simian sarcoma virus-transformed normal rat kidney cells [27]. Nevertheless, it should be pointed out that suramin is also a potent inhibitor of the reverse transcriptase of a number of retroviruses [28] and very recently has been found [29] to block *in vitro* the infectivity and cytopathic effect of a member of the human T-cell leukemia virus family (HTLV-III), which has

been linked to the acquired immune deficiency syndrome (AIDS). Thus, suramin is likely to receive wider attention in the future.

ACKNOWLEDGMENTS

I thank Maryse Broussé for excellent technical assistance and Elaine W. Raines, Drs. Hans Rudolf Baumgartner, Russell Ross, and Bernd Weimann for a critical reading of this paper.

REFERENCES

1. Balk SD: Proc Natl Acad Sci USA 68:271–275, 1971.
2. Ross R, Glomset JA, Kariya B, Harker L: Proc Natl Acad Sci USA 71:1207–1210, 1974.
3. Kohler N, Lipton A: Exp Cell Res 87:297–301, 1974.
4. Westermark B, Wasteson A: Adv Metab Disord 8:85–100, 1975.
5. Ross R, Glomset JA: Science 180:1332–1339, 1973.
6. Ross R, Glomset JA: N Engl J Med 295:369–377, 420–425, 1976.
7. Heldin CH, Westermark B, Wasteson A: Proc Natl Acad Sci USA 78:3664–3668, 1981.
8. Bowen-Pope DF, Ross R: J Biol Chem 257:5161–5171, 1982.
9. Ek B, Heldin CH: J Biol Chem 257:10486–10492, 1982.
10. Nishimura J, Huang JS, Deuel TF: Proc Natl Acad Sci USA 79:4303–4307, 1981.
11. Cooper JA, Bowen-Pope DF, Raines E, Ross R, Hunter T: Cell 31:263–273, 1982.
12. Heldin CH, Wasteson A, Westermark B: J Biol Chem 257:4216–4221, 1982.
13. Williams LT, Tremble PM, Lavin MF, Sunday ME: J Biol Chem 259:5287–5294, 1984.
14. Raines EW, Ross R: J Biol Chem 257:5154–5160, 1982.
15. Vogel A, Raines E, Kariya B, Rivest MJ, Ross R: Proc Natl Acad Sci USA 75:2810–2814, 1978.
16. Bowen-Pope DF, Ross R: Methods Enzymol (in press).
17. Huang JS, Huang SS, Kennedy B, Deuel TF: J Biol Chem 257:8130–8136, 1982.
18. Olenick JG: Antibiotics 3:699–703, 1975.
19. Huang TS, Huang SS, Deuel TF: Proc Natl Acad Sci USA 81:342–346, 1984.
20. Raines EW, Ross R: Proc Natl Acad Sci USA 81:3424–3428, 1984.
21. Heldin CH, Wasteson A, Westermark B: Exp Cell Res 109:429–437, 1977.
22. Müller WE, Wollert U: Biochim Biophys Acta 427:465–480, 1976.
23. DiCorleto PE, Poptic EJ: J Cell Biochem (Suppl)8a:252.
24. Huang JS, Nishimura J, Huang SS, Deuel TF: J Cell Biochem 26:205–220, 1984.
25. Hawking F: Adv Pharmacol Chemother 15:289–291, 1978.
26. Duke BOL: Bull WHO 39:157–163, 1968.
27. Garret JS, Coughlin SR, Niman HL, Tremble PM, Giels GM, Williams LT: Proc Natl Acad Sci USA 81:7466–7470, 1984.
28. De Clercq E: Cancer Lett 8:9–22, 1979.
29. Mitsuya H, Popovic M, Yarchoan R, Matsushita S, Gallo RC, Broder S: Science 226:172–174, 1984.