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Suppression of Polymorphonuclear Leukocyte Bactericidal Activity by Suramin

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Suramin is a polyanionic compound with potent antineoplastic properties. Because polymorphonuclear leukocytes (PMNs) are a crucial component of host defenses against bacteria and fungi, the effects of suramin on PMN function were studied in vitro. PMNs from healthy donors were incubated with concentrations of suramin of 1 to 1,000 μg/ml (within and exceeding the therapeutic range) for 30 min, and PMN functional parameters were subsequently assessed. Suramin had no effect on viability, chemotaxis to *N*-formylmethionyl leucyl phenylalanine, phagocytosis of *Candida albicans*, or superoxide anion production in response to phorbol myristate acetate and formylmethionyl leucyl phenylalanine. Fungicidal activity against *C. albicans* blastoconidia was unaffected at a suramin concentration of <500 μg/ml, whereas at higher concentrations a slight suppression was observed ($P = 0.04$). Bactericidal activity against *Staphylococcus aureus* was significantly suppressed by concentrations of ≥100 μg/ml ($P < 0.01$). Phagocytosis of *S. aureus* was also significantly impaired at ≥10 μg/ml ($P < 0.05$). The presence of 10% human serum during pretreatment did not abrogate the suramin-induced suppression of bactericidal activity. Treatment of PMNs with granulocyte colony-stimulating factor (4,000 U/ml) for 30 min prior to the addition of suramin (250 μg/ml) improved the bactericidal defect ($P = 0.02$). The PMN functional impairment may be related to increased susceptibility to bacterial infections, and granulocyte colony-stimulating factor may improve the defect.

Suramin is a polyanionic compound that was originally synthesized for use as an antiparasitic agent (8, 21). Because it possesses inhibitory properties against reverse transcriptase, it was also among the first agents to be studied as an antiretroviral agent in the treatment of human immunodeficiency virus infection (12). Suramin also has been shown to have potent growth inhibitory properties against tumor cell lines and, therefore, has recently undergone clinical evaluation as an antineoplastic agent (21).

Polymorphonuclear leukocytes (PMNs) play a crucial role in host defenses against bacteria and fungi. A number of chemotherapeutic agents, including antineoplastic and antimicrobial agents, can significantly impair the function of PMNs in vitro, potentially increasing the risk for infectious complications. Specifically, investigators have found that antineoplastic agents such as adriamycin, vinblastine, and nitrosourea and antibiotics such as amphotericin B can suppress various activities of PMNs (1, 4, 13, 16, 17, 20). To determine whether suramin has an adverse impact on PMN function, the effects of a wide range of concentrations of the compound within and exceeding clinically relevant dosages were studied.

(Preliminary results of this study were presented at the Infectious Diseases Society of America Annual Meeting, Anaheim, CA, 10 to 12 October 1992 [18a].)

MATERIALS AND METHODS

Subjects. Twenty-seven healthy adult volunteers served as donors for testing the effects of suramin on PMN function. Ten of them donated blood more than once (two to four times) during the course of the studies.

Suramin. Suramin was obtained as a sterile powder from the Investigational Drug Branch of the National Cancer Institute, Bethesda, Md. Stock solutions of 100 mg/ml were prepared by dissolving the compound in distilled H_2O and were kept frozen at −20°C. Final dilutions were made in Hanks' balanced salt solution (HBSS) prior to each experiment.

Microorganisms. The strains of *Staphylococcus aureus* SA and *Candida albicans* 86-21 used in the present studies were clinical isolates, both of which have been well characterized and used extensively for previous in vivo and in vitro studies (18–20). They were preserved in aliquots of skim milk at −70°C and were grown on Trypticase soy agar plates (*S. aureus*) or Sabouraud agar plates (*C. albicans*) for 18 to 24 h at 37°C before each experiment. Under these conditions, *C. albicans* grows exclusively as blastoconidia. *S. aureus* bacteria were taken from Trypticase soy agar plates and were grown in Trypticase soy broth for 2 to 3 h at 37°C before each experiment, to obtain log-phase growth. They were then washed with HBSS and adjusted to 2×10^7 CFU/ml (bactericidal assay) or 2.5×10^8 CFU/ml (bacterial phagocytosis) by spectrophotometric measurement. Blastoconidia were obtained directly from Sabouraud agar plates and were suspended in HBSS at a final concentration of 10^7 CFU/ml.

Separation of PMNs. The PMNs were prepared from heparinized whole blood (preservative-free heparin, 5 to 10 U/ml) by dextran sedimentation of the whole blood and then Ficoll centrifugation of the leukocyte-rich supernatants as described previously (2, 18). Contaminating erythrocytes were removed by hypotonic lysis. The PMNs were then resuspended in HBSS without Ca^{2+} or Mg^{2+}. This consistently resulted in preparations of PMNs with >95% purity and >95% viability, as judged by trypan blue exclusion.

Pretreatment of PMNs with suramin. Before the assays, 2.5×10^6 or 1×10^6 PMNs suspended in HBSS were incubated at 37°C for 30 min in the presence of buffer or concentrations of suramin ranging from 1 to 1,000 μg/ml. In

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recent clinical trials, levels of suramin in plasma have been targeted to range between 100 to 300 μg/ml (11). The concentrations of suramin were maintained during the PMN functional assays. In those bactericidal experiments in which the effect of suramin on PMNs was assessed in the presence of serum, 10% type AB human serum (GIBCO Laboratories, Grand Island, N.Y.) was added before the addition of suramin.

Recombinant human granulocyte colony-stimulating factor (G-CSF) produced in *Escherichia coli* was obtained from Amgen Inc., Thousand Oaks, Calif., and had a specific activity of 2×10^8 U/mg of protein, as determined by granulocyte-macrophage colony formation of nonadherent bone marrow cells in semisolid medium. The preparation contained <0.1 ng of protein endotoxin per mg, as tested by the *Limulus* amebocyte lysate assay. Sixty minutes before the initiation of the bactericidal assay, G-CSF was added to the PMNs and the cells were incubated at 37°C. Thirty minutes later, suramin was added at appropriate concentrations and the incubation was continued until the initiation of the assay with the addition of the organism.

Chemotaxis. Chemotaxis of PMNs was assessed by the method of Harvath et al. (7). Forty-five microliters of suspensions containing 2×10^6 PMNs per ml in HBSS (9×10^4 PMNs per well) was added to the upper wells of a 48-well chemotaxis chamber (Neuroprobe, Inc., Cabin John, Md.). Polyvinylpyrrolidone coating-free polycarbonate filters with 3-μm pores were used to separate the upper from the lower chambers in order to measure the entire population of chemotactically active cells. The wells of the lower chambers were filled with either 10^{-8} M *N*-formylmethionyl leucyl phenylalanine (FMLP; Sigma Chemical Co., St. Louis, Mo.), or control buffer (HBSS). To assess PMN migration, the chamber was incubated at 37°C in a humidified atmosphere with 5% CO_2 for 60 min. The filters were then removed, air dried, fixed, and stained with modified Wright-Giemsa stain (Diff-Quick; Diagnostic Systems Inc., Gibbstown, N.J.). Migrating cells were adherent to the lower surface of the filter (7). The number of cells per ×400 magnification field that were adherent on the lower surface of the filters was counted by direct microscopy. A chemotactic index was calculated as the ratio of the number of cells which were attracted to FMLP divided by the number of randomly migrating cells.

Fungicidal assay. The fungicidal activity of PMNs was assessed by a CFU assay. Briefly, *C. albicans* blastoconidia were preopsonized by incubation at 37°C for 30 min in the presence of 50% type AB human serum (GIBCO) and with a subsequent wash of the excess serum, mixed with 10^6 PMNs in a 1/1 ratio in 1 ml of HBSS containing 0.1% albumin, and incubated at 37°C for 1 or 2 h on a shaker. After complete lysis of PMNs with sterile H_2O, dilutions were made, plated in duplicate onto Sabouraud plates, and incubated at 37°C for 18 h. Colonies were counted and fungicidal activity was calculated by using the following formula: percent survival of *C. albicans* at $T_x = (C_x/C_0) \times 100$, where C_x is the number of CFU at time T_x, and C_0 is the number of CFU in the initial inoculum.

Bactericidal assay. The bactericidal activity of PMNs was also assessed by a CFU assay that measured the total intracellular and extracellular survival of bacteria. *S. aureus* bacteria were mixed with 2.5×10^6 PMNs in a 1/1 ratio in 1 ml of HBSS containing 10% normal type AB human serum. Preparations were incubated at 37°C on a shaker, and samples were obtained at time zero and after 1 and 2 h. The PMNs were completely lysed in sterile H_2O containing 0.01% albumin, serial dilutions were made, the serial dilutions were plated in duplicate onto Trypticase soy agar plates, and the plates were incubated for 18 h at 37°C. Colonies were counted, and the bactericidal activity was assessed by calculating the percent survival of bacteria at specific time points compared with the initial number of bacteria. The formula used for that assessment was as follows: percent survival of *S. aureus* at $T_x = (C_x/C_0) \times 100$, where C_x is the number of CFU at time T_x, and C_0 is the number of CFU in the initial inoculum.

Phagocytosis of *C. albicans*. PMN phagocytosis of *C. albicans* was assessed by direct microscopy. *C. albicans* blastoconidia were opsonized with serum, mixed with 10^6 PMNs in a 1/1 ratio in 1 ml of HBSS, and incubated at 37°C for 15 min on a shaker. Samples were then obtained, cytocentrifuged, and stained with modified Wright-Giemsa stain. Percent phagocytosis was calculated as the proportion of PMNs containing one or more blastoconidia after counting 100 PMNs. A phagocytic index was also calculated as the average number of blastoconidia associated with each phagocytosing PMN. No attempt to distinguish between attachment on PMN surfaces or actual ingestion of blastoconidia was made.

Phagocytosis of *S. aureus*. Phagocytosis of *S. aureus* by PMNs was also assessed by direct microscopy as described previously (5, 19). Briefly, 10^8 *S. aureus* bacteria preopsonized with serum were mixed with 10^6 PMNs that were previously treated with buffer or various concentrations of suramin in 1 ml of HBSS; the final effector-to-target cell ratio was 1/100. Preparations were rocked at 37°C. Samples were obtained after 15 min and were immediately mixed with cold *N*-ethylmaleimide (0.1 mM; Sigma) to inhibit additional phagocytosis. They were then incubated with lysostaphin (20 U/ml; Sigma) at 37°C for an additional 10 min to lyse the nonphagocytosed, extracellular bacteria. Preparations were cytocentrifuged and stained with modified Wright-Giemsa. Direct microscopy was used to measure phagocytosis. Percent phagocytosis was calculated as the proportion of PMNs containing one or more bacteria among 100 PMNs. A weighted phagocytic index was calculated by multiplying the number of PMNs with 1 to 10, 11 to 20, 21 to 30, 31 to 40, or >40 ingested organisms by 1, 2, 3, 4, or 5, respectively, and dividing the total score by the number of PMNs examined (usually 100).

Superoxide anion assay. Superoxide anion production in response to FMLP was assessed by spectrophotometry. One million PMNs were mixed with 5×10^{-5} M cytochrome *c* (Sigma) and either 0.5 μg of phorbol myristate acetate (PMA; Sigma) per ml or 5×10^{-7} M FMLP in 1 ml of HBSS, and the mixture was then incubated at 37°C for 5 min. The change in A_{550} was measured on a Gilford 260 spectrophotometer equipped with a thermostatted cuvette holder (CIBA-Corning Diagnostics Corp., Oberlin, Ohio). The superoxide produced was then calculated by using the millimolar extinction coefficient for reduced cytochrome *c*.

Statistics. The paired *t* test was used to compare PMN activity as it was affected by various concentrations of suramin with PMN activity at the baseline. No adjustment for the multiplicity of tests was made because of the correlations between them. All reported P values are two-tailed.

RESULTS

Toxicity. The viability of PMNs remained at >90%, as determined by trypan blue exclusion, when they were incubated with suramin at concentrations of 1 to 1,000 μg/ml.

TABLE 1. Chemotaxis of neutrophils (PMNs) pretreated with various concentrations of suramin[a]

Suramin concn (μg/ml)	No. of PMNs migrating		Chemotactic index
	Random	Directed	
Control	12 ± 3	52 ± 17	4.6 ± 1.2
1	18 ± 6	44 ± 18	3.1 ± 0.9
10	22 ± 7	41 ± 9	3.8 ± 1.7
100	16 ± 4	42 ± 11	5.6 ± 3.3
500	21 ± 6	34 ± 9	2.3 ± 0.6
1,000	14 ± 4	34 ± 13	3.9 ± 1.5

[a] Number of PMNs per ×400 magnification field migrating randomly or in response to 10^{-8} M FMLP (mean ± SEM of seven experiments). The Chemotactic index is the ratio of the number of cells which were attracted to FMLP divided by the number of randomly migrating cells. No differences between suramin concentrations and controls were significant ($P > 0.05$).

Chemotaxis. No significant effect on the chemotaxis of PMNs in response to FMLP was observed at any of the concentrations of suramin that were studied (Table 1). The chemotactic index of the untreated PMNs was 4.6 ± 1.2 (mean ± standard error of the mean [SEM] derived from seven experiments) and was not clearly affected by concentrations of suramin that were two to five times greater than those achieved in serum when this agent is used clinically. Random migration of PMNs was also essentially unaffected by pretreatment of the PMNs with suramin.

Fungicidal activity. There was no significant effect of suramin on PMN-induced growth inhibition of *C. albicans* blastoconidia at concentrations of less than 500 μg/ml. At concentrations exceeding those that are clinically tolerable (i.e., 500 and 1,000 μg/ml), there was a relatively small but statistically significant decreased killing after 2 h of PMN-blastoconidia incubation compared with that at the baseline ($P = 0.04$ and 0.03, respectively; Fig. 1). No decreased killing was observed after 1 h of PMN-blastoconidia incubation, even at the highest concentration of suramin (1,000 μg/ml). In parallel control experiments, suramin at concentrations of up to 1,000 μg/ml had no direct effects on the growth of *C. albicans* blastoconidia.

Bactericidal activity. In contrast, pretreatment of PMNs with suramin caused a profound impairment of the bactericidal activity of PMNs (Fig. 2). After both 1 and 2 h of incubation of PMNs with *S. aureus*, a highly significant

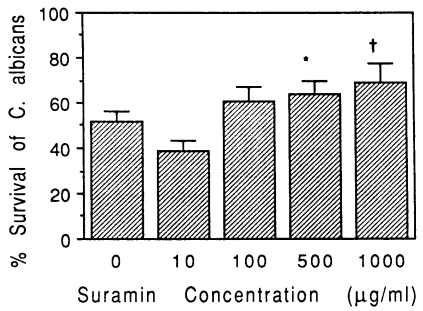

FIG. 1. Effects of suramin on growth inhibition of *C. albicans* by neutrophils (PMNs). PMNs were preincubated with buffer or with various concentrations of suramin for 30 min at 37°C. Equal numbers of PMNs and serum-preopsonized *C. albicans* blastoconidia (10^6) were mixed and were incubated at 37°C for 2 h. The results are expressed as means ± SEMs of seven experiments. Symbols indicate significance of differences from the baseline (untreated PMNs): *, $P = 0.04$; †, $P = 0.03$.

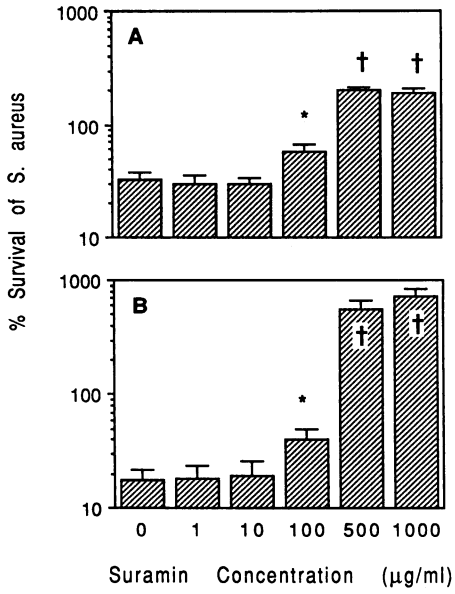

FIG. 2. Effects of suramin on growth inhibition of *S. aureus* by neutrophils (PMNs). The PMNs were pretreated with buffer or with various concentrations of suramin at 37°C for 30 min, and equal numbers of PMNs and *S. aureus* (2.5×10^6) were mixed and incubated for 1 h (A) and 2 h (B) at 37°C. The data are expressed as means ± SEMs of the percent survival of *S. aureus* derived from 9 to 11 experiments. Symbols indicate significance of differences from the baseline (untreated PMNs): *, $P < 0.01$; †, $P \leq 0.001$.

increase in the percent survival of bacteria was observed in comparison with that of bacteria treated with control PMNs. While the percent survival of *S. aureus* bacteria incubated with untreated PMNs was only 32.5% ± 5.1% and 17.8% ± 3.9% after 1 and 2 h, respectively, this was significantly increased to 56.5% ± 9.6% and 40.3% ± 9.0% at 1 and 2 h after pretreatment of PMNs with 100 μg of suramin per ml (for both time points, differences from the baseline [no drug] were significant at $P < 0.01$). At the higher concentration of 500 μg/ml, suramin pretreatment of PMNs totally ablated the PMN bactericidal capacity, allowing *S. aureus* to grow uninhibitedly (at 1 and 2 h, percent survival was 204% ± 12% and 547% ± 117%, respectively; $P \leq 0.001$). These increases were not due to an effect on the organism per se, because at concentrations of up to 1,000 μg/ml, suramin had no direct enhancing effect on the growth of *S. aureus*.

The experiments described above were performed in the absence of serum during pretreatment. Since suramin is very highly bound to the serum proteins, the bactericidal activity of PMNs was also studied in the presence of serum. In parallel experiments with or without the presence of serum during the pretreatment period, it was found that the presence of 10% normal serum did not significantly alter the PMN bactericidal defect caused by suramin (Table 2).

Phagocytosis. To explore the mechanism of differential suppression of PMN function, the phagocytic activity of suramin-pretreated PMNs was assessed by using *C. albicans* or *S. aureus* as a target. None of the concentrations of suramin that were tested significantly affected the phagocytosis of *C. albicans* blastoconidia by normal PMNs (Table 3). The percent phagocytosis and the phagocytic index were not affected by even the highest drug concentrations used (1,000 μg/ml).

In contrast, suramin significantly impaired the phagocytic

TABLE 2. Effect of serum on suramin-induced suppression of bactericidal activity of neutrophils (PMNs)

Suramin concn (μg/ml)	% Survival of *S. aureus*[a]			
	No serum		With serum	
	1 h[b]	2 h	1 h	2 h
0	28.8 ± 7.9	23.7 ± 9.2	44.1 ± 15.5	26.9 ± 12.4
100	59.9 ± 16.9	42.0 ± 18.5	39.3 ± 12.1	29.1 ± 12.9
500	172 ± 29	712 ± 259	123 ± 19	1113 ± 935

[a] Values are means ± SEMs of four experiments. There were no significant differences between survival with or without serum.
[b] Incubation time of PMNs with *S. aureus*.

activity of PMNs against *S. aureus*. At concentrations as low as 10 μg/ml, the drug significantly decreased both percent phagocytosis and especially the weighted phagocytic index ($P < 0.01$; Fig. 3).

Superoxide anion production. To examine the contribution of oxidative metabolic burst on the impaired bactericidal activity of PMNs, the superoxide anion produced by PMNs was assessed upon stimulation with either PMA or FMLP. None of the suramin concentrations tested (1 to 1,000 μg/ml) had a significant effect on the production of superoxide anion in response to the two stimuli (Table 4).

Effect of G-CSF on suramin-induced suppression of PMN bactericidal activity. G-CSF has been shown to enhance the bactericidal activities of normal as well as defective PMNs, restoring the activity of the latter cells to normal values in vitro (19). To test the ability of G-CSF to prevent the suramin-induced bactericidal defect, PMNs were treated with either G-CSF (4,000 U/ml) or buffer before suramin (250 or 500 μg/ml) was added to the PMNs. The concentration of G-CSF given above was chosen on the basis of previous experience that it is the optimal concentration to enhance the bactericidal activity of PMNs (19). Suramin pretreatment of PMNs was followed by a bactericidal defect, as indicated by the increased percent survival of *S. aureus* (Table 5). G-CSF alone enhanced the bactericidal activity of untreated PMNs at 1 h ($P = 0.037$). In addition, it appeared to improve the bactericidal defect of suramin-pretreated PMNs. This was particularly evident at 250 μg/ml (p = 0.023) but was less obvious at the high concentration of 500 μg/ml.

DISCUSSION

In the present study, in vitro treatment of human PMNs with suramin was shown to cause an impairment in the bactericidal activity of the cells against *S. aureus* bacteria. This bactericidal impairment of PMNs was associated with a defect in phagocytosis of *S. aureus* but not with a defective oxidative burst in response to PMA or FMLP. Thus, suramin can cause a differential acquired defect of the function of PMNs that is similar to those of the antimicrobial and antineoplastic agents such as amphotericin B, adriamycin, vinblastine, nitrosourea, and others that have previously been shown to cause defects in various PMN functions (1, 4, 13, 16, 17, 20).

It is possible that the suppressive effect of suramin on phagocytosis of *S. aureus* is mediated through its binding on specific peptides of the cytoplasmic membrane of PMNs, including the Fc and C′ receptors that play a very important role in the attachment and ingestion of bacteria by PMNs as well as the subsequent inclusion of the organisms in phagosomes. This hypothesis may explain the finding that the

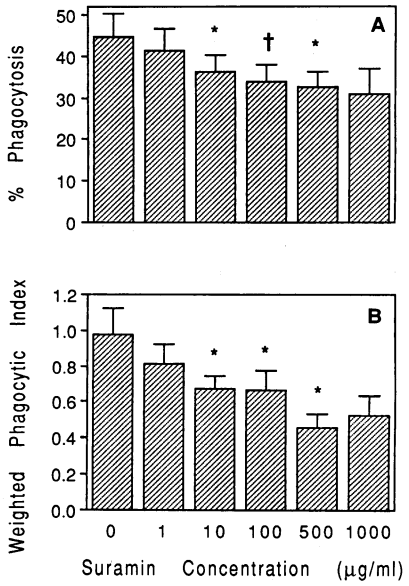

FIG. 3. Effects of suramin on phagocytosis of *S. aureus* by neutrophils (PMNs). The PMNs were pretreated with buffer or various concentrations of suramin at 37°C for 30 min, and they were then incubated with preopsonized *S. aureus* for 15 min. The data are expressed as means ± SEMs of 8 to 10 experiments. Percent phagocytosis (A) and weighted phagocytic index (B) were calculated. Symbols indicate significance of differences from the baseline (untreated PMNs): *, $P < 0.05$; †, $P < 0.01$.

TABLE 3. Phagocytosis of *C. albicans* by neutrophils (PMNs) pretreated with various concentrations of suramin[a]

Suramin concn (μg/ml)	%[b]	PI[c]
Control	77.8 ± 3.4	2.26 ± 0.19
1	80.0 ± 4.5	2.26 ± 0.22
10	76.0 ± 3.4	2.15 ± 0.16
100	79.3 ± 3.8	2.20 ± 0.18
500	80.8 ± 3.1	2.11 ± 0.25
1,000	79.0 ± 3.1	2.19 ± 0.18

[a] Values are means ± SEMs of four experiments.
[b] Percent phagocytosis was calculated as follows: (number of phagocytosing PMNs divided by the total number of PMNs counted) × 100.
[c] PI phagocytic index, which was calculated as follows: total number of PMN-associated *C. albicans* blastoconidia divided by the number of PMNs with blastoconidia phagocytosed or attached on them.

TABLE 4. Superoxide anion production by neutrophils pretreated with various concentrations of suramin[a]

Suramin concn (μg/ml)	Superoxide anion production after pretreatment with:	
	PMA	FMLP
Control	7.7 ± 2.3	6.6 ± 1.0
1	6.8 ± 2.0	6.6 ± 1.1
10	7.4 ± 1.7	6.5 ± 1.0
100	6.8 ± 0.6	7.6 ± 1.1
500	9.9 ± 2.0	7.3 ± 1.4
1,000	9.9 ± 1.9	6.9 ± 1.3

[a] PMA (0.5 μg/ml) and FMLP (5×10^{-7} M) were used as stimuli. The results are expressed as the means ± SEMs of six experiments. There were no significant differences between suramin concentrations and control PMNs.

TABLE 5. Effect of G-CSF on suramin-induced suppression of PMN bactericidal activity[a]

Suramin concn (μg/ml)	% Survival of *S. aureus*			
	No G-CSF		With G-CSF	
	1 h[b]	2 h	1 h	2 h
0	64.6 ± 9[c]	51.8 ± 13.3	40.8 ± 6.7[c]	41.7 ± 9.2
250	137 ± 12	251 ± 58[d]	136 ± 13	180 ± 49[d]
500	262 ± 46[e]	1373 ± 557	150 ± 14[e]	747 ± 238

[a] The results are expressed as the means ± SEMs of seven experiments.
[b] Incubation time of PMNs with G-CSF.
[c] Between experiments with or without G-CSF at 1 h, $P = 0.037$.
[d] Between experiments with or without G-CSF at 2 h, $P = 0.023$.
[e] Between experiments with or without G-CSF at 1 h, $P = 0.104$.

suramin-treated PMNs generate normal amounts of superoxide anion when stimulated by, for example, FMLP and PMA, but they do not ingest bacteria efficiently. These two stimuli were specifically selected, since PMA stimulates the oxidative mechanism of PMNs, bypassing the plasma membrane receptors, and FMLP is a bacterial peptide analog that binds to specific plasma membrane receptors to stimulate the same mechanism.

Suramin has previously been reported to be a lysosomatropic agent (6, 9). It inhibits the formation of phagolysosomes that occurs by fusion of the membranes of phagosomes and lysosomes after ingestion of *Saccharomyces cerevisiae* (3) but not after ingestion of the intracellular bacterium *Listeria monocytogenes* (15). Perhaps a similar inhibition of plasma membrane fusion by suramin accounts for the phagocytic impairment observed in the present study.

In the present study, the nonoxidative metabolic bactericidal activity was not explored as an additional component of PMN function that may be impaired by suramin. Pesanti (15) has previously found that the intracellular killing of *S. aureus* remains unaffected after the influence of suramin. This finding is consistent with our finding that oxidative metabolic burst is not affected by suramin but that phagocytosis is impaired. Taken together, the findings of the two studies suggest that the observed defect in the overall bactericidal activity may be due to the increased number of bacteria that remain extracellularly and that multiply without inhibition, even though the intracellular killing of the phagocytosed bacteria is intact. The bactericidal assay used in the present study measured the total microbicidal activity of phagocytes but did not distinguish between intracellular killing and extracellular inhibition of growth.

The defect in phagocytosis was observed only with bacterial targets but not with blastoconidia of *C. albicans*, even after exposure to very high concentrations of suramin. The reasons for this discordance are not clear. Although fungicidal activity was somewhat impaired only at the prolonged incubation period of 2 h, this defect was small in comparison with the bactericidal impairment.

The profound bacterial phagocytic and bactericidal defects might contribute to a heightened susceptibility of suramin-treated patients to bacterial infections. This susceptibility may be clinically realized when an additional risk of infection exists (e.g., defects of physical barriers with a central venous catheter).

G-CSF appeared to enhance the bactericidal activities of both normal and suramin-treated PMNs to a lesser degree. Although the function of suramin-treated PMNs was not completely restored, it was improved, especially at the clinically relevant concentration of 250 μg/ml. G-CSF has been shown to have immunoenhancing properties on the function of mature normal and defective PMNs, augmenting the phagocytosis of bacteria, oxidative burst, and the release of microbicidal oxygen metabolites as well as bactericidal activity (10, 19). Thus, our finding of the prevention of the bactericidal defect caused by suramin is not surprising. The concentration of G-CSF used in the present study (4,000 U/ml) is within the range of concentrations achievable in the serum of patients treated with the cytokine (14, 22). This finding suggests that G-CSF may also act on defective PMNs of suramin-treated patients, enhancing their function. The role of G-CSF in preventing or treating bacterial infections in patients receiving suramin is worthy of further study.

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