ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1985, p. 667–674
0066-4804/85/110667-08$02.00/0

Vol. 28, No. 5

Uptake, Intracellular Activity, and Influence of Rifampin on Normal Function of Polymorphonuclear Leukocytes

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Received 31 December 1984/Accepted 2 July 1985

Quinone and hydroquinone forms of rifampin accumulated in normal polymorphonuclear leukocytes (PMN) (maximal cellular to extracellular concentration ratio [C/E_{max}] ± standard error of the mean, 9.36 ± 0.54 and 8.82 ± 0.65, respectively, after 5 to 10 min) and chronic granulomatous disease PMN (C/E_{max}, 13.76 ± 0.77 and 14.29, respectively). Uptake of rifampin was influenced by incubation temperature and extracellular pH but not by phorbol myristate acetate stimulation or metabolic inhibitors. At extracellular concentrations between 0.06 and 5.0 mg/liter, rifampin significantly reduced the number of staphylococci surviving inside chronic granulomatous disease PMN, thus compensating for the bactericidal defect inherent with this disease. Spontaneous migration and chemotaxis of normal PMN were unaffected by rifampin. However, phagocytosis of yeast particles and oxygen consumption of stimulated PMN were moderately depressed, and O_2^- production and chemiluminescence were significantly depressed in a dose-dependent manner. The bactericidal activity of normal PMN was not impaired. Inhibition of chemiluminescence and O_2^- release were also observed in a cell-free system. We conclude that rifampin possesses favorable characteristics for the effective elimination of intracellular microorganisms. Further studies are needed to evaluate the in vivo significance of ion scavenging by rifampin, which could be hazardous to immunocompromised patients.

The use of rifamycins has been limited to the treatment of tuberculosis for nearly two decades. In recent years, they were found to be active against a number of other intracellular bacteria (legionella, chlamydia, meningococci). Owing to favorable tissue penetration, they are also considered useful for the treatment of certain staphylococcal and hemophilus infections (4). Rifamycins belong to a group of naphthalenic ansamycins and inhibit the DNA-dependent RNA polymerase in bacteria. Among several hundred chemical modifications of the original molecule, only rifamide, rifamycin SV, and rifampin [3-(4-methyl-1-piperazinyliminomethyl)rifamycin-SV] have been used clinically. The latter is the form most widely used in Europe.

Rifampin has been shown to accumulate and retain its bactericidal activity in granulocytes (13, 22, 24). However, an immunosuppressive potential of rifampin has been suggested (20). Conflicting data exist about negative side effects of rifampin on granulocyte function (5, 7, 8, 16, 21, 23, 25). Although patients might benefit from the intracellular activity of the agent, impairment of granulocyte function could be detrimental to patients suffering from immunodeficiencies.

In an attempt to reevaluate these findings, we tested intracellular accumulation and antistaphylococcal activity of rifampin in normal granulocytes and granulocytes from patients with defective intracellular killing (chronic granulomatous disease [CGD]). In this study we demonstrate that the quinone of rifampin (formed by the spontaneous oxidation of the hydroquinone form of rifampin) does not differ from the hydroquinone with respect to uptake. Furthermore, we studied the influence of rifampin at different concentrations on phagocytosis, chemotaxis, oxygen consumption, formation of superoxide anion, and chemiluminescence of stimulated normal polymorphonuclear leukocytes (PMN).

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MATERIALS AND METHODS

Isolation of PMN. Granulocytes were isolated with informed consent from peripheral blood of 20 healthy volunteers (age, 20 to 55 years; both males and females) and 14 patients with CGD (age, 5 to 20 years; males). Antibiotics were discontinued at least 24 h before samples were taken. Heparinized blood was sedimented with dextran, and the leukocyte-rich plasma was centrifuged across a Ficoll-Hypaque gradient as described previously (3). Remaining erythrocytes in the sediment were lysed hypotonically. The final suspension contained ≥95% PMN. Viability of the cells assessed by the Trypan blue dye exclusion test was ≥95%.

Intracellular accumulation of rifampin. Radioactive rifampin (specific activity, 4 μCi/mg; CIBA GEIGY AG, Basel, Switzerland), either the quinone or hydroquinone form (Fig. 1), was added for 10 min to a suspension of PMN (3×10^7/ml of Hanks balanced salt solution [HBSS]) preincubated at 37°C. Test preparations contained more than 85% of the quinone and between 90 and 36% of the hydroquinone (loss due to spontaneous oxidation), by thin-layer chromatography. The relative contents of the hydroquinone did not measurably affect the results as shown by direct comparison. At intervals (0.5, 1.5, 3, 5, 10, 20, and 30 min), fractions from the suspension were taken and centrifuged across a silicone oil gradient (density 1.05 g/liter; Versilube F50; General Electric, Bergen op Leven, The Netherlands) to determine the PMN/buffer coefficient as described previously (9, 19). After lysis of the cells in a tissue solubilizer (Soluene 100; Packard Instrument Co., Inc., Rockville, Md.), cell-associated radioactivity (pellet) and extracellular activity (supernatant) were separately counted in a beta-counter (MR 300; Kontron, Birsfelden, Switzerland). After determination

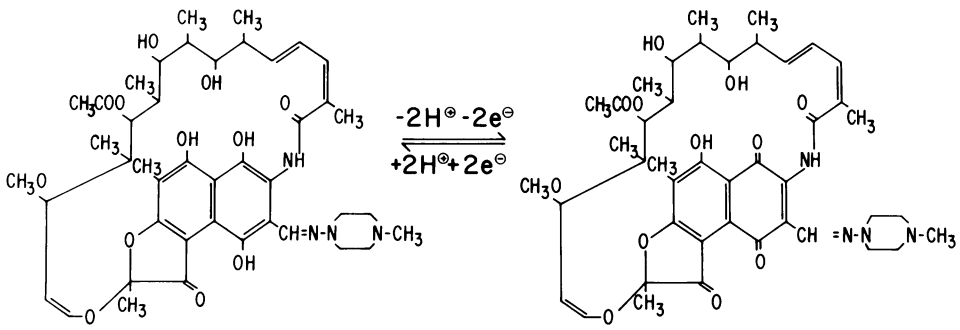

FIG. 1. Molecular structure of rifampin [3-(4-methyl-1-piperazinyliminomethyl)] and oxidation of hydroquinone.

of the cell volume with radiolabeled inulin and water (New England Nuclear Corp., Boston, Mass.), the accumulation rate of the antibiotics in PMN (cellular to extracellular concentration [C/E] ratio) was calculated as described previously (9).

To assess the influence of stimulation, PMN were preincubated in HBSS plus phorbol myristate acetate (PMA; final concentration, 1 μg/ml; Sigma Chemical Co., St. Louis, Mo.) for 10 min at 37°C before antibiotic uptake was measured.

To test the influence of metabolic inhibitors, PMN were preincubated (10 min, 37°C) in HBSS with sodium fluoride (final concentration, 20 mM), deoxyglucose (DOG; 1 mM), potassium cyanide (KCN; 10 mM), or sn-glycero-3-phosphate (sn-g-3-p; 5 μM) (all reagents were reagent grade and were obtained from Sigma) before rifampin uptake was assessed as desribed above.

The influence of temperature was tested by preincubating PMN in HBSS for 10 min at 2, 10, 20, 25, 30, 37, and 41°C before the addition of [^{14}C]rifampin. Ten minutes later, the C/E ratios were determined.

To measure the influence of pH, PMN were preincubated (10 min, 37°C) in HBSS adjusted to pH 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 with NaOH (0.5 M) or HCl (0.5 M). Ten minutes after the addition of [^{14}C]rifampin, fractions were taken for determination of ratios.

For the assessment of efflux of intracellular rifampin, PMN were preincubated (10 min, 37°C), and the radiolabeled rifampin was then added. After 10 min, a fraction was taken, and the C/E ratio was determined. The remaining suspension was centrifuged (10 min, 500 × *g*, 4°C), the supernatant was discarded, and the pellet was suspended in HBSS. The suspension was incubated again at 37°C, and fractions were taken at the time points described above to determine the decrease of C/E ratios after removal of the drug. Results were expressed as the percentage of the intracellular [^{14}C]rifampin activity at the beginning.

Intracellular activity of rifampin. The killing assay with intracellular *Staphylococcus aureus* Wood 46 has been described previously (9). Briefly, bacterial density was adjusted to 5×10^8 CFU/ml. After preopsonization in HBSS-serum (10 min, 37°C), the staphylococci were added to a suspension of CGD PMN in HBSS-serum (10^7 ml) at a bacteria to PMN ratio of 1:2. Subsequent to incubation (30 min, 37°C) and washing (three times in cold saline), the PMN were again suspended in HBSS-serum. Addition of lysostaphin (10 mg/liter; Sigma) did not result in further reduction of extracellular bacteria. Rifampin sodium salt (Rimactan [intravenous]; CIBA GEIGY) was added at the following concentrations: 0.06, 0.6, 1.25, 2.5, 5.0 mg/liter. MICs and MBCs of rifampin were 5×10^{-4} and 8×10^{-4} mg/liter, respectively.

Fractions were taken at the beginning and after 1, 2, 3, and occasionally 4 h of the final incubation. They were washed with HBSS and lysed in bidistilled water (15 min, room temperature). Virtually all PMN were destroyed by this procedure, which was controlled by phase-contrast microscopy. The samples were diluted serially and plated onto Mueller-Hinton agar. CFUs were counted after 2 days of incubation.

PMN from healthy donors (with and without rifampin), CGD PMN (with bacteria but without rifampin), and extracellular staphylococci (with and without rifampin) served as controls.

To investigate whether rifampin exerts negative effects on the respiratory burst of PMN, the killing assay was repeated with a rifampin-resistant mutant of *S. aureus* Wood 46 (MIC > 500 mg/liter). Killing of intracellular staphylococci by normal human PMN after 60, 90, 120, and 180 min was measured without rifampin and in the presence of 1, 10, and 100 mg of rifampin per liter.

Influence of rifampin on normal granulocyte functions. Rifampin sodium salt (hydroquinone form; the quinone form is not feasible) was dissolved in HBSS and added to PMN suspensions from healthy donors at the following final concentrations: 0, 5, 10, 20, and 50 mg/liter. Levels in serum during treatment range between 5 and 20 mg/liter (11).

Saccharomyces cerevisiae (2.5×10^8 cells per ml) and PMN (5×10^6/ml) were incubated in HBSS-serum (30 min, 37°C). After phagocytosis was stopped by the addition of 10 ml of ice-cold saline, the suspension was centrifuged (5 min, 150 × *g*, 4°C). The pellet was washed in HBSS and stained with Ziehl-Fuchsin stain. Extracellular and attached yeast cells stained red, while ingested yeast cells did not. The number of ingested yeast particles per 100 PMN was counted by light microscopy (Zeiss, Wetzlar, Federal Republic of Germany).

After incubation (37°C, 2 h, 5% CO_2) activated (chemoattractant, zymosan-activated serum; Sigma) and spontaneous (HBSS) migrations of PMN (10^8/ml) under agarose were measured with a light microscope as previously described (14). Results were expressed in micrometers.

PMN (5×10^6/ml) were stimulated either with PMA or with opsonized zymosan (OPZ). Oxygen consumption was recorded with an oxygraph (no. 5/6; Gilson Medical Electronics, Middleton, Ill.) as described previously (17). Results were expressed in nanomoles of O_2 per minute per 5×10^6 PMN.

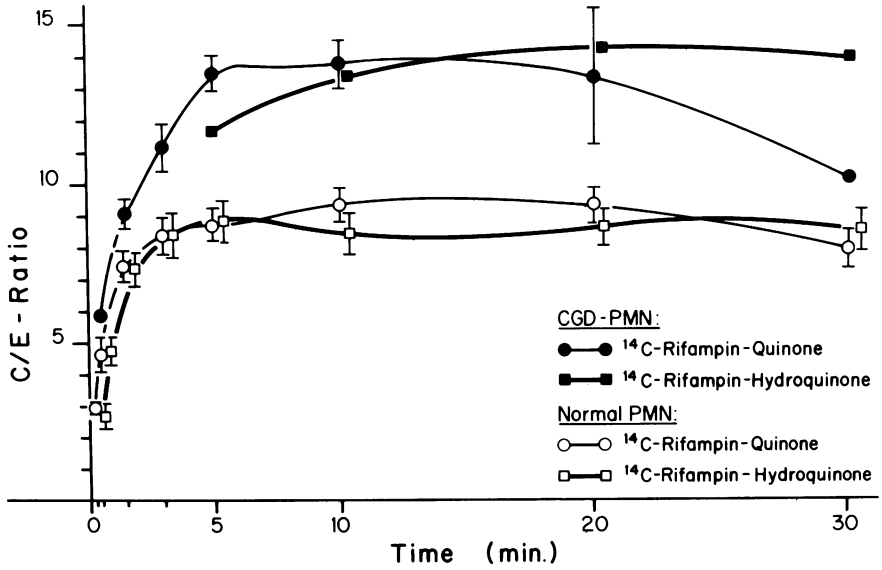

FIG. 2. Uptake of rifampin (quinone and hydroquinone) into normal and CGD PMN. For normal PMN, values are the mean ± SEM of 10 to 13 single determinations (three at 15 s) in 13 healthy blood donors. For CGD PMN, values are the mean ± SEM of two to three determinations in three patients for quinone and in one patient for hydroquinone.

Production of superoxide anion by PMA- or OPZ-stimulated PMN (3×10^7 ml) was assayed spectrophotometrically (Cary 251; Varian Inc., Victoria, Australia) by the reduction of ferricytochrome *c* as reported previously (2) and expressed in nanomoles of O_2^- per minute per 10^6 PMN. Maximal absorption of cytochrome *c* (oxidized) was at 550 nm. Rifampin had its maximal absorption at 473 nm.

Parallel experiments were done in a cell-free system (xanthine, 2×10^{-3} M, grade V; xanthine oxidase, 2×10^{-7} M, grade II; both from Sigma) in the same fashion (6).

PMN produce O_2^-, H_2O_2, OH^-, $^1O_2$, and other active O_2 derivatives upon adequate stimulation. This so-called respiratory burst can be measured in terms of emitted light impulses after enhancement with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; final concentration, 5×10^{-4} M; Sigma) as described previously (1). PMN (10^7/ml) were stimulated with PMA or OPZ. Tests were performed in a luminometer (LKB Wallac, Turku, Finland) and calculated in millivolts per minute per 10^6.

Parallel experiments were done in a cell-free system (xanthine, 4×10^{-2} M; xanthine oxidase, 10^{-7} M) in the same way.

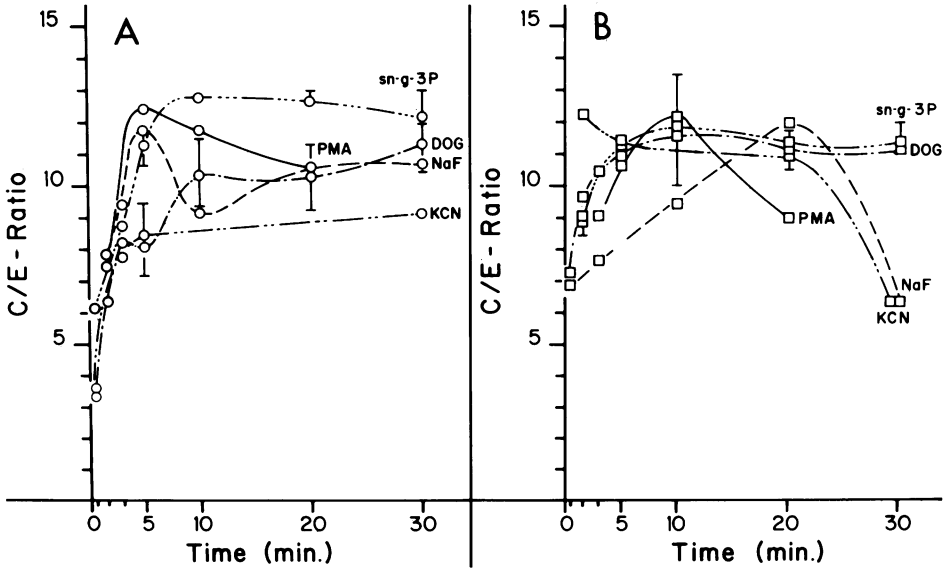

FIG. 3. Influence of PMA and metabolic inhibitors on [^{14}C]rifampin quinone (A) and [^{14}C]rifampin-hydroquinone (B) uptake into normal PMN. (A) For PMA and NaF, values are the mean of one to two determinations. For DOG and sn-g-3-p, values are the mean ± SEM of one to four determinations. There was a single determination for KCN. (B) For PMA, NaF, and DOG, values are the mean ± SEM of one to four determinations. For sn-g-3-p, values are mean ± SEM of three to four determinations. There was a single determination for KCN.

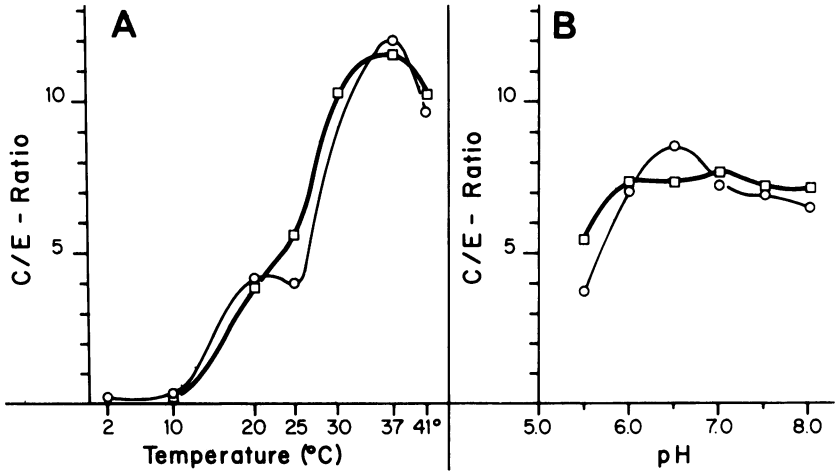

FIG. 4. Influence of temperature (A) and pH (B) on [^{14}C]rifampin quinone (○) and [^{14}C]rifampin hydroquinone (□) uptake into normal PMN. Values are the mean ± SEM of one to three determinations.

Statistical analysis. Significance between different test series was assessed by means of Student's *t* test.

RESULTS

Intracellular accumulation of rifampin. Both [^{14}C]rifampin quinone and hydroquinone forms accumulated well within normal and CGD PMN (Fig. 2). There was no significant difference in rate or kinetics of uptake between both forms. The initial accumulation phase was very rapid. About 80% of the maximal intracellular accumulation was reached within 90 s. The maximal C/E ratio (C/E_{max}) in normal human PMN was 9.36 ± 0.54 (mean ± standard error of the mean [SEM] of 13 determinations of quinone uptake after 10 min) and 8.82 ± 0.65 (two determinations of hydroquinone uptake after 5 min).

The intracellular concentration declined slowly and was 85 and 97% of C/E_{max} after 30 min.

In CGD PMN, the intracellular accumulation of both isomers was even higher, reaching C/E_{max} values of 13.17 (quinone; $n = 3$) and 14.29 (hydroquinone; $n = 1$). The difference was statistically significant for the quinone form only ($P < 0.05$).

To analyze whether rifampin enters the cell by an active or passive process, we tested the effect of PMA and several inhibitors of metabolism. PMA binds directly to the PMN membrane and activates the NADPH oxidase system. NaF is a potent inhibitor of protein synthesis and glycolysis, which is inhibited by DOG as well. KCN interferes with intramitochondrial oxidation processes and inhibits the myeloperoxidase. sn-Glycero-3-phosphate has been shown to competitively inhibit the uptake of fosfomycin (9). None of these agents was able to impede the intracellular entry of rifampin quinone (Fig. 3A) or hydroquinone (Fig. 3B), nor did PMA influence uptake of the agents.

Uptake of rifampin into PMN was related to incubation temperature (Fig. 4A). An increase of accumulation was observed between 10 and 37°C, the temperature at which C/E_{max} was reached. At 41°C, C/E ratios decreased again. Uptake kinetics of the quinone and hydroquinone again were very similar.

The highest intracellular accumulation was observed at

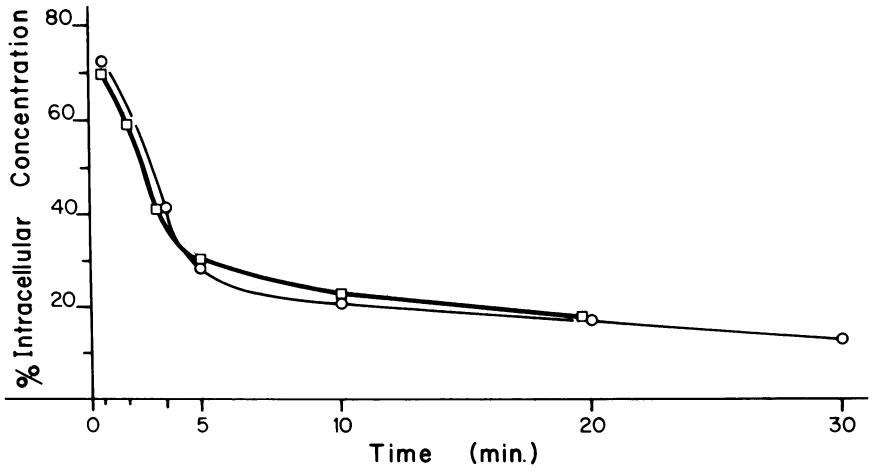

FIG. 5. Efflux of [^{14}C]rifampin quinone (○) and [^{14}C]rifampin-hydroquinone (□) from normal PMN. Single determinations; 100% denotes C/E ratio before elimination of extracellular rifampin.

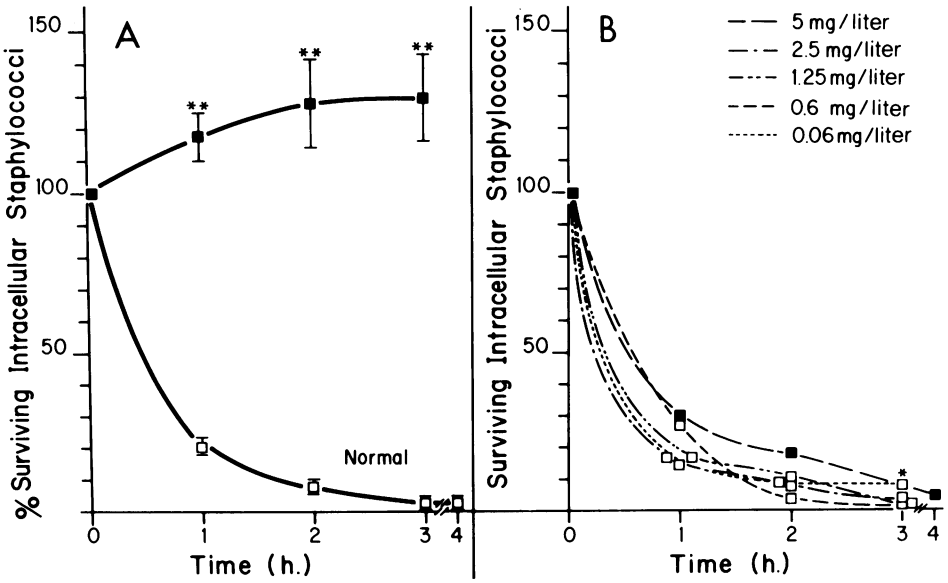

FIG. 6. Killing of intracellular *S. aureus* Wood 46 by rifampin in CGD PMN. (A) For normal PMN without rifampin, values are mean ± SEM of 14 to 18 (at 4 h, three determinations) in 18 different healthy blood donors. For CGD without rifampin, values are the mean ± SEM of 13 to 14 determinations in 14 different patients with CGD. (B) Means of two determinations at each concentration in eight different patients with CGD. *, $P < 0.05$; **, $P < 0.001$.

pH 6.5 (quinone) and 7.0 (hydroquinone). The difference between both isomers was not significant (Fig. 4B).

After removal of the extracellular rifampin, both isomers rapidly eluted from the cell (Fig. 5). About 17% of the original intracellular rifampin quinone and hydroquinone forms remained in the PMN 20 min later.

Intracellular activity of rifampin. At extracellular concentrations between 0.06 and 5.0 mg/liter, rifampin effectively killed intracellular staphylococci in CGD PMN. While the number of intracellular bacteria in CGD PMN without the antibiotic constantly increased (Fig. 6A), in the presence of different concentrations of rifampin between 71 and 86% of the staphylococci were killed within the first hour of incubation (Fig. 6B). This is comparable to the results achieved with normal PMN, which killed 79% in the first hour (Fig. 6A). After 3 h, only 1.66 to 4.87% of the bacteria survived in CGD PMN at rifampin concentrations of 0.6 to 5.0 mg/liter (normal PMN without antibiotic, 2.88%; $P < 0.40$). No dose dependence could be observed at these concentrations, while at a concentration of 0.06 mg/liter, slightly more intracellular staphylococci survived (7.9%; $P < 0.05$).

Rifampin did not negatively influence the killing of rifampin-resistant staphylococci by normal PMN (Table 1).

TABLE 1. Killing of *S. aureus* Wood 46 Rif^r by normal PMN in the presence of increasing concentrations of rifampin

Rifampin concn (mg/liter)	Reduction (%) of viable bacteria after incubation for the following times (min)[a]:			
	30	60	90	180
0 (control)	45.5 ± 2.7	77.5 ± 0.6	90.2 ± 3.3	92.8 ± 2.8
1	41.1 ± 12.7	79.6 ± 3.5	93.0 ± 1.3	94.6 ± 1.3
10	50.3 ± 10.7	78.5 ± 3.2	92.5 ± 3.1	94.9 ± 1.3
100	52.6 ± 22.6	83.8 ± 0.6	92.0 ± 1.7	95.9 ± 0.9

[a] Values are means ± standard deviation of two independent experiments. MIC of the *S. aureus* Wood 46 mutant, >500 mg/liter (determined in Trypticase [BBL Microbiology Systems, Cockeysville, Md.]).

Influence of rifampin on normal PMN function. As shown in Fig. 7A, rifampin significantly depressed the rate of yeast phagocytosis by normal PMN ($P < 0.001$). At 50 mg/liter, only 41.4% of the phagocytosis rate without rifampin was observed.

At concentrations between 5 and 50 mg/liter, rifampin did not affect activated or spontaneous migration of PMN ($n = 6$ determinations with normal PMN from different donors at each concentration). Spontaneous migration without rifampin was 379 ± 12 μm over 2 h as compared with 365 ± 20 μm (at 5 mg of rifampin per liter) to 412 ± 23 μm (50 mg/liter). Activated migration without rifampin was 622 ± 49 μm over 2 h and ranged between 716 ± 59 μm (5 mg/liter) and 573 ± 32 μm (50 mg/liter) in the presence of rifampin.

Rifampin revealed no statistically significant influence on the consumption of O_2 by PMA- or OPZ-stimulated PMN (Fig. 7B), although a slight reduction of OPZ-stimulated O_2 consumption was observed at high rifampin concentrations.

Production of superoxide anion by activated PMN was depressed by rifampin in proportion to the extracellular concentration of the agent (Fig. 7C). Absorption of light by rifampin could be excluded because rifampin and cytochrome *c* had different absorption maxima (473 and 550 nm, respectively). The same effect of rifampin on O_2^- release was observed in the xanthine-xanthine oxidase system (6) (Table 2).

Similar to O_2^- production, rifampin significantly reduced the chemiluminescence response of stimulated PMN (Fig. 7D) and the cell-free xanthine-xanthine oxidase system (Table 2) in a dose-dependent manner. The possibility that rifampin absorbed light could not be excluded in this assay, however (21).

DISCUSSION

Antibiotics that are active in the cell offer advantages over antibiotics that do not enter the cell. After phagocytosis, bacteria are to a certain extent protected against the antibacterial action of penicillins and aminoglycosides (9, 13,

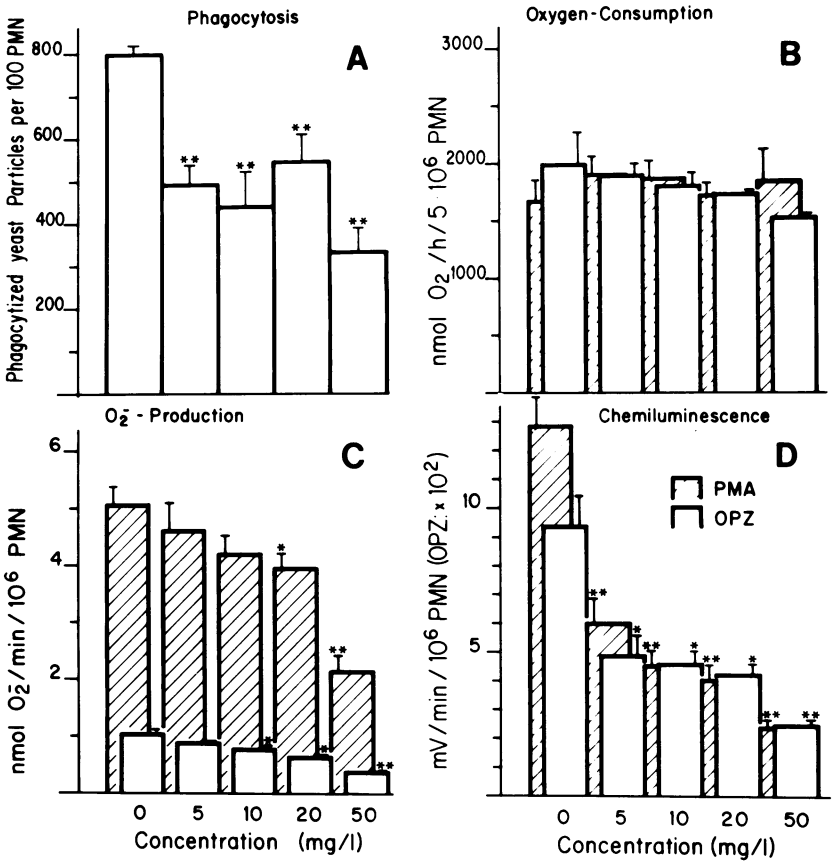

FIG. 7. Influence of rifampin on normal PMN function in vitro. (A) Phagocytosis of yeast particles. Values are the mean ± SEM of 6 to 14 determinations per concentration in PMN from six healthy blood donors. (B) O_2 consumption. Values are mean ± SEM of six to seven determinations. (C) O_2^- production. Values are the mean ± SEM of three to five (stimulus OPZ) and 7 to 12 (stimulus PMA) determinations. (D) Chemiluminescence. Values are the mean ± SEM of 6 to 7 (OPZ) and 8 to 12 (PMA) determinations. *, $P < 0.05$; **, $P < 0.001$.

22). Rifampin has been shown to be effective against mycobacteria as well as against gram-positive bacteria, such as *Neisseria* spp., *Hemophilus* spp., *Chlamydia trachomatis*, and *Legionella pneumophila* (4).

Results of this study confirm previous findings that rifampin is indeed accumulated within PMN. No difference in extent or kinetics of uptake was observed between the quinone and hydroquinone form. Therefore, the oxidation state of the molecule does not affect its intracellular accumulation. Rapid transformation of one form to the other, however, cannot be assessed by our present methodology. No data exist about the role and relationship of both molecular forms of rifampin in vivo. The observed C/E_{max} of both forms, however, were significantly higher than previously reported (15). Different technical procedures (e.g., rapid dissolution of PMN in formic acid and freezing of disrupted cells as described previously [15]) may account for lower intracellular concentrations of the agent observed in previous studies. Intracellular accumulation of rifampin is mainly due to its good lipid solubility (13). We extended methods used in previous studies by using a number of substances which would have interfered with an active transport process. DOG, KCN, NaF, and sn-g-3-p were unable to impede rifampin uptake (quinone and hydroquinone; Fig. 3). PMA did not stimulate uptake. Rifampin even accumulated within dead PMN (after fixation with glutaraldehyde; data not shown). These data and the rapid efflux of rifampin from PMN after withdrawal of extracellular substance (Fig. 5) favor a passive more than an active process of accumulation. However, fosfomycin, another antibiotic with intracellular activity, is actively transported into the cells, although to a somewhat lesser extent (9).

In CGD PMN, the C/E_{max} of both isomers was higher than in normal PMN (Fig. 2). This may be attributed to the lower pH within CGD PMN (18) which leads to intracellular protonization of the zwitterionic rifampin (11) and subsequent ion trapping within the cell. The same mechanism has been hypothesized for trimethoprim-sulfamethoxazole (19). Positive and negative charges on the agent at lower and

TABLE 2. Influence of rifampin on chemiluminescence and O_2^- release by the xanthine-xanthine oxidase system

Rifampin concn (mg/liter)	Chemiluminescence (mV/min)	O_2^- release (maximum nmol of O_2^-)
0	1,178.7 ± 317.4	26.2 ± 1.37
10	262 ± 90.51[a]	25.8 ± 1.89
20	162 ± 25.4[a]	23 ± 0.49[a]
50	56 ± 15.9[b]	12 ± 5.5[a]

[a] $P < 0.05$.
[b] $P < 0.001$.

higher extracellular pHs, respectively, may account for reduced uptake at pH 6.5 and 7.5 (Fig. 4B) because of the parallel decrease in lipid solubility. The optimal temperature for uptake (Fig. 4A) was 37°C. In Fig. 4A, physical alterations of the granulocyte membrane probably are reflected, because biological membranes crystallize below 10 to 15°C and may become leaky beyond 37°C. A significant reduction of rifampin uptake at 4°C has been shown previously (15). The in vivo significance of this observation, e.g., in case of elevated body temperature, is unclear at present, however.

Results of our experiments with CGD PMN demonstrate that after accumulation, rifampin is highly active against phagocytized staphylococci (Fig. 6), thus compensating for the bactericidal defect inherent in CGD PMN. Due to its high intracellular concentration, rifampin could be active against intracellular bacteria, even if they were found to be only intermediately sensitive to the agent. In addition to conventional tests for antimicrobial sensitivity at the extracellular level, intracellular accumulation seems to be an important criterion for the effectiveness of an antibiotic against intracellular pathogens. This is demonstrated in CGD, in which catalase-positive bacteria and fungi survive within PMN. The administration of rifampin alone or in combination (4) appears to be useful, especially in case of staphylococcal infections. Patients may benefit from the synergistic antifungal activity of rifampin with amphotericin B as well (4).

On the other hand, intracellular accumulation of rifampin inside PMN could be hazardous to their normal function. Several antibiotics possess immunosuppressive properties (25). In previous in vitro studies it has been postulated that rifampin has an immunosuppressive effect on lymphocyte function (20) which could not be confirmed in vivo (10). In our study, we paid special attention to the effect of rifampin on normal PMN function. Due to a lack of standardization of laboratory techniques, it is difficult to compare the results of studies dealing with side effects of antibiotics on granulocyte functions (25).

Rifampin has been found by some researchers (5) to reduce chemotaxis (migration of normal PMN toward *Escherichia coli* culture filtrate). Others observed stimulated chemotaxis in the presence of rifampin (migration of PMN from patients with rheumatoid arthritis toward casein [23]). Our own findings are in accordance with the results of Gray et al. (7), who found that even at high concentrations rifampin has no influence on the chemotaxis of normal PMN toward zymosan.

However, rifampin inhibited phagocytosis of yeast cells, most markedly at high doses (Fig. 7A). Depression of phagocytosis (of *S. aureus*) by rifampin has been observed by others (16). These findings are not in accordance with results of an earlier report (8), in which, by another method, no influence of rifampin on the phagocytosis of *E. coli* was found.

Inhibition of phagocytosis of yeasts and bacteria has been reported for tetracyclines as well (5). While these probably act by calcium chelation, rifampin could act by sterical inhibition or blocking (7) of C3 receptors on the PMN membrane which must be activated before phagocytosis can begin. In our experiments, depression of yeast phagocytosis was partly paralleled by a decrease of O_2 consumption after stimulation with opsonized zymosan (94.6 to 75.8% that of normal controls; Fig. 7B), but not after stimulation with PMA, which bypasses surface receptors. The latter difference, however, was not significant. Further work is needed to clarify this point.

The formation of bactericidal O_2 derivatives during the respiratory burst is essential for the intracellular killing of pathogens in PMN (Fig. 6A). Rifampin depressed chemiluminescence and O_2^- release in normal, stimulated PMN (Fig. 7C and D), as well as in a cell-free system (Table 2). However, in our in vitro killing assay, it did not lead to an impaired bactericidal activity of normal PMN against a rifampin-resistant mutant of *S. aureus* Wood 46 (Table 1). Therefore, rifampin appears to either interact directly with extracellular and possibly intracellular O_2 metabolites by a radical scavenging mechanism (12) or interfere with the assay system by light absorption (21) or quenching. The lack of a negative effect on bactericidal activity of normal PMN suggests that it did not inhibit the respiratory burst, but further studies are necessary to evaluate the relevance of these findings in immunocompromised patients.

ACKNOWLEDGMENTS

The skillful technical assistance of Bernadette Hollenstein and Ingrid Weissbrodt is gratefully acknowledged.

This study was supported in part by grant no. 3.884-0.81 from the Swiss National Science Foundation.

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