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Influence of Etoposide and Cyclophosphamide on the Efficacy of Cloxacillin and Erythromycin in an Experimental Staphylococcal Infection

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The effect of monocytopenia and granulocytopenia on the outgrowth of *Staphylococcus aureus* as well as on antibiotic efficacy was studied in an experimental thigh infection in mice. Pretreatment with etoposide reduced monocyte numbers in blood to 14% and those of granulocytes to 54% at the time of infection. Monocytopenia did not affect the proliferation of bacteria in the infected thigh or the reduction of bacterial numbers after treatment with cloxacillin or erythromycin. Pretreatment with cyclophosphamide reduced monocyte numbers to 15% and granulocyte numbers to 3%. This resulted in a marked increase in the number of bacteria at the site of infection and a decrease in the efficacy of antibiotic treatment.

Cytostatic drugs have several effects that can impair host resistance (3), but the decrease in the number of circulating granulocytes is probably one of the most important factors, since it promotes infections and reduces the efficacy of the antibiotic treatment applied. Previous work has shown that in an experimental infection in irradiated mice, the outgrowth of bacteria is inversely related to the number of granulocytes in peripheral blood (4, 8). Moreover, the efficacy of antibiotics is adversely affected by granulocytopenia. Although it is generally thought that monocytes do not play a major role in the defense against acute infections, the lack of experimental evidence led us to undertake the present study to compare the effects of two cytostatic drugs—etoposide, which induces monocytopenia, and cyclophosphamide, which induces both granulocytopenia and monocytopenia—on the antibiotic efficacy of cloxacillin and erythromycin in an experimental infection with *Staphylococcus aureus*.

Etoposide (VP 16-213), which was kindly donated by Bristol Myers (Weesp, The Netherlands), was dissolved in a specified vehicle and further diluted in phosphate-buffered saline (PBS; pH 7.2). The vehicle without etoposide was prepared by our hospital pharmacy according to the prescription of the manufacturer. Standard solutions of cyclophosphamide (Montedison, Rotterdam, The Netherlands) were prepared in PBS.

A strain of *S. aureus* that was isolated from clinical material and that was serum resistant was stored in brain heart infusion broth (Oxoid Ltd., Basingstoke, England) at −70°C in a suspension containing about 10^9 bacteria per ml. Just before the start of each experiment, a vial of this suspension was rapidly thawed at 37°C. Cloxacillin (MIC, 0.25 μg/ml; 90.5% activity; Beecham, Amstelveen, The Netherlands) and erythromycin (MIC, 0.25 μg/ml; 98.0% activity; Abbott N.V., Amstelveen, The Netherlands) were dissolved in PBS.

Short-term growth experiments were performed in vitro as described elsewhere (1). Male specific-pathogen-free Swiss mice (weight, 20 to 30 g) were used. Monocytopenia was induced by injecting a dose of 16 mg of etoposide per kg into a tail vein on days 1 and 3. Control animals received the same volume of vehicle alone. To induce granulocytopenia and monocytopenia, cyclophosphamide was injected intraperitoneally at a dose of 150 mg/kg on day 1 and 100 mg/kg on day 4 (2). The same volume of PBS was given to the control animals. The numbers of granulocytes, lymphocytes, and monocytes were determined as described by Sluiter et al. (7).

Approximately 5×10^6 CFU of *S. aureus* suspended in brain heart infusion broth to a volume of 100 μl were injected into a thigh muscle. After 1 h the various dosages of the antibiotic under study were administered subcutaneously. Four hours later, the animals were killed by cervical dislocation, the thigh muscle was isolated and homogenized, and appropriate dilutions were plated onto Difco sensitivity test agar (Oxoid Ltd.). The experiments were performed on day 4 (etoposide-pretreated animals) or day 5 (cyclophosphamide-pretreated animals).

In the etoposide-pretreated animals, concentrations of etoposide in blood and tissues were determined in a microbiological assay, with *S. aureus* 42D used as the test organism. The level of detection was 6.25 μg/ml. For both antibiotics, the concentrations in plasma after the subcutaneous injection of 10 mg/kg and the binding to murine plasma were determined as described by Hoogeterp et al. (4), with *Bacillus subtilis* ATCC 6633 used as the test organism for cloxacillin and *Sarcina lutea* ATCC 9341 used as the test organism for erythromycin.

Both cloxacillin and erythromycin had a bactericidal effect in vitro; for cloxacillin this was at concentrations above 0.075 μg/ml, and for erythromycin it was at levels above 0.5 μg/ml. Concentrations of cloxacillin higher than 0.25 μg/ml did not lead to any further reduction in the numbers of *S. aureus*, whereas erythromycin was maximally effective at 2.0 μg/ml. After 3 h of incubation, the maximal reduction of the numbers of CFU was from 5×10^6 to approximately 1×10^4 for both antibiotics.

Pretreatment with either etoposide or cyclophosphamide had a significant effect on the numbers of leukocytes present in the peripheral blood when the infection was induced. Etoposide pretreatment reduced the number of monocytes to 14% and the number of granulocytes to 54% (Table 1). The number of monocytes increased throughout the 5-h period of infection, but in the etoposide-treated mice, it only reached

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TABLE 1. Effect of etoposide on leukocyte numbers at the start of and 5 h after an experimental infection with *S. aureus*

Cell type	Time (h) after infection	No. of leukocytes/mm³ after treatment with[a]:		P^b
		Etoposide	Vehicle	
Monocytes	0	19	138	<0.01
	5	147	388	<0.01
Granulocytes	0	857	1,586	<0.05
	5	3,369	3,951	>0.05

[a] Each value represents the mean for six mice.
[b] According to Student's *t* test.

38% of that in the controls (Student's *t* test; $P < 0.01$), whereas the number of granulocytes rose to the same level in the etoposide-treated animals and the controls.

Cyclophosphamide led to a decrease of both cell types not only at the start of the infection but also 5 h later (Table 2). The effect on monocyte numbers at the start of the infection was similar to that of etoposide, i.e., a reduction to 15%, but 5 h later the number of monocytes dropped to 6% of that in the controls. The effect of cyclophosphamide on granulocyte numbers was much stronger than that of etoposide: at the end of the experiment the number of granulocytes was only 0.9% of that in the controls.

When the final number of CFU was taken as a parameter of the effect, cloxacillin was slightly, but not significantly, less effective in mice pretreated with etoposide than in the controls. At dosages higher than 40 mg/kg, which was the maximally effective dose, this difference was not seen. At dosages below 40 mg/kg, the effect was significantly dose dependent ($P < 0.025$). The results of treatment with erythromycin were also very similar in etoposide-pretreated and control mice, except that the effect was maximal at the relatively low dose of 10 mg/kg. Only at a dose of 5 mg/kg was the number of CFU significantly higher in the etoposide-pretreated than in the normal animals.

In mice made granulocytopenic and monocytopenic by cyclophosphamide, cloxacillin was much less efficacious than it was in the control animals ($P < 0.01$) (Fig. 1). The maximal antibiotic effect in the granulocytopenic animals tended to be obtained at about 80 mg/kg, a higher dose than that in the controls. For both groups the effect of cloxacillin was significantly ($P < 0.01$) dose dependent at concentrations below 80 mg/kg. In the control animals the effect of cloxacillin on the number of bacteria became manifest at a dosage level that had no effect on the cyclophosphamide-pretreated mice, and this gave a significant difference ($P < 0.005$) between the slopes for the dose-effect relationship in

TABLE 2. Effect of cyclophosphamide on leukocyte numbers at the start of and 5 h after an experimental infection with *S. aureus*

Cell type	Time (h) after infection	No. of leukocytes/mm³ after treatment with[a]:		P^b
		Cyclophosphamide	Saline	
Monocytes	0	18	120	<0.001
	5	24	407	<0.001
Granulocytes	0	37	1,202	<0.001
	5	58	6,639	<0.001

[a] Each value represents the mean for six mice.
[b] According to Student's *t* test.

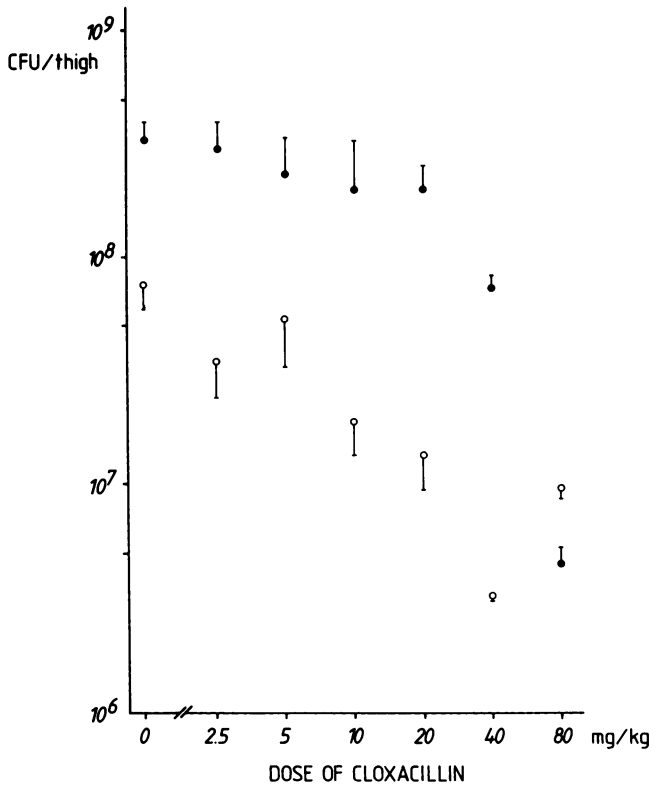

FIG. 1. Numbers of CFU of *S. aureus* 42D in an experimental infection in relation to the dose of cloxacillin in cyclophosphamide-pretreated mice (●) and controls (○). Each symbol represents the mean ± standard error of the mean for six animals.

the cyclophosphamide-pretreated and the control animals. Cyclophosphamide pretreatment also had a distinct effect on the efficacy of erythromycin (Fig. 2); i.e., there was a significant difference ($P < 0.01$) in the numbers of CFU between the cyclophosphamide and the control groups. The effect of erythromycin in the controls was already maximal at a dose of 10 mg/kg, whereas in the cyclophosphamide-pretreated mice it was only reached at a dose of 80 mg/kg. The effect of the antibiotic on the number of CFU was significantly dose dependent in both groups ($P < 0.02$), and the slopes of the dose-effect curves did not differ significantly.

At a dose of 10 mg/kg given subcutaneously, cloxacillin reached a maximal concentration in plasma of 8.4 μg/ml at 10 min and had a half-life of approximately 15 min. Erythromycin given in the same dose reached its peak concentration of 1.7 μg/ml after 20 min and had a half-life of 24 min. Protein bindings were 80% for cloxacillin and 33% for erythromycin in the concentration range that occurred in vivo.

The results of this study confirm that granulocytopenia caused by treatment with cytostatic drugs affects the outgrowth of *S. aureus*. The decrease in antibiotic efficacy was very similar to that seen when granulocytopenia was induced by irradiation (4, 8). This supports the view that granulocytopenia is the main determinant of antibiotic efficacy in the treatment of an acute staphylococcal infection. A decrease in monocyte numbers had no influence on the numbers of *S. aureus*. In the short-term infection model, the dominant role of the granulocytes compared with the role of monocytes was explained by the more rapid migration of the former to the site of inflammation (5, 6, 9).

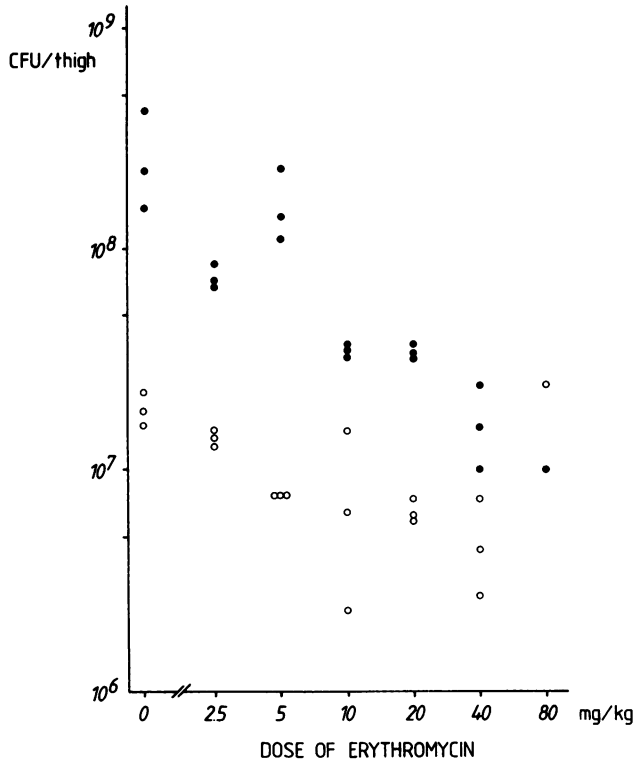

FIG. 2. Numbers of CFU of *S. aureus* 42D in an experimental infection in relation to the dose of erythromycin in cyclophosphamide-pretreated mice (●) and controls (○). Each symbol represents a single observation.

At relatively high doses of antibiotics there was less or no difference between the granulocytopenic mice and controls with respect to the numbers of bacteria. The effect of cytostatic treatment on the proliferation of bacteria during antibiotic treatment can be quantitated as the dose increase required to obtain the same final number of bacteria at the site of infection as that in the controls. In cyclophosphamide-pretreated mice the dose of cloxacillin had to be four to eight times higher to obtain this result. This difference was even greater for erythromycin, despite the high susceptibility of the microorganism in vitro: the dose had to be about 16 times higher in the cyclophosphamide-pretreated mice to equal the number of bacteria in the controls. In animals made granulocytopenic by irradiation, the required dose increase was also higher for bacteriostatic than for bactericidal antibiotics (4).

Although etoposide itself has antistaphylococcal activity (1), no detectable concentrations of this drug were found in either homogenized thigh tissue or blood (data not shown).

When the degree of protein binding was taken into account, concentrations of drug in plasma were high enough to be maximally effective, according to the in vitro growth curves. At 10 mg of cloxacillin per kg, the concentrations in plasma remained effective for approximately 45 min, and those of erythromycin remained effective for approximately 60 min. Higher doses led to a longer duration of effective concentrations in plasma, for instance, 1.5 h after administration of 80 mg cloxacillin per kg. At the latter dose the shortage of granulocytes was apparently compensated for by the activity of the antibiotic, which led to the same number of bacteria as that in the control mice.

Although concentrations in plasma may be higher in patients treated with cloxacillin or erythromycin than they are in experimental animals, it may be necessary to increase the dose for patients during periods of granulocytopenia caused by cytostatic drugs. This might be practically impossible for reasons of toxicity, at least for erythromycin. No increase is indicated if cytostatic treatment does not lead to granulocytopenia.

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