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Serum Bactericidal Activity as a Therapeutic Guide in Severely Granulocytopenic Patients with Gram-negative Septicemia*

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Abstract—*The peak and trough levels of bactericidal activity of the serum of 74 severely granulocytopenic patients (≤500 polymorphonucleates per μl) with hematologic malignancies and Gram-negative septicemia were measured using the patient's infectious organism and serum containing the given antibiotics. When the peak titer of bactericidal activity in the serum was >1:8 the septicemia was cured in more than 90% of the cases. However, in order to achieve a satisfactory rate of cure, patients with <100 polymorphonucleates/μl required higher peak levels than patients with 100-500 polymorphonucleates/μl. Serum bactericidal activity was influenced by the* in vitro *susceptibility of the offending pathogen and by the presence of* in vitro *synergism between the given antibiotics. These two variables showed a correlation with the clinical outcome that proved to be increasing with the degree of granulocytopenia. Furthermore, synergistic combination of the antibiotics appeared essential when the* in vitro *susceptibility shown by the offending pathogen was moderate. These data suggest (i) that determination of the bactericidal activity of the serum may prove to be a useful method to predict the clinical outcome in severely granulocytopenic patients with Gram-negative septicemia; and (ii) under the same conditions, antibiotic combinations that have demonstrable* in vitro *synergy against the offending pathogen should be given the utmost consideration.*

INTRODUCTION

GRAM-NEGATIVE septicemia is a frequent complication and cause of death in cancer patients, especially when severe granulocytopenia—due to the underlying disease or to the cytostatic treatment—is present [1]. The rate of cure in these patients is strongly correlated to the precocity and intensity of the bactericidal effect of the antimicrobial treatment. For this purpose, empiric therapy with combinations of antibiotics is suggested when a presumptive diagnosis of septicemia is made [2, 3]. Once the organism responsible for bacteremia has been isolated, the measurement of the antibacterial activity of the serum has been used by some investigators as a suitable method to predict the efficacy of the therapy [4]. It is the aim of our paper to further test the usefulness of such a technique in predicting the efficacy of the therapy in the case of septicemic patients with hematologic malignancies and severe granulocytopenia, receiving various combinations of antibiotics.

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MATERIALS AND METHODS

Patients

Seventy-four patients with Gram-negative septicemia and hematologic malignancy were given combinations of two antibiotics (in no case was therapy with single or more than two antibiotics used). In particular, they received i.v. one of the following combinations of antibiotics: cefamandole, 150 mg/kg/day, plus tobramycin, 5 mg/kg/day; carbenicillin, 300-600 mg/kg/day, plus sisomicin, 5 mg/kg/day; carbenicillin, 300 mg/kg/day, plus amikacin, 15 mg/kg/day; azlocillin, 300 mg/kg/day, plus amikacin; cefotaxime, 150 mg/kg/day, plus amikacin; and

piperacillin, 300–400 mg/kg/day, plus amikacin. Most of them had a rapidly fatal disease such as acute non-lymphocytic leukemia (51 patients) and acute lymphocytic leukemia (17 patients); six patients had an ultimately fatal disease such as non-Hodgkin's lymphoma (four patients), myeloma (one patient) and aplastic anemia (one patient). All of them had a granulocyte count of ≤ 500 polymorphonucleates (PMN)/μl at the onset of septicemia. Fifty patients had persistently less than 100 PMN/μl during antimicrobial therapy. Twenty-four patients had 100–500 PMN/μl for at least 7 days of antimicrobial treatment.

In all cases three blood cultures, one urine culture and culture of infected sites, if present, were obtained before starting empiric therapy. Blood cultures were again performed every day, and infected sites were re-cultured at least once during therapy. The diagnosis of septicemia rested on the isolation of an organism from at least two blood cultures obtained from a patient with fever (≥38.5°C) and/or other clinical signs of sepsis (ectima gangrenosum, severe arterial hypotension).

Response to therapy was assessed on microbiological and clinical grounds: eradication of the pathogen from blood and, if evaluable, from the infected sites, disappearance of fever and/or of the other clinical signs of sepsis in 48–72 hr without relapse until 3 days after cessation of therapy. Antimicrobial treatment was continued for a maximum of 11 days, or for 7 days after the patient became afebrile. The cases in which no improvement was observed after 72 hr of empiric antimicrobial treatment were considered failures, and the results of further adjustment of antibiotic therapy were not included in the study. In all patients treated in this study serum was obtained on the second day of therapy 1 hr after the administration of the antibiotics and 1 hr before the next dose. Serum samples were stored at 4°C until used, but the period of storage did not exceed 24–48 hr.

In vitro *studies*

Characterization of bacteria was accomplished by standard methods. *In vitro* checkerboard studies were determined for each organism, using the antibiotics given to the patients from whom the bacteria had been isolated. Each test was performed in microtiter plates, using Mueller-Hinton broth, supplemented with calcium and magnesium, when testing *Pseudomonas aeruginosa* [5]. Organisms were inoculated with a multipoint inoculator to give a final concentration of 10^4–10^5 organisms/ml. Fractional inhibitory concentration indices (FIC indices) were calculated from the formula:

$$\text{FIC index} = \frac{\text{MIC of drug A in presence of B}}{\text{MIC of drug A alone}} + \frac{\text{MIC of drug B in presence of A}}{\text{MIC of drug B alone}},$$

where MIC = minimal inhibitory concentration. According to Elion an index value of ≤0.5 was considered to be consistent with significant synergism [6].

Isolates were considered susceptible if they were inhibited at or below the peak serum level, which is usually obtained after intravenous administration of antibiotics at the previously mentioned doses: 128 μg/ml for carbenicillin, azlocillin and piperacillin, 32 μg/ml for cefotaxime, cefamandole and amikacin, and 4 μg/ml for tobramycin and sisomicin [7–9]. In order to relate results of susceptibility tests to achievable blood levels, the 'therapeutic ratios' were calculated for each antimicrobial agent tested. The 'therapeutic ratio' was defined as the ratio between the mean peak serum level of each antimicrobial agent and the MIC required to inhibit each organism.

Determination of serum bactericidal activity (SBA)

Peak and trough levels of SBA of each bacteremic patient under antimicrobial therapy were measured by diluting serum samples in tubes, each containing 0.5 of a 1/1 mixture of pooled human serum, which was heat-inactivated before use, in Mueller-Hinton broth supplemented with calcium and magnesium [10]. Each serum sample was tested against patient's microorganism at a final concentration of 5×10^5 bacteria. SBA was defined as the highest dilution of serum which yielded less than five colonies on subculture to sheep blood agar (99.9% kill) [10]. A 0.01-ml calibrated pipette was utilized to transfer the inoculum.

Statistics

Statistical evaluation of the data was carried out by means of the Yates method of chi-square determination.

RESULTS

The relation between the bactericidal activity of the sera of patients with Gram-negative septicemia and the clinical outcome is shown in Fig. 1. Peak and trough titers >1:8 were both correlated with a favorable outcome in more than 90% of the cases. With trough titers <1:8 the rate of cure was 45%, while with peak titers of SBA <1:8 the rate of cure was 26%. This seems to suggest that the peak titer of SBA is a more important indicator for a favorable clinical outcome than the trough titer.

Table 1 shows the relationship between peak

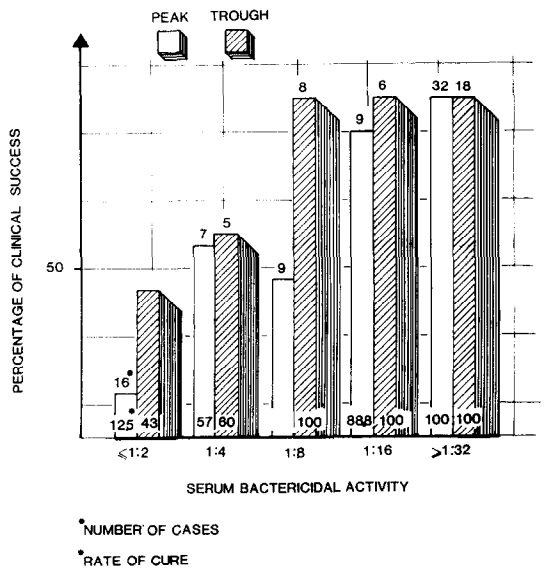

Fig. 1. Peak and trough levels of SBA as related to the rate of cure in 74 granulocytopenic patients with Gram-negative septicemia.

levels of SBA, granulocyte count and clinical outcome. When the granulocyte count was <100 PMN/μl, 31/50 (62%) patients were cured; when the granulocytes were 100-500/μl, 20/24 patients (80%) had a clinical response. Peak levels of SBA ≥1:32 were necessary to cure 100% of Gram-negative septicemias when the granulocyte count was <100 PMN/μl. With lower levels of SBA the rate of cure was 29.6%. This difference is highly significant ($\chi^2 = 23.20$; $P < 0.0005$). On the other hand, in patients with 100-500 PMN/μl no significant difference was observed between the rate of cure with levels of SBA≥1:32 (100%) and that with levels <1:32 (71.4%). In fact, in this group of patients, when peak levels ≥1:8 were achieved the rate of cure was 100%. With peak levels of SBA <1:8, when comparing patients with a granulocyte count <100 PMN/μl to patients with a granulocyte count of 100-500 PMN/μl, significantly lower rates of cure were observed in the first group (0 vs 60%; $\chi^2 = 7.67$; $P < 0.01$).

Table 2 shows that median peak levels of SBA and *in vitro* sensitivity of the pathogens to one or both antibiotics used in combination therapy are strictly related. However, SBA appeared to be more important than combination therapy in patients with <100 PMN/μl in predicting clinical outcome. When peak levels of SBA were <1:8 no patient was cured regardless of the *in vitro* sensitivity of the pathogen to the combination therapy. At the other extreme, with the same levels of SBA and 100-500 PMN/μl the susceptibility to both the given antibiotics was correlated with a 100% rate of cure; when only one agent inhibited the organism, the rate of cure was 20%.

Table 1. Relationship between peak levels of SBA, granulocyte count and clinical outcome

SBA (peak level)	<100 PMN/μl No.	% of successes			100-500 PMN/μl No.	% of successes		
<1:8	13	0	A	B	10	60	a	b
1:8	7	28.5			2	100		
1:16	7	85.7		C	2	100		c
≥1:32	23	100			10	100		
Total	50	62			24	80		

No. = number of cases; A vs a, $\chi^2 = 7.67$, $P < 0.01$; B vs b $\chi^2 = 8.72$, $P < 0.005$; C vs c, $\chi^2 = 4.9$, $P < 0.05$.

Table 2. Relationship between in vitro *sensitivity, peak levels of SBA, granulocyte count and clinical outcome*

*In vitro** sensitivity	SBA (median)	SBA <1:8 <100 PMN/μl No.	%	SBA <1:8 100-500 PMN/μl No.	%	SBA ≥1:8 <100 PMN/μl No.	%	SBA ≥1:8 100-500 PMN/μl No.	%
SS	1:64	5	0	5	100	34	85.3	12	100
RS	1:4	5	0	5	20	3	66.6	2	100
RR	1:2	3	0	-	-	-	-	-	-

*SS = inhibition by both drugs; RS = inhibition by aminoglycoside only, except in three cases, when inhibition was due to beta-lactam antibiotic; RR = not inhibited by two drugs; No. = number of cases; % = percent of successes.

Table 3. Relationship between bacterial sensitivity, synergism in vitro, peak levels of SBA, granulocyte count and clinical outcome

Bacterial sensitivity	Synergism	SBA (median)	PMN/μl <100 No.	<100 %	100-500 No.	100-500 %	total No.	total %
SS	+	1:128	14	92	12	100	26	96
RS	+	1:16	1	100 (C)	2	100 (E)	3	100 (D)
SS	-	1:64	25	64	5	100	30	70 (A)
RS	-	1:2	7	14	5	20	12	16 (B)
RR	-	1:2	3	0	-	-	3	0

No. = number of cases; % = percentage of response; + = synergism; - = no synergism; SS, RS, RR = see Table 2; A = $P < 0.0005$; B = $P < 0.01$; C = $P < 0.02$; D = $P < 0.05$; E = $P < 0.1$ (not significant).

Table 3 shows that SBA is also related to the presence of synergism. When the offending pathogen was susceptible to one (RS) or both (SS) of the antibiotics used, higher mean levels of SBA (1:16 and 1:128 vs 1:2 and 1:64) were obtained whenever *in vitro* antibacterial synergism was found. Of all patients, 25/26 (96%) who received a synergistic combination of two antibiotics active against the pathogens (SS+) were cured, whereas only 21/30 (70%) patients who were given two active non-synergistic antibiotics (SS-) recovered (D, $\chi^2 = 4.83$; $P < 0.05$). Patients treated with synergistic combinations had significantly more satisfactory rates of response than those who received non-synergistic combinations (A, $\chi^2 = 12.81$; $P < 0.0005$). The major contribution to the overall statistical significance was provided by patients with <100 PMN/μl (C, $\chi^2 = 5.67$; $P < 0.02$). With less severely granulocytopenic patients (100-500 PMN/μl) the trend approached but did not achieve significance (E, $\chi^2 = 3.09$; $P < 0.1$). Furthermore, among non-synergistic combinations the rates of cure were correlated to the susceptibility to one or both antibiotics used (16 vs 70%; B, $\chi^2 = 7.80$; $P < 0.01$).

Regardless of the bacterial species isolated from septicemic patients treated with combinations of antibiotics, the use of synergistic combinations was consistently associated with better clinical results (Table 4). The major contribution to the overall statistical significance was given by septicemias caused by *Pseudomonas aeruginosa* ($\chi^2 = 11.12$; $P < 0.001$). For this organism the means of 'therapeutic ratios' of beta-lactam and aminoglycoside antibiotics were consistently lower than those for *Klebsiella-Enterobacter-Serratia* spp. (K.E.S.) and *Escherichia coli*. Furthermore, higher mean peak levels of SBA were achieved when synergistic combinations were used (1:16 vs 1:4). With K.E.S. and *E. coli*, which showed a marked susceptibility to the antibiotics given to the patients, the differences between the use of synergistic vs non-synergistic combinations did not achieve significance.

DISCUSSION

The determination of SBA has originally been used to assess the adequacy of antimicrobial therapy for infectious endocarditis [11-13] and has subsequently been recommended as a guide for antimicrobial therapy in other serious infections such as osteomyelitis, septic arthritis and empyema [14, 15], as well as severe infections in cancer patients [14]. All of these studies have shown that a peak level of SBA ≥1:8 is strongly related to a favorable clinical outcome.

The aim of our work has been to testing the validity of such a method in predicting the efficacy of the therapy in the case of severely granulocytopenic patients with Gram-negative septicemia. Under these circumstances, the prognosis is severe and the chances of a favorable outcome crucially depend on the intensity of the bactericidal effect of antimicrobial treatment [2, 3].

Our data confirm previous observations about the usefulness of SBA determination in assessing the efficacy of antimicrobial therapy. According to Klastersky and co-workers [4], peak levels of SBA were more clearly related to the clinical outcome than trough levels. However, we found evidence that while less granulocytopenic patients (100-500 PMN/μl) needed bactericidal titers (≥1:8) equal to those required by non-neutropenic ones [4], patients with <100 PMN/μl required higher peak levels (≥1:32) to achieve a rate of cure of 100%.

Peak levels of SBA proved to depend on *in vitro* bacterial sensitivity; however, SBA appeared more important in predicting a favorable outcome than *in vitro* sensitivity in patients with a higher degree of granulocytopenia. Our data seem to confirm that *in vitro* synergistic combinations of antibiotics are associated with a more favorable clinical outcome, and this is probably due to the higher levels of SBA which can be achieved [16]. In our series 96% of patients under synergistic treatment vs 48% of patients under non-synergistic treatment ($P < 0.0005$) responded favorably. The

Table 4. Relationship between in vitro *sensitivity of the offending pathogen, occurrence of* in vitro *synergism, levels of SBA and clinical outcome*

Microorganism	Mean of 'therapeutic ratio'* Beta-lactam	Aminoglycoside	Synergism	SBA Trough Median	Trough Range	SBA Peak Median	Peak Range	% of response
Ps.aeruginosa (33)	7.5	8.6	+(11)	1:2	<1:2-1:8	1:16	1:2-1:64	91†
			-(22)	1:2	<1:2-1:16	1:4	<1:2-1:32	22†
E. coli (31)	974	23.5	+(15)	1:32	1:4-1:128	1:128	1:4-1:512	100‡
			-(16)	1:16	<1:2-1:64	1:128	1:2-1:256	87.5‡
K.E.S. (10)	259	51	+(3)	1:16	1:2-1:64	1:32	1:16-1:64	100§
			-(7)	1:8	<1:2-1:16	1:32	1:4-1:64	57§
Total (74)			+(29)	1:32	<1:2-1:128	1:128	1:2-1:512	96‖
			-(45)	1:8	<1:2-1:64	1:32	<1:2-1:256	48‖

( ) = number of cases; + = synergism; - = no synergism.

* = $\frac{\text{Mean peak serum level of each antibiotic.}}{\text{MIC required to inhibit each pathogen}}$

† $\chi^2 = 11.13$, $P < 0.001$.

‡ Not significant.

§ Not significant.

¶ $\chi^2 = 15.39$, $P < 0.0005$.

difference was especially striking in the patients with <100 PMN/μl and/or in those with a *Pseudomonas aeruginosa* septicemia. Anderson and co-workers [17] have reported that the favorable influence of *in vitro* synergism was especially remarkable in septicemias occurring in granulocytopenic patients (<2000 PMN/μl). Our data confirm this conclusion in the case of more severe granylocytopenic patients (≤500 PMN/μl). Furthermore, it seems that the importance of synergism increases with the decrease in granulocyte count (<100 PMN/μl). The above-mentioned study also reported that the major contribution to the overall statistical significance of association of *in vitro* synergism with clinical outcome was provided by septicemias caused by *Pseudomonas aeruginosa* rather than by those due to other Gram-negative bacilli. On the basis of our data, it seems to us that, as illustrated in Table 4, the distinguishing feature of *Pseudomonas aeruginosa* is that it showed the lowest *in vitro* susceptibility to the given antibiotics. On the other hand, the use of synergistic combinations was associated with a higher antipseudomonal activity of the serum and with a more favorable clinical outcome than the use of non-synergistic combinations of antibiotics. Conversely, the high *in vitro* susceptibility of K.E.S. and *E. coli* strains isolated from our septicemic patients did not result in a significant relationship between synergism and clinical outcome. In other words, our data suggest that the use of synergistic combinations may be crucial when the organism is not highly susceptible *in vitro* to the given antibiotics.

To conclude, our results show that a satisfactory correlation seems to exist between SBA levels and clinical outcome. We are aware that it has been suggested that single or even triple antibiotic therapies may be correlated with satisfactory rates of clinical successes when SBA values ≥1:16 were obtained [18]. However, as mentioned previously, the present study only deals with combinations of two antibiotics. According to our data the use of synergistic combinations of antibiotics, by increasing the bactericidal activity in the serum, may be important in curing any septicemia caused by organisms with a slight *in vitro* susceptibility to the given antibiotics. In terms of practical patient management, these data suggest that Gram-negative septicemias occurring in patients with <100 PMN/μl should, at least initially, be treated with combinations of antibiotics, and that they should be closely monitored through SBA determination. When the peak level of SBA is <1:16, antimicrobial therapy should be changed to a new combination of antibiotics that is more inclined to be not only active *in vitro* but also synergistic against the offending pathogen.

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