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Administration of Granulocyte Colony Stimulating Factor (G-CSF) in Experimentally Infected Rats*

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Abstract: We investigated the effect of antibiotic or G-CSF plus antibiotic therapy given to *E. coli* septic rats. In our study, 80 rats were inoculated transperitoneally with 1 ml of a solution containing 10^7 colony forming units per milliliter of *E. coli*. Rats were divided into four treatment groups before being inoculaded with *E. coli* in a random fashion. Therapy was then started with cefotaxime (150 mg/kg intraperitonelally) and either Granulocyte-Colony Stimulation Factor (G-CSF; 30 µg/kg intarperitoneally) or placebo 24 hours after inoculation of *E. coli* for 3 consective days. Group A_1, sepsis-antibiotics group, had a mortality rate of 65%; Group A_2, sepsis+G-CSF plus antibiotics group, had a mortality rate of 40% ($p>0.05$); Group B1, neutropenic sepsis+antibiotics group, had a mortality rate of 85%; B_2, neutropenic sepsis+G-CSF plus antibiotics group, had a mortality rate of 55% ($p<0.05$). By day 7 after *E. coli* incoculation, there was a significant difference in the mortality rate between the two neutropenic groups. No significant differences were found in the hemoglobin levels and thrombocyte counts between the groups but a significant increase in white blood cell (WBC) and absolute neutrophil counts (ANC) were found in the G-CSF treated groups. This study shows that administration of G-CSF, in addition to cefotaxime, in neutropenic rats with *E. coli* sepsis significantly decreased mortality rate compared with antibiotics alone.

Key Words: G-CSF, *E. coli* sepsis, neutropenia.

Introduction

Despite the increasing use of broad spectrum antibiotics, sepsis remains a leading cause of mortality in neonatal care units (1). Under the circumstances several new agents have been used to improve management of gram negative sepsis (2). The neutrophils are the first cells at the site of infection, atlacking the invading pathogen and simultaneously producing chemoattractants to call in additional phagocytic cells (3). Clinical trials show that G-CSF proved to be of value in preventing the neutropenia that is a poor prognostic factor among septic patients (4).

The purpose of our study was to assess the effectiveness of antibiotics and G-CSF plus antibiotics for reducing the mortality rate of *E. coli* sepsis with or without neutropenia.

Materials and Method

Eighty female albino rats weighing 100-150 g and 1.5-2 months old and *E. coli*, type ATCC 25922, were used. Concentration of bacteria was standardized and a suspension of $1x10^7$ organisms/ml was used. *E. coli* sepsis was confirmed with positive blood culture and neutropenia was defined as fewer than 500 circulating neutrophils per cubic millimeter in the prepiheral circulation, which was proven in a preliminary study (5). *E. coli* sepsis was developed by givin 1 ml of bacterial concentration and neutropenia was accomplished by giving cyclophosphamide (300 mg/kg). G-CSF, 30 µg/kg once a day and cefotaxime, 150 mg/kg/day q 12 hours was started 24 hours after infection with *E. coli* (6-8). *E. coli,* cyclophosphamide (Endoksan, Astra, İE), antibiotics (Claforan, Hoechst) and G-CSF (Neupogen, Roche) were injected intraperitoneally with a sterile tuberculin syringe. In the main experiment, animals were randomly assigned to four treatment groups (20 animals/group, observation period 7 days).

A_1. Animals received antibiotics after 24 hours of *E. coli* inoculation.

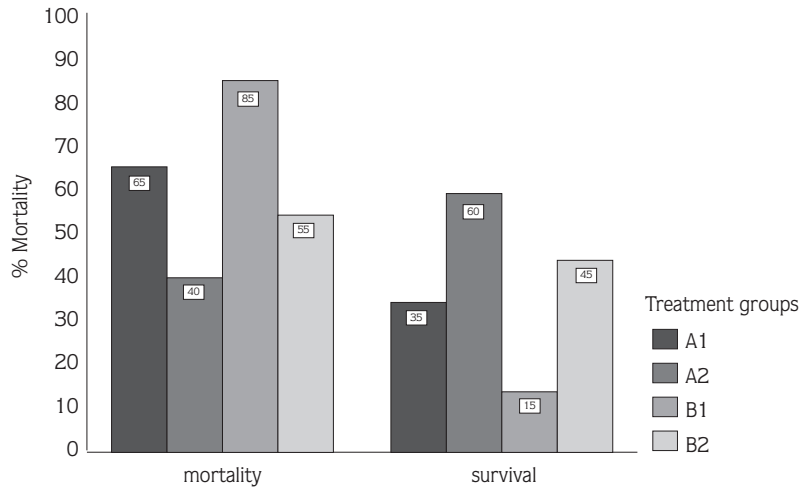

Figure. Mortality and survival percentage according to treatment group.

Table 1. Mortality rate, hemoglobin level and leukocyte, absolute neutrophil, thrombocyte count of the treatment groups of rats infected with *E. coli*.

Treatment	A (n=40) Group A_1 (Antibiotics)	Group A_2 (G-CSF plus antibiotics)	
Mortality rate(%)	13/20(65)	8/20(40)	p>0.05
Leukocyte count	3230±1967	6050±3026	p<0.01
Absolute neutrophil count	757±547	2514±1908	p<0.01
Hemoglobin level	12.3±1.9	12.3±1.6	p>0.05
Thrombocyte count	986200±92245	991000±72437	p>0.05

A_2. Rats were given G-CSF and antibiotics after inoculation of *E. coli.*

B_1. Rats obtained neutropenia by taking cyclophosphamide one day before and were given antibiotics after 24 hours of *E. coli* inoculation.

B_2. In addition to *E. coli* inoculation, neutropenic rats received G-CSF plus antibiotics simultaneously.

Mortality was monitored during the one week observation period and the animals were sacrificed on the 7th day. Serum samples from every rat were tested for complete blood count and blood smears were prepared on the 7th day or just before death.

Statistical analyses of clinical and laboratory data were done by using the χ^2 and Mann-Whitney U test for nonparametric variables. All results are expressed as mean values±SD and p values <0.05 were considered statistically significant.

Results

In the A_1 and A_2 groups, 13 rats (65 percent) and 8 (40 percent) died during the study period, respectively. At the end of study, 17 rats (85 percent) of the B_1 and 11 rats (55 percent) of the B_2 group died (Figure). Mortality rates were lowest in the non-neutropenic septic rats and highest in the neutropenic septic rats. Although the rats given antibiotics plus G-CSF had a low mortality rate, a significant difference was not observed between the A_1 and A_2. In group B, there was a significant reduction in mortality rate in rats receiving both antibiotics and G-CSF ($p<0.05$). WBC and ANC increased significantly in G-CSF treated rats. No significant differences were found in the hemoglobin level and thrombocyte count between the study groups treated with antibiotics plus G-CSF and antibiotics only (Tables 1 and 2).

Discussion

Despite the increasing use of newer therapeutic regimens, sepsis is still a common cause of morbidity and mortality. Most sepsis are caused by gram negative bacilli and *E. coli* is the most commonly isolated pathogen (2, 9-12). Neutrophils are the primary host defense mechanism

Table 2. Mortality rate, hemoglobin level and leukocyte, absolute neutrophil, thrombocyte count of the treatment groups of neutropenic rats infected with *E. coli*.

Treatment	B (n=40) Group B1 (neutropenia +antibiotics)	Group B2 (neutropenia +G-CSF plus antibiotics)	
Mortality rate (%)	17/20(85)	11/20(55)	p<0.05
Leukocyte count	920±684	4590±2423	p<0.01
Absolute neutrophil count	326±133	1905±1622	p<0.01
Hemoglobin level	13.8±0.5	13.6±0.9	p>0.05

in invasive bacterial infections. The increased use of immunosuppressive drugs enhances susceptibility to infection and mortality rate by reducing leukocyte counts (5, 10, 11). Perhaps the best studied hematopoietic growth and differentiation factor is G-CSF, which has a specific range of effect on the neutrophils (1, 13-15). G-CSF enhanced both neutrophil number and function (1, 5, 8, 9, 11, 15-17). It has potential benefit as an accelerator of neutropenia healing and has applications in this regard in sepsis or neutropenic sepsis (5, 9, 11, 15, 17-19).

G-CSF has been reported to be an effective adjunct to therapy in sepsis (9, 17-21). Studies published since 1980 have suggested that prophylactic or therapeutic use of G-CSF reduced the mortality rates during the courses of sepsis (13, 15, 21). In clinical practice, physicians are frequently treating patients for active infection or sepsis rather than trying to prevent infection. In vitro and animal experiments have shown a protective efficacy of G-CSF preparations against various bacterial infections (9, 13, 15, 17-20, 22). In this study, therapeutic administration of G-CSF also augmented the effect of antibiotics against *E. coli* sepsis. There were significant differences in mortality in G-CSF+antibiotics group than in the antibiotics group, especially in the neutropenic group (Figure). When we gave G-CSF after cyclophosphamide, the neutropenia produced by this agent was completey abrogated. We also found a life-prolonging effect in nonneutropenic *E. coli* infected rats that were given G-CSF therapy. In nonneutropenic rats, there was only a 25% difference in the mortality rate favoring the G-CSF treated group that did not achieve statistical significance. The differences which are not statistically significant may have been the result of the small size of the study group. These findings can be explained by the fact that the neutrophil count was also higher and the mortality rate was lower in nonneutropenic rats after treatment with G-CSF. We can speculate that G-CSF given in early sepsis can preempt the death of rats which are neutropenic and augment the effect of antibiotics treatment. This correlates with neonatal studies that demonstrate that neutropenia is a halkmark of a poor prognosis in sepsis. It was thought that therapeutic use of G-CSF in sepsis may shorten stays in neonatal intensive care units.

Neutrophils are short-living and are renewed constantly during life. Several studies have reported that an ANC is an important factor in septic patients (20, 23). The main role in defense against bacterial infections such as *E. coli* is played by neutrophils (1, 13, 20, 22). Clinically, the neutropenia of the pediatric patients usually show the worst prognoses (1, 10, 20, 21). Neutropenic patients and animals often have persistent infections because of the decrease in neutrophils, which are important in the early stage of host defense (1, 10, 20, 22). In rats specifically immune-suppressed by cyclophosphamide, the numbers of neutrophils are significantly reduced. G-CSF has no direct effect on the growth of bacterial cells, its activity is solely based on the production of new neutrophils and on the increasing activity granulocytes already formed (14, 15, 18, 22). We noted a significant increase in WBC and ANC in both groups treated with G-CSF plus antibiotics compared with antibiotics treatment alone. The mortality rate in our study also paralleled the presence of leukopenia and neutropenia (Table 1 and 2).

The result of this study demonstrates that administration of G-CSF with antibiotics in septic rats with or without neutropenia leads to a reduction in mortality and may have a synergistic and protective effect on survival in chemotherapy induced neutropenic patients.

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