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ORIGINAL PAPER

Recombinant human Granulocyte-Colony Stimulating Factor (rh-G-CSF) improves neutrophil phagocytic capability, without enhancing the systemic inflammatory response in septic patients with severely impaired neutrophil function

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Abstract: In the present pilot study recombinant human Granulocyte Colony Stimulating Factor (rh G-CSF) was adjunctively administered in septic ICU patients with severe structural abnormalities of their Polymorfonuclear Leucocytes (PMNL) and a phagocytic activity (NBT-test) lower than 50% of the normal value. The drug was administered subcutaneously in a dose of 0.5 IU (5 μg)/kg B.W./ day until the phagocytic activity of the circulating PMNL increased over 80%. This was done after 3-12 days (median 5) of administration. The following changes (means ± SD) have been occured during rh G-CSF administration: The number and the phagocytic activity of the circulating PMNL increased significantly from 13.454 ± 7.158 x 10^9/L and 34 ± 15% to 31.987 ± 16.150 x 10^9/L and 81 ± 6% respectively ($p < 0.001$), while the morphological picture of the peripheral PMNL experienced a drastic improvement. Platelets number increased from 122 ± 30.7 to 220 ± 45,25 x 10^9/L ($p < 0.001$). Cardiac index decreased significantly from 4.9 ± 1,9 to 3,4 ± 0,6 L/min/m^2 ($p < 0.001$) and systemic vascular resistance increased significantly from 642 ± 141 to 1034 ± 220 dyn.sec.cm^{-5} ($p < 0.001$). The APACHE II score decreased from 21,5 ± 1,8 to 16,2 ± 4,5 ($p < 0.001$), due to regression of fever, tachycardia, hypotensive episodes and hypernatremia. The serum iron concentration raised from 40 ± 10.6 to 82 ± 22 μg/dL ($p < 0.001$). These changes indicate a limitation of the inflammatory process. It is concluded that the hematopoietic growth factor G-CSF reverses the structural and functional derrangements of the PMNL in critically ill patients. This effect may imply a limitation of the infectious stimulus and hereby the host's inflammatory response, contributing to reduction of hospitalization and mortality in septic patients. A prospective randomized clinical trial could show the impact of adjunctive G-CSF therapy on the outcome of these septic patients.
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Nosocomial infections are directly related to hospitalisation and the outcome of critically ill patients[1,2]. Sepsis remains the leading cause of morbidity and mortality in critically ill patients despite the use of appropriate antibiotics and supportive therapy in competent intensive care units. Gram negative sepsis is associated with a mortality rate of 25-32%[3,4], which reaches to 40-77% when septic shock develops[5]. The incidence of gram-negative bacteremia has increased dramatically during the last 40 years, essentially during the development and broad clinical application of antibiotics[6]. Therapy with antibiotics is often ineffective, while endotoxemia is sometimes sustained or even worsened during this therapy[7]. These data redirected the search for new therapeutic modalities that are adjunctive to the traditional supportive and antimicrobial therapy of severe infections[5,7].

Critically ill patients present impaired and down-modulated immunological response[8-11]. Normally polymorphonuclear leukocytes

(PMNL) play both quantitatively and qualitatively a primary role in the host defence mechanism against bacterial invasion and proliferation[12-14]. Increased numbers and improved functional capacity of circulating PMNL consequently augment host defences against infections[15], whereas leucopenia increases mortality related to gram-negative bacteremia[3,16]. On the other hand, even adequate number of circulating PMNL during sepsis may be insufficient to defend against overwhelming bacterial invasion because their functional capacity may be impaired[17].

Transfusion of leukocytes from healthy volunteers to septic patients was suggested and performed twenty years ago[18]. Granulocyte-Colony Stimulating Factor (G-CSF) is a normally occuring hematopoietic growth factor, which stimulates proliferation and maturation of bone marrow (BM) progenitor cells committed to the granulocyte lineage and increases the number as well as the functional activity of peripheral PMNL[19-22]. This factor, – purified and characterised in recent years – has been recently cloned and recombinantly expressed in Escherichia coli[19,23,24]. The clinical application of the recombinant human Granulocyte Colony Stimulating Factor (rh G-CSF) in cancer patients with neutropenia results in increased BM cellularity and increased circulating PMNL numbers as well as in an improvement in the functional capabilities of mature neutrophils[25-28]. The clinical correlate of these BM and peripheral PMNL changes was a significant reduction in the time proportion free of fever, antibiotics and duration of hospitalisation during chemotherapy[28]. The application of G-CSF against infectious diseases has been recently considered. Animal models of infection and sepsis showed that rh G-CSF increases host defence and survival[14,29-33]. These results and the absence of any significant adverse effects in humans[27,28] support the clinical application of rh G-CSF in septic patients with neutropenia or those with poorly functioning neutrophils, in the absence of neutropenia.

In the present pilot study we have undertaken adjunctive therapy with rh G-CSF in septic patients, which presented morphological and functional disturbances of their peripheral PMNL.

PATIENTS AND METHODS

Patients: Eighteen consecutive septic patients treated in our ICU and presenting severe structural and functional alterations in their peripheral PMNL entered the study. The criteria for sepsis were set according to the definitions of the ACCP/SCCM Consensus Conference[34]. The study was approved by our Institutional Review Board as well as by the National Administration of Drugs.

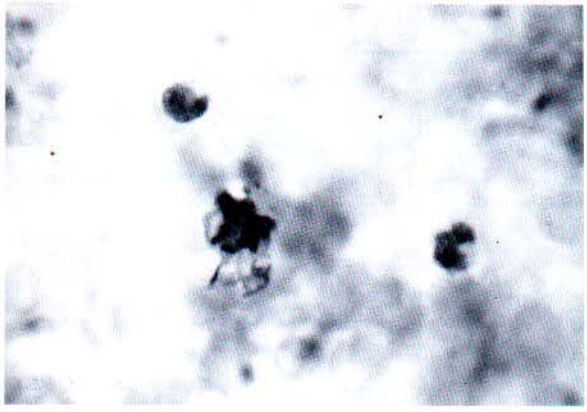
All patients received the standard therapeutic regimen given in our ICU for septic patients. This regimen includes mechanical ventilation, crystalloids and colloids to maintain a wedge pressure of about 12-18 mmHg, blood transfusions to maintain an Hematocrit (Ht) at or above 33%, antibiotics (according to antibiograms, when avalaible), combined enteral plus parenteral nutrition (30 non protein kcal/kg B.W./d.), human immunoglobulin i.v. (0.4 g/kg BW/d for 2 days and 0.2 g/kg BW/d for the next 3 days), pentoxifylline (25 mg/kg BW/d, i.v.), vit E (400 mg/d, i.m.) and Dopamine alone or in combination with Dobutamine when hemodynamic instability persists despite adequate fluid administration. Norepinephrine has to be administered at hypotensive episodes, to maintain the mean arterial blood pressure $\geq$80 mmHg.

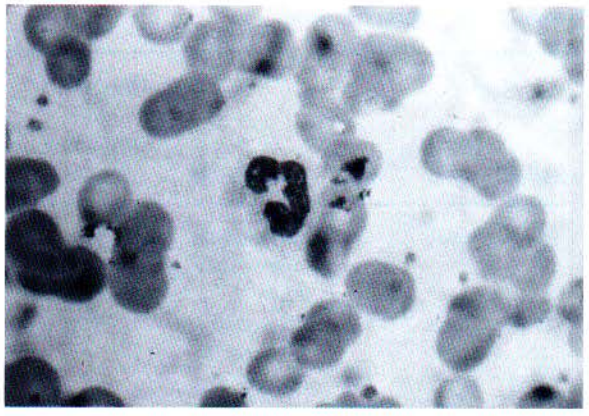
Monitoring included systemic and pulmonary artery pressures. The cardiac output was determined (thermodilution) once daily as well as during fluid or catecholamine therapy. The patients were clinically evaluated as required and the APACHE II score was ascertained daily, taking into account the worst values of the day.

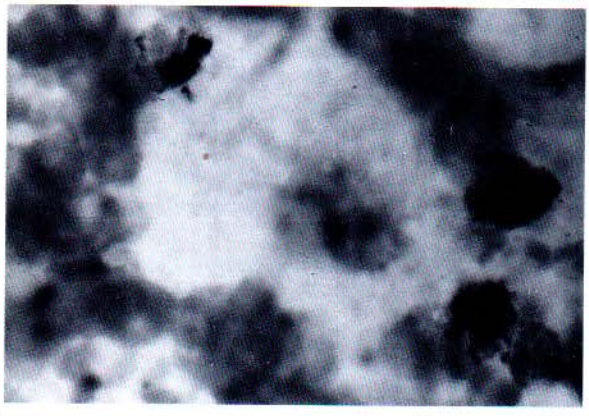
Estimation of Leukocytes. As soon as the clinical signs of sepsis appeared, daily laboratory control of the peripheral leukocytes begun. This evaluation was also undertaken during rh-G-CSF administration, as well as after its discontinuanuce, until PMNL numbers returned to a level of around 10-15 $\times$ 10^9/L. This control included: 1. Total leukocyte counts, by automatic analyser (counter S Plus IV). 2. Microscopic evaluation of blood smears stained by May-Gruenwald-Giemsa. 3. Determination of the phagocytic activity of the PMNL, using the reduction of dye Nitroblue-Tertazolium (NBT-test) (Fig. 1,2).

Standards for rh G-CSF administration. 1. Morphological alterations of the PMNL, consisting of absence of granules, decreased cell volume and suppression of the lobulation of the nuclei in a ratio $\pm$50% (Fig. 3,4). 2. Phagocytic activity lower than 50% of the normal values. Administration of the drug was ceased as soon as morphology improved and the phagocytic activity reached a level of 80% (or more) of the normal value. The Drug was administered subcutaneously in a dose of 0.5 I.U. (5 μg)/kg BW/day), as an adjunct to the above mentioned therapeutic regimen.

Other laboratory controls. Specimens for culture: 1. Blood, sampled repetitive every day. 2. Bronchial secretions, sampled with the protected catheter technique once daily. 3. Urine, open wound smear and percutaneous drain secretion every two days. 4. Catheter tip (central venous, urinary, drainage) at removing. 5. Peritoneal fluid, obtained by chance of laparotomy and Cerebrospinal Fluid (CSF), sampled by spinal punction when meningitis was suspected.







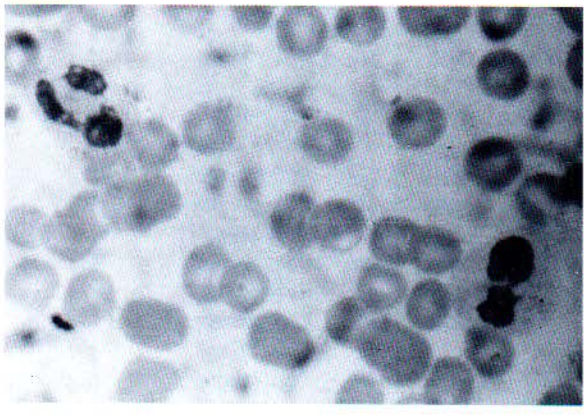


Fig.1,2. The phagocytic activity of the PMNL using the reduction of the dye Nitroblue Tetrazolium (N.B.T. test).

Fig. 3,4. Morphological alterations of the PMNL before the rh-G-CSF administration.

Clinical chemistry and Haematology. Daily routine biochemical and hematological tests including the above mentioned procedures for leucocyte estimation and platelet counts.

Definitions of infection. 1. The diagnosis of respiratory infection required the existence of purulent sputum, the isolation and the heavy growth of the same pathogen in two consecutive cultures from bronchial secretion and pulmonary infiltrates in the chest X-ray. 2. The diagnosis of urinary tract infection required the isolation and growth of a pathogen from urine cultures at a concentration greater than 10^5 CFU/mL. 3. The diagnosis of peritonitis required isolation of microorganisms in culture peritoneal fluid obtained directly (laparotomy) or from a percutaneous peritoneal drainage. 4. The diagnosis of bacteremia required the isolation of a microorganism in one or more blood cultures.

Statistical analysis. The values on the day that therapy with rh G-CSF was started, were considered as the pre-treatment values and those on the day next to therapy were interrupted as the post-treatment values.

The paired Student -t test was used to compare the post-treatment to the pre-treatment values of the monitored laboratory and clinical parameters. Statistical significance was considered at $p < 0.05$.

RESULTS

An overall of 18 patients, 16 male and 2 female, aged 12-76 years (median 38ys) have entered the study (Table 1). Seventeen patients were admitted due to multiple trauma with an injury severity score (ISS) 20-43 (median 32). Two of them, who were admitted from other wards, presented acute renal failure. Nine multiple trauma patients had moderate (GCS $\geq$ 8) to severe (GCS $<$ 8) head injury among other injuries. One patient was admitted postoperatively because of peritonitis with severe sepsis due to a perforated duodenal ulcer. The APACHE II score on the initial ICU day ranged 12-36 (median 20).

All patients received adequate i.v. fluid therapy and broad spectrum antibiotics and were ventilator dependent until the administration of rh-G-CSF began.

A respiratory infection alone or in combina-

Table 1. Demographic data

Patient	Sex	Age	Initial insult	APACHE II score at the first ICU day	Indication for G-CSF (ICU day)	Duration of administration of G-CSF (days)	ICU stay (days)	Outcome at the 28th day after the initial insult	Outcome at the 60th day after the initial insult
M.H.	male	29	Polytrauma-severe head injury	19	19th	7	41	Survived	Survived
M.P.	male	25	Polytrauma-AR (enal)F	14	5th	6	24	Survived	Survived
P.N.	female	12	Polytrauma-AR (enal)F	21	7th	5	23	Survived	Survived
N.C.	male	41	Polytrauma-severe head injury	16	5th	5	36	Survived	Died
P.P.	male	36	Polytrauma-injury	18	18th	5	25	Survived	Survived
S.S.	male	53	Peritonitis severe sepsis	22	2th	12	20	Survived	Survived
T.K.	male	63	Pollytrauma	23	12th	5	23	Survived	Survived
O.T.	male	16	Polytrauma-head injury	26	17th	8	27	Survived	Survived
S.I.	male	76	Polytrauma	22	4th	3	14	Survived	Survived
P.S.	male	48	Polytrauma	17	6th	5	16	Survived	Survived
K.I.	male	19	Polytrauma-head injury	13	7th	4	12	Survived	Survived
T.A.	male	37	Polytrauma-severe head injury	12	13th	5	23	Died	-
M.A.	male	60	Polytrauma	23	11th	3	20	Survived	Survived
P.A.	male	65	Polytrauma	35	13th	4	59	Survived	Died
T.D.	male	17	Polytrauma-head injury	18	10th	4	23	Survived	Survived
T.G.	male	39	Polytrauma	36	2th	6	10	Survived	Survived
K.S.	male	39	Polytrauma-severe head injury	35	11th	4	61	Survived	Died
B.L.	female	15	Polytrauma-severe head injury	18	15th	7	45	Survived	Died
	M/F: 16/2	median :38 range: 12-76	Polytrauma:17 Peritonitis:1	median:20 range:12-36	median:10 range:2-19	median:5 range:3-12	median: 2-3 range: 10-61	Survivors 17/18 (94,4%)	Survivors 13/18 (72%)

ARF: Acute Renal Faillure.

tion with urinary tract infection was identified in 13 patients (72%) (Table 2). Four patients developed bacteremia (22%). Gram negative bacteria were isolated in all but one cases with a prevalence of pseudomonas aeruginosa (72%) and klebsiella pneumoniae (25%).

The criteria for rh-G-CSF therapy were fulfilled on day 2-19 (median 10) after admission and the duration of administration ranged 3-12 (median 5) days (Table 1).

Both total leukocyte count and PMNL phacocytic activity increased significantly about 2.5 fold during rh-G-CSF administration ($p < 0.001$) (Table 3, Fig 5). The morphology of the neutrophils showed a characteristic improvement (Fig. 6). These changes began as soon as 24

Table 2. Isolated bacteria species and distribution of infection sites in the 18 septic

Patient	Pseudomonas Aeruginosa	Akinetobacter Anitratus	Klebsiella Pseumoniae	Serratia Marcencens	Strepto-coccus	Entero-bacter	E.Coli	Staphylo-coccus	Bacteriodes Fragilis
M.H.	+								
M.P.	+		+						
P.N.	≠	(*)							
N.C.			+						
P.P.	+								
S.S.				&,(*)	(*)				
T.K.	+								
O.T.	+,x								
S.I.			+						
P.S.	+								
K.I.	+								
T.A.	+					+			
M.A.	+								
P.A.	x						+		
T.D.	≠▷x		x					≠	
T.G.			&		&				
K.S.								(*)	
B.L.	+,x								

+: Respiratory, x: Urinary, (*): Blood, ≠: Wound, &: Peritoneal Drainage.

Table 3. Changes in hematologic variables during G-CSF treatment

Variable	Pre-treatment values (x ± SD)	Post-treatment values (x ± SD)	p value
Total Leycocyte Count (x 10^9/L)	13,454 ± 7,158	31,987 ± 16,150	<0.001
Phagocytic activity (% of the normal value)	34 ± 15	81 ± 6	<0.001
Platelets Count (x10^9/L)	122 ± 30,7	220 ± 45,25	<0.001
Hematocrit (Vol-%)	33 ± 3,2	36 ± 3,6	NS
Hemoglobin (gr/dL)	11,1 ± 1,3	11,8 ± 1,2	NS

NS = non significant.

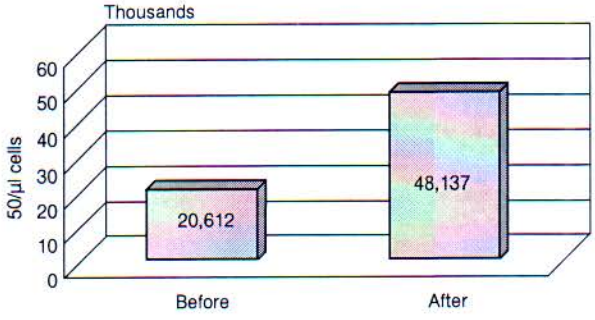


Fig.5. Leucocyte numbers before and after administration of G-CSF.

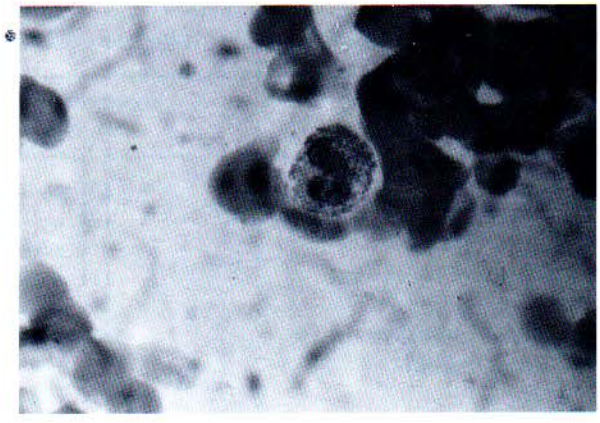


Fig. 6. Morphology of the PMNL after the rh-G-CSF administration.

hours after the first administration of rh-G-CSF and were progressive. In 75% of the patients, the total leucocyte numbers returned to a value around 10-15 × 10^9/L four days after rh-G-CSF administration was ceased. The platelet count increased parallel to the increase in leukocyte number about 2 fold ($p < 0.001$) (Table 3). Haemoglobin and haematocrit values (didnt's change) remained the same (Table 3).

The APACHE II score decreased significantly from 21.5 ± 1.8 points to 16.2 ± 4.5 points ($p < 0.001$) (Table 4) despite the two points gained by the increase in leucocyte count. This change was due to the subsidence of fever, tachycardia, hypotensive episodes and hypernatremia.

Cardiac index decreased significanty from 4.9 ± 1.9 to 3.4 ± 0.6 L/min/m^2 ($p < 0.001$) and systemic vascular resistance increased significantly from 642 ± 141 to 1034 ± 220 dyn.-sec.cm^{-5} ($p < 0.001$) (Table 4).

Among the biochemical parameters, the serum enzyme activities of alkaline phosphatase and g-GT and the concentration of iron raised significantly (Table 5), whereas the serum enzym activity of aminotransferases and the values of creatinine, bilirubin and uric acid didn't change. In the two patients with acute renal failure who received daily hemodialysis, creatinin serum concentration decreased from 9.5 and 5.2 to 7.1 and 4.9 mg/dl respectively. In the patient with peritonitis and initial bilirubinaemia, bilirubin serum concentration decreased from 10.7 to 4.7 mg/dL.

The level of endogenous G-CSF in the serum of the studied patients was at 100 ± 16,5 pg/mL, lying in the range of levels found in infected patients[35].

The mortality rate was 5,6% at the 28th and 28% at the 60th day after the onset of sepsis (Table 1). Four of the nonsurvivors were trauma patients with associated severe brain injury, who never exceeded the GCS of 8. One 65 years old nonsurvivor had suffered a severe blunt thoracic and abdominal trauma associated with protracted shock and a high first day APACHE II score (patient P.A., Table 1). This patient succumb to repeated septic episodes developing ARDS and MOSF. In an historical group of 16 comparable patients receiving the same therapeutic regimen except rh G-CSF, who were studied last year in our ICU to evaluate the therapeutic efficacy of

Table 4. Changes in hemodynamic variables and the APACHE II score during G-CSF

Variable	Pre-treatment values (x ± SD)	Post-treatment values (x ± SD)	p value
Cardiac Index (L/min/m^2)	4,9 ± 1,9	3,4 ± 0,6	<0.001
Systemic vascular resistance (dyn.sec.cm^{-5})	642 ± 141	1034 ± 220	<0.001
APACHE II score (points)	21,5 ± 1,8	16,2 ± 4,5	<0.001

Table 5. Changes in Biochemical variables during G-CSF treatment

Variable	Pre-treatment values (x ± SD)	Post-treatment values (x ± SD)	p value
Alkaline Phosphatase (IU/L)	125 ± 48	208 ± 82	<0.001
γ-GT (IU/L)	102 ± 60	164 ± 121	<0.01
SGOT (IU/L)	98 ± 55	93 ± 75	NS
SGPT (IU/L)	111 ± 96	94 ± 54	NS
Bullirubin (mg/dL)*	0,8 ± 0,6	0,6 ± 0,2	NS
Creatinine (mg/dL)**	1,04 ± 0,29	0,94 ± 0,17	NS
Uric acid (mg/dL)	2,7 ± 1,9	3,2 ± 2,5	NS
Fe (μg/dL)	4 0 ± 11	82 ± 22	<0.001

* except the value from the patient with peritonitis (patient S.S.) and initial bilirubinemia

** except the values of the two patients with initial acute renal failure (patients M.P. and P.N.)

adjunctive high dose human immunoglobuline, on 28th day mortality rate reached 35%.

DISCUSSION

Major surgery, anaesthesia, severe trauma or burns and severe infections are frequently associated with structural and functional disturbances of the PMNL[10,17,29,36]. In the present pilot study of critically ill patients, significant structural and functional alterations of the circulating PMNL have been identified. This finding suggests insufficient host defences against pathogens, despite the presence of normal or even elevated numbers of leucocytes[17].

This study showed that rh-G-CSF administered to critically ill patients with nosocomial infections increases significantly the number as well as the phagocytic capability of the circulating PMNL, already 24 hours after the first injection. These findings are in accordance with those in the animal model of sepsis as well as in patients with malignancy, receiving rh-G-CSF[14,24-33]. The morphological and functional improvement in the peripheral leukocytes, following rh-G-CSF administration are a result of: 1) Mobilization of the bone marrow pool of mature PMNL. 2) Stimulation of the proliferation and maturation of bone marrow progenitor cells committed to the granulocyte lineage and 3) Enhancement of the functional properties of the mature PMNL, including phagocytic activity[19-22,25-27,37,38].

The increased number of circulating PMNL enhances host defence against infection[13-15] and potentiates the effects of antibiotics in vivo[30,31]. The increased number combined with the improved functional activity (e.g. migration, chemotaxis, production of superoxide anion, phagocytosis, antibody dependent cytotoxicity and bacterial killing) of the PMNL in vivo[27,29,37,39] entail increased capability in approaching and neutralising pathogens[13]. Nelson and colleagues[14] found that rh-G-CSF prophylactically administered in rats intoxicated with alcohol and infected with Klebsiella pneumoniae, markedly increases the influx of PMNL to the site of infection (lungs) and significantly decreases the number of viable pathogens at this site. Consequently the survival rate of the treated rats was significantly higher in comparison to the survival rate of the untreated animals[14]. Similarly, Yasuda and col.[31] found that, pseudomonas aeruginosa infected mice markedly increased PMNL influx to the site of infection (muscle) and decreased bacterial proliferation at this site as well as in blood, when rh-G-CSF was administered therapeutically. In addition, the efficacy of antibiotics and the survival rate were significantly increased in treated compared to untreated animals[31]. Lang et al[33] measured a three fold increase in the number of leucocytes at the site of infection and a significant decrease in the number of viable bacteria at this site, when rh G-CSF was prophylactically administered in rats infected with Escherichia Coli.

These data could explain the stepwise regression of fever and the towards normal hemodynamic changes in our patients as a consequence of limitation of the infectious stimulus and the host's systemic inflammatory response, after rh G-CSF administration. Pre-treatment of septic rats with rh G-CSF did not modify the sepsis-induced changes in hemodynamics or body temperature[33]. To our knowledge, the interactions between G-CSF and other cytokines during infection has not been clarified yet. In uninfected patients with malignancy, no modification in the plasma levels of TNF-α and IL-1β have been detected, whereas the amount of soluble IL-2 receptors was increased[27]. The clinical improvement in our patient population is in our opinion equivalent to the decrease in days with neutropenia, fever, antibiotics and hospitalization found by Crawford et al. in patients with malignancy receiving chemotherapy and rh G-CSF[28]. Our patients were not neutropenic, but their circulating neutrophils were markedly altered in structure and function, a condition, which could be potentially equivalent to neutropenia.

The dose of rh G-CSF used in the present study is low and similar to that administered in cancer patients receiving chemotherapy[28]. This dose and even larger doses have been administered in cancer patients, without any serious adverse effects[27,28]. The increased activities of the enzymes LDH and alkaline phosphatase as well as the increased uric acid concentration detected in serum in these studies, correlated with the changes in the neutrophil turnover and were dose dependent and transient[27,28]. The increase in serum enzyme activity of alkaline phosphatase found in our patient population was associated with an increase in the serum enzyme activity of

γ-GT (Table 5). This finding suggests to a worsening of the liver function. This is not indispensable a result of the rh G-CDF application, given that other factors could also be responsible, for example participation of the organ in the septic process, long term mechanical ventilation and medication.

The use of immunomodulatory therapy in critically ill patients is presently associated with difficulties[5,11]. Activated PMNL adhere to stimulated proinflammatory capillary endothelium and secrete proteolytic enzymes and cytotoxic oxygen metabolites causing increased permeability and microcirculatory disturbances contributing to the pathogenesis of organ dysfunction or failure[40,41]. Hematopoietic growth factors enhance the production of cytotoxic oxygen metabolites[27,37] as well as the activity of lysosomal enzymes[27]. Consequently, administration of these factors encompasses the potential of tissue damage by the increased number and function of the circulating PMNL. However, it is not well known whether G-CSF will promote a generalized neotrophil infiltration into all tissues or a more selective recruitment of neutrophils into the focus of infection. The existing experimental data point at a more selective recruitment of neutrophils into the site of infection[14,31,33], whereas remote tissues did not show a detectable influx of neutrophils[33]. The decrease in the number of viable bacteria at the site of infection as well as in the blood found in experimental studies[14,31,33] suggests limitation of the inflammatory stimulus and control rather than an enhancement of the systemic inflammatory response. Prophylactic or therapeutic amdinistration of rh G-CSF in septic animals[14,29-33] as well as in cancer patients with or without neutropenia[27,28] did not cause enhancement of the host's inflammatory response. In contrast the survival of septic animals improved[14,30-32], and the number of days with fever, antibiotic requirement and hospitalization of cancer patients decreased significantly[28]. The findings of the present study, e.g. the changes in hemodynamics and APACHE II score (Table 4) and the behaviour of the serum iron (Table 5) signify a regression of the septic process.

It is hard to conclude about hospitalization and mortality in a mixed patient population. Patients with severe head injury have a long term ICU stay underlying repeated infectious insults. Brain injury is a major determinant of outcome in these patients. It is only the similar composition of the two groups and the same therapeutic regimen (except G-CSF) that we have untertaken comparison of mortality rates in the present versus the historical group. A prospective randomized clinical study taking into account the underlying condition is required to bring conclusions about the impact of adjunctive therapy with rh G-CSF on the outcome of septic patients in the ICU.

In conclusion, the present study showed that sepsis induced structural and functional abnormalities of the PMNL can be reversed by rh G-CSF. It is also suggested that the improvements in number and phagocytic activity of the circulating PMNL limits the septic process and associates with clinical improvement and better outcome of septic patients. A multicenter clinical trial addressing the last possibility is required to document that adjunctive therapy with rh G-CSF improves survival in sepsis.

ΠΕΡΙΛΗΨΗ

Μακρυγιαννάκη Ε, Χατζηνικολάου Κ, Βαγδατλή Ε, Φραγκοπούλου Ε, Τσιότρας Χ, Οικονόμου Μ. **Ο ανασυνδυασμός ανθρώπειος παράγοντας ενεργοποιήσεως αποικιών κοκκιοκυττάρων (rh-GCSF) αυξάνει τη φαγοκυτταρική ικανότητα των πολυμορφοπυρήνων, χωρίς να επιδεινώνει τη συστηματική φλεγμονώδη αντίδραση, σε σηπτικούς αρρώστους με βαρειά διαταραχή της λειτουργικότητας των πολυμορφοπυρήνων τους.** Hipokratia 1997, 1: 83-92.

Τα πολυμορφοπύρηνα λευκοκύτταρα (ΠΜΛ) παριστούν πρωταρχικό αμυντικό μηχανισμό του οργανισμού ενάντια στην εισβολή και τον πολλαπλασιασμό των μικροβίων. Βαρειές προσβολές του οργανισμού, συμπεριλαμβανομένων των λοιμώξεων, προκαλούν σημαντικές δομικές και λειτουργικές αλλοιώσεις των ΠΜΛ, με αποτέλεσμα την ανεπαρκή αντιμικροβιακή άμυνα ακόμα και επι φυσιολογικού ή και αυξημένου αριθμού κυκλοφορουμένων ΠΜΛ. Ο παράγοντας Granulocyte Colony Stimulating Factor (G-CSF) αυξάνει τον αριθμό αλλά και την δραστικότητα των ΠΜΛ, ενισχύοντας έτσι την αμυντική ικανότητα του οργανισμού. Στην παρούσα μελέτη-πιλότο χορηγήθηκε ο βιοτεχνολογικά παρασκευασθείς παράγοντας (rh G-CSF) σε σηπτικούς αρρώστους με βαρειές μορφολογικές αλλοιώσεις των ΠΜΛ τους και φαγοκυτταρική ικανότητα (N B T test) μικρότερη του 50% της φυσιολογικής. Το φάρμακο χορηγείτο υποδορίως σε δό-

ση 0,5 IU (5 μg)/kg Β.Σ/24ωρο, μέχρι η φαγοκυτταρική ικανότητα των κυκλοφορούντων ΠΜΛ να αυξηθεί σε επίπεδα 80% και πάνω της φυσιολογικής. Αυτό προέκυπτε μετά 3-12 ημέρες (διάμεσος 5 ημ.) χορηγήσεως. Κατά την χορήγηση του rh G-CSF προέκυψαν οι εξής μεταβολές (x ± SD): Ο αριθμός των λευκοκυττάρων και η φαγοκυτταρική ικανότητα των ΠΜΛ του αίματος αυξήθηκαν σημαντικά από 13,454 ± 7,158 x 10^9/L και 34 ± 15% σε 31,987 ± 16,150 x 10^9/L και 81 ± 6% αντίστοιχα ($p < 0,001$), ενώ η μορφολογική εικόνα των ΠΜΛ του αίματος βελτιώθηκε θεαματικά. Ο αριθμός των αιμοπεταλίων αυξήθηκε από 122 ± 30,7 σε 220 ± 45,25 x 10^9/L ($p < 0,001$). Ο καρδιακός δείκτης ελαττώθηκε από 4,9 ± 1,9 σε 3,4 ± 0,6 L/min/m^2 ($p < 0,001$) και οι συστηματικές αγγειακές αντιστάσεις αυξήθηκαν από 642 ± 140 σε 1034 ± 220 dyn.sec.cm^{-5} ($p < 0,001$). Το APACHE II score μειώθηκε από 21,5 ± 1,8 σε 16,2 ± 4,5 ($p < 0,001$), εξαιτίας υποχωρήσεως του πυρετού, της ταχυκαρδίας, των υποτασικών επεισοδίων και της υπερνατριαιμίας. Η συγκέντρωση του σιδήρου στον ορό αυξήθηκε από 40 ± 11 σε 82 ± 22 μg/dL ($p < 0,001$). Οι μεταβολές αυτές στην αιμοδυναμική στις παραμέτρους του APACHE II score και στην συγκέντρωση του σιδήρου είναι ενδεικτικές περιορισμού της φλεγμονώδους διαδικασίας. Συμπεραίνεται ότι ο ανασυνδυασμένος αυξητικός παράγοντας rh-G-CSF αναστρέφει τις μορφολογικές και λειτουργικές βλάβες των ΠΜΛ των βαρέως πασχόντων. Η δράση αυτή μπορεί να συνοδεύεται από περιορισμό του λοιμώδους ερεθίσματος και της φλεγμονώδους αντιδράσεως του οργανισμού, συμβάλλοντας στην μείωση της νοσηρότητας και της θνησιμότητας των σηπτικών αρρώστων. Μια προγραμματισμένη πολυκεντρική μελέτη θα μπορούσε να δείξει την επίδραση του rh-G-CSF στην έκβαση των ασθενών αυτών.

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