Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: Effect on survival in patients with septic shock*

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Objective: **To assess the safety and efficacy of the nitric oxide synthase inhibitor 546C88 in patients with septic shock. The predefined primary efficacy objective was survival at day 28.**

Design: **Multiple-center, randomized, two-stage, double-blind, placebo-controlled, safety and efficacy study.**

Setting: **A total of 124 intensive care units in Europe, North America, South America, South Africa, and Australasia.**

Patients: **A total of 797 patients with septic shock diagnosed for <24 hrs.**

Interventions: **Patients with septic shock were allocated to receive 546C88 or placebo (5% dextrose) for up to 7 days (stage 1) or 14 days (stage 2) in addition to conventional therapy. Study drug was initiated at 0.05 $mL \cdot kg^{-1} \cdot hr^{-1}$ (2.5 $mg \cdot kg^{-1} \cdot hr^{-1}$ 546C88) and titrated up to a maximum rate of 0.4 $mL \cdot kg^{-1} \cdot hr^{-1}$ to maintain mean arterial pressure between 70 and 90 mm Hg while attempting to withdraw concurrent vasopressors.**

Measurements and Main Results: **Hemodynamic variables, organ function data, microbiological data, concomitant therapy, and adverse event data were recorded at baseline, throughout treatment, and at follow-up. The primary end point was day-28 survival. The trial was stopped early after review by the independent data safety monitoring board. Day-28 mortality was 59% (259/439) in the 546C88 group and 49% (174/358) in the placebo group ($p < .001$). The overall incidence of adverse events was similar in both groups, although a higher proportion of the events was considered possibly attributable to study drug in the 546C88 group. Most of the events accounting for the disparity between the groups were associated with the cardiovascular system (e.g., decreased cardiac output, pulmonary hypertension, systemic arterial hypertension, heart failure). The causes of death in the study were consistent with those expected in patients with septic shock, although there was a higher proportion of cardiovascular deaths and a lower incidence of deaths caused by multiple organ failure in the 546C88 group.**

Conclusions: **In this study, the nonselective nitric oxide synthase inhibitor 546C88 increased mortality in patients with septic shock. (Crit Care Med 2004; 32:21–30)**

KEY WORDS: human; severe sepsis; septic shock; nitric oxide; nitric oxide synthase inhibitor; N^G-methyl-L-arginine hydrochloride; placebo-controlled study; norepinephrine; dopamine; dobutamine; nitrate; resolution of shock

Septic shock is the leading cause of death in intensive care units (ICUs) (1, 2). Although the clinical features may vary between patients, vasodilatation resulting in hypotension is a hallmark of patients with septic shock (1). Nitric oxide has been found to play an important role in the development of vasodilatation during septic shock (3, 4). In the healthy state, nitric oxide is continuously produced at low concentrations by a calcium-dependent nitric oxide synthase (NOS1 and NOS3, cNOS) from the substrate L-arginine. This enzyme resides in the vascular endothelial cells and plays an important role in the control of normal vascular tone (5). Endotoxin and cytokines, released during the host response to infection, induce a calcium-independent NOS (NOS2, iNOS). Induction of iNOS can result in the sustained production of nitric oxide for a prolonged period of time (up to 10 hrs), despite the presence of negative feedback mechanisms (6).

Increased production of nitric oxide has been demonstrated in both experimental and clinical sepsis (7–10). The increased production of nitric oxide has subsequently been associated with hypotension (11–13), decreased responsiveness to vasoconstrictors (14, 15), and development of multiple organ dysfunction (16). Reducing the overproduction of nitric oxide by partial inhibition of NOS could be postulated as a beneficial intervention in the treatment of septic shock. Previous experimental studies have produced conflicting results from the use of

***See also p. 282.**

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NOS inhibitors in models of septic shock provoked by either endotoxin or bacterial challenge (17–20). However, several small clinical studies have shown that the administration of NOS inhibitors (e.g., methylarginine, L-NMMA; nitroarginine, L-NNA) to patients with septic shock can restore hemodynamics and the vascular responsiveness to vasoconstrictor therapy without significant adverse effects (21, 22).

The safety and efficacy of 546C88 (N^G-methyl-L-arginine hydrochloride), a NOS inhibitor, was previously assessed in an international, multiple-center, randomized, double-blind, placebo-controlled, phase II clinical study (144-002) of its administration to patients with septic shock (23, 24). In that study, 546C88 was given by intravenous infusion and titrated up to a maximum rate of 20 $mg \cdot kg^{-1} \cdot hr^{-1}$ for up to 72 hrs to maintain the mean arterial pressure (MAP) at a target value of 70 mm Hg. Data from 312 patients with septic shock demonstrated that: 1) there was a significant increase in the proportion of patients who achieved resolution of shock at 72 hrs in the 546C88 treatment group compared with the placebo group ($p = .004$); 2) there was a significant decrease in the proportion of patients requiring vasopressors at 72 hrs in the 546C88 treatment group compared with the placebo group ($p = .044$); 3) the treatment groups had a similar incidence of complications of septic shock (e.g., acute renal failure, thrombocytopenia, disseminated intravascular coagulopathy, respiratory failure, dysrhythmias, acute hepatic failure, altered mental status), adverse events, and deaths up to day 28; 4) although more patients receiving 546C88 were reported to experience an increase in pulmonary artery pressure, the outcome of these patients was similar to patients in the placebo treatment group with this event and to the patients who were not reported to experience such an increase; and 5) the median plasma nitrate concentration at baseline was elevated in both treatment groups. There was a reduction in plasma nitrate concentration over time in the 546C88 treatment group, with no apparent change in the placebo group.

The current study was undertaken to assess the safety and efficacy of the NOS inhibitor 546C88 in patients with septic shock, with the primary efficacy objective being survival at day 28.

MATERIALS AND METHODS

This was an international, multiple-center, randomized, double-blind, placebo-controlled clinical study of the NOS inhibitor 546C88 administered to patients with septic shock. The study was conducted in accordance with the principles of Good Clinical Practice, including the Declaration of Helsinki. The protocol and procedure for obtaining informed consent were reviewed and approved by the local ethics committee/institutional review board of each participating center. A data and safety monitoring board (DSMB) was convened, and its responsibilities were determined before initiation of the study. The members included a biostatistician and five senior physicians with extensive practical experience in the practice of intensive care medicine or infectious diseases. They conducted interim reviews of the available safety data (including organ function variables, adverse events, and survival status) during (after the inclusion of 100 and 200 patients) and at the end of stage 1 and at scheduled enrolment milestones during stage 2. The DSMB had free access to the data and were able to recommend changes to the study design or to request suspension of recruitment if there were safety concerns.

Patients admitted to the participating ICUs were screened for study eligibility based on the following entry criteria: 1) age of ≥18 yrs; 2) severe sepsis diagnosed <72 hrs before randomization; 3) septic shock for <24 hrs (defined as a syndrome characterized by severe sepsis [Table 1]—a modification of the criteria proposed by Bone et al. (25)) associated with either a MAP consistently <70 mm Hg for at least 30 mins (despite fluid resuscitation) or a requirement for vasopressor support. Vasopressor support was defined as a continuous norepinephrine or dopamine infusion rate at or above 0.05 or 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively (or an equivalent dose of another vasopressor), administered for at least 30 mins to

Table 1. Study Definition of Severe Sepsis

1. Clinical evidence of infection and the introduction or change of systemic antimicrobial therapy within the previous 72 hrs
2. A systemic inflammatory response as evidenced by two or more of the following:
   a) Core temperature of <36°C (96.8°F) or >38°C (100.4°F).
   b) Tachycardia: defined by a heart rate of >90 beats/min.
   c) Tachypnea: defined by a respiratory rate of >20 breaths/min or an arterial CO_2 tension of <32 mm Hg (4.3 kPa) during spontaneous ventilation or the requirement for mechanical ventilation.
   d) A white blood cell count of $>12 \times 10^9$/L or $<4 \times 10^9$/L or >10% immature (band) forms.
3. Acute onset of end-organ dysfunction in the preceding 24 hrs, unrelated to the primary septic focus and not explained by any underlying chronic disease as indicated by one or more of the following:
   a) Acute deterioration in mental state, not due to sedation or primary underlying disease of the central nervous system.
   b) Acute hypoxemic respiratory failure: defined by a Pao_2/Fio_2 ratio of <300 mm Hg (40 kPa) in the absence of primary underlying pulmonary disease.
   c) Acute renal failure: defined by either oliguria (a urine output of $<0.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$) for at least two consecutive hours or a rise in serum creatinine concentrations ≥177 μmol/L (2.0 mg/dL) within the previous 48 hrs in the absence of primary underlying renal disease.
   d) Thrombocytopenia: defined by either a platelet count of $<75 \times 10^9$/L or an acute decrease of >50% within the previous 24 hrs in the absence of primary underlying bone marrow disease.
   e) Acute hepatic failure: defined by at least two of the following in the absence of primary underlying hepatic disease:
      i) A serum bilirubin concentration of >43 μmol/L (2.5 mg/dL).
      ii) Serum alanine transaminase concentration more than twice the upper limit of normal range.
      iii) Prothrombin time of >1.5 times the control value or an International Normalized Ratio of >1.5 in the absence of systemic anticoagulation.
   f) Disseminated intravascular coagulopathy: defined by at least two of the following:
      i) platelet count of $<75 \times 109$/L or an acute decrease of >50% within the preceding 24 hrs
      ii) Prothrombin time of >1.5 times the control value or an International Normalized Ratio of >1.5 in the absence of systemic anticoagulation.
      iii) D-dimer titer of >0.5 μg/mL or fibrin split product titer of >10 μg/mL.
   g) A plasma lactate of >2 mmol/L or a base deficit of >5 mmol/L.

The diagnostic elements of severe sepsis---1) clinical evidence of infection, 2) a systemic inflammatory response, and 3) the acute onset of end-organ dysfunction---must have been documented within a 24-hr period such that the diagnosis was confirmed no more than 72 hrs before randomization for study eligibility.

maintain a MAP of ≤90 mm Hg. 4) If the cardiac index was <5.0 $L \cdot min^{-1} \cdot m^{-2}$, pulmonary artery occlusion pressure had to be between 8 and 18 mm Hg, inclusive, and in the opinion of the investigator, the patient had to be adequately fluid resuscitated. 5) The patient had to have systemic and pulmonary arterial catheters in place with continuous pressure monitoring. 6) The clinician was prepared to provide full life-support measures for the duration of the study; and 7) in female patients, a history of a negative pregnancy test during the study hospital admission unless she was either postpartum, had undergone previous tubal ligation, had a hysterectomy, or was postmenopausal.

A variety of demographic data were recorded, including severity of illness on the day of ICU admission using the Simplified Acute Physiology Score (SAPS) II (26), date and time of onset of severe sepsis and septic shock, date and time of ICU admission and the treatment location before admission. The primary site of the causative infection (supported by the available microbiological data) was recorded for all patients.

The study was conducted in two stages that were designed prospectively. Individual patients were enrolled in only one stage of the study. Stage 1 was designed to monitor the safety and feasibility of treating with 546C88 for up to 7 days because previous experience was limited to 72 hrs of treatment in patients with septic shock. Using an allocation ratio of 2:1 between 546C88 and placebo and the results from the phase II study (144-002), it was estimated that approximately 250 patients would need to be recruited into the study to obtain data for 50 patients treated with 546C88 for >72 hrs. In consultation with regulatory authorities, 50 patients were agreed to be an appropriate number to identify any serious safety concerns associated with the extension of the treatment duration. Stage 2 permitted treatment for up to 14 days and was prospectively intended to provide the data for the primary efficacy evaluation. The stages were conducted consecutively.

Scheduled assessments were made before treatment, during treatment, and during the posttreatment period. Baseline data were recorded either immediately before the start of the study drug infusion or at 4 hrs from the time of randomization if the criteria for initiating the study drug had not been met (see below). Hemodynamic measurements were performed according to local institutional guidelines. Derived variables were calculated using standard formulas. Biochemical, hematologic, and coagulation variables were analyzed at either a central laboratory or by the local laboratory services at each institution.

The hydrochloride salt of N^G-methyl-L-arginine (546C88) was manufactured and supplied to the investigators by Glaxo Wellcome in 100-mL infusion bags containing an aqueous solution at a concentration equivalent to 50 mg/mL of the base. The placebo was supplied in identical bags containing the same volume of 5% dextrose solution.

Treatment Regimen. Throughout the study, all patients received conventional therapy according to local standard practice, including intravenous fluids, parenteral antibiotics, enteral or parenteral nutrition, and surgery when indicated. The choice of conventional vasoactive drug infusions that could be administered during the treatment period was limited to norepinephrine, dopamine, epinephrine, phenylephrine, dopexamine, and dobutamine.

Treatment with the study drug was initiated within 4 hrs of randomization when the following predefined starting criteria were met: 1) MAP of <70 mm Hg for ≥30 mins or MAP of ≤90 mm Hg with a requirement for vasopressor support. 2) If the cardiac index was <5 $L \cdot min^{-1} \cdot m^{-2}$, the pulmonary artery occlusion pressure was to be between 8 and 18 mm Hg. If the starting criteria were not achieved within 4 hrs after randomization, baseline assessments were made, and the treatment period was considered to have started. Treatment with the randomly allocated study drug could then be initiated at any time within the subsequent 14 days if the starting criteria were satisfied. The rationale for the hemodynamic criteria described above was that 546C88 was only to be used to treat shock due to severe sepsis and not to treat hypovolemic or cardiogenic shock.

The initial dose rate of the study drug for all patients was 0.05 $mL \cdot kg^{-1} \cdot hr^{-1}$ (equivalent to 2.5 $mg \cdot kg^{-1} \cdot hr^{-1}$ 546C88). The infusion rate was then reviewed and adjusted, if necessary, every hour according to a predefined dosing algorithm. Concomitant vasopressor therapy was to be reduced and, if possible, withdrawn while maintaining the patient's MAP between 70 and 90 mm Hg by using the minimum dose rate of the study drug that achieved this target. The infusion rate of the study drug could be titrated in sequential dose increments or decrements equivalent to 546C88 dose rates of 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 $mg \cdot kg^{-1} \cdot hr^{-1}$. Reduction of the study drug was only to begin when conventional vasopressors had been discontinued, except for reasons of patient safety. The infusion rates of the concomitant vasopressors could be adjusted at any time as clinically indicated but were assessed at least every 4 hrs. During the treatment period, the cardiac index was to be maintained at >3 $L \cdot min^{-1} \cdot m^{-2}$ using dobutamine or dopexamine, as was clinically appropriate. The study drug was either temporarily or permanently discontinued (according to clinical judgment) if either the MAP exceeded 100 mm Hg at any time in the absence of norepinephrine or dopamine (>3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and without any other obvious cause or if the patient developed a serious adverse experience that had a reasonable possibility of being caused by the study drug. Otherwise, the study drug was administered as required until the end of the treatment period, at which time it was discontinued, and the patients were subsequently managed with conventional therapy alone. Study drug could be adjusted, discontinued, or restarted at any time during the treatment period.

Safety Evaluation. Safety was assessed during the treatment and posttreatment period by a variety of biochemical indices of organ function, blood counts, coagulation variables, serum troponin I, and urine output. Adverse experiences that occurred or worsened during the treatment or posttreatment periods of the study were recorded. Predefined disease-related events were recorded separately from adverse experiences. All patients were followed-up at day 90 (day 28 in stage 1) to determine their survival status.

Efficacy Objectives and Sample Size Determination. The prospectively designed primary efficacy objective for this study was to determine whether 546C88, compared with placebo, resulted in a statistically significant decrease in all-cause mortality before the end of day 28. Secondary objectives were to determine whether 546C88 infusion compared with placebo: 1) resulted in a statistically significant decrease in day-14 mortality; 2) resulted in a higher proportion of patients who achieved resolution of shock at 72, 168, and 336 hrs; and 3) resulted in an increased survival time measured until day 90 after study entry. Resolution of shock was defined by a patient simultaneously meeting all of the following criteria: 1) epinephrine, norepinephrine, phenylephrine, and dobutamine infusion rates of 0 $\mu g \cdot kg^{-1} \cdot min^{-1}$; 2) dopamine infusion rate of ≤3 $\mu g \cdot kg^{-1} \cdot min^{-1}$; 3) dopexamine infusion rate of ≤1 $\mu g \cdot kg^{-1} \cdot min^{-1}$; 4) 546C88/placebo infusion rate of 0 $mL \cdot kg^{-1} \cdot hr^{-1}$; and 5) systemic MAP of ≥70 mm Hg.

Randomization Process and Statistical Analysis. Geographic territory and SAPS II score category (<40, 40–60, >60) were used as stratification variables in the randomization. All patients were counted as being in the treatment group to which they were randomized, regardless of whether the assigned drug was actually administered. All patients randomized into the study were included in the intention-to-treat primary efficacy analysis.

Survival Analysis. Using an allocation ratio of 1:1 between 546C88 and placebo in stage 2, a fixed sample size of approximately 4,400 patients would provide 90% power at a significance level of .05 (two-sided hypothesis) to detect a 10% relative reduction in day-28 mortality in the 546C88-treated patients from a predicted placebo mortality rate of 50%. Day-28 mortality was assessed using the triangular test with Christmas-tree correction at the stopping boundaries (Whitehead (27), pp

Table 2. Patients' Demographics

Variable	546C88 n = 439	Placebo n = 358
Age, yrs [mean (range)]	64.0 (18–94)	64.0 (18–90)
Sex, n (%)		
Male	276 (63)	216 (60)
Female	163 (37)	142 (40)
Weight, kg [mean (range)]	75.0 (33.6–160.0)	75.0 (30.8–161)
SAPS II score, mean (range)		
First ICU day	49.0 (5–106)	52.0 (18–99)
At randomization	52.0 (12–121)	52.0 (22–112)
Type of ICU admission, n (%)		
Scheduled surgery	15 (3)	28 (8)
Medical	250 (57)	185 (52)
Unscheduled surgery	174 (40)	145 (41)
Co-morbidity, n (%)		
Hypertension	113 (26)	104 (29)
Alcoholism	66 (15)	49 (14)
COPD	66 (15)	47 (13)
Diabetes mellitus	46 (10)	47 (13)
Cancer	38 (9)	39 (11)
Chronic heart failure	26 (6)	25 (7)
Chronic renal failure	25 (4)	14 (4)
Cirrhosis	19 (4)	15 (4)
Other	190 (43)	163 (46)

SAPS, simplified acute physiology score; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease.

Table 3. Summary of Septic Episodes

	546C88 n = 439	Placebo n = 358
Origin of infection, n (%)		
Community	262 (60)	201 (56)
Hospital	135 (31)	112 (31)
Intensive care unit	21 (5)	24 (7)
Unknown	21 (5)	21 (6)
Primary site of infection, n (%)		
Blood	17 (4)	12 (3)
Gastrointestinal tract	50 (11)	34 (9)
Intra-abdominal	100 (23)	96 (27)
Respiratory tract	172 (39)	133 (37)
Skin/soft tissue	21 (5)	20 (6)
Urinary tract	28 (6)	23 (6)
Other/missing	51 (12)	40 (11)
Confirmed infection, n (%)		
No	123 (28)	100 (28)
Yes	315 (72)	258 (72)
Missing	1 (<1)	0
Type of infection, n (%)	n = 315	n = 258
Gram negative	133 (42)	105 (41)
Gram positive	119 (38)	100 (39)
Mixed bacterial	40 (13)	41 (16)
Atypical	6 (2)	4 (2)
Fungal	8 (3)	2 (<1)
Other/missing	9 (3)	14 (5)

80–91), adjusted for SAPS II score at randomization (Whitehead (27), pp 212–221), and stratified by geographic territory (Whitehead (27), pp 209–212) (28). Mortality was treated as a binary response variable, SAPS II score was treated as a continuous variable, and geographic territory was treated as a nominal variable for this analysis. This test was to be carried out after approximately 1,300 patients had been enrolled into stage 2 and then again after approximately every 500 patients.

When each interim analysis was performed, if the corrected upper boundary was crossed, the trial was to be stopped due to efficacy. If the corrected lower boundary was crossed, the trial was to be stopped due to futility. Otherwise, the trial was to continue until 4,800 patients had been enrolled into stage 2 of the trial. Once the trial had been discontinued for any of the reasons listed above, and all data for the remaining patients had been collected, the final p value and 95% confidence interval on the log-odds ratio of mortality between the treatment groups was to be calculated such that values greater than zero corresponded to differences in favor of the 546C88 group.

RESULTS

Demographics. A total of 797 patients (215 in stage 1 and 582 in stage 2) recruited by 126 centers located in 24 countries worldwide were enrolled into the study, with 439 patients allocated to the 546C88 group and 358 to the placebo group. The greater number that received 546C88 was due to the 2:1 randomization that applied during stage 1 of the study. The first patient was enrolled on June 9, 1997, and the last on April 17, 1998. Patient demographics are summarized in Table 2. There were no significant differences between the groups with respect to age, sex, or severity of illness on the day of ICU admission. Both groups had a similar probability of survival as determined using the SAPS II customized probability model for severe sepsis in adult ICU patients (26). The data in Table 2 confirm that the randomization procedure resulted in good balance between the two groups with respect to SAPS II score categories at the time of randomization.

A causative organism considered to be responsible for the primary underlying infection was identified in 315 (72%) and 258 (72%) of the patients in the 546C88 and placebo groups, respectively (Table 3). The majority of patients presented with a community-acquired infection. Of the patients with confirmed infection, the frequency of Gram-positive, Gram-negative, mixed bacterial, and fungal isolates was similar in the 546C88 and placebo groups (Table 3).

Exposure to Study Drug and Other Vasoactive Agents. The study drug (546C88 or placebo) was administered to 98% of the patients in both groups. The principal reasons for discontinuation were 1) the study drug was not required to maintain the MAP at >70 mm Hg (42% vs. 54%); 2) MAP exceeded 100 mm Hg (6% vs. 5%); 3) adverse experience (5% vs. 2%); and 4) death (39% vs. 31%) in the 546C88 vs. placebo groups, respectively. At baseline, vasoactive drugs were used in comparable proportions and infusion rates between groups (Table 4).

Survival Profile. The survival status of

all patients was determined up to the end of day 28. The Kaplan-Meier survival profile for stages 1 and 2 combined shows a significantly worse outcome for the 546C88 group, with a separation of the curves by day 3 (log-rank, $p = .001$) (Fig. 1). At day 28, 259 of 439 patients (59%) in the 546C88 group and 174 of 358 patients (49%) in the placebo group had died (both stages combined). In stage 1 at day 28, 80 of 147 patients (54%) in the 546C88 group and 40 of 68 patients (59%) in the placebo group had died. Refractory shock followed by multiple organ failure were the most frequent reported causes of death in the 546C88 group. Multiple organ failure was the most frequent reported cause of death in the placebo group. Cardiac disorder and respiratory failure were other important causes of death (Table 5). Survival over the 90-day study period was lower in patients administered 546C88 compared with those patients administered placebo ($p < .001$).

A number of *post hoc* analyses were performed (Fig. 2). Patients who received a lower maximum dose rate (≤ 5 mg·kg^{-1}·hr^{-1}) of 546C88 had an improved survival rate relative to the placebo group, whereas patients administered higher doses of 546C88 (>5 mg·kg^{-1}·hr^{-1}) had a poorer outcome relative to the placebo group. Patients with a baseline cardiac index of <3 L·min^{-1}·m^{-2} who were treated with 546C88 had a poorer outcome than those patients with a cardiac index of ≥ 3 L·min^{-1}·m^{-2}. Survival was compared in patients with a baseline pulmonary artery pressure of ≤ 20, >20 to 35, and >35 mm Hg. Across the range of pressures, placebo-treated patients had consistently higher survival rates. At higher baseline pulmonary artery pressures, there was a smaller difference in survival between the 546C88 and placebo-treated groups.

*DSMB Reviews.*The DSMB reviewed the safety data that were available after the inclusion of 100 and 200 patients and at the end of recruitment into stage 1 (n = 215). At the first and second safety reviews, the DSMB reviewed a summary of the adverse experiences and deaths. At the review at the end of stage 1, the DSMB reviewed the following data: mortality, resolution of shock, organ dysfunction, pulmonary and systemic hemodynamics, clinical laboratory data, and adverse experiences. The information in these summaries was unblinded at the request of the DSMB. At the end of stage 1, the Kaplan-Meier survival plots showed a trend for increased mortality at day 14 in the 546C88-treated group. This trend was not maintained, however, so that by day 28, the curves crossed. At the next DSMB review (119 patients in stage 2), there was increased mortality at day 14 (stage 1 and 2 patients combined, $p = .031$), but no difference at day 28, and so the DSMB requested expedited collection of mortality data. *Ad hoc* review of the data by the project statistician (523 patients, 215 patients recruited into stage 1 and 308 patients recruited into stage 2) during preparation for the next DSMB review revealed a statistically significant difference at day 14 ($p = .002$ for stage 1 and 2 patients) and a trend at day 28 ($p = .109$ for stage 1 and 2 patients). The DSMB convened by telephone, and at this

Table 4. Baseline Vasoactive Drugs

Drug, n (%) Infusion Rate, μg·kg^{-1} · min^{-1} [Median (Range)]	Study Group 546C88 n = 439	 Placebo n = 358
Norepinephrine	293 (67) 0.26 (0.01–112.00)	219 (61) 0.22 (0.01–25.00)
Dopamine	231 (53) 6.17 (0.80–41.02)	208 (58) 5.53 (0.99–60.60)
Dobutamine	91 (21) 7.4 (0.98–33.30)	72 (20) 5.9 (0.19–45.45)
Epinephrine	89 (20) 0.18 (0.01–9.24)	84 (23) 0.19 (0.03–3.00)
Phenylephrine	19 (4) 1.6 (0.16–330.00)	14 (4) 1.9 (0.50–10.8)
Dopexamine	7 (2) 0.6 (0.48–1.57)	0

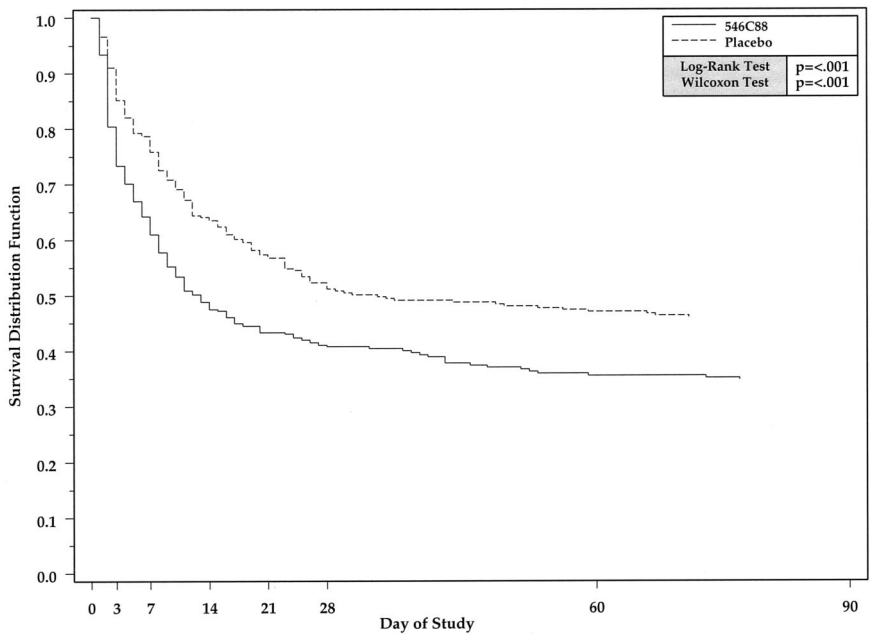

Figure 1. Kaplan-Meier survival plots up to day 90 for the 546C88 and placebo groups; stages 1 and 2 combined.

Table 5. Causes of Death

Cause of death, n (%)	Study Group 546C88 n = 275	 Placebo n = 189
Refractory shock	98 (36)	48 (25)
Multiple organ failure	75 (27)	78 (41)
Cardiac disorder	38 (14)	12 (6)
Respiratory failure	32 (12)	23 (12)
Craniocerebral injury	8 (3)	10 (5)
Metabolic disorder	5 (2)	1 (<1)

Table 6. Summary of Disease-Related Events

Event, n (%)	Study Group: 546C88 n = 439 Total	546C88 n = 439 Serious	Placebo n = 358 Total	Placebo n = 358 Serious
Acute circulatory failure	217 (49)	184 (42)	152 (42)	126 (35)
Cardiac dysrhythmia	190 (43)	115 (26)	136 (38)	74 (21)
Acute respiratory failure	185 (42)	134 (31)	141 (39)	106 (30)
Metabolic acidosis	181 (41)	109 (25)	125 (35)	69 (19)
Thrombocytopenia	172 (39)	37 (8)	136 (38)	63 (18)
Acute renal failure	163 (37)	104 (24)	136 (38)	94 (26)
DIC	89 (20)	41 (9)	60 (17)	32 (9)
Acute deterioration in mental status	74 (17)	32 (7)	70 (20)	33 (9)
Acute hepatic failure	69 (16)	37 (8)	67 (19)	32 (9)

DIC, disseminated intravascular coagulopathy.

final review, the DSMB recommended that enrollment into the study should be discontinued. Final analyses of the data showed a statistically significant difference in mortality at days 14, 28, and 90 (stage 1 and 2 patients, $p = <.001$, .003, and $<.001$, respectively).

Resolution of Shock. At 72 hrs, a greater proportion of patients in the 546C88-treated group had resolution of shock than in the placebo group (27% and 16%, respectively). The proportion of patients at 72 hrs who had died, however, was higher in the 546C88-treated group compared with the placebo group (29% and 17%, respectively). At 168 hrs, there was no difference in the proportion of patients whose shock was resolved, and by 336 hrs, the proportion of patients with shock resolved was lower in the 546C88-treated group compared with the placebo-treated group (25% and 32%, respectively). The proportion of patients in the 546C88-treated group who had died at 168 and 336 hrs remained higher than in the placebo group at these time points.

Hemodynamic Effects. The hemodynamic status of both groups was comparable at baseline (Fig. 3). Within an hour of introducing the study drug, there was a substantial increase in the median systemic vascular resistance in the 546C88 cohort. This had returned toward baseline after 24 hrs; however, it remained persistently higher than the control group value, which showed little change throughout the duration of the study. The increase in systemic vascular tone was accompanied by a reduction in cardiac index, which was associated with a fall in stroke index and heart rate. These effects in the 546C88-treated patients were associated with a significant increase in the baseline mean systemic arterial blood pressure, which was maximal after 1 hr and remained persistently higher than the placebo group during the treatment period. In contrast, the average blood pressure for the placebo group remained relatively constant throughout most of the treatment period.

The baseline mean pulmonary artery pressure was increased above normal in both groups and was somewhat more so in the 546C88 group, indicative of modest pulmonary hypertension. The higher pulmonary artery pressure in the 546C88 group persisted.

Organ Dysfunction. Almost all patients required mechanical ventilation during their ICU admission. There was no apparent effect of treatment with 546C88 on arterial pH or oxygen tension. The Pao_2/Fio_2 ratio tended to be higher in the 546C88 group during the treatment period of the study.

The incidence of renal failure requiring renal replacement therapy was similar in both groups. There was no detectable difference over time between the two treatment groups for any of the measured indices of renal or hepatic function.

There were no treatment-emergent differences in hemoglobin concentration, white cell count, or platelet count. Similarly, there were no detectable effects on indices of coagulation. No difference in serum troponin I was observed.

Disease-Related Events and Other Adverse Events. One or more predefined disease-related event that either occurred or worsened during the treatment period was recorded for 354 (81%) and 270 (75%) of the patients in the 546C88 and placebo groups, respectively. The nature, seriousness, and frequency of these events are summarized in Table 6. The adverse experiences that were reported to be possibly attributable to the study drug in at least 2% of the patients (in either treatment group) are summarized in Table 7. The overall number of patients who experienced one or more adverse experience was similar between groups. In general, a greater proportion of patents in the 546C88 group (n = 22, 5%) experienced serious events characterized by low cardiac output (decreased cardiac output, cardiac failure, and cardiogenic shock) compared with the placebo treated group (n = 3, <1%).

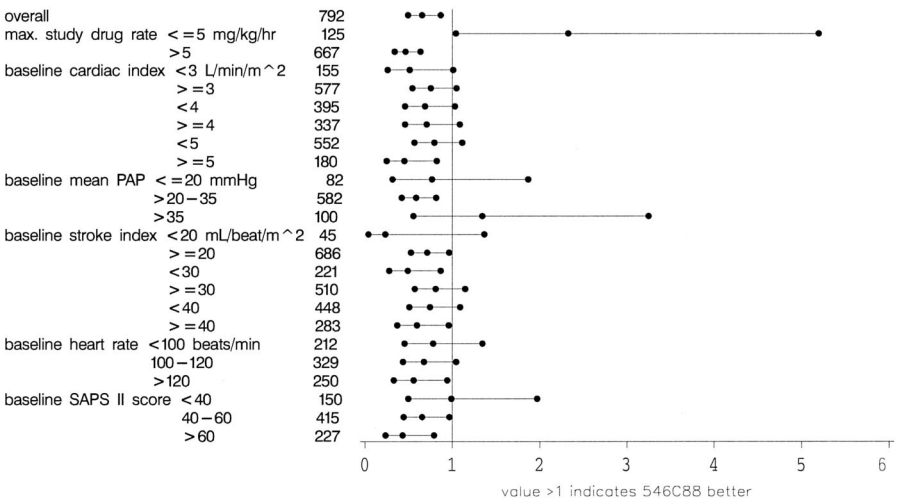

Figure 2. Odds ratio for day-28 survival by maximal (*max.*) study drug infusion rate and baseline characteristics (cardiac index, mean pulmonary artery pressure [*PAP*], stroke index, heart rate, and Simplified Acute Physiology Score [*SAPS*] II categories).

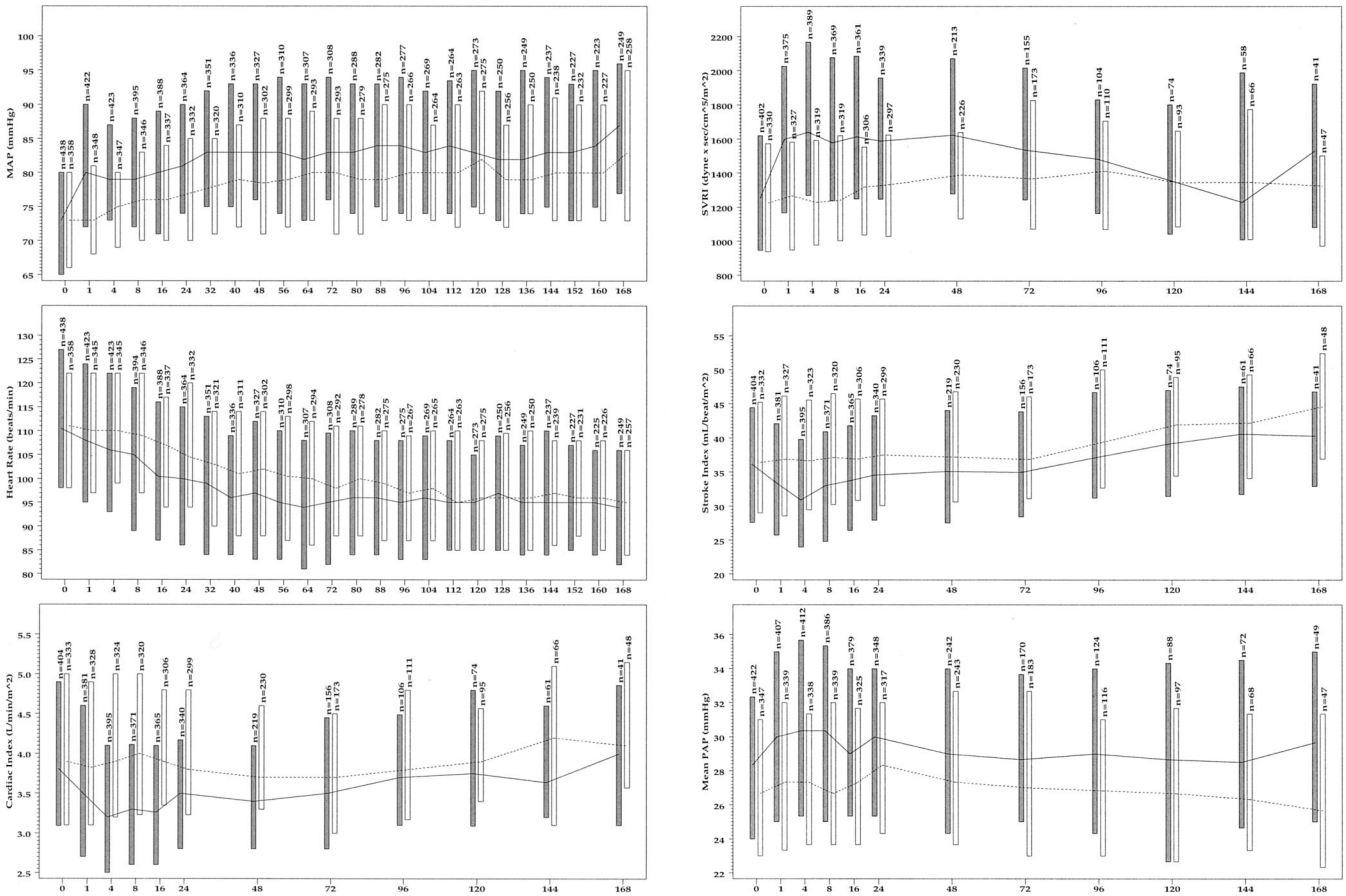

Figure 3. Mean arterial blood pressure, heart rate, cardiac index, systemic vascular resistance index (*SVRI*), stroke index, and mean pulmonary artery pressure (*PAP*) over time (from 0 to 168 hrs). *Vertical bars* represent the 25th to 75th percentile range.

Table 7. Summary of Adverse Events Possibly or Reasonably Caused by Study Drug

	Study Group			
	546C88 n = 439		Placebo n = 358	
Event, n (%)	Total	Serious	Total	Serious
Any event	81 (19)	42 (10)	29 (8)	17 (5)
Pulmonary hypertension	26 (6)	14 (3)	3 (1)	2 (<1)
Decreased cardiac output	19 (4)	9 (2)	2 (<1)	2 (<1)
Cardiac failure	9 (2)	9 (2)	0	0
Cardiac arrest	5 (1)	5 (1)	2 (<1)	2 (<1)

DISCUSSION

This randomized, double-blind, placebo-controlled phase III clinical study of patients with septic shock detected an increase in overall mortality associated with treatment with the NOS inhibitor 546C88. This effect is in contrast to the trend toward reduced mortality (which was measured as a safety variable) observed in the previous phase II study (study 144-002) (23). Given the relative sample sizes of the two studies, the obvious initial conclusion is that the larger phase III study has simply revealed an adverse outcome caused by treatment with 546C88 that was not detected by the smaller, exploratory, phase II trial. Although the design of the phase III study was based on the earlier study, there were several differences between the studies. It is not possible to determine in retrospect which differences in the studies contributed to the unexpected outcome of the phase III study. Nevertheless, it is apparent that in the 546C88-treated group, relatively high exposure to the drug (in terms of maximum dose rate and total quantity of drug administered) was associated with a poorer outcome. The reduction in the minimum time between increases in dose rate from 4 hrs to 1 hr, combined with the change in target MAP from 70 mm Hg to a range of 70–90 mm Hg, could both have inadvertently promoted the use of higher doses of study drug. A higher proportion of patients in the 546C88 treatment group received the maximum permitted dose rate of 20 $mg \cdot kg^{-1} \cdot hr^{-1}$ in the phase III compared with the phase II study (56% and 35%, respectively). By contrast, the increased duration of the treatment period from 72 hrs up to 336 hrs does not seem to have had any obvious adverse consequence because, retrospectively, the excess mortality seemed to be evident by 72 hrs. The other notable difference between the studies was that the phase III protocol

Nonselective nitric oxide synthase inhibitor 546C88 increased mortality in patients with septic shock.

permitted the inclusion of patients with hypodynamic septic shock (i.e., cardiac index of <3 L·min^{-1}·m^{-2}). Although these patients provided only 20% of the total study population, it is apparent that they were particularly intolerant of exposure to higher dose rates of 546C88 (i.e., >5 mg·kg^{-1}·hr^{-1}). Interestingly, *post hoc* analysis suggests that low doses of 546C88 (i.e., ≤ 5 mg·kg^{-1}·hr^{-1}) may have provided an overall survival benefit even in patients with a low baseline cardiac index. However, it should be emphasized that inferences based on *post hoc* analyses are less reliable than primary analyses.

The increase in mortality was evident early in the course of treatment. This increase reflected a higher proportion of cardiovascular deaths but a reduced proportion of deaths due to multiple organ failure. This may be explained by the fact that multiple organ failure tends to occur later in the course of septic shock, and the earlier deaths were due to cardiovascular causes. In addition, there was a higher proportion in the 546C88 group with adverse experiences that were considered to be attributable to the study drug. These events were typically hemodynamic in nature, for example, pulmonary hypertension, decreased cardiac output, or heart failure.

Overproduction of nitric oxide is central to the hemodynamic disturbance characteristic of established human septic shock. Preclinical and the phase II clinical data suggested that the suppression of nitric oxide activity could represent a novel and potentially effective approach to the treatment of septic shock (3, 4, 22–24). Patients with septic shock have circulatory abnormalities characterized by increased cardiac output, reduced ejection fraction, peripheral vascular vasodilatation, and capillary leak (29, 30). The typical hemodynamic profile of survivors is characterized by a modestly hyperdynamic circulation that sustains both organ blood flow and perfusion pressure. Conventional cardiovascular support for patients with septic shock includes fluids and vasopressors. Adrenoceptor agonists increase systemic vascular tone and also have inotropic effects that may not be manifest by an increase in cardiac output due to the concomitant increase in ventricular afterload. The primary cardiovascular effect of 546C88 is a reversal of peripheral vascular failure. 546C88 has little or no direct effect on myocardial contractility but augments the inotropic effect of beta adrenoceptor agonists (e.g., dobutamine).

The mechanism underlying the overall adverse effect of 546C88 in the phase III study remains unknown. It is interesting to note the apparent absence of adverse effects on noncardiovascular organ function, the preponderance of possibly attributable cardiovascular adverse events (e.g., low cardiac output, pulmonary hypertension), and the increased proportion of deaths due to refractory shock in the 546C88 treatment group. These observations suggest that in some patients, treatment with 546C88 provoked a paradoxic worsening of their acute circulatory failure or perhaps, more specifically, myocardial dysfunction. This may have been due to either overcorrection of vascular tone resulting in an excessive increase in ventricular afterload compounded by inadequate inotropic support or a previously unrecognized primary effect of 546C88 on myocardial performance.

Finally, the continuous and close involvement of the independent DSMB ensured that the emergent safety profile of the study was closely monitored. This preserved the integrity of the study but also enabled it to be terminated when the adverse outcome of the 546C88-treated group became apparent.

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REFERENCES

1. Parrillo JE, Parker MM, Natanson C, et al: Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. *Ann Intern Med* 1990; 113:227–242
2. Rangel-Frausto M, Pittet D, Costigan M, et al: The natural history of the systemic inflammatory response syndrome (SIRS): A prospective study. *JAMA* 1995; 273:117–123
3. Rees DD, Moncada S: Induction by endotoxin of a Ca-independent nitric oxide synthase in the vasculature and its pathological role in endotoxin shock. *In*: Clinical Relevance of Nitric Oxide in the Cardiovascular System. Moncada S, Higgs EA, Barrazueta JR (Eds). Madrid, Edicomplet, 1991, pp 69–87
4. Kilbourn RG, Griffith OW: Overproduction of nitric oxide in cytokine-mediated and septic shock. *J Natl Cancer Inst* 1992; 84:827–831
5. Moncada S, Palmer RM, Higgs EA: Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; 43:109–142
6. MacNaul KL, Hutchinson NI: Differential expression of iNOS and cNOS mRNA in human vascular smooth muscle cells and endothelial cells under normal and inflammatory conditions. *Biochem Biophys Res Commun* 1993; 196:1330–1334
7. Cobb J, Danner R: Nitric oxide and septic shock. *JAMA* 1996; 275:1192–1196
8. Lorente JA, Landin L, de Pablo R, et al: L-arginine pathway in the sepsis syndrome. *Crit Care Med* 1993; 21:1287–1295
9. Landry DW, Oliver JA: The pathogenesis of vasodilatory shock. *N Engl J Med* 2001; 345: 588–595
10. Booke M, Meyer J, Lingnau W, et al: Use of nitric oxide synthase inhibitors in animal models of sepsis. *New Horiz* 1995; 3:123–138
11. Palmer RMJ: The discovery of nitric oxide in the vessel wall: A unifying concept in the pathogenesis of sepsis. *Arch Surg* 1993; 128: 396–401
12. Moncada S, Higgs A: The L-arginine-nitric oxide pathway. *N Engl J Med* 1993; 329: 2002–2012
13. Brady AL, Poole-Wilson PA: Circulatory failure in septic shock: Nitric oxide, too much of a good thing? *Br Heart J* 1993; 70:103–105
14. Wylam ME, Samsel RW, Umans JG: Endotoxin *in vivo* impairs endothelium-dependent relaxation of canine arteries *in vitro*. *Am Rev Respir Dis* 1990; 142:1263–1267
15. Julou-Schaeffer G, Gray GA, Fleming I: Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway. *Am J Physiol* 1990; 259(4 Pt 2):H1038–H1043
16. Geng YJ, Hansson GK, Holme E: Interferon-gamma and tumor necrosis factor synergize to induce nitric oxide production and inhibit mitochondrial respiration in vascular smooth muscle cells. *Circ Res* 1992; 71: 1268–1276
17. Kilbourn RG, Cromeens, DM, Chelly FD, et al: N^G-methyl-L-arginine, an inhibitor of nitric oxide formation, acts synergistically with dobutamine to improve cardiovascular performance in endotoxemic dogs. *Crit Care Med* 1994; 22:1835–1840
18. Cobb JP, Natanson C, Quezado ZM, et al: Differential hemodynamic effects of L-NMMA

in endotoxemic and normal dogs. *Am J Physiol* 1995; 268:H1634–H1642
19. Rees DD, Monkhouse JE, Cambridge D, et al: Nitric oxide and the haemodynamic profile of endotoxin shock in the conscious mouse. *Br J Pharmacol* 1998; 124:540–546
20. Schlag G, Redl H, Gasser H, et al: Protective effects of NO synthase inhibition in a baboon model of severe sepsis. *Crit Care Med* 1997; 25(Suppl A3):153
21. Grover R, Zaccardelli D, Colice G, et al: An open-label dose escalation study of the nitric oxide synthase inhibitor N^G-methyl-L-arginine hydrochloride (546C88) in patients with septic shock. *Crit Care Med* 1999; 27: 913–922
22. Avontuur JA, Tutein-Nolthenius RP, van Bodegom JW, et al: Prolonged inhibition of nitric oxide synthesis in severe septic shock: A clinical study. *Crit Care Med* 1998; 26: 660–667
23. Bakker J, Grover R, McLuckie A, et al: Administration of the nitric oxide synthase inhibitor N^G-methyl-L-arginine hydrochloride (546C88) by intravenous infusion for up to 72 hours can promote the resolution of shock in patients with severe sepsis: Results of a randomized, double-blind, placebo-controlled, multicenter study (study no. 144-002). *Crit Care Med* 2004; 32:1–12
24. Watson D, Grover R, Anzueto A, et al: Cardiovascular effects of the nitric oxide synthase inhibitor N^G-methyl-L-arginine hydrochloride (546C88) in patients with septic shock: Results of a randomized, double-blind, placebo-controlled multicenter study (study no. 144-002). *Crit Care Med* 2004; 32:13–20
25. Bone RC, Fisher CJ, Clemmer TP, et al: Sepsis syndrome: A valid clinical entity. *Crit Care Med* 1989; 17:389–393
26. Le Gall JR, Lemeshow S, Leleu G, et al: Customized probability models for early severe sepsis in adult intensive care patients: Intensive Care Unit Scoring Group. *JAMA* 1995; 273:644–650
27. Whitehead J: The Design and Analysis of Sequential Clinical Trials. Chichester, UK: Ellis Horwood, 1983
28. Whitehead J, Stratton I: Group sequential clinical trials with triangular continuation regions. *Biometrics* 1983; 39:227–236
29. Kilbourn RG, Jubran A, Gross SS, et al: Reversal of endotoxin-mediated shock by N^G-methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem Biophys Res Commun* 1990; 172:1132–1138
30. Parrillo JE: Pathogenetic mechanisms of septic shock. *N Engl J Med* 1993; 328:1471–1477

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