Efficacy of Treatment With the Iron (III) Complex of Diethylenetriamine Pentaacetic Acid in Mice and Primates Inoculated With Live Lethal Dose 100 *Escherichia coli*

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Abstract

The iron (III) complex of diethylenetriamine pentaacetic acid (DTPA iron [III]) protected mice and baboons from the lethal effects of an infusion with live LD_{100} *Escherichia coli.* In mice, optimal results were obtained when DTPA iron (III) was administered two or more hours after infection. Prevention of death occurred in spite of the fact that the adverse effects of TNF-α were well underway in the mouse model. The half-life of DTPA iron (III) was 51±9 min in normal baboons; primary clearance was consistent with glomerular filtration. In septic baboons, survival was observed after administration of two doses of DTPA iron (III) at 2.125 mg/kg, the first one given before, or as late as 2 h after, severe hypotension. Administration of DTPA iron (III) did not alter mean systemic arterial pressure, but did protect baboons in the presence of high levels of TNF-α and free radical overproduction. Furthermore, exaggerated production of nitric oxide was attenuated. The mechanism of protection with DTPA iron (III) is not obvious. Because of its ability to interact in vitro with free radicals, its poor cell permeability, and its short half-life, we postulate that DTPA iron (III) and/or its reduced form may have protected the mice and baboons by sequestration and subsequent elimination of free radicals (including nitric oxide) from their systems. (*J. Clin. Invest.* 1996. 98:192–198.) Key words: baboons • free radical scavengers • nitric oxide • pharmacokinetics • septic shock

Introduction

Septic shock is an extreme inflammatory condition that results from acute invasion of the bloodstream by microorganisms or their toxic products, such as bacterial endotoxin. Once in circulation, bacterial endotoxin induces a variety of factors, including the production of TNF-α (1–3), IL-1 (4–6), and platelet-activating factor (7, 8). Consequently, current experimental approaches to treating septic shock include the use of monoclonal antibodies against endotoxin components and TNF and the use of platelet-activating factor antagonists (9). Despite the use of antibiotics and intensive supportive care, mortality due to septic shock varies from 20 to 60%, depending on the specific population (10–12).

Bacteremia and septic shock also are associated with overproduction of free radicals such as hydroxyl, superoxide, and carbon- and oxygen-centered radicals (13–17). In addition, nitric oxide (NO)[1] overproduction is at least partly responsible for the vasodilation that causes a reduction in mean systemic arterial pressure (MSAP) and organ perfusion pressure during septic shock. This overproduction of NO likely results from early activation of the endothelial constitutive form of NO synthase via agonists that increase intracellular calcium (7, 8, 18–20), followed by induction of the inducible form of NO synthase via TNF and IL-1 (21, 22).

The simultaneous increase and further reaction of NO with superoxide, which yields the oxidant peroxynitrite anion, occurs in cellular systems in response to inflammatory mediators (23). In addition, in sepsis-associated adult respiratory distress syndrome (ARDS), the presence of nitrotyrosine residues (formed by reaction of peroxynitrite and the tyrosine residues of proteins) are apparent throughout the lung (24).

Recently, we showed that administration of iron chelates (25), and in particular the iron (III) complex of diethylenetriamine pentaacetic acid (DTPA iron (III), Fig. 1 *A*), prevented death in *Corynebacterium parvum* + LPS-treated mice (25, 26). We also have shown, using electrochemistry, the binding of NO to DTPA iron (II). The DTPA iron (II) form can be easily formed by common biological reductants, because the potential for the iron (III/II) couple is $E_{1/2} = -0.22$ (26). We have expanded our observations to mice (27) and baboons (28) challenged with lethal doses of live bacteria and dosed with antibiotics and have confirmed the efficacy of DTPA iron (III) in these two acute models of sepsis. In addition, we determined the half-life of DTPA iron (III) in baboons and observed low micromolar plasma levels of the compound in the treated septic animals.

Methods

Reagents. DTPA iron (III) disodium salt dihydrate, diethylenetriamine pentaacetic acid (Fig. 1 *B*), acetic acid (≥ 99.7%), and phosphoric acid (85%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Potassium phosphate monobasic was obtained from Mallinckrodt Inc. (Paris, KY). HPLC-grade water and acetonitrile were purchased from EM Science (Gibbstown, NJ). PIC A reagent (tetrabutylammonium hydrogen sulfate) was obtained from Waters

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1. *Abbreviations used in this paper:* ARDS, adult respiratory distress syndrome; DTPA iron (III), iron (III) complex of diethylenetriamine pentaacetic acid; L-NMA, L-N_6-monomethyl-L-arginine; MSAP, mean systemic arterial pressure; NO, nitric oxide.

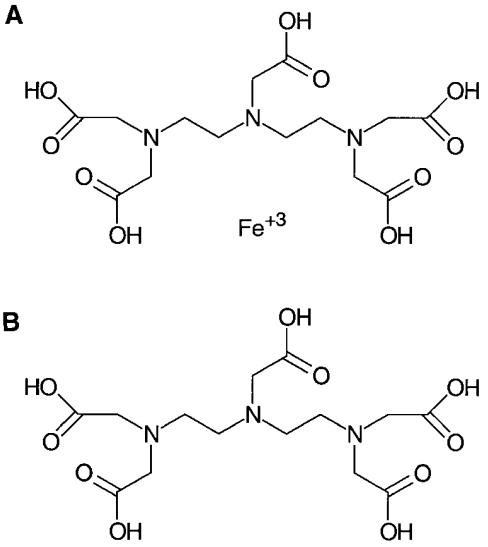

Figure 1. Structures of (*A*) DTPA iron (III) complex, and (*B*) free ligand.

Associates, Millipore Corp. (Milford, MA). Normal rat plasma (sodium citrate) was purchased from Pel-Freez Biologicals (Rogers, AR). L-NMA (L-N_6-monomethyl-L-arginine) was synthesized at Wellcome Industries (Beckenham, UK).

Bacteria. Escherichia coli B7 serotype 086A:K61 was obtained from American Type Culture Collection (Rockville, MD). A frozen stock at −80°C was used to prepare fresh cultures for injection. The stock culture was grown in BactoR Nutrient Broth (Difco Laboratories Inc., Detroit, MI) and incubated with shaking at 37°C for 15 h, harvested by centrifugation at 2,500 *g* for 10 min, and suspended in physiologic saline. The bacteria were counted by measuring the optical density at 600 nm and using standard curves previously determined by serial dilution and plating.

Mice. Male B6D2F1/CrlBR mice (6–8-wk old, 25–30 g) were purchased from Charles River Laboratories (Raleigh, NC). Mice were inoculated intraperitoneally with 5×10^9 *E. coli.* Then 0.1 mg/kg of Butorphanol (Torbugesicr; Fort Dodge Laboratories, Fort Dodge, IA) was administered intraperitoneally as a single dose 1 h after bacteria administration. Gentamicin sulfate injection (Solopak Laboratories) was administered intraperitoneally 2 h after bacteria administration in a single dose of 2 mg/kg. DTPA iron (III) and DTPA were dissolved in sterile saline and administered intravenously at 10 mg/kg at indicated times. Mice were observed for 7 d; survival was evaluated at 6 and 48 h.

Baboons. Experiments were carried out on young adult baboons (*Papio cynocephalus*) captured in East Africa and obtained through the Charles River Primate Center (Houston, TX). Animals were quarantined for at least 30 d in the University of Oklahoma Health Sciences Center Animal Resource Facility (Oklahoma City, OK), were free of infections, parasites, and tuberculosis, and had hematocrits > 36%. Baboons were examined regularly by the staff veterinarian and maintained in acceptable physical condition during the quarantine period, as evidenced by appearance, behavior, and dietary intake. Animals were fed regularly with monkey chow (Ralston Purina Co., St. Louis, MO) and given water ad libitum. Animals were fasted overnight before each experiment. They were sedated with ketamine hydrochloride, 14 mg/kg i.m., on the morning of the study. A percutaneous catheter was introduced into a cephalic vein to administer sodium pentobarbital (anesthesia), and animals were maintained at a light surgical level for 8 h. This catheter also was used to administer the drug treatment. Another percutaneous catheter was inserted in the saphenous vein for infusion with *E. coli* and antibiotic.

Table I. E. coli-induced Septic Shock in Mice

Compound	Dose schedule* (hours after infection)	Number of survivors/ total number	
		6 h	*48 h*
DTPA iron (III)	+1	10/20	0/20
	+2	14/20	4/20
	+3	15/20	3/20
	+4	18/20	5/20
	+5	20/20	15/20
DTPA	+5	15/20	1/20
Saline	−	10/20	0/20

*Compounds dosed i.v. at 10 mg/kg in saline at indicated times after injection of live bacteria.

Baboons were intubated orally and allowed to breathe spontaneously. Femoral vessels were cannulated aseptically for measurements of blood pressure and heart rate and for blood sampling for various biochemical parameters (29–31). During an 8-h period in the operating room, isotonic saline was infused at 3.3 ml/kg per h to compensate for insensible fluid loss. DTPA iron (III) was given as a slow bolus infusion for 30 min, according to the schedules presented below in Table IV. Baboons were monitored for 8 h, observed continuously for 34 h, and evaluated daily for 7 d. The antibiotic gentamicin (9 mg/kg) was infused intravenously for 60 min after all *E. coli* organisms were delivered, and two doses of antibiotic, 4.5 mg/kg, were infused at 300 and 540 min after the initiation of the *E. coli* infusion. An intramuscular dose of gentamicin, 4.5 mg/kg, was given at the end of the 8-h monitoring period and daily for 3 d.

Animals surviving more than 7 d were considered permanent survivors (30, 31). Postmortem examinations were conducted at death or when killed after the end of the 7th or 14th d; organ tissues were sectioned and placed in 10% formalin to be examined by light microscopy and evaluated by a designated veterinary pathologist. The pathologist was not informed of the content of any experimental protocol and, therefore, served in a blinded capacity.

Pharmacokinetics. Two portions of DTPA iron (III) were weighed independently and diluted to 1 mM with water. Calibration standards and spiked controls were prepared by combining equivalent portions of normal rat plasma and independent dilutions of the 1-mM stock solutions. The range of the calibration standards was from 1.0 to 100 μM (0.446 to 44.6 μg/ml). Calibration standards and spiked controls were prepared before analysis, and baboon plasma samples were stored at −20°C until analyzed.

Plasma samples (kinetics, baboons 14, 18, and 20; sepsis, baboons 10–13, 16, 19, and 21) were thawed, vortexed, and ultracentrifuged for 5 min before analysis. Separation of DTPA iron (III) from plasma was accomplished by solid-phase extraction (Accell Plus QMA Sep-Pak cartridges; Waters Associates, Millipore Corp.). Portions of the calibration standards, spiked controls, and baboon plasma samples

Table II. Pharmacokinetic Parameter Estimates after Intravenous Administration of DTPA Iron (III) (12.75 mg/kg) to Baboons

Baboon number	Weight	$t_{1/2}$	CL/F
	kg	*min*	*ml/min per kg*
14	10.0	45	2.58
18	7.7	50	2.67
20	14.1	58	6.34
AVG±SD		51±9	3.86±2.14

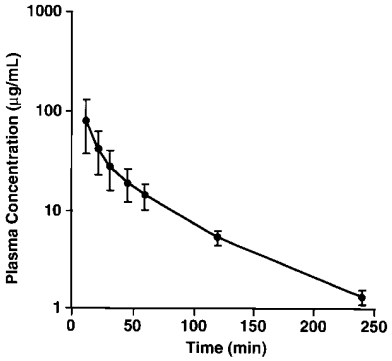

Figure 2. Baboon plasma concentration–time profile of DTPA iron (III). The profile represents the average concentration (±SD, $n = 3$) after intravenous administration of DTPA iron (III) at 12.75 mg/kg.

Table III. Average Plasma Concentration of NO_2^- + NO_3^- Measured at Various Times after Onset of E. coli Infusion in Baboons

Time	Control	DTPA iron (III)-treated
hours	*Concentration*	
	μM	
0	8.6±5.6	15.4±10.9
2	12.8±2.4	13.9±6.8
4	17.5±6.7	16.3±5.2
6	35.6±19.8	28.2±4.2
8	88.7±6.2	47.4±6.6

(200 μl) were combined with acetic acid (3% in water, 400 μl) and vortexed. Extraction cartridges were conditioned with water (3 vol) and loaded with acidified plasma. Cartridges were washed with water (3 vol), and DTPA iron (III) was eluted with 400 μl of 100 mM citric acid (pH 9.1).

Plasma concentrations of DTPA iron (III) were determined by HPLC. A 100-μl portion of each filtrate was injected onto a Symmetry C_{18} analytical column (4.6 × 250 mm, Waters Associates, Millipore Corp.) in line with a Symmetry guard column (3.9 × 20 mm) and a 0.5-mm precolumn filter (Upchurch Scientific, Oak Harbor, WA). The mobile phase consisted of 5 mM potassium phosphate buffer: 5 mM PIC A (pH 6.5, phosphoric acid) and acetonitrile (85%:15%, vol/vol) delivered at 1.0 ml/min. The HPLC system consisted of a 600E system controller, 717 autosampler, and 490E programmable multiwavelength detector set at 254 nm (Waters Associates, Millipore Corp). Data were collected and analyzed with VG Multichrom software and a VMS operating system (Fisons Instruments Inc., Beverly, MA). Plasma concentration-time profiles and pharmacokinetic parameter estimates after intravenous administration of DTPA iron (III) to baboons were accomplished by noncompartmental analyses.

Nitrite + nitrate determination. Plasma NO_2^- + NO_3^- levels were determined according to recent work (32), and TNF-α was deter-

Table IV. Survival Data of Baboons Treated with DTPA Iron (III)

Baboon number	Weight	Sex	Dose *E. coli*	Survival time	Time and MSAP	Time of treatment	Hisological evaluation
	kg		*CFU/kg × 10^{10}*		*min mmHg*	*min*	
10	8.0	F	7.5	> 7 d	T_o = 125 +120 = 75 +240 = 77 +360 = 94	30 and 300	No multiorgan pathology; mild lung lesion (ARDS)
11	8.2	M	7.0	> 7 d	T_o = 123 +120 = 87 +240 = 87 +360 = 98	30 and 300	No multiorgan pathology; mild lung lesion (ARDS)
12	9.1	F	6.9	> 14 d	T_o = 93 +120 = 49 =240 = 69 +360 = 84	30 and 300	No multiorgan pathology; lungs normal
13	7.3	M	7.1	> 14 d	T_o = 110 +120 = 45 +240 = 80 +360 = 85	60 and 300	No multiorgan pathology; mild lung congestion
16	10.0	F	7.4	> 14 d	T_o = 105 +120 = 68 +240 = 69 +360 = 48	120 and 300	No multiorgan pathology; mild lung congestion
21	7.7	F	4.5	> 14 d	T_o = 110 +120 = 62 +240 = 60 +360 = 65	180 and 300	No multiorgan pathology; mild lung congestion
19	12.5	F	6.6	54 h	T_o = 75 +120 = 30 +240 = 45 +360 = 64	240, 300, and 450	Severe lung pathology (ARDS); no adrenal/renal damage

Table V. Control Baboons (E. coli and Placebo Treated)

Baboon number	Weight	Sex	Dose *E. coli*	Survival time	Time and MSAP	Histological evaluation
	kg		*CFU/kg* $\times 10^{10}$	*hours*	*min mmHg*	
1	9.3	F	5.7	18	$T_o = 123$ $+120 = 75$ $+240 = 65$ $+360 = 60$	Multiorgan pathology with generalized thrombosis; severe lung pathology
2	9.6	M	5.2	34	$T_o = 113$ $+120 = 85$ $+240 = 70$ $+360 = 90$	Multiorgan pathology with generalized thrombosis; severe lung pathology
3	9.6	M	4.8	66	$T_o = 95$ $+120 = 80$ $+240 = 62$ $+360 = 80$	Multiorgan pathology; severe lung adrenal and renal change
4	6.8	F	6.2	100	$T_o = 100$ $+120 = 88$ $+240 = 80$ $+360 = 95$	Multiorgan pathology; severe lung and marked renal damage
5	8.6	M	6.1	100	$T_o = 120$ $+120 = 85$ $+240 = 95$ $+360 = 105$	Multiorgan pathology; severe lung and renal damage
6	12.9	F	8.8	14	$T_o = 110$ $+120 = 62$ $+240 = 60$ $+360 = 65$	Multiorgan pathology; severe renal and adrenal damage
Mean	9.5 (0.8)		6.2 (0.6)	55 (16)		

mined as previously described (33), with an ELISA assay from Endogen, Inc. (Boston, MA).

Results

Effect of DTPA iron (III) in mice subjected to systemic E. coli infections. We studied the action of DTPA iron (III) in mice infected with live *E. coli* and treated with antibiotics. In this model, in the absence of any other treatment, some mice died after 5 h and 100% mortality occurred at 48 h after initiation of the bacterial infusion. The animals were infused with bacteria at the beginning of the experiment. Gentamicin was administered 2 h after infusion of bacteria, and cohorts of 20 mice received a single dose of DTPA iron (III), 10 mg/kg, at different time points from 1 to 5 h (Table I). Survival was evaluated at 6 and 48 h (Table I), and no deaths were noted between 48 h and 7 d. As shown in Table I, treatment with DTPA iron (III) resulted in a significant decrease in mortality compared to the untreated controls. The efficacy of DTPA iron (III) increased when given to mice 2 h or more after infection. The best results were observed when DTPA iron (III) was given 5 h after infection.

Our previous observations on the effect of late administration of DTPA iron (III) and DTPA (in the *C. parvum* + LPS mouse model of shock, (references 25, 26) showed that DTPA was less effective than DTPA iron (III). We confirmed this observation in the live bacteria model in mice where administration of DTPA 5 h after onset of infection did not provide protection, whereas DTPA iron (III) was very effective (Table I).

Plasma concentrations and pharmacokinetics of DTPA iron (III) in baboons. In preparation to study the action of DTPA iron (III) in a baboon model of septic shock, we determined the pharmacokinetics of the compound in untreated baboons. The average plasma concentration–time profile of DTPA iron (III) after intravenous administration (12.75 mg/kg) in the baboon is shown in Fig. 2. Pharmacokinetic parameter estimates determined by noncompartmental analyses are shown in Table II. DTPA iron (III) exhibited rapid distribution and elimination. The estimated plasma half-life was 51±9 min; plasma concentrations dropped below the lower limit of quantitation (1 μM, 0.446 mg/ml) between 4 and 6 h. Apparent clearance (CL/F) was 3.86±2.14 ml/min per kg.

Effect of DTPA iron (III) on survival of baboons and organ protection. In a previous publication, we postulated that the protective effect of DTPA iron (III) in mice resides in its ability to bind free radicals, and in particular NO, after reduction of the iron (III) present in the molecule (26). We decided to test DTPA iron (III) in the same baboon model of sepsis used to determine whether free radical overproduction indeed occurred (measured between 90 and 150 min after onset of *E. coli* infusion) (17). We found that circulating NO_2^- + NO_3^- levels (which are a reflection of NO overproduction) increased from a baseline value of 15.4±10.9 μM at time 0 to 88.7±6.2 μM 8 h after onset of *E. coli* infusion (Table III); these levels

Table VI. Plasma Concentrations of DTPA Iron (III) after Intravenous Administration of 2.125 mg/kg to Septic Baboons

Time	Concentration				
hours	*mg/ml*				
	12*‡	13§	16‖	19¶	21**
0	ND‡‡	ND	ND	ND	ND
2	1.50	3.62	ND	ND	ND
4	0.53	NS	1.98	ND	7.70
6	3.28	NS	5.40	12.9	17.9
8	8.42	2.83	2.13	15.7	16.8
12	NS§§	NS	ND	NS	NS
24	ND	ND	NS	NS	NS

*Animal/experiment identification number.
‡30-min infusions of DTPA iron (III) (2.125 mg/kg) at 0.5 and 5 h.
§30-min infusions of DTPA iron (III) (2.125 mg/kg) at 1 and 5 h.
‖30-min infusions of DTPA iron (III) (2.125 mg/kg) at 2 and 5 h.
¶30-min infusions of DTPA iron (III) (2.125 mg/kg) at 4, 5, and 7.5 h.
**30-min infusions of DTPA iron (III) (2.125 mg/kg) at 3 and 5 h.
‡‡Not detected (LLOQ 0.45 mg/ml).
§§No sample.

are similar to those observed in human sepsis (34). To target free radical and NO overproduction, the dosing scheme with DTPA iron (III) included two doses. One dose was given as a slow infusion over 30 min, initially between 30 and 60 min after onset of *E. coli* infusion. The second dose was given between 300 and 330 min in a similar manner. The timing of the second dose was selected based on the observation of the increase in $NO_2^- + NO_3^-$ levels. This event occurred between 4 and 8 h in the control group (Table III). The efficacious dose of DTPA iron (III) at 10 mg/kg in mice was adjusted according to surface area (35) to a value of 2.125 mg/kg for further use in baboons. The results of this initial treatment are summarized in Table IV; baboons 10, 11, and 12 survived the challenge with bacteria.

In the septic baboon model, the untreated animals became hypotensive between 1 and 2 h after onset of bacterial infusion. Administration of DTPA iron (III) did not prevent hypotension observed at 120 min after onset of bacterial infusion (Table IV). To explore the effect on survival of a dose of DTPA iron (III) during severe hypotension, the first dose was given to the baboons at 60, 120, and 180 min after onset of bacterial infusion, while the second dose was given at 300 min (Table IV). Administration of the compound did not significantly affect the change in MSAP over time; however, septic baboons 13, 16, and 21 survived (Table IV). Finally, when the first dose of DTPA iron (III) was given at 240 min, baboon 19 died in spite of the administration of a second dose at 300 min and a third dose at 450 min (Table IV). Interestingly, the average plasma level of $NO_2^- + NO_3^-$ in the drug-treated group was 47.4±6.6 μM measured at 8 h (Table III). The results in the controls, six placebo-treated animals, are shown in Table V. An average survival time of 55 h for the placebo-treated animals reflects the acuteness of our model; all six placebo-treated animals died as a result of multiple organ damage, with the lungs, adrenals, and kidneys being the most severely affected organs. The most prominent lung lesion was ARDS, characterized by capillary leakage and intraalveolar fibrin deposits. Adrenal and kidney damage was characterized primarily by cortical hemorrhage, disseminated intravascular coagulation, and necrosis. The only significant finding in the group of surviving treated animals was the mild ARDS present in the two animals that survived for 7 d and were killed. All four animals that survived and were killed after 14 d appeared to be essentially normal. Interestingly, baboon 19, which was dosed late with DTPA iron (III), was the only drug-treated animal that died and presented only lung pathology; the kidneys and adrenals appeared to be normal. A comparison between the drug-treated and untreated groups of baboons with respect to the number of survivors at day 7 showed that the beneficial effects of treatment with DTPA iron (III) were highly significant ($P = 0.005$; two-tailed Fisher's exact test).

The plasma concentration–time profiles of DTPA iron (III) after intravenous administration (2.125 mg/kg) to septic baboons are shown in Table VI. Plasma concentrations of DTPA iron (III) in septic baboons were dependent on the infusion regimen for each animal. Plasma concentrations were generally low (1.18–39.8 μM, 0.53–17.9 μg/ml) and were below the lower limit of quantitation by 12 to 24 h.

Discussion

To be useful in the treatment of septic shock, a pharmacological agent must produce an effect when given after the bacterial challenge. For instance, in mice that received an LD_{90} of *E. coli* followed by antibiotics, TNF-α rose by 90 min and returned to baseline at 5 h (27). A monoclonal antibody developed against TNF-α prevented death when given 90 min before or 30 min after challenge (27). A similar time course of TNF-α production was observed in our mouse model in the presence or absence of treatment with DTPA iron (III) (not shown). Interestingly, treatment with DTPA iron (III) was most effective 5 h after infection (after TNF-α level had peaked and returned to the baseline value) rather than earlier after initiation of bacterial infusion. This finding suggests that prevention of death occurs with DTPA iron (III) therapy even when septic shock is advanced and when the documented effects of TNF-α on vascular endothelium, hematopoietic elements, adrenals, and liver are well underway (36).

As mentioned earlier, NO overproduction is associated with septic shock. In spite of the fact that inhibition of NO synthesis would seem a rational approach for the treatment of sepsis, the use of NO synthase inhibitors has been questioned (37, 38). To compare the action of a free radical scavenger such as DTPA iron (III) with the action of an inhibitor of NO synthesis, we dosed septic mice with L-NMA (1 mg/kg) from 1 to 5 h after infection. All the L-NMA–treated mice died within 48 h (not shown). This contrasts with the 75% survival rate obtained when DTPA iron (III) was administered 5 h after infection (Table I). Importantly, DTPA iron (III), at a concentration of 25 μM, showed no inhibition of human inducible, endothelial, or neuronal NO synthase in vitro when L-arginine was well below the enzyme's respective K_m values (Garvey, E., personal communication). Thus, we confirmed both the value of the iron chelate therapy (25, 26) and previous observations supporting the lack of activity of nonselective NO synthase inhibitors such as L-NMA in septic shock mice models (38–40).

The pharmacokinetic study carried out in baboons showed that DTPA iron (III) exhibits a short plasma half-life in untreated animals. Noncompartmental pharmacokinetic parameter estimates determined in these baboons support a primary

role for renal clearance of DTPA iron (III). Glomerular filtration rate in baboons is ~ 3 ml/kg per min (41), which is similar to the estimated value of apparent clearance of the compound (3.86±2.14 ml/kg per min, Table II). The disposition of [^{14}C]DTPA in several species also is in agreement with these data (42).

The rationale behind the use of baboons as animal models for septic shock was reviewed recently (28). In these animals, the changes in hemodynamic, respiratory, and metabolic parameters are characteristic of the changes in humans (43). In addition, the morphological lesions in adrenals, kidneys, lung, spleen, and liver observed in baboons (44) correlated well with the multiple organ failure described in clinical septic shock in humans (11, 12).

Early administration of anti–TNF-α antibody was effective in preventing morbidity and mortality in baboons given lethal intravenous injections of live *E. coli.* (43). This model was characterized by an abrupt increase of TNF-α concentration 2 h after onset of infection. As in the mouse model, administration of DTPA iron (III) to baboons did not alter the time course or the amount of TNF-α produced at 2 h (not shown). In spite of that, all animals but baboon 19 survived. The time of administration of the first dose appeared to be crucial for outcome. Also, at the dose of DTPA iron (III) used in these experiments (2.125 mg/kg), the time span for successful intervention in this model was between 30 min and 3 h after onset of infection. After that time, the extent of tissue damage was so severe that survival was not possible. Our results in Table III clearly showed a reduction in NO_2^- + NO_3^- plasma levels in the drug-treated group. After the results obtained in vitro, and assuming that the activity of NO synthase was not affected in vivo by DTPA iron (III), the possibility arises that some of the NO was scavenged by DTPA iron (II) formed in vivo by reduction of DTPA iron (III).

As stated above, plasma concentrations of DTPA iron (III) were low in septic baboons, suggesting that the compound was fairly potent. The observed plasma concentrations at later time points (> 4 h) were often greater than predicted values based on the estimated half-life. These data suggest that later stages of sepsis may compromise renal fuction as well as affect the elimination of the compound and, possibly, its efficacy.

DTPA iron (III) has little tendency to cross the cell membrane (26). It is likely that the protection observed in mice and baboons in our experiments was the result of an extracellular action of DTPA iron (III), rather than an intracellular effect. Interestingly, this extracellular action took place even during severe hypotension, when organ perfusion was highly compromised.

The mechanism of protection with DTPA iron (III) is not obvious. Other NO scavengers (45, 46), low molecular weight superoxide dismutase mimics (47), and superoxide dismutase (15) protected rats and mice against the deleterious effects of NO and oxygen-derived free radicals, preventing endotoxin-induced mortality. DTPA iron (III) reacts in vitro with hydroxyl radical (48), dismutates superoxide (49), and binds NO after reduction (26). Whether one or a combination of these activities play a role in vivo remains unknown. It is possible to speculate that scavenging of NO (26) and catalytic superoxide dismutase activity (49) occurring in the bloodstream in the proximity of vital organs would prevent formation of the oxidant peroxynitrate (23, 24), increasing organ/tissue protection. In total, the results of this study encourage continued investigation of the mechanism of action, as well as further evaluation of the clinical applicability, of DTPA iron (III). Although adverse effects were not noticed in our studies, a future clinical evaluation may have to take into account the potential ability of DTPA iron (III) to participate in Fenton chemistry.

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