Treatment of Experimental Gram-Negative and Gram-Positive Bacterial Sepsis with the Hematoregulatory Peptide SK&F 107647


SK&F 107647, a novel synthetic low-molecular-weight peptide, has demonstrated potent antinfec-
tive activities in murine models of fungal and viral infection. To determine if the hematoregulatory ac-
tivities of SK&F 107647 could offer protection over conventional antibiotic therapy or as a single agent in animal models of bacterial sepsis, rats were implanted intraperitoneally with a live bacteria-
containing fibrin-thrombin clot. Rats pretreated subcutaneously or orally with SK&F 107647 and
then infected with either a gram-negative (Escherichia coli) or a gram-positive (Staphylococcus
aureus) bacterium-containing clot demonstrated significantly improved survival over control formula-
tion–treated animals. Treated animals showed increased effector cell activation, measured by CD11b
expression on neutrophils and monocytes, and up to 1000-fold reduction in the number of E. coli
recovered from blood. Thus, the hematoregulatory activities of SK&F 107647 can increase natural
cell resistance to infections caused by both gram-negative and gram-positive bacteria.

Despite considerable research, sepsis and septic shock have
remained an important cause of patient morbidity and mortality,
with present therapies remaining primarily supportive [1, 2].
Although a variety of endotoxemia and sepsis animal models
have been developed, many models are not representative of
the clinical septic syndrome [3, 4]. Some models use a bolus
intravenous injection of bacteria or endotoxin [5–7]; others
use immunosuppressive regimens, such as cyclophosphamide
or vinblastine, or hepatotoxins such as galactosamine [8, 9].
However, all of the above models produce pathophysiologic
responses that are frequently dissimilar to the clinical septic
syndrome [3].

Several laboratories have described models of sepsis produ-
ced by implanting infected fibrin-thrombin clots into the perito-
neal cavity [10, 11]. In contrast to other experimental
models of sepsis, the fibrin-thrombin clot model produces an
evolving septic process from a focus of infection that more
closely mimics the human septic syndrome [12–14].

The hematoregulatory peptide SK&F 107647 has been shown to be a potent stimulator of granulocyte-macrophage colony-forming units. SK&F 107647 also increases serum col-
ony-stimulating activity [15, 16] and peritoneal macrophage
superoxide-candidacidal activity [17]. In addition, SK&F
107647 has been shown to protect mice from lethal Candida
albicans and herpes simplex virus infections [18, 19]. It was
unknown if the hematoregulatory activities elicited by SK&F
107647 could offer protection in animal models of bacterial
sepsis. To determine this potential, we used the rat fibrin-
thrombin clot model to evaluate SK&F 107647 in gram-nega-
tive and gram-positive bacterial sepsis.

Materials and Methods

Rats. Male Fischer 344 rats (Tacomin Farms, Germantown,
NY) weighing 180–210 g were used. The rats were housed singly
in standard plastic caging and fed laboratory chow and water ad
libitum.

SK&F 107647. SK&F 107647 (figure 1) was prepared by stan-
dard peptide synthesis procedures. The purity of the peptide was
analyzed by high-performance liquid chromatography and the
structure confirmed by amino acid analysis and fast atom bombard-
ment mass spectroscopy [20]. The compound was formulated in
a biodegradable microsphere formulation of poly-L-lactide [21].
Release of drug from this formulation is controlled by diffusion
through channels in the spheres and hydrolysis of ester linkages.
SK&F 107647 in vivo doses from this formulation were deter-
mined by in vitro release into 25 mM TRIS HCl, pH 7.0, with 125
mM NaCl. From a single injection, this formulation of SK&F
107647 releases uniform amounts of compound for >2 weeks. In
studies using this formulation, control animals were dosed with
empty (non–SK&F 107647–containing) microsphere formulation
prepared in the same manner.

Escherichia coli. Five separate E. coli clinical isolates were
individually animal-passaged in mice and subsequently recovered
and plated onto MacConkey’s agar. The reisolated organisms were
grown overnight in brain-heart infusion broth, then stored frozen
at −70°C. The animal-passaged organisms were then evaluated
for their ability to cause deaths in the rat fibrin-thrombin clot
model. From these studies, an isolate from sputum was selected
(provided by D. McDonald, Magainin Pharmaceuticals, Plymouth
Meeting, PA). This strain caused a significant death rate in rats.
by turbidimetry, and the concentration was adjusted with normal saline. The number of organisms was quantified on a rotary shaker (120 rpm) at 37°C. The organisms were harvested by centrifugation, washed three times, and finally resuspended in normal saline. All inoculum sizes were based on viable counts determined in Mueller-Hinton agar pour plates (Becton Dickinson, Franklin Lakes, NJ). In organ culture studies, individual rats were sacrificed and both kidneys removed and homogenized for CD11b analysis was collected in the presence of EDTA from placebo- and SK&F 107647-treated rats before and at various time points after infection. The number in all test groups was 25, except for certain groups of controls, which had 35 animals each. In peripheral blood and kidney culture studies, the number of rats at each time point was 10.

Antibiotics. In studies using antibiotic therapy, rats were treated subcutaneously with either gentamicin sulfate (Elkins-Sinn, Cherry Hill, NJ) or ceftazidime (SmithKline Beecham, Philadelphia, PA). 5 mg/kg twice daily. Antibiotics were tested for antibiotic sensitivity using an automated antimicrobial susceptibility system (BioMerieux Vitek, Hazelwood, MO) and found to be sensitive to gentamicin (MIC, 1 μg/mL) and ceftazidime (<2 μg/mL) and resistant to penicillin G, erythromycin, and vancomycin.

Staphylococcus aureus. Six separate S. aureus clinical isolates were animal-passaged in mice and recovered on sheep red blood cell trypticase soy agar, then evaluated for their ability to cause death in the rat fibrin-thrombin clot model. Two isolates were found to cause significant decreases in survival in this model, ATCC 13565 and 27664 (American Type Culture Collection, Rockville, MD). From these preliminary studies, an isolate (ATCC 13565) recovered from a food poisoning outbreak that was linked to 40 deaths was selected [22]. In rats not treated with antibiotics, this isolate had an LD_{50} of 1.0 × 10⁸ cfu. It was found to be sensitive to gentamicin (MIC, <1 μg/mL), ceftazidime (8 μg/mL), and vancomycin (<2 μg/mL) and as a β-lactamase producer was resistant to penicillin G.

To seed the fibrin clot, organisms from thawed stocks were inoculated into brain-heart infusion broth and incubated overnight on a rotary shaker (120 rpm) at 37°C. The organisms were harvested by centrifugation, washed three times, and finally resuspended in normal saline. The number of organisms was quantified by turbidimetry, and the concentration was adjusted with normal saline. All inoculum sizes were based on viable counts determined by scoring colony-forming units on sheep red blood cell trypticase soy agar.

Fibrin clot. Infected fibrin clots were prepared by the method of Ahrenholz and Simmons [10]. Briefly, clots were made from a 1% solution of bovine fibrinogen (type I-S; Sigma, St. Louis) in sterile saline. The clot was formed by sequentially adding a 1% solution of bovine thrombin (Parke-Davis, Morris Plains, NJ), bacteria, and fibrinogen solution to 24-well plastic plates. The resulting mixture was incubated at room temperature for 30 min before implantation.

Bacterial dose. Preliminary bacterial dose-response studies indicated that if the rats were not treated with antibiotics, the implantation of fibrin-thrombin clots inoculated with 1.0–2.0 × 10⁶ cfu resulted in no animals surviving at 24 h. However, when antibiotic therapy was started 2 h after infection, 30%–40% of the animals survived. If antibiotic treatment was combined with a lower bacterial inoculum (5 × 10⁵ cfu), 100% survival was achieved. Higher inocula (5 × 10⁶ cfu) resulted in no animals surviving at 12 h despite antibiotic treatment. From these preliminary studies, a bacterial dose of 1.0–2.0 × 10⁶ cfu was selected.

Animal model. Rats were anesthetized with ketamine (40 mg/kg; Fort Dodge Labs, Fort Dodge, IA) and xylazine (5 mg/kg; Miles, Shawnee Mission, KS), the abdominal surfaced was shaved, and a midline laparotomy was done. Bacterial peritonitis was induced by implanting a fibrin-thrombin clot containing either E. coli or S. aureus into the abdominal cavity. After implantation, the muscle layers were closed with a 4-0 silk suture (Ethicon, Somerville, NJ) and the wound closed with surgical staples (Becton Dickinson, Sparks, MD). Starting 2 h after infection, rats were dosed subcutaneously twice daily with antibiotics. Animals were closely observed, and any animals obviously moribund were euthanatized. In survival studies, animals were followed until day 14 after infection. The number in all test groups was 25, except for certain groups of controls, which had 35 animals each. In peripheral blood and kidney culture studies, the number of rats at each time point was 10.

Figure 1. Chemical structure of SK&F 107647, (S)-5-oxo-L-prolyl-L-α-glutamyl-L-α-aspartyl-N⁶(5-amino-1-carboxypentyl)-8-oxo-N⁵-[N-[(S)-5-oxo-L-prolyl]-L-α-glutamyl]-L-α-aspartyl]-L-threo-2,7,8 triamino-octanoyl-lysine.

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Results

Effect of gentamicin therapy on survival in Escherichia coli– and Staphylococcus aureus–induced sepsis. Rats were infected with either an E. coli– or S. aureus–containing (1–2 × 10⁶ cfu) fibrin-thrombin clot. Beginning 2 h after infection, animals were administered diluent or 5 mg/kg gentamicin twice daily and followed for survival. Control rats infected with either organism were all alive 12 h after infection; however, there were no survivors at 24 and 48 h among E. coli– and S. aureus–infected animals, respectively (figure 2). When infected rats were treated with gentamicin, a significant increase in survival was seen in both groups of infected animals (P < .01).

SK&F 107647 dose response in E. coli– and S. aureus–induced sepsis. SK&F 107647 was tested for its ability to improve the survival of bacteria-challenged rats over that observed with antibiotic treatment alone. Animals received a single subcutaneous injection of biodegradable microsphere–formulated SK&F 107647 (0.01–100 ng/kg/day) or control formulation 6 days before infection with either E. coli (figure 3A) or S. aureus (figure 3B). After infection, animals were treated with gentamicin. Rats treated with control formulation followed by gentamicin therapy had similar survival rates when challenged with either E. coli or S. aureus (38% and 36%, respectively). The rats pretreated with the lowest dosage of SK&F 107647, 0.01 ng/kg/day, did not demonstrate significant protection. However, pretreatment with higher doses of SK&F 107647 followed by gentamicin therapy resulted in a statistically significant increase in survival of both E. coli– and S. aureus–challenged rats over control rats given only therapeutic gentamicin (0.1 ng/kg/day, P < .04; 1.0 ng/kg/day, P < .003; and 10 ng/kg/day, P < .001, respectively). SK&F 107647 demonstrated a “bell-shaped” dose response that is characteristic of this type of hematoregulatory compound with the 100 ng/kg/day dosage failing to significantly protect.

We also evaluated SK&F 107647 in combination with the antibiotic ceftazidime. Animals were prophylactically treated with biodegradable microsphere–formulated SK&F 107647 in the same manner and doses as in the previous studies. Rats were treated subcutaneously with ceftazidime beginning 2 h after infection, then twice daily thereafter. Rats treated with control formulation followed by ceftazidime had a survival rate of 48%. In contrast, rats pretreated with SK&F 107647 at 1.0 ng/kg/day (76% survival; P < .05) or 10 ng/kg/day (84% survival; P < .02) followed by ceftazidime after infection demonstrated a significant increase in survival over antibiotic therapy alone. The two lowest dosages of SK&F 107647 (0.01 and 0.1 ng/kg/day) did not show significant protection. As in the gentamicin studies, rats receiving 100 ng/kg/day SK&F 107647 did not show significant protection, with 64% of the rats surviving.

Pretreatment with SK&F 107647 without the benefit of any conventional antibiotic therapy was also evaluated in the E. coli model. Animals were prophylactically treated with SK&F 107647 (1.0, 3.3, 10.0, 33.0, or 100.0 ng/kg/day) in the same manner as the animals treated with either gentamicin or ceftazidime and then infected with a reduced number of E. coli (5.0 × 10⁶). Without the benefit of conventional antibiotic treatment, only 17% of rats treated with non–peptide containing control formulation survived, even with the reduced number of organisms. Pretreatment with SK&F 107647 at the lowest dosage, 1.0 ng/kg/day, did not show significant protection. The number of survivors in the groups receiving SK&F 107646 at 3.3 ng/kg/day (48% survival; P < .05), 10 ng/kg/day (64% survival; P < .02), or 33 ng/kg/day (44% survival; P < .05) demonstrated statistically significant protection over
the control formulation-dosed rats. As before, rats dosed with 100 ng/kg/day SK&F 107647 did not show significant protection, with 28% of the rats surviving.

Length of SK&F 107647 pretreatment necessary in E. coli- and S. aureus-induced sepsis. To determine the length of pretreatment necessary, rats were given a single subcutaneous injection of biodegradable microsphere-formulated SK&F 107647 at the optimal dosage (10 ng/kg/day) or control formulation 6 days, 3 days, 1 day, 6 h, or 2 h before infection (table 1). An additional group of E. coli-challenged animals was dosed with SK&F 107647 2 h after surgery. Animals were infected with either E. coli or S. aureus, and beginning 2 h after infection, rats were administered gentamicin and followed for survival. Rats pretreated with SK&F 107647 for 6 days (P < .001), 3 days (P < .003), or 1 day (P < .04) demonstrated statistically significant improvement in survival over their respective controls. Although rats pretreated for 6 or 2 h demonstrated an increase in survival, this improvement was not statistically significant. Rats infected with an E. coli-containing fibrin-thrombin clot and therapeutically treated with SK&F 107647 2 h after infection were offered some benefit over gentamicin alone, but as with the animals treated 2 and 6 h before infection, this protection was not statistically significant. The length of pretreatment necessary to achieve statistical significance was the same whether the animals were infected with E. coli or S. aureus.

Effect of oral administration of SK&F 107647 in E. coli-induced sepsis. To determine the oral activity of SK&F 107647 in this model, rats were prophylactically treated for 6 days with SK&F 107647 or control diluent administered orally with food. SK&F 107647 or control diluent was administered by placing dissolved compound in a commercially prepared tablet (Bio-serv; A. Holton, Frenchtown, NJ). Tablets were placed with the animals, and consumption of the tablet was confirmed after 20 min. Animals were infected with an E. coli-containing fibrin-thrombin clot, then treated with gentamicin and followed for survival. Animals dosed orally with the control diluent showed a 44% survival rate (figure 4). SK&F 107647 dosed orally demonstrated the characteristic bell-shaped dose response, with dosages of 10 ng/kg/day (P <

Table 1. Effect of length of SK&F 107647 treatment (10 ng/kg/day) on percentage of survival in Escherichia coli- or Staphylococcus aureus-induced sepsis.

<table>
<thead>
<tr>
<th>Bacterium, treatment</th>
<th>-6 days</th>
<th>-3 days</th>
<th>-1 day</th>
<th>-6 h</th>
<th>-2 h</th>
<th>+2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>36</td>
<td>24</td>
<td>32</td>
<td>36</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK&amp;F 107647</td>
<td>88*</td>
<td>76*</td>
<td>64*</td>
<td>48</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>S. aureus</td>
<td>36</td>
<td>36</td>
<td>48</td>
<td>44</td>
<td>32</td>
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</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK&amp;F 107647</td>
<td>84*</td>
<td>80*</td>
<td>80*</td>
<td>72</td>
<td>56</td>
<td>ND</td>
</tr>
</tbody>
</table>

NOTE. * n = 25/group. % survival was determined on day 14. ND, not done. Compared with control, * P < .001; † P < .03; ‡ P < .003; § P < .04; other comparisons were not significant.
In addition, organ cultures were done on the kidneys of *E. coli*–infected animals. Rats were prophylactically treated, infected with an *E. coli*–containing clot, and dosed with gentamicin as before. Groups of 10 rats were sacrificed 27 and 42 h after infection and both kidneys removed and homogenized. At 27 h after infection, rats pretreated with SK&F 107647 showed a decrease in the number of bacteria in the kidneys (not significant; figure 6). However, by 42 h after infection, rats treated with SK&F 107647 showed a statistically significant decrease in the number of *E. coli* in the kidneys over control formulation–treated animals.

**Ex vivo analysis of CD11b expression.** As a measure of monocyte and neutrophil activation [24, 25], CD11b expression on the surface of PBL from rats receiving SK&F 107647 (10 ng/kg/day, biodegradable microsphere formulation) or control formulation starting on day 6 before infection was measured before and at 18, 20, 24, 42, 44, and 48 h after implantation of an *E. coli*–containing fibrin-thrombin clot. Administration of SK&F 107647 resulted in significantly enhanced levels of CD11b expression (compared with controls) on both neutrophils and monocytes (figures 7A and B, respectively) after infection. Immediately before infection (time 0), levels of CD11b expression were 2- to 3-fold greater on cells from animals receiving SK&F 107647 than from control-treated rats. After infection, the levels of receptor expression on neutrophils from SK&F 107647–treated rats remained significantly elevated (1.5- to 2.5-fold) above that measured on cells from the control group for 20 h. Monocytes from treated animals were significantly activated (2- to 9-fold) at all but the last time point (48 h after infection). No differences in cell population differentials between treatment groups were observed, indicating that increases in bound anti-CD11b antibody on PBL from SK&F 107647–treated rats reflects increases in individual cell receptor expression. Overall, the percentage of monocytes expressing surface CD11b was comparable between the SK&F 107647 and control groups, while the percentage of neutrophils positive for CD11b expression at times was 25%-50% greater in control rats than in SK&F 107647–treated rats (data not shown). When measured in uninfected rats that had undergone sham abdominal surgical procedures, CD11b expression on neutrophils and monocytes remained at 2- to 3-fold and 5- to 11-fold greater levels, respectively, on cells from SK&F 107647–treated animals than on cells from controls for the duration of the study (data not shown).

**Serum TNF-α analysis.** At all time points taken, there were no detectable levels of TNF-α in rats implanted with a sterile clot (table 2). Rats challenged with an *E. coli*–containing fibrin-thrombin clot demonstrated a peak in TNF-α 4 h after infection. By 8 h, levels had decreased to 69 pg/mL for control-treated animals and 62 pg/mL for SK&F 107647–treated animals. At 12 h after infection and at all time points taken thereafter (25, 27, 32, 37, 42, and 44 h), there was no measurable TNF-α in serum. Rats challenged with *S. aureus* showed low levels of TNF-α in serum at 4, 8, and 12 h (table 2) and, as was seen in *E. coli*–challenged animals, no measurable TNF-α at any later time point.

Figure 4. Effect of orally dosed SK&F 107647 in gram-negative (*Escherichia coli*) sepsis. *P < .05; **P < .001.

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.001), 33 ng/kg/day (P < .001), and 100 ng/kg/day (P < .04) providing a statistically significant improvement in survival over their respective controls. Oral dosages of 3.3 or 333 ng/kg/day failed to offer protection.

**Effect of SK&F 107647 pretreatment on number of *E. coli*.** Femorally cannulated rats were injected subcutaneously with a single injection of biodegradable microsphere–formulated SK&F 107647 (10 ng/kg/day) or with control formulation. Animals were infected with an *E. coli*–containing (1 × 10⁹ cfu) fibrin-thrombin clot and dosed with gentamicin as before. Beginning at 18 h after infection, blood samples were taken from the femoral vein cannula. The number of rats sampled at each time point was ≥10. At early time points, 18–25 h, control animals and SK&F 107647–pretreated animals demonstrated low but similar numbers of organisms in blood (figure 5). However, after the 25-h time point, control animals demonstrated a steady increase in the number of *E. coli* cultured from blood, with little or no response to antibiotic therapy. In contrast, rats pretreated with SK&F 107647 followed by gentamicin therapy demonstrated a statistically significant reduction in the number of recoverable organisms from the blood at each time point after 25 h (27, 32, 37, 42, and 44 h; P < .01).
Figure 5. Effect of SK&F 107647 (○) on numbers of *Escherichia coli* in blood. ▲, rats pretreated with control formulation. Each point represents mean ± SD of data from ≥10 rats/time point. * P < .01.

Discussion

Despite considerable research into the understanding of sepsis and advances in antimicrobial therapy, sepsis and septic shock remain a major cause of mortality in surgery and trauma patients. Traumatized and surgical patients have been shown to have changes in the hematopoietic system [26], CD11b expression [27], MHC type II expression [28], effector cell functions [29], and levels of effector proteins [30]. The decreases in immune status of traumatized patients has been shown to precede sepsis by several days [31, 32]. An important aspect in treating sepsis is its seemingly intractable resistance to a variety of antimicrobial agents, and despite the development of many new therapeutic agents, the overall incidence of sepsis has increased. Clearly, other therapies in addition to antibiotics need to be evaluated if the incidence of infection and sepsis is to be reduced. We have used the rat fibrin-thrombin clot model to evaluate SK&F 107647 to determine if the novel hematoregulatory activities of this compound could offer protection in bacterial sepsis.

When evaluating the activities of an immunoregulatory compound, a key issue is that a true infection model rather than a bacterial intoxication model is used. Such a bacterial infection model demands the selection of virulent stains of organisms [33]. In preliminary studies, we evaluated several strains of *E. coli* and *S. aureus* for virulence. While relatively avirulent bacterial strains could elicit deaths in the model, high inocula (10^10–10^11 cfu) were required, and very little protection was demonstrated with appropriate antibiotic therapy. In contrast, the virulent bacterial strains selected for these studies caused
significant deaths with fewer organisms, and therapeutic administration of antibiotics (gentamicin or ceftazidime) improved survival in rats challenged with either gram-negative or gram-positive bacteria. Although the protection seen with antibiotics was not complete, antibiotics not only slowed the rate of death but also improved the overall survival of the animals. These results indicate that in contrast to the bolus intraperitoneal injection of live organisms, placement of the bacteria into a fibrin-thrombin clot created a focus of evolving infection that remained partially responsive to antibiotic therapy. The failure of antibiotics to give complete protection in this model is consistent with the lack of uniform protection provided in clinical sepsis and makes it possible to evaluate other pharmacologic interventions in conjunction with a conventional antibiotic regime in an attempt to improve survival over that provided by an antibiotic alone.

Pretreatment with SK&F 107647 protected rats infected with either E. coli or S. aureus in a dose-responsive manner, with dosages as low as 0.1 ng/kg/day offering significant protection. In both the gram-negative and gram-positive sepsis models, SK&F 107647 demonstrated a characteristic bell-shaped dose response, with a dosage of 10 ng/kg/day resulting in optimal protection. Dosages as high as 1.0 µg/kg/day of SK&F 107647 were evaluated in the sepsis model and in noninfected rats with no observable toxic effects (data not shown). In infection studies at these nonprotective higher dosages (≥100 ng/kg/day), the number of surviving animals never fell below that of the control groups. This indicates that the bell-shaped dose response is not due to a toxic effect. The mechanism is not known but could be related to feedback suppression pathways or to receptor desensitization, as has been proposed as a mechanism for the bell-shaped dose response demonstrated with some of the chemokines [34–36]. In addition, SK&F 107647 has demonstrated protection used prophylactically as a single agent; however, additional protection was demonstrated in combination with conventional antibiotic therapies—gentamicin or ceftazidime.

When rats were dosed orally with SK&F 107647 and then infected with an E. coli—containing fibrin-thrombin clot, significant protection was also demonstrated. SK&F 107647 has previously demonstrated activity after oral or parenteral dosing in the induction of hematoregulatory factors [16] and in protecting C. albicans—infected mice [18]. These data indicate the potential for SK&F 107647 to be used clinically either parenterally or orally.

In initial studies using Alzet pumps (Alza, Palo Alto, CA) to deliver SK&F 107647, protection was similar to that seen with the biodegradable microsphere—formulated peptide described here. Additionally, a control peptide with no hematoregulatory activity that differs from SK&F 107647 by a single methylene unit failed to offer any activity in rats challenged with an E. coli—infected fibrin-thrombin clot (data not shown). Therefore, the activities demonstrated in SK&F 107647—treated animals are not due to a nonspecific peptide effect but attributable to the specific hematoregulatory activities of this peptide.

SK&F 107647 offered significant protection against either gram-negative or gram-positive infections with as little as 1

<table>
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<tr>
<th>Infection, treatment</th>
<th>Tumor necrosis factor (pg/mL)</th>
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<tr>
<td>None/sterile clot, control</td>
<td>4 h</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
</tr>
<tr>
<td>SK&amp;F 107647</td>
<td>497 ± 159*</td>
</tr>
<tr>
<td>S. aureus</td>
<td>456 ± 88*</td>
</tr>
<tr>
<td>SK&amp;F 107647</td>
<td>21 ± 56</td>
</tr>
</tbody>
</table>

NOTE: Data are mean ± SD (n = 7 at each time point). Additional samples taken at 25, 27, 32, 37, 42, and 44 h were all <10 pg/mL.

* P < .001 vs. sham surgery/no infection at same time point, Student’s t test.
† P < .05 vs. sham surgery/no infection at same time point, Student’s t test.
day of pretreatment. Although courses of pretreatment shorter than 1 day showed increased survival over diluent-treated control animals, this protection did not reach statistical significance. When animals were infected with *E. coli* and then therapeutically treated with SK&F 107647 and gentamicin, some benefit was also demonstrated, although the increase in survival was not statistically significant.

When the number of organisms in the blood and kidneys was determined from rats infected with an *E. coli*–containing fibrin-thrombin clot, rats pretreated with SK&F 107647 demonstrated significantly reduced numbers of organisms in both the peripheral blood and kidneys. The mechanism by which SK&F 107647 lowers the number of organisms in *E. coli*–infected animals is under investigation. However, the activities demonstrated in this model could be due to activation of mature effector cells by the cytokine(s) elicited by this compound [16, 17]. CD11b is the α-subunit of the heterodimer (CD11b/CD18; CR3) adhesion molecule on neutrophils and monocytes. In addition to being involved in the process of phagocyte adhesion and diapedesis, this receptor is also a major component of the complement-mediated bacterial phagocytosis process [37, 38].

In *E. coli*–infected rats, the data indicate that SK&F 107647–induced activation of neutrophils and monocytes may be at least one mechanism responsible for the increased bacterial clearance and killing observed in the culture studies. Peripheral blood monocytes and neutrophils collected from these animals possess significantly greater levels of surface-expressed CD11b at the time of infection and throughout a critical period of time after infection. This time frame corresponds to the period after infection during which organism burden declines; beyond 20 h, the number of circulating organisms is significantly less in SK&F 107647–treated rats than in controls. In the case of placebo-treated infected rats, increases in activation of both the neutrophil and monocyte populations did not occur before 20 h after infection, which is apparently too delayed to be of benefit to the animals. Since binding of ligand at CR3 has been shown to be a potent trigger for intracellular microbialic mechanisms such as superoxide production and degranulation [39], it is probable that the effector cell activation induced by prophylactic treatment with SK&F 107647 sets up a favorable environment in which the animals can more efficiently ward off the advancement of the infection.

Positive blood and kidney cultures were also found in animals challenged with an *S. aureus*–containing fibrin-thrombin clot, and animals pretreated with SK&F 107647 had reduced numbers of organisms at both sites; however, the numbers of recoverable organisms were not consistent with large variations from individual animals from time point to time point (data not shown), and the reductions seen with SK&F 107647 were not statistically significant. It is unclear if the lack of reproducibility in *S. aureus* culture studies was due to variation in the number of organisms or in our ability to reproducibly culture them. This is currently under investigation along with cultures from other sites. However, since the *S. aureus* that was used produces both enterotoxin A and β-hemolysin [24], it is possible that organisms from the peritoneum are the source of toxic factors that lead to the death of the animals.

Rats infected with a gram-negative bacterium (*E. coli*) showed a short burst of TNF-α at the 4-h time point, with all 14 rats demonstrating TNF-α in serum. The level of TNF-α had dropped to just above the level of detection by 8 h, and at every time point after 8 h, the TNF-α levels were <10 pg/mL (level of detection). Even when rats went on to die, there was no measurable TNF-α in the serum after 8 h. These results are similar to those demonstrated in the rat cecal ligation and puncture model [40] and in *E. coli*–infected mice [41]. While there were strong differences in survival between control treated rats and those pretreated with SK&F 107647, serum TNF-α levels in the groups were identical.

In rats infected with a gram-positive bacterium (*S. aureus*), 5 of 14 rats were positive for TNF-α at 8 h. By 12 h, only 3 of the 14 rats were positive, with low levels of TNF-α in the serum. As in *E. coli*–challenged rats, pretreatment with SK&F 107647 increased survival but did not have a measurable effect on serum TNF-α levels in the animals. These studies indicate that the unique hematoregulatory activities of SK&F 107647 can prevent the decreased immune status that may compromise the host's ability to effectively clear an overwhelming bacterial challenge and therefore may represent an important new approach to the prevention of sepsis and septic shock. Clinical studies to determine if the hematoregulatory potential of SK&F 107647 can offer protection from infectious complications in myelosuppressed patients are currently ongoing.

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References


