Therapeutic efficacy of the magainin analogue MSI-78 in different intra-abdominal sepsis rat models

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Objectives: This study was designed to investigate the antimicrobial and anti-endotoxin activity of MSI-78, a synthetic cationic peptide analogue of magainin 2.

Methods: The in vitro antimicrobial activity of MSI-78 was investigated against the commercially available quality control strain Escherichia coli ATCC 25922. In addition, three rat models of septic shock were investigated: (i) rats were injected intraperitoneally with 1 mg Escherichia coli 0111:B4 LPS; (ii) rats were given an intraperitoneal injection of 2 × 10^10 cfu of Escherichia coli ATCC 25922; (iii) intra-abdominal sepsis was induced via caecal ligation and puncture. All animals were randomized to receive after 360 min intravenously isotonic sodium chloride solution, 1 mg/kg MSI-78, or 60 mg/kg piperacillin. Main outcome measures were: abdominal exudate and plasma bacterial growth, plasma endotoxin and tumour necrosis factor α (TNF-α) concentrations, and lethality.

Results: Our in vitro data showed that MSI-78 possesses a strong activity against Escherichia coli. The in vivo studies showed that all compounds reduced the lethality when compared to controls. MSI-78 showed a slightly higher antimicrobial activity than piperacillin and achieved a substantial decrease in endotoxin and TNF-α plasma concentrations than the β-lactam.

Conclusions: Because of its strong double anti-endotoxin and antimicrobial activities MSI-78 could be an interesting compound for Gram-negative septic shock treatment.

Keywords: septic shock, endotoxin, peptides

Introduction

Despite intense efforts to improve survival, septic shock remains a serious clinical problem in a variety of human patients, including all immunocompromised patients, individuals with burns and patients in critical care units.1-3 Intra-abdominal sepsis is characterized by a massive leucocyte infiltration into the peritoneum, followed by an overwhelming systemic response via the massive production of inflammatory mediators, including release of cytokines, increased expression of adhesion molecules, chemotactic recruitment of lymphoid cells and increased phagocytic activity of macrophages. This cascade may result in life-threatening complications including shock or multiple organ failure.4,5 Lipopolysaccharide (LPS), a major structural and functional component of Gram-negative bacteria, is one of the major toxins responsible for initiating this pathophysiological phenomenon.6-8 For this reason, over the last decade, the idea of its inhibition after administration of endotoxin-blocking agents as therapy for sepsis caused by Gram-negative bacteria has been thoroughly explored with various animal models and in clinical trials.9-12 In particular, the rat model of intra-abdominal sepsis has been used extensively to study the roles of several microorganisms during the infectious process and to test the therapeutic efficacy of a variety of antimicrobial and anti-endotoxin agents. In fact,
the predictive value of this model for human sepsis has been well documented.\textsuperscript{4,18} LPS is composed of an O-polysaccharide chain, a core sugar, and a lipophilic fatty acid: lipid A. It produces the signal responsible for the induction of cytokine genes and possesses an anionic and amphipathic character. Antimicrobial peptides are positively charged and amphipathic molecules that have been discovered in animals ranging from insects to humans.\textsuperscript{16,17} They represent components of the system of host defence commonly termed ‘innate immunity’ and are used by animals to effectively deal with microbes in their environment.\textsuperscript{18} In recent years, antimicrobial peptides have received increased attention as they possess a broad spectrum of activity against bacteria, fungi and protozoa and anti-endotoxin activity.\textsuperscript{19,20}

Among these animal-derived antimicrobial agents are the magainins, discovered in the skin of the African clawed frog \textit{Xenopus laevis} more than 15 years ago.\textsuperscript{21} Similar to other polycationic peptides, which are \alpha-helical ionophores, they possess two important activities: a broad antimicrobial spectrum and anti-endotoxin activity. Through a series of amino acid deletions and substitution, MSI-78 was created. It is a 22 residue magainin analogue that showed an enhanced \textit{in vitro} and \textit{in vivo} potency relative to that of magainin 2 against both Gram-positive and Gram-negative bacteria.\textsuperscript{22–24}

The present experimental study was designed to investigate the \textit{in vivo} efficacy of MSI-78 in three rat models of septic shock.

**Materials and methods**

**Animals**

Adult male Wistar rats weighing 200–250 g were used for all the experiments. All animals were housed in individual cages under constant temperature (22°C) and humidity with a 12-h light/dark cycle, and had access to chow and water \textit{ad libitum} throughout the study. The study was approved by the animal research ethics committee of the I.N.R.C.A.—I.R.R.C.S., Polytechnic University of Marche, Ancona, Italy.

**Organisms and reagents**

The commercially available quality control strain of \textit{Escherichia coli} ATCC 25922 was used. Endotoxin (\textit{Escherichia coli} serotype 0111:B4; Sigma–Aldrich S.r.l., Milan, Italy) was prepared in sterile saline, and stored in aliquots at –80°C for short periods.

**Agents**

MSI-78 (Gly-Ile-Gly-Lys-Phe-Leu-Lys-Lys-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Lys-Ile-Leu-Lys-Lys-NH$_2$) was synthesized by 9-fluorenlymethoxycarbonyl (Fmoc) solid-phase chemistry according to the following procedure: (i) 2 and 20 min deprotection steps using 20% piperidine in dimethylformamide (DMF) in the presence of 1% Triton; (ii) the coupling reactions were carried out with 1% Triton with using diisopropylcarbodiimide (DIC) as the coupling reagent in the presence of 1-hydroxybenzotriazole (HOBT) for 2 h. The completeness of each coupling reaction was monitored by the chloranil test.\textsuperscript{25,26} The protected peptide resin was treated with the mixture: 95% trifluoroacetic acid (TFA), 2.5% water and 2.5% triisopropylsilane (TIS) for 2 h. After cleavage, the solid support was removed by filtration, and the filtrate was concentrated under reduced pressure. The cleaved peptide was precipitated with diethyl ether and lyophilized. Pexiganan was purified by high-pressure liquid chromatography (HPLC) on a Knauer K501 two-pump system. The resulting fractions with purity greater than 97–98% were tested by HPLC. The peptide was analysed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF).

Piperacillin (Sigma–Aldrich) powder was diluted in accordance with manufacturer’s recommendations. Solutions were made fresh on the day of assay.

**Susceptibility testing**

Susceptibility testing was carried out by microbroth dilution method according to the procedures outlined by the National Committee for Clinical Laboratory Standards.\textsuperscript{27} However, since cationic peptides bind polystyrene, polypropylene 96-well plates (Sigma–Aldrich) were substituted for polystyrene plates. The MIC was taken as the lowest antibiotic concentration at which observable growth was inhibited. Experiments were carried out in triplicate.

**Experimental design**

Septic shock was induced under three different experimental conditions: (i) intraperitoneal administration of LPS, (ii) \textit{E. coli} induced peritonitis, (iii) caecal ligation and puncture.

**Model (i)**. Three groups, each containing 30 animals, were anaesthetized by intramuscular injection of ketamine (30 mg/kg of body weight) and injected intraperitoneally with 1.0 mg \textit{E. coli} serotype 0111:B4 LPS in a total volume of 500 lL of sterile saline. At 360 min after injection, the animals received intravenous isotonic sodium chloride solution (control group C0), 1 mg/kg of MSI-78, or 60 mg/kg of piperacillin, respectively.

**Model (ii)**. \textit{E. coli} ATCC 25922 was grown in brain–heart infusion broth. When bacteria were in the log phase of growth, the suspension was centrifuged at 1000 g for 15 min, the supernatant was discarded, and the bacteria were resuspended and diluted in sterile saline. All animals (three groups, each containing 30 animals) were anaesthetized as mentioned above. The abdomen of each animal was shaved and prepared with iodine. The rats received intraperitoneally 1 mL of saline containing 2 x 10$^{10}$ cfu of \textit{E. coli} ATCC 25922 and intravenously, 360 min after bacterial challenge, 1 mL of isotonic sodium chloride solution (control group C0), 1 mg/kg MSI-78, or 60 mg/kg piperacillin, respectively.

**Model (iii)**. All animals (three groups, each containing 30 animals) were anaesthetized as described above. The abdomen of each animal was shaved and prepared with iodine. Through a midline laparotomy, the caecum was filled with faeces by milking the stools back from the descending colon and then ligated just below the ileocecal valve with a 3–0 silk ligature. The antisenseenteric caecal surface was punctured twice with a 23-gauge needle below the ligature, the bowel was placed back into the peritoneal cavity, and the abdomen was closed in two layers. The operative procedure was done under aseptic conditions. For administration of antibiotics, a catheter was placed into the jugular vein and was sutured to the back of the rat. The drugs were given 360 min after the surgical procedure. The rats received isotonic sodium chloride solution (control group C0), 1 mg/kg of MSI-78, or 60 mg/kg of piperacillin, respectively.

For each animal model, toxicity was evaluated on the basis of the presence of any drug-related adverse effects, i.e. local signs of inflammation, anorexia, weight loss, vomiting, diarrhoea, fever
and behavioural alterations. In particular, to evaluate the physiological effects of MSI-78, rectal temperature, pulse, blood pressure, breathing rate and oxygenation were monitored in a supplementary MS-78-treated group without infection or LPS.

**Serum antibiotic concentration measurements and kinetics**

Preventive experiments were carried out to measure serum MSI-78 and piperacillin levels in uninfected animals. Blood samples were obtained from the tail vein of 12 rats (six rats for each agent) at 0.50, 1, 2 and 8 h after a single intravenous dose of MSI-78 (1 mg/kg) and piperacillin (60 mg/kg). Drug levels were measured by bioassay: a spore suspension of Bacillus subtilis ATCC 6633 suspended in tryptic soy agar was used. The plates were read after incubation at 30°C for 18 h.

**Evaluation of treatment**

On the basis of the type of experiment, at the end of the study, the rate of blood culture positivity, the quantities of bacteria in the intra-abdominal fluid, the rate of lethality, and plasma endotoxin and tumour necrosis factor α (TNF-α) levels were evaluated. The animals were monitored for the subsequent 72 h. In models (i) and (iii), the presence of sepsis 6 h after bacterial challenge was defined by analogy to the criteria applied for humans. Each animal was considered to be septic if it satisfied at least two of the following criteria: a) increased pulse rate; b) rectal temperature above 38°C or below 36°C; c) increased breathing rate.

The surviving animals (models (ii) and (iii)) were killed with chloroform, and blood samples for culture were obtained by aseptic percutaneous transthoracic cardiac puncture. In addition, to perform quantitative evaluations of the bacteria in the intra-abdominal fluid, 10 mL of sterile saline was injected intraperitoneally, samples of the peritoneal lavage fluid were serially diluted, and a 0.1 mL volume of each dilution was spread onto blood agar plates. The limit of detection was ≤1 log10 cfu/mL. The plates were incubated both in air and under anaerobic conditions at 35°C for 18 h.

For blood cultures and determination of endotoxin and TNF-α in plasma, 0.2 mL blood samples were collected from a tail vein 0, 2, 6 and 12 h after injection into a sterile syringe and transferred to tubes containing ethylenediaminetetraacetic acid (EDTA-K3).

**Biochemical assays**

Endotoxin measurements were determined by a Limulus amoebocyte lysate test. Plasma samples were serially two-fold diluted with sterile endotoxin-free water and were heat-treated for 5 min in a water bath at 75°C to destroy inhibitors that can interfere with the activation. Endotoxin standards (0, 0.015, 0.03, 0.06, 0.125, 0.25 and 0.5 EU/mL) were tested in each run, and the concentration of endotoxin in the test samples was calculated by comparison with the standard curve. TNF-α levels were measured with a solid phase sandwich enzyme-linked immunosorbent assay. The standards and samples were incubated with a TNF-α antibody coating 96-well microtitre plate. The wells were washed with buffer and then incubated with biotinylated anti-TNF-α antibody conjugated to streptavidin-peroxidase. This was washed away and colour was developed in the presence of chromogen (tetrathymethylbenzidine) substrate. The intensity of the colour was measured in a MR 700 Microplate Reader (Dynatech Laboratories, Guernsey, UK) by reading the absorbance at 450 nm. The results for the samples were compared with a standard curve to determine the amount of TNF-α present. All samples were run in duplicate. The lower limit of sensitivity for TNF-α by this assay was 0.05 ng/mL.

**Statistical analysis**

MIC values are presented as the average values obtained in triplicates on three independent measurements. Mortality rates between groups were compared using Fisher’s exact test. Qualitative results for blood cultures were analysed by the χ² test with Yates’ correction or Fisher’s exact test, depending on the sample size. Quantitative evaluations of the bacteria in the intra-abdominal fluid cultures were presented as means ± standard error of the mean (S.E.M.). Statistical comparisons between groups were made by analysis of variance. Multiple comparisons were carried out by Tukey’s test. As a result of the presence of several values below the lower limit of sensitivity, plasma endotoxin levels were compared between groups by Kruskal–Wallis non-parametric test, adjusted for ties, followed by Dunn’s procedure for multiple comparisons. Each comparison group contained 30 rats. Significance was accepted when the P value was ≤0.05.

**Results**

**In vitro studies**

According to the broth-microdilution method, E. coli ATCC 25922 was similarly susceptible to MSI-78 (MIC 0.5 mg/L) and to the control agent piperacillin (MIC 1 mg/L).

**In vivo studies**

Model (i). Intraperitoneal administration of LPS. MSI-78 administration resulted in a marked decrease (P < 0.05) of TNF-α and endotoxin plasma levels compared with the control group (C0), whereas no substantial differences in the plasma levels of both LPS and TNF-α were observed between the piperacillin-treated group and the controls (Table 1).

Model (ii). E. coli induced peritonitis. Lethality in control group C1 was 100%. All antibiotic treatments were better than no treatment (P < 0.05). In detail, survival was 76.7% and 70.0% in MSI-78 and piperacillin-treated groups, respectively (Table 2). Bacteriological evaluation showed 100% positive blood and intra-abdominal fluid cultures in C0: bacterial counts in the peritoneal fluid demonstrated 7.1 × 10⁸ ± 1.6 × 10⁶ cfu/mL. MSI-78 showed the highest antimicrobial activity and therapeutic efficacy. In the results from the quantitative bacterial cultures, there were significant differences when the data obtained from the piperacillin-treated groups were compared with those obtained from the peptide-treated group (P < 0.05). Endotoxin and TNF-α

**Table 1. Endotoxin and TNF-α plasma levels in a rat model 12 h after intraperitoneal administration of 1.0 mg E. coli serotype 0111:B4 LPS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Endotoxin (EU/mL)</th>
<th>TNF-α (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group C0</td>
<td>24.87 ± 4.10</td>
<td>123.34 ± 14.09</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>11.9 ± 0.81*</td>
<td>43.78 ± 10.30*</td>
</tr>
<tr>
<td>PIP, 60 mg/kg</td>
<td>23.65 ± 3.67</td>
<td>112.34 ± 12.2</td>
</tr>
</tbody>
</table>

PIP, piperacillin. Data are presented as means ± s.d.

*P < 0.001 versus the control group C0 and the piperacillin-treated group.
concentrations increased constantly in the control and treated
groups with mean peak levels at 6 h post-injection. After the
antibiotic administration, the MSI-78-treated group showed a
significant reduction in plasma endotoxin and TNF-α
levels when compared to the control and the piperacillin-treated group.
In contrast, no significant difference in plasma endotoxin and
TNF-α concentrations was observed between the piperacillin-
treated group and the control group C 1. The results are summar-
ized in Tables 3 and 4.

Model (iii). Caecal ligation and puncture. The rate of lethality
in control group C 2 was 100%. All antibiotic treatments led to
decreased mortality (P < 0.05). Specifically, survival rates of
66.7% and 63.3% were observed for the MSI-78- and piperacil-
lin-treated groups (Table 5). Bacteraemia was detected in all
animals of C2 and 7.7/C2 108 ± 2.0/C2 108 cfu/mL were counted in
their intra-abdominal fluids. Gram-negative and Gram-positive
bacteria were simultaneously isolated from more than 90% of
the rats. The microorganisms isolated from both blood and the
abdominal fluid were primarily Enterobacteriaceae including
Escherichia coli, Enterobacter spp., Klebsiella spp., and
Gram-positive cocci. The most frequent anaerobic isolates were
Bacteroides spp. Organisms were identified by the API systems
(BioMérieux Italia S.p.A, Rome, Italy). MSI-78 showed the
highest antimicrobial activity. As shown in models (i) and (ii),
before the drug administration constant increases in plasma,
endotoxin and TNF-α concentrations were found with mean
peak levels achieved at 6 h after surgical procedure. Similar to
the other models, MSI-78 produced a significant reduction in
plasma endotoxin and TNF-α levels compared to control and
piperacillin-treated groups. The results are summarized in Tables
6 and 7.

Overall, the septic status was documented in controls by an
increase in pulse rate (from 251.5 ± 18.0 to 305.3 ± 23.6), breath-
ing rate (from 70.2 ± 10.4 to 83.4 ± 15.4) and a decrease in rectal
temperature (from 36.5 ± 1.1 to 35.3 ± 1.2°C). Thirty minutes
after a single intravenous injection, MSI-78 and piperacillin
reached peak levels of 2.7 and 26.8 mg/L, respectively.
Finally, none of the treated animals had clinical evidence of
drug-related adverse effects and no changes in physiological
parameters were observed in the supplementary 1 mg/kg MSI-
78-treated group without infection.

Discussion
Treatment of sepsis still constitutes a clinical challenge and
de spite advances in supportive care, the mortality rate of sepsis-
related diseases remains high. 1–3 Current treatments are based on
prompt institution of antimicrobial agents to control the infection
and intensive care support to correct the dysfunction of the main
organ systems. 1,14,28 In recent years, great progress has been
made in understanding the pivotal role of LPS in the pathogen-
esis of Gram-negative septic shock as a potent prototypical
stimulus of the immune response to bacterial infection. Using
this knowledge, a number of agents capable of inhibiting or

### Table 2. Efficacy of intravenous administration at 360 min of MSI-78 and piperacillin in a rat model of E. coli induced peritonitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>no. dead/total</th>
<th>%</th>
<th>Qualitative blood culture (no. positive/total)</th>
<th>Bacterial count in peritoneal fluid (cfu/mL)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (control group C 2)</td>
<td>30/30</td>
<td>100</td>
<td>15/15</td>
<td>7.1 × 10&lt;sup&gt;8&lt;/sup&gt; ± 1.6 × 10&lt;sup&gt;6&lt;/sup&gt;**</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>7/30&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>23.3</td>
<td>7/30&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>4.4 × 10&lt;sup&gt;8&lt;/sup&gt; ± 0.5 × 10&lt;sup&gt;6&lt;/sup&gt;**</td>
</tr>
<tr>
<td>PIP, 60 mg/kg</td>
<td>9/30&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>30.0</td>
<td>10/15&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>5.8 × 10&lt;sup&gt;7&lt;/sup&gt; ± 2.0 × 10&lt;sup&gt;5&lt;/sup&gt;**</td>
</tr>
</tbody>
</table>

PIP, piperacillin. <sup>a</sup>Lethality was monitored for 72 h following the challenge. <sup>b</sup>Data are presented as means ± S.D. <sup>‡</sup>P<0.05 versus the control group C 2. **P<0.001 for all the possible comparisons.

### Table 3. Effect of administration at 360 min of MSI-78 and piperacillin on endotoxin plasma levels in a rat model of E. coli induced peritonitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Endotoxin (EU/mL) at time (h) after antibiotic administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No treatment (control group C 2)</td>
<td>0.88 ± 0.02**</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>0.77 ± 0.01**</td>
</tr>
<tr>
<td>PIP, 60 mg/kg</td>
<td>0.99 ± 0.02**</td>
</tr>
</tbody>
</table>

PIP, piperacillin. Data are presented as means ± S.D. <sup>*</sup>P<0.001 versus the control group C 2 and the piperacillin-treated group. **P<0.001 for all the possible comparisons.
binding LPS have been developed.Interestingly, among these compounds, the polycationic peptides might offer the opportunity to inhibit not only the biological activity of the endotoxin but also the bacterial growth. The polycationic peptides are an important component of the innate defences of all species of life. They have good activities against a broad range of bacterial strains, kill very rapidly and do not easily select resistant mutants. In addition, the polycationic peptides generally have a high affinity for LPS and are able to inhibit the production of cytokines such as TNF-α and interleukin-6 by macrophages stimulated with the endotoxin.

Like the other polycationic peptides, MSI-78 demonstrated broad spectrum in vitro antimicrobial activity against most of the common pathogens responsible for intra-abdominal sepsis. Interestingly, since it acts directly on the anionic phospholipid of the bacterial cell membrane and not on membrane receptors, the development of resistance is theoretically less likely to occur.

A growing body of research has been devoted to studying animal models of septic shock and a single dose of endotoxin or a single inoculum of one Gram-negative species has been the most used for screening of anti-endotoxin and antimicrobial drugs. On the other hand, Gram-positive and Gram-negative infections are also common in humans. Therefore, in this study, we used not only the animal models of intraperitoneal administration of LPS or E. coli inoculum but also the model of caecal ligation and puncture to resemble the clinical condition of a bowel perforation and mixed bacterial infection. Moreover, in our experiments, we administered the drugs 360 min after the bacterial challenge or the surgical procedure to mimic the clinical situation where an interval between the onset of sepsis and the initiation of therapy is present. Piperacillin was used as control agent. It acts against most of the pathogens that can cause intra-abdominal infections including anaerobes, Gram-positive cocci and Enterobacteriaceae.

Data analysis for our three experiments did not show any important different impacts on parameter evaluation. In fact, it was evident that the efficacies of the compounds were not affected by the animal models used and that these were retained regardless of the system used. Overall, the administration of all agents yielded a lower rate of mortality than was seen in saline-treated controls. MSI-78 was effective against all parameters considered, regardless of the animal model utilized. Importantly, single intraperitoneal or intravenous doses of MSI-78 produced a significant reduction in the TNF-α plasma levels, compared with both control and piperacillin-treated groups.

However, in models (ii) and (iii), MSI-78 showed the lowest lethality rate but no significant differences were observed in the rate of lethality among MSI-78- and piperacillin-treated groups. It is important to point out that the greatest significant differences in defining the efficacy of the compounds were observed

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (control group C₁)</td>
<td>79.4 ± 82*</td>
<td>101.3 ± 10.3</td>
<td>215.8 ± 28.6**</td>
<td>201.9 ± 16.3</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>66.8 ± 4.3*</td>
<td>20.1 ± 2.0*</td>
<td>22.8 ± 2.2**</td>
<td>21.0 ± 2.0*</td>
</tr>
<tr>
<td>PIP, 20 mg/kg</td>
<td>80.7 ± 8.2</td>
<td>99.0 ± 6.3</td>
<td>197.7 ± 12.4**</td>
<td>191.4 ± 15.5</td>
</tr>
</tbody>
</table>

**P<0.001 for all the possible comparisons.

Table 5. Efficacy of administration at 360 min after the operative procedure of MSI-78 and piperacillin in a rat model of caecal ligation and puncture induced peritonitis

| Treatment | No. dead/total | % | Qualitative blood culture (no. positive/total) | Bacterial count in peritoneal fluid (cfu/mL)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (control group C₂)</td>
<td>30/30</td>
<td>100</td>
<td>30/30</td>
<td>7.7×10⁸±2.0×10⁸**</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>10/30*</td>
<td>33.3</td>
<td>10/30*</td>
<td>6.7×10⁸±0.8×10⁸**</td>
</tr>
<tr>
<td>PIP, 60 mg/kg</td>
<td>11/30*</td>
<td>36.7</td>
<td>11/30*</td>
<td>4.0×10⁸±0.6×10⁸**</td>
</tr>
</tbody>
</table>

**P<0.001 for all the possible comparisons.

Table 4. Effect of administration at 360 min of MSI-78 and piperacillin on TNF-α plasma levels in a rat model of E. coli induced peritonitis

### Notes

- *P<0.001 versus the control group C₁ and the piperacillin-treated group.
- **P<0.001 for all the possible comparisons.

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Table 6. Effect of administration at 360 min after the operative procedure of MSI-78 and piperacillin on endotoxin plasma levels in a rat model of caecal ligation and puncture induced peritonitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Endotoxin (EU/mL) at time (h) after antibiotic administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No treatment (control group C2)</td>
<td>0.136 ± 0.01</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>0.132 ± 0.03/*</td>
</tr>
<tr>
<td>PIP, 60 mg/kg</td>
<td>0.129 ± 0.02</td>
</tr>
</tbody>
</table>

**P**< 0.001 versus the control group C2 and the piperacillin-treated group. **P**< 0.001 for all the possible comparisons.

Table 7. Effect of administration at 360 min after the operative procedure of MSI-78 and piperacillin on TNF-α plasma levels in a rat model of caecal ligation and puncture induced peritonitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNF-α (ng/mL) at time (h) after antibiotic administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No treatment (control group C1)</td>
<td>103.8 ± 11.1</td>
</tr>
<tr>
<td>MSI-78, 1 mg/kg</td>
<td>94.4 ± 7.3*</td>
</tr>
<tr>
<td>PIP, 20 mg/kg</td>
<td>111.8 ± 10.3</td>
</tr>
</tbody>
</table>

**P**< 0.001 versus the control group C1 and the piperacillin-treated group. **P**< 0.001 for all the possible comparisons.

in their capacity to inhibit the release of LPS, while no statistically significant differences were noted in their antimicrobial activity. The relevance of this pivotal role of antimicrobial activity has been clearly shown by the third experimental model where various Gram-negative and Gram-positive bacterial species were involved in the pathogenesis of the sepsis. In fact, lethality data demonstrated that the strong activity and broad antimicrobial spectrum of MSI-78 prevailed against the other agents. Although the extrapolation of results from animal models to human pathology should be regarded with caution, our results highlight the high therapeutic potential of MSI-78. As a result of its strong antimicrobial activity and its ability to neutralize the biological effects of endotoxin, MSI-78 may play a crucial role in the treatment of intra-abdominal sepsis.

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**References**

A. Giacometti et al.