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Original Article

Effect of antimicrobial peptides on mice with acute peritonitis and its possible mechanism

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Abstract: Objectives: We aimed to investigate the effect of antimicrobial peptide (AMP) on a mouse model of acute peritonitis (AP) and its possible mechanism in order to examine the feasibility of using AMP for treating AP. Methods: An AP model was established in forty-five adult BALB/c mice by intraperitoneal injection of methicillin-resistant staphylococcus aureus (MRSA), and the mice were divided to three groups of 15 mice each, which were group A (no treatment), group B (treated with intraperitoneal injection of AMP), and group C (control, treated with intraperitoneal injection of normal saline). The behavior and body temperature of the mice were observed before and after modeling and treatment, and the blood was collected from the tail vein before and after modeling and treatment to measure the white blood cell (WBC) count and the levels of interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α. Mortality was recorded. Results: After model creation, the mice in group A displayed abnormal behavior, elevated body temperature (the temperature increased with time, all $P<0.05$), and increased levels of the WBC, IL-6, IL-1β, and TNF-α (all $P<0.05$). After treatment, the mice in group B presented more normal behavior and lower body temperature compared with those in groups A and C; but the body temperature in group B was still higher than that before modeling (all $P<0.05$). Moreover, the mice in group B had lower WBC count and lower levels of IL-6, IL-1β, and TNF-α than those in groups A and C after treatment (all $P<0.05$). No differences were found between groups A and C in the levels of the above markers after treatment (all $P>0.05$). Conclusions: An AP mouse model can be established by intraperitoneal injection of MRSA, and AMP can achieve good results in treating this model. This may be because AMP can reduce the levels of the inflammatory factors IL-6, IL-1β, and TNF-α in mice. This finding may provide some new insights into the treatment of drug-resistant AP.

Keywords: Antimicrobial peptide, acute peritonitis, methicillin-resistant staphylococcus aureus

Introduction

Acute peritonitis (AP) is a common acute abdominal disease that is usually caused by acute appendicitis and gastrointestinal perforation [1]. In some cases, the disease can also be a spontaneous bacterial infection induced by internal diseases [2]. Major symptoms of AP include abdominal pain, nausea, and vomiting. The condition can progress quickly, and at the late stage, there can be septic shock and multiple organ failure, leading to poor prognosis and high mortality [1, 3]. Clinically, patients with AP are often treated with surgical methods and antimicrobial agents in clinic [2, 3]. The majority of AP patients are senile with poor body conditions, and the most critical part of the treatment is infection control. Since many patients with AP also have other diseases, an operation is usually required for cleaning the infected area. However, conventional exploratory laparotomy can cause surgical trauma, and the postoperative pain can significantly hinder the recovery of patients. In recent years, the laparoscopic technique has been advancing. This method causes little trauma and less pain, but cannot achieve thorough cleaning in the abdominal cavity in the AP patients, which may affect the surgical outcome negatively. Therefore, using antibiotics is essential in the treatment of AP. However, due to the increasing antibiotic-resistant bacteria arising from antibiotic misuse an effective alternative to the antibiotics needs to be discovered urgently for the treatment of this disease.

Antimicrobial peptides (AMPs), a group of minor polypeptides with biological activities, are key components in the innate immune system [4]. AMP is known to have activity against ba-

cteria, fungus, viruses, parasites, and tumors [5]. Compared to traditional antibiotics, AMP has a broader spectrum, acts more stably and effectively, and is harder to produce drug-resistance, which can be a ideal option for treating infection caused by drug-resistant bacteria [6]. Lycosin-I is a cationic AMP isolated from animals. Some studies have demonstrated that lycosin-I can inhibit the growth of some gram-negative and positive bacteria, including escherichia coli and staphylococcus, as well as fungus, including candida albicans and aspergillus flavus. Moreover, some researchers have documented that lycosin-I does not damage eukaryotes when exerting antibacterial effect in the mouse model of infection [7, 8]. Therefore, in this study, we used lycosin-I as an AMP to investigate whether it can treat AP induced by drug-resistant bacteria, with the hopes of providing some new insight into the clinical treatment of this disease.

Materials and methods

Study subjects

Forty-five BALB/c male mice (7-8 weeks of age, 20-35 g) were selected for the study. They were raised in a quiet germ-free room (22±0.5°C, light cycle 12/12) and had adlibitum access to food and water. Before model creation, the mice were housed for over 3 days to help them to be adapted to the environment. An AP model was established in mice by intraperitoneal injection of methicillin-resistant staphylococcus aureus (MRSA). The mice were randomly divided to three groups of 15 mice each, which were group A (AP model, no treatment), group B (AP model, treated with intraperitoneal injection of AMP), and group C (AP model, control, treated with intraperitoneal injection of the same volume of normal saline). The study was approved by the Animal Ethics Committee of Jiaozhou Central Hospital of Qingdao.

AP model creation

MRSA solution at a density of $1.4*10^{10}$ CFU/mL was prepared by mixing MRSA strain in normal saline. Some studies have documented that this density is the optimal concentration of bacteria to induce infection that is used in experiments for observing the efficacy of drugs. All the mice were injected with 0.1 mL MRSA solution intraperitoneally at 8 AM [9].

Treatment method

The mice in group A did not receive any other treatment after injection of MRSA solution. The mice in group B were injected with 200 μg/mL lycosin-I (Hybio Pharmaceutical, Shenzhen, China) immediately following the injection of MRSA solution. The mice in group C were injected with 0.5 mL 0.9% sodium chloride immediately after the injection of the MRSA solution.

Outcome measures

Body temperature: The body temperatures of the mice were measured before and on the 1st, 3rd, and 7th day after modeling. During the measurement, a digital thermometer (Alcott Biotech, Shanghai, China) was gently inserted 2 cm deep into the anus of the mouse at 9 AM every day after adequate lubrication, and the temperature was recorded when the reading was stabilized.

Mouse behavior and body weight: Mouse behavior and weight changes were observed and recorded at 8 AM before and on the 1st, 3rd, and 7th day after modeling.

Inflammatory markers

Blood from the tail vein was collected at 3 PM before and on the 1st, 3rd, and 7th day after modeling for measuring white blood cell (WBC) count and the levels of interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α.

Mortality rate: Mortality was also recorded during the study. If the mice died within three days after the modeling, the deaths of the mice were counted in the mortality on the 3rd day; if the mice died between the 4th and 7th day after modeling, the deaths of the mice were counted in the mortality on the 7th day.

Statistical analysis

Statistical software SPSS 19.0 and GraphPad Prism 5.0 were applied for data analysis and plotting. Measurement data are expressed as mean ± standard deviation, and comparisons between two groups were performed by independent samples t-test or repeated measures analysis of variance (ANOVA). Measurement data at different time points were compared by Bonferroni post hoc test between the two groups, and comparisons among three groups were conducted by ANOVA. Count data

Table 1. Weight changes in the three groups

Day	Group A (g)	Group B (g)	Group C (g)	F	P
Before modeling	22.51±2.45	22.63±2.18	21.87±3.14	4.643	0.654
1st day after modeling	20.44±2.23	20.57±2.73	19.56±2.61	6.758	0.375
3rd day after modeling	17.21±3.52$^{**,\#}$	19.23±3.37*	17.11±2.89$^{**,\#}$	15.753	0.0236
7th day after modeling	14.13±3.74$^{***,\#\#}$	18.88±3.64*	15.15±2.76$^{***,\#\#}$	18.656	0.002

Note: *P<0.05, **P<0.01, and ***P<0.001 vs. before modeling; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. group B.

Table 2. Changes in the body temperature in the three groups

Day	Group A (°C)	Group C (°C)	Group B (°C)	F	P
Before modeling	36.33±1.12	36.19±1.35	35.98±2.07	7.654	0.351
1st day after modeling	39.81±1.76$^{*,\#}$	40.66±1.27$^{*,\#}$	37.53±1.25*	16.341	0.028
3rd day after modeling	41.62±1.57$^{**,\#\#}$	41.88±1.69$^{***,\#\#}$	38.12±1.12**	20.432	0.004
7th day after modeling	42.87±1.17$^{***,\#\#\#}$	42.05±1.92$^{***,\#\#}$	37.98±1.54*	23.563	<0.001

Note: *P<0.05, **P<0.01, and ***P<0.001 vs. before modeling; $^{\#}$P<0.05, $^{\#\#}$P<0.01, and $^{\#\#\#}$P<0.001 vs. group B.

were analyzed using χ^2 test. P<0.05 was considered to indicate a statistically significant difference.

Results

General condition in the three groups

No abnormal behavior was observed in the three groups before model creation. The mice were adapted to the environment and could eat by themselves. On the 1st day after modeling, the mice in groups A and C became listless. They were curled up and motionless, did not want to eat or drink, and trembled. Moreover, their hair became messy and their eyeballs were congested. The mice in group B also showed a lack of energy, but the symptoms were not as severe as those in groups A and C. The mice in group B could still eat and drink, but they displayed poor motor ability and were often in a resting state; they also had slightly congested eyeballs and occasional tremors. On the 3rd and 7th day after modeling, the mice in groups A and C experienced severe listlessness, hair loss, and were near death or even died; the mice in group B presented similar conditions to that on the 1st day, and only a few mice exhibited similar conditions as those in groups A and C.

Weight changes in the three groups

No intergroup differences were observed in the weight of the mice among the three groups before model creation (P>0.05). On the 1st day after modeling, the weight reduced in all three groups but was not significantly different from that before modeling. On the 3rd day, the weights in the groups A and C were much lower than that before modeling (both P<0.05); the weights in group B also reduced, but the reduction was not as significant as those in the groups A and C (all P<0.05). On the 7th day after modeling, the weights in the groups A and C were much lower than that on the 3rd day (both P<0.05), whereas the weight in group B was similar to that on the 3rd day (P>0.05). See **Table 1**.

Body temperature changes in the three groups

No intergroup differences were observed in the body temperature among the three groups before model creation (P>0.05). On the 1st day after modeling, the body temperatures increased in all the three groups (all P<0.05); however, the temperature in group B was lower than those in groups A and C (both P<0.05). On the 3rd and 7th day after modeling, the temperatures in groups A and C kept increasing (all P<0.05), whereas the temperatures in group B were similar to that on the 1st day (P>0.05). See **Table 2**.

Mortality in the three groups

On the 1st day after modeling, death occurred in groups A and C, and no death occurred in group B. On the 3rd day, nearly half of the mice died in groups A and C, while few mice died in group B. On the 7th day, most of the mice died

Table 3. Mortality rates in the three groups

Day	Group A	Group C	Group B	F (A/B)	P	F (B/C)	P
1st day after modeling (n)	4	5	0				
Mortality rate (%)	26.67	33.33	0.00	16.645	0.003	17.436	0.002
3rd day after modeling (n)	7	8	2				
Mortality rate (%)	46.67	53.33	13.33	25.476	<0.001	23.656	<0.001
7th day after modeling (n)	14	13	5				
Mortality rate (%)	93.33	86.67	33.33	28.756	<0.001	27.157	<0.001

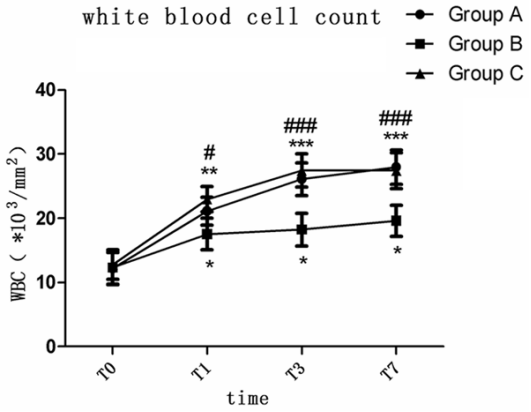

Figure 1. Changes in the WBC count in the three groups. WBC, white blood cell; T0, T1, T3, and T7 represent before modeling and 1st, 3rd, and 7th day after modeling, respectively. *P<0.05, **P<0.01, and ***P<0.001 vs. before modeling; #P<0.05 and ###P<0.001 when the groups A and C were compared with the group B.

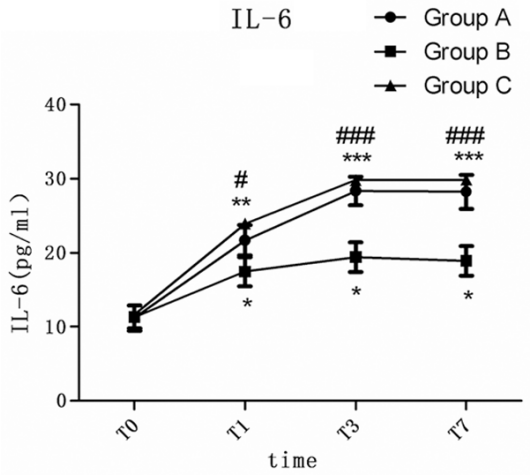

Figure 2. Changes in the IL-6 level in the three groups. IL, interleukin; T0, T1, T3, and T7 represent before modeling and 1st, 3rd, and 7th day after modeling, respectively. *P<0.05, **P<0.01, and ***P<0.001 vs. before modeling; #P<0.05 and ###P<0.001 when the groups A and C were compared with the group B.

in groups A and C, whereas only a few mice died in group B. The mortality rates in groups A and C were higher than those in group B (all P<0.05). See **Table 3**.

Levels of the inflammatory markers in the three groups

No intergroup differences were observed among the three groups in WBC count and the levels of IL-6, IL-1β, and TNF-α before modeling (all P>0.05). On the 1st day after modeling, the levels of these markers all increased in the three groups compared to those before model creation, and the magnitudes of the increases in the groups A and C were greater than those in group B (all P<0.05). On the 3rd and 7th day after modeling, the levels of the inflammatory markers in the groups A and C kept increasing with time (all P<0.05), whereas the levels in the group B remained similar to those on the 1st day (all P>0.05). See **Figures 1-4**.

Discussion

AP is a common acute abdominal disease. It can result from surgical diseases and intestinal flora disturbance, or it can be a spontaneous bacterial infection induced by internal diseases. Due to antibiotic misuse in recent years, many bacteria have become antibiotic-resistant. The MRSA investigated in this study is also called a superbug, as it is resistant to all antibiotics except for vancomycin [10-12]. MRSA is a common pathogen; its infection can be found especially in patients who are treated in an intensive care units or patients undergoing peritoneal dialysis [13, 14]. Currently, MRSA-induced AP is hard to be treated and has a high mortality rate. Therefore, in this study, we investigated the effect of AMP in treating MRSA-induced AP, with hopes of providing some new insight into the clinical treatment of this disease. The

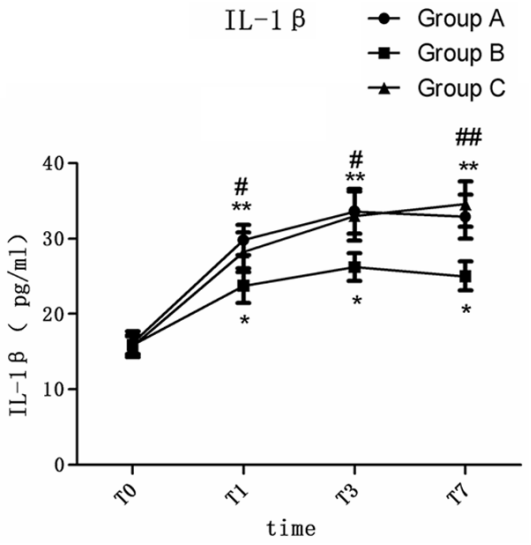

Figure 3. Changes in the IL-β level in the three groups. IL, interleukin; T0, T1, T3, and T7 represent before modeling and 1st, 3rd, and 7th day after modeling, respectively. *P<0.05 and **P<0.01 vs. before modeling; #P<0.05 and ##P<0.01 when the groups A and C were compared with group B.

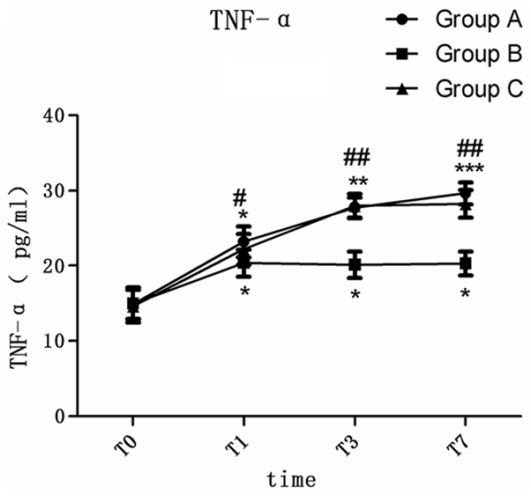

Figure 4. Changes in the TNF-α level in the three groups. TNF, tumor necrosis factor; T0, T1, T3, and T7 represent before modeling and 1st, 3rd, and 7th day after modeling, respectively. *P<0.05, **P<0.01, and ***P<0.001 vs. before modeling; #P<0.05 and ##P<0.01 when the groups A and C were compared with the group B.

AP mouse model was successfully created by intraperitoneal injection of MRSA. After model creation, the mice became listless and drowsy, had hair loss, and did not want to eat or drink; moreover, they also had increased body temperature and WBC count and reduced body weight. As compared to the mice injected with normal saline, the mice treated with AMP had more energy and less weight reduction; their body temperature, WBC count, and mortality rate were also much lower. These results revealed the effectiveness and feasibility of AMP therapy.

IL-6, IL-1β, and TNF-α are inflammatory cytokines. IL-6 comes from monocytes and macrophages; the increase in its level in blood is closely associated with infection and complications of inflammation [15]. IL-6 is a key acute phase response factor and serves an essential role in the assessment of severity of inflammation and prognosis [16]. TNF-α can induce other inflammatory factors to produce a cascade reaction, and IL-1β can release proteinases including matrix metallopeptidases and calmodulin to promote the release of other inflammatory factors for a cascade reaction [17-19]. All these three factors have critical roles in trauma, infection, and immune-mediated inflammation. It has been documented that the levels of these pro-inflammatory factors rise markedly when there is a local infection or inflammatory response, which promotes inflammation progression. In contrast, when the infection or inflammation is controlled, the expression levels of these factors are lowered, and the pro-inflammatory effect is reduced [20, 21]. In the present study, we found that the expression levels of IL-6, IL-1β, and TNF-α in the blood samples of mice all increased significantly after AP model creation, but the levels of these markers reduced in mice after treatment with AMP. Since inhibiting the expression of these three factors can suppress the cascade reactions of other pro-inflammatory factors, we inferred that the effect achieved by AMP in treating MRSA-induced AP in mice may be due to AMP inhibition of the expression levels of the pro-inflammatory factors IL-6, IL-1β, and TNF-α. However, the specific pathway through which AMP inhibits these factors still needs to be further investigated.

The present study only demonstrated that AMP lycosin-I can achieve positive effects in the mouse model of MRSA-induced AP, but did not prove that lycosin-I can exert the same effects on peritonitis caused by other bacteria. Moreover, the efficacy was only ob-

served for the first 7 days after treatment since most of the mice had already died after that period of time. As a result, we cannot determine how long the treatment effect can last and whether the peritonitis can progress during the later period. Therefore, more studies need to be carried out in the future for further verification.

In conclusion, AMP lycosin-I can achieve marked effects in treating mice with MRSA-induced AP in the short term and can significantly reduce the mortality of mice. This finding can provide some new insights into the clinical treatment of MRSA-induced peritonitis.

Disclosure of conflict of interest

None.

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