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Influence of *Pseudomonas aeruginosa* Autoinducer of Las on Murine Macrophage *In vitro*

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Abstract Quorum-sensing systems is critical regulator of the expression of virulence factors of various organisms, including *Pseudomonas aeruginosa*. Las and Rhl are two major quorum-sensing components, and they are regulated by their corresponding autoinducers, N-3-oxododecanoyl homoserine lactone (3-oxo-C_{12}-HSL) and N-butyryl-L-homoserine lactone (C_4-HSL). Recent progress has demonstrated the potential of quorum-sensing molecules, especially 3-oxo-C_{12}-HSL, for modulation of the host immune system. Here we show the specific ability of 3-oxo-C_{12}-HSL to induce apoptosis in mice-derived macrophages (RAW264.7) and reduce phagocytosis of the cell. When RAW264.7 cells were incubated with synthetic 3-oxo-C_{12}-HSL, the significant loss of viability was observed in a concentration (6.25 to 100 μmol/L) and incubation time (2 to 24 h) dependent manner. The cytotoxic activity of 3-oxo-C_{12}-HSL was also observed in RAW264.7 cells. The cells treated with 3-oxo-C_{12}-HSL revealed morphological alterations indicative of apoptosis. Acceleration of apoptosis in 3-oxo-C_{12}-HSL-treated cells was confirmed by multiple criteria (caspases 3, 8 and 9, mitochondrial depolarization, phosphatidylserine expression). Phagocytosis of neutral red assay demonstrated that higher concentration of 3-oxo-C_{12}-HSL significantly reduced phagocytosis of RAW264.7 cells ($P < 0.05$). High concentration of 3-oxo-C_{12}-HSL also obviously lowered RAW264.7 cells for gobbling up of *P. aeruginosa* capability ($P < 0.001$). These data suggest that 3-oxo-C_{12}-HSL specifically promotes induction of apoptosis and reduces the phagocytosis of RAW264.7 cells, which may be associated with 3-oxo-C_{12}-HSL-induced cytotoxicity in RAW264.7 cells. Our data suggest that the quorum-sensing signal molecule 3-oxo-C_{12}-HSL has critical roles in the pathogenesis of *P. aeruginosa* infection, not only in the induction of bacterial virulence factors, but also in the modulation of host responses.

Key words *Pseudomonas aeruginosa*, quorum-sensing systems, Las, apoptosis, phagocytosis
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Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen that causes a wide range of acute and chronic infections, including sepsis and wound and pulmonary infections. In particular, this organism is a major cause of pulmonary damage and death in patients with cystic fibrosis, diffuse panbronchiolitis, and other forms of bronchiectasis [1]. This organism is known to produce a variety of virulence factors, such as exoproteases, hemolysins, exotoxin A, exoenzyme S, pyocyanin, cyanide, and the cytotoxic lectins PA-IL and PA-IIL. The synthesis of these factors is regulated by a cell-to-cell signaling mechanism referred to as quorum sensing[2], which was originally described in *Vibrio fischeri* as a LuxR/LuxI-type system[3]. In *P. aeruginosa*, swarming motility and biofilm maturation are also controlled by quorum sensing. So an active quorum-sensing system is crucial for full pathogenicity [4]. *P. aeruginosa* utilizes two N-acyl-homoserine lactone (AHL)-dependent quorum-sensing systems, termed Las and Rhl, which together regulate an extensive set of cell population density and growth-phase-dependent virulence factors [5]. This mechanism enables bacteria to coordinately turn genes on and off in a density-dependent manner by the production of small diffusible molecules called autoinducers [6]. The Las and the Rhl quorum-sensing systems employ two autoinducers, N-3-oxododecanoyl

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homoserine lactone (3-oxo-C_{12}-HSL) and N-butyryl-L-homoserine lactone (C_4-HSL)[7]. The expression of these autoinducer-regulated virulence factors directly contributes to bacterial colonization and dissemination, which may determine the course and outcome of the disease in individuals infected with *P. aeruginosa*. Recently, it has become apparent that *Pseudomonas* HSLs are not only important in the regulation of bacterial virulence genes but also interact with different eukaryotic cells and modulate immune responses[8]. An increasing body of evidence shows that the *P. aeruginosa* quorum-sensing signal molecule 3-oxo-C_{12}-HSL, but not C_4-HSL, can regulate inflammatory cytokines expression and promote apoptosis[9].

Macrophage cells are a group of immune cells that have been shown not only to function as key effector cells of allergic inflammation, but also to play a major role in infectious diseases. However, only a few of studies have explored the interaction between *P. aeruginosa* and macrophages. Macrophages play a pivotal role in the phagocytosis and killing of *P. aeruginosa* and subsequently die through the onset of apoptosis[10]. The physiological process of apoptosis at the site of infection is regulated not only by host factors but also by pathogens themselves. In *Pseudomonas*-induced apoptosis, a variety of virulence determinants, such as pyocyanin, exoenzyme S, and cell surface porin, have been reported to manipulate the host apoptosis cascade. Moreover, an *in vitro* study has shown that *P. aeruginosa* biofilms can produce 3-oxo-C_{12}-HSL at a concentration approximately 600 μmol/L, which is 6- to 10-fold higher than that of 3-oxo-C_{12}-HSL used to exhibit immune response to host cells[11]. Therefore, it is reasonable to speculate that macrophages cells in the vicinity of infection region are frequently exposed to quorum-sensing signals.

We wonder if 3-oxo-C_{12}-HSL directly influences macrophages cells, so here we investigated the potential of *Pseudomonas* autoinducers to induce apoptosis in RAW264.7 cells. Our data demonstrate that 3-oxo-C_{12}-HSL accelerated apoptosis in RAW264.7 cells and reduced the ability of phagocytosis. We provide preliminary evidence of further study of the interaction between bacteria and the host by observing the basic biological effects of chemically synthesized 3-oxo-C_{12}-HSL molecule in the mouse macrophage cell line RAW264.7 cells.

1 Materials and methods

1.1 Cell culture

RAW264.7, a mouse macrophage cell line, was obtained from Institute of Immunology, Huazhong University of Science and Technology (Wuhan, China) and was cultured with DMEM medium containing 10% fetal serum at 37℃ in a 5%(v/v) CO_2 atmosphere.

1.2 3-oxo-C_{12}-HSL synthesis

3-oxo-C_{12}-HSL was synthesized by acylating Meldrum's acid with decanoyl chloride and then reacting the resulting product with D-HSL as reported previously[12]. The reaction was carried out in 10 mmol/L HCl at room temperature for 3 h. The 3-oxo-C_{12}-HSL product was purified by chromatography over silica gel. Purity and identity were confirmed by thin-layer chromatography and ^{1}H nuclear magnetic resonance.

1.3 The morphological changes

RAW264.7 cells were seeded into 6-well plates at a density of 10^6 cells/ml, and cultured for overnight and then incubated in the presence or absence of 3-oxo-C_{12}-HSL(100, 50, 25, 12.5, 6.25 μmol/L) at 37℃ for 4 h in a humidified 5% CO_2 and 95% air incubator. Then, the morphological changes were observed by microscope and photographed.

1.4 Cell viability assay

Viability of cells treated with 3-oxo-C_{12}-HSL was measured using the MTT assay. RAW264.7 cells were seeded into 96-well plates at a density of 2×10^5 cells/ml, and cultured for overnight and then incubated in the presence or absence of 3-oxo-C_{12}-HSL (100, 50, 25, 12.5, 6.25 μmol/L) at 37℃ for 2 or 4 h in a humidified 5% CO_2 and 95% air incubator(n=6). After treatment, 20 μl of MTT solution (5 g/L in PBS) and 180 μl of DMEM medium only were added to each well. The viable cells were stained with MTT for 4 h. Media were removed and the formazan crystal was dissolved by adding 150 μl of dimethylsulfoxide (DMSO). The absorbance at 490 nm was monitored. The percent viability of cells was calculated by comparison to that of untreated control cells.

1.5 Apoptosis assay

Assessment of apoptosis were seeded into 6/96-well plates at a right density and cultured for overnight and then incubated in the presence or absence of 3-oxo-C_{12}-HSL(100, 50, 25, 12.5, 6.25 μmol/L) at 37℃ for 2 or 4 h in a humidified 5% CO_2 and 95% air incubator. After treatment, cell apoptosis was evaluated by flow cytometry utilizing Annexin V-FTIC

apoptosis Kit and caspase 3, 8 and 9 activities were determined by colorimetric assays, according to the manufacturer's instruction. The data are expressed as fold increases compared to the values of control cells (n = 5). JC-1 probe was employed to measure mitochondrial depolarization. We compare the apoptosis of RAW264.7 cells incubated in the presence or absence of 3-oxo-C_{12}-HSL of different concentration through these three different assay methods.

1.6 Phagocytic assay by neutral red

The phagocytic ability of macrophage was measured by neutral red uptake. After cells were cultured with 3-oxo-C_{12}-HSL or control(DMSO) for 2 h, 200 μl neutral red solutions (dissolved in 10 mmol/L PBS with the concentration of 0.075%) was added and incubated for 1h. The supernatant was discarded and the cells in 96-well plates were washed with PBS twice to remove the neutral red that was not phagocytized by RAW264.7 cells. Then cell lysate (ethanol and 0.01% acetic acid at the ratio of 1∶1, 200 μl/well)was added to lyse cells. After cells were incubated at 4℃ overnight, the optical density at 490 nm was measured by a microplate reader.

1.7 Phagocytic assay by *P. aeruginosa*

Phagocytosis experiments were performed using PAO1. Assessment of phagocytic ability were seeded into 6-well plates at a right density and cultured for overnight and then incubated in the presence or absence of 3-oxo-C_{12}-HSL (100, 50, 25, 12.5, 6.25 μmol/L) at 37℃ for 2 h in a humidified 5% CO_2 and 95% air incubator. After treatment, cells were incubated with Saline suspension of PAO1 for 1 h, the supernatant was discarded and the cells in 6-well plates were washed with PBS three times to remove the PAO1 that was not phagocytized by RAW264.7 cells. Phagocytic rate and phagocytic index could be obtained by Wright staining observed in the oil microscope. Statistic analysis was conducted based on 5 parallel tests.

1.8 Statistical analysis

Data are expressed as $\bar{x} \pm s$ representing three times of independent experiments performed in duplicate. Statistical significance between means was evaluated by the one-way analysis of variance (ANOVA). Multiple groups compared with the control were performed with Dunnett's test. $P < 0.05$ was taken as statistically significant.

2 Results

2.1 The morphological changes

The morphological changes of cells with all concentrations show below (Figure 1). As shown in Figure 1, the morphology of the control is normal. After incubated with 3-oxo-C_{12}-HSL, aggregation of cells (6.25 μmol/L) and detachment and shrinkage of cells (100 μmol/L) were observed. The transparency of the cells was significantly decreased. The gap of cells widened. And even some cells floated on the culture medium and swelled.

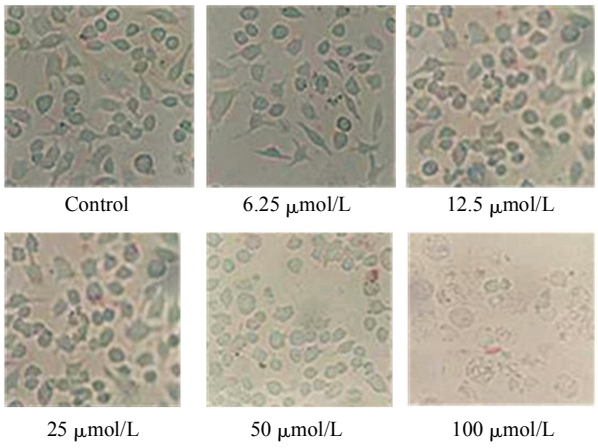

Fig. 1 Microscopic images of RAW264.7 cells after treatment with different concentrations of 3-oxo-C_{12}-HSL

Microscopic images (200×) of RAW264.7 cells after treatment with different concentrations of 3-oxo-C_{12}-HSL for 2 h. Cells were incubated with or without 3-oxo-C_{12}-HSL for 2 h. Then cell morphology was examined.

2.2 Cell viability assay

For quantitative analysis, changes in cell viability were determined and compared to the viability of control cells 2 to 4 h after addition of 6.25 μmol/L to 100 μmol/L 3-oxo-C_{12}-HSL(Figure 2). 3-oxo-C_{12}-HSL drastically induced loss of viability. At 2 h and 4 h of incubation, significant loss of viability was observed from 6.25 to 100 μmol/L 3-oxo-C_{12}-HSL in a concentration-dependent manner. These data demonstrate that *Pseudomonas* 3-oxo-C_{12}-HSL induces loss of viability in macrophages RAW264.7 cells.

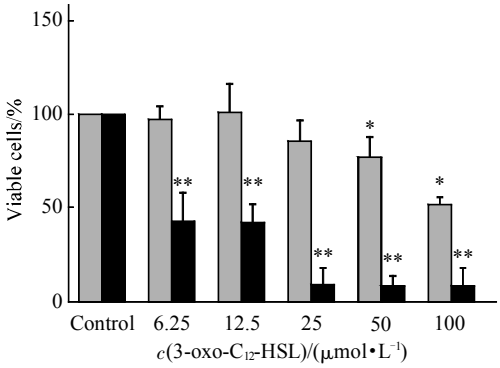

Fig. 2　Cell viability of RAW264.7 after treatment with 3-oxo-C_{12}-HSL

Cell viability of RAW264.7 after treatment with 3-oxo-C_{12}-HSL for 2 h or 4 h. Cells were incubated with various concentrations 3-oxo-C_{12}-HSL for 2 h or 4 h, and then cell viability at each time point was examined and compared to that of nontreated cells (n=6). *P < 0.001; **P < 0.05 compared with nontreated control group. □: 2 h; ■: 4 h.

2.3　Apoptosis analysis

The observation of these morphological changes caused by 3-oxo-C_{12}-HSL prompted us to examine apoptotic markers in these cells. RAW264.7 cells were incubated with 6.25 to 100 μmol/L 3-oxo-C_{12}-HSL for 2 h or 4 h, and then apoptotic markers, such as caspases 3, 8 and 9, phosphatidylserine expression and mitochondrial depolarization, were examined (Figure 3, 4, 5). A significant change in these factors was demonstrated in RAW264.7 cells treated with 3-oxo-C_{12}-HSL. Furthermore, phosphatidylserine expression on the cell surface, which is a specific marker of cells entering apoptosis, was examined by flow cytometry (Figure 3b). With the concentration of 3-oxo-C_{12}-HSL (not for 100 μmol/L)rised, the rate of apoptosis increases. Taken together, these data

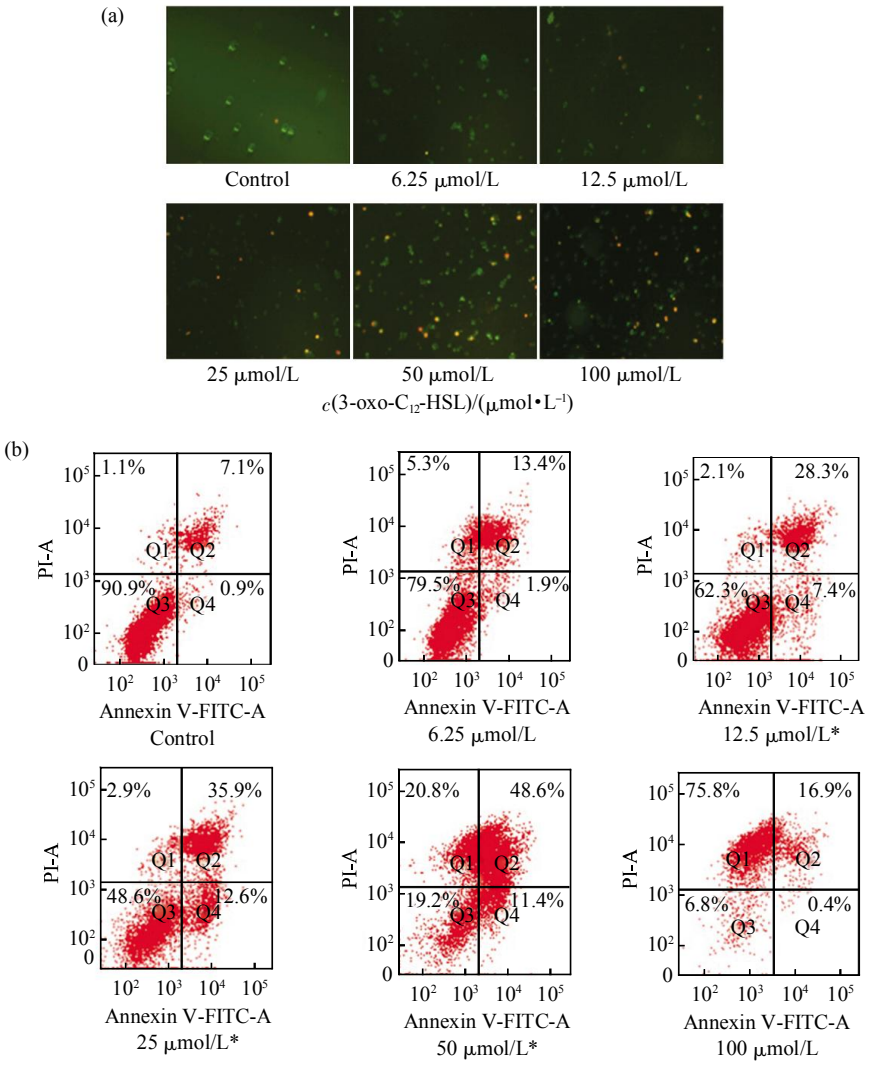

Fig. 3　Apoptosis after treated with different concentrations 3-oxo-C_{12}-HSL by means of V-FITC and PI double staining

(a) Fluorescent microscopic images (100×) of RAW264.7 cells after treatment with 3-oxo-C_{12}-HSL for 2 h. (b) Dose-dependent induction of apoptosis detected by flow cytometry ananlysis with annexin V-FITC and PI double staining in RAW264.7 after exposure to various concentrations of 3-oxo-C_{12}-HSL for 2 h, and then expression of annexin V-fluorescein isothiocyanate and propidium iodide was examined as described in **Materials and methods**. *P< 0.05 compared with untreated control group (n=3).

strongly suggest that *Pseudomonas* 3-oxo-C_{12}-HSL accelerates apoptosis, which may be associated with the loss of viability seen in macrophages RAW264.7 when they were exposed to 3-oxo-C_{12}-HSL.

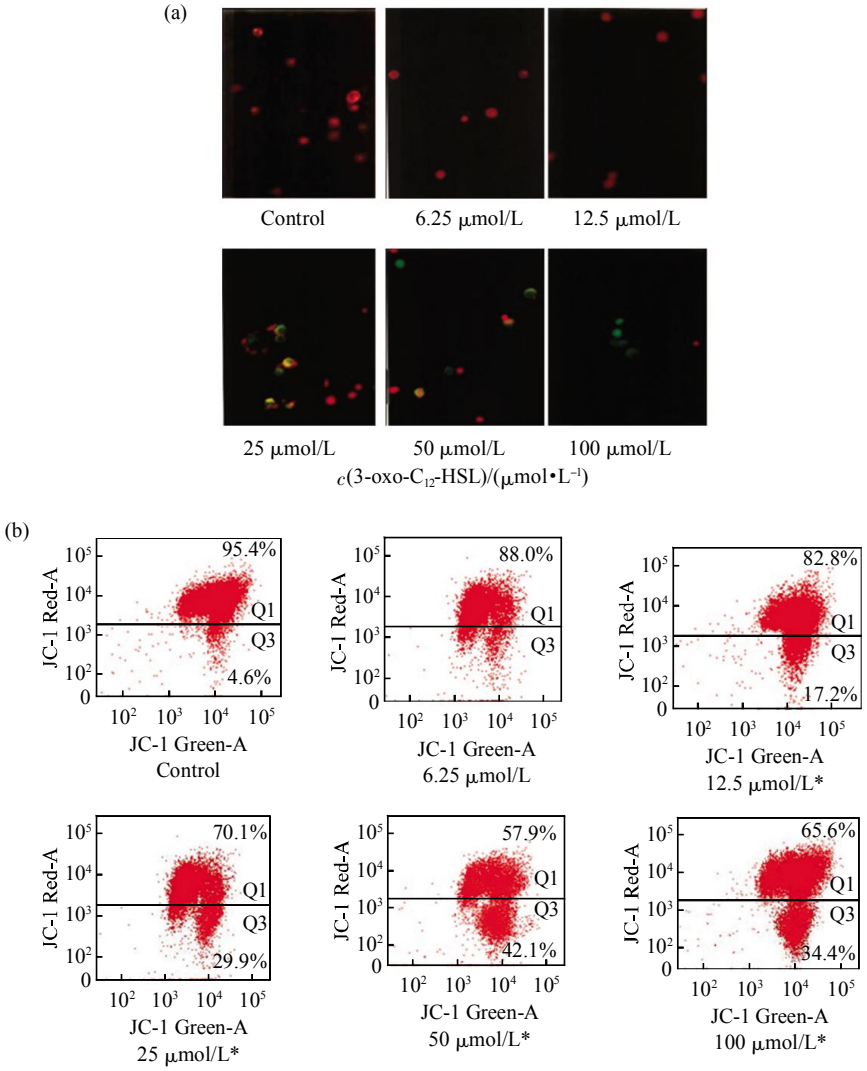

Fig. 4 Apoptosis after treated with different concentrations 3-oxo-C_{12}-HSL by means of JC-1 staining

(a) Fluorescent microscopic images (200×) of RAW264.7 cells with JC-1 staining after exposure to various concentrations of 3-oxo-C_{12}-HSL for 2 h. (b) Dose-dependent induction of apoptosis detected by flow cytometry ananlysis with JC-1 staining in RAW264.7 cells after exposure to various concentrations of 3-oxo-C_{12}-HSL for 2 h. *P< 0.05 compared with untreated control group (n=3).

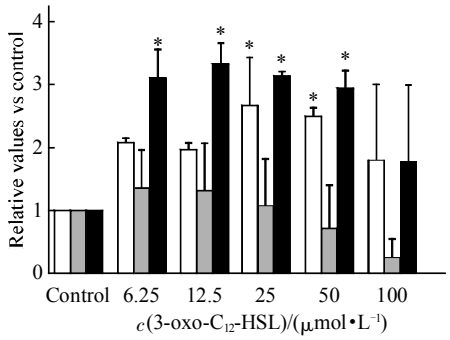

Fig. 5 The alteration of caspase-3, -8, -9 activitiy after exposure to various concentrations of 3-oxo-C_{12}-HSL

Dose-dependent induction of caspase-3, -8, -9 activities in RAW264.7 cells after exposure to various concentrations of 3-oxo-C_{12}-HSL for 4 h. (n =3). *P < 0.05 compared with the nontreated control group. □: Caspase-3; ▒: Caspase-8; ■ : Caspase-9.

2.4 Effect of 3-oxo-C_{12}-HSL on phagocytosis

Phagocytosis of neutral red assay demonstrated that higher concentration of 3-oxo-C_{12}-HSL significantly reduced phagocytosis of RAW264.7 cells (P < 0.05) (Figure 6). High concentration of 3-oxo-C_{12}- HSL also obviously lowered RAW264.7 cells for gobbling up of *P. aeruginosa* capability (P < 0.001) (Figure 7).

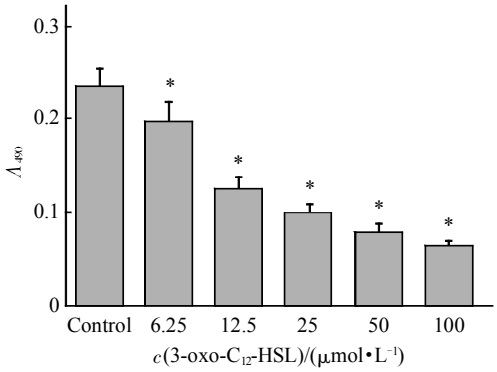

Fig. 6 Effect of 3-oxo-C_{12}-HSL on phagocytosis measured by neutral red uptake assay

After treated with 3-oxo-C_{12}-HSL for 2 h, cells were used to test the phagocytosis. *P < 0.05 compared with untreated group (DMSO). n = 6.

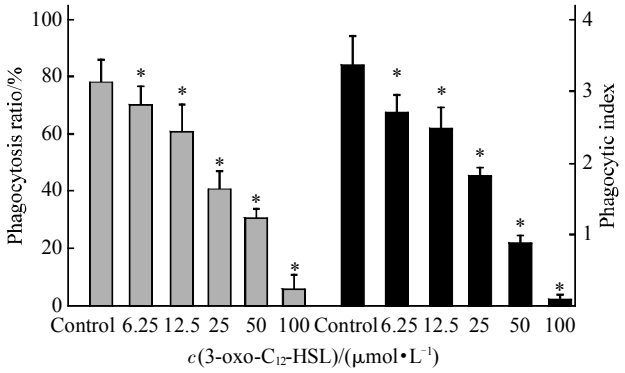

Fig. 7 Effect of 3-oxo-C_{12}-HSL on phagocytosis measured by PAO1 uptake

Phagocytic rate and phagocytic index of *P. aeruginosa* phagocytized by RAW264.7 cells treated with various concentration of 3-oxo-C_{12}-HSL for 2 h; *P < 0.001 compared with untreated group (DMSO). n = 12. □: Phagocytosis; ■: Phagocytic index.

3 Discussion

P. aeruginosa is a versatile Gram-negative bacterium that grows in soil, marshes and coastal marine habitats, as well as on plant and animal tissues. It is an opportunistic pathogen that causes a wide range of acute and chronic infections. However, the most prominent are the pulmonary infections. Due to the ubiquitous nature of *P. aeruginosa* and its ability to develop resistance to many antibiotics, it has become the major cause of nosocomial pneumonia. These infections are impossible to eradicate, in part because of the natural resistance of the bacterium to antibiotics, and ultimately lead to pulmonary failure and death. This encouraged us working on the mechanism of infection so as to find an effective treatment.

Recent study showed bacteria monitor the quantity change of itself or others in the surroundings according to concentration of special signal molecules. They adjust related genes to respond to such changes when signal shows molecules concentration has reached certain threshold value. It is called quorum-sensing system which universally exists in most of Gram-negative and Gram-positive bacteria. More researches of quorum-sensing system showed it is relevant to many nosogenesis. The research of quorum-sensing system of *P. aeruginosa* was very deep in Gram-negative pathogenic bacterium and we have known that quorum-sensing system plays a key role in adjusting various virulence factors. Virulence is controlled in *P. aeruginosa* by a number of quorum-sensing signal molecules, of which the AHLs (3-oxo-C_{12}-HSL and C_4-HSL) have been the most extensively investigated. Recent publications indicate that, depending on experimental condition, from 2.9% to over 10% of all genes in the genome of *P. aeruginosa* are under AHL-dependent quorum-sensing control [13–15]. More researches on quorum-sensing system confirmed that cell-to-cell signaling system is controlled by multiple genes and most of the genes are relevant to bacterial virulence. And also identified that the gene group which controlls cell-to-cell signaling system is a complicated cascade system. In this cascade system, except two main transcription regulation factors LasR and RhlR, it includes some other composition elements.

Las system is on the top of the cell-to-cell signaling cascade system. In cell-to-cell signaling system of *P. aeruginosa*, *Pseudomonas* quinolone signal PQS is a hinge between Las system and Rh1 system. Las system is in the central. 3-oxo-C_{12}-HSL will be produced in basal level when bacterial density is low, and if bacterial density increases, the density of 3-oxo-C_{12}-HSL will rise up to the threshold value as well. At this time, 3-oxo-C_{12}-HSL can combine with LasR, LasR-3-oxo-C_{12}-HSL compound can control the network regulation and activate transcription of the number of lower genes. Accordingly, expression of a series of causative agents including pyocyanin can be activated too. Recently, it has become apparent that 3-oxo-C_{12}-HSL is not only important in the regulation of bacterial virulence genes but can also interact with

eukaryotic cells and reduce an immune response. Recent progress has demonstrated the potential of quorum-sensing molecules, especially 3-oxo-C_{12}-HSL, for inhibition of the host immune system. Also it may be an important regulate factor of the host immune response.

Recent data have demonstrated that *Pseudomonas* N-3-oxododecanoyl-L-homoserine(3-oxo-C_{12}-homoserine lactone, 3-oxo-C_{12}-HSL), but not N-butanoyl-L-homoserine lactone (C_4-HSL), induces apoptosis in macrophages and neutrophils[16–18]. Cell apoptosis plays a central role in the balance between host defense and the invading pathogen in the normal phase of inflammation [19]. These results further reinforce the current concept that 3-oxo-C_{12}-HSL not only regulate bacterial virulence factors but also modulate eukaryotic cell functions, suggesting that this molecule has a pivotal role in the pathogenesis of *P. aeruginosa* infection [20]. These data strongly suggest that the *Pseudomonas* autoinducer 3-oxo-C_{12}-HSL is a potent immunomodulator of macrophage RAW264.7. Although the concentration of autoinducers in the lungs of *P. aeruginosa*-infected patients remains unknown, C_4-HSL and 3-oxo-C_{12}-HSL, in addition to mRNAs for these autoinducers and autoinducer-regulated virulence factors, were detected in the sputum of these patients [21–22]. These data indicate that a functional cell-to-cell signaling mechanism occurs in the airways of these patients. It has been shown that biofilms of *P. aeruginosa* grown *in vitro* can produce approximately 600 μmol/L 3-oxo-C_{12}-HSL, a concentration that is significantly higher than what has previously been measured in planktonic cultures[23]. Considering the fact that most of the *P. aeruginosa* bacteria are present in a biofilm in the lungs of patients with chronic infections, the concentration of autoinducers, at least in some areas of the active site of infection, may be equivalent to or higher than 12 to 50 μmol/L, concentrations at which we consistently observed induction of apoptosis in susceptible cells. Apoptosis is important in the normal resolution phase of inflammation, since it leads to functional down-regulation [24] and to the recognition and clearance of apoptotic neutrophils by macrophages [25]. Since apoptotic death is less proinflammatory, inappropriate induction of apoptosis rather than necrosis could confer a further advantage on an invading pathogen. A wide range of pathogens have been reported to interfere with the apoptosis cascade as a survival strategy by means of an array of pathogen-encoded virulence determinants[26–27].

The immune system consists of immune organs, immune cells, as well as immune molecules. In the course of infection immunity, the immune organs, tissues, cells and immune molecules collaborate with each other and check each other closely together to complete complex function of the immune defense. After the bacteria invades the host, the first encounter is against the innate immune function.Innate immunity mainly consist of tissue barriers and certain immune cells, immune molecules and other components. The macrophage as an important innate immune response cells which plays the role of secretion of cytokines, has the role of engulfing particulate antigen and the role of pinocytosis of soluble antigen. After treatment with different concentration of 3-oxo-C_{12}-HSL, pinocytosis of neutral red and phagocytosis of *Pseudomonas aeruginosa* standard strain PAO1 both declined compared with the control group. As the major phagocytic cells, macrophages are responsible for eliminating necrosised tissue, cell debris and pathogens at body injury in the phase of inflammation, which have important roles of healing process [28]. While macrophage apoptosis is observed in certain diseases. Studies have found that many factors can cause macrophage apoptosis, as some stress the role of the invasion of pathogens and pathogen components and cytokine stimulation[25].

Therefore, the activation of macrophages of the early stage play a certain role in anti-infective immunity [29]. So the behavior study of the change of apoptosis and phagocytosis of macrophages is new strategies for screening and development of new immunomodulatory drugs. Given that 3-oxo-C_{12}-HSL-induced apoptosis plays a crucial role in the pathogenesis of *P. aeruginosa* infection, the search for and identification of a eukaryotic molecular target for 3-oxo-C_{12}-HSL is a promising research subject.

In conclusion, we observed the morphology, mitochondrial depolarization, changes of three key caspase and phagocytic capacity of the mouse macrophage cell line RAW264.7 cells cultured with different concentrations of 3-oxo-C_{12}-HSL. At the same time, various indicators of apoptosis and phagocytosis were detected. Our data indicated that *P. aeruginosa* Las signal molecule 3-oxo-C_{12}-HSL cultured with the mouse macrophage cell line RAW264.7 cells has immunoinhibited activity and can reduce the

macrophage immune response to infection. The results would provide primary evidence for further study in the interaction between bacteria and host. At last, it has laid a good fundation fort he design of durgs for anti-microbe.

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铜绿假单胞菌 Las 系统信号分子对小鼠巨噬细胞影响的体外研究

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摘要 对于包括铜绿假单胞菌在内的众多微生物而言，群体感应系统是细菌表达毒力因子的重要调节子．Las 和 Rhl 是群体感应两个主要组成部分．Las 和 Rhl 分别受自诱导剂 N-3- 氧化十二烷酰基 -L- 高丝氨酸内酯(3-oxo-C_{12}-HSL)和 N- 丁酰 -L- 高丝氨酸内酯(C_4-HSL)的影响．最近的研究进展显示群体感应分子尤其是 3-oxo-C_{12}-HSL 具有调节宿主免疫系统的能力．本实验展示了 3-oxo-C_{12}-HSL 可以诱导鼠源巨噬细胞(RAW264.7)的凋亡和吞噬作用．把合成的 3-oxo-C_{12}-HSL 加入 RAW264.7 细胞培养基中，发现细胞生活力以一种依赖于 3-oxo-C_{12}-HSL 的浓度(6.25 to 100 μmol/L)和培养时间(2 to 24 h)的方式逐渐丢失．同样，我们观察到 3-oxo-C_{12}-HSL 的细胞毒活性，用 3-oxo-C_{12}-HSL 处理的细胞出现细胞形态上的改变，这一改变表明 3-oxo-C_{12}-HSL 处理的细胞加速凋亡，这一点同时也被其他多个标准(caspases3、8 和 9，线粒体膜电位，磷脂酰丝氨酸的表达)所证实．中性红吞饮实验证明，3-oxo-C_{12}-HSL 会显著地减小 RAW264.7 细胞的吞噬能力($P < 0.05$)．同时，高浓度的 3-oxo-C_{12}-HSL 会降低 RAW264.7 细胞对铜绿假单胞菌的吞噬作用($P < 0.001$)．这些数据表明 3-oxo-C_{12}-HSL 能特异性地促进细胞凋亡的诱导和 RAW264.7 细胞吞噬能力的减小．这可能和 3-oxo-C_{12}-HSL 诱导的细胞毒性有关．最终我们的实验数据证明，群体感应信号分子 3-oxo-C_{12}-HSL 在铜绿假单胞菌感染的致病机理中扮演着重要的角色．

关键词 铜绿假单胞菌，Las 系统信号分子，群体感应系统，细胞凋亡，吞噬作用

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