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Mechanism underlying N-(3-oxo-dodecanoyl)-L-homoserine lactone mediated intracellular calcium mobilization in human platelets

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ABSTRACT

Acyl-homoserine lactones (AHLs), are the key autoinducer molecules that mediate *Pseudomonas aeruginosa* associated quorum sensing. *P. aeruginosa* produces two types of AHLs; N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C_{12} HSL) and N-butyryl-L-homoserine lactone (C4 HSL). AHLs are not only regulating the virulence gene of bacteria but also influence the host cell functions by interkingdom signaling. In this study, we explored the mechanism of AHLs induced calcium mobilization in human platelets. We found that 3-oxo-C_{12} HSL but not C4 HSL induces intracellular calcium release. 3-oxo-C_{12} HSL induced calcium mobilization was majorly contributed from the dense tubular system (DTS). Furthermore, 3-oxo-C_{12} HSL also stimulates the store-operated Ca^{2+} entry (SOCE) in platelet. Intracellular calcium rise was significantly lowered in rotenone, and bafilomycin pre-treated platelets suggesting partial involvement of mitochondria and acidic vacuoles. The significant effect of 3-oxo-C_{12} HSL on calcium mobilization can alter the platelet functions that might results in thrombotic disorders in individuals infected with *P. aeruginosa*.

1. Introduction

Platelets, also known as thrombocytes, are the second most abundant cells present in the blood. They are highly sensitive and respond quickly to any external stimuli. They play an important role in blood clot formation following blood vessel injury (hemostasis) and also participate in thrombotic disorders (thrombosis) [1]. The unwanted activation of platelets in blood circulation can cause pathological thrombus formation, which leads to myocardial infarction, atherosclerosis, and other cardiovascular complications [2]. Infection-induced platelet adhesion and aggregation also contribute to the development of thrombotic disorders [3].

Calcium is an essential secondary messenger which regulates a wide range of cell functions. In platelets, the rise in intracellular Ca^{2+} contributes to the regulation of different functions like activation, adhesion, actin reorganization, shape change, and degranulation [4]. The elevation of intracellular Ca^{2+} is caused by the release from intracellular calcium reserves as well as the entry of Ca^{2+} from the extracellular environment through plasma membrane [5]. Two separate Ca^{2+} stores have been reported in platelets; first, the Dense Tubular System (DTS), an analogue of the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) and second lysosomal like acidic organelles [4,6–8]. In addition to these stores, mitochondria also play an important role in the maintenance of Ca^{2+} homeostasis and regulate various functions of different eukaryotic cells [9,10], including platelets [11–14]. It is widely established that stimulation of platelet surface receptors leads to the activation of phospholipase C (PLC) which further hydrolyzes the phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor present on the surface of intracellular stores and release intracellular calcium, while DAG helps in the entry of Ca^{2+} from extracellular compartment [5,15]. Prolong elevation of intracellular Ca^{2+}

Abbreviations: AHL, acyl-homoserine lactone; 3-oxo-C_{12} HSL, N-(3-oxo-dodecanoyl)-L-homoserine lactone; C4 HSL, *N*-butyryl-L-homoserine lactone; PLC, phospholipase C; QS, quorum sensing; SOCE, Store-operated Ca^{2+} entry; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol trisphosphate; DAG, diacylglycerol; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; DTS, dense tubular system

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concentration causes cellular damage [16]. In order to maintain the cytosolic calcium level, a calcium pump is present on endoplasmic reticulum surface, i.e., sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) which helps in restoration of cytosolic Ca^{2+} into ER lumen [17]. SERCA isoform SERCA2b (100 kDa), highly sensitive to thapsigargin, is expressed on DTS whereas thapsigargin insensitive SERCA isoform present on acidic organelles are known as SERCA3(97 kDa) helps to maintain the cytosolic Ca^{2+} level in platelets [18–21].

In immuno-compromised individuals, *Pseudomonas aeruginosa* causes a wide range of acute and chronic infection, including wound infection, pulmonary infection, and development of sepsis condition [22]. Besides, *P. aeruginosa* also produces a wide variety of virulence factors such as exotoxins, proteases, hydrogen cyanide, and pigments, etc. Synthesis of these virulence molecules is commonly regulated by autoinducers known as Acyl Homo-serine Lactones (AHLs) [23]. *P. aeruginosa* mainly produces two types of these autoinducers viz. 3-oxododecanoyl homoserine lactone (3-oxo-C_{12} HSL) and N-butyryl homoserine lactone (C4 HSL) [23]. Recently, it has been documented that these AHL molecules also interact and influence the host immune responses by interkingdom signaling [24,25]. Moreover, it has been evident that 3-oxo-C_{12} HSL has immunomodulatory activity in murine peritoneal macrophages [26]. Besides, 3-oxo-C_{12} HSL inhibits vasoconstrictor tone in porcine coronary and pulmonary arteries, that might exert a regional hemodynamic effect to benefit the invading bacteria [27]. 3-oxo-C_{12} HSL enhances the neutrophil chemotaxis by the reorganization of the actin cytoskeleton and phosphorylation of PLCγ1 and Rac1/Cdc42 [28,29] and disrupts the integrity of epithelial junction [30]. 3-oxo-C_{12} HSL also modulates the function of NIH3T3 fibroblast cells through intracellular calcium rise [31]. Our recent report demonstrated that 3-oxo-C_{12} HSL activates platelet through intracellular calcium-mediated ROS generation [32]. In this study, we explored the 3-oxo-C_{12} HSL-mediated calcium mobilization in human platelets and elucidated pathways and stores involved in intracellular calcium release.

2. Materials and methods

2.1. Reagents

Purified N-3-oxo-dodecanoyl-L-homoserine lactone (3-oxo-C_{12} HSL), *N*-butyryl-L-homoserine lactone (C4 HSL), DMSO, thapsigargin, ethylene diamine tetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), U73122, 2-Aminoethoxydiphenyl borate (2-APB), and Aspirin were purchased from Sigma-Aldrich. Rotenone and bafilomycin A1 are the product of Santacruz Biotechnology. Fluo-4 AM was procured from Thermo Fisher Scientific. All the reagents utilized in this study were of analytical grade, and Type 1 deionized water (18.2 MΩ cm, Millipore) was used for the preparation of solutions.

2.2. Preparation of platelet-rich plasma and washed platelets

Human venous blood was collected in citrate phosphate dextrose from healthy volunteers (drug-free and non-smokers) with their written informed consent as per the guidelines of Institutional Ethical Committee, Motilal Nehru National Institute of Technology Allahabad, India (Ref. No. IEC/16–17/017). Platelets were isolated by differential centrifugation method, as previously described [33]. Briefly, blood was centrifuged for 10 min at 180 × *g*, and platelet-rich plasma (PRP) was collected in the fresh falcon tube and incubated with aspirin (1 mM) at 37 °C in a water bath for 15 min. After the addition of EDTA (5 mM), PRP was centrifuged at 600 *g* for 10 min. Cells were further washed in buffer A (20 mM HEPES, 138 mM NaCl, 2.9 mM KCl, 1 mM $MgCl_2$, 0.36 mM NaH_2PO4 and 1 mM EGTA, supplemented with 5 mM glucose). Platelets were resuspended in buffer B, which was the same as buffer A lacking EGTA. The final count was adjusted to 1–3 × 10^8 cells/ml and mixed carefully to avoid activation.

2.3. Measurement of intracellular calcium

Washed platelets were incubated with 5 μM of Fluo-4 AM for 45–50 min at 37 °C in the dark. After incubation, the Fluo-4 AM loaded platelets were washed and resuspended in buffer B. Fluorescence intensity of Fluo-4 AM loaded platelets were recorded in a fluorescence spectrophotometer (Perkin Elmer model LS45). The samples were excited at λ = 495 nm, and emission was recorded at λ = 525 nm. The fluorescence intensity for the first 60 s was recorded to achieve the basal level, after 60 s 3-oxo-C_{12} HSL or C4 HSL was added to the samples in a different set of experiments. DMSO was used as vehicle control, and fluorescence intensity was further recorded for 300 s.

2.4. Measurement of SOCE

SOCE measurements were performed by the method as described elsewhere [34]. Briefly, platelet cells were incubated with 5 μM Fluo-4 AM for 45–50 min at 37 °C in the dark. After 60 s of baseline, 3-oxo-C_{12} HSL (100 μM), or DMSO as vehicle control were added in a different set of samples, and after 300 s 1 mM of $CaCl_2$ was added, and fluorescence intensity was recorded up to 600 s.

2.5. Statistical analysis

All the given data are represented as means ± SEM of at least three independent experiments. The one-way ANOVA and student's *t*-test was used for data analysis. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. 3-oxo-C_{12} HSL but not C4 HSL induces platelet intracellular calcium

The platelet activation mainly depends on Ca^{2+} signaling, which plays a crucial role in many essential cellular processes, including secretion, aggregation, and shape changes [35,36]. In this study, we found that 3-oxo-C_{12} HSL induces intracellular calcium in a dose-dependent manner, and the maximum rise was obtained at 100 μM (Fig. 1 a, b). In contrast, another important QS molecule C4 HSL does not affect the intracellular calcium levels (Fig. 1c, d). To explore the origin of observed cytosolic calcium rise, we further incubated the platelets separately with EGTA (1 mM) or $CaCl_2$ (1 mM) for 10 min, followed by treatment with 3-oxo-C_{12} HSL (100 μM). DMSO was served as vehicle control. 3-oxo-C_{12} HSL induced Fluo-4 fluorescence intensity was higher in the presence of $CaCl_2$ than EGTA (Fig. 2 a, b). The result suggests that 3-oxo-C_{12} HSL induces both the release of intracellular Ca^{2+} and the entry of Ca^{2+} from extracellular space to the cytoplasm.

Role of the Dense Tubular System (DTS) in 3-oxo-C_{12} HSL induced intracellular calcium rise.

The two major calcium reserves in platelets, viz. dense tubular system (DTS) and acidic organelles, are categorized on the basis of expression of different isoforms of SERCA on their surface. SERCA 2ab is present on the dense tubular system (DTS), which is highly sensitive to thapsigargin, a specific inhibitor of SERCA. In order to understand the source of intracellular calcium rise induced by 3-oxo-C_{12} HSL, Fluo4-AM loaded platelets were preincubated with thapsigargin (1 μM) for 20 min and then stimulated with 3-oxo C_{12} HSL. Results showed that thapsigargin prevented 3-oxo- C_{12} HSL induced intracellular Ca^{2+} rise (Fig. 3 a, b) which suggests that 3-oxo-C_{12} HSL mobilizes calcium majorly from DTS store. Intracellular calcium release from DTS majorly follows the PLC-IP3 pathway [4]. So, to investigate the involvement of PLC-IP_3 axis in 3-oxo C_{12} HSL-induced calcium rise, platelets were preincubated with a PLC–β inhibitor U73122 (10 μM) and IP_3 receptor antagonist 2-APB (100 μM) separately in Ca^{2+} free buffer, followed by

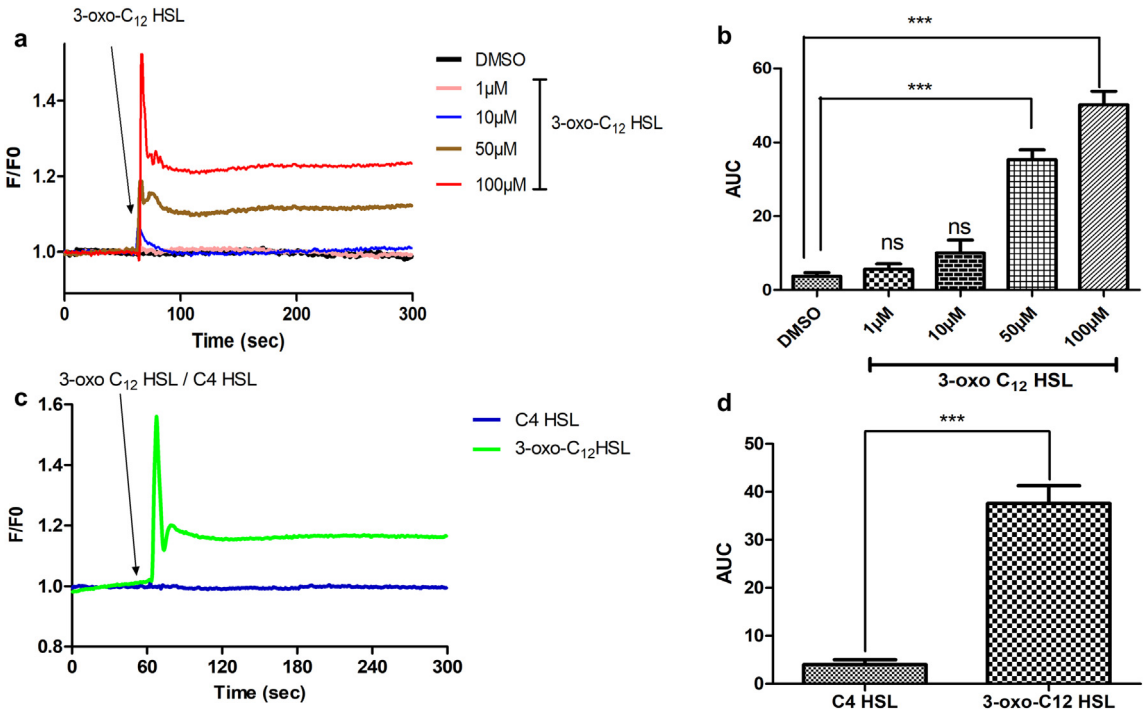

Fig. 1. 3-oxo-C_{12} HSL but not C4 HSL evokes an increase in intracellular calcium in blood platelets.
The blood platelets were loaded with Fluo-4 AM an intracellular calcium indicator that gives strong fluorescence when it is bind with calcium, and fluorescence intensity was measured in a fluorescence spectrophotometer.
(a) The fluorescence intensity of Fluo-4 AM loaded Platelet was recorded for the first 60 s to achieve the baseline, and then stimulated with varying concentration of 3-oxo-C_{12} HSL. **(b)** The bar graph shows an average of area under curve (AUC) of three independent experiments from (a). **(c)** The calcium traces show the platelet treated with 100 μM of either 3-oxo-C_{12} HSL or C4 HSL. **(d)** The bar graph represents the average of AUC of three independent experiments from (c). In control experiments, 0.05% DMSO was used as vehicle control.
F = fluorescence at 525 nm, F0 = average fluorescence of first 60 s. Data represent Mean ± SEM of at least three independent experiments; ns = not significant, p*** < 0.001.

Ca^{2+} measurement. The results demonstrated that both the inhibitors significantly reduced the free intracellular calcium rise in the platelets (Fig. 3c, d). Altogether, these results indicate that 3-oxo-C_{12} HSL increases the intracellular calcium levels by mobilization of calcium, majorly from DTS stores through PLC-IP_3 signaling cascade.

3.2. 3-oxo-C_{12} HSL Stimulate SOCE in platelet

During platelet activation, the rise in intracellular Ca^{2+} is mediated mainly by two mechanisms: Ca^{2+} release from intracellular stores or by the entry of extracellular Ca^{2+} into the cytosol [37]. The decrease in ER calcium pool directly induces SOCE to refill the ER lumen through the entry of Ca^{2+} from extracellular space [38]. To investigate the effect of

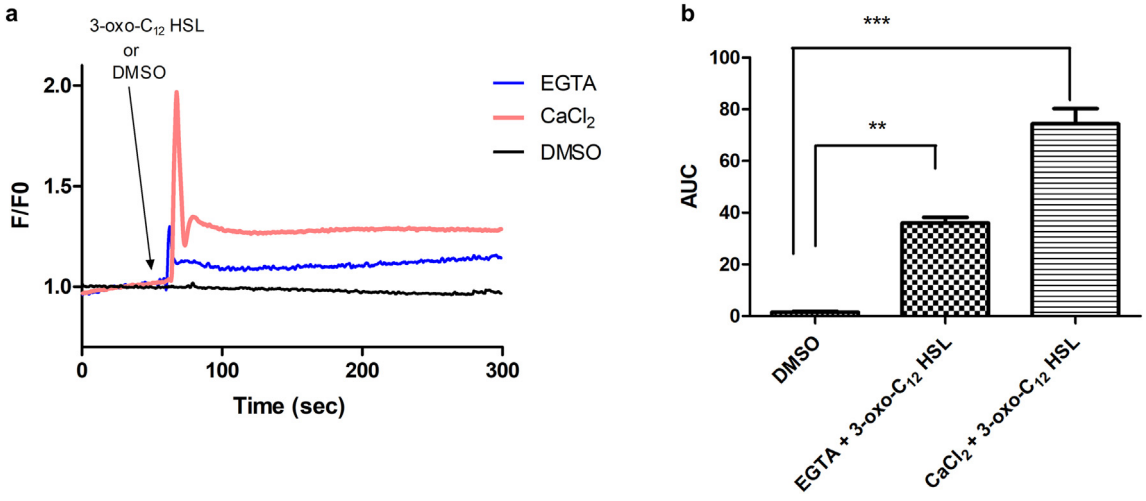

Fig. 2. 3-oxo-C_{12} HSL raises free intracellular Ca^{2+} in platelet by intracellular calcium release and induces the entry of extracellular calcium to the cytoplasm. **(a)** Fluo-4 AM loaded Platelets were preincubated with either 1 mM $CaCl_2$ or 1 mM EGTA for 10 min, after achieving the baseline, 100 μM of 3-oxo-C_{12} HSL was added in the sample, and fluorescence intensity was measured for further 300 s. **(b)** The bar graph represents the average of AUC of three independent experiments, from (a). In these experiments, 0.05% DMSO was used as vehicle control. Data represent Mean ± SEM of at least three independent experiments; p** < 0.01, p*** < 0.001.

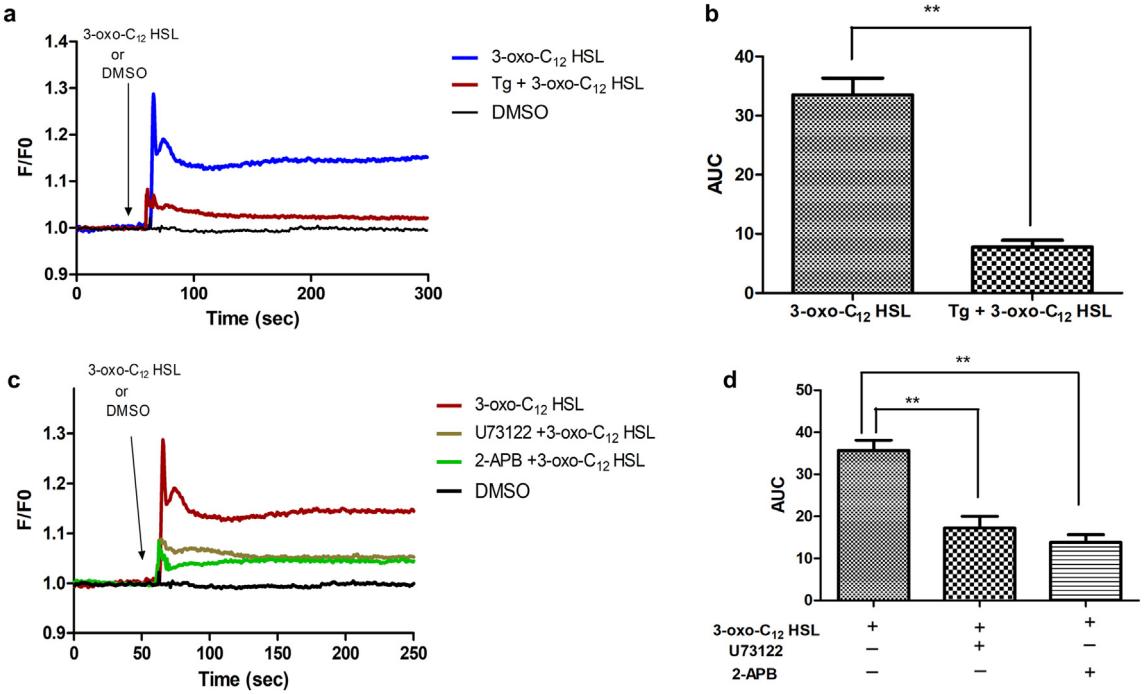

Fig. 3. 3-oxo-C_{12} HSL induces calcium mobilization majorly from the DTS to the cytosol by PLC-IP3 axis in. platelet.
(a) Fluo-4 AM loaded Platelets were pre-treated with 1 μM of Thapsigargin (Tg) in the presence of 1 mM EGTA to deplete the DTS store, after achieving the baseline, 100 μM of 3-oxo-C_{12} HSL was added in the sample pre-treated with or without Tg, and fluorescence intensity was measured for further 300 s. **(b)** The bar graph represents the average of AUC of three independent experiments, from (a). **(c)** Fluo-4 AM loaded platelets incubated in EGTA were pre-treated with or without U73122 or 2-APB, followed by addition of 3-oxo-C_{12} HSL (100 μM). **(d)** The bar diagram represents the average of AUC of three independent experiments, from (c). In these experiments, 0.05% DMSO was used as vehicle control. Data represent Mean ± SEM of at least three independent experiments; $p^{**} < 0.01$.

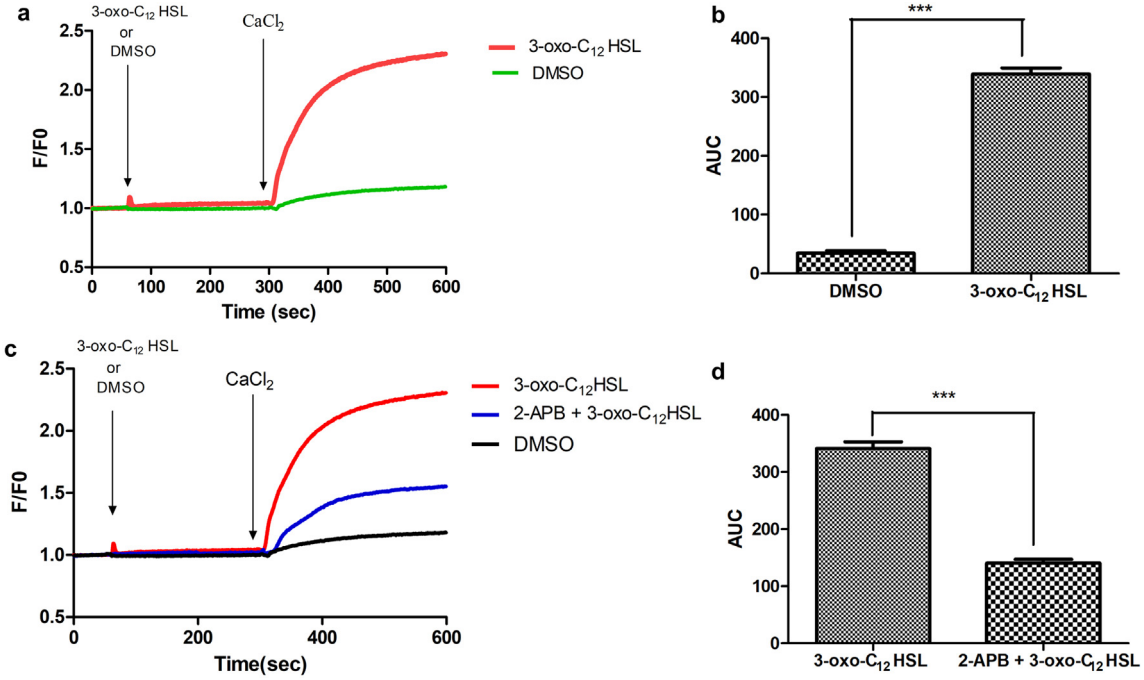

Fig. 4. 3-oxo-C_{12} HSL stimulate Store-operated Ca^{2+} in platelet. **(a)** After achieving the baseline, 100 μM of 3-oxo-C_{12} HSL or DMSO vehicle control was added in Ca^{2+} free medium, 300 s later after addition of 3-oxo-C_{12} HSL $CaCl_2$(1 mM) was added to the medium to initiate Ca^{2+} entry, and fluorescence intensity was measured up to 600 s. **(b)** The bar diagram represents the average of AUC of three independent experiments, from (a). **(c)** Fluo-4 AM loaded platelets were incubated with 2-APB (a potent SOCE blocker) for 10 min at 37 °C and then stimulated with 3-oxo-C_{12} HSL (100 μM) in Ca^{2+} free medium. After 300 s of the addition of 3-oxo-C_{12} HSL, $CaCl_2$(1 mM) was added to the medium to initiate Ca^{2+} entry, and fluorescence intensity was measured up to 600 s. **(d)** The bar diagram represents the average of AUC of three independent experiments, from (c). Data represent Mean ± SEM of at least three independent experiments; $p^{***} < 0.001$.

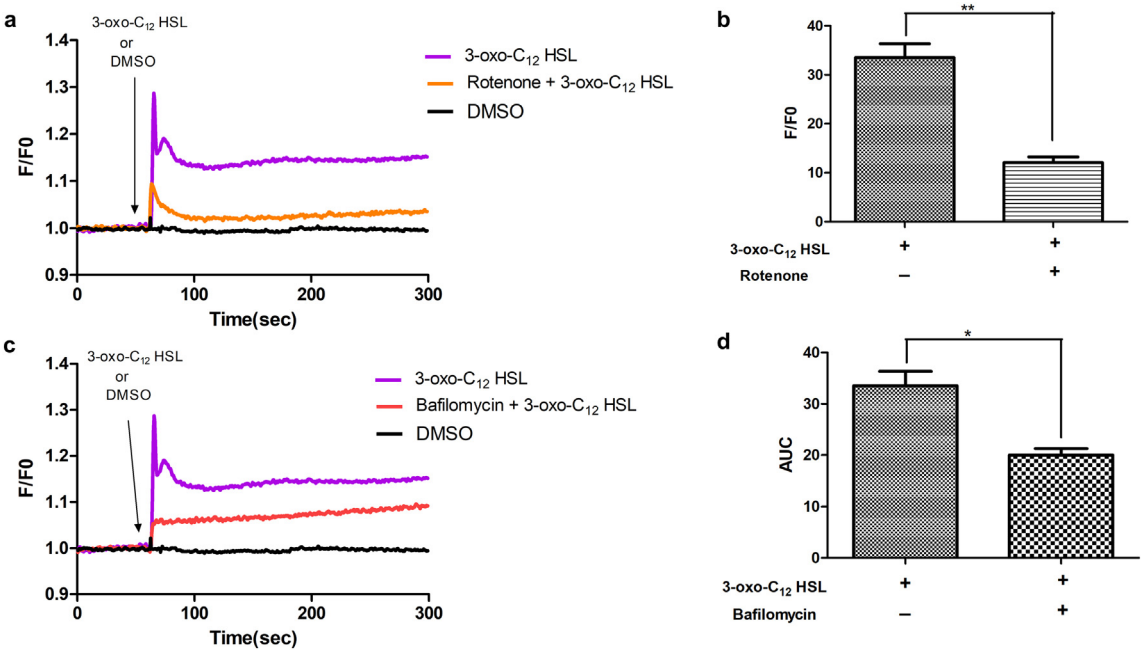

Fig. 5. Cytosolic calcium influx in 3-oxo-C_{12} HSL treated platelet was partially contributed by both mitochondrial and acidic stores of calcium source. **(a)** Platelets incubated in EGTA were pre-treated with rotenone for 10 min, followed by addition of 3-oxo-C_{12} HSL (100 μM), fluorescence was measured for 300 s. **(b)** The bar graph represents the average of AUC of three independent experiments, from (a). **(c)** Fluo-4 AM loaded platelets incubated in EGTA were pre-treated with bafilomycin A for 10 min, followed by addition of 3-oxo-C_{12} HSL (100 μM), fluorescence was measured for 300 s. **(d)** The bar diagram represents the average of AUC of three independent experiments, from (c). In these experiments, 0.05% DMSO was used as vehicle control. Data represent Mean ± SEM of at least three independent experiments; p* < 0.05, p** < 0.01.

3-oxo-C_{12} HSL on SOCE activation, the fluorescence of Fluo-4 AM loaded platelets were recorded, and after 1 min 3-oxo-C_{12} HSL was added; subsequently, $CaCl_2$ (1 mM) was exposed to the extracellular medium. A prolonged elevation in intracellular calcium was observed in the presence of 3-oxo C_{12} HSL indicating SOCE activation (Fig. 4 a, b). Further, 2-APB, a known SOCE blocker, significantly reduced the 3-oxo-C_{12} HSL induced extracellular Ca^{2+} entry (Fig. 4 c, d). In these experiments, DMSO served as vehicle control. These findings indicate that 3-oxo-C_{12} HSL stimulates the SOCE in platelets.

3-oxo-C_{12} HSL induced Ca^{2+} rise is partially contributed by mitochondria and acidic organelles.

Next, we investigated the contribution of other intracellular calcium stores such as mitochondria and acidic vacuoles behind 3-oxo-C_{12} HSL-induced intracellular calcium mobilization. To examine the possible role of mitochondria in 3-oxo-C_{12} HSL induced calcium rise, platelets were pre-incubated with rotenone (20 μM), that disintegrates mitochondrial transmembrane potential and releases intracellular calcium from mitochondria. The result showed that 3-oxo-C_{12} HSL induced calcium rise was significantly lowered in the presence of rotenone (Fig. 5 a, b). Furthermore, to inspect the involvement of acidic granules, intracellular calcium was measured in the presence of Bafilomycin A1 (1μM), a potent and specific blocker of vacuolar-type H^+-ATPase, that releases Ca^{2+} from acidic granules. It was found that 3-oxo-C_{12} HSL induced calcium rise was diminished in the presence of Bafilomycin A1 (Fig. 5 c, d). These results suggest that mitochondria and acidic granules partially contribute to 3-oxo-C_{12} HSL induced Ca^{2+}rise.

4. Discussion

The interaction between bacteria and host cells does not only depends upon the cell to cell contact but also on different toxins or diffusible QS molecules which regulates several virulence factors of bacteria, including biofilm formation. Although different families of QS molecules have now been described, the AHLs of gram-negative bacteria have been studied most extensively. These AHLs primarily differ in length (from 4 to 18 carbon) and saturation level of their acyl chain having oxo or hydroxy substitutions at the C-3 position. *P. aeruginosa*, a gram-negative bacterium, mainly produces two types of AHLs, i.e., 3-oxododecanoyl homoserine lactone (3-oxo-C_{12} HSL) and N-butyryl homoserine lactone (C4 HSL). Several studies have shown that the QS molecule 3-oxo-C_{12} HSL modulates the function of different mammalian host cells by the phenomenon of interkingdom signaling [24,25]. Bacteria may enter to the circulatory system by indwelling medical devices, catheters, and other surgical processes [39] and it interacts with the blood cells, including platelets by direct or indirect mode of contacts and modulates their functions [40].

Recently, we have shown that *P. aeruginosa* quorum sensing molecule 3-oxo-C_{12} HSL but not C4 HSL induces platelet activation by calcium-regulated ROS generation [32]. In this study, we have explored the pathway and stores involved behind 3-oxo-C_{12} HSL mediated Ca^{2+} mobilization in platelets.

We found that 3-oxo-C_{12} HSL induces intracellular calcium release in a dose-dependent manner, while C4 HSL did not show such activity. In platelets, the elevation in cytosolic calcium is either due to release from intracellular compartments or by the entry of Ca^{2+} from extracellular space through channels present on the plasma membrane. To understand the origin of 3-oxo-C_{12} HSL induced intracellular Ca^{2+} rise in platelets, we performed the experiment in the presence of EGTA and $CaCl_2$ separately. It was observed that 3-oxo-C_{12} HSL (100 μM) induced calcium elevation was higher in $CaCl_2$ than in the EGTA-treated sample. These results suggest that the 3-oxo-C_{12} HSL induces both extracellular as well as intracellular calcium mobilization in platelets. In non-excitable cells, mobilization of Ca^{2+} in the cytoplasm is predominantly mediated by PLC-IP_3 pathway [15]. To confirm the involvement of PLC-IP_3 axis in 3-oxo-C_{12} HSL induced calcium mobilization, the platelets were preincubated separately with U73122, an antagonist of PLCβ, and 2-APB, an IP_3, receptor antagonist. Treatment of these antagonists significantly reduced the calcium mobilization caused by 3-oxo-C_{12} HSL. The activation of PLC hydrolyzes the PIP_2 into IP_3 and DAG [15]. IP_3 acts as a secondary messenger and binds to the IP_3 receptor and

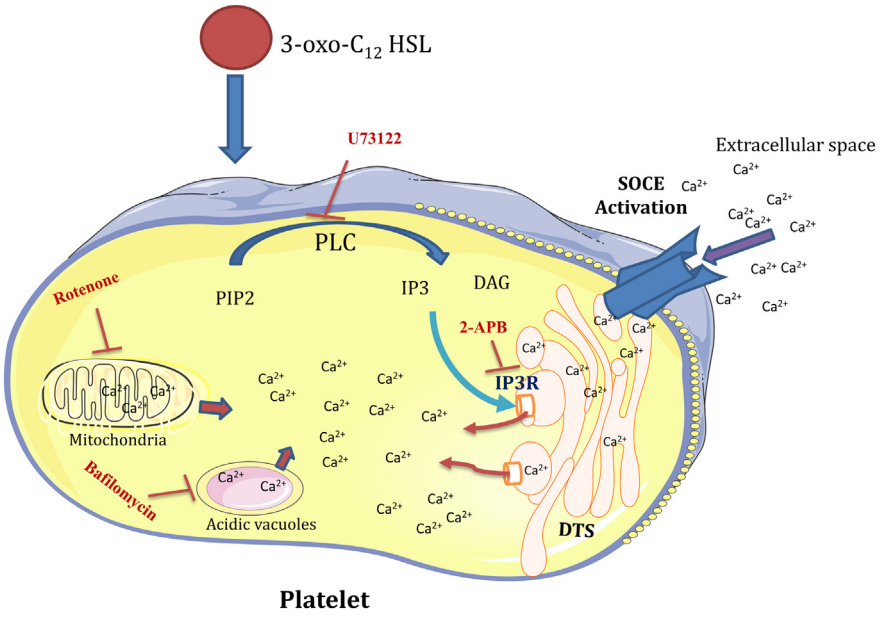

Fig. 6. A summary of 3-oxo-C_{12} HSL induced calcium mobilization in platelets.
3-oxo-C_{12} HSL activates the phospholipase C (PLC) which further hydrolyzes the phosphatidylinositol 4,5-bisphosphate (PIP_2) into Inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor on Dense tubular system (DTS) and releases intracellular calcium into the cytoplasm. 3-oxo-C_{12} HSL also potentiates the influx of calcium from extracellular space through the plasma membrane by SOCE activation. Furthermore, Mitochondria and acidic granules partially contribute to 3-oxo-C_{12} HSL induced Ca^{2+} rise in platelets.

stimulate the Ca^{2+} release from DTS store to the cytoplasm [15]. Based on IP3 binding and expression of two different SERCA isoforms (100 kDa and 97 kDa), two calcium stores are present in platelets [18]. Thapsigargin sensitive 100 kDa isoform of SERCA (SERCA2b) is found in the DTS membrane [18]. In order to assess the role of DTS store in 3-oxo-C_{12} HSL induced Ca^{2+} mobilization, platelets were preincubated with thapsigargin for 20 min at 37 °C and then stimulated with 3-oxo-C_{12} HSL (100 μM). Thapsigargin abrogated the 3-oxo-C_{12} HSL induced Ca^{2+} rise, which confirms the significant contribution of DTS in 3-oxo-C_{12} HSL stimulated Ca^{2+} mobilization. The depletion of intracellular calcium stores stimulate the entry of Ca^{2+} from extracellular space via plasma membrane channels and activate SOCE to refill these stores [41]. The SOCE activation mechanism is mainly regulated by the two protein families known as STIM and ORAI [42]. After depletion of the store, STIM moves to the DTS-plasma membrane and interacts with ORAI1 and thus induces calcium flow from extracellular compartments [42]. Since 3-oxo-C_{12} HSL majorly induces the calcium rise though DTS stores, we further investigated whether 3-oxo-C_{12} HSL is able to induce SOCE activation. For this purpose, the calcium influx in the Fluo-4 AM loaded platelets were monitored after the treatment with 3-oxo-C_{12} HSL (100 μM) or DMSO and subsequent addition of $CaCl_2$ to the extracellular medium. The addition of $CaCl_2$ in the 3-oxo-C_{12} HSL treated sample evokes a strong calcium influx compared to DMSO, suggesting the SOCE activation. Further, 2-APB, a well-known SOCE blocker, significantly reduced the calcium influx induced by 3-oxo-C_{12} HSL. Thus, results suggest that 3-oxo-C_{12} HSL depletes DTS calcium stores, which further stimulates the SOCE in the platelets. In order to examine the involvement of other probable calcium sources such as mitochondria and acidic stores in 3-oxo-C_{12} HSL induced calcium mobilization, platelets were pretreated separately with rotenone and bafilomycin A1. The calcium mobilization induced by 3-oxo-C12 HSL was significantly reduced in the presence of rotenone or bafilomycin A1, suggesting the partial contribution of the mitochondria and acidic vacuoles in 3-oxo-C_{12} HSL induced calcium mobilization.

To conclude, our data indicate that 3-oxo-C_{12} HSL mobilizes the intracellular calcium from DTS through PLC-IP_3 axis. Additionally, 3-oxo-C_{12} HSL also potentiates the influx of extracellular calcium through the plasma membrane by SOCE activation. Mitochondria and acidic granules partially contribute to 3-oxo-C_{12} HSL induced Ca^{2+} rise. A graphical summary of the current findings is represented in Fig. 6. These findings suggest that 3-oxo-C_{12} HSL induced intracellular calcium mobilization can alter platelet functions. Thus, this study may further be utilized for the development of new therapeutic drugs intended to reduce cardiovascular complications in *P. aeruginosa* infected individuals.

Author contributions

V.K.Y, V.A, and S.K.S conceived and designed the experiments; V.K.Y and P.K.S performed the experiments and analyzed the data; S.K.S and D.S provided comments and technical support; V.K.Y, S.K.S, and V.A wrote the paper.

Declaration of Competing Interest

No competing interest exists among the authors.

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