

ARACHIDONIC ACID CORRELATION WITH SUSPENDED AND ATTACHED NEUTROPHILS

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ABSTRACT

Background: Neutrophils are the blood cells for which adhesion is one of the main functions. Neutrophils adhered to biological surfaces show different characteristics of activation compared with neutrophils in suspension. The respiratory burst in suspended neutrophils in response to a chemotactic factor is lower when compared with the cells attached to the surface. **Objectives:** This study aimed to study the role of arachidonic acid release in suspended and attached neutrophils following stimulation of human neutrophils with LPS and PAF. **Materials and methods:** The suspended and attached neutrophils were primed by LPS and PAF, and inhibited by Anti-CD 14 (MY4). **Results:** The arachidonic acid release in attached cells is more than in suspended cells. This release is rapid with the increase of incubation time and dose-dependent. The Fmet-Leu-Phe (FMLP) potentiates arachidonic acid release in attached cells pretreated with LPS and serum. Arachidonic acid release was less in suspended cells in comparison to attached cells where the addition of low concentration of LPS in presence of serum or platelet activating factor (PAF) to suspended cells produce increase in arachidonic acid release. On the other hand LPS in combination with PAF produce a large and significant increase in arachidonic acid release. The potentiative effect of LPS is mediated via CD14 receptors. The monoclonal antibodies against CD14 had no effect on arachidonic acid release in PAF treated cells where it inhibited greatly the potentiation by LPS or LPS-serum complex with PAF. **Conclusion:** LPS or PAF alone produced small increase in arachidonic acid release in both suspended and attached human neutrophils, where LPS in combination with PAF induced significant arachidonic acid release in suspended and attached human neutrophils.

Keywords: Arachidonic acid, Neutrophils, LPS, PAF, CD14.

INTRODUCTION

The neutrophils release arachidonic acid (AA) in response to stimulation. The fatty acid is then metabolized to generate one or more biologically active eicosanoids such as prostaglandins, prostacycline, thromboxanes and leukotrienes (Astudillo et al., 2012).

Platelet activating factor (PAF) possesses multiple biological activities which encompasses the field of allergic and inflammatory reactions as well as the regulation of the cardiovascular system.

The effect of PAF on neutrophils includes chemotaxis, degranulation, aggregation, oxidative burst and superoxide release (Gill et al., 2015).

Lipopolysaccharide (LPS), from gram negative bacteria, has profound effects on the host immune system (Moco et al., 2014). The exposure of neutrophils to LPS primes the cells for enhanced release of microbial metabolites. The increase in oxidative burst might permit resistance to bacterial infection, yet predispose the host to increased oxidative tissue damage and release of various inflammatory

mediators. While the generated inflammatory mediators are crucial to the host defense role of the neutrophils, they can cause severe tissue damage when excessively generated. LPS-induced injury has been implicated in the pathogenesis of many diseases processes such as septic shock, myocardial infarction and adult respiratory distress syndrome (**Taddonio et al., 2014**).

Initiation of these responses depends on LPS interaction with a number of immune cells such as mononuclear phagocytes and neutrophils. The neutrophils bind the LPS-lipopolysaccharide binding proteins (LBP) complex through the CD14 receptor, and thus mediate the release of a wide range of inflammatory mediators. Release of these mediators appears to be beneficial but under certain circumstances may be harmful, resulting in the systemic inflammatory response syndrome (**Bozena 2016**).

The present studies aimed to study the role of arachidonic acid release following stimulation of human neutrophils with PAF.

MATERIAL AND METHODS

Materials:

1. Lipopolysaccharide (LPS) [**Difco laboratories, USA**]
2. Platelet-activating factor (PAF) [**Calbiochem, USA**].
3. Anti-CD14 monoclonal antibody (MY4) [Coulter Corporation, USA].
4. Biocoat cell ware human fibronectin 24 well [**Collaborative Biomedical products, USA**].

Isolation of human neutrophils: Neutrophils were isolated from 50 normal healthy adult donors. They were students

from Al Azhar Faculty of Medicine (Assuit), with exclusion criteria of no history of any infectious disease and administration of antibiotic and common cold medicine for at least two weeks, using a histopaque gradient method described by **English and Anderson (1974)** and **Aida and Pabst (1990)**. The contaminating red blood cells were lysed by hypotonic shock (**Vuorte et al., 2001**). The neutrophils were resuspended in Hanks buffered salt solution (HBSS) without Ca^{++} or Mg^{++} . The last supernatant of cell suspension was checked for the presence of endotoxin by the limulus amoebocyte lysate test.

Arachidonic acid release in suspended cells (**DiPersio et al., 1988**)

Arachidonic acid in adherent cells was released and counted (**Ashour, 1995**).

Written consents were obtained from the subjects after their information about the work.

Statistical analysis: Data were analyzed using the statistical package for social science (SPSS) software version 20. Mean and standard deviation, and percentages were used for data summarization. P value < 0.05 was considered significant.

RESULTS

The arachidonic acid release from pre-labeled cells was stimulated with LPS, LPS+PAF. LPS only did not produce a significant increase in arachidonic acid release. However, LPS, in combination with PAF produced a significant increase in arachidonic acid release. The most appropriate time for the highest increase in arachidonic acid release was found 45 minutes (**Figure 1**).

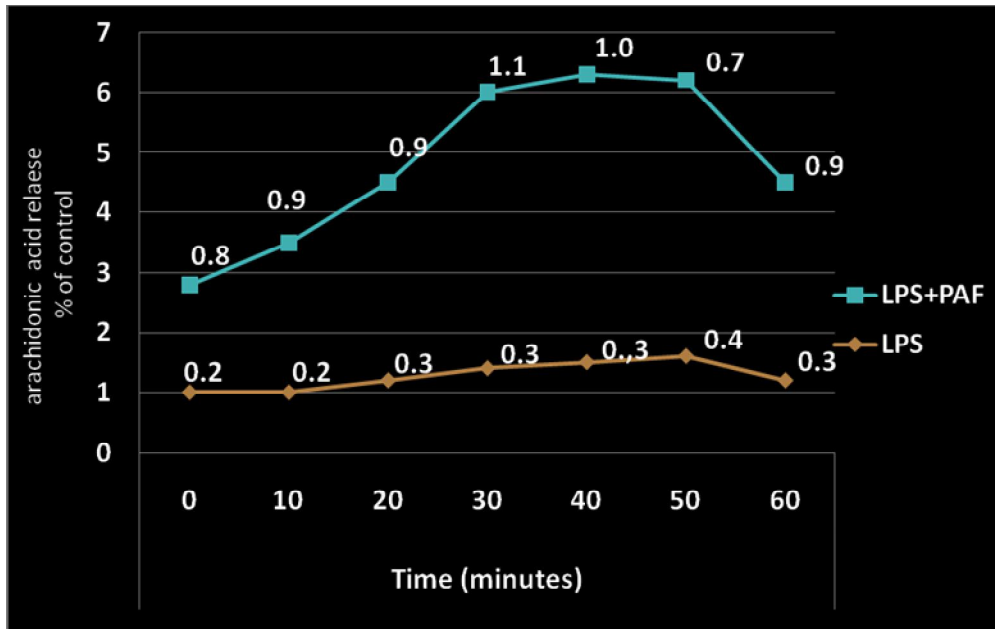


Figure (1): Time course of suspended neutrophils priming with LPS or LPS+PAF on arachidonic acid release.

LPS did not produce a large increase a large increase in arachidonic acid release. However, LPS in combination with PAF produced a significant increase in arachidonic acid release. A dose response

of priming of neutrophils with LPS demonstrated that 100-200 ng/ml resulted in the maximum release of arachidonic acid (**Figure 2**).

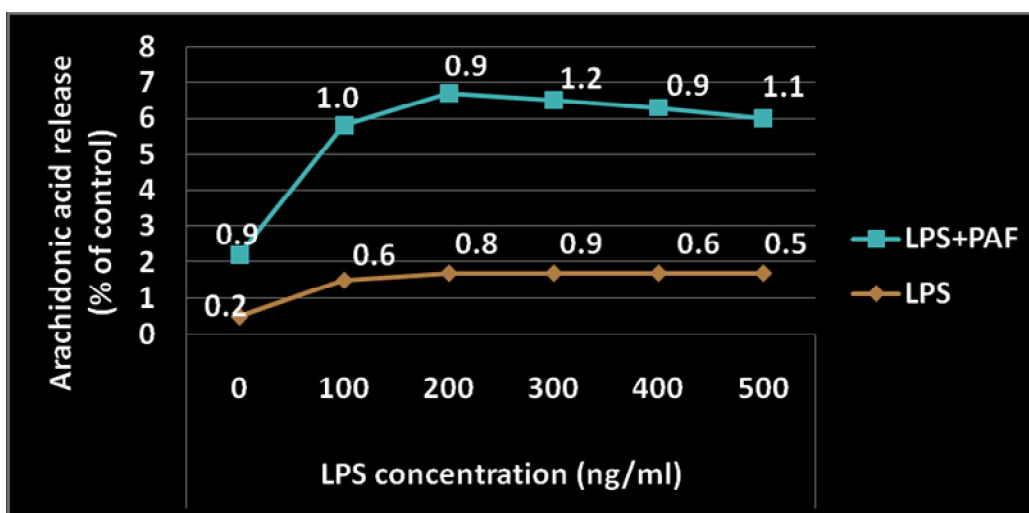


Figure (2): Effect of LPS concentration (dose- response) on potentiation of PAF induced on arachidonic acid.

LPS, PAF and LPS=PAF in absence of serum caused a significant increase in arachidonic acid release as LPS and PAF, in presence of serum. However, LPS+PAF

in presence of serum caused a large significant increase in arachidonic acid release (**Figure 3**).

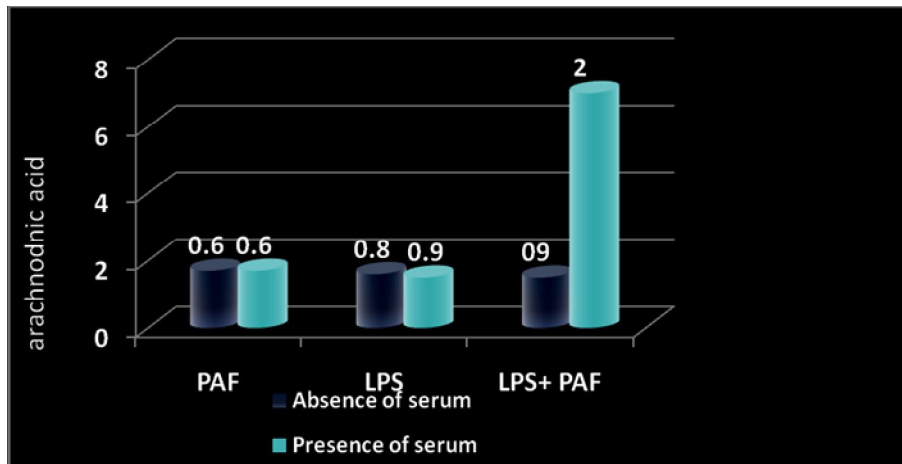


Figure (3): Effect of absence or presence of serum on LPS potentiating of PAF induced on arachidonic acid release.

LPS alone did not produce significant increase in [³H] arachidonic acid release. However, LPS+ serum produced a slight increase in [³H] arachidonic acid release, where LPS+SERUM+FMLP produced a significant increase in [³H] arachidonic

acid release. The most appropriate time for the highest increase in [³H] arachidonic acid release was found after 40 minutes (**Figure 4**).

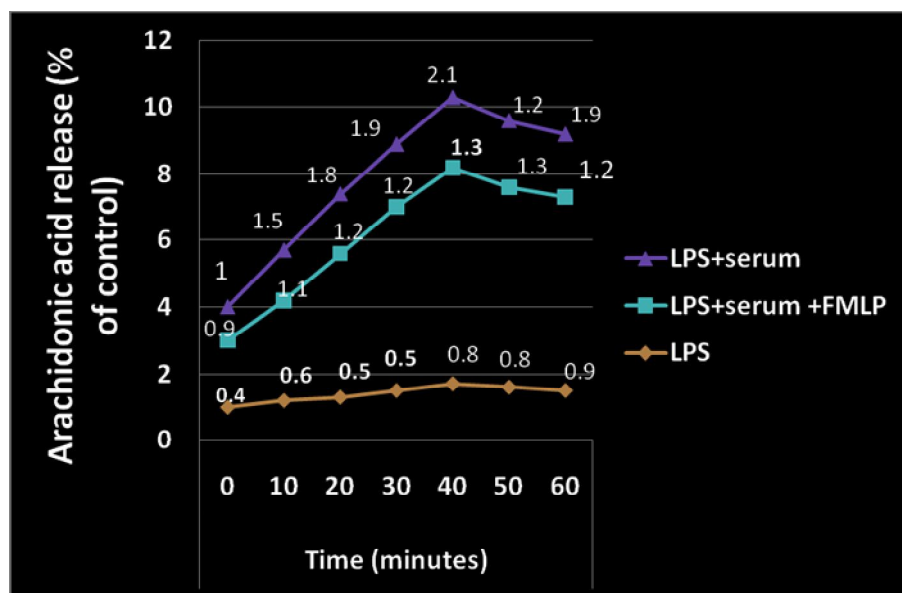


Figure (4): Time course of attached neutrophils.

LPS alone, LPS with serum or LPS-FMLP did not produce a large increase in arachidonic acid release from attached cells. However, LPS in combination with serum and FLMP produced a significant increase in

arachidonic acid release. It was found that a dose response a dose response of neutrophils to LPS has a maximum release of arachidonic acid with 100-200 ng/ml of LPS (**Figure 5**).

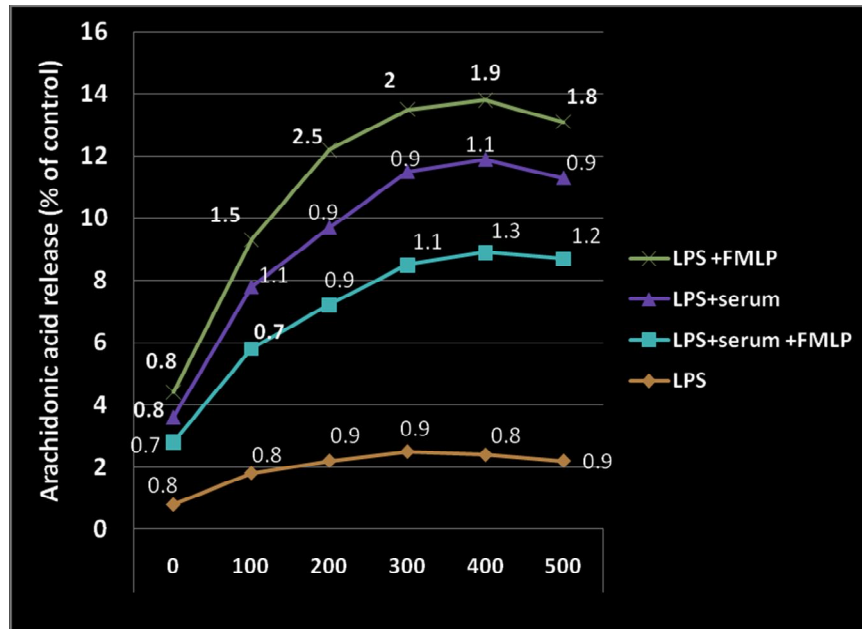


Figure (5): Effect of LPS concentration and its potentiation on FLMP on arachidonic acid release.

MY4 has no significant effect on arachidonic acid release in control, LPS, PAF or FMLP treated cells. It inhibited greatly the

potentiation by LPS-serum complex of PAF induced arachidonic acid release (**Figure 6**).

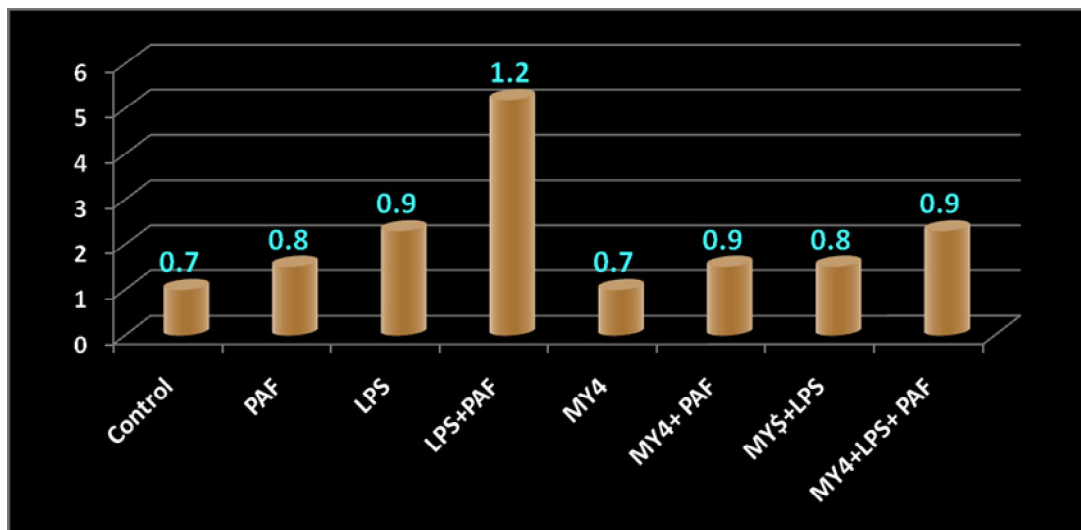


Figure (6): Effect of anti CD14 (MY4) on LPS potentiated of arachidonic acid from neutrophils stimulated by PAF.

DISCUSSION

Many different cell types, including neutrophils, release arachidonic acid in response to various stimuli. Neutrophils stimulated with granulocyte-macrophage colony – stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF- α) primes neutrophils for arachidonic acid release induced by FMLP, PAF and LTB₄ (**Berry et al., 2008**). In response to certain infections, the human body initiates various complex reactions to fight the bacteria. These reactions activate arachidonic acid cascade and generate several biologically active substance (**Markiewski and Lambris, 2007**).

LPS alone released arachidonic acid from suspended neutrophils but not too much as in case of LPS+PAF which caused significant increase of arachidonic acid release, and the main appropriate time was found 45 minutes. It was found also that most appropriate dose of LPS to prime neutrophils to release arachidonic acid in suspended cells was 100-200 ng/ml, and these results showed clearly that LPS alone did not cause a significant increase in arachidonic acid release where, it has a priming effect on suspended neutrophils to release arachidonic acid, and their priming effects were concentration and time dependent. The suspended neutrophils need another agonist to prime the cells for arachidonic acid release (**Lee et al., 2016**).

Attachment of human neutrophils to surfaces produce significant changes in the behavior of these cells, e.g. adherence of neutrophils to surfaces caused an increase in F-actin, a rise in the intracellular concentration of free calcium, an increase in affinity of TNF-

receptors, up regulation of adhesion molecular concentration of cyclic nucleotides (**Sintsova et al., 2014**).

MY4 in different concentration has no significant effect on the arachidonic acid release in control, LPS or PAF-treated cells, it inhibited greatly the potentiation by LPS-serum complex of PAF induced arachidonic acid release. Inhibition of the potentiation was observed at an antibody concentration of 1 μ g/ml but the inhibition of the potentiation at 10 μ g/ml was greater. Thus LPS potentiated PAF-induced arachidonic acid release through CD14 receptors, where DC14 bonded LPS-LBP complex on the surface of neutrophils to start the signal transduction cascade. Similar results were reported by (**Marcos et al., 2010**). It was reported that several effects of LPS (in the presence of serum) on neutrophils were inhibited by MY4 (**Pieterse et al., 2016**). LPS-LBP complex binds to membrane CD14 on human monocytes. Soluble CD14 seems to play a role in the LPS- mediated activation of 14- negative cells (**Illarregui et al., 2016**).

CONCLUSION

LPS or PAF alone produced small increase in arachidonic acid release in both suspended and attached human neutrophils, where LPS in combination with PAF induced significant arachidonic acid release in suspended and attached human neutrophils. This action was via CD14 receptor in neutrophils cell membrane. There was a difference in response between suspended and attached neutrophils, where the attached neutrophils released more arachidonic acid in response to LPS, PAF and FMLP.

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علاقة حمض الأراكيدونيك في خلايا النيتروفيل الملتصقة والمعلقة

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خلفية البحث: خلايا النيتروفيل هي واحدة من خلايا الدم و يعتبر التصاقها بأسطح خلايا الجسم واحدة من المهام الرئيسية. وعندما تلتصق النيتروفيل بأسطح الخلايا البيولوجية تظهر خصائص مختلفة مقارنة بخلايا النيتروفيل المعلقة في محلول، وعلى وجه الخصوص في ظاهرة النشاط التنفسي في الخلايا

الهدف من البحث: دراسة العلاقة بين إنتاج حمض الأراكيدونيك من إستئارة خلايا النيتروفيل الملتصقة والمعلقة.

مواد وطرق البحث: تم استئارة خلايا النيتروفيل الملتصقة والمعلقة بمواد إل بي اس نتي سيدي فورتين فورى مادة ام واى.

نتائج البحث: تم خروج حمض الأراكيدونيك بعد التحفيز من خلايا النيتروفيل الملتصقة والمعلقة. وخروج حمض الأراكيدونيك من الخلايا الملتصقة كان اكثر من الخلايا المعلقة، وهذا الناتج من الحمض يكون اكثر مع زيادة فترة الحضانه، وكذلك زيادة كمية المواد التى تستخدم فى الإستئارة (اف ام تى - ل بي اس) وقد وجد أن مادة اف ام تى تسبب خروج الحمض من خلايا النيتروفيل الملتصقة المعاملة ب ل بي اس أكثر من الخلايا المعلقة. وعند إضافة تركيز منخفض لكل من إل بي اس و وباف، سيدي فورتين. فلم يكن هناك أي تأثير للأجسام المضادة ضد سيدي فورتين على حمض الأراكيدونيك.

الخلاصة: إل بي إس و وباف كل منهما على حده ينتج عنه زيادة طفيفة في اطلاق سراح حمض الأراكيدونيك في كل من خلايا النيتروفيل المعلقة والملتصقة، لكن عند إضافة إل بي إس إلى وباف تم خروج كمية كبيرة من الحمض من الخلايا الملتصقة والمعلقة وذلك عن طريق مستقبلات سيدي فورتين في غشاء خلايا النيتروفيل.