



# The Role of Purinergic Signaling in Endothelial Inflammatory Responses

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## Introduction

### An Overview of Atherosclerotic Cardiovascular Disease (ASCVD) and Endothelial Activation

- Endothelial activation, characterised by increased vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, increased secretion of pro-inflammatory cytokines and chemokines including interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), tumour Necrosis Factor-  $\alpha$  (TNF- $\alpha$ ), monocyte recruitment, and plaque formation, is a critical early event in ASCVD pathogenesis.<sup>1-3</sup>

### Purinergic Signalling and Adenosine Deaminase

- Extracellular adenosine exerts potent anti-inflammatory effects mainly via  $A_2A$  receptors by inhibiting NF- $\kappa$ B, reducing adhesion molecules and cytokines.<sup>4</sup>
- Adenosine deaminase (ADA) degrades adenosine to inosine.<sup>5</sup>

### Clinical Implications

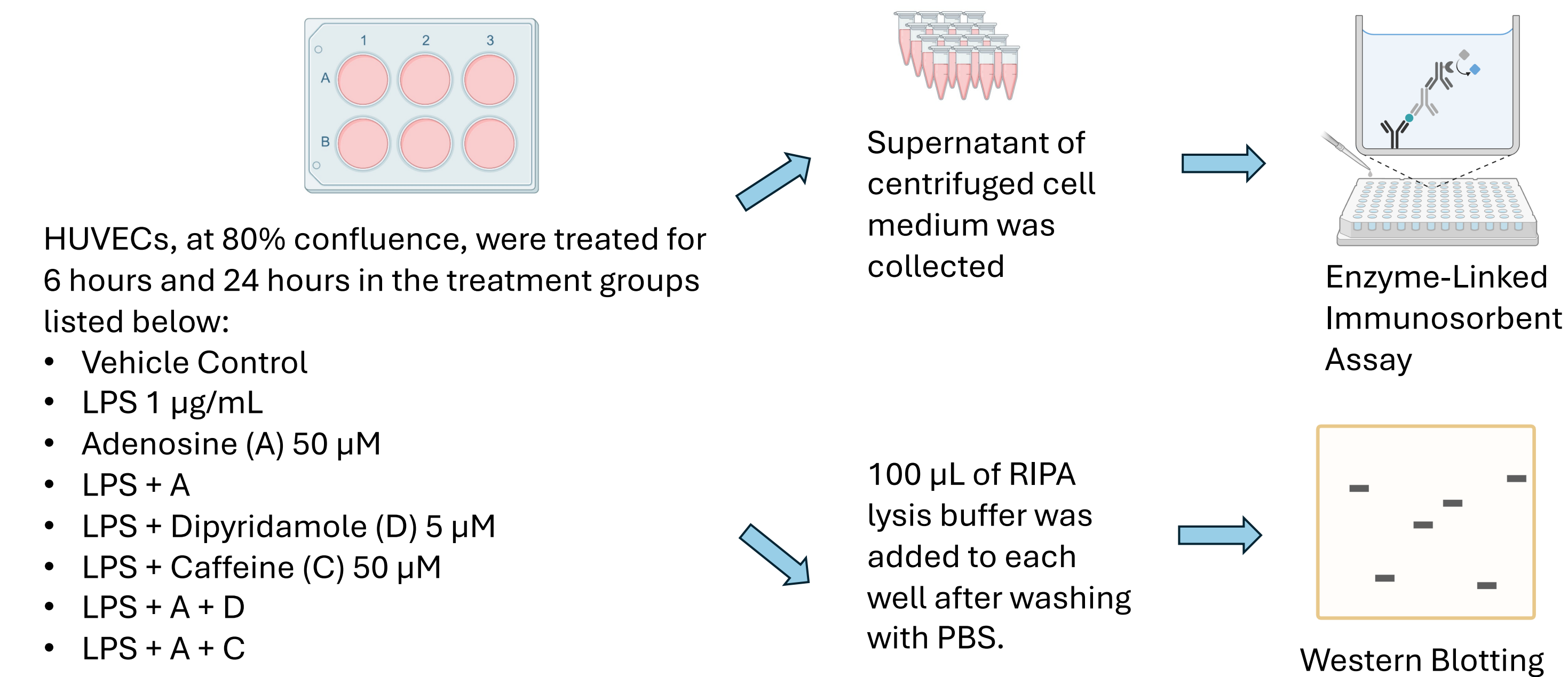
- Recent literature highlights its expression is markedly upregulated in human atherosclerotic lesions on endothelial cells and macrophages.<sup>6</sup>
- Elevated ADA depletes protective adenosine, amplifies inflammation, and promotes plaque progression; serum ADA levels correlate with ASCVD severity.<sup>7</sup>

## Aims

- To investigate the role of ADA in endothelial inflammation induced by lipopolysaccharides (LPS) in human umbilical vein endothelial cells (HUVECs).
- To evaluate whether pharmacological modulation of extracellular adenosine attenuates the pro-inflammatory phenotype.

## Methods

### 1. Cell Treatment of HUVECs



## Results

### 1. Effects of LPS and adenosine, alone or in combination, on the expressions of ADA and inflammatory mediators

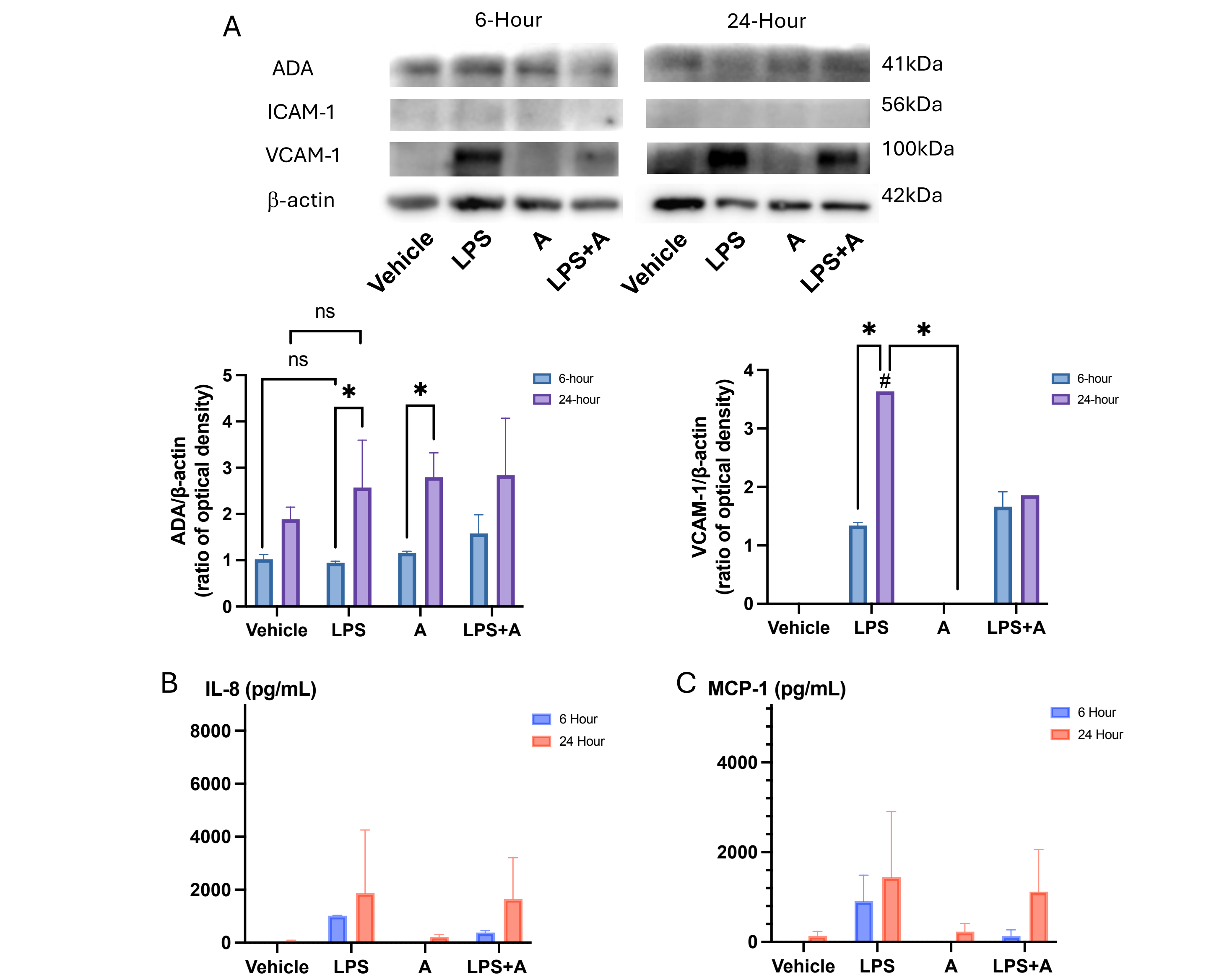


Figure 1. (A) LPS and adenosine did not significantly affect the protein level of (A) ADA in HUVECs after incubation for 6 or 24 hours, although a higher level was observed after 24 hours than after 6 hours. By contrast, LPS increased VCAM-1 expression after 24 hours. While adenosine alone did not affect VCAM-1 expression, it prevented the increase induced by LPS at 24 hours. Neither LPS nor adenosine significantly affected the secretion of (B) IL-8 and (C) MCP-1 by HUVECs. Data are expressed as (A, upper) representative images of western blotting displaying protein bands for ADA, VCAM-1 and  $\beta$ -actin, and those for ICAM-1 were undetectable; and (A, lower; B & C) mean  $\pm$  SD (n=2); ns: not significant ( $p>0.05$ ); \* $p<0.05$ ; # $p<0.05$  compared to Vehicle group of the same time point, Two-way ANOVA with Tukey's multiple comparisons test

## Results

### 2. Effects of dipyridamole and caffeine on expressions of ADA and inflammatory mediators in HUVECs treated with LPS without or with adenosine

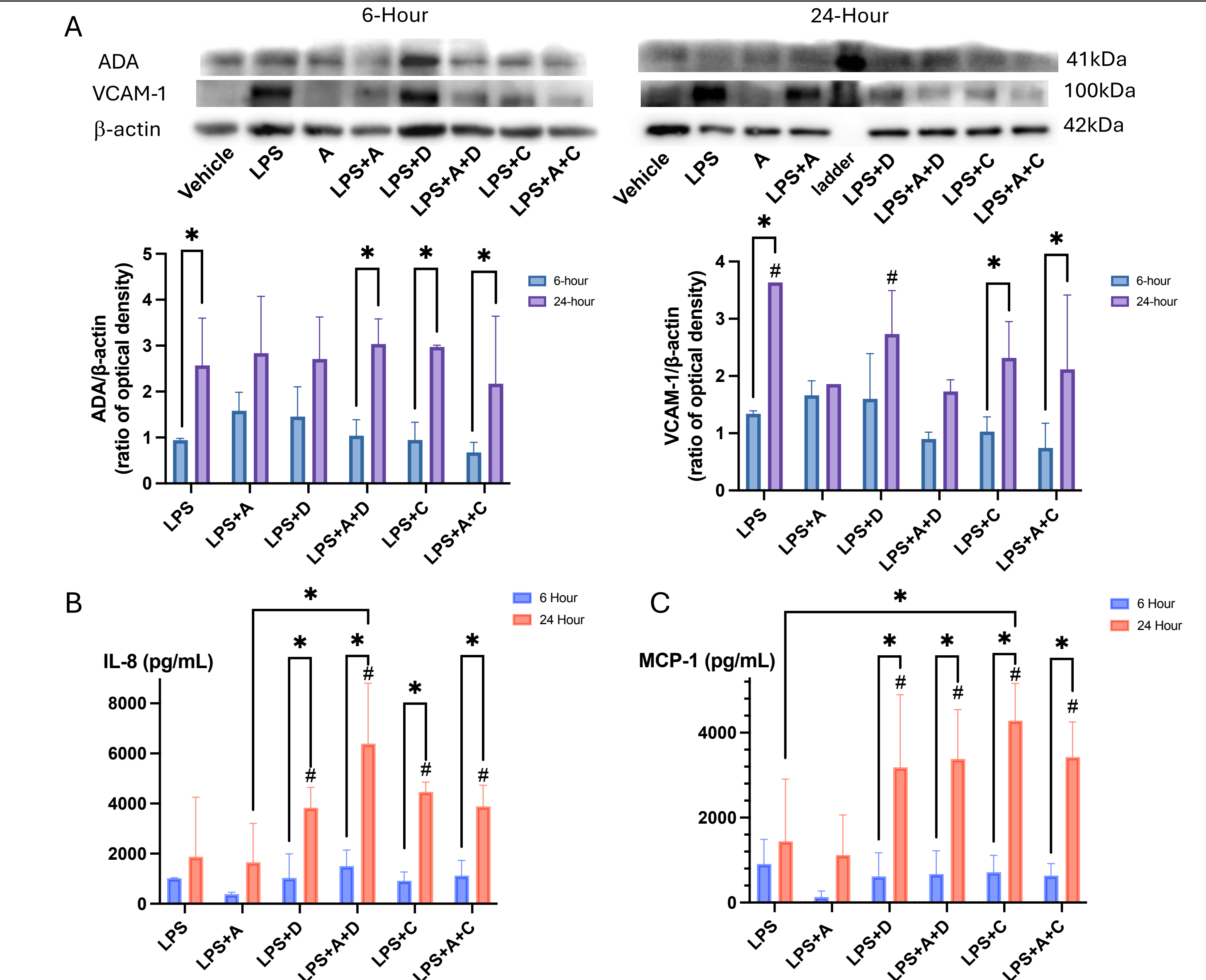


Figure 2. Dipyridamole did not significantly affect (A) ADA and VCAM-1 expressions in HUVECs treated with LPS, without or with adenosine, at 6 and 24 hours; but increased the secretion of (B) IL-8, and (C) likely also MCP-1, by HUVECs treated for 24 hours with LPS and adenosine. Caffeine did not significantly affect (A, lower left) ADA and (A, lower right) VCAM-1 expressions in HUVECs treated with LPS, without or with adenosine, at 6 and 24 hours. While caffeine did not affect VCAM-1 expression, it prevented the increase induced by LPS at 24 hours. Caffeine likely increased the secretion of (B) IL-8 and (C) MCP-1, by HUVECs treated for 24 hours with LPS and adenosine, and significantly increased MCP-1 secretion in HUVECs treated with LPS at 24 hours. Data are expressed as (A, upper) representative images of western blotting; and (A, lower; B & C) mean  $\pm$  SD (n=2); \* $p<0.05$ ; # $p<0.05$  compared to Vehicle group of the same time point, Two-way ANOVA with Tukey's multiple comparisons test.

## Discussions

### 1. ADA expression and inflammatory responses:

- The higher ADA expression in HUVECs treated with LPS or adenosine for 24 hours than at 6 hours suggests that prolonged exposure is required for ADA upregulation.
- LPS significantly increased VCAM-1, but not ADA, expression after 24 hours, when compared with the vehicle, thus suggesting that ADA does not play a major role in LPS-induced inflammatory responses.

### 2. Protective effect of adenosine

- Adenosine prevents the LPS-induced VCAM-1 expression at 24 hours and appears to reduce LPS-induced secretion of IL-8 and MCP-1 at 6 hours.
- Suggesting that it protects endothelial cells from LPS-induced inflammation.

### 3. Inhibition of adenosine receptors by caffeine:

- Inhibition of  $A_2A$  and  $A_2B$  adenosine receptors by caffeine unmasks the induction of IL-8 and MCP-1 release by HUVECs after being treated with LPS for 24 hours.
- Confirming that activation of purinergic signaling protects against LPS-induced endothelial activation.

### 4. Unexpected pro-inflammatory effect of dipyridamole:

- Increasing extracellular adenosine level by dipyridamole, which inhibits the uptake of adenosine into cells, increases the secretion of IL-8 and MCP-1 by HUVECs treated with LPS, alone or in combination with adenosine, for 24 hours.
- The pro-inflammatory effect of dipyridamole can be explained by the high extracellular adenosine level
  - (1) activating the low-affinity pro-inflammatory  $A_2B$  receptors, and/or
  - (2) activating ecto-ADA on the cell surface, leading to pro-inflammatory downstream signaling.<sup>5,8</sup>

### Limitations

- Small sample size (n=2) limits statistical power
- HUVECs at high passage numbers (P24-31) may have undergone phenotypical changes
- Measuring surface ecto-ADA enzyme activity, instead of total ADA protein (in the current experiment), would provide a better indication of the effects of ADA on endothelial inflammation.

## Conclusion

This study shows that LPS does not upregulate ADA protein expression in HUVECs and that dipyridamole and caffeine at the concentration tested do not attenuate endothelial inflammation. These findings highlight the complex, context-dependent role of the ADA-adenosine axis in inflamed endothelium

## Acknowledgement

I would like to express my gratitude to the PhD students of Prof. Susan W.S. Leung, Ms. Bella Liu and Ms. Sinnie Dong, for their professional guidance and support of this project. I would also like to acknowledge the support of the Department of Pharmacology and Pharmacy, The University of Hong Kong.

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