



Application Note I100

Immunology: Immunomodulation; Statins; Sepsis

Objectives

Investigation of T-cell adhesion to ICAM-1 with a mixed sepsis model treated with a range of statins under physiological shear stress using the Cellix VenaFlux platform.

Introduction

Sepsis continues to be an increasingly frequent and acute medical problem in critically ill patients in the intensive care unit despite significant advances in management and treatment. Statins are potent lipid-lowering agents that act on the mevalonate pathway by inhibiting HMG-CoA the rate-limiting enzyme for cholesterol synthesis. Recent clinical trials have shown that statins have anti-inflammatory properties that are independent of their lipid lowering capability. Patients with cardiovascular disease or patients prior to surgery who were receiving statin therapy had a lower incidence of sepsis and with fewer progressing to severe sepsis and death. The mechanisms through which statins influence sepsis associated inflammation are poorly understood.

Methods

1. Cell Harvesting and Sample Treatments

Peripheral blood was donated by 6 healthy subjects. Following mononuclear cell isolation, monocytes were allowed to adhere to the culture vessels before B-cells were removed from the T-cell population using nylon wool adhesion. T-cells were then co-cultured in the presence of monocytes. Cells were treated with 10 nM meva-, lova- or simvastatin dissolved in

ethanol (0.1% v/v final) or prava- or fluvastatin dissolved in water. Cells were then stimulated with 2 µg/ml lipopolysachharide (LPS) and 20 µg/ml peptidoglycan G (PepG). Control cells were treated with 0.1% v/v ethanol ± LPS/PepG and incubated a humidified 37°C incubator containing 5% CO₂ for 18 hrs.

2. Biochip Coating Procedure

Vena8 Fluoro+ biochip (400 µm wide, 100 µm deep) was coated overnight in humid conditions at 4°C with rhICAM-1 (10 µg/ml), before being coated with BSA, 10 µg/ml. Two additional channels were coated with BSA for 2 hrs at room temperature. Prior to shear experiments, all channels were washed thrice with media.

3. Adhesion Profiles

Isolated T cells were infused into the rhICAM-1 and BSA coated channels under a defined shear stress of 0.5 dyne/cm² for a time period of 5 min in CO₂ independent media. Images were captured using the using VenaFlux assay software.

4. Image Analysis

T cell adhesion profiles of single cells were recorded using Image Pro Premier software. Cell images were captured from three microscopic fields from each channel. Data was exported into Excel to allow further analysis.

5. Statistics

Data obtained from this experiment were not normally distributed and are reported as median [range] adherent cells and analysed using the Wilcoxon's signed-rank test using Graphpad Prism® 4 software.

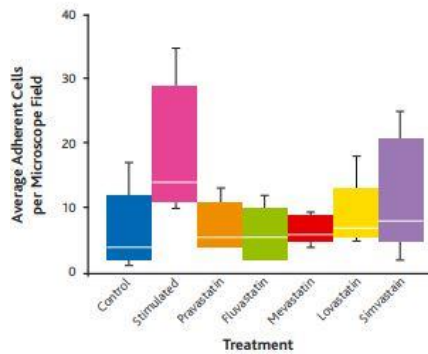


Figure 1

Figure 1: Adhesion of T cells to ICAM. T-cells devoid of B-cells were co-cultured with monocytes in media containing 10nM prava-, fluva-, meva-, lova- or simvastatin \pm 2 μ g LPS and 20 μ g PepG. Controls cells were cultured in media containing 0.1% v/v ethanol \pm LPS/PepG. The number of adherent T-cells was measured under constant shear stress of 0.5 dyne/cm² for 5 mins. Box and whisker plot show the median, interquartile and full range from data obtained from 6 healthy individuals is shown. $p < 0.05$, $n = 6$.

Results

Co-cultured T cells stimulated with LPS/PepG caused a statistically significant increase in adhesion to ICAM, 13.7 [10 to 36] adherent cells when compared to the unstimulated control 4.5 [1 to 17] adherent cells, $p < 0.05$. However, results showed that LPS/PepG stimulated cells treated with all statins used in the study caused a statistically significant reduction in adhesion when compared to the LPS/PepG stimulated control, 6.1 [3 to 13]

for pravastatin, 6 [2 to 12] for fluvastatin, 6.5 [4 to 9] for mevastatin, 8 [5 to 18] for lovastatin and 9 [2 to 24] adherent cells for simvastatin, $p < 0.05$. (Figure 1). However, simvastatin treated cells nearly always tended to aggregate (Figure 2) even at pM concentrations (data not shown) and made assessment of adherence more difficult.

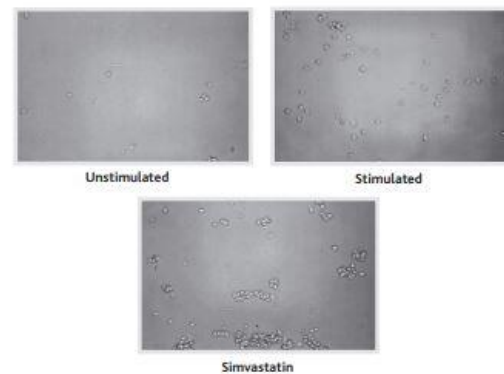


Figure 2

Figure 2: representative microscope image (20x magnification) showing unstimulated and LPS/PepG stimulated T-cell adhesion to rhICAM-1 and T-cell adhesion profile of LPS/PepG stimulated cells treated with 10 nM simvastatin. The T cells were subjected to a shear stress of 0.5 dyne/cm² for 5 mins.

Discussion

Several studies have recognised the benefit of patients receiving statin therapy in reducing the incidence of sepsis in ICU patients, while other studies have documented a benefit in reducing mortality [1]. The potential protective effect of statin therapy reducing the incidence of sepsis is probably due to the immunomodulatory mechanisms of these compounds that are capable of inhibiting the inflammatory pathways involved in sepsis. LFA-1 is an integrin that is critical for T-cell adhesion, T-cell priming and cytokine secretion. Statins inhibit LFA-1 interaction with ICAM by either directly binding to a novel site that is distant from the ICAM binding site (not pravastatin) or inhibit the prenylation of small GTPases such as Ras, Rap, Rho and Rac. Rap1, a Ras-related GTPase, appears to be central to LFA-1 function and is highly expressed in T-cells [2]. Our results have shown that physiologically relevant concentrations of fluvastatin, mevastatin, lovastatin, simvastatin or pravastatin markedly inhibit T-cell adhesion to ICAM equally, irrespective of direct LFA-1 binding. Therefore, the reported clinical outcome of septic patients receiving statin therapy can be at least be partially associated with T-cell-ICAM binding activities. In conclusion the Cellix Microfluidic Platform allows for T-cell adhesion to be accurately determined using physiological shear stress with physiologically relevant concentrations of statins.

Acknowledgements

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References

1. Chua D, Tsang RS and Kuo I. The Role of Statin Therapy in Sepsis. *The Annals of Pharmacotherapy*; 41: 647, 2007.
2. Mor A, Dustin ML, Philips MR. Small GTPases and LFA-1 reciprocally modulate adhesion and signaling. *Immunological Reviews*; 218: 114, 2007.

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