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ICAM-1 OVEREXPRESSION INDUCED BY ABACAVIR IS MEDIATED BY P2X7 RECEPTORS

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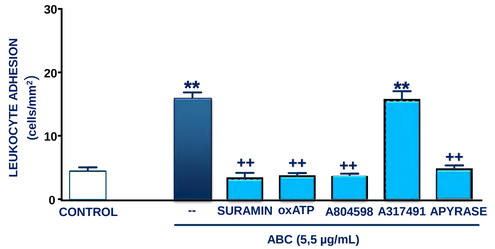
INTRODUCTION

The use of abacavir has been associated with cardiovascular diseases. Specifically, it has been observed an increased risk of myocardial infarction (MI), aterosclerosis and thrombosis in abacavir treated patients ([1, 2]), but the mechanism remains unclear. In earlier studies, we have demonstrated that abacavir induces an increase in endotelial intercellular adhesion molecule-1 (ICAM-1) expression and promotes leukocyte recruitment through Mac-1/ICAM-1 interaction ([3,4,5]) . Given the chemical structure of abacavir, we have previously explored the link between abacavir and its proinflammatory effects by interfering in the purine signalling pathway, and have seen that ATP and its P2X7 receptors are involved in the leukocyte accumulation induced by abacavir (Figure 1).

Figure 1.Role of ATP receptors on leukocyte accumulation induced by abacavir on HUVEC



Abacavir produced a significant increase on leukocyte adhesion on HUVEC. A non-selective antagonist (Suramin), selective antagonist of P2X7 receptors (oxATP and A804598) and the agent responsable of ATP hydrolisis (Apyrase) prevented the increase on leukocyte adhesion.

REFERENCES

[1]Sabin et al. Lancet 2008;26;371(9622):1417-26. [2]AIDS 2008;22: F17-F24. [3]De Pablo et al. AIDS 2010;24(9):1259-1266.
[4]De Pablo et al. Antiviral therapy 2012;17(8):1615-1619. [5]De Pablo et al. The Journal of Infectious Diseases 2013;208(9):1448-53.

AIM

The aim of the present study was to evaluate the role of ATP and its receptors on the endothelial ICAM-1 overexpression induced by abacavir.

METHODS

FLOW CYTOMETRY

The expression of the endothelial adhesion molecule ICAM-1 was evaluated using an FACScalibur cytometer (BD Biosciences). ICAM-1 was analyzed in HUVEC/HUAEC incubated with the anti-ICAM-1 antibody conjugated with phycoerythrin (PE).

EXPERIMENTAL PROTOCOL

To study the effects of blocking P2X7 receptors on ICAM-1 expression, human umbilical vein or arterial endothelial cells (HUVEC or HUAEC, respectively) were pre-treated with the following reagents:

	[Concentration]	FUNCTION
SURAMIN	100 µM	P2X-Y Antagonist
oxATP	600 µM	P2X7 Antagonist
A804698	1 µM	P2X7 Antagonist
A317491	25 nM	P2X2/3 Antagonist
APYRASE	1 UI/ml	ATP Hydrolisis

Subsequently, the cells were treated with abacavir (4h) and finally ICAM-1 expression was measured by flow cytometry.

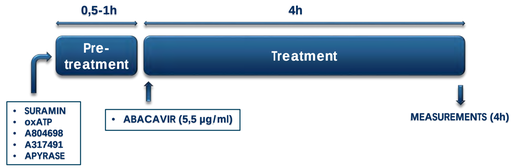
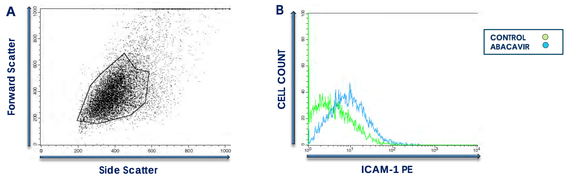


Figure 2A shows the HUVEC population gated by Side Scatter and Forward Scatter histogram. Figure 2B represent the number of cells with a determined fluorescence of ICAM-1, in the absence (green) or presence (blue) of abacavir (5,5 µg/ml).

Figure 2. Representation of the histograms of ICAM-1 expression on HUVEC.



STATISTICAL ANALYSIS

Data represent the percentage of median fluorescence intensity vs. control group (100%) and are expressed as media±SEM. Statistical analysis was performed with one-way ANOVA and a Newman-Keuls post-hoc test, with significance *p<0.05 (vs. control) and +p<0.05 (vs. abacavir), n≥4.

RESULTS

Figure 3. Role of ATP receptors on ICAM-1 expression-induced by abacavir on HUVEC.

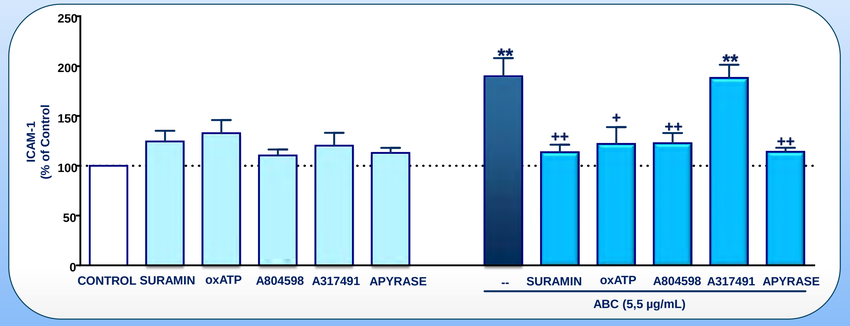
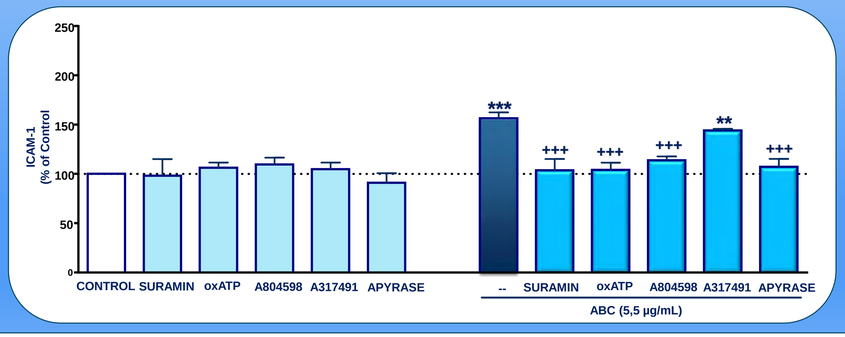


Figure 4. Role of ATP receptors on ICAM-1 expression-induced by abacavir on HUAEC.



Abacavir promoted a significant increase on endothelial adhesion molecule ICAM-1 on HUVEC (Fig 3) and on HUAEC (Fig 4). A non-selective antagonist of ATP receptors (Suramin), the selective antagonists of P2X7 receptors (oxATP and A804598) and the agent responsible for ATP hydrolisis (Apyrase), but not the selective antagonist of P2X2/3 receptors (A317491) prevented ICAM-1 overexpression on HUVEC and on HUAEC. Significance *p<0.05 (vs. control) and +p<0.05 (vs. abacavir), n≥4.

CONCLUSIONS

Our results suggest that the activation of P2X7 receptors promote overexpression of ICAM-1 in the venular and arterial endothelium. This process may be responsible for the leukocyte recruitment observed in the vascular damage associated with atherosclerosis and myocardial infarction in HIV patients treated with abacavir.