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ABC Induces Leukocyte Accumulation through the Interaction of ATP and $P2X_7$ Receptors

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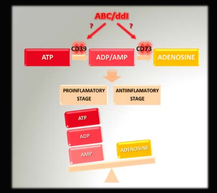
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INTRODUCTION

The use of some NRTI, particularly abacavir (ABC) and didanosine (ddI), has been linked with cardiovascular side effects, though the mechanism implicated remains unclear. Leukocyte accumulation in the vessel wall is considered the first phase in the onset of cardiovascular diseases with an inflammatory component, such as myocardial infarction, atherosclerosis and thrombosis.

Using *in vitro* and *in vivo* models, we have demonstrated that the cyclic purine analogues, ABC and ddI, induce leukocyte-endothelial cell interactions through Mac-1/ICAM-1 interaction. However, this early vascular side effect is not produced by pyrimidine analogues (lamivudine, zidovudine, emtricitabine) or the acyclic nucleotide tenofovir[1,2,3].

Due to the analogy of ABC and ddI with the substrates of the enzymes involved in the degradation of ATP, these drugs are likely to interfere with this route, leading to an increase in the levels of proinflammatory ATP/ADP/AMP and a reduction of those of the antiinflammatory molecule adenosine.



[1]de Pablo et al. AIDS 2010;24(9):1259-1266. [2]de Pablo et al. Antiviral therapy 2012;17(8):1615-1619. [3]de Pablo et al. The Journal of Infectious Diseases 2013;208(9):1448-53.

AIM

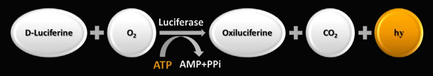
To explore whether ABC´s proinflammatory effects are a result of the interference of its structure with the purine signalling pathway.

METHODS

Human umbilical vein endothelial cells (HUVEC) and polymorphonuclear leukocytes (PMN) were treated with clinical concentrations of ABC (0.5-15 µM, 4h) to determine:

1. ATP levels:

Intracellular ATP levels were determined by a luciferase bioluminescence assay.

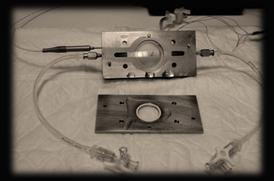


2. CD73 and CD39 expression:

Expression of CD73 and CD39 (enzymes responsible for ATP degradation) was evaluated by Western blot.

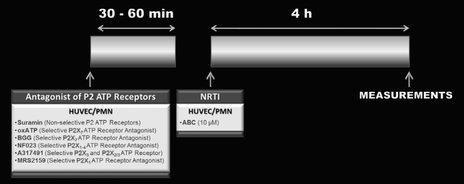
3. Dynamic adhesion assay:

Coverslips containing confluent HUVEC were inserted into a parallel plate flow chamber and adhesion assays were performed at 37ºC. The entire chamber was mounted on an inverted microscope connected to a video camera. PMN cells were drawn across the HUVEC monolayer at an estimated shear flow of 0.7 dyne/cm².



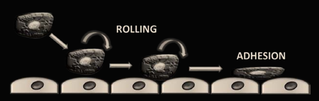
a) Experimental protocol:

To analyze the role of ATP and its receptors in the leukocyte accumulation induced by ABC, HUVEC and PMN were pre-treated with different P2 ATP receptors prior to ABC administration and leukocyte-endothelium interactions were then measured using a flow chamber system.



b) Parameters determined:

Leukocyte rolling velocity: µm/s.
Leukocyte rolling flux: cells/min.
Leukocyte adhesion: cells/mm²

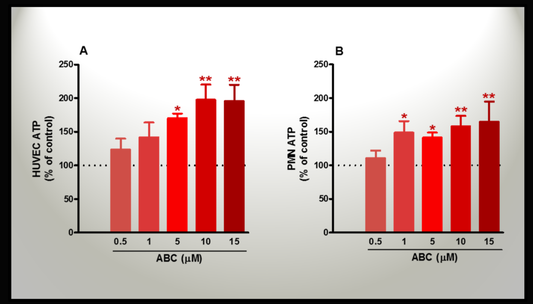


Statistical analysis

*All data are expressed as mean±SEM. Data were analyzed using a one-way-ANOVA followed by a Newman-Keuls post hoc test, n≥4. *p<0.05, **p<0.01 vs. control and ++p<0.01 vs. ABC (10µM).*

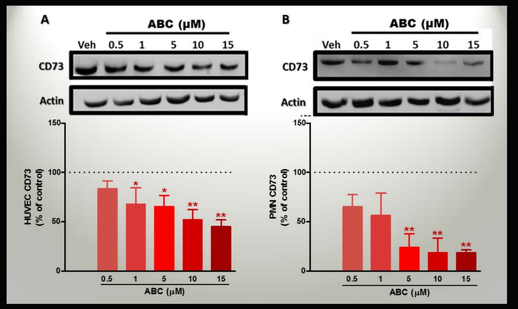
RESULTS

Fig.1: Effect of ABC in ATP levels in HUVEC and PMN.



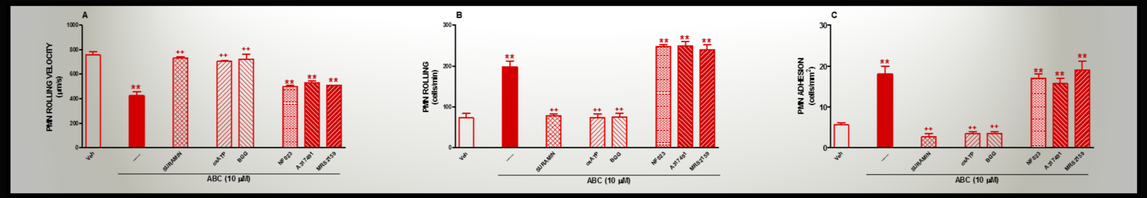
A significant dose-dependent increase in ATPi levels was observed in HUVEC (A) and PMN (B) treated ABC .

Fig.2: Action of ABC on CD73 expression in HUVEC and PMN.



ABC reduced the expression of CD73 in HUVEC (A) and PMN (B). CD39 expression was not modified (data not shown).

Fig.3: Role of ATP receptors in leukocyte recruitment induced by ABC.



ABC promoted a significant reduction in PMN rolling velocity (A) and an increase in PMN rolling flux (B) and PMN adhesion (C) on HUVEC. A non-selective antagonist of ATP receptors and selective antagonists of $P2X_7$ ATP receptors prevented the PMN accumulation induced by ABC in HUVEC.

CONCLUSIONS

Our results suggest a structure-activity relationship in the effects of ABC on leukocyte accumulation through the interaction of ATP with its $P2X_7$ receptors. This proinflammatory mechanism may be especially relevant for understanding the vascular damage observed in ABC-treated patients.