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LEUKOCYTES ARE KEY TO THE PRO-THROMBOTIC EFFECTS OF ABACAVIR

Angeles.alvarez.@uv.es

Victor Collado-Diaz[1], Ainhoa Sanchez-Lopez[1], Samuel Orden[2], Maria Amparo Blanch-Ruiz[1], Maria Angeles Martinez-Cuesta[1], Isabel Andujar[2], Angeles Alvarez[1], Juan V. Esplugues[1]

[1] University of Valencia, [2]FISABIO, Valencia, Spain, Spain

INTRODUCTION

- Abacavir (ABC) has been linked to vascular toxicity but its mechanism of action remains unclear. ABC, a purine analogue, shares structural similarities with endogenous purines (e.g. ATP and ADP; **Figure 1**), major signaling molecules capable of triggering pro-inflammatory and pro-thrombotic programs by interacting with P2-nucleotide receptors on vascular structures.

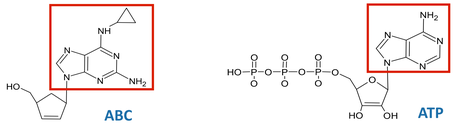

Figure 1. (A) Chemical structures of ABC (left) and ATP (right).

- ABC induces platelet-leukocyte-endothelial cell interactions and pro-thrombotic effects through a mechanism involving interference with the purinergic system, specifically with ATP-P2X7 receptors[1-5] (**Figure 2**).

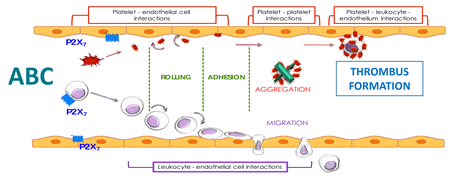

Figure 2. Process of vascular inflammation and thrombi formation induced by ABC. ABC induces the recruitment of leukocytes by the endothelium, one of the earliest features of inflammation-associated cardiovascular disease, through a mechanism involving the activation of P2X7 receptors[2-4]. ABC does not affect aggregation, but it promotes the adherence of platelets to both endothelial cells and leukocytes[5], processes that could be implicated in thrombi formation[1].

- The ATP-P2X$_7$ receptors implicated in the leukocyte-endothelial cells interactions induced by ABC are located primarily in leukocytes)[1].
- The recruitment of leukocytes, mainly neutrophils, by platelets is an important phase in the formation of thrombi.

OBJECTIVE

To evaluate the role of white cells in the pro-thrombotic effects of ABC in an animal model of thrombosis.

METHODS

- Mouse strains used: **C57BL/6** Wild-type (WT).

Model of leukopenia

- Leukopenia was induced by cyclophosphamide (CPM,150 mg/kg, i.p, 96 h)[6].
- Kimura and Wright staining were employed to quantify total leukocytes and to differentiate leukocyte populations, respectively.

Model of thrombosis

- Thrombosis was induced with the endothelium damaging agent Ferric chloride (**FeCl$_3$**)[7] at a concentration of 25 mM, which does not modify blood flow but predisposes arterioles to thrombosis in the presence of other deleterious vascular agents.
- Rofecoxib, a selective COX-2 inhibitor and a well characterized vascular deleterious vascular effects, was used as positive control[8].

Experimental protocol

Treatments

CPM or saline (veh)

96h

DRUG	Concentration
Saline (veh)	
Abacavir (ABC); 4h, i.s	2.5 - 7.5 µg/mL
Rofecoxib (Rof); 2h, i.p	0.1 mg/mL

- Microcirculation in cremasteric arterioles was observed by **intravital microscopy**. The parameter measured was **time to occlusion** (s). Videos were recorded until flow cessation or during a 8-min period (480 seconds, if no occlusion occurred).

2-4h → Surgery → 0.5h → Intravital Microscopy

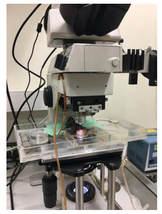

Figure 3. Intravital microscopy.

- **Statistical analysis**: one-way ANOVA analysis followed by Newman-Keuls). n≥4.

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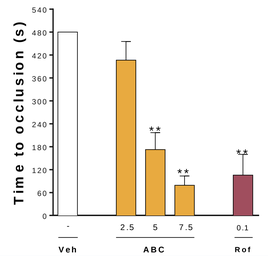
RESULTS

1. ABC induced dose-dependent vessel occlusion in non-leukopenic mice.



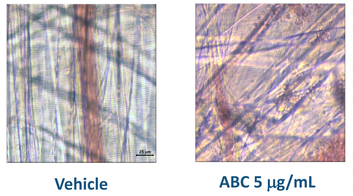


Figure 4. Thrombus formation induced by ABC. **(A)** Mice were treated with saline (vehicle, veh), abacavir (ABC, 2.5-7.5 µg/mL, intrascrotally, 4h) or rofecoxib (rof, 0.1 mg/kg,i.p, 2h). Following surgery, the cremasteric artery was superfused with a ferric chloride solution ($FeCl_3$,25 mM) and the time to occlusion of the arterioles was determined. There was a dose-dependent acceleration of vessel occlusion as a consequence of thrombi formation in ABC treated mice, the same effect was observed with after the addition of rof. Results are mean ± SEM, n≥4. **p ≤ 0.05 corresponding value in vehicle treated group (ANOVA followed by Newman-Keuls) **(B)** Representative video stills of cremasteric arterioles of animals treated with vehicle (left) or ABC 5 µg/mL (right).

2. CPM reduced the number of leukocytes by almost 90%.

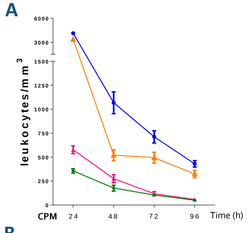
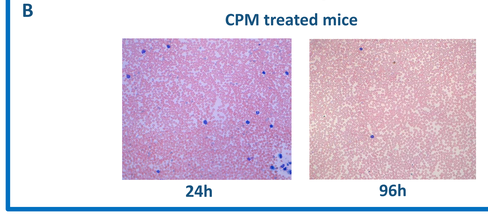


Figure 5. CPM causes a reduction in the number of leukocytes. **(A)** Effect of CPM on the total number of leukocytes, and on the number of each leukocyte population (lymphocytes, neutrophils and monocytes) at 24-96h. **(B)** Representative images of blood samples of animals treated with CPM at 24h (left) or 96h (right) with Wright staining.



3. The pro-thrombotic effects of ABC were absent in leukopenic mice.

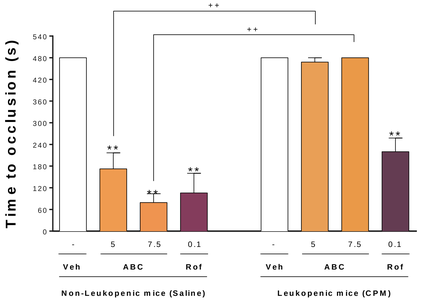


Figure 6. Leukocytes have a role in the pro-thrombotic effect exerted by Abacavir. Mice were treated with saline (vehicle, veh), abacavir (ABC, 5–7.5 µg/mL, intrascrotally, 4h), or rofecoxib (rof, 0.1 mg/kg, i.p., 2h). To generate leukopenia, some mice were pre-treated with cyclophosphamide (CPM, 150 mg/kg, 96 h). Following surgery, the cremasteric artery was superfused with a ferric chloride solution ($FeCl_3$,25 mM) and the time to occlusion of the arterioles was determined. Results are mean ± SEM, n≥4. **p<0.01 vs. corresponding value in vehicle-treated group and ++p<0.01 vs. corresponding value in non-leukopenic group (ANOVA followed by Newman-Keuls test).

CONCLUSION

The pro-thrombotic effect of ABC in vivo depends on the presence of leukocytes, thus demonstrating a key role of these cells in the deleterious vascular effects of this drug. These results support previous research suggesting that ABC induces thrombi formation through a specific mechanism involving leukocyte purinergic P2X7 signalling. This may explain the cardiovascular toxicity associated with the use of ABC in humans.