Antiretrovirals inhibit arginase in human microglia

Lucia Lisi\textsuperscript{1*}, Emilia Laudati\textsuperscript{1*}, Teresa F. Miscioscia\textsuperscript{2}, Cinzia Dello Russo\textsuperscript{1}, Alessandra Topai\textsuperscript{2} and Pierluigi Navarra\textsuperscript{1}.

\* These authors equally contributed to this paper

\textsuperscript{1} Institute of Pharmacology, Catholic University Medical School, L.go F. Vito 1, 00168 Rome, Italy
\textsuperscript{2} Colosseum Combinatorial Chemistry Centre for Technology, Via della Ricerca Scientifica, 00133, Rome Italy

ARG activity in human PBMC.

Here we have investigated the effects of ARVs (namely, DRV, ATV, EFV and NVP) on urea production (taken as a marker of arginase activity) by primary cultures of human peripheral blood mononuclear cells (PBMC).

Eparine peripheral blood samples were obtained from healthy volunteers. PBMC were obtained by density gradient centrifugation using the Ficoll-Paque Premium as published in Lisi et al 2013. Briefly, after two washes with balanced salt solution, the PBMC fraction was re-suspended in DMEM/F12 culture medium supplemented with 100 UI/ml penicillin/streptomycin and 1% heat inactivated FCS (Gibco Brl, Invitrogen Corporation, Paisley, UK) and were plated at density of 4.2x10\textsuperscript{5} cells/well. This population of cells contains CD3, CD8 and CD4 cells, with CD4 T-cells ranging between 30 and 60% of the total number. PBMC were treated with medium alone or with ARV drug for 48 h. All analytical tests carried out on PBMC have been described in the main paper, except lactate dehydrogenase (LDH) assay, which was conducted measuring LDH activity in incubation media with a CytoTox 96\textsuperscript{®} Assay (Promega, Madison, WI, USA). In no case LDH levels were exceeding the toxicity thresh-old limit.

At variance with CHME-5 microglia, human PBMC express both ARG-I and ARG-II gene (Fig Suppl 1A) under basal conditions. Looking at the product of ARGs, after 24 hours of incubation cells produce about 20 µg/ml of urea, a far larger amount compared to CHME-5; no significant increase are detected in the time frame 24 - 72h (Fig Suppl 1B). The specific ARG-I inhibitor, Nor-NOHA (Tenu 1999) reduces ARG activity by about 20% after 48h exposure; such decrease does not attain statistical significance compared to controls (Fig. Suppl 1C). In these conditions, ARVs (namely 100 pM DRV, 100 pM ATV, 1 nM EFV and 1µM NVP) tend to reduce the release of urea in the incubation medium by about 10% with respect to the control; likewise, such decrease was not significant (Fig Suppl 1D).
**Figure 1.** Effects of ARVs on ARG activity in human PBMC. (A) ARG-I and ARG-II gene expression was evaluated in PBMC culture under basal condition. (B) PBMC were incubated for different time (24-48-72) and urea levels were measured. (C) PBMC were incubated with 100 µM nor-NOHA for 48h. (D) PBMC were incubated with ARV drugs, as indicated in figure, for 48h. The data were expressed as the means ± SEM of (5) replicates. The experiments were repeated twice with similar results.

Here we used PBMC cells as a control cell population, to test the hypothesis that the inhibitory effects of ARVs on ARG activity in CHME-5 microglia can be observed in other immune-competent cells as well. We found that both tested ARVs and the specific ARG-I inhibitor Nor-NOHA failed to elicit significant decreases in urea production in this experimental setting. Such discrepancy is most probably due to the fact that urea production in PBMC is accounted for by both ARG isoforms, and therefore the total amount of urea produced is far higher. Since ARVs, similar to nor-NOHA, appear to selectively inhibit ARG-I isoform, urea production by ARG-II is unaffected, thereby masking the decrease associated to ARG-I blockade. We conclude that, because of the different profile of ARG isoform expression, PBMC do not prove useful as control cell population in human microglia experiments.

**References:**
