In addition to antiviral properties, some antiretrovirals have an anti-NLRP3 inflammasome activity

0233

Elisa Teyssou[1], Gilles Peytavin[2], Cathia Soulié[1], Minh Lê[2], Sophie Sayon[1], Karen Zafilaza[1], Anne-Genevieve Marcelin[1] & **Vincent Calvez**[1]

[1] AP-HP Pitié-Salpêtrière, Sorbonne Université, INSERM, iPLESP, Paris, France

[2] AP-HP Bichat, Sorbonne Université, INSERM, Paris, France

BACKGROUND

- Chronic low-grade inflammation has emerged as a central biological driver of aging and age-related diseases, a phenomenon commonly referred to as “inflammaging”. Among the various innate immune pathways implicated in this process, persistent activation of the NLRP3 inflammasome plays a particularly important role by promoting the sustained production of pro-inflammatory cytokines such as IL-1β, thereby contributing to tissue damage, metabolic dysregulation, and functional decline across multiple organs. Chronic inflammation is an issue that increasingly needs to be considered in people living with HIV. HIV infection itself is a driver of persistent inflammation and represents an inherent risk factor for the development of several chronic diseases. As a result, managing inflammation has become a central component of long-term care in this aging HIV population.

- Several classes of drugs, statins for example, beyond the primary indication for which they were originally developed, have subsequently been found to possess significant anti-inflammatory properties, including activity against the NLRP3 inflammasome.

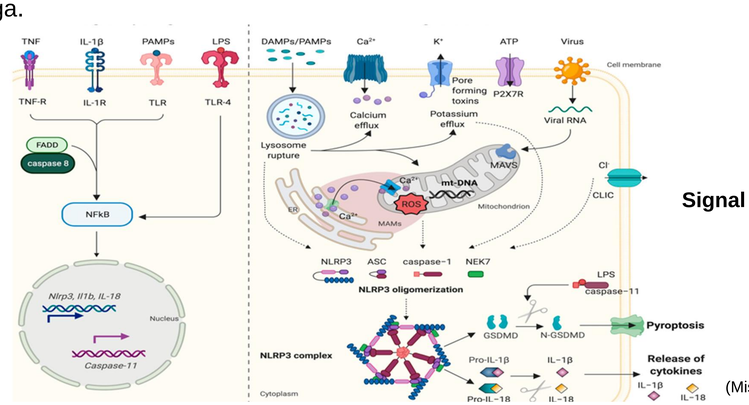
The objective of this study was to evaluate the potential intrinsic inhibitory or activating effects on the NLPR3 inflammasome of the main antiretroviral drugs currently used and for some of them anti NF-kB activity

METHODS

- Antiretrovirals (ARVs) were tested on macrophages at the mean Cmax concentrations observed in clinical practice: Tenofovir (TDF), Lamivudine (3TC), Emtricitabine (FTC), Abacavir (ABC), Rilpivirine (RPV), Cabotegravir (CAB), Dolutegravir (DTG), Bictegravir (BIC), Raltegravir (RAL), Doravirine (DOR), Lenacapavir (LEN), Islatravir (ISL).

- THP-1 monocyte cells were differentiated in macrophage cells with 10nM of phorbol 12-myristate-13-acetate for 24h. To measure only the NLRP3 activity independently of the first step, which is NF-κB production, macrophages were first primed with 1µg/ml LPS for 3h **(signal 1)** and incubated with ARVs for 2h. Nigericin NLRP3 inflammasome activator was added for 1h **(signal 2),** and IL-1β secretion was measured in the supernatant. To measure anti-NF-κB activity (phosphorylated p65 protein), the ARVs are added before TNF-α stimulation to give them time to block the cascade that leads to NF-κB formation after stimulation by **signal 1**. Protein expression was measured by Lumit® technology, Promega.

Signal 1



Signal 2

(Missiroli et al, 2021)

DOR, LEN, DTG and BIC have anti-NLRP3 inflammasome activity

RESULTS for NLRP3 inhibition

- None of the ARVs tested activate the NLRP3 inflammasome
- However, some of them have a statistically significant anti-NLRP3 inflammasome effect: DOR (-25.5%), LEN (-24.5%), BIC (-19.5%) and DTG (-19%), even when using nigericin that is a highly potent NLRP3-mediated inflammasome inductor (Figure1).

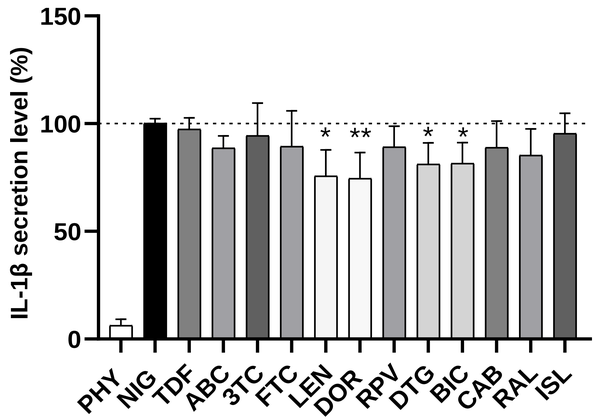


Figure1: IL-1b secretion in NLRP3 induced macrophages treated with antiretroviral drugs.
NIG: positive control, PHY: IL-1β basal expression.*p<0.05 ; **p<0005

RESULTS for NF-kB inhibition

- None of the ARVs tested activates NF-κB production measured by the concentration of phosphorylated p65 protein (signal 1), but only LEN significantly inhibits it (−20%) (Figure2).

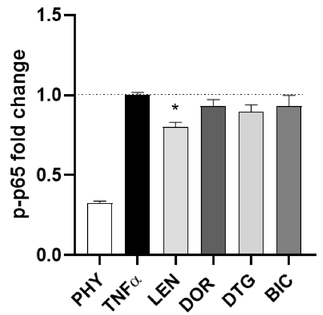


Figure2: phosphorylated p65 (p-p65) protein expression in macrophages treated with ARVs.
TNFα: positive control, PHY: p-p65 basal expression.*p<0.05 (Anova, multiple comparaison)

CONCLUSIONS

- Accumulating experimental evidence indicates that statins can attenuate NLRP3 inflammasome activation in macrophage models, including primary monocyte-derived macrophages and THP-1 cells. In vitro studies using classical inflammasome triggers such as LPS plus ATP or nigericin, as well as metabolic stimuli including cholesterol or oxidized LDL, show that several statins (notably simvastatin, rosuvastatin, atorvastatin, and pitavastatin) decrease downstream markers of NLRP3 activation (caspase-1 cleavage and IL-1β release), and in some contexts also lower NLRP3 protein levels. Quantitatively, these inhibitory effects in macrophage and THP-1 models have been reported in the approximate range of 20 % to 65 % reduction in IL-1β secretion or caspase-1 activity

- But, in contrast to statins, the anti-NLRP3 effects observed in this study with some ARVs are likely to be more pronounced and pharmacologically relevant, as they are detectable at substantially lower concentrations (observed in treated patients). Whereas statin-mediated inhibition of NLRP3 in vitro generally requires micromolar exposures that exceed typical plasma levels in treated patients, some ARVs exhibit comparable or greater suppression of NLRP3 activation, caspase-1 cleavage, and IL-1β release at concentrations much closer to those achieved clinically.

- In conclusion, some ARVs possess anti-NLRP3 inflammasome activity that could have a clinically protective impact against certain age-related pathologies. This warrants further investigation both at a fundamental and at a clinical level.

Funding: this study was funded by ANRS MIE

PLAIN LANGUAGE SUMMARY

Our study suggest that some antiretrovirals recommended for HIV infection treatment have been shown in vitro to have a beneficial anti-inflammatory effect on NLRP3-mediated inflammasome this could potentially have a beneficial effect in the long term