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Abacavir does not have a direct cardiotoxic effect compared to other antiretroviral agents: Effects on cell death, mitochondrial membrane potential, intracellular calcium and free radical generation

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Although long term use of protease inhibitors (PIs) is associated with increased cardiovascular risk, a recent subgroup analysis of the DAD study showed abacavir (ABC) to increase the risk of myocardial infarction (Friis-Moller *et al.*, 2007), a finding previously unassociated with non-nucleoside reverse transcriptase inhibitors (NNRTI). Understanding the effects of ARV (antiretrovirals) on cellular mechanisms central to the development of cardiac dysfunction is critical to our understanding of the pathogenesis of cardiovascular disease in treated HIV patients. Using a high content screening system, we investigated the relationship between PIs (RTV, NFV, SQV, IDV, FOS), NNRTIs (EFV, NVP) and NRTIs (ABC; TFV) on cellular biochemistry likely to affect cardiac function in cardiomyoblasts (H9C2).

Cells were incubated in the presence of the ARV agents (0.01–50μM) for 24 hrs. Dimethylsuphoxide (0.1%) and doxorubicin were used as controls. Cells were analysed (INcell 1000) following staining with Hoechst, Fluo-4, TMRM and DCF to assess cell death, intracellular Ca^{2+} levels, mitochondrial membrane potential (MMP) and free radical generation, respectively. Data were analysed by ANOVA with *post hoc* analysis and expressed as mean±s.e.m.; *P<0.05 indicates significance vs untreated controls.

[Drug] 10μM ~Clinical Concentration	Cell Death (% increase)	Calcium (Pixel intensity)	MMP (Δ intensity)	Free radicals (Pixel intensity)
ABC (abacavir)	1.9±3.2	120.5±2.6	-2.2±6.7	1.9±0.4
TFV (tenofovir)	4.1±3.2	122.6±6.9	-3.3±1.3	10.7±3.1
FOS (fosamprenavir)	3.7±4.5	104.9±7.6	9.8±2.6	16.9±7.4
RTV (ritonavir)	18±2.1*	101.6±8.5	6.2±3.7	51.6±23
SQV (saquinavir)	35.2±3.3*	122±11.6	-1.3±2.0	24.1±15.3
NFV (nelfinavir)	44.3±8.7*	284±41.7*	-16.1±6.8*	9.7±3.3
IDV (indinavir)	0.3±4.2	109.3±4.4	6.9±1.9	5.6±1.9
EFV (efavirenz)	36.4±11.2*	113.3±8.1	12.6±3.9	33.6±7.6
NVP (nevirapine)	7.0±2.9	101.7±5.2	9.8±1.4	10.7±4.6
Untreated	0±6.9	100±6.8	0±3.9	13.4±3.1

NFV decreased cell survival by 45%, increased intracellular calcium levels by 2 fold and reduced MMP by 16% (P<0.05). In contrast, RTV, SQV and EFV only caused cell death (P<0.05). ABC and the other ARV agents had no effect on any of the parameters studied at this concentration. At higher concentrations (50μM), NVP increased intracellular calcium levels, SQV and EFV decreased MMP, while RTV, SQV and EFV induced free radical generation (P<0.05).

In conclusion, in cardiomyoblasts ABC at approximate clinical concentrations and above had no effect on the parameters studied. NFV was the most toxic ARV. Effects on cell death, MMP and intracellular calcium levels may have functional consequences with regard to the cardiac contractility.

Friis-Moller,N., et al (2007). *New Eng. J. Med.,* 356, 1723-1735.