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Effects of antiretroviral drugs on carcinogenesis in human breast and cervical cells

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ABSTRACT

Highly active antiretroviral therapy (HAART), a multidrug combination regimen, commonly consisting of Nucleoside Reverse Transcriptase Inhibitors, non- Nucleoside Reverse Transcriptase Inhibitors and Protease Inhibitors, has radically decreased mortality and morbidity rates among people living with HIV/AIDS and is currently the treatment of choice in South Africa and around the world. The emphasis of the original development of the antiretroviral drugs was on clinical effectiveness (reducing mortality), before all other considerations. Presently, emphasis has shifted from the initial short-term considerations to the long-term undesirable or harmful effects induced by this treatment regimen. Studies on the effects of HAART on the incidence and progression of HIV/AIDS associated cancers, non-Hodgkin's lymphoma, cervical cancer and Kaposi's sarcoma have provided contrasting data. While there has been a decrease in the incidence of Kaposi's sarcoma, HAART has reportedly not had a significant impact on the incidence of the other two AIDS defining malignancies, while some evidence even suggests an increase in these cancers. It has also been extensively reported that the widespread use of HAART has increased the risk of non-AIDS defining malignancies, including breast cancer.

Whether individual antiretroviral compounds or their combinations are oncogenic is therefore widely speculated. These speculations led to the investigation of the effects of some of the antiretroviral drugs used in the South African treatment guidelines on the expression of key apoptotic regulatory genes, *BAX* and *BCL-2* in two human breast, MCF-7 and MCF-10A and two human cervical cell lines, HCS-2 and NCE16IIA by Real Time qPCR gene expression and immunofluorescence. This is because cancer is initiated when there is an up-regulation of anti-apoptotic genes (e.g. BCL-2,) and down regulation of pro-apoptotic genes (e.g. BAX).

Because the formation of new blood vessels from pre-existing vasculature (angiogenesis) is required for cancer growth and development, this study also investigated the effects of the antiretroviral drugs on the expression levels of key angiogenic regulatory genes; hypothesising that the antiretroviral compounds might up-regulate pro-angiogenic $VEGF_{165a}$ and/or down-regulate anti-angiogenic $VEGF_{165b}$ gene expression. This study also evaluated the cytotoxicity of the antiretroviral drugs in normal and cancer cell lines of the breast and cervix at clinically relevant concentrations of the drugs and at different time points – 24, 48, 72 and 96 hours, employing the Neutral Red Toxicology/Viability Assay. In addition, the potential anti-apoptotic effects of the protease inhibitors - LPV/r were investigated by cell death detection ELISA and acridine orange staining.

This study shows that the antiretroviral drugs; tenofovir disoproxil fumarate, emtricitabine, efavirenz, lopinavir, ritonavir and the two triple combinations (all at clinically relevant concentrations which reflect their steady-state peak plasma concentrations in patients receiving these drugs) demonstrated varying degrees of cytotoxicity in the normal breast and cervical cells. The resulting DNA damage associated with cytotoxicity is strongly implicated in the processes of tumor initiation. The results of the qPCR data demonstrated differences in the response of the two tissue types used in this study, which, though is not statistically significant, showed trends and often opposite trends between the breast and cervical and between the normal and cancer cell lines. All the antiretroviral drugs and combinations tested did not significantly alter *BAX* and *BCL-2, $VEGF_{165a}$* and *$VEGF_{165b}$* gene expression in both cell lines. The protein localisation of BAX, BCL-2 and $VEGF_{165b}$ were also not altered.

The protease inhibitors - LPV/r exhibited significant ($p<0.05$) inhibition of Camptothecin induced apoptosis in the cervical cancer HCS-2 cell line but not in the normal immortalised NCE16IIA or the two breast cell lines. This anti-apoptotic property of HIV protease inhibitors, although shown here not to involve BAX or BCL-2 protein and RNA synthesis might promote the development of cervical cancer.