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Research Article

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Antiretroviral Therapy and Cancers: Dolutegravir, Ritonavir and Zidovudine Have Toxic Effects on the Viability of Cal-27 Cancer Cell Line

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Abstract

Introduction: *Effective HIV management through Highly Active Antiretroviral Therapy (HAART) is a crucial step in managing AIDS-related Kaposi sarcoma, as emphasized by Kat [1]. Treatment for non-small cell lung cancer (NSCLC) in HIV patients significantly improves clinical outcomes Rengan et al. [2]. According to the American Cancer Society (2019), NSCLC encompasses squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, constituting about 85% of lung cancer cases.*

Methods: *This study addressed the question of whether both DTG and RTV have the ability to inhibit mitosis of CAL-27 cell line when compared to zidovudine.*

Results: *A review of the dose response pattern versus efficacy against cancer cell viability of the selected ARDS revealed that these drugs have anti-mitotic properties. and may relieve patients of not only HIV infection, but also the additional burden of HIV-cancer. One-way Analysis of Variance (ANOVA) was used to compare the efficacies of the drugs, at 0.05 significance level ($p \leq 0.05$).*

Conclusion: *The antiretroviral compounds, (Zidovudine, Dolutegravir and Ritonavir) significantly suppress the viability of Cal-27 oral cancer cells, when compared to the control groups.*

Keywords: HIV-cancers, Cal-27, Dolutegravir, Ritonavir, Zidovudine

Introduction

CAL 27 cell line is derived from oral adenosquamous carcinoma, and is relevant to oral squamous cell carcinoma studies [3]. The cells are characterized by their epithelial traits, featuring a polygonal shape and a cytoplasm that exhibits a highly granular appearance. Immunocytochemical analysis has confirmed strong positive staining with anti-keratin antibodies in these cells. Oral cancers account for 3% of total cancer incidence in the USA, with over 90% being squamous cell carcinoma (SCC), as communicated by the American Cancer Society [4] and Myers et al. [5]. Incidence and mortality patterns vary geographically, driven by factors like smoking, alcohol, DNA oncogenic viruses, and habits such as betel nut consumption, as highlighted by Ghantous and Abu Elnaaj [6]. While incidence has diminished in certain regions, some countries (primarily low-income nations) and female populations have seen an increase [7].

Ritonavir (RTV) plays a crucial role in the treatment of lung cancer by inhibiting a protein called Survivin [8]. Survivin is involved in regulating multiple pathways related to cancer and is particularly notable for its role in inhibiting apoptosis, which provides RTV with an advantage in treating T cell leukemia as well [9]. Moreover, it has been observed that RTV effectively induces cell cycle arrest and apoptosis in ovarian cell lines MDH-2774 and SKOV-3, with

its effects being dose-dependent. The conclusion drawn from this study was that ritonavir has the potential to be used in ovarian cancer treatment, potentially enhancing the effects of conventional chemotherapeutic regimens [10]. Hendrikx et al. [11] discovered that the antitumor activity of intravenously administered docetaxel, used in a mouse model for breast cancer, can be significantly enhanced through the co-administration of orally administered ritonavir. Another study by Rauschenbach et al. [12] demonstrated that RTV monotherapy leads to a selective antineoplastic response, showing both cytostatic and anti-migratory effects at clinical plasma peak levels. This study suggested that RTV could be a valuable candidate for further exploration as an adjunct therapeutic in the clinical care of glioblastoma.

Dolutegravir (DTG), along with its derivatives also displays anticancer effects [13] by inhibit the proliferation and migration of multiple human cancer cell lines through the suppression of Human endogenous retrovirus K (HERV-K) Proteins; the viral envelope glycoprotein has been reported to induce cell-cell fusion in melanoma and so could contribute to tumorigenesis. Zidovudine or azidothymidine (AZT) is azide analog of deoxythymidine(3′-azido-3′-deoxythymidine) and like all compounds that block nucleic acid synthesis (such as purine and pyrimidine nucleoside analogs) has proven antibacterial, antiviral, and anticancer potentials [14-16].

Methods

Zidovudine is hereby tested alongside DTG and RTV, but our focus is on DTG and RTV. While both DTG and RTV have demonstrated beneficial effects in cancer treatment, their impact on oral squamous cell carcinoma remains unexplored. This gap in knowledge prompted the need for the conducted study.

Experimental Groups

Test Groups cells were treated with AZT, DTG and RTV at various concentrations:
Test Group 1: 4 µg/mL each.
Test Group 2: 2 µg/mL each.
Test Group 3: 1µg/mL each.
Test Group 4: 0.5 µg/mL each.
Test Group 5: 0.25 µg/mL each.
Solvent Control groups were treated with equal volumes of physiological saline while positive Control Groups were not subjected to any experimental intervention; cells in these groups underwent normal growth processes.

Drug Treatment and Cell Incubation

Stock solutions of AZT, DTG and RTV at a concentration of 10 µg/mL were prepared and subsequent serial dilutions were then carried out using physiological saline, and the resulting solutions were added to respective culture flasks containing CAL-27 cells to support cell health and growth, each culture media received 2-3 drops of penstrep solution containing penicillin (50 U/ml) and streptomycin (50 µg/ml). The cell cultures were placed in a controlled environment with 5% CO2 at a temperature of 37°C. Over a period of 10 days, daily observations were conducted using an inverted microscope. On the 10th day, the cell cultures were harvested for the final assessment.

Cell Dissociation with Trypsin and Mycoplasma Contamination Test

The process of cell dissociation with trypsin was initiated when the cell growth reached a confluence of 90% of the total volume within the culture flask. This step ensured the controlled separation of cells from the culture substrate, allowing for further manipulation and analysis. To ensure the integrity of the cell lines, a test for mycoplasma contamination was carried out. This was performed on both day 5 and day 10 of the experiment using Hoechst dye from Sigma-Aldrich, St. Louis, USA. The presence of mycoplasma, a type of bacterial contamination, was assessed through this procedure, contributing to the reliability and accuracy of our experimental results. To assess the viability of CAL27 cells, a trypan blue assay was performed 48 hours after treatment. At harvest, any cell with confluence of less than 90% was eliminated from the study. To determine the concentration of cells per milliliter (ml) in the culture media, we adopted the approach outlined by Srivastava et al. [17]; utilization of hemocytometers and trypan blue staining-we took a 10µL sample from the cell culture and combined it with 90 µL of trypan blue solution in an autoclaved micro centrifuge tube. It was thorough mixed after which a 10µL aliquot of the blended mixture was placed on an automated hemocytometer. By tallying the cell count across all the squares on the hemocytometer, an average cell count was calculated. And to convert this average count into cells per milliliter, it was multiplied by the dilution factor (which, in this case, is 10). This calculation yielded the number of cells in millions per milliliter of the cell culture, providing a valuable metric for assessing cell concentration in the experimental setup.

Counting of Cells

As a part of the experimental process, a portion of the freshly prepared cell suspension was extracted from each experimental group and the cell viability (the essential stain trypan blue was employed) thus selectively labeling and identifying dead cells. An automated cell counting method was employed (Luna-FL™ dual fluorescence cell counter developed by Logos Biosystems in the USA). This automated cell counter applies the same foundational principles as counting chambers or hemocytometers but integrates the use of fluorescent staining for enhanced accuracy.

Data Analysis

Results obtained were analyzed using the one-way Analysis of Variance (ANOVA) and obtained values used to compare the efficacies of the drugs, at 0.05 significance level ($p \leq 0.05$).

Results

The impact of AZT, DTG, and RTV on the viability of Cal-27 oral cancer cell line, as shown in Figures 1A-C. All three antiretroviral compounds showed significant anti-mitotic effects on the viability of the cells (Figures 1A: Zidovudine: $F_{(6,20)} = 4.388$, $p = 0.0106$, Fig.

1b-c; Norvir and Myltega: $F_{(6,20)} = 8.641$, $p = 0.0005$,). However, at a dose of 2.0 µg/ml only Zidovudine significantly suppressed cell activities when compared to the control ($p<0.05$, Figure 1A). On the other hand, Norvir and Myltega showed toxic effects on the viability of Cal-27 cancer cell line at doses of 0.25, 2.0, and 4.0 µg/ml, decreasing active cancer cell count, when compared to the control cells ($p<0.05$, Figure 1B and C). Notably, the highest dose (4.0 µg/ml) of Norvir and Myltega exhibited the most potent antiproliferative effects against the cells (Figures 1B and C).

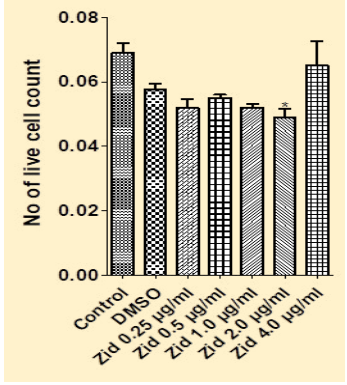


Figure 1A

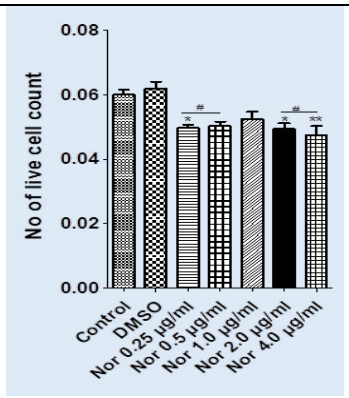


Figure 1B

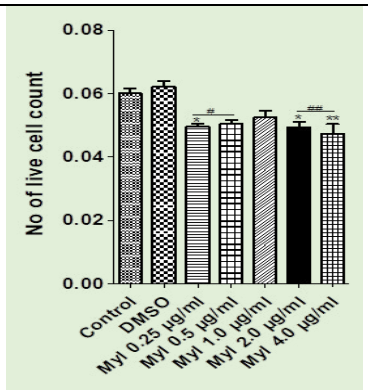
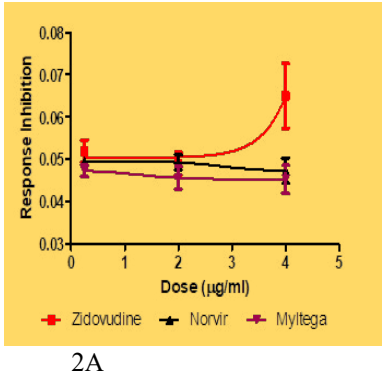


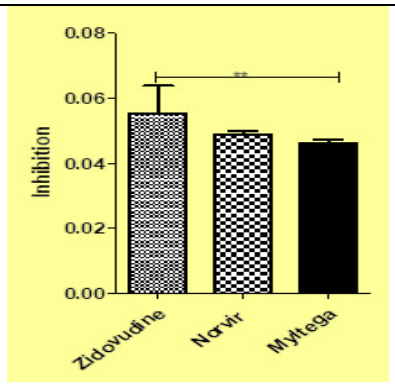
Figure 1C

Figure 1 A-C: These figures above demonstrate the antiproliferative effects of some ARVs. (a-c) the effects of Zidovudine, Norvir, and Myltega on viability of Cal-27 cancer cell line. Data shown represents mean±SEM; n=3 per group. *P<0.05, **P<0.01 compared to control, #P<0.05, ##P<0.01 compared to other treatment groups; One-way ANOVA, followed by Bonferroni post hoc comparison test.

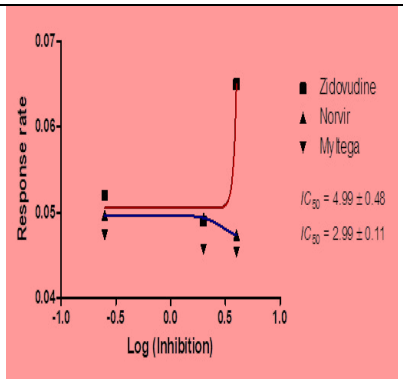
Figures 2 A-C below, show the dose response pattern versus efficacy against cancer cell viability:



2A



2B



2C

Figures 2A: Tumor suppressive action and (2B) comparison of non-linear regression analyses of potent doses of NNRTIs-dose response curve; Log (inhibition) vs. response variable slope. Non-linear Goodness of fit curve. Data shown represents mean±SEM or LogIC50, where applicable; n=3 per group.

Discussion

Sample Size Determination

In the study of cell biology, manufacturers provide specifications for various sizes of plates, dishes, and flasks used in cell culture. These specifications include the recommended seeding density and the number of cells per vessel type. This differentiation arises because the number of cells that can reach confluence on a given plate, dish, or flask will vary depending on the specific cell type being cultured. Consequently, the volume of a flask becomes a determining factor in the relative number of cells it can accommodate, essentially shaping the process of sample size determination. Following the insights of Lazic et al. [18], when a flask achieves at least 90% confluence, it is deemed to have an equal and sufficient sample size. Notably, each experimental group and subgroup in this study encompassed three flasks. To ensure the precision of results, the experiment was repeated three times. Furthermore, as outlined by Pollard et al. [19], the mean of results obtained from cell culture experiments gets progressively closer to the true mean of the entire population as the sample size increases through repeated growth. In the context of this descriptive analytical study, statistical significance can be achieved through the cloning of experimental cells from the same cell group. This practice was

followed in the study. Moreover, each experimental group was done in triplicates, with all the experimental cell groups achieving a minimum of 90% confluency. Two separate studies, Lazic [20] and Lazic et al. [18], shared the consensus that the determination of sample size in cell culture need not be more intricate than the approach described. This is due to the concurrent application of treatments to all cells within a well, rather than individual cells being treated independently. Importantly, the interactions between cells within a flask or well, including cell-to-cell connections, release of signaling molecules, and competition for nutrients in the culture media, contribute to the rationale behind this approach.

Current Incidence of OSCC

Across Africa, increasing smoking habits, westernized lifestyles, dietary shifts, HIV/HPV infections, and unfavorable health policies are identified as predominant etiological factors, as discussed by Adeola et al., [21]. At age 45, 7.3% of oral cancers are diagnosed in males, and 7.8% in females [22]. A concerning rise in OSCC incidence among human individuals under 45 years old has therefore been observed, as noted by Coletta et al., [23]. In specific regions of the world; places characterized by limited access to oral healthcare, delayed OSCC diagnosis has contributed to reduced survival rates. In South Africa, OSCC prevalence spans a broad geographical scope, impacting males as the fifth most common cancer. In females it is the tenth most common type [22].

Conclusion

HIV-cancer patients with CD4 counts of over 200 who received chemotherapy exhibited comparable survival rates in comparison to untreated patients [24-27]. In this work we have shown that among the three antiretroviral compounds, Zidovudine (AZT) at a dose of 2.0 µg/ml has a significant suppressive effect on the number of live cancer cells compared to the control. Both ritonavir (marketed as Norvir) and Dolutegravir (marketed as Myltega) showed toxic effects to viability of Cal-27 cancer cells at three doses/dilutions (0.25 µg/ml, 2.0 µg/ml, and 4.0 µg/ml); by decreasing the number of active cancer cells, when compared to the control, and as seen within the drug treatment groups. It was dose-dependent: notably, the highest dose of 4.0 µg/ml of ritonavir and Dolutegravir exhibited the most potent antiproliferative potency against the cancer cells.

This study provides experimental evidence that antiretroviral compounds, and particularly Zidovudine (AZT), Ritonavir (Norvir), and Dolutegravir, all possess anti-mitotic and thus anti-cancer properties against human squamous cell carcinoma of the tongue. These ARDS therefore hold a future promise among conventional anti-cancer agents.

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