TRANSFORMATION OF MONOCYTES TO MACROPHAGES BY COMBINATION ANTIRETROVIRAL TREATMENT

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1. INTRODUCTION

- The increased use of highly active antiretroviral therapy (HAART) has resulted in a dramatic increase survival of HIV-infected individuals. A downside of the success of anti-retroviral therapy (ART) is the premature onset and increased risk of certain inflammatory age-related diseases associated with low levels of chronic immune activation. Such activation is thought to contribute to what is described as to serious non-AIDS events (SNAEs)(1). HAART entails combining two nucleoside reverse transcriptase inhibitors (NRTIs) with either one or two protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs), in this study usually nevirapine (NVP)(2). In the present study the impact of different combinations of anti-retroviral drugs (ARD) was assessed on monocyte cells.

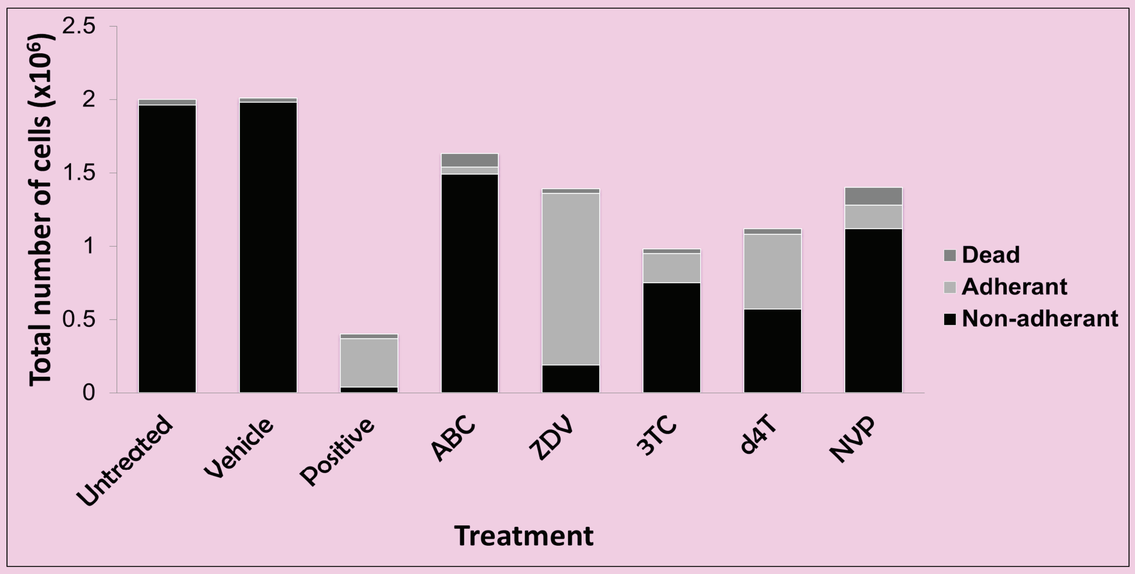

Fig 1: Differential conversion of U937 monocytes to adherent cells by single antiretroviral treatment. U937 cells were treated with 10ng/ml PMA as the positive control (+ve) or treated with single antiretroviral drugs or left untreated (-ve) for 72hrs. The numbers shown represent the estimated total cell count (x10^6 cells) for three independent experiments.

2. METHODOLOGY: U937 cells (a histolytic lymphoma monocyte line) were exposed to a range of drugs used for HAART therapy and differentiation to macrophage morphology assessed for 72 hours in 24 well plates. Cells were assessed for viability, adherence to the place and differentiation to macrophage morphology.

3. RESULTS

Individual and combinations of ARD reduced the viability of U937 cells and also increased the numbers of cells with macrophage morphology (Figure 1). Table1 and Table2 depict the number of cells transformed to macrophage by single and combination antiretroviral treatment respectively while Figure 2 and Figure 3 summarize the total numbers of viable and transformed cells.

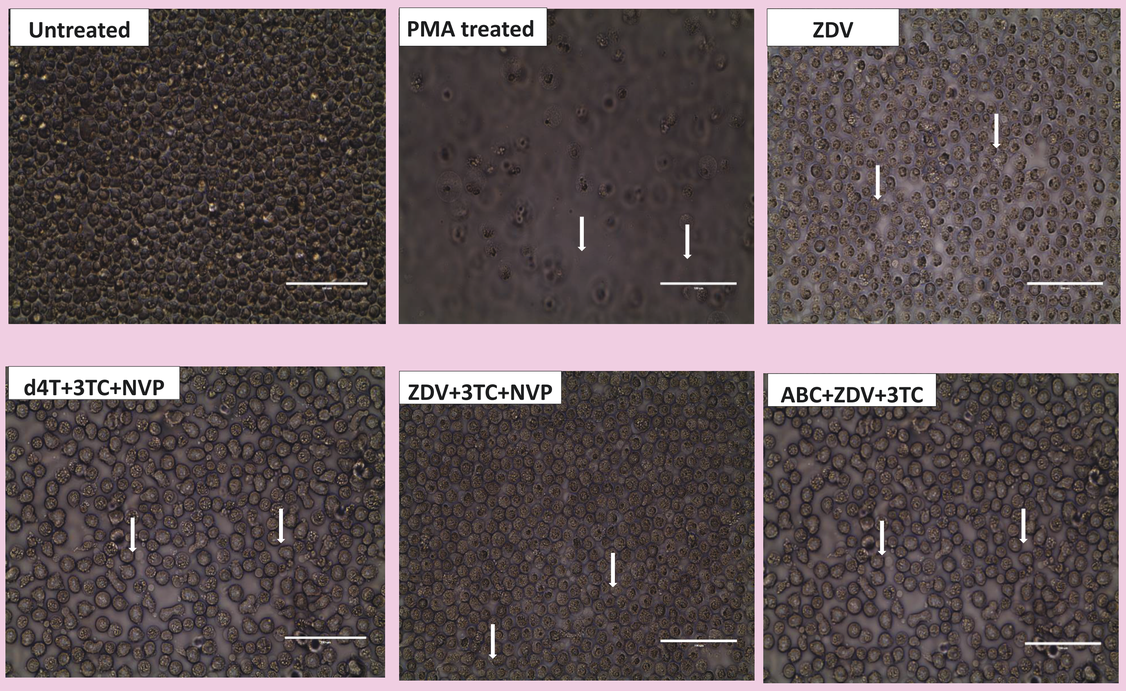

Fig 1: Morphological appearance of cells after 72hr treatment with ZDV and combination antiretroviral treatment. Cells were treated with ZDV or combination drugs at a final concentration of 50µg/ml for each drug and incubated at 37^{O}C and 5% CO2 for 72hrs. A high degree of granulation was observed for ZDV, d4T+3TC+NVP and ZDV+3TC+NVP treated cells. ABC+ZDV+3TC treatment resulted in a mixture of granular and non-granular cells. Arrows indicate typical transformed cells with some distinct granulated morphology.

Table 2: Descriptive statistics for U937 cells pre-treated with combination antiretroviral drugs (n=3, mean ± SD)

Cell type (x10^6)	-ve	+ve	ABC+ 3TC	ZDV+ 3TC	ABC+ZDV +3TC	ZDV+3TC +NVP	d4t+3TC +NVP
Non-adherent	2.03 ±0.12	0.07 ±0.06	1.60 ±0.08	1.40 ±0.12	1.12 ±0.08	0.84 ±0.12	1.07 ±0.14
Adherent	0.00 ±0.00	0.28 ±0.04	0.25 ±0.06	0.12 ±0.04	0.75 ±0.06	0.61 ±0.08	0.71 ±0.17
Dead	0.04 ±0.02	0.04 ±0.04	0.11 ±0.02	0.21 ±0.16	0.09 ±0.02	0.19 ±0.06	0.08 ±0.04

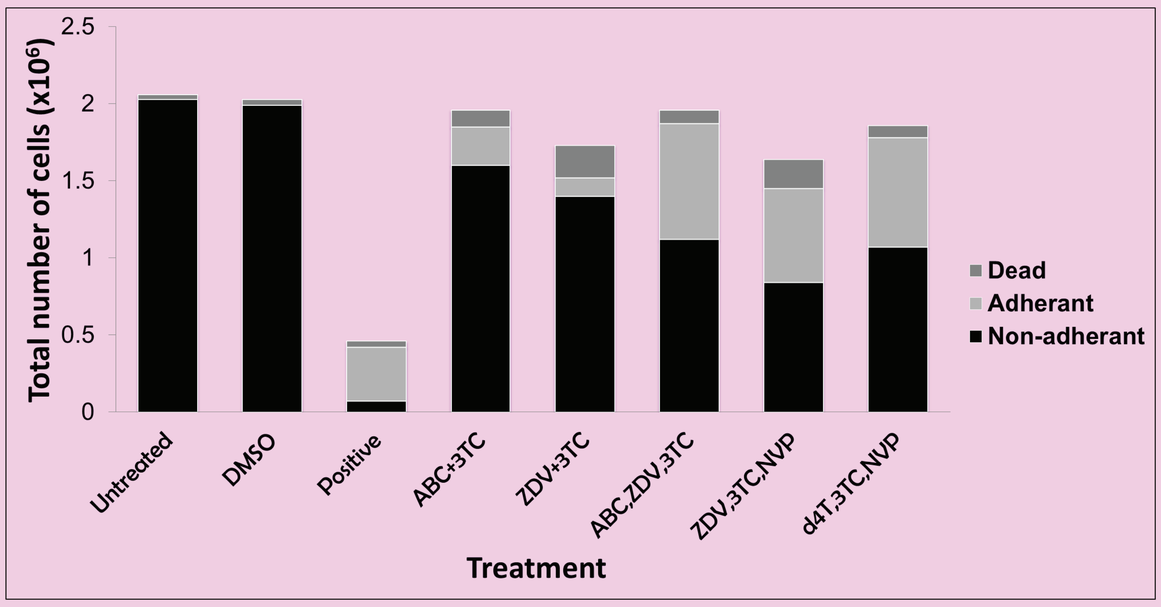

Fig 3: Differential conversion of U937 cells to adherent cells by combination antiretroviral treatment. U937 cells were treated with 10ng/ml PMA as the positive control (+ve) or treated with combination antiretroviral drugs or left untreated (-ve) for 72hrs. The numbers represent the estimated total cell count (x10^6 cells) for three independent experiments.

Table 1: Descriptive statistics for U937 cells pre-treated with single drugs

Cell (x10^6)	-ve	+ve	ABC	ZDV	3TC	d4T	NVP
Non-adherent	1.96 ±0.17	0.04 ±0.04	1.49 ±0.08	0.19 ±0.18	0.75 ±0.08	0.57±0. 06	1.12 ±0.08
Adherent	0.00 ±0.01	0.33 ±0.06	0.05 ±0.02	1.17 ±0.06	0.20 ±0.04	0.51 ±0.08	0.16 ±0.04
Dead	0.04 ±0.02	0.03 ±0.07	0.09 ±0.02	0.03 ±0.02	0.03 ±0.02	0.04 ±0.04	0.12 ±0.04

U937 cells were treated with 10ng/ml PMA as the positive control (+ve) or treated with single antiretroviral drugs or left untreated (-ve) for 72hrs. The numbers represent the estimated total cell count (x10^6 cells) ± SD for three independent experiments.

Conclusion

Overall, adherence of single drug treatment was as follows: 3.25% for a ABC, 86.03% for ZDV, 21.05% for 3TC, 47.22% for dT4 and 12.50% for NVP. The effect of treatment with combination antiretroviral was as follows: 13.51% for ABC+3TC, 7.89% for ZDV+3TC, 40.11% for ABC+ZDV+3TC, 42.07% for ZDV+3TC+NVP and 39.89% d4T+3TC+NVP. Morphological changes including an increase in the cell size were noted predominantly for ZDV and triple antiretroviral drug combination stimulated cells. These initial observations suggest that HAART drugs are capable of stimulating the differentiation of monocyte (pre-macrophage) into cell with some characteristics of differentiated macrophages. If replicated in vivo could go some way to explaining the SNAEs seen with long term HARRT therapy.

References

1. Hsu et al. (2013). AIDS Research and Therapy: 10:29 Availble from: http://aidsrestherapy.biomedcentral.com/articles/10.1186/1742-6405-10-29
2. http://www.who.int/topics/antiretroviral_therapy/en/