CALCITRIOL AND SIDE-CHAIN MODIFIED VITAMIN D ANALOGUES: THEIR IMPACT ON HEALTHY AND MALIGNANT B CELLS

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INTRODUCTION

- Novel vitamin D analogues (VDAs) have potential as therapy for acute myeloid leukaemia (AML).
- Some of the VDAs have been shown to exert potent pro-differentiating and anti-proliferative effects on AML cells.
- This project investigated the impact of VDAs upon healthy and malignant B cells (e.g. diffuse large B cell lymphoma DLBCL).

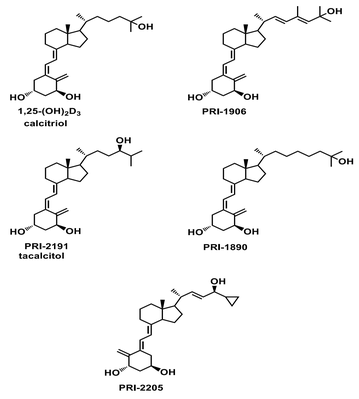

Fig. 1 Molecular structures of calcitriol (1,25D) and some VDAs

AIMS

- To test impact of calcitriol (1,25D) and some analogues upon malignant heamatological cell lines and primary cells from healthy donors and patients
- To investigate the effect of vitamin D and some analogues on viability, proliferation and differentiation of cells
- To assess vitamin D receptor (VDR) expression in the cells under investigation
- To assess the therapeutic potential of vitamin D and some analogues in combination with established and developmental drugs to search for potential synergies to enhance the potential of therapeutic strategies *in vitro*

References:
1. Marcinkowska et al. Steroids 7 3 (2 0 0 8), 1359–1366
2. Sloane et al., Br. J. Cancer 68, (1993), 668-672
3. Kraft et al., Cancer Res. 71(2) (2001), 506–515
4. Chamba et al., Leukemia Research 34 (2010), 1103-1106

METHODS

- Peripheral blood mononuclear cells were isolated from healthy donors by Ficoll gradient centrifugation
- DOHH2 cells were grown in RPMI-1640 at 37ºC with 4% CO_2
- Flow cytometry experiments (PhiPhiLux/PI and proliferation assays) were performed using a ADP Cyan flow cytometer
- SDS-PAGE/Western blotting was performed on the nuclear fractions of cells

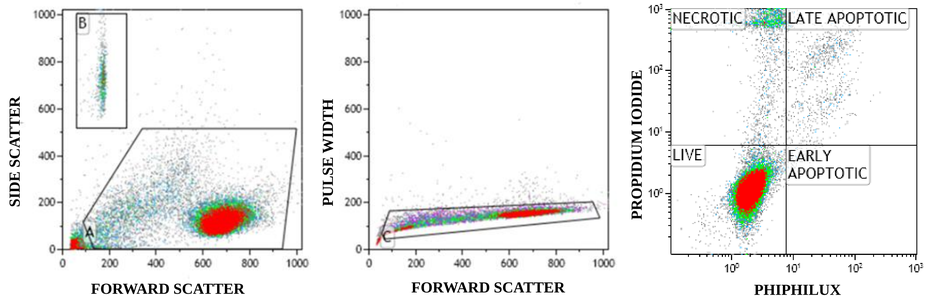

Fig. 2 PhiPhiLux/PI assay gating strategy.

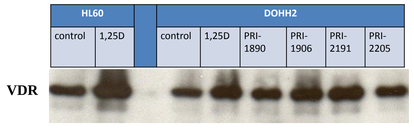

Fig. 3 SDS-PAGE/Western blot analysis of VDR protein upregulation in DOHH2 cells (DLBCL) treated with either calcitriol or VDAs. The highest VDR upregulation in DOHH2 cells has been observed in cells treated with 1,25D, PRI-1906 and PRI-2191. HL60 (AML) cells were used as a positive control.

RESULTS

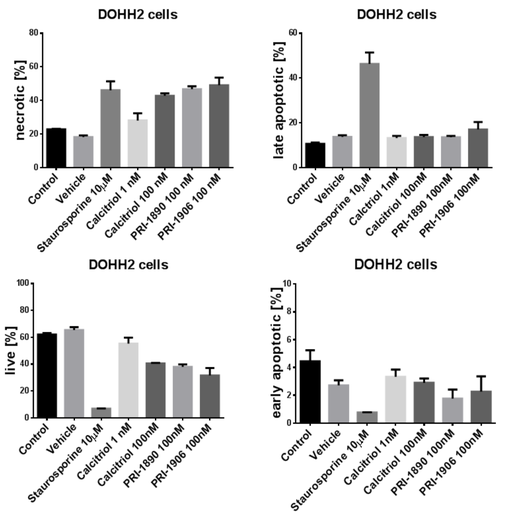

Fig. 4 Impact of vitamin D and some analogues upon viability of DOHH2 cells. Cells were treated for 24 hours. 1,25D and its 2 analogues exhibit cytotoxic effect on DOHH2 Data represented as mean ± SEM.

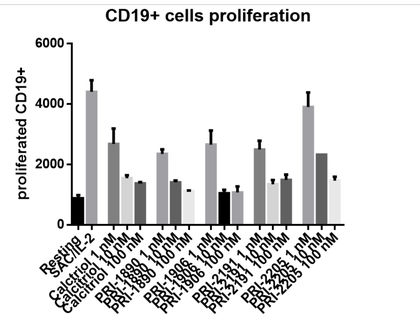

Fig. 5 Inhibitory impact of vitamin D and the analogues on proliferation of *Staphylococcus aureus* Protein A and Interleukin 2 (SAC/IL-2) stimulated CD19+ B cells. PBMCs were stimulated for 7 days and labelled with CD19 antibody. Data represented as mean ± SEM.

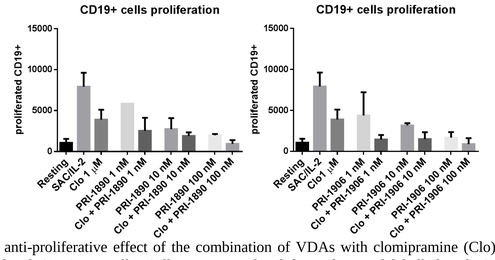

Fig. 6 Additive anti-proliferative effect of the combination of VDAs with clomipramine (Clo) on SAC/IL-2 stimulated CD19+ B cells. Cells were stimulated for 7 days and labelled with CD19 antibody. Data represented as mean ± SEM.

CONCLUSIONS

- DOHH2 express VDR and can be used as a model line to study VDAs effects on DLBCL
- Side-chain modified analogues exhibit moderate cytotoxic and pro-apoptotic activities in DLBCL DOHH2 cells
- VDAs inhibit proliferation of SAC/IL-2 stimulated CD19+ cells

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