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1,25-OH_2 vitamin D_3 and AKT-inhibition increase glucocorticoid induced apoptosis in a model of T-cell acute lymphoblastic leukemia (ALL)

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ABSTRACT

In acute lymphoblastic leukemia (ALL), steroid resistance and hypovitaminosis D are both associated with a poor prognosis. We show that methylprednisolone, calcitriol and the AKT-inhibitor MK-2206 have a synergistic effect on the apoptosis of steroid resistant T-ALL cells. Compared to methylprednisolone monotherapy, calcitriol increases methylprednisolone induced apoptosis dose-dependently (1.37–1.92-fold; $p < 0.05$). Pre-incubation with calcitriol increases the apoptotic effect of MK-2206 even further (3.6-fold; $p < 0.05$). It also potentiates synergism between MK-2206 and methylprednisolone (vehicle control 38% vs. calcitriol 58%, $p < 0.01$). The combination of calcitriol and AKT inhibition should be investigated further as treatment options for steroid resistance in T-ALL.

1. Introduction

Glucocorticoids (GC) are a core component of current treatment protocols in T-cell acute lymphoblastic leukemia (T-ALL) and act mainly through the induction of apoptosis [1]. Nevertheless, GC-resistance is common in T-ALL, which negatively impacts the overall prognosis [2,3]. In addition to GC-resistance, also hypovitaminosis D appears to be associated with a decreased treatment response and a reduced prognosis in patients with hematological malignancies [4]. More than 70% of children with ALL have subnormal levels of 1,25-OH_2 vitamin D_3 (calcitriol), which is the active form of vitamin D [5]. Using primary human T-cells, we recently demonstrated that 1,25-OH_2 vitamin D_3 upregulates the GC receptor and increases GC induced apoptosis [6]. In this study, we aimed to investigate whether there is a synergistic action of calcitriol on GC-induced apoptosis of a steroid resistant T-ALL cell line (Jurkat). Since steroid resistance is also associated with defective IL-7 signaling trough JAK/STAT, PI3K/AKT and MEK [7], we furthermore investigated inhibitors of AKT (MK-2206), JAK 1/2 (ruxolitinib) and MEK (CI-1040) for possible additional synergisms between GC and calcitriol.

2. Methods

Jurkat cells (Clone: E 6-1, kindly provided by the Department of Virology, University of Bochum, Germany; 1×10^7 cells/ml) were cultured in RPMI 1640 (Invitrogen, Carlsbad, USA), 1% penicillin/streptomycin (Invitrogen), 300 mg/l L-Glutamine (Invitrogen) with 10% FCS (Sigma-Aldrich, St. Louis, USA) at stable ambient conditions (37 °C/5% CO_2). First, cells were treated with calcitriol (100 nM, 1 μM; Medchem Express, Monmouth Junction, USA) dissolved in DMSO (final DMSO

Table 1
Methylprednisolone induced apoptosis after 24 h of incubation.

Condition	Mean percentage of apoptotic cells (SEM)	P-value (MP vs. control)
Control	5.9 (0.5)	
MP 6.3 μM	6.3 (0.3)	> 0.05
MP 63 μM	6.7 (0.3)	> 0.05
MP .63 mM	12.1 (0.8)	< 0.05
MP 2.5 mM	42.3 (5.0)	< 0.05
MP 3.75 mM	77.4 (2.9)	< 0.05

Abbreviations: MP: Methylprednisolone, SEM: Standard Error of Mean.

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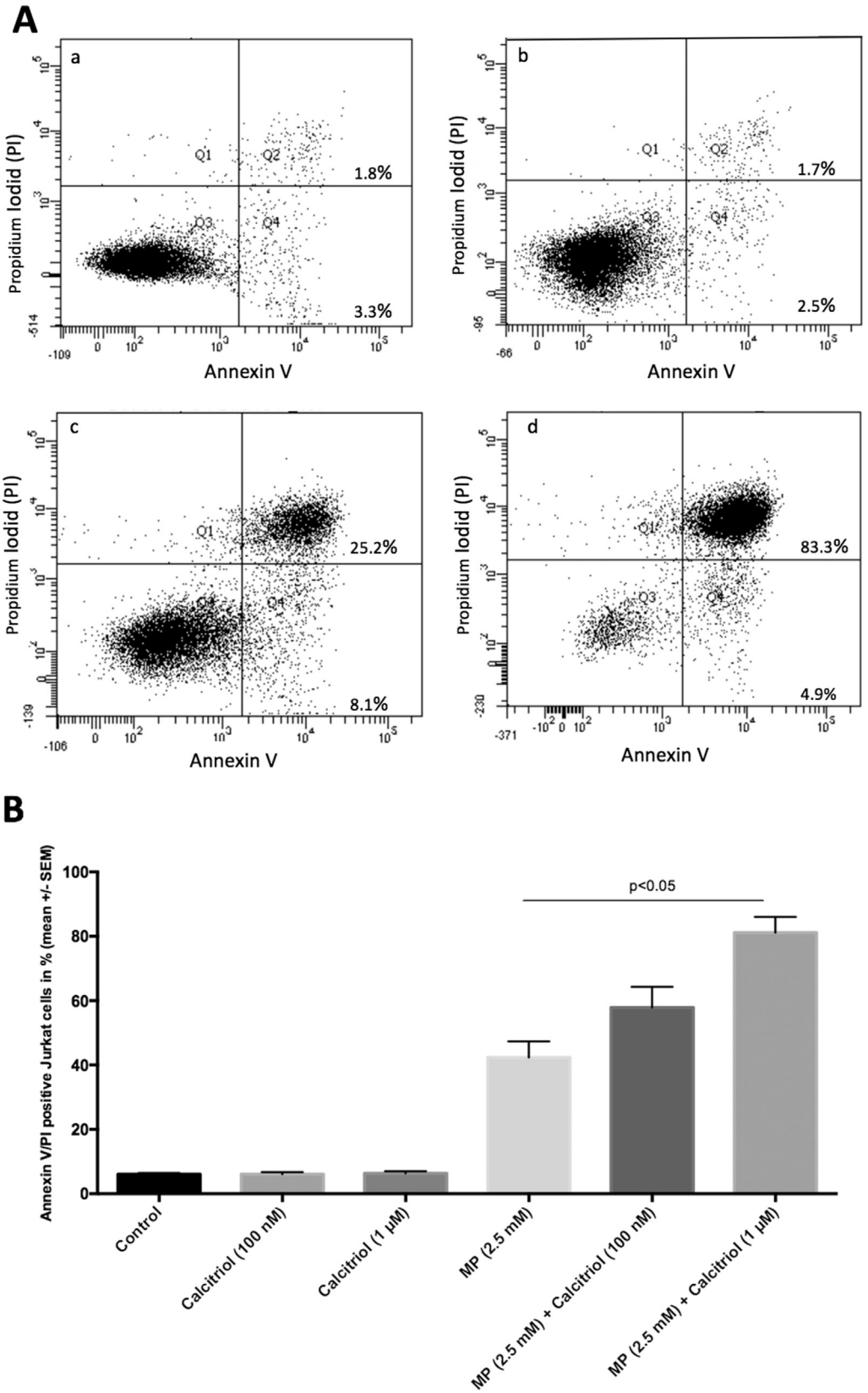

Fig. 1. Synergistic effect between calcitriol and methylprednisolone on Jurkat apoptosis. **A)** Representative dot plot diagram of Jurkat cell apoptosis after 24 h incubation with DMSO-control (a), 1 µM 1,25-OH_2 vitamin D_3 (b), 2.5 mM MP (c) and combination therapy (d). Annexin V/PI flow cytometry staining. **B)** Percentage of apoptotic Jurkat cells (Annexin V/PI) with 1.37 (VD 100 nM) to 1.92 (VD 1 µM) fold increase of MP-induced apoptosis compared to the untreated control. n = 5, WSRT. MP: Methylprednisolone; SEM: standard error of the mean; WSRT: Wilcoxon Signed Rank Test.