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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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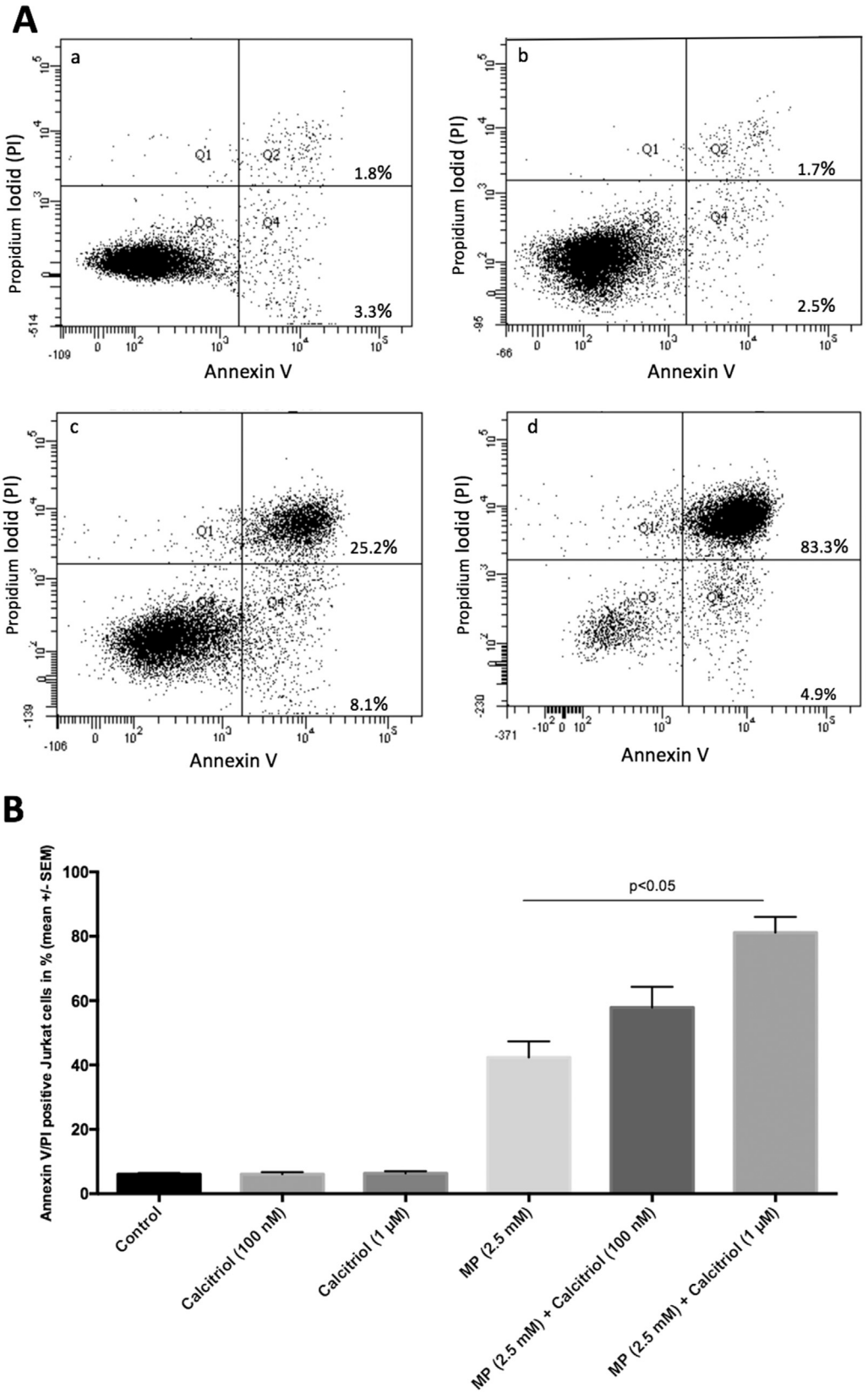

Fig. 1. Synergistic effect between calcitriol and methylprednisolone on Jurkat apoptosis. **A)** Representative dot plot diagram of Jurkat cell apoptosis after 24 h incubation with DMSO-control (a), 1 µM 1,25-OH_2 vitamin D_3 (b), 2.5 mM MP (c) and combination therapy (d). Annexin V/PI flow cytometry 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.

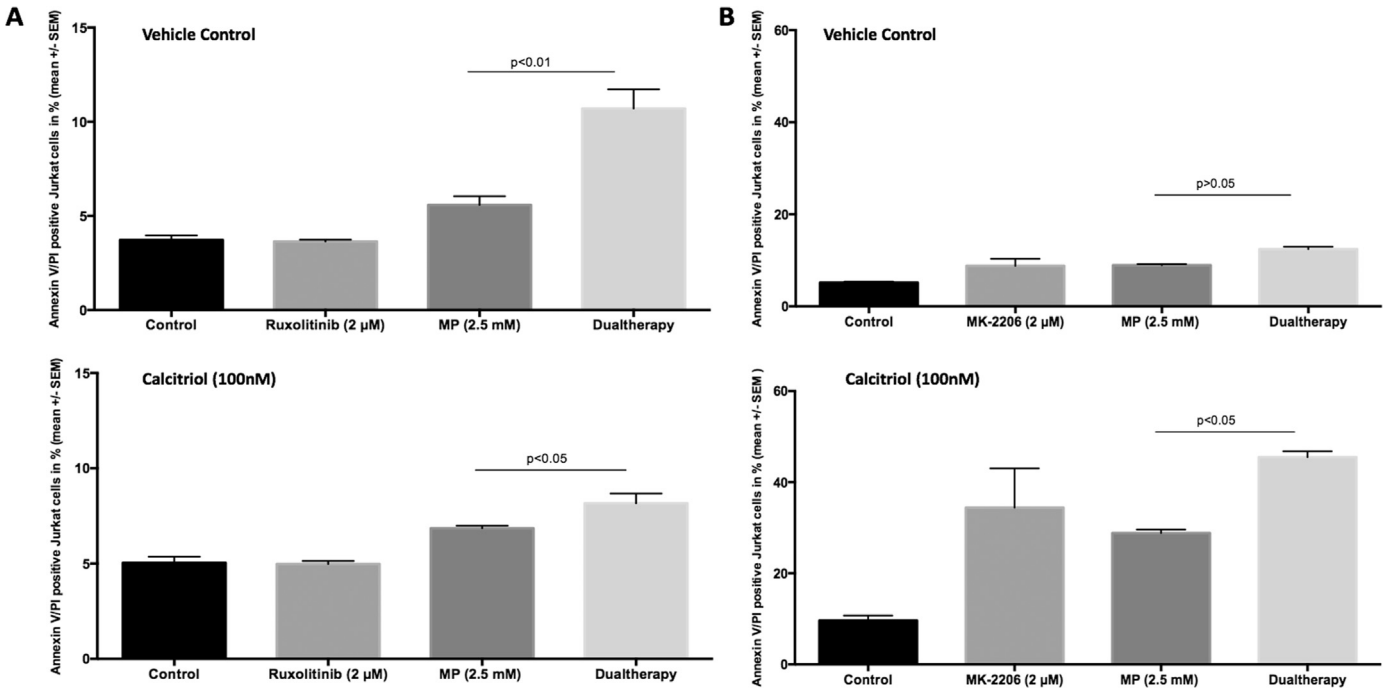

Fig. 2. Ruxolitinib's and MK-2206 synergism with methylprednisolone and the effect of additional vitamin D supplementation. **A)** Ruxolitinib increases MP-induced apoptosis after 24 h pre-incubation with calcitriol (19% increase, $p < 0.05$) or vehicle control (91% increase, $p < 0.01$); n = 10, WSRT. **B)** AKT inhibition through MK-2206 treatment increases MP-induced apoptosis after pre-incubation with calcitriol (58%; $p < 0.05$) or vehicle control for 24 h (38%; $p > 0.05$); n = 5, WSRT. Abbreviations: MP: methylprednisolone; SEM: standard error of the mean. WSRT: Wilcoxon Signed Rank Test.

concentration in all conditions 0.25%), methylprednisolone (MP; 2.5 mM)/dexamethasone (800 µM, 1 mM; Mibe, Brehna, Germany) or the respective vehicle control for 24 h. Second, Jurkat cells were pre-incubated with calcitriol (100 nM) or vehicle control for 24 h. Jurkat cells were subsequently treated with the respective control, MP (2.5 mM), ruxolitinib (2 µM, Selleckchem, Houston, USA)/ MK-2206 (2 µM, Selleckchem)/ CI-1040 (5 µM, Selleckchem), or a combination (inhibitor + MP). After 24 h, apoptosis was evaluated by flow cytometry using Annexin V/PI staining (BD, San Jose, USA). All experiments were repeated 5–10 times. Data are presented as mean and standard error of the mean (SEM).

3. Results

We first investigated whether calcitriol induces apoptosis in Jurkat cells. Calcitriol treatment with up to 1 µM over 24 h did not induce apoptosis in Jurkat cells (1 µM: 6.1% and 100 nM: 6.3% vs. control: 5.9%, each $p > 0.05$). In contrast, MP concentrations above 0.63 mM significantly increased Jurkat cell apoptosis after 24 h (Table 1) compared to the untreated control condition. To evaluate a possible synergism, Jurkat cells were co-incubated with MP (2.5 mM) and calcitriol (100 nM; 1 µM). This dual therapy resulted in a calcitriol dose-dependent 1.37–1.92-fold increase of cell death (Fig. 1 A and B). This effect was not specific for MP since calcitriol also increased dexamethasone (800 µM, 1000 µM) induced apoptosis by 1.51–1.58 fold synergistically with calcitriol (1 µM, $p < 0.05$; Supplementary Fig. 1).

Subsequently, we investigated whether the inhibition of signaling pathways involved in steroid resistance in T-ALL and treatment with MP/calcitriol have synergistic effects on the induction of apoptosis. Jurkat cells were pre-incubated with calcitriol (100 nM) or vehicle control for 24 h. They were subsequently treated with the respective control, MP, the MEK inhibitor CI-1040, the JAK 1/2 inhibitor ruxolitinib and the AKT inhibitor MK-2206 or a combination thereof.

First, CI-1040 failed to demonstrate additional effects on GC apoptosis irrespective of calcitriol supplementation (data not shown). In contrast, the JAK 1/2 inhibitor ruxolitinib showed synergistic effects with MP. However, this effect could not be increased by calcitriol pre-incubation (Fig. 2A). The inhibition of the AKT signaling pathway using MK-2206 also demonstrated a synergistic effect with MP, which further increased through calcitriol pre-incubation (Fig. 2B). Interestingly, also a synergism between calcitriol and inhibition of the AKT pathway was found, which exceeded synergistic effects of MK-2206 and MP (Fig. 2B).

4. Discussion

In our study, we demonstrated that the observed synergism of calcitriol and GC in primary human T-cells [6] can be transferred to a model of T-ALL. This is an intriguing finding as it connects two important observations in ALL patients: (I) a reduced overall prognosis of patients with a poor response to steroids and (II) the deficiency of serum vitamin D_3, especially of serum 1,25-OH_2 vitamin D_3 [3,5]. We additionally investigated to what extent calcitriol acts synergistically with the inhibitors of JAK 1/2, AKT and MEK pathways, which are also relevant for steroid resistance in T-ALL [7]. We identified a synergistic action of calcitriol with the AKT inhibitor MK-2206 alone as well as in combination with MP. Several vitamin D analogs have already shown the ability to inhibit AKT. Therefore, the treatment with MK-2206 and calcitriol could lead to a dual AKT inhibition, which might explain our finding [8]. The presented study bears several weaknesses and should only be interpreted as a pilot investigation. 1,25-OH_2D_3/MP concentrations used in our in vitro analysis are higher than achieved in therapeutic situations. Additionally, conflicting evidence exists concerning the effects of 1,25-OH_2 vitamin D_3 on dexamethasone efficacy in several pre-B ALL cell lines [9]. Nevertheless, our study clearly argues for additional research to investigate the effect of calcitriol on the therapeutic efficacy of glucocorticoids and AKT-inhibition in T-ALL patients and might have clinical implications for steroid resistant T-ALL.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.lrr.2018.01.003.

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