1,25-OH$_2$ vitamin D$_3$ and AKT-inhibition increase glucocorticoid induced apoptosis in a model of T-cell acute lymphoblastic leukemia (ALL)

Maximilian Pistor$^{a, \ast}$, Lisa Schrewe$^b$, Steffen Haupeltshofer$^a$, Andrei Miclea$^c$, Simon Faissner$^d$, Andrew Chan$^b$, Robert Hoepner$^b$

$^a$Department of Neurology, Neuroimmunology Lab, St. Josef Hospital, Ruhr University Bochum, Universitätsstraße 150, 44801 Bochum, Germany
$^b$Department of Neurology, Inselspital, University Hospital Bern, Switzerland
$^c$Medical Faculty, Ruhr-University Bochum, Germany
$^d$Department of Neurology, St. Josef Hospital, Ruhr University Bochum, Germany

**ARTICLE INFO**

**Keywords:**
Calcitriol
MK-2206
Ruxolitinib
Steroid resistance
Jurkat

**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; $1 \times 10^7$ 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

| Condition | Mean percentage of apoptotic cells | P-value (MP vs. control) |
|-----------|-----------------------------------|--------------------------|
| Control   | 5.9 (0.5)                         | > 0.05                   |
| 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.
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-OH2 vitamin D3 (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.
contrast, the JAK 1/2 inhibitor ruxolitinib showed synergistic effects irrespective of calcitriol supplementation (data not shown). In contrast, the JAK 1/2 inhibitor ruxolitinib and the AKT inhibitor MK-2206 or a combination thereof. 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 D3, especially of serum 1,25-OH2 vitamin D3 [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-OH2D3/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-OH2 vitamin D3 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.
References

[1] M. Stanulla, M. Schrappe, Treatment of childhood acute lymphoblastic leukemia, Semin. Hematol. 46 (2009) 52–63, http://dx.doi.org/10.1053/j.seminhematol.2008.09.007.

[2] S. Goossens, P. Van Vlierberghe, Overcoming steroid resistance in T cell acute lymphoblastic leukemia, PLOS Med. 13 (2016) e1002208, http://dx.doi.org/10.1371/journal.pmed.1002208.

[3] H. Inaba, C.-H. Pui, Glucocorticoid use in acute lymphoblastic leukemia, Lancet Oncol. 11 (2010) 1096–1106, http://dx.doi.org/10.1016/S1470-2045(10)70114-5.

[4] A.C. Hall, M.B. Juckett, The role of vitamin D in hematologic disease and stem cell transplantation, Nutrients 5 (2013) 2206–2221, http://dx.doi.org/10.3390/nu5062206.

[5] J.M. Haltun, S.A. Atkinson, L. Fraher, C. Webber, G.J. Gill, S. Dawson, R.D. Barr, Altered mineral metabolism and bone mass in children during treatment for acute lymphoblastic leukemia, J. Bone Miner. Res. 11 (1996) 1774–1783, http://dx.doi.org/10.1002/jbmr.5650111122.

[6] R. Hoepner, S.M. Pittlik, A. Salmen, F. Azakrak, X. Pedreiturria, R. Gold, H.M. Reichardt, F. Löhder, A. Chan, P 1067 - Key regulatory function of vitamin D for response to glucocorticosteroids in multiple sclerosis, Mult. Scler. 21 (2015) 547, http://dx.doi.org/10.1177/1352458515602642.

[7] Y. Li, J.G.C.A.M. Buijs-Gladdines, K. Canté-Barrett, A.P. Stubbs, E.M. Vroegindeweij, W.K. Smits, R. van Marion, W.N.M. Dinjens, M. Horstmann, R.P. Kuiper, R.C. Buijsman, G.J.R. Zaman, P.J. van der Spek, R. Pieters, J.P.P. Meijerink, IL-7 receptor mutations and steroid resistance in pediatric T cell acute lymphoblastic leukemia: a genome sequencing study, Pediatr Med. 13 (2016), http://dx.doi.org/10.1371/journal.pmed.1002200.

[8] A. Datta Mitra, S.P. Raychaudhuri, C.J. Abria, A. Mitra, R. Wright, R. Ray, S. Kundu-Raychaudhuri, 1 alpha,25-dihydroxyvitamin-D3-3-Bromoacetate regulates AKT/mTOR signaling cascades: a therapeutic agent for psoriasis, J. Invest. Dermatol. 133 (2013) 1556–1564, http://dx.doi.org/10.1038/jid.2013.3.

[9] R. Antony, X. Sheng, E.A. Ehsanipour, E. Ng, R. Pramanik, L. Klemm, B. Ichihara, M.S. D., vitamin D protects acute lymphoblastic leukemia cells from dexamethasone, Leuk. Res. 36 (2012) 591–593, http://dx.doi.org/10.1016/j.leukres.2012.01.011.