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Type 4 cAMP phosphodiesterase (PDE4) inhibitors augment glucocorticoid-mediated apoptosis in B cell chronic lymphocytic leukemia (B-CLL) in the absence of exogenous adenylyl cyclase stimulation

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Abstract

cAMP-mediated signaling potentiates glucocorticoid-mediated apoptosis in lymphoid cells, but an effective means by which to take advantage of this observation in the treatment of lymphoid malignancies has not been identified. The primary objective of the current study was to determine whether PDE4 inhibitors, a class of compounds in late clinical development that raise intracellular cAMP levels by inhibiting type 4 cyclic nucleotide phosphodiesterases (PDE4), increase the efficacy of glucocorticoid-mediated apoptosis in leukemic cells from patients with B cell chronic lymphocytic leukemia (B-CLL). Rolipram, a prototypic PDE4 inhibitor, synergized with glucocorticoids in inducing B-CLL but not T cell apoptosis. Rolipram also augmented glucocorticoid receptor element (GRE) transactivation in B-CLL cells. In contrast, inhibition of protein kinase A (PKA) with the cAMP antagonist Rp-8Br-cAMPS reversed both glucocorticoid-induced apoptosis and GRE transactivation. CCRF-CEM cells, a well-studied model of glucocorticoid and cAMP-induced apoptosis, differed from B-CLL cells in that stimulation of adenylyl cyclase with the diterpene forskolin was required to increase both glucocorticoid-mediated apoptosis and GRE activation, while PDE4 inhibition had no effect. Consistent with these results, inhibition of PDE4 induced cAMP elevation in B-CLL but not CCRF-CEM cells, while forskolin augmented cAMP levels in CCRF-CEM but not B-CLL cells. While rolipram treatment up-regulated PDE4B in B-CLL, forskolin treatment up-regulated PDE4D in CCRF-CEM cells. These studies suggest that PKA is required for and enhances glucocorticoid-induced apoptosis in B-CLL by modulating glucocorticoid receptor signal transduction. Clinical trials that examine whether PDE4 inhibitors enhance the efficacy of glucocorticoid-containing chemotherapy regimens in B-CLL are indicated.

Keywords: Glucocorticoid therapy; B-CLL; PDE4 inhibitors; cAMP; PKA; GRE

1. Introduction

Specific subsets of normal and malignant B and T lineage lymphoid cells are unique in their sensitivity to the induction of apoptosis by agents that increase intracellular levels of the second messenger cAMP [1–3]. The same subsets of lymphoid cells are unusually sensitive to the induction of apoptosis by glucocorticoids [4–6]. Several groups have identified similarities in the signaling pathways activated by these two stimuli in such cell types. Early studies demonstrated that certain genes were up-regulated in lymphoid cells by both glucocorticoids and cAMP analogs [7]. Subsequent studies by McConkey and co-workers demonstrated that in CCRF-CEM cells, a human lymphoid cell line derived from a patient with T-ALL, loss of glucocorticoid receptor (GR) led to loss of sensitivity to cAMP-induced apoptosis [8]. Glucocorticoid

Abbreviations: B-CLL, B cell chronic lymphocytic leukemia; PDE4, type 4 cAMP phosphodiesterase

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and protein kinase A (PKA) signaling pathways have also been shown to synergize in inducing apoptosis in glucocorticoid-resistant CCRF-CEM cells [9–11]. Interestingly, cAMP-mediated potentiation of glucocorticoid-induced apoptosis has been reported to be independent of cAMP response element (CRE)-associated transcriptional activation [12]. Most recently, the catalytic subunit of PKA was found to associate with the GR [13].

Although the studies noted above suggest that cAMP-mediated apoptosis in lymphoid cells may be mediated through the GR, the mechanism by which glucocorticoids themselves induce lymphoid apoptosis remains unclear. GR signaling both positively and negatively regulates transcription. While positive regulation of gene transcription is mediated through palindromic GRE elements, several mechanisms for negative regulation of gene transcription by the GR have been described including negative GREs, composite elements and tethering [14–16]. Surprisingly, most of the clinically beneficial activities of glucocorticoids, such as inhibition of lymphoid proliferation and inflammatory cytokine secretion, appear to be mediated by a tethering mechanism, in which GR suppresses NFκB or AP1-mediated transcription in a manner independent of the ability of the GR to bind to DNA itself [17].

The majority of studies examining glucocorticoid and cAMP-mediated apoptosis have utilized leukemic cell lines as the experimental model. Primary leukemic cells differ in numerous ways from such immortalized cell lines, most strikingly in that primary cells fail to proliferate to any significant degree in tissue culture. In this study, we have performed parallel studies of GC and cAMP-induced apoptosis in freshly isolated B-CLL cells and the CCRF-CEM cell line. To stimulate PKA signaling, we have utilized inhibitors of type 4 cAMP phosphodiesterase (PDE4), the predominant cAMP PDE in lymphoid cells [18–20]. Cyclic nucleotide phosphodiesterases (PDEs) are a diverse group of 11 or more enzyme families that catabolize cAMP and/or cGMP [21,22]. PDE4 phosphodiesterases are derived from four genes, PDE4A–PDE4D, each of which generates a variety of PDE4 isoforms as a result of alternative splicing in their amino termini [23,24]. As a result of differential expression and subcellular localization, PDE4 isoforms vary in their signal transduction properties. In some instances, differential localization results from association of distinct splice isoforms of PDE4 with A-kinase-anchoring proteins (AKAPs) which tether PKA holoenzyme along with some PDE4 splice isoforms and other associated proteins into a signaling complex [25].

Our prior studies have demonstrated that rolipram, a prototypic PDE4-specific inhibitor, induces apoptosis in B-CLL cells but not peripheral blood T cells, by a mitochondrial pathway and in a PKA-dependent manner [26–29]. Here, we report that in B-CLL cells, PDE4 inhibitors synergize with glucocorticoids to induce apoptosis and transactivate GRE-containing reporter constructs in the absence of exogenous adenylyl cyclase activation.

2. Materials and methods

2.1. Materials

The following reagents were obtained from commercial sources: cilostamide and rolipram (Calbiochem); forskolin, 1,9-dideoxyforskolin, phenazine methosulfate (PMS) (Sigma); Hoechst 33342 and $DiOC_6(3)$ (3,3′-dihexyloxacarbocyanine iodide) (Molecular Probes); MTS and St-Ht31 AKAP inhibitor peptide (Promega); RO20-1724 (Biomol), (R_p)-8-Br-cAMPS (Biolog).

2.2. Patient selection

Blood samples were obtained by IRB-approved consent from flow cytometry-confirmed B-CLL patients that were either untreated or for whom at least 1 month had elapsed since chemotherapy. Patients with active infections or other serious medical conditions were not included in this study.

2.3. Cell purification and culture

CCRF-CEM cells were obtained from ATCC [31]. Leukemic or normal mononuclear cells were obtained by centrifugation over Histopaque 1077 (Sigma). For purification of T cells, whole mononuclear cells from normal subjects were incubated with magnetic beads coated with appropriate antibodies, then positively purified using a magnet (Miltenyi). Cells were cultured in RPMI 1640 media (Biowhittaker) supplemented with 10% fetal calf serum, 50 μmol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, 10 mM Hepes pH 7.4, 100 μg/ml penicillin, and 100 U/ml streptomycin (Sigma). For isolation of glucocorticoid resistant clones, parental CCRF-CEM cells were treated with 1 μM dexamethasone for 10 days, and surviving cells diluted to $\leq$1 cell/well in a 96-well flat-bottom tissue culture plate, grown for 3 weeks in media supplemented with 20% fetal calf serum and 1% insulin–transferrin–selenium (GIBCO), and then transferred to regular growth medium. A glucocorticoid-resistant subclone (CEM-R8) was completely resistant to dexamethasone-induced apoptosis up to at least 10 μM dexamethasone. For isolation of glucocorticoid-sensitive clones, the same procedure was used, except that treatment with dexamethasone was omitted. A glucocorticoid-sensitive subclone (CEM-S2) was inhibited in its survival by dexamethasone with an $IC_{50} = 0.007$ μM.

2.4. Apoptosis and cell survival assays

Hoechst 33342 and $DiOC_6(3)$ apoptosis assays were performed as previously described [28,32]. For CCRF-CEM cell survival assays, cells were plated at a density of 3×10^4/well in 96-well flat-bottom plates in the presence of test reagents or vehicle in 0.1 mL media. Following