Modulation of \( GILZ \) and \( Erg \) upon Non-Steroidal Treatment in Chicken Leukemic Cells

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ABSTRACT

Background and aims: Glucocorticoids (GCs) have been successfully employed in the treatment of human leukemia; however, those effects can be deteriorated as the metabolic side effects occurred due to the activation glucocorticoid-induced leucine zipper (\( GILZ \)) leading to the induction of the GC resistance which is initiated via the Ets-related gene (\( Erg \)). In the present work, we recruited this antagonist influence on \( Erg \) for aiming to understand the physiological potential that might be of benefit in the treatment of leukemia using a non-steroidal compound (CPDA).

Methods: We examined the effect of dexamethasone, as a steroid, and CPDA, as a non-steroid substance, on the \( GILZ \) and \( Erg \) receptor gene expression in The chicken leukemia cells (CLCs), DT40 cells, after treating them with the drug at 1\( \mu \)M followed by a 24-hour incubation. A real-time-polymerase chain reaction (RT-PCR) method was used to measure gene expression.

Results: An increase (two-fold) in the expression of \( GILZ \) receptor was unveiled. This enhanced-\( GILZ \) model presents the steroidal positive actions and refers to the CPDA ability to mimic this action of steroids. The results also demonstrated a downregulation in the mRNA expression level of \( Erg \), indicating of antagonism of steroids in responding cells and as a positive finding for the effect of the non-steroidal compound A tested.

Conclusions: Steroid-induced upregulation in the \( GILZ \) receptor provides proof of GC undesirable effects leading to correlated \( Erg \)-initiated resistance to the steroids used in exposed cells. Interestingly, CPDA has a GC-based treating activity low \( GILZ \) expression.

Keywords: \( GILZ \), Glucocorticoids, Leukemia.


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Conflict of interest: None

INTRODUCTION

Steroids are the classical therapeutic agents that are used in the treatment of leukemia. They work through the GC receptors (GR)-based activation of the glucocorticoid-induced leucine zipper (\( GILZ \)) gene; however, the therapeutic activities of these GCs can be faced with resistance in humans which can also be seen in an animal through the activation of \( Erg \). The activation of the downstream gene cascades via the GR stimulation indicates the occurrence of GC work. One of the affected genes regulated in a sequent of events occurred in cancer cells is the \( GILZ \), which modulates different physiological functions in cancer cells such as metabolism, especially of lipids and proteins.1 There is accumulating evidence that \( GILZ \), among others, is associated with GC therapy thereby with GR activity.2

Ets-related gene (\( Erg \)) gene encodes a family of ETS transcription factors. Those factors play important roles in regulating the development of embryos, a proliferation of cells, cellular differentiation, the process of angiogenesis, inflammatory responses, and apoptosis. The \( Erg \) protein is expressed and present in the nucleus. The protein has an ETS DNA-binding domain and a PNT (pointed) domain related to the self-association of oncoproteins of a chimeric origin. The process of platelet adhesion to the subendothelium needs the presence of this protein performing remodeling of vascular cells. The protein activity is critical in hematopoiesis regulation and megakaryocytic maturation and differentiation. This \( Erg \) gene participates in the translocation of chromosomes showing the ability to generate proteins from different fusion genes such as NDRG1-\( Erg \) as present in prostate cancer and FUS-\( Erg \) in acute myeloid leukemia. This factor has been found in high levels in mutated tumors,3 and contributes to cancer progression in the affected cells.4 Interestingly, it is well known that in leukemic cell line treated with GCs, the \( Erg \) factor is downregulated in apoptotic cells,5 which gives a strong indicator of unresponsiveness to steroid therapy.

The GCs have been successfully employed in the treatment of human leukemia; however, those effects can be deteriorated as the metabolic side effects occurred due to the activation \( GILZ \)

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leading to the induction of the GC resistance, which is initiated via the Ets-related gene (Erg). In the present work, we recruited this antagonist influence on ERG for aiming to understand the physiological potential that might be of benefit in the treatment of leukemia using a non-steroidal compound (CPDA).

**MATERIALS**

**Treatments**

Steroid: Dexamethasone, PubChem id 5743, Non-steroid compound A (CPDA) MW 264.146100% ethanol 9838147 purchased from enzo. Chicken leukemia Cells, DT40, were derived from chicken infected with avian leucosis virus (ALV) and purchased from ATCC. The duration of treatment was 24h at a dose of 1µM

**Cell Culture**

DT40 cells were grown in DMEM media with antibiotics and FCS and incubated in incubator at a 5% at 37°C.  

**Real-time Polymerase Chain Reaction (PCR) Assay**

One step real-time-PCR (RT-PCR) was carried out according to 7 in which treated cells were harvested, and RNA was extracted by RNeasy Plus Mini Kit (Qiagene-cat 74134). Qiagene RNeasy plus Mini kit was followed by Precision One-Step PLUS TM qRT-PCR for amplification of DNA in Engine Opticon 2 System (Bio Rad). The mRNA levels were expressed as \((2^{-\Delta\Delta Ct}}\).

RNeasy plus Mini kit (Qiagene-cat 74134) was used to extract RNA from DT40 cells, and the amplification protocol of the manufacturer was followed. The primer design; Reverse transcription for 10 minutes at 55°C, enzyme activation set for 2 min at 95°C, Cycling x40 (Denaturation for 10s at 95°C, DATA collection for the 60s at 60°C), and finally the melt curve was managed.

**RESULTS**

**RNA Levels of Investigated Receptors**

Erg levels are decreased upon steroid treatment of 1um for 24h, fig.1A. Non-steroid therapy contributed in a doubled effect of steroids on LL isolated cells, fig 1B. The trend line revealed sharp decline in the amount of indicted gene, Figure 1C.

GILZ levels are increased upon steroid treatment of 1um for 24h, fig.1A, while the non-steroidal therapy contributed to a short increase in GILZ in the LL isolated cells, Figure 1B. The trend line revealed that GILZ was up-regulated upon steroidal treatment twice than the amount of indicted gene upon CPDA use, Figure 1C.

The results revealed that CPDA clearly decreased the expression of the ERG and the GILZ proteins more than that in the case of dexamethasone-treated leukemia cells, Figure 3.

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Figure 1: ERG mRNA levels of lymphoid leucosis infected bursal cells measured by RT-PCR techniques
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DISCUSSION

GILZ upregulation via the use of GC in the treatment of the leukemia cell line agrees with studies who have demonstrated GC related activity due to the presence of GC receptor (GR) gene, which enhances the accumulation of high levels of steroids in adipose tissues. It is well understood that cancer cells show elevated levels of Erg. The demonstrated effect that a downregulation in the mRNA expression level of Erg indicating of antagonism of steroids in responding cells and as a positive finding for the effect of the non-steroidal compound A tested, and this completely agrees with the study that have discovered that Erg deactivation and GR upregulation can elevate the rate of apoptosis in the GC resistant and sensitive leukemia cells.

An increase (two-fold) in the expression of GILZ receptor was unveiled. This enhanced-GILZ model presents the steroidal positive actions and refers to the CPDA ability to mimic this action of steroids. As a rule of thumb, GCs had widely been understood to play big roles only as an anti-inflammatory and immunosuppressive agents producing those effects via a cascade of genes; however, those beneficial influences soon were challenged due to the later occurrence of detrimental activities of those drugs. In cancer cell treatment, it is well-established that GCs represent an important anti-leukemia medicine, and ant-chemo/radiotherapy resulted in inflammatory side effects generated in patients with solid tumors. Those outcomes of using GCs depend on the type of tissue, type of tumor type, type of GC, GR level of expression, and immune cell based signals which may increase the survival or death of the treated cells. One of those GRs is the GILZ gene that provides the human body defense system with functions such as GC based anti-inflammatory and immunosuppressive detrimental outputs due to managing cell differentiation, cycling, and apoptosis. Moreover, it deactivates Raf22/Ras23 downstream pathway genes resulting in providing the system with anti-proliferative activity. This completely approves the present findings related to the effects of the used compound in inhibiting the leukemic cell lines via the activity of the GILZ gene.

CONCLUSION

Dexamethasone is one of the drugs known to generate apoptosis in leukemia cells in acute lymphoblastic leukemia.
However, the vital therapeutic action of this substance is challenged via the extra activation of GILZ receptor that results in metabolic-based side effects finalized by the induction of GC resistance leaving the drug with less or no treating effects. In our results of the current experiment, this profile is not true for CPDA use, which has induced a tiny increase of this GILZ receptor in chicken cells; perhaps, due to posttranscriptional modification. Contrarily, ERG concentrations show an opposite profile for both of them, but with a severe down-regulation upon a non-steroidal treatment, which gives insight to the potential use of CPDA in the treatment of leukemia cells for animals and humans with fewer side effects.

REFERENCES