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Original article

Cationic lipid-conjugated dexamethasone as a selective antitumor agent

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ABSTRACT

Dexamethasone (Dex) is one of the highly potent synthetic glucocorticoids. It exhibits prominent anti-inflammatory but moderate anti-proliferative activities. It is widely used along side chemotherapy to alleviate toxic side effects. Additionally, Dex is also a potent inducer of gluconeogenesis. However, its overuse critically desensitizes cells against chemotherapy. Herein, we report on the development of a new class of cationic lipid–Dex conjugates in which the C-8 carbon chain analogue (DX8) exhibited glucocorticoid receptor (GR)-mediated, caspase-3-assisted, cancer cell-selective anti-proliferative activity. Melanoma tumors in DX8-treated mice exhibited significantly reduced tumor aggressiveness with respect to tumors in Dex-treated mice. Tumor lysates prepared from DX8-treated group showed elevated levels of p53. DX8-treated cancer cells showed clear degradation of kinase JAK3/STAT3 protein levels. Additionally, DX8-treatment decreased the level of VEGFR2 in tumor-endothelial cells implying DX8's anti-proliferative roles in both tumor cells and tumor neovascular cells. Collectively, our results demonstrate potent anti-angiogenic, and selective JAK3/STAT3 down-regulating anticancer characteristics of DX8, a new dexamethasone-based antitumor molecule.

1. Introduction

Glucocorticoid receptors (GR) are nuclear hormone receptors ubiquitously expressed in both cancer and non-cancer cells. GRs play many crucial roles in various cellular mechanisms. These include cellular energy metabolism involving non-carbohydrate precursors for glucose homeostasis, regulation of protein and fat metabolism and various anti-inflammatory and immunosuppressive responses [1,2]. With structural elucidation of ligand-binding domain (LBD) of GR an upsurge of studies ensued to develop selective GR-ligands. These studies led to better understanding of the conformation of ligand–receptor complexes and thereby the fate & functions of GR [3,4]. These also helped in optimizing ligands' anti-inflammatory activities with reduced side-effect profile.

Classically, glucocorticoids (GC) exert their function by binding to the intracellular GR. Upon ligand-binding GR translocates to the nucleus. Therein, it interacts with specific DNA sequences (popularly known as glucocorticoid response elements, GREs), i.e., with promoter sequences of target genes, resulting in their transcriptional activation [5]. For GR-targeting and transactivation, we chose to use the synthetic GC ligand Dex instead of natural ligand cortisol due to its superior activity in transactivating glucocorticoid responsive genes than cortisol. Additionally, Dex exhibits effective binding with even mutated LBD [6].

Dexamethasone (Dex) is a widely used synthetic GC for inducing apoptotic cell deaths in malignant lymphoid and in other cancer cells [7,8]. In general, these GCs are used in patients during and post-chemotherapeutic treatment of solid tumors to control dehydration, nausea, acute toxicity, edema, and pain towards protecting normal tissues of cancer patients against long-term effects of genotoxic drugs [9]. Dex also exhibits contrasting pharmacological properties. In one hand, Dex exhibits anticancer activity [8]. One of the possible anticancer mechanisms elucidated so far involves the regulation of STAT kinases [10]. On the other hand, Dex induces insensitivity in cancer cells against anticancer drugs [11,12]. So the restricted and carefully regulated use of Dex is needed for effective anticancer treatments.

Previously, we showed that a Dex-associated cationic liposomal gene delivery system could selectively transfect cancerous cells and tumor *in vivo* via GR [13]. This selectivity was observed in spite of

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GR's ubiquitous presence in both cancer and non-cancer cells. In this liposomal system Dex was associated as a co-lipid with other lipids. We questioned: if, instead of mere association as a co-lipid in a liposome, Dex is conjugated with a cationic lipid will it be able to exhibit selective anticancer effect? To this end, we undertook chemical conjugation of 'cationic, twin carbon chain-length lipid' with Dex. Lipids with cationic head-groups have inherent affinity toward negatively charged cell membranes resulting in enhanced interaction with trans-membrane proteins [14]. Thus, cationic lipid-conjugated Dex is expected to have more availability and local concentration inside the cells than free Dex. We found that this cationic Dex derivative could maintain uncompromised GR-transactivation property of Dex.

Recently we reported that similar cationic lipid-conjugation to estrogen, the natural ligand for estrogen receptor (ER), converted the estrogen molecule into a very potent anti-breast cancer agent. The molecule acts through induction of apoptosis and autophagy in breast cancer cells [15]. The cationic lipid-conjugation also converted haloperidol into a potent anticancer agent [14]. Subsequently, others have also shown the pharmacologic benefit of cationic lipid conjugation to benzamide [16]. These prior studies clearly exemplify that cationic lipid conjugation (or 'lipidation') of known pharmacophores holds promise toward developing new class of drug molecules. On the basis of these recent observations (Scheme 1A), we sought to develop newer cancer targeted molecules through conjugation of cationic lipid to GR ligand, Dex. We hypothesized that the modified Dex-molecule will resurrect programmed cell death machinery against aggressive cancer. Herein we report on the development of a new kind of cationic Dex-derivatives. We show that the lead molecule DX8 exhibited GR-mediated, effective tumor growth inhibition presumably through down-regulation of JAK-STAT pathway with concomitant up-regulation of p53.

2. Results and discussion

2.1. Chemistry

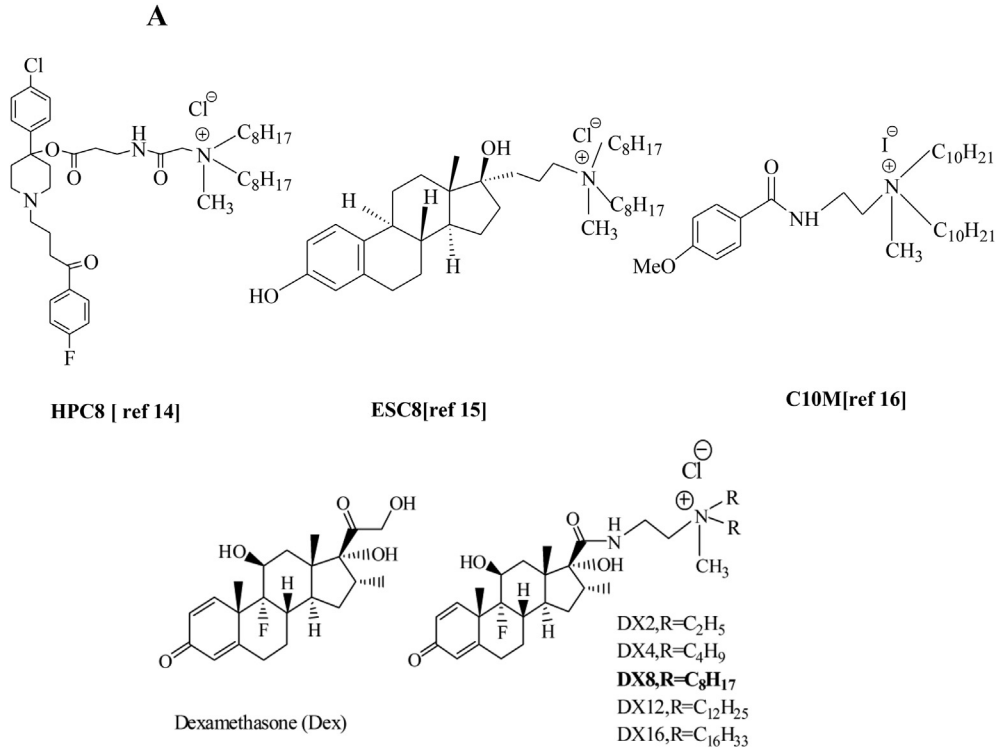
2.1.1. Syntheses of Dex-derivatives

In the present study, we chemically conjugated cationic twin-carbon chain lipid with Dex. Since different Dex derivatives differed by respective lengths of carbon chain linked to quaternary Nitrogen atom, a general procedure was used to synthesize them (Scheme 1B). At first the amino group of *tert*-butyl N-(2-aminoethyl)carbamate was reacted with alkyl bromides of different carbon chain lengths to obtain respective tertiary amines (**1a**–**e**). This was followed by N-deprotection to give free 1^0 amines (**2a**–**2e**). Separately, Dex was oxidized using $NaIO_4$ to produce free carboxylic acid functionality-containing compound **3**, which contains one less carbon than the Dex [17]. The compound **3** was coupled to free 1^0 amine linked to dialkyl amine moiety (**2a**–**2e**) using EDCI/HOBT/DMAP, to produce amide products (**4a**–**4e**). The resulting tertiary amine upon quaternization with methyl iodide followed by chloride ion exchange, afforded pure target lipids (**5a**–**5e**) [DXn, where $n = 2$, 4, 8, 12, and 16 numbers of carbon-containing long chains] (Scheme 1A, B). All Derivatives were characterized using mass and NMR spectrometry (S1–S43). The purities of the respective compounds were determined and confirmed by HPLC (S44–S48).

2.2. Biology

2.2.1. Screening of Dex-derivatives for selective cytotoxicity

For screening the anticancer property of the newly synthesized Dex-derivatives we chose cancer cell lines (B16F10, MCF-7, and A549) and non-cancer cells or cells of primary origin (CHO, HEK293T and mouse skin fibroblast, FB). Both cancer and non-



Scheme 1. A: Chemical structures of cationic lipid conjugated anticancer agents, dexamethasone (Dex) and newly synthesized cationic Dex-derivatives. B: syntheses of dexamethasone derivatives.