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Research paper

Cationic lipid-conjugated hydrocortisone as selective antitumor agent

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ABSTRACT

Hydrocortisone, the endogenously expressed steroidal, hormonal ligand for glucocorticoid receptor (GR), is body's natural anti-inflammatory and xenobiotic metabolizing agent. It has both palliative as well as adverse effects in different cancer patients. Herein, we show that conjugation product of C16-carbon chain-associated cationic lipid and hydrocortisone (namely, HYC16) induces selective toxicity in cancer (e.g. melanoma, breast cancer and lung adenocarcinoma) cells with least toxicity in normal cells, through induction of apoptosis and cell cycle arrest at G2/M phase. Further, significant tumor growth inhibition was observed in syngeneic melanoma tumor model with considerable induction of apoptosis in tumor-associated cells. In contrast to hydrocortisone, significantly higher anti-angiogenic behavior of HYC16 helped in effective tumor shrinkage. This is the first demonstration to convert natural hormone hydrocortisone into a selective bioactive entity possessing anti-tumor effect.

1. Introduction

Hydrocortisone (HYD) or cortisol is the natural, stress-induced, steroidal hormone ligand for one of the nuclear hormone receptors namely, glucocorticoid receptor (GR). Upon HYD binding GR classically acts as transcription factor regulating the expression of various glucocorticoid responsive genes by acting on respective promoter elements called glucocorticoid responsive element (GRE) [1–3]. This is grossly called 'GR-transactivation'. HYD as a naked drug and synthetic glucocorticoids are used as key clinical components for the treatment of autoimmune disorders, inflammatory diseases and specific hematologic cancers [1,4,5]. Synthetic glucocorticoids are also associated with various delivery systems to reduce inflammation around the device towards imparting biocompatibility [6–8]. However, regarding the treatment of cancer their effect is truly limited due to the induction of refractory effects following their long-term use in cancer patients. In contrast, HYD or its oxidized natural derivative cortisone is clinically known for long as one of the palliative medicines for cancer patients [5]. But, late evidences indicate that HYD-expression level increases during cancer growth and may have pro-malignancy effects [9–14]. GR is also linked to various cellular proliferating factors related to cancer development [15–17]. Not limited to these but all these examples indicate that HYD and its receptor (i.e., GR) are critically involved in various human cancer pathophysiologies. Therefore, GR and its hormone are pharmacologically important targets for clinical interventions against cancer. However, structure-activity study related with structural modification of HYD is critical as any irrationality in the design may cause inadvertent, non-specific side effects.

Recently we found that as to GR-functioning, cancer cells behave differently than non-cancer cells. Upon treating dexamethasone (i.e., Dex, a synthetic, anticancer, GR ligand)-associated cationic liposomal gene delivery agent to different GR-expressing cells, GR-mediated transfection was observed selectively in only cancer cells. As a result a new form of selective anticancer therapeutic was developed [18]. Further we showed that even short chain (C8) cationic lipid-dexamethasone conjugate could induce selective anticancer activity [19]. Similar cationic lipid conjugation of estradiol (endogenous ligand for another nuclear hormone receptor called estrogen receptor) also yielded highly selective anti-breast cancer agent [20]. All these studies generalize that cancer cell-associated nuclear hormone receptors such as GR can be selectively targeted by properly designed molecules, more so if it is derived from endogenous ligands such as HYD. However, unlike Dex, unambiguous anticancer effect of naturally occurring HYD is

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not established. There is also no past precedence about development of new therapeutic molecule especially of anticancer in nature, bychemical modification of HYD as such. In the present study we demonstrate cationic lipid modification of HYD (Scheme 1) towards exhibiting highly selective anticancer property. It was challenging because of HYD's dubious role in cancer. Moreover, its receptor GR is also non-specifically expressed in most, if not all cells of cancer and non-cancer lineage [21].

2. Results and discussion

2.1. Synthesis of hydrocortisone derivatives

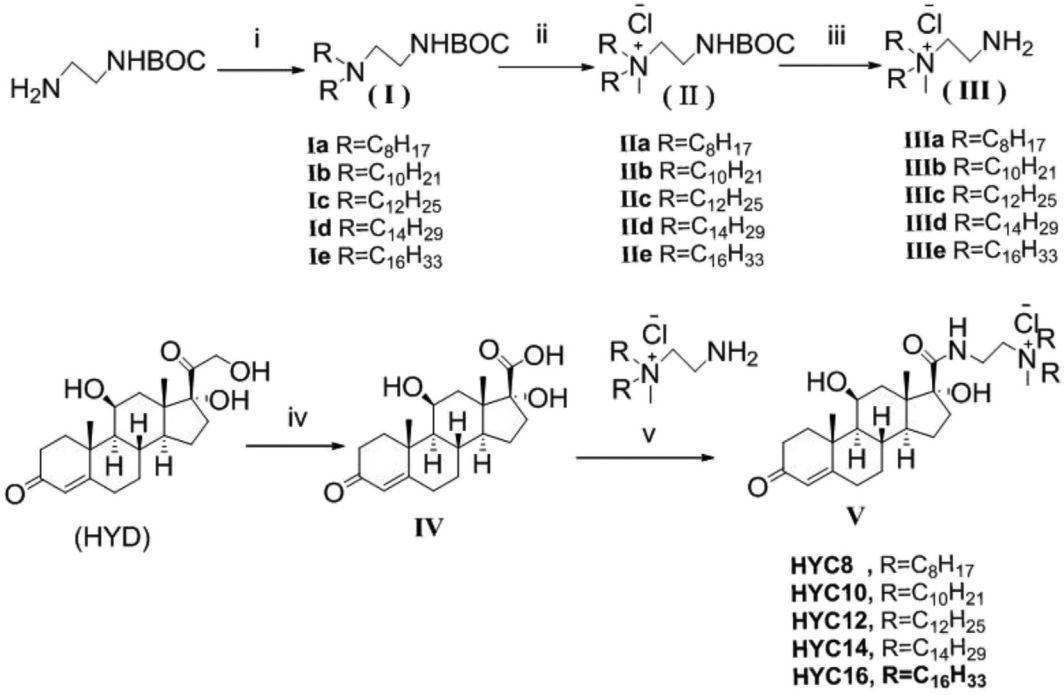
As shown in Scheme 1, the synthesis was easily accomplished for hydrocortisone-conjugated cationic lipid derivatives of carbon chain lengths between 8 and 16 using a convergent synthetic scheme. Firstly, the syntheses of various precursor cationic lipids were accomplished, sequentially by incorporating twin aliphatic chains of various lengths to a mono-BOC (tert-butyloxycarbonyl) protected ethylenediamine. This was followed by quaternization with methyl iodide and then converting its anion into chloride. Finally, the precursor was readied for further reaction after BOC-deprotection (**III**). On the other hand, HYD was oxidized into carboxylic acid form (**IV**) [22], which was then reacted with cationic lipid derivatives with free amine (**III**) to give respective amides and final five products of varying carbon chain lengths (**V**). Following column chromatography and preparative thin-layered chromatography the desired products were isolated, purified and characterized.

2.2. Selective toxicity of HYC16 against cancer cells

Next, the final products were then tested for their cellular toxicities against various cells. Mouse melanoma (B16F10) cells, human cancer cells of lung (A549), breast (MCF-7), ovarian (SKOV3), and cervical (Hela) origins were used. Side-by-side, non-cancer cells such as mouse macrophage (RAW 264.7), mouse fibroblast (NIH3T3) and monkey kidney (COS-1) cells were also included. Table 1 exhibits IC50s (concentration of molecules inducing 50% killing of cells) of respective molecules in cancer and in non-cancer cells. HYC10 and HYC12 (except in A549) induced efficient killing of both cancer and non-cancer cells, and hence were not considered as cancer selective. The toxic effects of HYC8 and HYC14 showed confusing trend as they showed cytotoxicity and also non-toxicity in both cancer and non-cancer cells. On the contrary, the parent molecule HYD exhibited no cell killing effect in the given experimental condition. But clearly, HYC16 not only showed lowest IC50s in cancer cells but also had relatively low cytotoxicities in non-cancer cells. This clearly exhibits HYC16's selective killing of cancer cells over non-cancer cells. The control C16 molecule [compound **IIe**] showed much lower cytotoxicity in either cancer or in non-cancer cells (Supplementary Fig. S1). Furthermore we found that the mixture of parent molecule HYD and C16 cationic lipid did not generate significant toxicity towards tested cancer cells (Supplementary Fig. S2). Treatment of cationic lipid either individually or in addition with HYD has only 20–35% toxicity at two different concentrations, whereas, HYC16 (conjugation product of both) has >90% toxicity in melanoma cells (Fig. S2). This indicates that it is the chemical conjugation of the two parent molecules which is responsible for the induced toxicity.

2.3. HYC16 selectively induces apoptosis in cancer cell

To explore the apoptosis inducing effects of the synthesized hydrocortisone-based derivatives in normal and cancer cells, Annexin V/Propidium iodide (PI) binding based flow cytometric apoptosis assay was performed. Incubation of A549 and B16F10 cells for 24 h with HYC16 (20 μM) significantly raised the



Scheme 1. Synthetic strategy for the syntheses of hydrocortisone derivatives (HYCn): Chemicals and reagents: (i) $C_nH_{2n+1}Br$, K_2CO_3, Ethyl acetate, 48 h reflux, (ii) CH_3I, K_2CO_3, 12 h, RT, followed by chloride ion exchange by Amberlite IRA 400Cl, Methanol. (iii) TFA/DCM (1: 2), 4 h, RT (iv) $NaIO_4$, H_2SO_4, H_2O, Ethanol stirring 12–14 h (v) EDCI, HOBT, DIPA, DCM, 12 h, RT.