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# Folate-cyclodextrin Conjugate for Targeted Chemotherapy

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# Folate-Cyclodextrin Conjugate for Targeted Chemotherapy

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## Abstract

A general disadvantage of chemotherapy is that the drugs cannot discriminate between fast-growing cancer cells and normal healthy cells which causes many side effects such as hair loss. An ideal solution to current methods of chemotherapy would be the development of a carrier for an anticancer drug which would be able to transport the drug and therefore target only cancer cells and release the drug molecules inside the cells. In this work folic acid (FA) is attached to an aminoalkane derivative of β-Cyclodextrin (CDEn) with a view to developing novel drug delivery systems for therapeutic purposes. Cytotoxicity of folate-conjugate (CDEnFA) is tested on folate over-expressing cell line (HeLa cells) and folate receptor deficient cell line (A549 cells). $EC_{50}$ values compared to standard (Cisplatin).

## Introduction

Folate receptors are highly expressed in a range of solid tumor including breast and prostate cancer[1]. The reported frequency of folate receptors overexpression to other cancer cells is 40-80%[1]. Folate receptor overexpression is an important factor in anticancer targeting development. Many researchers worldwide suggest that the introduction of a substance that has a high affinity to folate receptors to an anticancer agent would increase drug selectivity and hence lead to a decrease of common side effects associated with chemoterapy[2]. Several drug conjugates are currently used in clinical cancer treatment, and more are in the pipeline for clinical development[2]. Many of them are a combination of folic acid and an anticancer agent. In this work Folic acid was covalently bonded to a cyclodextrin derivative (CDEn) via a peptide bond to give a Folate-CDEn conjugate. Once the conjugate was prepared and characterized its toxicity needed to be tested. Number of cytotoxicity tests for accurate assay are available. These assays depend on the cell death mechanism[3]. MTT (3-(4,5-dimethylthylthiazole-2-yl)-2,5-diphenyltetrazoli umbromide) is a widely used method to assay cell viability. The MTT substance is reduced by mitochondrial dehydrogenases in living cells to a blue-mageta coloured formazan precipitate. The absorbance of dissolved formazan in the visible region correlates with the number of intact active cell[3]. Cytotoxic compounds such as cisplatin are able to damage and destroy cells, and thus decrease the reduction of MTT to formazan. Using MTT assay the toxicity of CDEnFA was determined and expressed as an EC50 value which was compared with the EC50 value of cisplatin.

## Materials and Method

The CDEn was prepared from β-Cyclodextrin as reported in Potter *at al* [5] .*The* Folate conjugate has been prepared by the reaction of folic acid with CDEn in pyridine as seen in Figure 1 below

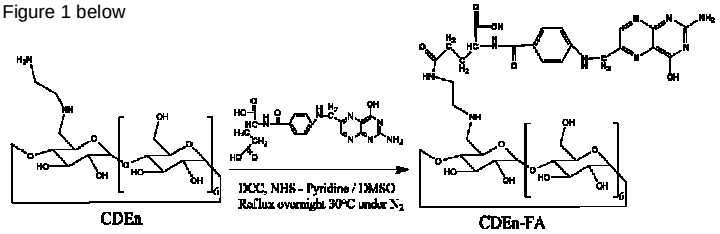


***Figure 1- synthesis of CDEnFA conjugate***

A lung cancer cell A549 and breast cancer HeLa cells were cultured in Mega Cell™ RPMI-1640 suplplemented with penecillin G (100U/ml), L-glutamine, and 15% fetal calf serum at 37ºC in incubator containing 5% $CO_2$. Cisplatin 5mM stock solution was prepared in 10% NaCl. CDEnFA 5mM stock solution was prepared in DMSO. Cells were seeded at density of 2000 cells per well of 96-well plates in 100µL medium. After 24 h drugs ( 0.5 – 50 µM) were introduced for 24h and 96 h of drug exposure.

## Discussion

A CDenFA conjugate for potential delivery of antitumor argent has been obtained employing DCC-NHS peptide bond formation. The reaction includes formation of the intermediate active ester (the product condensation of the carboxyl group and N-hydroxysuccinimide) that further reacts with the amine function to yield an amide bond. MTT assay was employed to determine whether CDEnFA conjugate has any toxicity effect on chosen tumour cells. Results were obtained and expressed in form of $EC_{50}$ values. This also refers to the concentration of a drug, antibody or toxicant that induces a response halfway between the baseline and maximum after some time of exposure. In this study the toxicity of Cisplatin (0.5-50µM) was also found and used as standard. Percentage of violability of A549 and HeLa cell line presented in Figure2 (B), and (D) respectively. According to calculated $EC_{50}$ values (Table 1) cisplatin has three times greater toxicity for HeLa cell line than for A549. These results agree favourably with Li Bai *et al*[1] who reported $EC_{50}$ of cisplatin (74h exposure) to be 0.7 µM and Giuliano Ciarimboli *et al*[4] who reported $EC_{50}$ of cisplatin (24h exposure) to be 100.4µM.
Results obtained for CDEnFA conjugate can be seen in Figure 2 A549cell line (A), and HeLa cell line (C). Calculated $EC_{50}$ values (listed in Table 1) suggest that conjugate itself has no toxicity effect for A549 or HeLa tumour cell. In both cases EC50 values were nearly ten times higher than those for cisplatin.

## Conclusion

Preliminary investigations suggest that the formation of peptide bond in CDEnFA conjugate was successful. Conjugates main function is to deliver anticancer agent specifically to cancer cells. The conjugate was tested in *Vitro* using MTT assay. Obtained $EC_{50}$ values suggest relatively low toxicity when compared to cisplatin ( ten times lower).

## Results

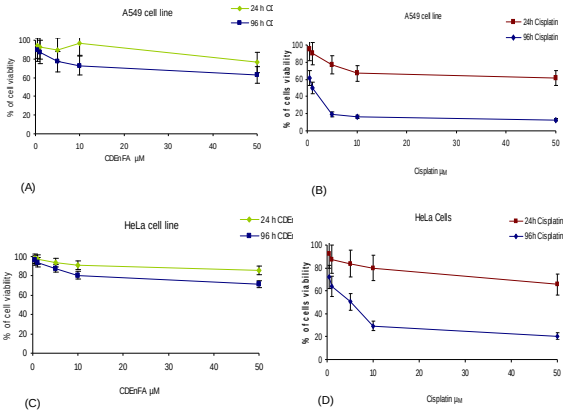


***Figure 2- Effect of cisplatin(0.5-50µM) and CDEnFA(0.5-50µM) on the viability of (A),(B) A549 cell line and (C),(D) HeLa cell line***

***Table 1- EC50 values of different drugs in A549 and HeLa cells(µM)[a] ± SD***

| | A549 24h | A549 96h | HeLa 24h | HeLa 96h |
|---|---|---|---|---|
| Cisplatin | 106.49±10.44 | 0.9±0.05 | 305.95±26.14 | 3.20±0.482 |
| CDEnFA | 1215.55±658 | 215.15±24.50 | 360.43±114 | 3604±1658 |

[a]$EC_{50}$ value correspond to the concentration (µM) of the compound necessary to reduce activity of to 50%

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