ADENOSINE RECEPTORS LIGAND MODULATED NANOPARTICLE TARGETING TO HUMAN BREAST CANCER CELLS VIA OVER EXPRESSED ADENOSINE RECEPTORS.

Rajan Swami¥, Indu Singh¥, Manish Kumar Jeengar¥, V.G.M. Naidu¥,Wahid Khan¥, Ramakrishna Sistla¥*

¥ National Institute of Pharmaceutical Education and Research (NIPER) Hyderabad, Andhra Pradesh, 500037, India

*Medicinal Chemistry and Pharmacology Division, Indian Institute of Chemical Technology (IICT), Hyderabad, Andhra Pradesh, 500007, India

Abstract

G protein coupled cell surface adenosine receptors (ARs) are found to be up regulated in various tumor cells like breast, prostate and brain tumors. The present study was performed to investigate the efficiency of adenosine receptors ligand to target human breast cancer cell lines MCF-7. Solid lipid nanopartlces (SLN) were prepared by solvent emulsification and evaporation process and further evaluated by various techniques like Dynamic light scattering (DLS). Drug loaded SLN were surface modified with ARs ligand using carbodiimide coupling. Conjugation was confirmed using Infrared spectroscopy (IR). *In vitro* drug release was performed by dialysis bag method and conjugated SLN were found to give better sustain drug release as compared to unconjugated nanoparticles and drug solution. Cell toxicity assay were executed and results were encouraging with remarkable decrease in IC50 values as compared to drug encapsulated unconjugated lipidic nanoparticles and drug control and these results were further substantiated by improved cell uptake assay. Hence, this novel ARs ligand has the capability to target breast tumors and incorporating this ligand on surface of SLN modulates the delivery of nanoparticles specifically to ARs overexpressed tumos.

Keywords: Adenosine, breast cancer, receptors, solid lipid nanoparticle, conjugation

INTRODUCTION

Breast cancer remained the most common cancer in women in 2013 and its incidence continues to rise. Nonetheless, mortality is falling, partly as a result of earlier diagnosis through mammographic screening,improved surgical techniques and attention to margins, improved delivery of radiotherapy, and better adjuvant medical therapies. Despite these improvements, breast cancer remains the second most common cause of death from cancer in women.

Over expression of certain receptor on cancer surface enable them to become hyper responsive to the ambient levels of growth factor that normally would not trigger proliferation. With this advancement of understanding of receptor's role in cancer biology it become mandatory to use such information to target and eradicate cancer [1], [2].

G protein coupled cell surface adenosine receptors (ARs) are found to be upregulated in various tumor cells like breast, prostate and brain tumors. ARs are over expressed on many tumor cell surfaces like prostate and breast melanoma etc. There are numerous reports of a potential role of adenosine and ARs in breast cancer. It was stated that ADN (natural agonist) and other agonists of AR may be useful for eradicating or suppressing the growth of tumor malignancies [3], [4].

Nanotechnology unlocked many gates to numerous problems associated with unfair delivery of anti cancer drugs to targeted area. Solid lipid nanoparticle (SLN) are efficient way to deliver such drugs because of their biocompatible nature, small size, higher encapsulation of hydrophobic drugs and sustain drug release kinetics [5], [6]. Solid lipid nanoparticles found renowned attention because of its lymph targeting potential which further helps in increasing bioavailability and targeting to breast cancers etc. [7].

Ligands can be conjugated on the surface of SLN w.r.t to surface groups, which can further be modified with proper use of charge modifying lipids like stearic acid or stearylamine etc. These nanoparticles then assist in delivery of therapeutic molecules by EPR effect or active targeting [8].

The present study was performed to investigate the efficiency of AR ligand i.e ADN to target AR over expressed cancer cell lines. Stearic acid was used as charge modifier and to create carboxylic groups on the surface of SLN. In addition, stearic acid also helps in physical stability of SLN.

MAIN TEXT (EXPERIMENTAL, RESULTS AND DISCUSSION)

SLN preparation

SLN were prepared by solvent emulsification and evaporation process using glyceryl monostearte, soy lecithin and stearic acid in lipid phase. Process optimization was done to get particles with good size and encapsulation efficiency.

ADN conjugation to SLN surface

ADN was covalently coupled to the SLN surface using EDC/NHS chemistry. The nanoconujugate using docetaxel (DTX) as a model drug was physicochemically characterized further.

Particle size and Zeta potential: Particle size and zeta potential of blank and drug loaded SLNs were measured by photon correlation spectroscopy using Zetasizer, Nano ZS (Malvern Instruments, UK). Particle size of DTX loaded SLNs and ADN-SLNs were respectively 76.43 ± 6.05 and 90.77 ± 2.50 nm. Increase in particle size was observed after ligand conjugation. However, the zeta potential of ADN-SLNPs was significantly decreased in comparison with other SLNPs proving surface group modification.

Conjugation characterization using IR Spectroscopy

Amide bond formation between the ligand and SLNs confirmed the conjugation using IR spectroscopy (Figure 1). This amide bond formation occurred between the ligand (ADN) and stearic acid of lipids.
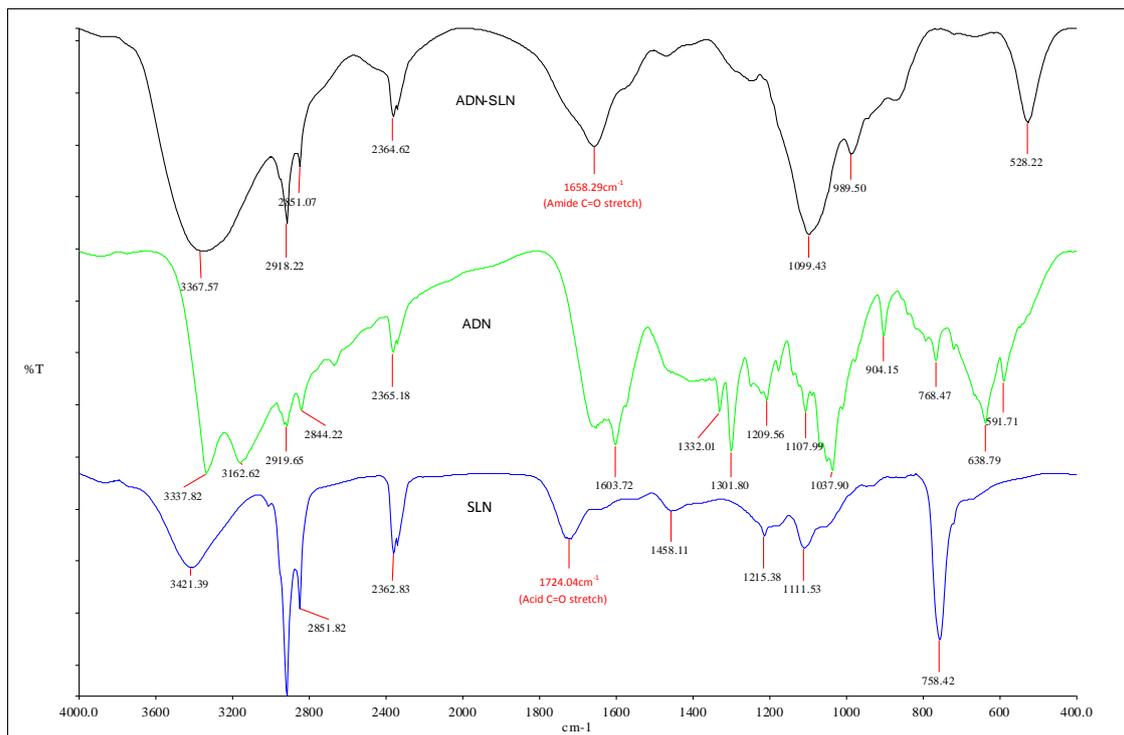
Figure 1: FTIR spectra of AND-SLN, SLN, and ADN.

Entrapment efficiency

The entrapment efficiencies of conjugated and unconjugated SLNs were estimated using validated high performance liquid chromatography (HPLC) method. The entrapment efficiency of DTX in ADN-SLNs was decreased notably in comparison with DTX loaded SLNPs. The entrapment efficiency of DTX in ADN-SLNPs was decreased notably in comparison with DTX loaded SLNPs. The entrapment efficiency of DTX is 99.8% in DTX loaded SLN, but after conjugation process the encapsulation decreased to 66%.

Differential scanning colorimetry (DSC) studies

DSC studies of drug, physical mixture of drug and SLN and ADN-SLN were carried out which evidenced the change of drug from crystalline to amorphous form in the SLNs. ADN-SLNs exhibited slower release than unconjugated drug loaded SLNs and both the SLN formulations showed sustained release s compared to the drug solution.

***In vitro* release studies**

In vitro release studies were carried out using dialysis bag method. ADN-SLN were found to give better sustain drug release as compared to unconjugated SLN and drug solution. In vitro release profile for DTX formulations is shown in figure 2.
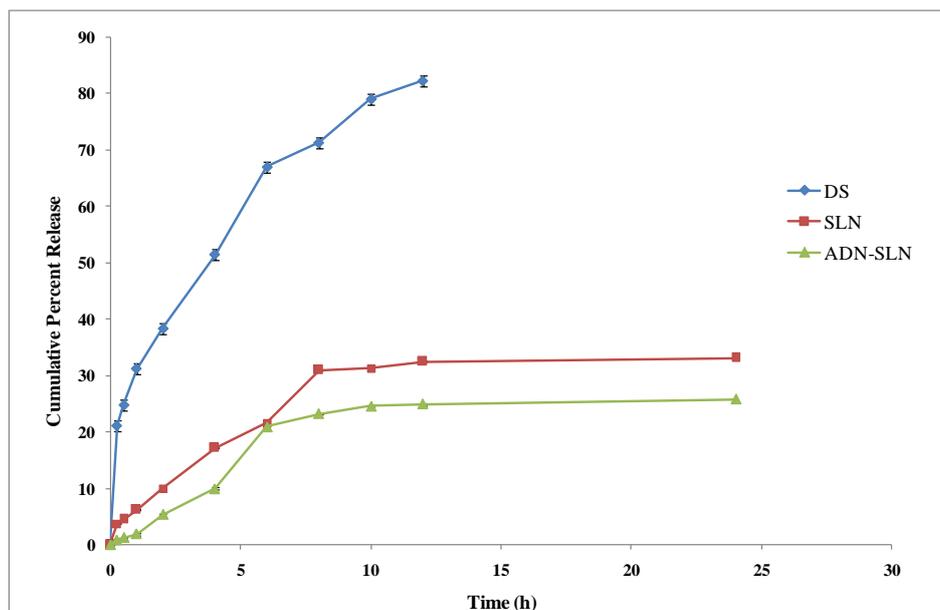
Figure 2: *In vitro* drug release studies of different DTX formulations presented as cumulative drug release vs time graph (mean ± SD; n = 3).

CYTOTOXICITY AND CELL UPTAKE STUDIES

Cytototoxicity and uptake studies were performed for drug solution, unjconjugated as well as for ADN-SLN using MCF-7 cell lines. Significantly higher cell toxicity and lower IC-$_{50}$ of ADN-SLN formulation in all the cell lines indicate that conjugating ADN on the surface of SLN increases the targeting efficiency of DTX in mentioned cells. However IC$_{50}$ values for the ADN SLNs were less in comparison with the unconjugated SLNs and drug solution showing better efficacy.

Conjugated ADN-SLN formulation showed higher uptake than unconjugated SLN. This study corroborated the higher cell cytotoxicity attribute of ADN-SLN as found from MTT cell viability and assay.

CONCLUSION

Breast cancers were reported to over express AR on their surface. So, this AR can be targeted by using ligand having affinity for these receptors, hence aids in internalization of the particle inside the cell. ADN (natural agonist) was reported to be used as ligand to target such cells. In this study adenosine was conjugated on the surface of solid lipid nanoparticles. DTX was released in a sustainable manner from ADN-SLN evident from i*n vitro* drug release studies. Further, these formulations were found to be effective against breast cancer cell lines of and this data was further substantiated by quantitative cell uptake studies on the same cells. Because of small size, sustained drug release and enhanced and targeted delivery, the developed drug delivery system has great potential to deliver anticancer drugs to adenosine receptor over-expressing cells in breast cancer.

ACKNOWLEDGEMENT

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