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Review Article

A REVIEW ON POLYMERIC MICELLES: *IN VITRO* CELL LINE STUDY AGAINST DIFFERENT CANCER CELL LINES

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ABSTRACT

Polymeric micelles have been the subject of many studies in the field of drug delivery. The interest has specifically been focused on the potential application of polymeric micelles in three major areas in drug delivery: drug solubilisation, controlled drug release and drug targeting. Polymeric micelles represent an effective delivery system for poorly water-soluble anticancer drugs. The present article is focusing the *in vitro* cell line study of different drug loaded polymeric micelles of various anticancer drug like ursolic acid, methotrexate, curcumin, doxorubicin and paclitaxel by various *in vitro* cytotoxic assay like MTT and CCK-8 against different cancer cell lines like HepG2, L-02, CT26, HT-29, Caco-2, MCF-7 and A549. Cell culture model are adequate for screening toxicity of several substances including polymeric micelles and the assays help to determine whether any of the anticancer drug loaded polymeric micelles has toxicity or proliferative activity. The data from those models can provide an indication of safety use of these particles in humans.

Keyword: *In vitro* cytotoxicity, polymeric micelles, cell lines

INTRODUCTION

Polymeric micelles is nanoscopic core shell type nanoparticle formed through the self-assembly of block copolymers which are composed by the hydrophobic blocks and hydrophilic blocks. The hydrophobic blocks of the polymer are separated from the aqueous exterior to form the inner core which solubilizes lipophilic drugs and hydrophilic blocks of the polymer forms the outer shell which makes the micelles water soluble. It is widely applied as a novel nano-sized drug delivery system due to its numerous advantages. For example, the nanosize (10-100 nm) and hydrophilic outer shells of polymeric micelles prevent their uptake by reticuloendothelial system (RES) and prolong their circulation time in blood.[1,2] Additional advantages of polymeric micelles are reduced side effect of the encapsulated drug, ability to slow down opsonization, easy and reproducible scale-up and the possibility of longer circulation times.[3]

Cancer is one of the serious health problems that threaten human life. Tumor cell proliferate quickly and uncontrollably. They are characterized by the formation of abnormalities and the combination of mutagenic stages.[4] After surgery, chemotherapy is the commonly used tumor treatment. Chemotherapeutic drugs suffer from poor pharmacokinetic and from an inappropriate bio distribution. Because of their high hydrophobicity and/or low molecular weight, the most of the routinely used chemotherapeutic agent are characterized by a large volume of distribution and short circulation time, leading to very low concentration at tumor sites and also prominent localization in healthy non-target tissues. It is resulting in significant toxicity. To improve the tumor concentration of chemotherapeutic agent and its circulation time and also decrease their accumulation in healthy tissues, many different nanosized drug delivery systems have been designed. One of the clinically relevant examples of nanosized carrier material is polymeric micelles (PM). Mechanism of action of this system is based on Enhanced Permeability and Retention (EPR) effect.[5,6] It is very important to evaluate and ensure that these chemotherapeutic drugs are effective and potent prior to patient administration. *In vitro* cell-based assays have been used to rapidly determine the cytotoxicity activity of several chemotherapeutic agents. This cell-based assay is also developed to determine variation in susceptibility of different target cells to several compounds. Several chemotherapeutic drugs are available in market. The efficacy of these products has been tested at the site of production and passed quality assurance and quality control requirements. Hence, it may be important to test randomly selected lots for their activity to ensure efficacy. The objective of the study is to evaluate potency and cytotoxicity activity of commercially available generic chemotherapeutic drugs in comparison with originator using various human cancer cell lines in an *in vitro* cell based assay.[7,8]

Advantage of Polymeric micelles

- Improved controlled release function.
- Multifunctional design possible.
- Suitable for intravenous administration[9]
- Capacity to solubilizes hydrophobic drug[10]
- Tunable chemical and physical properties
- Protecting drug from environmental condition

Disadvantage of Polymeric micelles

- Lack of stability in blood
- Limited number of polymers for use.
- Lack of suitable methods for scale up

- It dissociates very slowly.
- Used only for lipophilic drug
- Low drug-loading capacity
- Dependency to critical micelle concentration.[11]

Types of Polymeric micelles

Based on the type of intermolecular forces leading the separation of the hydrophobic segment such as core from the aqueous environment, polymeric micelles can be classified into;

- Conventional
- Polyion complex micelles
- Non-covalently connected polymeric micelles.[12,13]

Conventional

These types of polymeric micelles are formed by hydrophobic interactions between the corona region and core segment in the aqueous environment. One of the amphiphilic block copolymer such as, poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide), forms micelles as a result of hydrophobic interactions.

Polyion Complex Micelles

Poly electrolytes enables for the generation of polymeric micelles. These poly electrolytes can penetrate in the corona of micelles and it brings poly ionic micelles. Such formed micelles are termed polyion complex micelles (PICMs). They have some particular features such as easy to assemble in aqueous solution, prolonged circulation in the blood, simple synthetic route and high-level drug loading capacity. The core of the polyion complex micelles can entrap many therapeutic agents such as hydrophilic compound, hydrophobic compound, charged macromolecules and metal complexes through hydrophobic, electrostatic, hydrogen bonding interactions and release them after receiving suitable trigger. Sizes of polyion complex micelles were about 50-200 nm and drug loading efficiency of micelles was greater than 80% (w/w).

Noncovalent Connected Polymeric Micelles

Novel "block-copolymer-free" technique can also be used for the preparation of polymeric micelles. Polymeric micelles obtained by random copolymer, homo polymer, graft polymer or oligomer for which inter polymer hydrogen bonding complexation serve as driving force. The core and shell of polymeric micelle is non-covalently linked to their homo polymer chain end through the specific intermolecular interaction such as H-bonding in the resultant structures, hence these are called as non-covalently connected micelles.

***In vitro* cell line study of different cancer cell lines**

***In vitro* Cytotoxicity Study of Cisplatin Loaded Polymeric Micelle in Lewis Lung Carcinoma Cells (LLC) and Colon Tumor Cell Line (C26) by 3-(4'5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Assay**

Cisplatin is a well-known chemotherapeutic drug. It has been used for numerous human cancer treatment including head and neck, bladder, ovarian, lung and testicular cancer. Cisplatin is clinically proven to effective against various types of cancer, including carcinomas, lymphomas, germ cell tumors and sarcoma.[14] The LLC cell line was derived from the lung of a C57BL mouse bearing a tumor of primary Lewis lung carcinoma. It is primarily used as syngeneic animal model as well as evaluating the efficacy of chemotherapeutic agents *in vivo*.[15] The colon tumor cells (C 26) were used to study the effect of various anti- cancer agents.[16] The study indicated that the cisplatin loaded polymeric micelles exhibit lower toxicity than free cisplatin at each incubation time (42 h and 72 h). This study reported that cisplatin loaded polymeric micelles after pre-incubation in physiological saline resulted in *in vitro* cytotoxicity nearly comparable with that of free cisplatin.[17]

***In vitro* Cytotoxicity Study of Methotrexate-Polymeric Micelles in Colon Carcinoma Cell Line CT26 by MTT Assay**

Methotrexate (MTX) is 4-amino-4-deoxy-N[10]-methyl-pteroylglutamic acid. MTX is a weak bicarboxylic acid. It essentially ionized and lipid insoluble at physiological pH.[18] MTX has an important role in the treatment of small lung cancer, lymphocytic leukemia, head and neck cancer, choriocarcinoma, breast cancer, osteosarcoma and intra thecal chemotherapy. It is one of the most studied and potent therapeutic agents to treat solid tumors. Methotrexate act as chemotherapeutic agent by inhibiting dihydrofolate reductase (DHFR) with high affinity.[19] CT26 colon carcinoma cell line was developed by exposing BALB/c mice to N-nitroso-N-methylurethane (NMU). It is resulting in a rapid-growing grade IV carcinoma that is easily implanted and readily metasizes. CT26 colon carcinoma is one of the widely used cell line in drug development.[20] The study indicated that methotrexate-incorporated polymeric micelles showed slightly lower cytotoxicity against cancer cells. MPEG is exposed on the surface of particle in aqueous environment. The stealth properties of polymeric micelles may delay the uptake of particle into tumor cell and much of drug-incorporated particle may remain in the media. This fact might be one of the reasons for the lower toxicity of polymeric micelles.[21]

***In vitro* Cytotoxicity Study of Curcumin Loaded Polymeric Micelle in Colon Carcinoma HT-29 and Caco-2 Cells by MTT Assay**

Curcumin is the active ingredient of the *Curcuma longa* plant. It has received great attention over as an antioxidant, anti-inflammatory and anticancer agent. Previous excellent studies have recommended that curcumin is a universal anticancer agent, defeating many types of cancers. Curcumin also has potential in overcoming multidrug-resistant cancers. It shows synergistic anticancer effect with other antitumor agents.[22] HT29 is a human colon cancer cell line used extensively in biological and cancer research.[23] Human colon adenocarcinoma cells (line HT29) was isolated by Fogh and Trempe from a human carcinoma of the colon.[24] The Caco-2 cell line of heterogeneous human epithelial colorectal adenocarcinoma cells are developed by Sloan-Kettering, Institute for Cancer research conducted by Dr. Jorgen fogh.[25] The study indicated that there is no significant difference in cytotoxicity effect of N-naphthyl-N, O-succinyl chitosan (NSCS) and N-octyl-N, O-succinyl chitosan (OSCS) micelles in the Caco-2 cells has been observed. The low molecular weight chitosan (3.8-13kDa; DDA 87-92%) with concentration lower than 5 mg/mL had low toxicity to Caco-2 cells after 2 h of incubation (24). This revealed that chitosan micelles had low cytotoxicity on Caco-2. The blank NSCS and OSCS micelles showed minimal cytotoxicity in HT-29 cells at the concentration up to 0.5 mg/mL. This study suggested that blank micelles may be regarded as a safe drug carrier.[26]

***In vitro* Cytotoxicity Study of Doxorubicin Loaded Polymeric Micelle in Cell Line MCF-7 and SMMC-7721 by CCK-8 Assay**

Doxorubicin is an anthracycline drug. It is first extracted from *Streptomyces peucetius* var in 1970's. Doxorubicin is commonly used in the treatment of various cancers including lung, breast, thyroid, ovarian, gastric, non-Hodgkin's and Hodgkin's lymphoma, sarcoma, multiple myeloma and pediatric cancer.[27] MCF-7 is mostly used breast cancer cell line. These cells were isolated from the pleural effusion of a 69-year-old woman with metastatic disease. MCF-7 cell line proves to be a suitable model cell line for breast cancer investigation worldwide, including those regarding anticancer drug.[28] In this study, cells were treated for 48 h at various doxorubicin concentration ranging from 0.2-25 μg mL^{-1} and both cells viability decreased as the micelles concentration increased at highest DOX concentration (25 μg mL^{-1}). The viability of both cells decreased to 57.9 ± 1.3% and 26.9 ± 1.1% respectively. The IC_{50} of DOX-loaded polymeric micelles are for MCF-7 cells and SMCC-7721 cells as approximately 6.3 μgmL^{-1} and 40 μgmL^{-1} respectively. The IC_{50} of "free" DOX for both MCF-7 cells and SMCC-7721 cells are approximately 40 μgmL^{-1} and 1 μgmL^{-1} respectively. This study indicated that blank micelles showed lower toxicity at this concentration because the PEG block overcomes the cytotoxicity of hyPEI.[29]

***In Vitro* Cytotoxicity Study of Paclitaxel Loaded Polymeric Micelles in Lung Cancer Cell Line A549 by MTT Assay**

Paclitaxel is a powerful chemotherapeutic agent. Paclitaxel assist polymerization of tubulin, thereby leading cell death by disrupting the normal microtubules dynamics needed for cell division and vital inter phase processes. Paclitaxel has action against a broad band of tumour types including breast, lung, head, ovarian, head and neck cancers.[30] A549 cell are adenocarcinomic human alveolar basal epithelial cells. A549 cells are used as models for the study of lung cancer and the development of drug therapies against it.[31] The study indicated that Stragen® and free PTX showed similar cytotoxicity in the range of 0.03 to 1.5 μg/mL of paclitaxel concentration. The cytotoxicity of the free drug did not change in concentrations higher than 1 μg/mL. Paclitaxel loaded polymeric micelles showed the highest cytotoxicity among studied group which was significant at concentration greater than 1 μg/mL. The empty micelles did not exhibit any measurable cytotoxicity at low concentration (< 1 μg/mL).[32]

CONCLUSION

Polymeric micelles have been extensively studied over the last decade as versatile and efficient drug delivery systems for cancer therapy. In the present study, the systematic steps in order to do the cell line study of different drug loaded polymeric micelles at different concentration by using different *in vitro* cell line study. The review discussed *in vitro* cell line study of various examples of polymeric micelles in different cancer cell lines like HepG2, L-02, CT26, HT-29, Caco-2, MCF-7 and A549 cell line. The assays significantly help to determine whether any of the anticancer drug loaded polymeric micelles has toxicity or proliferative activity.

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