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Research Article

Formulation and Characterization of Hyaluronic Acid Anchored Polycaprolactone Nanoparticles for Delivery of Anticancer Drugs

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ABSTRACT

Methotrexate (MTX), a stoichiometric inhibitor of dihydrofolate reductase, is a chemotherapeutic agent for treating a variety of neoplasms. The objective of the present study was to synthesize nanoparticles of MTX using hyaluronic acid-polyethyleneglycol-polycaprolactone (HA-PEG-PCL) copolymer for tumor targeting. Targeting efficiency of HA-PEG-PCL nanoparticles was compared with non-HA-containing nanoparticles polycaprolactone. The copolymers were chemically synthesized and characterized by IR and Nuclear Magnetic Resonance (NMR) spectroscopes. The nanoparticles were characterized for shape and morphology by transmission electron microscopy (TEM), particle size, percentage of drug entrapment and *in-vitro* drug release profile. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies were also performed to appraise the crystalline or amorphous nature of MTX inside the polymer matrix. Formulations were prepared using different MTX: polymer ratios (1:0.05-3:0.15 w/w) and the optimum formulation with the drug: polymer ratio of 1:0.05 showed the mean particle size of 185 ± 2.21 nm and entrapment efficiency of 93.16 ± 2.1% in the case of HA-PEG-PCL nanoparticles, while the values were 140 ± 1.24 nm and 90.12 ± 1.3%, respectively, in the case of PCL nanoparticles. The nanoparticles prepared using copolymers HA-PEG-PCL showed their potential to sustain the MTX release. MTX-HA-PEG-PCL nanoparticles showed 95.08 ± 2.50% release of MTX in 96 hours while MTX-PCL nanoparticles released almost 100% of the drug in the same duration. The stability was evaluated in terms of particle size and residual drug content, the nanoparticles formulations were found to be more stable at 5 ± 3°C and then 40 ± 2°C. These results suggest that HA-PEG-PCL nanoparticles could be an efficient delivery system for MTX for targeted therapy of cancer.

Introduction

Tumor is a heterogenic, mutagenic architectural unit, a pathological state with properties which are still incompletely comprehended.[1,2] Targeting of anti-cancer bioactives at the tumor cells is desirable for two reasons: first, to maximize cytotoxic effect throughout the tumor growth phase during which the majority of the cells remains sensitive to pharmacotherapy and secondly, to protect the surrounding healthy cells from exposure to the cytotoxic agents. In order to obtain better tumor regression, it is also desirable to maintain a steady infusion of the drug into the tumor interstitium. Advances in nanobiotechnology have resulted in the evolution of several novel colloidal carriers such as liposomes, polymeric micelles, nanoparticles and nanoemulsions to achieve these above-mentioned objectives. Polymeric nanoparticles are the most attractive colloidal carriers owing several merits such as ease of purification and sterilization, drug targeting possibility and sustained release action.[3] Polymeric nanoparticles made from natural or synthetic polymers have drawn

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major attention due to higher stability, maneuverability for industrial manufacture and opportunity for further surface nanoengineering. They can be tailor made to achieve both controlled drug release and tumor targeting by tuning the polymer characteristics and surface chemistry. Long circulatory nanoparticles have been developed over the past years. Conceiving long circulatory nanoparticles that would have controlled degradation, high entrapment, prolong release of bioactive and possess a hydrophilic chain to allow easy coupling of ligands at controlled surface densities, without degrading either the ligands or the nanoparticles or provoking an uncontrolled release of the entrapped bioactive is a big challenge. Long circulation and ligand coupling can be achieved using polyethyleneglycol (PEG)-coupled copolymers. Ligands can also be covalently linked to the block copolymer containing PEG segments prior to nanoparticle formation by nanodeposition or solvent evaporation. Alternatively, ligands can also be coupled to preformed nanoparticles possessing reactive groups at the PEG terminus. In the recent years, the preparation of targeted drug delivery systems has attained much enthusiasm due to higher therapeutic index of antitumor drugs achieved by increase in target/non-target drug ratio. In order to develop the targeted drug delivery systems, identification of such ligands that are able to recognize specific cancer cells as target sites has grown enormously. The targeting agents directed to membrane-bound tumor-associated antigens or receptors include vitamins, hormones and antibody derivatives.[4] MTX, a folic acid antagonist, is a hydrophilic small molecule (log P, -1.8, MW, 454.5 and poor aqueous solubility (0.01 mg/mL) which is commonly used as clinical chemotherapy agent and highly efficacious antineoplastic drug.[5,6] MTX inhibits dihydrofolate reductase (DHFR) and interfere with tumor cell DNA, RNA and consequently protein synthesis, leads to inhibition of the proliferation of tumor cells.[7] However, using methotrexate has been restricted because of limitations such as low half-life in plasma, low penetration into the brain and dose-dependent systemic side effects.[8] To overcome this problem, MTX can be loaded in various NPs such as liposomes, solid lipid NPs, cationic bovine serum albumin, poly (butyl cyanoacrylate), nanogels, dendrimers. Hyaluronic acid (HA), a naturally occurring linear polysaccharide and chemically classified as a glycosaminoglycan, is composed of repeating disaccharide units of D-glucuronic acid and (1-b-3)-N-acetyl-D-glucosamine. The polysaccharide contains between 500 and 50,000 monosaccharide residues per molecule; thus, its molecular mass ranges from 104 to 107Da.[9] The unique physicochemical and biological properties of HA have highlighted it as a potential targeted macromolecular carrier of drugs to solid tumors. More specifically, the amphiphilic nature of the HA enables it to form a large coiled meshwork at low concentrations,[10,11] which makes it an ideal vehicle for the solvation and entrapment of smaller molecules. Activated HA receptors are over-expressed by CD44 and receptor for hyaluronan-mediated motility (RHAMM) on tumor cells, which are lacking in their non-tumorigenic counterparts.[12] The unique ability of CD44 to internalize HA[13] reinforced its potential as a targeted drug transport vehicle. HA produces an important signal for activating kinase pathways[14] and regulating angiogenesis in tumors.[15] This application of HA has been previously recognized and several preclinical studies have chemically conjugated HA to cytotoxicagents with an end result of highly effective anticancer agents *in-vitro*.[16-18] The approach that we pursued in this study used a drug carrier into which caprolactone-linked oligomers of the HA repeat units were attached by diamine PEG as a spacer. The resultant nanoparticles were supposed to have increased exogenous HA concentrations in ascites tumor fluid. High molecular weight of HA is believed to be involved in inhibiting tumor metastasis. MTX-loaded hyaluronic acid-polyethyleneglycol-polycaprolactone (HA–PEG–PCL) nanoparticles were able to deliver MTX within the tumor by enhanced permeability and retention (EPR) effect and binding to proteins (receptors) of the cell membrane.

Materials and Methods

Materials

Methotrexate was received as a gift sample from Fresenius Kabi Ltd. Gurgaon (Haryana, India).HA and PCL were procured from Sigma-Aldrich (Bangalore, India). Pluronic F-68 was purchased from Himedia Lab, Mumbai, India. N-hydroxysuccinimide (NHS), ethylene diamine (EDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Acetone, isopropyl alcohol, NaOH, DCM (Dichloromethane), DMSO and acetonitrile were all purchased from Merck Limited, Mumbai India. PEG and dialysis sacs (MW cut-off 12000-15000 Da) was purchased from Sigma Aldrich (New Delhi, India). All other chemicals used were of the highest analytical grade.

Copolymer HA-PEG-PCL Synthesis and Characterization

Diamine PEG was synthesized as per the method reported by Zalipsky *et al.*[19] For the synthesis of HA–PEG– PCL copolymers, diamine PEG (0.125 g) was dissolved in 10 mL of anhydrous DMSO and stirred overnight at room temperature. Activated HA (0.574 g of HA in distilled water and a 1.2- fold molar quantity of 0.23 g of EDC was added) was then added to the reaction flask. The reaction was allowed to continue for 48 hours at room temperature. The product was isolated from the organic (DMSO) phase by dialysis molecular weight cutoff (MWCO) 12,000–14,000 Da) against water for 24 hours and vacuum dried. Free amine groups were determined by ninhydrin test. The free amine groups in HA-PEG-NH_2 were found to be 40–45%.

HA-PEG-NH_2 (0.136 g) was reacted under anhydrous conditions with 0.38 g of PCL in 4 mL toluene for 5 hours at 110°C. The copolymer thus obtained was vacuum dried. The synthesized copolymer was characterized by fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopies.

Preparation of MTX-Loaded HA-PEG-PCL Nanoparticles

MTX-loaded HA-PEG-PCL nanoparticles were prepared by the nanoprecipitation method. Briefly, 0.1 g of MTX in the presence of 6.2 mL of NaoH was stirred on a magnetic stirrer for 24 hours at room temperature under nitrogen atmosphere. Then 0.005 g of HA-PEG-PCL copolymer was dissolved in 5 mL of DMC and to this MTX solution was added with stirring. This organic solution containing the drug and the polymer was added dropwise to 10 mL distilled water containing different concentrations of Pluronic F-68 under magnetic stirring. The nanoparticles were formed immediately. The organic solvent was removed by simple evaporation by keeping the mixture at room temperature, followed by centrifugation at 10,000 rpm for 30 minutes. The supernatant was discarded and the pellet containing the nanoparticles was washed thrice with water by repeated re-suspension in water followed by centrifugation to discard the supernatant. The final pellet was lyophilized to obtain a dry powder of nanoparticles. The process variables like the amount of copolymers and Pluronic F-68 concentration were optimized. The amount of copolymer was varied from 5 to 15 mg, while keeping Pluronic F-68 concentration constant (0.5% w/v). Similarly, the nanoparticles were also prepared using different concentrations of Pluronic F-68 (0.5–1.5% w/v), while keeping the amount of copolymer constant (5 mg).

Preparation of DOX-Loaded PCL Nanoparticles

MTX loaded PCL nanoparticle were prepared by the nanoprecipitation method. In briefly 0.1 g of MTX in the presence of 6.2 mL of NaoH was stirred on a magnetic stirred for 24 hours at room temperature under nitrogen atmosphere. Then 0.005 g of PCL polymer dissolved in 5 mL of dimethylchloride and to this MTX solution was added with stirring. This organic solution containing the drug and polymer was added in to drop wise in 10 mL of distilled water containing different concentration of Pluronic F-68 under magnetic stirring. The nanoparticle was formed immediately.

Optimization

There are various parameters which affect the preparation, property and stability of nanoparticle. This formulation parameter were identified and optimized to get uniform preparation and highest encapsulation efficiency. Different type of studies includes concentration of polymer, amount of surfactant concentration and process variables include stirring time and sonication time. All these parameter were optimized by taking effect on particle size, entrapment efficiency.

Characterization of Nanoparticles

Shape, Morphology and Particle Size

The shape and the morphology of the prepared nanoparticles were investigated by transmission electron microscopy (TEM; Morgagni 268 D, Fei, The Netherlands) of the nanoparticles in aqueous medium after negative staining with 1% phosphotungstic acid. Particle size was determined using laser diffraction-based particle size analyzer (Malvern- Zetasizer 3000hs, Malvern, UK).[20]

Differential Scanning Calorimetry

The transition temperatures of pure MTX, copolymers (HA-PEG-PCL and PCL) and MTX loaded nanoparticles of the copolymers (HA-PEG-PCL and PCL) were measured by differential scanning calorimetry (DSC; TA Instrument 2910 MDSC V4.4E, Shin, Osaka, Japan) under a flow of nitrogen between 0 and 500°C at a scanning rate of 108°C per minute.

X-ray Diffraction Study

X-ray diffractograms of MTX, HA-PEG-PCL, MTX loaded HA-PEG-PCL nanoparticles, were obtained with a Rigaku D/Max 1200 diffractometer (Rigaku Denki Co. Ltd, Tokyo, Japan) using Ni-filtered Cu-Ka radiation (35 kV, 15 mA).

Estimation of Drug Entrapment Efficiency

The drug entrapment efficiency was determined using a dialysis method for separating unloaded MTX from the nanoparticles. It is a method to estimate indirectly determines the amount of drug bound with the nanoparticles. Taken 5 mL of MTX loaded nanoparticle dispersion was placed into a dialysis bag MWCO 1200–1500 KDa and dialyzed against 50 mL of 0.1 N NaoH for 15 minutes with magnetic stirring 100 rpm, after 15 minutes. Dialysis bag were taken out and drug in reservoir were estimate in UV spectrophotometer.

In-vitro Drug Release Profile

Nanoparticulate formulations (10 mg) were suspended in 2 mL phosphate-buffered saline (PBS) (pH 7.4) and subsequently transferred into a dialysis tube (MWCO 2000 g/mole). The dialysis tube was placed into a 100 mL bottle containing 50 mL PBS (pH 7.4), maintained at 37°C, and the medium was stirred at 100 rpm. A whole media change method was used for prevention of drug saturation in the drug release study. At specific time intervals, the whole medium (50 mL) was taken and replaced with the same volume (50 mL) of fresh PBS (pH 7.4). The concentration of released MTX in the PBS was determined by the UV spectrophotometer (GBC Citra 10) at 300 nm.

Stability Study

Stability study of optimized PCL nanoparticle and hyaluronic acid anchored PCL nanoparticles formulation

were stored in screw caped, amber colored and white transport small glass bottles at 5 ± 3°C and 40 ± 2°C (as per ICH guideline). Analysis of sample was made for particle size and residual drug content after a period of 30 days.

Results and Discussion

Nanoparticles that had no targeting group (*i.e.,* PCL, MPEG–PLGA) containing hydrophobic drugs have been by reported by numerous research groups.[21,22] Some researchers have attempted to increase the tissue specificity of the drug carriers by coupling targeting agents, such as monoclonal antibodies, peptide, biotin[23] and folic acid,[24] to achieve tumor-specific targeting. In order to attach the targeting agents on the nanoparticles, the HA-PEG-PCL conjugates were prepared by attaching one amino group of diamine PEG with the carboxylic group of HA and attaching the second free amino group of diamine PEG with carboxylic acid of polycaprolactone. This approach was based on the idea that the HA present in the supernatant of ascites fluid and the HA receptors are also over-expressed in most of the tumor cells. In the current study, we prepared the nanoparticles from by conjugating the biodegradable polymers HA, PCL and PEG and from PCL alone. These polymers are well known for biodegradability, biocompatibility and shows the targeting potential to the cancer cells.[25,26] MTX loaded nanoparticles then optimized on the basis of copolymer, dug, surfactant concentration, stirring time and sonication time, which affect characterization of developed nanoparticles such as particle size, surface morphology and entrapment efficiency. The nanoparticles were prepared by varying the concentration of copolymer while keeping the other variables constant. The effect of polymer concentration was directly observed on particle size and %EE. The maximum %EE 90.8 ± 2 and optimum size 120 ± 2 nm was observed when the 5 mg of copolymer was used, this might be due to aggregation of the particles, increasing the concentration did not affect the %EE. While at maximum concentration of copolymer (15 mg) the average size of particle was found to be 168 ± 3 nm and 82.2 ± 3% EE. The surfactant concentration showed great impact on %EE and particle size while all other variables were kept constant. When it was increased from 0.5 to 1.0% the particle size was decreased from 185 ± 2 nm to 158 ± 4 nm. When the concentration of surfactant was increase (1, 1.5%) so the particle size was increase (158 ± 4, 190 ± 5) and entrapment efficiency was decrease (90.3 ± 4, 88.3 ± 2). When the concentration of drug was increase from (100, 200 mg) so the particle size was decrease (164 ± 2, 122 ± 1) and entrapment efficiency was increase (72.5 ± 3, 90.0 ± 1). When the concentration of drug was increase

Table 1: Optimization of formulation and process variables in preparation of nanoparticles

F. code	*Copolymer concentration (mL)*	*Drug concentration (mg)*	*Surfactant concentration (%)*	*Stirring time(hr)*	*Sonication time (Sec)*	*EE (%)*	*Average particle size* (nm)*
Copolymer concentration							
F1	5	100	0.5	10	30	90.8 ± 2	120 ± 2
F2	10	100	0.5	10	30	85.1 ± 1	145 ± 2
F3	15	100	0.5	10	30	82.2 ± 3	168 ± 3
Drug concentration							
F1	5	100	0.5	10	30	72.5 ± 9	164 ± 2
F2	5	200	0.5	10	30	90.5 ± 9	122 ± 1
F3	5	300	0.5	10	30	54.3 ± 7	180 ± 6
Surfactant concentration							
F1	5	100	0.5	10	30	72.3 ± 5	185 ± 2
F2	5	100	1.0	10	30	80.5 ± 7	158 ± 4
F3	5	100	1.5	10	30	90.3 ± 2	190 ± 5
Stirring time							
F1	5	100	0.5	10	30	70.2 ± 7	182 ± 4
F2	5	100	0.5	12	30	80.3 ± 4	160 ± 2
F3	5	100	0.5	14	30	90.2 ± 3	144 ± 3
Sonication time							
F1	5	100	0.5	10	30	60.5 ± 4	180 ± 5
F2	5	100	0.5	10	60	80.3 ± 2	155 ± 7
F3	5	100	0.5	10	90	90 ± 3	140 ± 2

Table 2: Optimization of particle size and entrapment efficiency of PCL NPs and HAPCLNPs

S.no.	*Formulation*	*Entrapment Efficiency*	*Particle size (nm)*	*Zeta potential (mV)*
1	PCL-NPs	90.12 ± 1.3	140 ± 1.24	-6.9 ± 0.2
2	HA-PCL-NPs	93.16 ± 2.1	185 ± 2.21	-18.4 ± 0.7

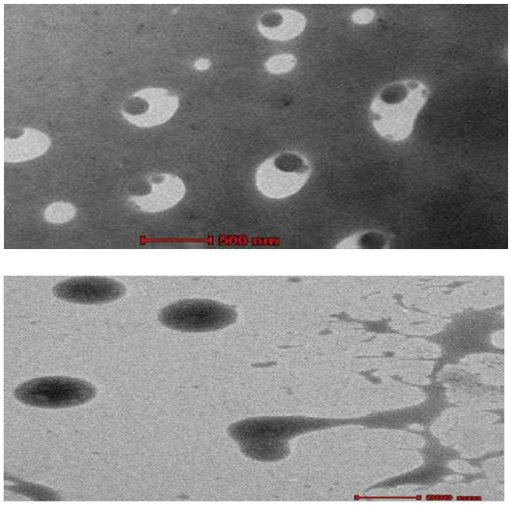

Fig. 1: TEM photomicrograph of (a) PCL nanoparticles (b) HA-PEG-PCL nanoparticles

(200, 300 mg) so the particle size was increase (122 ± 1, 180 ± 4) and entrapment efficiency was decrease (90.0 ± 1, 54.4 ± 1). When the increase the string time of the sample from (10, 12, 14 hrs) the particle size was decrease (182 ± 4,160 ± 2,144 ± 3) and entrapment efficiency was increase (70.2 ± 2, 80.0 ± 1, 90.1 ± 3). When the sonication time increase from (30, 60, 90sec) the particle size was decrease (180 ± 2,155 ± 3,140 ± 1) and entrapment efficiency was increase (62.0 ± 3, 80.3 ± 2, 90 ± 4) (Table 1). The shape and morphology of the prepared nanoparticles was investigated by TEM, and was found to be spherical, irrespective of the polymer/surfactant concentration used Fig. 1(a) and (b). Differential scanning calorimetries measure the heat loss or gain result from physical and chemical changes within a sample as a function of temperature. DSC thermogram of MTX, PCL, MTX loaded PCL-NPs and MTX loaded HA-PCL-NPs was perform and thermogram shown in Fig. 2(a-e) in case of MTX peak was found at 105°C, in case of PCL 61.7°C in case of HA-PCL-NPs peak observed at 69°C HA ,51°C PCL, 99°C MTX. XRD of MTX, HA are shown in Fig. 3 (a, b) in case of HA intense peak were found at between 2 value of 10–30 which are very collates. The average size of the PCL-NPs was found to be around 140 ± 1.24 nm. While the size of the HA-PEG-PCL NPs was 185 ± 2.21 nm, the increase in size might be due to the use of polymer conjugate for NPs development. The zeta potential of PCL NPs and HA-PEG-PCL NPs was found to -6.9 ± 0.2 and -18.4 ± 0.7 mV, respectively (Table 2). The NPs prepared using copolymers such as HA-PEG-PCL showed their potential to sustain the MTX release (Fig. 4).

Initially burst release of MTX was observed which might be due to the release of surface adsorbed drug. MTX-HA-PEG-NPs showed 95.08 ± 2.50% release of MTX in 96 hours while MTX-PCL NPs released almost 96.43 ± 1.28% of the drug in 72 hours. The sustained release of drug might be due to their hydrophobicity and entrapment in the inner core of co polymeric NPs. The stability of MTX-HA-PEG-PCL NPs was observed by storing the NPs formulations at 5 ± 3°C and 40 ± 2°C; the stability was evaluated in term of particle size and residual drug content. Storage stability studies were completed to examine the integrity of formulations under varied environmental conditions. The stability was determined by storing formulations for 30 days. At 5 ± 3°C the particle size was not significantly affected while the size was found to be increased at 40 ± 2°C due to aggregation of the polymer matrix. Similarly, significant alteration in residual drug content was noticed at 40 ± 2°C compared to formulation when stored at 5 ± 3°C, this might be due to the leakage or degradation of the drug at this temperature.

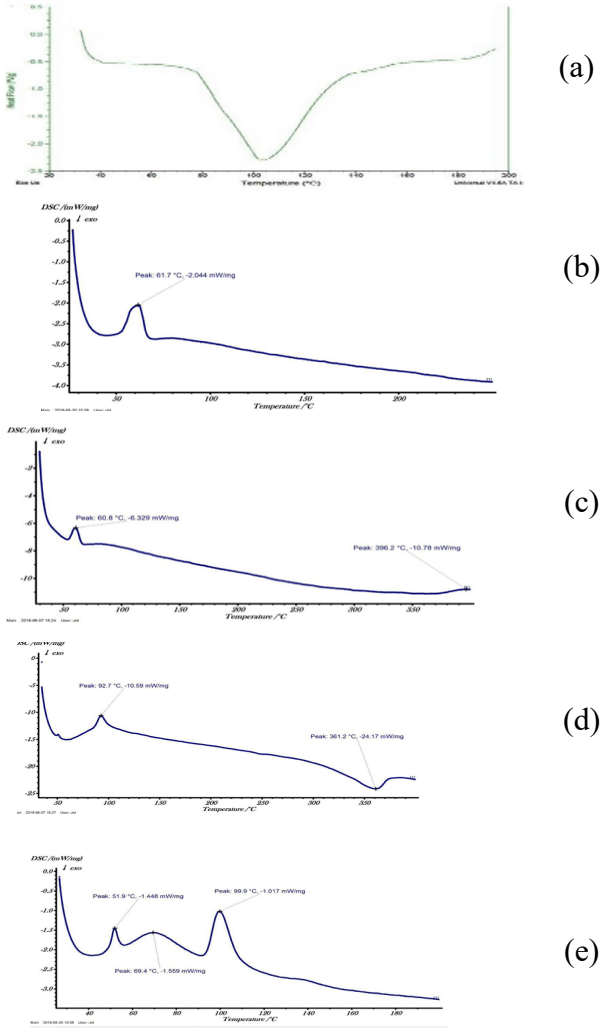

Fig. 2: DSC thermogram of (a) MTX, (b) PCL, (c) MTX loaded PCL NPs, (d) HA–PEG–PCL nanoparticles (without MTX), (e) MTX-loaded HA–PEG–PCL nanoparticles

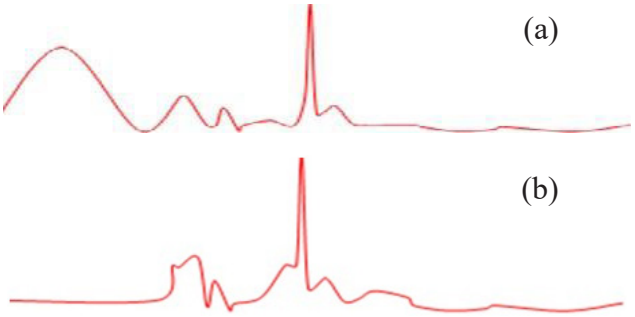

Fig. 3: (a) XRD Thermogram of MTX, (b) HA.

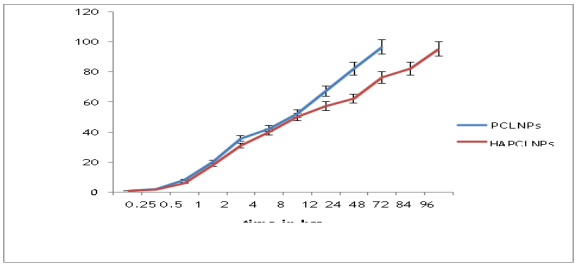

Fig. 4: *In-vitro* MTX release from MTX-PCL NPs and MTX-HA-PEG-PCL NPs

Conclusion

In order to deliver MTX in the tumor cell, HA-PEG-PCL copolymers were synthesized and used to prepare MTX loaded nanoparticles. The nanoparticulate system was found to be safe for administration, more hemo-compatible and biocompatible. On the basis of the above studies, it can be concluded that HA-PEG-PCL nanoparticles can serve as efficient tools to ferry large doses of anti-cancer drug to tumor sites, with reduced access to non-tumor tissues. These observations suggest that the present NPs offer an exciting mode of target delivery for potent MTX.

References

1. Agarwal A, Asthana A, Gupta U, Jain NK. Tumour and dendrimers: a review on drug delivery aspects. J Pharm Pharmacol. 2008; 60(6):671-688.
2. Agarwal A, Saraf S, Asthana A, Gupta U, Gajbhiye V, Jain NK. Ligand based dendritic sytems for tumor targeting. Int J Pharm. 2008; 350:3-13.
3. Allemann E, Gurny R, Doelker E. Drug–loaded nanoparticles preparation method and drug targeting tissues. Eur J Pharm Biopharm. 1993; 39:173-191.
4. Cavallaro G, Mariano L, Salmaso S, Caliceti P, Gaetano G. Folate mediated targeting of polymeric conjugates of gemcitabine. Int J Pharm. 2006; 307:258-269.
5. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. Pharmaceutical Press, London. 2011.
6. Zhang Y, Jin T, Zhuo RX. Methotrexate-loaded biodegradable polymeric micelles: Preparation, physicochemical properties and in vitro drug release. Colloids Surf B Biointerfaces 2005; 44: 104-109.
7. Goldman ID, Matherly LH. The cellular pharmacology of methotrexate. Pharmacol Ther 1985; 28: 77-102.
8. Khan ZA, Tripathi R, Mishra B. Methotrexate: A detailed review on drug delivery and clinical aspects. Expert Opin Drug Deliv. 2012; 9: 151-169.
9. Fransson LA. Mammalian polysaccharides. In: Aspinall O, editor. The polysaccharides. Vol. 3. New York: Academic Press.1985; p 347-348.
10. Laurent TC. Structure of hyaluronic acid. In: Balazs EA, editor. Chemistry and molecular biology of the intercellular matrix. New York: Academic Press. 1970; p 703-721.
11. Scott JE, Cummings C, Brass AY, Chen Y. Secondary and tertiary structures of hyaluronan in aqueous solution, investigated by rotary shadowing-electron microscopy and computer stimulation. Biochem J. 1991; 274:699-705.
12. Wang C, Thor AD, Moore DH, Zhao Y, Kerschmann R, Stern R, Watson PH, Turley EA.The over expression of RHAMM, a hyaluronan-binding protein that regulates ras signalling, correlates with over-expression of mitogen-activated protein kinase and is a significant parameter in breast cancer progression. Clin Cancer Res. 1998; 4:567-576.
13. Hall CL, Yang BH, Yang XW, Zhang SW, Turley M, Samuel S, Lange LA, Wang C, Curpen GD, Savani RC, Greenberg AH, Turley EA. Overexpression of the hyaluronan receptor RHAMM is transforming and is also required for h-ras transformation. Cell. 1995; 82:19-28.
14. Rooney P, Kumar S, Ponting J, Wang M. The role of hyaluronan in tumour neovascularization (review). Int J Cancer. 1995; 60:632-636.
15. Akima K, Ho H, Iwata Y, Matsuo K, Watari N, Yanagi M, Hagi H, Oshima K, Yagita A, Atomi Y, Tatekawa I. Evaluation of antitumor activities of hyaluronate binding antitumor drugs:
16. Synthesis, characterization and antitumor activity. J Drug Target. 1996; 4:1-8.
17. Coradini D, Pellizzaro C, Miglierini G, Daidone MG, Perbellini A. Hyaluronic acid as drug delivery for sodium butyrate: Improvement of the anti-proliferative activity on a breast-cancer cell line. Int J Cancer. 1999; 81:411-416.
18. Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid–antitumor bioconjugate. Bioconjug Chem. 199910:755–763.
19. Zalipsky S, Gilon C, Zilkha A. Attachment of drugs to polyethylene glycols. Eur Polym J. 1983; 19:1177-1183.
20. Jo Y-u, Lee CB, Bae SK, Na K. Acetylated hyaluronic acid-poly (L-lactic acid) conjugate nanoparticles for inhibition of doxorubicinol production from doxorubicin. Macromolecular Res. 2020;28(1):67-73.
21. Kim SY, Lee YM. Taxol-loaded block copolymer nanospheres composed of methoxy poly (ethylene glycol) and poly (1-caprolactone) as novel anticancer drug carriers. Biomaterials. 2001; 22:1697-1704.
22. Shuai X, Ai H, Nasongkla N, Kim S, Gao J. Micellar carriers based on block copolymers of poly (1-caprolactone) and poly (ethylene glycol) for doxorubicin delivery. J Control Rel 2004;. 98:415-426.
23. Gref R, Couvreur P, Barratt G, Mysiakine E. Surfaceengineered nanoparticles for multiple ligand coupling. Biomaterials. 2003; 24:4529-4537.
24. Park EK, Lee SB, Lee YM. Preparation and characterization of methoxy poly(ethylene glycol)/poly (1-caprolactone) amphiphilic block copolymeric nanospheres for tumor-specific folatemediated targeting of anticancer drugs. Biomaterials. 2005; 26:1053-1061.
25. Prajapati SK, Jain A, Jain A, Jain S. Biodegradable polymers and constructs: A novel approach in drug delivery. Eur Polym J. 2019; 120:109191.
26. Qindeel M, Ahmed N, Shah KU, Ullah N. New, environment friendly approach for synthesis of amphiphilic PCL–PEG–PCL triblock copolymer: an efficient carrier for fabrication of nanomicelles. J Polym Environ. 2020;28(4):1237-51.

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