Available online on 15.10.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

IN-VITRO AND IN VIVO STUDIES OF CETUXIMAB LOADED POLYMERIC NANOPARTICLES

Ajinder Kaushik, Hemant Kumar Sharma

College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Pachama, Sehore (M.P.) 466001

ABSTRACT

Nanoparticles speak to one of the appealing choices in the compelling treatment of tumor chemo-treatment. In the present work, definition and improvement of a novel Cetuximab (MTX)- stacked biodegradable nanoparticles utilizing poly(D,L-lactide-co-glycolide) (PLGA) was done. The arranged nanoparticles were assessed for physicochemical properties, for example, molecule measure, zeta potential, discharge thinks about, and so forth. Molecule size of upgraded definition was < 200 nm. Our essential outcomes exhibit that the created Cetuximab-stacked PLGA nanoparticles discharging the medication for delayed timeframe.

Keywords: Cetuximab; PLGA 50:50; nanoparticles

Article Info: Received 27 Aug, 2018; Review Completed 27 Sep 2018; Accepted 29 Sep 2018; Available online 15 Oct 2018

Cite this article as:

Kaushik A, Sharma HK, In-vitro and In vivo studies of Cetuximab Loaded Polymeric Nanoparticles, Journal of Drug Delivery and Therapeutics. 2018; 8(5-s):184-188 DOI: http://dx.doi.org/10.22270/jddt.v8i5-s.1941

***Address for Correspondence:**

Dr. Hemant Kumar Sharma, Prof & Dean, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Pachama, Sehore (M.P.) 466001

INTRODUCTION

After cardiovascular ailments, malignancy is the biggest reason for death around the world (http://www.cdc.gov.in). The word malignancy originated from a Greek word "karkinos" to depict carcinoma tumors by a doctor Hippocrates (460– 370 B.C) [1]. Malignancy can be characterized as an uncontrolled development of typical cells in a specific piece of the body[2-5].When growth spreads to alternate parts of the body through the circulation system or lymphatic framework, this is called metastasis. A solitary malignant cell encompassed by solid tissue will reproduce at a higher rate than alternate cells. Once a little tumor mass has shaped, the sound tissue won't have the capacity to rival the growth cells for the insufficient supply of supplements from the circulatory system. The tumor cells will dislodge solid cells until the point that the tumor achieves a dispersion constrained maximal size. (www.cancer.gov.in).

The most widely recognized malignancies are anticipated to be lung and bronchus disease, bosom tumor, prostate growth, colon and rectum growth, bladder growth, melanoma of the skin, non-hodgkin lymphoma, thyroid disease, kidney and renal pelvis growth, endometrial disease, leukemia, and pancreatic malignancy. The quantity of new instances of disease (tumor frequency) is 454.8 for every 100,000 people for each year (in light of 2008-2012 cases) (www.nih.gov.in). The quantity of growth passings (malignancy mortality) is 171.2 for each 100,000 people for every year (in view of 2008-2012 passings). The quantity of individuals living past a growth finding achieved about 14.5 million out of 2014 and is required to ascend to very nearly 19 million by 2024. In 2014, an expected 15,780 kids and youths ages 0 to 19 were determined to have tumor and 1,960 passed on of the infection. National uses for disease mind in the United States totaled almost $125 billion out of 2010 and could reach $156 billion of every 2020 (www.nih.gov.in). Nanoparticles stacked with anticancer operators can effectively build sedate focus in malignancy tissues and furthermore act at the cell level, improving antitumor viability. The principle points of interest of nanoparticles incorporates, the amazing tumor focusing on and can escape from the vasculature through the cracked endothelial tissue that encompasses the tumor and afterward aggregate, bringing about improved porousness impacts. These medication conveyance frameworks enhance bioavailability by improving fluid

solvency, expanding protection time in the body and focusing on medication to particular area in the body. Different medications utilized as a part of a specific sort of disease, for example, erlotinib, geftinib, docetaxel and so on are utilized as a part of the treatment of lung tumor, while, Cetuximab, doxorubicin, paclitaxel and so on. are utilized as a part of the treatment of bosom cancer.The point of the present work is to define the medication stacked polymeric nanoparticles of anticancer medication utilizing pharmaceutical trial plan. The goal behind the work is to contemplate the impact of definition factors on the molecule estimate, sedate exemplification and % aggregate medication arrival of nanoparticles. [6-10] Study the in vitro release character of Cetuximab from the prepared nanoparticles. Study the stability of prepared nanoparticles for optimized formulations Perform biodistribution and pharmacokinetic studies in mice for the Tc^{99m} labeled Cetuximab and nanoparticle formulations after intravenous and oral administration. Carryout tumor uptake studies for radio labeled Cetuximab and formulations along with biodistribution studies. Gamma scintigraphic imaging was proposed to be used for the study.

MATERIAL AND METHODS

Materials

Poly (lactide-co-glycolide), PLGA (Purasorb R 85/15, Mol.wt. 10000) was liberally skilled by Purac chemicals, The Netherlands. Poly-ε-caprolactone (PCL) (atomic weight of 40000) and Pluronic F 68 were bought from Sigma-Aldrich Chemicals, (Milwaukee, WI, USA) . Cetuximab was blessing from Dabur Research Foundation (Sahibabad, U.P. India). Tc-99m was naturally eluted from molybdenum, stannous chloride dihydrate was bought from Sigma Chemicals. Triple refined water was utilized as a part of the readiness of nanoparticles.

Preparation of Nanoparticles [11-12]

In the present investigation nanoparticles of MTX were set up by emulsification dissolvable dissipation strategy (Xua et al 2005). In a word, polymer and MTX were broken up in $CH_3)_2CO$ under rapid homogenization (Polytron Mixer, Kinematica) at 1000 rpm. The polymeric arrangement was test sonicated for 2 min and gradually added to the watery stage containing surfactant arrangement utilizing fast homogenization at 7000 rpm for 10 min. coming about O/W emulsion was again test sonicated at 40 W adequacies for 5 min in ice water shower. The emulsion was kept for mixing on an attractive stirrer at 1200 rpm for finish dissipation of natural dissolvable. After entire vanishing of the dissolvable, the suspension was centrifuged at 20,000 rpm for 30 min. The pellet at the base was reconstituted in an answer containing mannitol as a cryoprotectant. The suspension was profound freezed at – 80 °C for 8 h and lyophilized with vacuum weight of < 50 mTorr and at a temperature of – 40 °C for 48 h. Supernatant was investigated with the expectation of complimentary medication (unentrapped) utilizing as a part of house created RP-HPLC strategy.

In vitro Drug Release

Drug release from nano-particles and subsequent biodegradation are important for developing the successful formulations. The release rate of drug from nano-particles depend upon: (i) desorption of the surface-bound /adsorbed drug; (ii) diffusion through the nano-particle matrix; (iii) diffusion through the nanocapsules polymer wall; iv) nanoparticle matrix erosion; and (v) combined erosion and diffusion process. Thus, diffusion and biodegradation govern the process of drug release. Methods to study the in vitro release are: (i) side-by-side diffusion cells with artificial or biological membranes; (ii) dialysis bag diffusion technique; (iii) reverse dialysis sac technique; (iv) ultra-centrifugation; (v) ultra-filtration; or (vi) centrifugal ultra-filtration technique.

Animal Models Used for *In-vivo* Studies

Strain mice (25 to 30 g) were used for biodistribution, pharmacokinetic, tumor development and tumor uptake studies. Pharmacokinetic studies of radio labelled preparations were conducted in male New Zealand, white rabbits of 2 to 2.5 kg. Animals were kept in cages at constant temperature and humidity. Water and feed were given *ad libitum*. The Social Justice and Empowerment Committee further approved by IAEC, Daksh Institute of Pharmaceutical Sciences, Chhatarpur, Madhya Pradesh (Ref. No. CPCSEA/2018/12-18) for all the animal experiments for the purpose of control and supervision of experimental animals, New Delhi, India.

Biodistribution and Pharmacokinetic Study in Healthy Mice

a) Intravenous Administration (*i. v.*):

Tc^{99}m labelled Cetuximab and formulations: containing around 200 µ Ci of radioactivity was injected into the tail vein of healthy mice. For each preparation injected, three mice were used per time point. The mice were sacrificed at 0.5, 1, 2, 4 and 24 h post administration. Before sacrificing mice, at specific time points, mice were anaesthetized with excess amount chloroform and blood samples were collected by cardiac puncture and placed in pre weighed plastic tubes. Various organs like, the heart, liver, lungs, muscle, bone (femur), kidneys, spleen, gastrointestinal tract (GIT) and brain were then isolated. In the case of GIT, the whole tract was excised and separated into stomach, small intestine and large intestine. All the organs/tissues collected were thoroughly rinsed with saline, placed in pre-weighed plastic tubes and weighed. The radioactivity was determined in a well type gamma scintillation counter along with three aliquots of the diluted standard representing 100% of the injected radioactivity. Mean of this radioactivity was used to obtain the total injected radioactivity into the animal. The radioactivity present in organs/tissues was interpreted as percentage of the injected radioactivity per gram of organ/tissue (% A/g). Pharmacokinetic parameters were calculated for the blood samples collected in the study.

b) Oral Administration:

Tc^{99m} labeled Cetuximab, ETNP/F68/17, ETNP/PCL/F68/03, ETNP3/F68 and NP/F68/17 (100 µ l) containing around 200 µ Ci of radioactivity were administered orally. All other conditions and procedures were kept same as mentioned in *i. v.* administration. The radioactivity present in all organs/tissues was determined using gamma scintillation counter along with the diluted standard representing 100% of the administered radioactivity. Mean of this radioactivity was used to obtain the total administered radioactivity into the animal. The radioactivity present in organs/tissues was interpreted as % A/g.

Lymphoma Tumor Bearing Mice

a) Tumor Implantation and Development:

The Dalton's Lymphoma solid tumor (DLS) cells were maintained in the peritoneum of Balb/C mice in the ascites form by serial weekly passages. Exponentially growing cells were harvested and tumor cells of 5 x 106 per mouse were injected subcutaneously in the thigh of right hind leg of the Strain A mice. After 8 to 10 days a palpable tumor in the volume range of 1.0 ± 0.1 cm 3 was observed and used for further studies.

b) Biodistribution, Pharmacokinetic and Tumor Uptake Study:

Tc^{99m} labeled Cetuximab (100 µ l) containing around 200 µ Ci of radioactivity were injected into the tail vein of tumor bearing mice. For each injected preparation three mice were used per time point. The mice were sacrificed 1, 4, and 24 h post injection. Before sacrificing those mice, at specified time points, mice were anaesthetized with excess amount chloroform and blood samples were obtained by cardiac puncture and placed in pre-weighed plastic tubes. The heart, liver, lungs, muscle, bone (femur), kidneys, spleen, and brain were isolated.Along with these organs, tumor was excised from the right hind leg. As a control; muscle from the right hind leg of a healthy animal, which was administered with the same preparation, was used. All the organs/tissues collected were thoroughly rinsed with saline, placed in pre-weighed plastic tubes and weighed. The radioactivity was determined as mentioned above.

Pharmacokinetic Study in Rabbits

a) Intravenous Administration:

Tc^{99m} labeled Cetuximab, ETNP/F68/17, ETNP/PCL/F68/03 and NP/F68/17 (250 µ l) containing around 6 mCi of radioactivity were injected into the marginal ear vein of rabbits. Study was conducted on grouping three rabbits per preparation. At specific time points, 5, 10, 15, 30 min, 1, 2, 3, 4, 6 and 24 h, blood samples were collected from the marginal vein of the ear and placed in pre weighed plastic tubes and weighed. Radioactivity was checked using gamma counter. Along with the blood samples, standard solution, which was injected, also checked for radioactivity as it gives total injected radioactivity and it was taken as 100%. The radioactivity present in blood samples was interpreted as percentage of the injected radioactivity per gram of blood (% A/g).

b) Oral Administration:

Tc^{99m} labeled Cetuximab, ETNP/F68/17 and NP/F68/17 (500 µ l) containing around 6 mCi of radioactivity were administered orally for pharmacokinetic studies. Blood samples were collected after 0.5, 1, 2, 3, 4, 6 and 24 h post administration of preparations. All other conditions and procedures were kept same as mentioned in i.v. administration. The radioactivity present in blood was determined as above and represented as % A/g.

Gamma Scintigraphic Imaging:

For gamma scintigraphic study of Tc99m labeled Cetuximab and ETNP/F68/17, 100 µl of preparations containing 200 µ Ci of radioactivity was injected through the tail vein of the tumor bearing and healthy mice. Healthy mice were kept as control. At 4 and 24 h of post injection, mice were fixed on animal fixing tray board and imaging was performed with Single Photon Emission Computed Topography gamma camera. Gamma imaging was also done for rabbits after administering Tc99m labeled Cetuximab and ETNP/F68/17 preparations containing 6 mCi of radioactivity through the marginal ear vein of rabbits.

RESULTS AND DISCUSSION

Animal Models Used for *In vivo* Studies

Strain mice (25 to 30 g) were used for biodistribution, pharmacokinetic, tumor development and tumor uptake studies. Pharmacokinetic studies of radio labeled preparations were conducted in male New Zealand, white rabbits of 2 to 2.5 kg. Animals were kept in cages at constant temperature and humidity. Water and feed were given ad libitum.

Biodistribution, tumor development, tumor uptake and pharmacokinetic studies were carried out at Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi after the prior approval (Sanction number: INM-302) and in accordance with the rules and regulations of the Animal Ethics Committee of INMAS, New Delhi. The Social Justice and Empowerment Committee further approved all the animal experiments for the purpose of control and supervision of experimental animals, New Delhi, India.

Bio-distribution and Pharmacokinetic Study in Healthy Mice

a) Intravenous Administration (i.v.)

Tc^{99}m labeled Cetuximab and formulations: ETNP/F68/17, ETNP/PCL/F68/03, ETNP3/F68 and NP/F68/17 (100 µ l) containing around 200 µ Ci of radioactivity were injected into the tail vein of healthy mice. For each preparation injected, three mice were used per time point. The mice were sacrificed at 0.5, 1, 2, 4 and 24 h post administration. Before sacrificing mice, at specific time points, mice were anaesthetized with excess amount chloroform and blood samples were collected by cardiac puncture and placed in pre weighed plastic tubes. Various organs like, the heart, liver, lungs, muscle, bone (femur), kidneys, spleen, gastrointestinal tract (GIT) and brain were then isolated. In the case of GIT, the whole tract was excised and separated into

stomach, small intestine and large intestine. All the organs/tissues collected were thoroughly rinsed with saline, placed in pre-weighed plastic tubes and weighed. The radioactivity was determined in a well type gamma scintillation counter along with three aliquots of the diluted standard representing 100% of the injected radioactivity. Mean of this radioactivity was used to obtain the total injected radioactivity into the animal. The radioactivity present in organs/tissues was interpreted as percentage of the injected radioactivity per gram of organ/tissue (% A/g). Pharmacokinetic parameters were calculated for the blood samples collected in the study.

b) Oral Administration

$Tc^{99}m$ labeled Cetuximab, ETNP/F68/17, ETNP/PCL/F68/03, ETNP3/F68 and NP/F68/17 (100 µ l) containing around 200 µ Ci of radioactivity were administered orally. All other conditions and procedures were kept same as mentioned in i.v. administration. The radioactivity present in all organs/tissues was determined using gamma scintillation counter along with the diluted standard representing 100% of the administered radioactivity. Mean of this radioactivity was used to obtain the total administered radioactivity into the animal. The radioactivity present in organs/tissues was interpreted as % A/g.

Data Analysis

Results of the in vivo biodistribution studies were statistically evaluated by Student's t-test with $P<0.05$ as the minimal level of significance. Study was done in between free Cetuximab and Cetuximab loaded formulations. Pharmacokinetic parameters were assessed using non-compartmental technique with the software program WinNonlin (version 2.1). The mean %A/g and time data was fitted to the model i. v. bolus for i.v. administration and extravascular for oral administration. Pharmacokinetic parameters like Area under the curve (AUC), Mean residence time (MRT), Clearance (Cl), t1/2, Tmax, Cmax were calculated using this software.

Table 1: Tissue distribution kinetics of Tc^{99m} labeled Cetuximab in healthy mice - after i.v.

Time (h)	% A/g ± SD				
	0.5	1.0	2.0	4.0	24.0
Stomach	0.021 ± 0.003	0.057 ± 0.008	0.047 ± 0.015	0.078 ± 0.025	0.038 ± 0.016
Small Intestine	0.759 ± 0.094	1.169 ± 0.307	1.736 ± 0.091	2.143 ± 0.055	0.637 ± 0.131
Large Intestine	0.237 ± 0.052	0.607 ± 0.092	0.394 ± 0.005	0.246 ± 0.074	0.076 ± 0.012

Table 2: Tissue distribution kinetics of Tc^{99m} labeled nanoparticle formulation (ETNP/F68/17) in healthy mice after i.v. administration.

Time (h) →	% A/g ± SD				
	0.5	1.0	2.0	4.0	24.0
Stomach	0.689 ± 0.063	0.870 ± 0.066	0.554 ± 0.104	0.403 ± 0.099	0.276 ± 0.101
Small Intestine	0.469 ± 0.021	0.540 ± 0.213	0.607 ± 0.254	0.962 ± 0.276	0.546 ± 0.327
Large Intestine	0.471 ± 0.074	0.860 ± 0.332	0.897 ± 0.143	0.587 ± 0.128	0.403 ± 0.07

Each value is the mean of 3 independent determinations

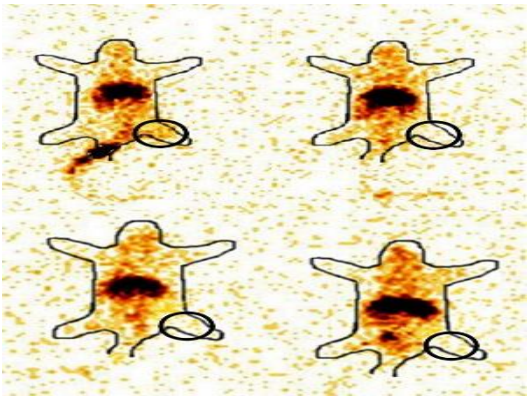

Figure 1: Gamma Scintigraphic image of DLS tumor induced and normal mice 24 h after i.v. administration

CONCLUSION

Biodistribution studies of radiolabeled Cetuximab and Cetuximab loaded nanoparticles in healthy mice after intravenous and oral administration produced different distribution profile compared with free drug. Higher concentrations of radio labeled Cetuximab loaded nanoparticles were observed in blood with increased residence time. There was preferential uptake by reticuloendothelial system with maximum amount of radioactivity observed in liver with lower clearance. Nanoparticles prepared with PLGA 85/15, PCL and their combination, were distributed more into liver, blood, lungs, bone and brain after i.v. and oral administration which might be useful in treatment of malignancies in

the respective organs. Lower distribution of nanoparticles to heart, kidneys could reduce the side effects and toxicity. Higher uptake of nanoparticles by gut wall of GI tract after oral administration indicates potential use of nanoparticles in treatment of GI tract malignancies. sites or organs with extended release of Cetuximab in the site. These systems can potentially avoid problems associated with conventional formulations of Cetuximab and demonstrated the promising potential of the Cetuximab loaded nanoparticles to improve the therapeutic efficacy of Cetuximab and reduce drug associated toxicity.However, further work is required to be done to enhance the drug loading in nanoparticles by varying different parameters or change of polymer. Also in vivo therapeutic efficacy need to be done in cancer induced animals. On the basis of animal studies, clinical trial may be made in human volunteers to study the effectiveness and specificity.

REFERENCES

1. Alpar H.O., Field W.N., Hyde R., Lewis D.A. The transport of microspheres from the gastrointestinal tract to inflammatory air pouches in the rat. J. Pharma. Pharmacol. 1989; 41:194-196.
2. Babbar A., Kashyap R., Chauhan U.P. A convenient method for the preparation of 99mTc-labelled pentavalent DMSA and its evaluation as a tumour imaging agent. J. Nucl. Biol. Med. 1991; 35:100-104.
3. Bhatnagar A., Singh A.K., Babbar A., Soni N.L., Singh T. Renal imaging with 99Tc (m)-dextran. Nucl. Med. Commun. 1997; 18:562-566.
4. Blok D., Feitsma R.I., Wasser M.N., Nieuwenhuizen, W., Pauwels E.K., 1989. A new
5. method for protein labeling with 99mTc. Int. J. Rad. Appl. Instrum. B. 16, 11-16.
6. Chervu L.R., Blaufox M.D. Renal radiopharmaceuticals-an update. Semin. Nucl.Med. 1982; 12:224-245.
7. Florence A.T. The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. Pharm. Res. 1997; 14:259-266.
8. Garron J.Y., Moinereau M., Pasqualini R., Saccavini J.C. Direct 99mTc labeling of monoclonal antibodies: radiolabeling and in vitro stability. Int. J. Rad. Appl. Instrum. B. 1991; 18:695-703.
9. Hillery A.M., Jani P.U., Florence A.T. Comparative quantitative study of lymphoid and non lymphoid uptake of 50 nm polystyrene particles. J. Drug. Target. 1994; 2:151-154.
10. Jani P., Halbert G.W., Langridge J., Florence, A.T. The uptake and translocation of the latex nanospheres and microspheres after oral administration to rats. J. Pharm. Pharmacol. 1989; 41:809-812.
11. Jani P., Halbert G.W., Langridge J., Florence, A.T. nanoparticle uptake by the rat gastrointestinal mucosa: Quantification and particle size dependency. J. Pharm. Pharmacol. 1990; 42:821-826.
12. Jani P.U., Florence A.T., McCarthy D.E., Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat. Int. J. Pharm. 1992; 84:245-252.