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Synthesis and characterization of Agiopep-2 anchored PEGylated poly propyleneimine dendrimers for targeted drug delivery to glioblastoma multiforme

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ABSTRACT

The present study was aimed at developing and exploring the use of Angiopep-2 anchored PEGylated Poly propyleneimine (PPI) dendrimers for targeted delivery of paclitaxel to the brain glioma. PPI dendrimers were synthesized and modified with PEG-2000 for surface neutralization. PEGylated PPI dendrimers were further conjugated with Angiopep-2 (ANG-PEG-PPP) for improved drug delivery across blood brain barrier (BBB) into the vicinity of brain glioma. Ligand conjugated PPI dendrimers were loaded with Paclitaxel (PAPP) and characterized for size, percentage drug loading, cumulative drug release and cell line studies. Drug loading was found to be 57.42±0.8% while in vitro release profile depicted an initial burst release followed by zero order kinetics (46.8±0.8% in 24hr). MTT assay and cellular uptake studies on PAPP dendrimers demonstrated an enhanced antiproliferative activity against C6 glioma cells. Targeting potential of PAPP was evaluated using in vitro co culture model of BCECs and C6 glioma cells. Our study concludes, Angiopep-2 conjugated PEGylated PPI dendrimers as a promising nanocarrier for targeted delivery of paclitaxel to the brain glioma.

Keywords: Dendrimer, Polyethylene glycol, paclitaxel, angiopep-2, drug targeting, glioblastoma multiforme

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INTRODUCTION

Human brain is the most delicate and protected organ of body surrounded by dense network of blood vessels and connective tissue membranes called meninges [1]. Several diseases and disorders like encephalitis, multiple sclerosis, neurological disorder, stroke and tumor may affect the normal functioning of brain. Brain glioma especially glioblastoma multiforme (GBM) a type of brain tumor is one of such serious diseases which adversely affects the brain's anatomy and physiology [2,3]. GBM is most frequently encountered aggressive type of grade IV brain tumor, which has poor prognosis and high rate of recurrence making it a major reason behind cancer death in adults less than 35 years of age. GBM cells easily diffuse from one site to the other nearby healthy tissues, posturing its complete eradication impossible using conventional chemotherapies or surgical method and thus tumor recurrence is more frequent [4,5]. Conventional drug delivery methods are incapable to deliver anticancer drugs effectively to the tumor as well as to migrating cells in brain however; they may often result in systemic side effects. The effectiveness of anticancer drugs is limited due to poor permeability of majority of drug molecules across the BBB either because of their larger size or due to low lipophilicity. Another problem in chemotherapy is maintaining a higher concentration of therapeutic agents at the tumor site and then preventing their spread into healthy tissue [6,7]. Thus to treat GBM effectively, limitations of conventional drug delivery methods should be overcome by novel drug delivery strategies. In the present era nanotechnology based drug delivery systems (NDDS) are increasingly applied in cancer therapy because of their site specific accumulation in tumor cells either by enhanced permeation and retention (EPR) effect or active targeting delivery [8-11].

In the present study we have designed a novel nano-carrier that can carry drugs cross the BBB and then target the tumor without affecting normal brain and body cells. We have synthesized poly propyleneimine (PPI) dendrimers as drug delivery system and coated with PEG-2000 before loading paclitaxel a powerful anti-neoplastic agent against malignant glioma cells [12,13]. However, it is crucial for PEGylated PPI dendrimers to deliver drugs across the BBB and therefore our strategy for brain glioma targeting, mainly focused on receptor mediated endocytosis (RME).

GBM bearing brain express large number of receptors on the luminal endothelial plasma membranes, including the transferring receptor, the insulin receptor, endothelial growth factors receptor, and low-density lipoprotein receptor [14,15]. Out of this low-density lipoprotein receptor are extensively present on BBB as well as human glioma cells. This makes LRP as a potential targeted moiety for effective drug delivery to the glioma cells [16,17]. Angiopep-2, is reported as a ligand of LRP which possesses a high brain penetration and perfusion capability in mice [18]. Angiopep-2 can be used to enhance the drug delivery across BBB along the targeting of brain glioma. Thus in the present study we have conjugated angiopep-2 with PEGylated PPI dendrimers to deliver paclitaxel site specifically to the glioma cells. Paclitaxel (PTX), has been selected as model anti cancer for the treatment of brain glioblastoma. However its poor aqueous solubility and low therapeutic index make it suitable for the delivery through PEGylated PPI dendrimers [19,20].

2. MATERIALS AND METHODS

2.1. Materials

Ethylenediamine (EDA) and acrylonitrile (ACN) were purchased from CDH, India. Cellulose dialysis bag (MWCO 12-14 KDa, Himedia, India), Fluorescein Isothiocyanate (FITC), MePEG2000 and Raney Nickel were obtained from Merck, India. Triethylamine, dioxan, succinic anhydride, N, N dicyclohexyl carbodiimide (DCC), 4 dimethyl amino pyridine were procured from sd-fine chemicals, India. Angiopep-2 was purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). Paclitaxel was a benevolent gift sample obtained from Panacea Biotech Ltd. (New Delhi). Penicillin, streptomycin mixture was purchased from Sigma, St. Louis, Missouri. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serums (FBS) were procured from HiMedia, Mumbai, India. All other chemicals were of analytical grade and used without any further modification.

2.2. Cell lines and culture conditions

The BCECs and C6 glioma cells were procured from Centre for Cellular & Molecular Biology (CCMB) Pune. The cells were routinely cultured in DMEM containing 10% FBS supplemented with 0.25% (w/v) trypsin, 1% penicillin streptomycin mixture. All the cells were cultured in incubators maintained at 37°C under a humidified atmosphere containing 5% CO_2. All experiments were performed on cells in the logarithmic phase of growth.

2.3. Synthesis of dual targeted delivery system

2.3.1. Synthesis of 4.0G EDA-PPI core dendrimers

The half generation EDA-dendrimer-(CN)4*n* (where *n* is generation of reaction or reaction cycle) was synthesized by double Michael addiction reaction between ACN (2.5 molar times per terminal NH_2 group of core amine moiety) and aqueous solution of EDA. After the initial exothermic phase, the reaction mixture was heated at 80ºC for 1hr to complete the addiction reaction. The excess of acrylonitrile was then removed by vacuum distillation (16 mbar, bath temperature 40ºC). The full generation EDA-dendrimer-(NH_2)4*n* was obtained by hydrogenation of half generation dendrimers in methanol at 40 atm hydrogen pressures and 70ºC for 1hr with Reney Nickel. PPI dendrimers up to 4.0G were prepared by repetition of all the above steps consecutively, with increasing quantity of ACN [21-23].

2.3.2. PEGylation of 4.0G PPI Dendrimers (PEG-PPI)

PEG2000 (8 g, 8 mmol), succinic anhydride (500 mg, 10 mmol), 4 dimethyl amino pyridine (488 mg, 8 mmol) and triethylamine (404 mg, 8 mmol) were dissolved in dioxane and stirred over night at room temperature. Dioxane was completely removed from the resulting solution and residue was dissolved in dichloromethane, filtered and concentrated. Resultant PEGCOOH2000 was precipitated out with ether and dried in oven to remove final traces of solvents. To a solution of 4.0G PPI dendrimer (0.01mmol) in dimethyl sulfoxide (DMSO) (10 ml), PEGCOOH2000 (0.32mmol) in DMSO (10 ml) and DCC (0.32 mmol) in DMSO (10 ml) were added and stirred at room temperature for 5 days. The product was precipitated by addition of water, filtered and dialyzed (MWCO 12-14 Kda, Himedia, India) against double distilled water for 24 hr to remove free PEGCOOH2000, DCC and partially PEGylated dendrimers followed by lyophilization (Heto drywinner, Germany) [24-28].

2.3.3. Preparation of angiopep-2 conjugated PEGylated PPI dendrimers (ANG-PEG-PPI)

ANG-PEG-PPI was prepared by reacting activated PEG-PPI dendrimers with Angiopep-2 in PBS (pH 7.4) for 8 hr under nitrogen flow at room temperature. The outer groups of activated PEG-PPI dendrimers were specifically reacted with the thiol groups of Angiopep-2. The reaction mixture was then centrifuged at 12,000 rpm for 60 min at 4°C and dialyzed (MWCO 12-14Kda) against PBS buffer (pH 7.4) for 24 hr to remove free ANG and PEG-PPI. Residue was re-suspended in PBS (pH 7.4) and kept at 4°C for further use [29].

2.3.4. PTX loading in ANG-PEG-PPI (PAPP)

Known molar concentration (1:1) of ANG-PEG-PPI was dissolved in methanol and mixed with methanolic solution of PTX. The mixed solutions were incubated with slow magnetic stirring (50 rpm) using teflon beads for 24 hr. This solution was twice dialyzed in cellulose dialysis bag (MWCO 1000 Da Sigma, Germany) against double distilled water to remove free drug. Concentration of free drug was estimated spectrophotometrically (λ_{max} 227 nm) (UV-1601, Shimadzu, Japan) to determine indirectly the amount of drug loaded within the system. The dialyzed formulations were lyophilized and used for further characterization. FITC loaded ANG-PEG-PPI dendrimers (FAPP) were prepared similar to the preparation of PTX loaded formulation [30].

2.5. Characterization of PAPP dendrimers

2.5.1. Morphological studies of the PAPP dendrimers

Transmission electron microscopy (TEM) was performed to investigate particle size and morphology of dendrimer formulation. The TEM studies were carried out using 3mm Forman (10.5% plastic powder in amyl acetate) coated copper grid (300 mesh) at 60 KV using negative staining by 2% phosphotungstic acid (PTA) for whole generation of dendrimers at 150,000X magnification on Philips CM-10 TEM and Fei-Philips Morayagni 268D with digital TEM image analysis system.

2.5.2. FTIR, ^{1}H NMR and Mass spectroscopies

4.0G PPI, PEG-PPI and ANG-PEG-PPI dendrimer were lyophilized and separately analyzed by FTIR (Perkin Elmer-Spectrum RX-I, PU, Chandigarh), ^{1}H NMR at 300 Hz (Avance-II (Bruker, Germany) and Mass spectroscopy by MALDI-TOF (Micromass Tof-Spec 2E instrument, USA).

2.5.3. Percentage drug loading

Percentage Drug-loading (DL%) was calculated by the following equation

$$DL\% = \frac{\text{Weight of (feeding drug} - \text{unbound drug)}}{\text{weight of ANGPEGPPI dendrimer} + \text{weight of (feeding drug} - \text{unbound drug)}} x100$$

2.5.4. In vitro drug release

PAPP dendrimer formulation (containing 5 mg of PTX) was dispersed in 2 ml of PBS (pH 7.4), and transferred into dialysis bag (MWCO 2000 Da). The dialysis bags was end-sealed and submerged into 100 ml of PBS (pH 7.4) with continuous stirring under sink condition at 37°C in dark. At definite time intervals, 1 ml of solution was withdrawn and replaced with an equal volume of fresh medium. The amount of PTX release was analyzed spectrophotometrically at λ_{max} 227 nm [31-33].

2.6. In vitro cytotoxicity assay

Cytotoxicity studies were carried out as per the standard procedure for methylthiazole tetrazolium (MTT) assay. C6 glioma cells were used to evaluate the cytotoxicity of PAPP dendrimers. C6 glioma cells were seeded in 96-well plates with a density of 10,000 cells/well, and allowed to adhere for 24 hr prior to assay. Then free PTX and PAPP dendrimers were added to the plates at a series of PTX concentrations from 0 to 10 μM. Blank ANG-PEG-PPI without PTX was used to test the cytotoxicity of carrier. After each treatment, the cells were incubated with 0.5 mg/ml MTT in DMEM for 4 hr in dark and then mixed with dimethyl sulfoxide after the supernatant was removed. The absorbance at 570 nm was detected using the microplate reader (Synergy TM2, BIO-TEK Instruments Inc. USA). Cell viability was determined by the percentage of OD value of the study group over the control group [34,35].

2.7. Cellular uptake of FAPP dendrimers

C6 glioma cells were placed on glass cover slips that were placed in 6-well plates. After 24 hr, cells were preincubated in DMEM for 30 min, and then treated with FAPP dendrimers (containing 10μg/ml FITC) and FITC loaded PEGylated PPI dendrimers (FPPD) in DMEM for 2 hr. Cells on coverslips were washed with PBS (pH 7.4). The samples were subjected to observe with fluorescent microscope (Leica DMI 4000B, Germany) [36].

In vitro drug targeting study

Drug targeting effect of PAPP dendrimers was studied in co-culture model of BCECs and C6 glioma cells. BCECs incubated in transwell for 4 days subsequently transferred into another 24 well culture plate containing previously cultured C6 glioma cells and incubated further for 24 hr. Co-culture model was exposed to serum free DMEM and Angiopep-2 for 30 min before treating with free PTX, PTX loaded PPI dendrimers (PPD), PTX loaded PEGylated PPI dendrimers (PPPD) and PAPP dendrimers. After 8hr of incubation period transwell was removed and C6 glioma cells were further cultured to determine the cell survival using MTT assay [37].

3. RESULTS

3.1. Synthesis and characterization of ANG-PEG-PPI (4.0G) dendrimer

In the present study 4.0G PPI dendrimers were synthesized according to the procedure reported by De Brabender-Van Den Berg and Meijer using EDA as initiator core and ACN as branching units [21]. Synthesis of 0.5G PPI was also confirmed by IR peaks, mainly of nitrile at 2249 cm^{-1}. All the nitrile terminal of 0.5G PPI converted into primary amine in 1.0G PPI and further confirmed by IR peak at 3432 cm^{-1} for primary amine (N—H stretch). Similarly synthesis of 4.0G PPI dendrimer was confirmed by IR peaks at C-C bend (1109 cm^{-1}); C-N stretch (1301cm^{-1}); C-H bend (1411 cm^{-1}, 1465 cm^{-1}); N-H deflection of amine (1667 cm^{-1}) and primary amine at 3400 cm^{-1} (N-H stretch). Important shifts in NMR spectrum of 4.0G dendrimer at 3.24–3.86 ppm confirmed the presence of terminal $-CH_2NH_2$ groups while Mass spectrum confirmed 3527 Da molecular weight of 4.0G PPI dendrimer. PEGylation of 4.0G PPI dendrimers was also confirmed by FTIR and NMR spectra. Characteristic peak of secondary amide stretch at 3397.7cm^{-1}, C-H stretch at 2972.0 cm^{-1} and C=O stretch near 1653.6 cm^{-1} confirmed the PEGylation of the amine terminated dendrimers. NMR spectrum shown the shift of CO-NH bond at 7.41ppm and CH_2-CH_2-O bond at 3.49, 3.47 ppm. This is due to the presence of amide and ether linkages in PEGylated dendrimers.

3.2. Characterization of PTX loaded ANG-PEG-PPI dendrimer

TEM photographs of PTX loaded ANG-PEG-PPI dendrimers displayed the spherical shape of dendrimers with average particle size of 47±0.20 nm (Figure 1). Mass spectroscopy and ^1H NMR studies confirmed the conjugation of ANG to PEG-PPI dendrimers. Percentage drug loading of PTX was found to be 57±0.8% which was higher than the 40±0.6% drug loading in plain 4.0G PPI dendrimers. Increased drug loading may be due to entrapment of PTX in dendrimer scaffold as well as in PEG network. In vitro cumulative release profile of PTX from drug loaded dendrimer formulations shown in Figure 2. PTX release from PEG-PPI dendrimers (PPPD) and ANG-PEG-PPI (PAPP) dendrimers was found to be 46.4±0.8% and 38.6±0.2% in 24hr respectively. However both formulation shown an initial burst release followed by zero order release profile.

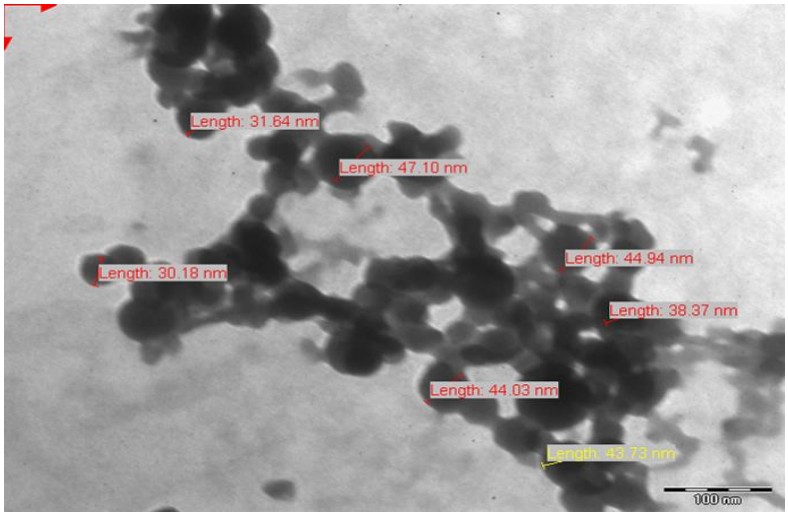

Figure 1: TEM images of ANG-PEG-PPI dendrimers

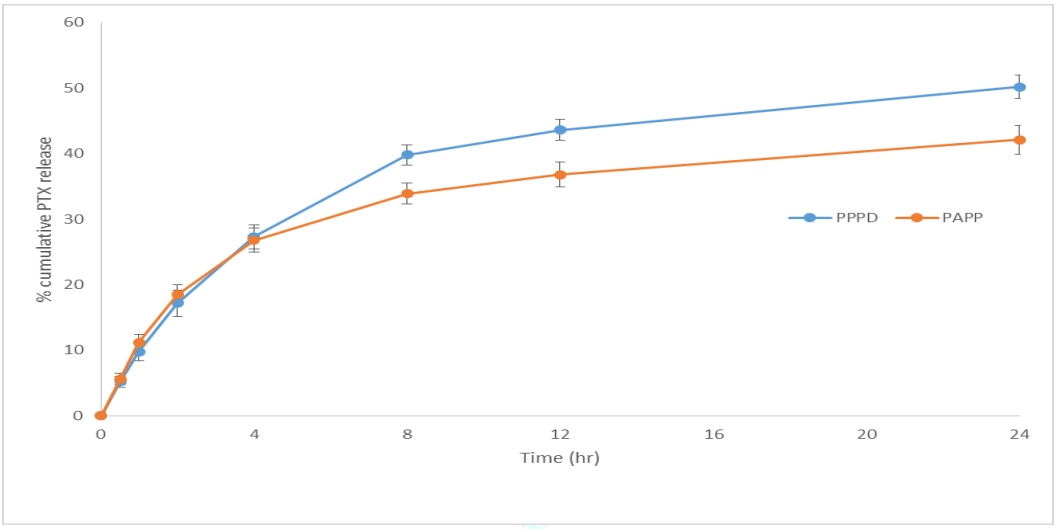

Figure 2: Cumulative PTX release from PTX loaded PEG-PPI dendrimers and PTX loaded ANG-PEG-PPI dendrimers (n=3)

3.3. Cytotoxicity assay

The cytotoxicity of free drug PTX and PTX loaded ANG-PEG-PPI dendrimer was studied on C6 glioma cells against blank ANG-PEG-PPI dendrimers. Blank dendrimer formulation exhibited low toxicity and good biocompatibility at concentrations (<0.1µg/ml) comparative to the free PTX and PAPP dendrimer. However at higher concentrations blank dendrimers elicited cell inhibition due to its own cytotoxic nature. At lower concentrations (<0.01µg/ml) of PAPP dendrimer, C6 glioma Cells viability was near 75% which drastically fall at higher concentrations. PAPP exhibited highest inhibitory effect of cell proliferation comparative to other formulations. The IC_{50} value of PAPP (0.088±0.002 µg/ml) was 3.2 times lower than the free PTX (0.282±0.06 µg/ml) indicating higher antiproliferative activity of PAPP dendrimers (Figure 3).

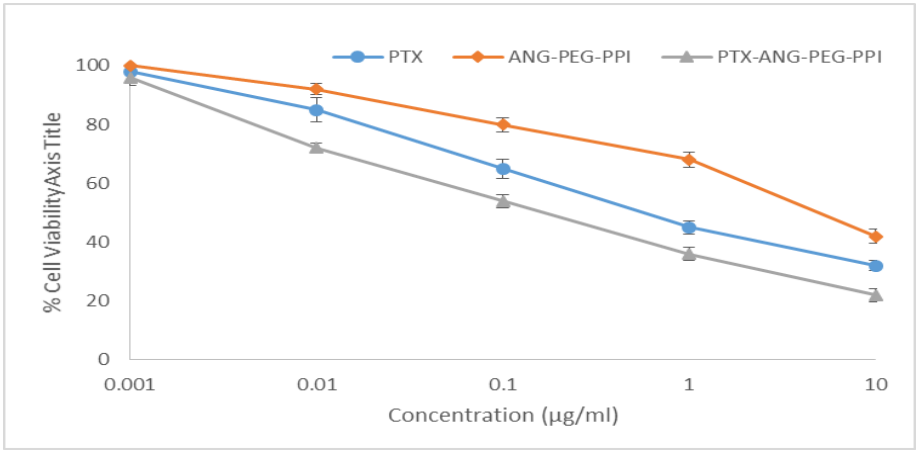

Figure 3: In vitro cytotoxicity of various formulations of PTX against C6 cells (n=3)

3.4. Cellular uptake of FAPP dendrimers

C6 glioma cells exhibited more intense green fluorescence when incubated with FAPP dendrimers comparative to FPPD dendrimer formulation indicating the higher cellular uptake of FAPP dendrimers. Higher fluorescence intensity of FAPP dendrimers corresponds to the presence of Angiopep-2 on the dendrimer surface and considered as the result of ligand receptor mediated cellular uptake of FAPP dendrimers. FPPD dendrimers uptake by the C6 glioma cell was very limited. However on increasing the exposure time an increased fluorescence was observed.

3.5 In vitro drug targeting study

In order to determine the targeting efficiency of FAPP dendrimers co-culture model of BCECs and C6 glioma cells was developed. The percentage cell viability of C6 glioma cells treated with PAPP dendrimer formulation was found to be least than that of free PTX, PTX loaded PPI dendrimers, PTX loaded PEGylated PPI dendrimers (Figure 4). The cell growth inhibitory activity of PAPP dendrimers was significantly reduced when C6 glioma cells were pretreated with angiopep-2 before incubating with PAPP dendrimers. Competitive binding of ligand with receptor may be responsible for this. It demonstrated the potential of angiopep-2 conjugated with PEGylated PPI dendrimers to deliver drugs across the BBB targeting specifically to C6 glioma cells.

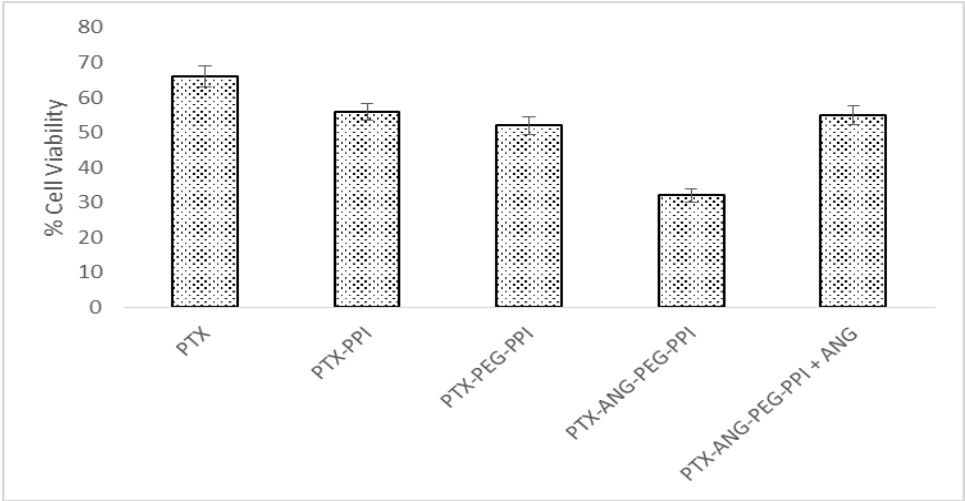

Figure 4: Targeting effect: cell viability of free PTX, PTX-PPI, PTX-PEG-PPI, PTX-ANG-PEG-PPI and PTX-ANG-PEG-PPI +Angiopep-2 against Co culture of BCECs and C6 glioma cells (n=3).

4. DISCUSSION

The success of chemotherapy in GBM treatment is largely depends on the specific drug delivery to the GBM cell vicinity leaving normal cells unaffected. However conventional drug delivery strategies has limited success due to their nonspecific drug delivery and greater side effects. Another big obstacle in GBM chemotherapy is BBB which restrict the adequate amount of drug delivery to the brain GBM. Hence a novel drug delivery strategy that could deliver significant amount of anticancer drug across the BBB with reduced side effects and improved patient's survival time is highly required. PPI dendrimers is considered one of the promising nanocarrier which are globular, hyperbranched, biocompatible systems with low toxicity profile. However highly positive charge at surface dendrimers can be reduced by surface modification using PEG molecules. These long circulating PEGylated PPI dendrimers alone are incapable of drug targeting and thus required to be targeted using ligand receptor interaction. It is reported that LRP receptors are overexpressed on BBB as well as GBM cells which show competitive affinity to bind with different lipoproteins. Angiopep-2 a novel peptide containing 19 amino acids possesses a higher LRP affinity to cross BBB than other proteins.

In the present study we have developed Angiopep-2 conjugated PEGylated PPI dendrimers as drug carrier for GBM specific drug delivery. In ANG-PEG-PPI dendrimers, Angiopep-2 was linked to PPI dendrimers using bifunctional PEG. PPI dendrimers were characterized by FTIR, NMR and Mass spectroscopies for confirming the synthesis and structural uniformity. The particle size of ANG-PEG-PPI dendrimer was found to be less than 100 nm, which was optimum for dendrimer endocytosis by brain capillary endothelial cells. Higher drug payload and controlled release profile of PTX loaded ANG-PEG-PPI dendrimers was further proved it's superiority in drug delivery.

PTX loaded ANG-PEG-PPI dendrimers has shown a better anti-glioma effect than PTX alone, which was proven by enhanced C6 glioma cell inhibition. The MTT assay demonstrated that PAPP dendrimer formulation resulted in greater cytotoxicity effects in C6 glioma cells. Reduced IC_{50} value for PAPP dendrimers comparative to free PTX, suggested its better antiproliferative activity against C6 glioma cells. Ligand receptor interaction understood to be responsible for increased endocytosis of PAPP dendrimers. Targeting efficiency of the PAPP dendrimer formulation was evaluated in BCECs and C6 glioma cells co-culture. The inhibitory effects on C6 cells were significantly enhanced when Angiopep-2 conjugated to PTX-loaded PEG-PPI dendrimers. However when the co-culture was exposed to the angiopep-2 before treating with PAPP, significant fall in cell inhibitory activity was observed. This strongly indicated the targeting efficacy of PTX loaded ANG-PEG-PPI dendrimers.

5. CONCLUSIONS

PPI dendrimers are well established monodisperse nanocarrier for drug delivery. However highly positive surface charge due to presence of amine group restrict their biomedical applications. In the present study we have PEGylated the PPI dendrimers rendering them neutral and

biocompatible. Further modification with Angiopep-2 improved their BBB crossing activity along with the site specific drug delivery to the brain tumor. Physical characterization for size, structural integrity, drug loading capacity and in vitro cell line studies for increased cytotoxicity, cellular uptake and targeting confirmed its superiority over the free paclitaxel drug. Hence targeting potential of Angiopep-2 conjugated PEGylated PPI dendrimers across the BBB and in the vicinity of tumor can be concluded by the present study.

REFERENCES

1. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. Neurobiology of disease. 2010; 37(1):13-25.
2. Gao JQ, Lv Q, Li LM, Tang XJ, Li FZ, Hu YL, Han M. Glioma targeting and blood–brain barrier penetration by dual-targeting doxorubincin liposomes. Biomaterials. 2013; 34(22):5628-39.
3. Huse JT, Holland EC. Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. Nature reviews cancer. 2010; 10(5):319-31.
4. Stiles CD, Rowitch DH. Glioma stem cells: a midterm exam. Neuron. 2008; 58(6):832-46.
5. Huse JT, Phillips HS, Brennan CW. Molecular subclassification of diffuse gliomas: seeing order in the chaos. Glia. 2011; 59(8):1190-9.
6. Friedmann-Morvinski D, Narasimamurthy R, Xia Y, Myskiw C, Soda Y, Verma IM. Targeting NF-kB in glioblastoma: A therapeutic approach.Science Advances. 2016; 2(1):1-11.
7. Glaser T, Han I, Wu L, Zeng X. Targeted nanotechnology in glioblastoma multiforme. Frontiers in Pharmacology. 2017; 8:166.
8. Allard E, Passirani C, Benoit JP. Convection-enhanced delivery of nanocarriers for the treatment of brain tumors. Biomaterials. 2009; 30(12):2302-18.
9. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: Overcoming blood–brain barrier to treat neurodegenerative diseases. Journal of Controlled Release. 2016; 235:34-47.
10. Agemy L, Friedmann-Morvinski D, Kotamraju VR, Roth L, Sugahara KN, Girard OM, Mattrey RF, Verma IM, Ruoslahti E. Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma. Proceedings of the National Academy of Sciences. 2011; 108(42):17450-5.
11. Kreuter J. Drug delivery to the central nervous system by polymeric nanoparticles: what do we know?. Advanced drug delivery reviews. 2014; 71:2-14.
12. Ong BY, Ranganath SH, Lee LY, Lu F, Lee HS, Sahinidis NV, Wang CH. Paclitaxel delivery from PLGA foams for controlled release in post-surgical chemotherapy against glioblastoma multiforme. Biomaterials. 2009; 30(18):3189-96.
13. Marupudi NI, Han JE, Li KW, Renard VM, Tyler BM, Brem H. Paclitaxel: a review of adverse toxicities and novel delivery strategies. Expert opinion on drug safety. 2007; 6(5):609-21.
14. Minchinton AI, Tannock IF. Drug penetration in solid tumours. Nature Reviews Cancer. 2006; 6(8):583-92.
15. Dhanikula RS, Argaw A, Bouchard JF, Hildgen P. Methotrexate loaded polyether-copolyester dendrimers for the treatment of gliomas: enhanced efficacy and intratumoral transport capability. Molecular Pharmaceutics. 2008; 5 (1):105-16.
16. Davis ME, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nature reviews Drug discovery. 2008; 7(9):771-82.
17. Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Advanced drug delivery reviews. 2008; 60(15):1615-26.
18. Bell RD, Sagare AP, Friedman AE, Bedi GS, Holtzman DM, Deane R, Zlokovic BV. Transport pathways for clearance of human Alzheimer's amyloid β-peptide and apolipoproteins E and J in the mouse central nervous system. Journal of Cerebral Blood Flow & Metabolism. 2007; 27(5):909-18.
19. Xin H, Jiang X, Gu J, Sha X, Chen L, Law K, Chen Y, Wang X, Jiang Y, Fang X. Angiopep-conjugated poly (ethylene glycol)-co-poly (ε-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. Biomaterials. 2011; 32(18):4293-305.
20. Regina A, Demeule M, Che C, Lavallee I, Poirier J, Gabathuler R, Beliveau R, Castaigne JP. Antitumour activity of ANG1005, a conjugate between paclitaxel and the new brain delivery vector Angiopep-2. British journal of pharmacology. 2008; 155(2):185-97.
21. de Brabander-van den Berg E, Meijer EW. Poly (propylene imine) dendrimers: large-scale synthesis by hetereogeneously catalyzed hydrogenations. Angewandte Chemie International Edition in English. 1993; 32(9):1308-11.
22. Parashar AK, Patel M, Jhariya AN, Nema RK. Synthesis and characterization of PEGylated PPI dendrimer for prolonged delivery of insulin. Techno Pharma. 2014; 2:01-10.
23. Gupta U, Agashe HB, Jain NK. Polypropylene imine dendrimer mediated solubility enhancement: effect of pH and functional groups of hydrophobes. J Pharm Pharm Sci. 2007; 10(3):358-67.
24. Zalipsky S. Chemistry of polyethylene glycol conjugates with biologically active molecules. Advanced Drug Delivery Reviews. 1995; 16(2-3):157-82.
25. Veronese FM, Caliceti P, Pastorino A, Schiavon O, Sartore L, Banci L, Scolaro LM. Preparation, physico-chemical and pharmacokinetic characterization of monomethoxypoly (ethylene glycol)-derivatized superoxide dismutase. Journal of controlled release. 1989; 10(1):145-54.
26. Olivier JC, Huertas R, Lee HJ, Calon F, Pardridge WM. Synthesis of pegylated immunonanoparticles. Pharmaceutical research. 2002; 19(8):1137-43.
27. Vijayaraj Kumar P, Agashe H, Dutta T, Jain NK. PEGylated dendritic architecture for development of a prolonged drug delivery system for an antitubercular drug. Current drug delivery. 2007; 4(1):11-9.
28. Chang B, Guo J, Liu C, Qian J, Yang W. Surface functionalization of magnetic mesoporous silica nanoparticles for controlled drug release. Journal of Materials Chemistry. 2010; 20(44):9941-7.
29. Xin H, Jiang X, Gu J, Sha X, Chen L, Law K, Chen Y, Wang X, Jiang Y, Fang X. Angiopep-conjugated poly (ethylene glycol)-co-poly (ε-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. Biomaterials. 2011; 32(18):4293-305.
30. Karthikeyan R, Sai Koushik O, Vijayraj Kumar P. Biodistribution Study of Pegylated Ppi Dendrimer Loaded with Prednisolone for Prolonged Release. Nano Research & Applications. 2016; 7:362.
31. Shen J, Zhan C, Xie C, Meng Q, Gu B, Li C, Zhang Y, Lu W. Poly (ethylene glycol)-block-poly (D, L-lactide acid) micelles anchored with angiopep-2 for brain-targeting delivery. Journal of drug targeting. 2011; 19(3):197-203.
32. Gajbhiye V, Kumar PV, Tekade RK, Jain NK. PEGylated PPI dendritic architectures for sustained delivery of H 2 receptor antagonist. European journal of medicinal chemistry. 2009; 44(3):1155-66.
33. Bhadra D, Bhadra S, Jain S, Jain NK. A PEGylated dendritic nanoparticulate carrier of fluorouracil. International journal of pharmaceutics. 2003 May 12;257(1):111-24.
34. Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials. 2003; 24(7):1121-31.
35. Béduneau A, Saulnier P, Benoit JP. Active targeting of brain tumors using nanocarriers. Biomaterials. 2007; 28(33):4947-67.
36. Ke W, Shao K, Huang R, Han L, Liu Y, Li J, Kuang Y, Ye L, Lou J, Jiang C. Gene delivery targeted to the brain using an Angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. Biomaterials. 2009; 30(36):6976-85.
37. Huang S, Li J, Han L, Liu S, Ma H, Huang R, Jiang C. Dual targeting effect of Angiopep-2-modified, DNA-loaded nanoparticles for glioma. Biomaterials. 2011; 32(28):6832-8.