Current Trends in Biotechnology and Pharmacy
Vol. 13 (2) 146-155 April 2019, ISSN 0973-8916 (Print), 2230-7303 (Online)

In Vitro Cytotoxicity and In Vivo Anti-Tumor Efficacy of CD13 Targeted Peptide – Monomethyl Auristatin E (MMAE) Conjugates

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Abstract

A series of novel peptide ligands that specifically bind to tumor vascular endothelial CD13 receptor were designed, synthesized and evaluated for in vitro binding. In this study, peptide - monomethyl auristatin E (MMAE) conjugates were evaluated for their in-vitro cytotoxicity and mice in-vivo anti-tumor efficacy. The peptides [PEP20, GYPAY; and PEP173 GYPAVYLF] were synthesized by standard solid phase peptide synthesis method. The drug-linker conjugate maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl-monomethyl auristatin E (mc-vc-PABC-MMAE) was used to prepare the peptide-drug conjugates (PDCs). Formation of the PDCs was confirmed by ESI-MS (PEP20-MMAE, 1062.5 $[M+H+Na]^{+2}$; PEP173-MMAE, 828.9 $[M+2H+Na]^{+3}$) and the % purities of the PDCs after purification were higher than 98%. For in-vitro cytotoxicity study, CD13 +ve HT1080, CD13 –ve MCF7 and HEK293 (normal cells) cells were incubated with various concentrations of PDCs/ MMAE. The drug, MMAE, showed very high potency (low IC_{50} values) across all three cell lines (HT-1080 cells, IC_{50} 0.09358 nM; MCF-7 cells, IC_{50} 0.4250 nM; HEK-293 cells, IC_{50} 0.8354 nM). PDCs (PEP20-MMAE and PEP173-MMAE) showed significantly lower cytotoxicity than MMAE in all cell lines. PEP20-MMAE showed 5.2 and 4.3 times lower cytotoxicity in CD13 negative MCF-7 and control normal HEK-293 cells, respectively, when compared to that in CD13 positive HT-1080 cells. PEP173-MMAE was found to have approximately 2.4 times less cytotoxicity in both MCF-7 cells and HEK-293 cells as compared to HT-1080 cells. For the anti-tumor efficacy study, 975 nmol/kg, which is equivalent to 0.70 mg/kg of MMAE, was selected as the treatment dose. In the mice treated with only PBS, the tumor grew rapidly and reached approximately 450 mm^3 by day 28 after tumor implantation (Figure 5). MMAE, PEP20-MMAE, and PEP173-MMAE, all showed almost complete tumor regression during the study. PEP20-MMAE and PEP173-MMAE showed slightly higher tumor regression than MMAE, but the difference was not statistically significant. However, the PDCs exhibited much lower weight loss in mice as compared to the drug MMAE indicating lower side effects in vivo. The limited effectiveness of peptide drug conjugates in in vivo mice tumor model suggested the need of further research to achieve the optimal chemical configuration of the conjugates for in vivo targeting.

Key words : CD13, aminopeptidase N, Knob-Socket model, peptide-drug conjugate

Introduction

In cancer therapy, the primary objective of targeted drug delivery is to transport drug to the cancer sites while minimizing their exposure to normal tissues. Two key strategies have been extensively studied to achieve this goal, both of which rely on modifying the pharmacokinetic properties of the drug. The first strategy uses a delivery vehicle, like nanoparticles, that carries

the drug and determines the drug biodistribution via its own physicochemical characteristics. The second one is the prodrug strategy, where covalent modification of the drug with a moiety that momentarily disguises the drug's bioactivity and confers desirable pharmacokinetic properties. The prodrug approach have several advantages over delivery vehicle approach: (a) significantly lower amount of inert materials that results in decreased metabolic burden of the patient (b) minimize premature drug release, (c) relatively straight forward and simple preparation/manufacturing (1, 2).

Peptide-drug conjugate (PDC) is an emerging type of prodrug (3). It is formed by covalent attachment of a specific peptide sequence to a drug through a linker. The use of peptides would enable the incorporation of many functionalities into PDCs. The amino acid sequences can be selected to regulate the physicochemical properties of the conjugate, as well as to impart active targeting towards a specific receptor expressed at the target cancer tissue. PDCs are biodegradable and generally show no or minimum undesired immunogenic responses because they are composed of amino acids and typically have short peptide sequences (3, 4). Different amino acid combinations allow simplistic preparation of different PDCs. A number of tumor targeting peptides have been developed till date for different types of cancers (5). The peptide sequence can be easily modified to facilitate drug conjugation and, to tune the conjugate molecule ionization and hydrophobicity, which in turn impact the bioavailability. Additionally, PDCs can be purified by simple HPLC technique because of their low molecular weight (3).

The main building blocks of a PDC include a cytotoxic drug, a targeting peptide ligand and a linker between them. The therapeutic efficacy of the PDC is primarily governed by the potency of the cytotoxic drug and the targeting efficiency of the conjugate. The process of synthesizing PDCs is generally fast and simple. Since an already approved drug can be selected as the therapeutic payload, the overall cost of production of PDCs is significantly lower than that of synthesizing a new drug (4).

Doxorubicin, chlorambucil, camptothecin, and paclitaxel are some of the chemotherapeutic drugs that have been used in PDC development (6). But these drugs are relatively low potency cytotoxic drugs. Currently, very potent cytotoxic agents, like auristatins, are used in drug conjugate development (7). Auristatins leads to cell apoptosis by inhibiting the polymerization of tubulin in dividing cells (8).

Design of a novel peptide-doxorubicin conjugate was reported by Soudy et al. They made two different conjugates having ester and amide bonds between doxorubicin and linker. The PDC with ester bond showed 4 times more toxicity than doxorubicin in MDA-MB-435 cells and 40 times better selectivity towards breast cancer cell lines when compared to normal cells (9). Polyak et al reported the development of integrin targeted cyclic RGD-PEG-Dox conjugate. The PDC inhibited the cell proliferation at lower IC_{50} as compared to doxorubicin or control conjugate without RGD peptide (10). An EGFR-binding peptide-doxorubicin conjugate was developed and evaluated in-vitro and in-vivo for anti-cancer efficacy. The study showed improved anticancer efficacy and lower systemic toxicity of PDC with EGFR upregulated tumor cells (11).

CD13, also known as aminopeptidase N (APN), is Zn+2 dependent cell surface ectopeptidase. CD13 consists of 967 amino acid residues. It has a short N-terminal intracellular domain, a single transmembrane region, and a large extracellular domain. CD13 is heavily glycosylated with carbohydrates that is at least 20% of the protein mass. It has at least five different isoforms with differential O-glycosylation sites (12-14). While there is very little or no CD13 expression in normal vessels, it is overexpressed in angiogenic vessels of the neoplastic tissues. Different tumor cells also express or overexpress CD13 receptor. In terms of malignant cell growth, CD13 is implicated in tumor cell invasion, differentiation, proliferation and apoptosis, motility

Vol. 13 (2) 146-155 April 2019, ISSN 0973-8916 (Print), 2230-7303 (Online)

and angiogenesis (15-20). CD13 is overexpressed in many cancers like breast, kidney, prostate, ovarian, colon, gastric, pancreatic and thyroid cancer (21).

In this study, two PDCs were prepared by conjugating previously designed CD13 targeted novel peptides (22) (PEP20, GYPAY; and PEP173, GYPAVYLF) to the drug monomethyl auristatin E (MMAE) via cleavable linker, and the in vitro cytotoxicity and in vivo anti-tumor efficacy of the PDCs were evaluated. The peptides (PEP20; and PEP173) were found to selectively bind to the CD13 receptor in vitro with significantly higher affinity as compared to CNGRC(C1-C5) peptide ligand (22). The linker-drug construct contains a spacer, maleimidocaproyl (mc); a protease degradable dipeptide, valine-citrulline (vc); a self-immolative moiety, para-amino benzyloxycarbonyl (PABC); and the antimitotic drug, MMAE. This construct is termed as mc-vc-PABC-MMAE. The linker-drug construct can be conjugated to a cysteine residue in the peptide sequence using the specific thiol-maleimide coupling reaction (23, 24).

Materials and Methods

Preparation of peptide-drug conjugates

Synthesis: First, the peptides (PEP20-Ahx-Cys, GYPAY-Ahx-C; and PEP173-Ahx-Cys, GYPAVYLF-Ahx-C) were synthesized by standard solid phase synthesis method (25-27). The amino acids and coupling reagents were obtained from Chem-Impex (Wood Dale, IL, USA). The synthesis was started with Fmoc-L-Cys(Trt)-2-chlorotrityl resin to place a cysteine residue at the C-terminal. Fmoc group was removed in each step by treating the resins with solution of 20% piperidine in DMF. A spacer (e.g. 6-aminohexanoic acid/ Ahx) was added between the designed peptide sequence and the C-terminal cysteine residue. Coupling of subsequent amino acids was performed with 1-hydroxy-benzotriazole (HOBt) and diisopropyl-carbodiimide (DIC). Boc protected amino acids were used only for the last N-terminal amino acids to eliminate the necessity of final Fmoc deprotection step. Cleavage of the peptides were performed by treating the resins with trifluoroacetic acid – water – triisopropylsilane (95: 2.5: 2.5) cocktail for 3 hours. The obtained TFA-peptide solution was cooled and evaporated under nitrogen flow until it became thick viscous oily liquid. Ice cold ether was added to the oily liquid to precipitate the peptide. The peptides are then freeze dried and stored in “20° C freeze until further use. The peptides were used to synthesize the PDCs without further purification. The PDCs were prepared using thiol-maleimide coupling reaction. To synthesize each PDC, 5mg of the peptide was dissolved in 2.5 ml of PBS-ACN (70:30) mixture, and equimolar amount of mc-vc-PABC-MMAE (MuseChem, Fairfield, NJ, USA) was dissolved separately in 2.5 ml PBS-ACN (70:30) mixture. The peptide solution and the drug-linker solution were mixed thoroughly by vortexing. The pH of the reaction mixture was adjusted to 6.5 – 7 using HCl (aq). The reaction mixture was shaken for 1 hour at room temperature, and solidified by freeze drying.

Characterization: All the synthesized molecules (PEP20-MMAE; and PEP173-MMAE) were characterized by Electron Spray Ionization Mass Spectrometry (ESI-MS, API 3000, SCIEX, Ontario, Canada). The purity of the synthesized molecules was determined using high pressure liquid chromatography (HPLC) (Agilent Technologies, Santa Clara, CA, USA). The column used was Agilent Zorbax C18, 5 ìm, 4.6×150 mm, and the wavelengths used for detection were 210, 254 and 280 nm. The samples were eluted with a mobile phase consisting of water (A) and acetonitrile (B) using a linear gradient from 10 to 90% B over 30 to 35 minutes, at 1.0 mL/min flow rate. The synthesized PDCs were purified by collecting the appropriate peaks. The purified compounds were freeze dried and stored at -80° C until further use.

*In vitro cytotoxicity of peptide-drug conjugates:*The cytotoxicity study of MMAE (MedKoo Biosciences, Morrisville, NC, USA) and peptide-drug conjugates was performed using CD13 overexpressing HT-1080 cells, CD13 non-expressing MCF-7 cells, and a control normal cell line HEK293. The cells were purchased from

ATCC (Manassas, VA, USA) and were grown in 75 cm^2 flasks in DMEM (high glucose, with L-glutamine, and sodium pyruvate) (Thermo Fisher Scientific, Waltham, MA, USA) with 10% FBS (Gemini Bio-Products, West Sacramento, CA, USA) and 1% penicillin-streptomycin (Gemini Bio-Products) at 37° C and 5% CO2. The cells were detached using TrypLE Express (Thermo Fisher Scientific) and the cell density was counted using Scepter Automated Handheld Cell Counter (MilliporeSigma, Burlington, MA, USA). The cells were seeded into clear 96 well plates at 5000 cells/well (0.2 mL cell suspension/well) and incubated overnight to allow them to attach to the wells. Then the cells were incubated with MMAE or PDCs at various concentrations ranging from 0.0000302 to 30200 nM for 72 h at 37° C and 5% CO2 (in complete growth media). The Sulforhodamine B (SRB) Cell cytotoxicity assays were performed following the company recommended protocol (SRB assay kit, Abcam, ab235935). The SRB absorbance was measured at 490 nm wavelength by using a microplate reader (BioTek Instruments, Inc., VT, USA).

Percent cytotoxicity was calculated by the following formula:

$$Cytotoxicity\,(\%) = \frac{O.D.(DMSO) - O.D.(Smaple)}{O.D.(DMSO)} \times 100$$

Where,

O.D. (DMSO) = absorbance of the DMSO control after background correction.

O.D. (Sample) = absorbance of the sample after background correction.

The IC_{50} values of the compounds were determined using Graph Pad Prism 7 software (GraphPad Software Inc., CA, USA) with nonlinear regression dose-response - inhibition curve fit (variable slope four parameter).

In vivo anti-tumor efficacy of peptide-drug conjugates: The in vivo studies were performed as per the animal protocol (No. 17R02) reviewed and approved by the Institutional Animal Care and Use Committee, University of the Pacific, Stockton, CA, USA. Four to six weeks old female athymic nude mice (nu/nu) were purchased from Simonsen Laboratories (Santa Clara, CA, USA).

Determining maximum tolerated dose (MTD) of the drug MMAE: The MTD of the drug MMAE was determined in four to six weeks old female athymic nude (homozygous, nu/nu) mice. Five healthy mice were given a single dose of 0.375 mg/kg, 0.5 mg/kg, 0.7 mg/kg, 1 mg/kg, and 1.5 mg/kg of MMAE, respectively via tail vein injection using 29 gauge needles. Following administration, the mice were observed daily for their general health and the body weight was measured every three days.

Xenograft model: CD13 overexpressing HT-1080 cells were cultured as described before. On the day of tumor transplantation, cells were detached using TrypLE Express and re-suspended in DMEM. The cell density was counted using Scepter Automated Handheld Cell Counter. The cells were centrifuged at 125xG for 5 minutes and the supernatant was discarded. The cell pellet was washed once with sterile PBS and re-suspended in 50:50 PBS – Matrigel (High Concentration) (Corning Life Sciences, Tewksbury, MA, USA) so that 0.1 mL of the suspension contains approximately 1,000,000 cells. The cell suspension was maintained on ice until injection. All mice were anaesthetized using isoflurane. 0.1 mL of cell suspension was injected subcutaneously on the right flank of each mouse using a 27 gauge needle. Following inoculation, the mice were observed daily for their general health and tumor appearance.

Anti-tumor efficacy: Tumors in mice were grown to reach an average of approximately 100 mm^3. The tumor bearing mice were then divided in to four groups each having four mice. The groups were PBS group, MMAE group, PEP20-MMAE group, and PEP173-MMAE group. Treatment was started 9 or 12 days after cancer cell transplantation and was administered intravenously via tail vein using 29 gauge needles every 4 days for a total of 4 doses (q4d X 4). The dose was 975 nmol/kg for each compound which

is equivalent to 0.7 mg/kg MMAE. The greatest longitudinal diameter (L) and the greatest transverse diameter (W) of the tumor were measured using Vernier caliper every four days from the day of first dosing. Tumor volume was determined using the formula $(L \times W^2)/2$. The body weights were also measured every four days.

Results and Discussion

Preparation of peptide-drug conjugates: The anticipated binding mode of the peptides (PEP20, GYPAY; and PEP173, GYPAVYLF) under study indicates that the N-terminal side binds deep inside the peptide binding channel of CD13 (22). Therefore, a cysteine conjugation site was incorporated at the C-terminal side of the peptides. A spacer (6-aminohexanoic acid, Ahx) was added in between the peptide sequence and the cysteine conjugation site to have spatial separation between the targeting peptide moiety and the linker-drug moiety.

The peptides (PEP20-Ahx-Cys; and PEP173-Ahx-Cys) were conjugated to monomethyl auristatin E (MMAE), a highly potent but non-selective tubulin polymerization inhibitor, through a maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl (mc-vc-PABC) linker. The maleimidocaproyl (mc) part of the linker-drug motif (mc-vc-PABC-MMAE) utilizes maleimide chemistry for cysteine linkage, which takes advantage of exceptional reactivity of maleimide towards sulfhydryl groups to form stable thioester bond. Valine-citrulline (vc) dipeptide is an intracellular protease, cathepsin B, sensitive linker. This protease sensitive approach uses the main proteases found in the tumor cell lysosome for identification and cleavage of a specific peptide sequence. The para-amino benzyloxycarbonyl (PABC) moiety in the linker-drug motif is a self-immolative spacer (24, 28).

Formation of desired molecules was confirmed by ESI-MS as shown in table 1. In the mass spectra PEP20-Ahx-Cys and PEP173-Ahx-Cys were observed as singly charged species whereas the peptide drug conjugates PEP20-MMAE (PEP20-Ahx-Cys-mc-vc-PABC-MMAE), and PEP173-MMAE (PEP173-Ahx-Cys-mc-vc-PABC-MMAE) were observed as double and triple charged. The HPLC analysis of the purified PDCs showed greater than 98% purity (Table 1).

In vitro cytotoxicity studies: Cytotoxicity studies were carried out to evaluate the potency of the free drug, and the peptide drug conjugates. Percentage cytotoxicity was calculated using sulphorhodamine B assay. MMAE showed very high potency (low IC_{50} values) across all three cell lines (Table 2). For all three cells lines, PDCs (PEP20-MMAE and PEP173-MMAE) showed significantly lower cytotoxicity than MMAE in the cell culture medium (Figures 1, 2 and 3, and Table 2).

PEP20-MMAE showed 5.2 and 4.3 times lower cytotoxicity in CD13 negative MCF-7 and control normal HEK-293 cells, respectively, when compared to that in CD13 positive HT-1080 cells (Figures 1, 2 and 3, and Table 2). PEP173-MMAE was found to have approximately 2.4 times less cytotoxicity both in MCF-7 cells and HEK-293 cells as compared to HT-1080 cells (Figures 1, 2 and 3, and Table 2). The PDCs seemed to have specificity trend towards CD13 positive HT-1080 cells as evidence by the lower IC_{50} in the HT-1080 cells. However, as compared to the HT-1080 cells, the drug MMAE was also found to be 4.5 and 8.9 times less cytotoxic (in terms of IC_{50}) in MCF-7 cells and HEK-293 cells, respectively (Figures 1, 2 and 3, and Table 2). This indicates that the conjugation of peptide-linker construct to the drug decreased the potency of MMAE but did not improve its selectivity.

There could be several reasons behind the lower cytotoxicity of the PDCs as compared to MMAE. One possibility could be higher protein binding of the PDCs or interference by fetal bovine serum present in the cell culture medium in which the cells were incubated with PDCs/drug. Liraglutide, a human glucagon-like peptide-1, was reported to exhibit approximately 99% serum protein binding in vitro (29). Hsiao IL and Huang YJ observed significantly lower in vitro cytotoxicity of ZnO particles in serum containing medium as

compared to the serum free medium (30). A dual targeting NGR-peptide"drug conjugate also showed significantly lower in vitro cytotoxicity than the drug itself in serum containing medium (31). The designed peptides might also lost their targeting ability after conjugating to the linker-drug construct due to the large size of the construct. The size of the drug-linker construct (mc-vc-PABC-MMAE) is bigger than the peptides. While, the molar weight of PEP20-Ahx-Cys and PEP173-Ahx-Cys are 785.91 and 1145.3 g/mol, respectively, the molar weight of the linker-drug construct is 1316.6 g/mol. It has been reported that conjugation induced perturbations in the peptide structural microenvironment can lead to diminished binding affinity to the target receptor (4). Peng ZH and Kopeèek J have shown that the cell penetrating cyclic peptide iRGD (CRGDKGPDC) lose its targeting ability after conjugating to linker-drug (valproic acid) construct (32).

In vivo anti-tumor efficacy: To select the therapeutic doses of the compounds in the in vivo study a maximum tolerated dose (MTD) was determined. MMAE was injected into healthy female athymic nude mice at doses ranging from 0.375 mg/kg to 1.5 mg/kg (n=1 for all doses, except for 0.70 mg/kg n=2). General health and body weight of the mice were monitored for 15 days after injection (Figure 4). Doses up to 0.70 mg/kg of MMAE were well tolerated with no apparent sign of toxicity. At 1.0 mg/kg dose, the mouse experienced around 20% weight loss within 6 days of injection and then started regaining weight. The mouse returned to initial body weight

Table 1. MS and HPLC data for the peptides and PDCs

Molecule	**MS (g/mol) Observed**	**HPLC Purity (%)**
PEP20-Ahx-Cys	808.8 [M+Na]+	80.5
PEP173-Ahx-Cys	1144.2 [M-H]-	80.9
PEP20-MMAE (PEP20-Ahx-Cys-mc-vc-PABC-MMAE)	1062.9 [M+H+Na]+2	98.1
PEP173-MMAE (PEP173-Ahx-Cys-mc-vc-PABC-MMAE)	828.9 [M+2H+Na]+3	98.3

Table 2. *In-vitro* cytotoxicity study data

Cell Line	IC50 (nM)		
	MMAE	PEP20-MMAE	PEP173-MMAE
HT-1080 (CD13 +ve)	0.09358 ± 0.01086	92.54 ± 13.38	72.68 ± 9.910
MCF-7 (CD13 -ve)	0.4250 ± 0.08300	477.9 ± 89.67	175.0 ± 21.30
HEK-293 (normal cell line)	0.8354 ± 0.1101	399.7 ± 51.89	172.8 ± 23.93

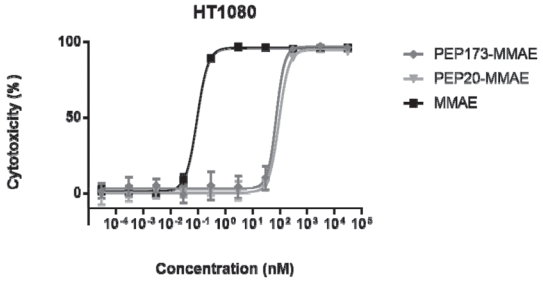

Fig. 1. Cytotoxicity of MMAE, PEP20-MMAE, and PEP173-MMAE in HT-1080 cells

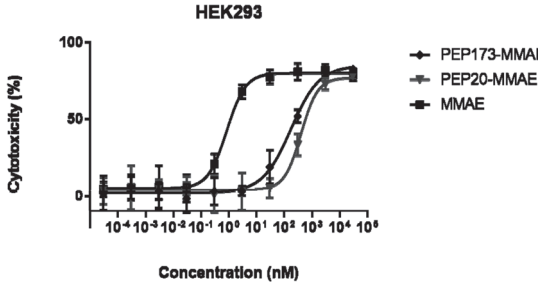

Fig. 3. Cytotoxicity of MMAE, PEP20-MMAE, and PEP173-MMAE in HEK-293 cells

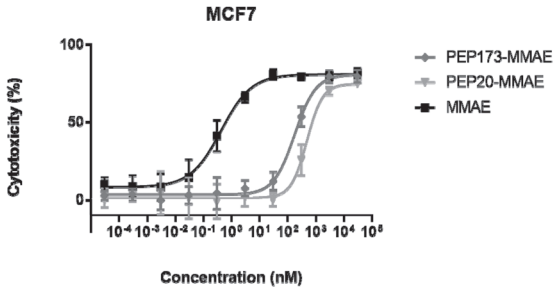

Fig. 2. Cytotoxicity of MMAE, PEP20-MMAE, and PEP173-MMAE in MCF-7 cells

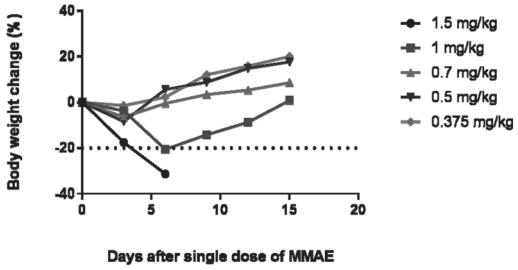

Fig. 4. MMAE tolerance in mice - MTD study

by day 15. The mouse receiving 1.5 mg/kg of MMAE lost more than 30% of the body weight by day 6 at which point the mouse was euthanized. Thus, the MTD of MMAE in female athymic nude mice (4-6 weeks old) was determined to be between 1.0 mg/kg and 1.5 mg/kg. The maximum tolerated dose (MTD) of dolastatin 10 in mice was reported to be approximately 0.45 mg/kg (33). Dolastatin 10 is a cytotoxic agent whose structure is similar to MMAE (34). Francisco et al reported the MTD of MMAE in SCID mice to be between 0.50 mg/kg and 1.0 mg/kg (23).

For the anti-tumor efficacy study, 975 nmol/kg, which is equivalent to 0.70 mg/kg of MMAE, was selected as the treatment dose (MMAE, PEP20-MMAE, and PEP173-MMAE). Treatment was started when the average tumor volume reached approximately 100 mm3 (9 or 12 days after tumor cell injection). Mice were administered with the drug, drug conjugates or PBS treatment every 4 days for a total of 4 doses (q4d x 4). In the mice treated with only PBS, the tumor grew rapidly and reached approximately 450 mm^3 by day 28 after tumor implantation (Figure 5). MMAE, PEP20-MMAE, and PEP173-MMAE all showed almost complete tumor regression during the study (Figure 5). PEP20-MMAE and PEP173-MMAE showed slightly higher tumor regression than MMAE, but the difference was not statistically significant. This insignificant difference in antitumor activity between the drug MMAE and PDCs may also be due to higher plasma protein binding of the PDCs or the diminished targeting ability of the peptides after conjugating with the larger linker-drug construct. Additionally, the degradation of PDCs in circulation by different enzymes may have contributed to this observation. Enzymatic degradation in systemic circulation has long been one of the major challenges for the peptide based drugs (35). van Hensbergen et al previously reported the CD13 targeted peptide-drug conjugate, Doxorubicin-CNGRC (C1-C5), to

Vol. 13 (2) 146-155 April 2019, ISSN 0973-8916 (Print), 2230-7303 (Online)

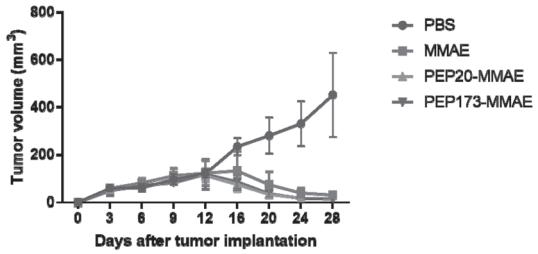

Fig. 5. Anti-tumor efficacy of the PDC's in mice

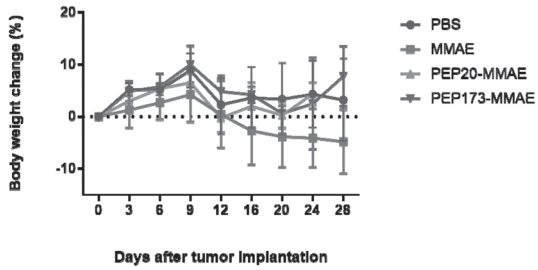

Fig. 6. Effect of PDC's on Body weight change (%) in mice

show no added advantage as compared to the drug, doxorubicin, in terms of in vitro cytotoxicity and in vivo antitumor effects (36).

Although, the PDCs have comparable anti-cancer efficacy in mouse as compared to the drug MMAE, the groups treated with PEP20-MMAE and PEP173-MMAE showed a body weight increase of 4% and 8%, respectively, even after fourth dose (25 and 28 days after tumor implantation) (Figure 6). On the other hand, the drug MMAE treated group lost body weight after dosing started and had a maximum weight loss of 10% after 28 days of tumor implantation (Figure 6). Weight loss is one of major adverse effects of anticancer agents due to high and non-selective cytotoxic potency (37). The PDCs (PEP20-MMAE and PEP173-MMAE) have shown significantly reduced side effects in terms of weight loss.

Conclusion

PDCs investigated showed limited effectiveness of peptide drug conjugates in vivo mice tumor model but reduced adverse effects, suggesting the need to improve the designed peptide-MMAE drug conjugates. Future research will focus on achieving the optimal chemical configuration of the conjugates for in vivo targeting and receptor mediated cellular uptake. The current computational simulation studies is limited to projecting binding of the peptides to the target protein. In future, peptide design with higher number of amino acids in the targeting moiety, and smaller linker-drug construct (especially small molecules) may be used to conserve the target binding ability of the PDCs.

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