ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

PH-Sensitive Polymeric Nanoparticles for Targeted Delivery of Doxorubicin

Yuzhe Yuan[1,a*], Shuyao Geng[2,b], Chenxi Li[3,c], Jiayuan Yang[4,d]

[1]*China Pharmaceutical University, Nanjing, Jiangsu, China*
[2]*Shandong University, Jinan, Shandong, China*
[3]*Beijing No.11 High School, Beijing, China*
[4]*Hangzhou Dianzi University, Hangzhou, Zhejiang, China*
[a]*905497516@qq.com,* [b]*shuyaogeng@gmail.com,* [c]*2937358467@qq.com,* [d]*yangjy1999@icloud.com*
**Corresponding author: 905497516@qq.com*
These authors contributed equally to this work

Abstract: *In order to improve the targeting of doxorubicin and prolong the action time of the drug, we synthesized a pH-responsive PEG-Schiff-DOX polymer prodrug loaded with nanoparticles. The nanoparticles were prepared by synthesis of PEG-CHO and condensation reaction of PEG-CHO and adriamycin. The release behavior of PEG-Schiff-DOX was tested at different pH. The morphology and particle size of these nanoparticles changed obviously after acid treatment(pH=5.0), and some of them had completely disintegrated. The narrowing of particle size distribution indicates that the heterogeneous nanoparticles disintegrate in the acidic environment of tumor cells, releasing the drug and finally achieving the maximum drug release at pH 5.0. The nanodrugs based on polymeric prodrug had advantages of simple preparation, high drug loading, good storage stability and achieve higher local drug concentration and longer drug action time under the condition of weak acid of tumor microenvironment to keep the curative effect and reduce the side effects. The advantages of offers choices for the development of new drugs and is expected to achieve good application in cancer therapy, has good prospects for development.*

Keywords: *pH-responsive, Prodrug, Nanometer carrier, Targeted drugs*

1. Introduction

In the past few decades, polymer nanomedicine has been extensively researched in cancer treatment. They have the potential to increase drug solubility and prolong the action time of drug as well as increase therapeutic effect and reduce toxic side effects. Comparing with small-molecule anti-cancer drugs, based on the EPR effect, nano-drugs can enhance its accumulation in tumor cells and also enhance penetration and retention [1,2]. These have the advantages of avoiding glomerular filtration, prolonging circulation time, and improving pharmacokinetic properties[3]. Among a variety of nano-drugs such as polymer nanoparticles, prodrugs, micelles, and vesicles, nanogels and prodrug-based nanoparticles can achieve higher local drug concentration and prolong the action time of drug due to their clear and simple structures [4]. The nanodrugs have great potential in cancer treatment and has attracted more and more attention.

Adriamycin is a spectral antitumor antibiotic. Mainly suitable for acute leukemia, acute lymphoblastic leukemia and granulocytic leukemia are effective, often combined with other anticancer drugs. Adriamycin has strong cytotoxicity, the main toxic reactions are leukopenia and thrombocytopenia and cardiotoxicity. These shortcomings can be ameliorated by decreasing doxorubicin solubility, improving targeting, and increasing circulation time. Current drug delivery systems showing great promise include polymer drug carriers such as liposomes, hydrogels and nanoparticles. However, the preliminary development of liposome drug delivery system still has some deficiencies. It shows signs of leakage and is easily recognized. Also, it is removed from the circulatory system through the reticuloendothelial system (RES) [5,6].A limitation of Chi/DPO hydrogels is the platform from which adriamycin is released over a relatively short period of time [7].

These doxorubicin-coupled nanoparticles show selective delivery to tumors. This increases the safety range and reduces side effects [8]. Another advantage of using nanoparticles as drug delivery systems is that they can be easily manufactured and have the added benefit of low cytotoxicity and

ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

biodegradability [9].

Adriamycin is one of the most commonly used anti-tumor chemotherapy drugs. However, the therapeutic dose and clinical efficacy of adriamycin in normal tissues were restricted and decreased by its toxic and side effects and low bioavailability. Therefore, the development of targeted nanoparticle carriers is the main task of DOX in the treatment of tumor cancer, and significant progress has been made [10-11]. DOX nanocarriers are reported to be more physically or chemically delivered in vivo to achieve better release. Although DOX liposomes have been clinically available, these nanocarriers still have some inherent disadvantages, such as low drug loading, early release and individual differences, as evaluated in clinical trials [12-15]. We speculate that these disadvantages are related to the structural properties of DOX molecule itself. This is challenging for clinical conversion of DOX nanocarriers. At this point, some chemical coupling methods were used to improve the DOX nanocarrier to achieve the effect of tumor treatment. DOX is combined with polymers to form polymer-DOX prodrugs such as Angiop-2 Modified and Doxorubicin loaded carbonnanodots (AN-PEG-DOX-CDS) [16], Glutathione-coated nanoparticles (NPs) [17], MUC1 Aptamer-functionalized nanosphere (DOX-apt-Sphere) [18], etc. For example, Chen and his colleagues found that DOX nanocarriers coated with LAPonite and modified with PEG-linked lactic acid (PEG-LA) could be used to target liver cancer cells. Lm-peg-la /DOX can not only release drugs stably for a long time, but also have pH responsiveness [19]. In addition, DOX exhibits significant toxicity when directly attached to a targeted target. For example, disrupting nucleic acid synthesis selectively eliminates the rapid division of cancer cells and is one of the most powerful ways to treat tumor cells. Obviously, many effective drugs fail to function as potential substrates for efflux [20]. However, doX-MPP ligating can inhibit DNA topoisomerase II [21-22] to achieve therapeutic effect. Peg-dox was studied in particular because polyethylene glycol (PEG) is one of the most widely used hydrophilic polymers approved by the FDA. PEG has negligible toxicity and low immunogenicity [23-24]. Peg-dox nanoparticles not only increase the residence time of drugs in vivo, but also achieve targeted movement through EPR effect [25]. The change of physical and chemical environment affects the controllable release of drugs, among which, one of the important factors is the change of pH value in tumor cell environment, which is a common adjustable factor of drug release in aqueous solution.

In this study, we designed and prepared a amphiphilic polymer drug coupling (PEG-Schiff-DOX) via acid-sensitive Schiff base bonds, which can self-assemble into ph-responsive nanoparticles in solution. The drug release degree of DOX nanocarrier is closely related to environmental PH. At PH=5.0 and PH=7.4, the results showed that the bridge schiff base could maintain the structural and functional integrity at PH=7.4, but would decompose at PH=5.0. DOX precursor drugs and DOX carrying nanoparticles have significant effects on pH responsive drug release.

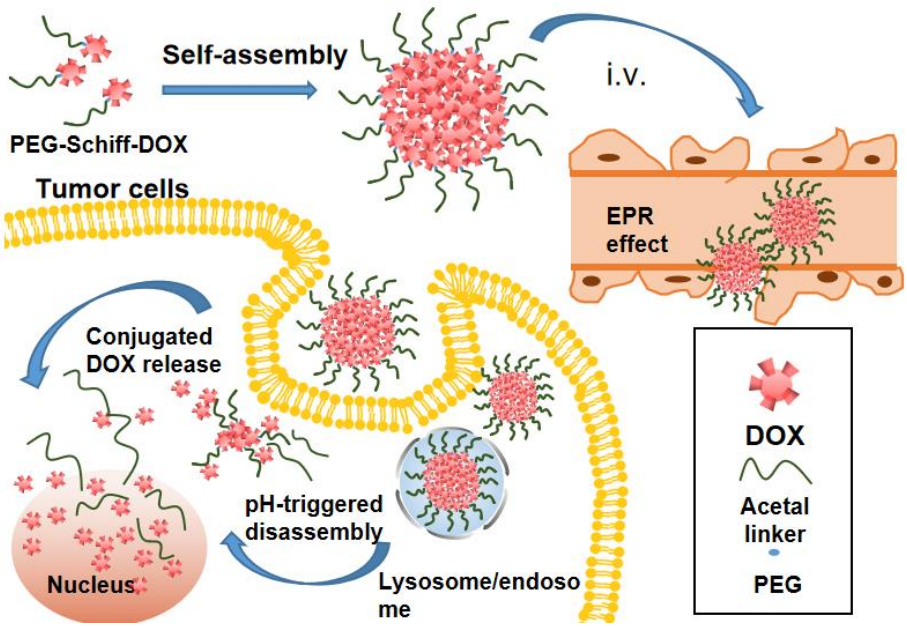

Fig. 1. Schematic illustration of formation and delivery of the DOX-loaded micelles.

ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

2. Materials and methods

2.1 Materials

P-carboxy benzaldehyde (98%, Energy Chemical), 4-dimethylaminopyridine (DMAP, Energy Chemical). Adriamycin hydrochloride was provided by Beijing 301 Hospital. Anhydrous N, N-dimethylformamide (DMF) and anhydrous dimethyl sulfoxide (DMSO) [26]were purchased from Anergic Chemical. The experimental water is ultrapure water. Other chemical reagents used are purchased from Beijing Chemical Plant and used directly.

2.2 Experimental instruments for characterization and testing

Nuclear magnetic resonance hydrogen spectrum (1H NMR): Avance Hocky 400 (400 MHz) spectrometer.

Deuterium reagents were deuterium chloroform (CDCl3), deuterium acetone (ACTone-D6) and deuterium dimethyl sulfoxide (DMSO-D6). TMS was the internal standard. The measurement was performed at 25 ℃.

Dynamic Light Scattering (DLS) : Malvern Zetasizer Nano ZS Dynamic light scattering granulometer. Equipped with a 633 nm he-Ne laser, the detection Angle is 173 °, and the particle size test sample pool is a quartz colorimeter.

UV-visible Spectrum (UV-VIS) : Shimadzu TU1901 UV-visible spectrophotometer.

Scanning electron microscope(SEM)

2.3 Synthesis of pH responsive prodrugs

The pH responsive prodrug was synthesized in two steps, as shown in the figure1 below.

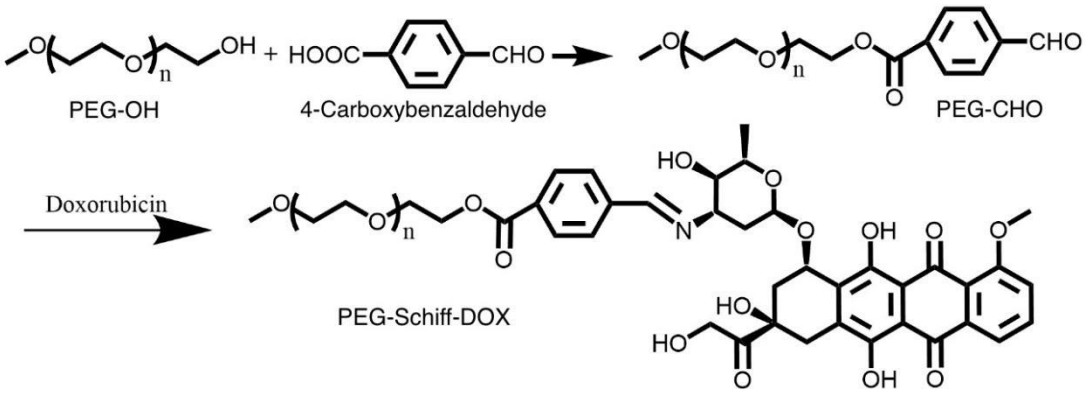

Fig. 2. Synthesis roadmap of PEG-Schiff-DOX

2.3.1 Synthesis of PEG – CHO

4-Carboxybenzaldehyde (150 mg, 1 mmol), EDCI (191.7 mg, 1 mmol) and DMAP (61 mg, 0.5 mmol) were dissolved in ultra-dry DCM, then PEG-OH (375 mg, 0.5 mmol) was added under the protection of nitrogen. The whole system was stirred at 37℃ for 24 hours, and then washed with 1 M HCl, saturated NaHCO3 and saturated salt water for three times. The organic phase was collected and dried, filtered and steamed by anhydrous magnesium sulfate. Ended up with a 90% yield.

2.3.2 Synthesis of PEG - Schiff – DOX

PEG-CHO (100 mg, 110 μmol), deionized doxorubicin (DOX, 50 mg, 90 μmol) and TEA (70 μL, 500 μmol) were dissolved overnight in 3 mL anhydrous DMF. After removing the solvent by rotary evaporation, it was dissolved with a large amount of DCM, extracted with saturated salt water for three times, and precipitated in cold ether.

2.4 Preparation of nano-drugs

Dissolve PEG-Schiff -DOX in water and stirred to obtain the nano drug solution.

ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

2.5 pH-responsive degradation of the nanoparticles

To investigate the pH-responsive degradation of the nanoparticles, nanoparticles were re-dispersed in PBS buffer solution with pH = 5.0 and a concentration of 0.5 mg/mL. After being in constant temperature water bath for 24 h, the particle size distribution was characterized by DLS [27-30].

3. Results and discussion

3.1 Synthesis of PEG-Schiff DOX

The 1H NMR spectrum of PEG-CHO is shown in Fig. 2. The analysis shows that the ratio of benzene ring hydrogen in the synthesized PEG-CHO corresponds to the methyl peak at the PEG end, which can be inferred as the successful synthesis of the synthesized PEG-CHO.

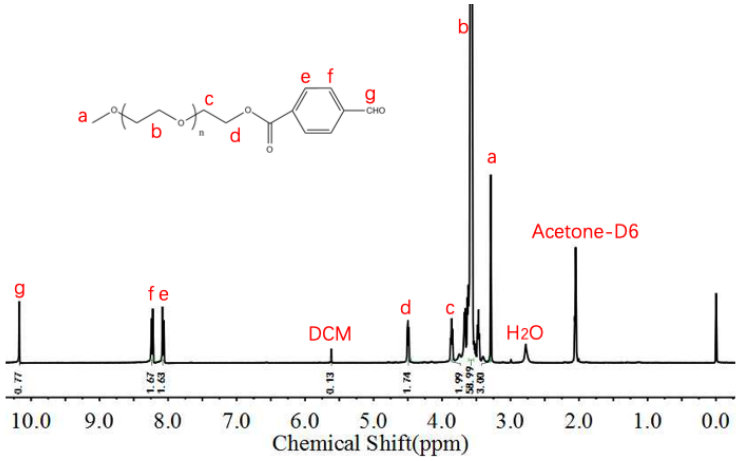

Fig. 3. 1 H NMR spectra of PEG-CHO

According to the 1H NMR spectrum of PEG-Schiff DOX (Fig. 3), the peak shapes of the molecular are chaotic and short. The methyl group at the end of PEG was used as the starting point of analysis, and the methyl peak is basically corresponding to the peak area of the main chain. In addition, it is believed that the peak in the low-field region came from the benzene ring and Schiff base bond for a total of 8 H. In particular, the appearance of the peak c at δ=8.1 PPM indicates the formation of the Schiff base bond, thus proving the successful preparation of PEG-Schiff DOX.

Fig. 4. 1 H NMR spectra of PEG-Schiff-DOX

3.2 Nano drug testing

The most important part of pharmaceutical formulations is pH responsiveness. DLS was used to

ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

determine the distribution of these nanoparticles to investigate their stability in vivo. The particle size changes of buffers with Ph=7.4 and Ph= 5.0 at medium 37 ℃ for 24 hours were shown in Figure 4 and figure 5. It can be seen from Figure 5 (A) that the size does not change significantly after 24 hours. It can be seen from Figure 5 (B) that the particle size of PEG-SchiFF-DOX drug-loaded nanoparticles varies significantly at pH=5.0, and some nanoparticles have completely disintegrated. The narrowing of particle size distribution indicates that heterogeneous nanoparticles disintegrate under the action of acid, releasing drugs. The large size is the degraded drug DOX aggregate, and the small size is the degraded PEG molecule size. The acid-resistant Schiff base bonding agent between DOX and PEG makes the nanoparticles vulnerable to decomposition under weak acid conditions. The pH responsive degradation behavior of PEG-SchiF-DOX drug-loaded nanoparticles at pH = 5.0 was monitored by DLS, and the results were shown in Figure 5. Small molecules and aggregates were detected after shaking for 24h with pH = 5.0, indicating the decomposition of the nanoparticles. Secondly, the large and small polymers and small molecules observed in TEM images (FIG.4), FIG.4 (A) is the morphology of pH=7.4, with relatively uniform size, and FIG.4 (B) is the morphology after immersion in pH5.0, which is fragmented, indicating that the structure has been degraded. This further confirms the responsive degradation of pH to nanoparticles.

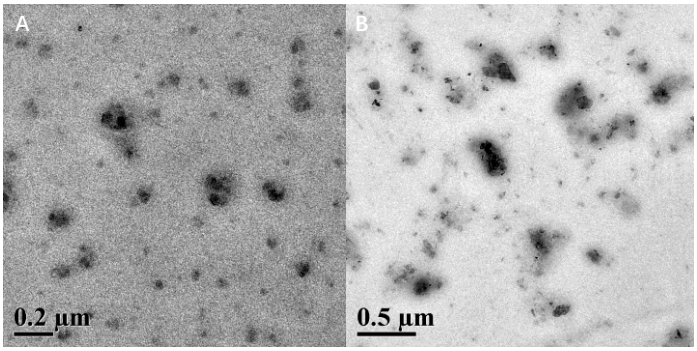

Fig. 5. Morphology of PEG-Schiff-DOX nanoparticles at pH = 7.4 and pH = 5.0

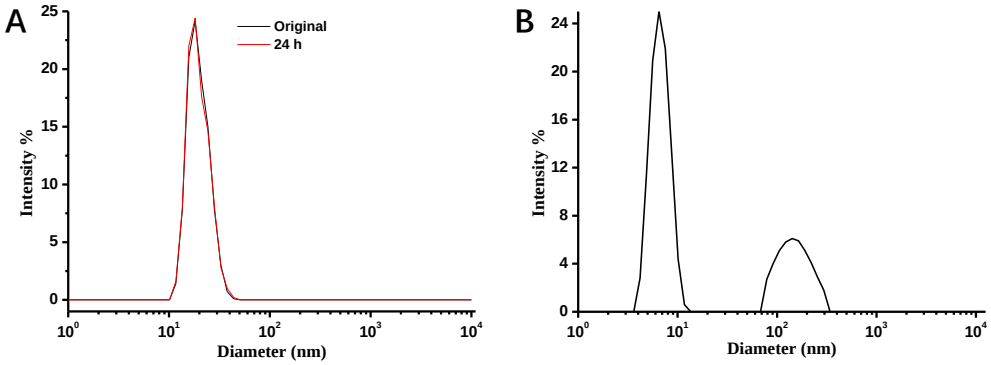

Fig. 6. Particle size of PEG-Schiff-DOX nanoparticles at pH = 7.4 and pH = 5.0

In pH-responsive prodrugs, DOX binds to PEG via schiff base bonds, and the ligand cleaves to release DOX under weakly acidic conditions in the tumor microenvironment. The degradation components of PEG-Schiff-DOX were detected by uv spectrometer to confirm the original release of DOX. The test results of drug loading in gel are more intuitive, as shown in FIG. 6. It can be seen that when pH=7.4, the maximum release rate is about 12%, and the drug-loaded nanoparticles almost release no drugs, and the release curve is almost horizontal. According to the content of free DOX in nanoparticles, it can be released by diffusion mechanism in neutral environment. However, when pH=5.0, the release behavior was significantly enhanced and the release amount increased. When pH = 5.0, the cumulative release rate of DOX was significantly increased, reaching 86%, respectively, indicating that almost all PEG-Schiff-DOX was released. As mentioned earlier, the decomposition of nanoparticles in acidic environments is due to the cleavage of schiff base bonds. In the early stages, DOX is released very quickly, leveling off after 40 minutes.

These results indicate that PEG-SchiFF-DOX, a polymer precursor of pH-responsive doxorubicin, can be released in a neutral environment with little release to reduce drug leakage, and can be released

ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

rapidly in acidic environment of tumor tissue to achieve rapid drug release In addition, PEG-SchiFF-DOX nanoparticles exhibit second-order drug release behavior, in which the encapsulated DOX is released quickly at an early stage to achieve higher drug concentrations that kill tumor cells, while the Schiff base bonded DOX is released over a longer period of time to improve potency, precision therapy, and therapeutic efficacy.

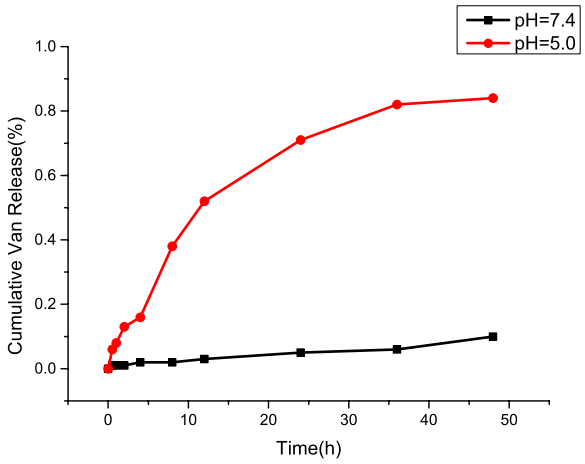

Fig 7. In vitro release curves of PEG-SchIFF -DOX drug-loaded nanoparticles

4. Conclusion

PEG-SchIFF -DOX polymer prodrug loaded with ph-responsive doxorubicin was prepared and its release behavior at different pH was tested. Through the multistage release system, the drug can successfully prolong the action time and make a timely response to the acid environment, which has a certain development potential. The preparation of these drug-loaded nanoparticles has the following advantages :(1) regular and clear chemical structure and simple preparation method; (2) High drug load; (3) Good storage stability; (4) Under the neutral condition, there is little or no drug release, while under the weakly acidic condition of tumor microenvironment, DOX is released to maintain the therapeutic effect and reduce the toxic and side effects; (5) Secondary programmed drug release can achieve higher local drug concentration and longer drug action time. Therefore, these nanomedicines based on polymer prodrugs provide a choice for the development of new pharmaceutical agents and are expected to achieve good application in cancer treatment.

References

[1] Oktay Tacar, Pornsak Sriamornsak, Crispin R Dass, Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems, Journal of Pharmacy and Pharmacology, Volume 65, Issue 2, February 2013, Pages 157–170, https://doi.org/10.1111/j.2042-7158.2012.01567.x
[2] H. Cabral, Y. Matsumoto, K. Mizuno, Q. Chen, M. Murakami, M. Kimura, Y. Terada, M.R. Kano, K. Miyazono, M. Uesaka, N.Nishiyama, K. Kataoka, Nat. Nanotechnol. 6 (2011) 815–823.
[3] J.L. Xian, J.O. Shi, T.T. Yin, H.T. Choo, Polym. Chem. 4 (2013) 2564–2574.
[4] K.A. Abdul, Q. Dou, Z. Li, X.J. Loh, Chem. Asian. J. 11 (2016) 1300–1321.
[5] Poste G et al. Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. Cancer Res 1982; 42: 1412–1422.
[6] Gabizon A et al. Pharmacokinetics of pegylated liposomal doxorubicin: review of animal and human studies. Clin Pharmacokinet 2003; 42: 419–436.
[7] qTa HT et al. Chitosan-diabasic orthophosphate hydrogel: a potential drug delivery system. Int J Pharmaceut 2009; 371: 134–141.
[8] Ta HT et al. A chitosan-dipotassium orthophosphate hydrogel for the delivery of doxorubicin in the treatment of osteosarcoma. Biomaterials 2009; 30: 3605–3613.
[9] Sun K et al. Dextran-grafted–PEI nanoparticles as a carrier for co-delivery of adriamycin and plasmid into osteosarcoma cells. Int J Biol Macromol 2011; 49: 173–180.
[10] Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev (2004) 56:185–

ISSN 2616-5791 Vol.2, Issue 1: 73-79, DOI: 10.25236/AJMHS.2021.020113

229. doi: 10.1124/ pr.56.2.6
[11] Lyass O, Uziely B, Ben-Yosef R, Tzemach D, Heshing NI, Lotem M, et al. Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. Cancer (2000) 89:1037– 47. doi: 10.1002/1097-0142(20000901)89:53.0.CO;2-Z
[12] Zhou N, Zhang N, Zhi Z, Jing XN, Liu DM, Shao YP, et al. One-pot synthesis of acid-degradable polyphosphazene prodrugs for efficient tumor chemotherapy. J Mater Chem B (2020) 8:10540–8. doi: 10.1039/D0TB01992E
[13] Zhu XH, Li C, Lu Y, Liu YJ, Wan D, Zhu DW, et al. Tumor microenvironment-activated therapeutic peptide-conjugated prodrug nanoparticles for enhanced tumor penetration and local T cell activation in the tumor microenvironment. Acta Biomater (2021) 119:337–48. doi: 10.10
[14] /j.actbio.2020.11.008 16. Wen W, Guo C, Guo J. Acid-responsive adamantane-cored amphiphilic block polymers as platforms for drug delivery. Nanomaterials (2021) 11:188. doi: 10.3390/nano11010188
[15] Cytryniak A, Nazaruk E, Bilewicz R, Górzyńska E, Ż elechowska-Matysiak K, Walczak R, et al. lipidic cubic-phase nanoparticles (cubosomes) loaded with doxorubicin and labeled with 177Lu as a potential tool for combined chemo and internal radiotherapy for cancers. Nanomaterials (2020) 10:2272. doi:10.3390/nano10112272
[16]Xin He, Dong Wang, Peng Chen, Youbei Qiao, Tiehong Yang, Zhe Yu, Chaoli Wang and Hong Wu(2020)Glioma cell-targeting doxorubicin delivery and redox-responsive release using angiopep-2 decorated carbonaceous nanodots Chem. Commun., 2020,56, 4785-4788
[17]Geldenhuys, W (Geldenhuys, Werner) Wehrung, D (Wehrung, Daniel) Groshev, A (Groshev, Anastasia) Hirani, A (Hirani, Anjali) Sutariya, V (Sutariya, Vijaykumar)(2015)PHARMACEUTICAL DEVELOPMENT AND TECHNOLOGY.,2015, 4, 497-506
[18] Chaithongyot, S (Chaithongyot, Supattra) Duangrat, R (Duangrat, Ratchanee) 1Wootthichairangsan, C (Wootthichairangsan, Chanida) Hanchaina, R (Hanchaina, Rattanavinan) Udomprasert, A (Udomprasert,Anuttara) Kangsamaksin, T (Kangsamaksin, Thaned) (2020) MATERIALS LETTERS, 260, 126952
[19] Chen, GX (Chen, Guangxiang) ,Li, D (Li, Du) ,Li, JC (Li, Jingchao) ,Luo, Y (Luo, Yu) ,Wang, JH (Wang, Jianhua), Shi, XY (Shi, Xiangyang) ,Guo, R (Guo, Rui) (2015)Targeted delivery of doxorubicin by lactobionic acid-modified laponite to hepatocarcinoma cells,JOURNAL OF CONTROLLED RELEASE 213,E34-E34
[20] Hembruff, S. L., Laberge, M. L., Villeneuve, D. J., Guo, B., Veitch, Z., Cecchetto, M., and Parissenti, A. M. (2008) Role of drug transporters and drug accumulation in the temporal acquisition of drug resistance BMC Cancer 8, 318
[21] Horton, K. L., Stewart, K. M., Fonseca, S. B., Guo, Q., and Kelley, S. O. (2008) Mitochondria-penetrating peptides Chem. Biol. 15, 375– 382[Crossref], [PubMed], [CAS], Google Scholar
[22] Yousif, L. F., Stewart, K. M., Horton, K. L., and Kelley, S. O. (2009) Mitochondria-penetrating peptides:sequence effects and model cargo transport ChemBioChem 10, 2081– 2088[Crossref], [PubMed], [CAS], [Google Scholar]
[23] R.B. Greenwald, J. Controlled, Release 74 (2001) 159–171.
[24] J.M. Harris, R.B. Chess, Nat. Rev. Drug Discov. 2 (2003) 214–221.
[25] T. Luo, C. Loira-Pastoriza, H.P. Patil, B. Ucakar, G.G. Muccioli, C. Bosquillon, R. Vanbever, J. Control. Release 239 (2016) 62–71.
[26] X. Wang, D. Li, F. Yang, H. Shen, Z. Li, D. Wu, Polym. Chem. 4 (2013) 4596–4600.
[27] S.S. Liow, Q. Dou, D. Kai, Z. Li, S. Sugiarto, C.Y.Y. Yu, R.T.K. Kwok, X. Chen,Y.L. Wu, S.T. Ong, A. Kizhakeyil, N.K. Verma, B.Z. Tang, X.J. Loh, Small 13 (2017) 1603404.
[28] D. Li, Y. Bu, L. Zhang, X. Wang, Y. Yang, Y. Zhuang, F. Yang, H. Shen, D. Wu,Biomacromolecules 17 (2016) 291–300.
[29] H. Gelderblom, J. Verweij, K. Nooter, A. Sparreboom, Eur. J. Cancer 37 (2001)1590–1598.
[30] C. Zheng, H. Gao, D.P. Yang, M. Liu, H. Cheng, Y.L. Wu, J.L. Xian, Mater. Sci. Eng. C74 (2017) 110–116.