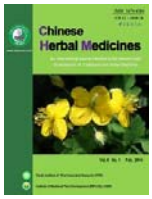

Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Review

Research Progress on Novel Carrier-modified Methods and Evaluation of Active Targeting Antitumor Preparation

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ARTICLE INFO

Article history

Received: July 10, 2013

Revised: September 11, 2013

Accepted: October 27, 2013

Available online:

January 24, 2014

DOI:

10.1016/S1674-6384(14)60002-2

ABSTRACT

This article mainly introduced novel carrier-modified methods for active targeting antitumor preparation as well as their evaluation methodology in recent years. By reviewing related domestic and overseas literatures, the up-to-date scientific researches concerning active targeting antitumor preparation were elaborated and the problems existing in present studies were discussed. Numerous valid vector-embellished methods had been discovered with excellent targeting effects, and the significant progress was acquired for the evaluation tools *in vitro* and *in vivo*. The active targeting agent would be a major direction in prospective tumor or cancer therapeutic regimen.

Key words

active targeting; antitumor; carrier-modified methods; evaluation

1. Introduction

Traditional cancer therapy could be successful in destroying tumors, but could also cause dangerous side effects (Daniels et al, 2012). Most of the side effects were caused by the lack of drug-specific affinity toward tumor tissues, thus led to the toxicity to normal tissues (Xu et al, 2011) and increased doses of the drugs. How to specifically deliver the anticancer drugs to tumor tissues was still a big challenge in the development of anticancer drugs (Maric et al, 2013). The active targeting preparation employed modified drug carrier as missile, which was transported to the target tissue, target organ, target cell, and specific target in cell, acting in a concentrated way. Compared with the passive targeting preparation such as nanoparticle, microemulsion, and microsphere lipidosome (Kedar et al, 2010; Annett et al, 2010), the active targeting preparation ornamented with special material had higher tissue selectivity, more secure property, lower toxicity, and the improvement of compliance of patients, which could actively delivery antitumor drugs into tumor tissue and decrease the distribution in normal tissue, therefore developing targeting drug delivery system was a promising orientation for tumor therapy (Kwon et al, 2012; Cukierman and Khan, 2010). It was well known that folate receptor (Wang et al, 2013a), transferrin receptor (TfR), apolipoprotein receptor, and tumstatin (Thevenard et al, 2013) widely overexpressed in several carcinomas (Qin et al, 2013). Hence the antitumor preparations which contained folic acids (Cheng et al, 2012a; Kawakami, Higuchi and Hashida, 2008), peptides (Pisal et al, 2010; Shahin et al, 2011; Mai et al, 2009), and monoclonal antibodies (Zakhari et al, 2012) could enhance the targeting ability, because they could recognize

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receptors and bind to specific ligands to tumor cells. With regard to modified new antitumor preparations, we could verify and evaluate them in physicochemical properties by FT-IR, ^{1}H-NMR, ^{13}C-NMR spectra, dynamic light scattering, and transmission electron microscopy (TEM). In order to explore how the antitumor drugs entered into tumor cells and caused toxicity to normal tissues, some *in vitro* and *in vivo* evaluations were used. The article mainly summarized novel carrier-modified methods and the evaluation of active antitumor targeting preparation.

2. Vector modification methods

2.1 Folate-decorated chitosan / poly (butyl) cyanoacrylate modification

It was reported that coating liposomes with chitosan not only could maintain the advantages of liposomes but also increase their stability. Moreover, there were abundant functional groups such as hydroxyl and amino groups on the backbone of chitosan, therefore some biologically active molecules such as folate, could be linked to the chitosan coated liposomes (CCLs) by covalent bonds (Wang et al, 2010a; Zhao et al, 2012; Su et al, 2012). It had been known that folate receptor widely overexpressed in several epithelial carcinomas including cancers of the ovary, breast, liver, kidney, uterus, testis, brain, colon, and lung, but rarely found on normal cell surface (Liu et al, 2007). As a result, folate and folate conjugates could bind to the folate receptor with high affinity and pass into cells by receptor-mediated endocytosis (Zhu et al, 2011; Zhao et al, 2010; Chen et al, 2012a), while it was highly restricted in normal tissues. Yang et al (2013) prepared folate-modified-chitosan-coated liposomes (FCCLs) for tumor-targeted drug delivery. FCCLs had larger size and higher Zeta potential, and could prolong the drug release behaviors with better physical stability. Compared with conventional liposomes stored at 25 ^{o}C, the coupling of folate into chitosan could significantly improve the internalization of FCCLs into the MCF-7 cells via the folate receptor-mediated endocytosis. Xu et al (2013) encapsulated gemcitabine with folate-chitosan as the polymeric coating material. The cellular uptake experiment confirmed that the folate conjugated core-shell nanoparticles had high uptake efficiency in pancreatic cancer BXPC3 cells. The cell cytotoxicity test displayed that they had remarkable cytotoxicity towards BXPC3 cells, which clearly indicated that the folate conjugated core-shell nanoparticles were highly effective as a pancreatic tumor-targeted delivery carrier. Duan et al (2012) synthesized a novel chitosan coated poly butyl cyanoacrylate (PBCA) nanoparticles-loaded doxorubicin (DOX) (Alyamkina et al, 2010) and then conjugated with folic acid surfacely to produce a folate-targeted drug carrier for tumor-specific drug delivery. The results demonstrated that folate-conjugated DOX-PBCA-nanoparticles (NPs) drug delivery system could provide the increased therapeutic benefit by delivering the encapsulated drug to the folate receptor positive cancer cells.

2.2 Transferrin modification

Transferrin widely existed in vertebrate body fluid as well as type II transmembrane glycoprotein family, which could assist plasma glycoprotein in carrying iron ions to cells, yet TfR could overexpress in the tumor cell surface (Kawamotol et al, 2011) and the tumor cells demanding for iron ions were relatively larger than normal cells (Zhang et al, 2012). Therefore transferrin had been used as drug carrier in tumor-targeted therapy. Curcin could inhibit the proliferation of tumor cells and promote the apoptosis of tumor cells, but had no selectivity for tumors or normal cells, while the transferrin-TfR-mediated drug delivery system could improve the uptake of drugs, reduce toxicity (Kawamotol et al, 2011), and improve the initiative targeting activities. In order to enhance the antitumor targeting ability of curcin, Zheng et al (2013) fused the TfR binding peptide (TfRBP9) with curcin, the curcin-TfRBP9 gene was cloned into pQE-30 and the recombinant vector pQE-30-curcin-TfRBP9 was established. Immunofluorescence analysis showed that TfRBP9 significantly enhanced the ability of curcin binding to HepG2, and was enriched in the cytoplasm. Some researchers (Tang et al, 2012; Zheng et al, 2010; Wang et al, 2010b; Hong et al, 2010) applied transferrin-modified polyamidoamine-polyethylene glycol (PAMAM-PEG) complex for anticancer drug camptothecin (CPT). Transferrin-modified PAMAM-PEG-CPT could enhance the half-life and mean residence time compared with that of CPT solution. The results illustrated that transferring-modified PAMAM-PEG-CPT had a better long-circulating effect in rats.

2.3 Magnetic and polymeric modification

2.3.1 Oxide PAMAM-PEG-PAMAM linear-dendritic copolymers modification

The interest in polymers antitumor agents had exponentially increased these years due to their special physicochemical properties and promising potential application (Ta et al, 2010). Among them, the temperature, pH value, magnetic field, and light had been considerably investigated because they were the most common used as external stimuli. Adeli et al (2013) applied new hybrid nanostructure-based magnetic drug delivery systems (HNMDDSs) for antitumor drug Doxorubicin. HNMDDSs consisted of three parts, carbon nanotubes, magnetic iron oxide nanoparticles (Shen et al, 2012; Liu et al, 2009), and linear-dendritic copolymers linked to anticancer drugs. Multiwall carbon nanotubes (MWCNT) played as a biocompatible platform for the delivery of magnetic iron oxide nanoparticles, therapeutic drugs, and diagnostics. PAMAM-PEG-PAMAM linear-dendritic copolymers (Xu and Zhu, 2012) acted as water soluble, biocompatible, and high functional hybrid materials with a linear polyethylene glycol part which caused a high solubility for MWCNT through supramolecular interactions and dendritic PAMAM parts which caused a high functionality for MWCNT. Magnetic iron oxide nanoparticles (Du et al, 2011) played as targeting,

imaging, or hyperthermia cancer treatment agents. The results demonstrated that HNMDDSs with hybrid properties of individual moieties were able to load anticancer drugs and kill cancer cells efficiently. In addition, HNMDDSs had the potential application in nanomedicine and possessed the capability as nano-excipients in biological systems, but they still were toxic against normal fibroblasts cells.

2.3.2 Poly (nisopro-pylacrylamide-co-acrylic acid)/ Fe_3O_4 modification

Fan et al (2011) had successfully prepared a novel thermo and pH responsive magnetic hydrogel nanosphere (Wei et al, 2010) poly (nisopro-pylacrylamide-co-acrylic acid)/Fe_3O_4 [poly (NIPAAm-co-AA)/Fe_3O_4]. The magnetic hydrogel nanospheres exhibited uniform sphere structures and superparamagnetic property. Finally, the drug loading capacities and the releasing behavior of the magnetic hydrogel nanospheres were investigated with doxorubicin (DOX) hydrochloride as an anticancer drug model. The resulting magnetic hydrogel nanospheres exhibited high encapsulation efficiency (95%) to DOX under an appropriate condition. *In vitro* release experiments revealed that magnetic hydrogel nanospheres could rapidly release at pH 5.3 and 37 ^{o}C. The DOX-loaded magnetic hydrogel nanospheres also showed the enhanced anticancer effect compared with the free drug *in vitro*. These presented results suggested that the magnetic hydrogel nanospheres were potential as tumor targeting drug carrier.

2.4 Cationic and heat-responsive nanocarriers modification

Developing selectively targeted and heat-responsive nanocarriers (Wei et al, 2010) held paramount promise in chemotherapy (Sanoj Rejinold, 2011; Yerriswamy et al, 2012). Dicheva et al (2013) designed the liposomes combining with cationic charged and thermo-sensitive lipids (Swamy et al, 2013) in the bilayer. Cationic thermo-sensitive liposomes (CTSL) specifically targeted angiogenic endothelial and tumor cells. Application of mild hyperthermia led to a rapid content release extra- and intra-cellularly in two crucial cell types in a solid tumor. CTSL bound to tumor vascular endothelial cells both *in vitro* and *in vivo*. PEGylated CTSL in combination with mild heat trigger (HT) could increase the drug delivery to tumors because of their selective targeting properties together with heat-triggered content release. Cheng et al (2012b) synthesized the thermally sensitive diblock copolymer {γ-2-[2-(2-methoxyethoxy) ethoxy]ethoxy-epsilon-caprolactone}-b-poly (γ-octyloxy-epsilon-caprolactone (PMEEECL-b-POCTCL) with the purpose of combining with polycaprolactone that possessed biocompatibility and biodegradability with the oligoethylene glycol substituted polymers that possessed thermo-responsive properties (Rezaei et al, 2012a). Thermo-responsive micelles were prepared from the amphiphilic diblock copolymer displaying a lower critical solution temperature of 39.7 ^{o}C. The results demonstrated that the cellular uptake and the cytotoxicity of PTX-loaded micelles increased prominently. These results indicated that these thermo-responsive micelles might offer a very promising carrier to improve the delivery efficiency and cancer specificity of hydrophobic chemo-therapeutic drugs.

2.5 Tetrahexyloxy-tetra-p-aminocalix [4] arene modification

Weeden et al (2012) developed and optimized a nano-particle delivery platform for the anticancer agent (paclitaxel) by using a novel amphiphilic carrier (Han et al, 2013; Liu et al, 2013; Lee et al, 2013), tetrahexyloxy-tetra-*p*-aminocalix [4] arene (A4C6). The nanoparticles were successfully prepared at pH 4 by an emulsion evaporation method. The drug-loaded nanoparticles had a mean size of (78.7 ± 20.7) nm, surface potential of (38.3 ± 7.67) mV, and paclitaxel loading and encapsulation efficiencies of (69.1 ± 5.3) mg drug/mg carrier and (50.4 ± 3.2)%, respectively. The TEM graphs showed discrete particles with no evidence of agglomeration. *In vitro* dissolution into phosphate buffered saline showed that (32.7 ± 3.9)%, (82.6 ± 5.3)%, and (91.0 ± 6.0)% of the encapsulated paclitaxel was released at 5, 72, and 120 h, respectively. This was the first report on the use of amino-substituted amphiphilic calixarenes for the encapsulation of anticancer agents. The nanoparticles were significantly smaller, but had comparable drug loads to the abraxane nanoparticles, and had the potential to achieve targeted delivery of paclitaxel to tumor tissues.

3. Evaluation methods

3.1 Evaluation of physiochemical properties

With regard to ornamenting the targeting preparation with special material, in order to test and verify whether the recorded carrier had been linked to the antitumor drugs or not, Rezaei et al (2012b) utilized FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra to verify, and utilized dynamic light scattering (DLS) and TEM to detect the mean particle size and size distribution (Chen et al, 2012b). Adeli et al (2013) utilized Raman spectra to test whether it was synthesized or not, employed TGA thermo grams to test the physical stability, and used Space vector curve to evaluate the velocity of blood. All the consequences demonstrated that the evaluation methods were feasible and favorable. Wang et al (2013b) determined the critical aggregation concentration (CAC) value by fluorescence spectroscopy using pyrene as the hydrophobic fluorescent probe to confirm the self-assembly of the copolymer poly (VAF-co-VSC-co-VGA).

3.2 In vitro evaluation methods

3.2.1 Dialysis method

Xu et al (2012) adapted the dialysis method to measure the targeted liposomes (Yan et al, 2010). The dialysis method was designed to mimic the two stages of *in vivo* delivery and

release the processes of drug from liposomes: (1) During the initial stage there was a minimal passive drug leakage (mimicking the phase when the liposomes were circulating in the body prior to uptake at the target site); and (2) During the second stage there was a triggered drug release (mimicking liposome breakdown at the target site). Elmowafy et al (2013) processed a slight modification to the dialysis method in order to minimize the influence of added surfactant on the UPLC analysis of silymarin. Silymarin had hepatoprotective properties and was used in the treatment of various liver diseases, but its bioavailability from oral products was very poor. In order to overcome its poor oral bioavailability, Elmowafy et al (2013) prepared silymarin-loaded hepatic targeting liposomes composed of hydrogenated soy phosphatidylcholine and cholesterol with or without distearoylphosphoethanolamine-(polyethyleneglycol)-2000 and various amounts of b-sitosterol b-*D*-glucoside (Sito-G) as the hepatic targeting moiety. These results suggested that Sito-G containing liposomes prepared in this work had the hepatic targeting capability and they were the promising candidates for delivering silymarin to the liver.

3.2.2 Fluorescence analysis

Fokong et al (2012) applied both hydrophilic (rhodamine-B) and hydrophobic (coumarin-6) model drugs efficiently and stably entrapped within the shell of polybutyl cyanoacrylate (PBCA) microbubbles (MB). The fluorescence microscopy analysis (Zou et al, 2013; CAO et al, 2013) of rhodamine-B and coumarin-6 accumulation in tumors and tumor blood vessels upon US-mediated indicated that model drug could significantly release from VEGFR2-targeted PBCA MB. Zheng et al (2013) applied immune-fluorescent staining for recombinant vector pQE-30-curcin-TfRBP9, the nuclei were counterstained with Hoechst 33342 and the visualization of the immunofluorescence was detected using a confocal laser scanning microscope. Immunofluorescence showed that the curcin could be mediated in binding to tumor cells by the TfRBP9 peptide and then taken up into tumor cells. Curcin-TfRBP9 had a higher cytotoxicity to the tumor cells over-expressing TfR than normal cells, and the TfRBP9 peptide significantly enhanced the targeting effects of curcin on tumor cells.

3.3 In vivo evaluation methods

3.3.1 Docking analysis

D'Souza et al (2013) designed hepatic targeted curcumin (CUR) nanoparticles using Gantrez (GZ) as a polymer. Three carbohydrate-based hepatocyte asialoglycoprotein receptor (ASGP-R) ligands, such as kappa carrageenan (KC), arabinogalactan (AG), and pullulan (P) (Sun et al, 2013; McVicker et al, 2013), were used in the study. AG and KC were galactose-based while P was a glucose-based polymer. Docking simulation was evaluated as a tool to predict ligand ASGP-R interactions, using grid-based ligand docking with energies (Glide). Monomers and dimers were used as representative units of polymer for docking analysis. The binding of ASGP-R was validated using *D*-galactose as monomer. The interaction of the ligands with the receptor was evaluated based on Glide scores and E_{model} values, both for monomers and dimers. The data of the docking study based on Glide scores and E_{model} values suggested higher affinity of AG and P to the ASGP-R, compared to KC. Docking analysis using dimers as representative stereochemical units of polymers provided a good indication of ligand receptor affinity and a useful tool for the preliminary screening of ligands for hepatic targeting.

3.3.2 Living optical imaging method

Optical imaging approaches were emerging as promising high-resolution modalities for tumor diagnosis. Among the optical imaging technologies, near-infrared fluorescence (NIRF) imaging was a desirable modality for tumor detection on account of its high sensitivity. The most common organic NIR fluorophores used for *in vivo* NIRF imaging were polymethines. NIRF optical imaging (Wang et al, 2013c; Li et al, 2011; Zheng et al, 2012) offered several advantages over conventional imaging modalities, it is extremely sensitive, inexpensive, and robust, involves no harmful radiation, and allows real-time visualization (Hengerer et al, 2005). Zou et al (2013) used NIRF optical imaging technique to investigate the *in vivo* biodistribution of micelles in MCF-7-bearing mice. The tumor-bearing mice were injected with cyanine 7-*N*-octyl- *O*,*N*-carboxymethyl chitosan (Cy7-OCC) micelles and cyanine 7-*N*-octyl-*O*,*N*-carboxymethyl chitosan-octreotide (Cy7-OCC-OCT) micelles, respectively. The result showed the real-time images of micelles in the tumor-bearing mice, in which the whole bodies of living mice were monitored at 1, 6, 12, and 24 h after administration, respectively. During the living imaging test, most of the Cy7 accumulated in liver. With time going on, obvious fluorescence was still observed in the tumor site, whereas weak or no fluorescence was observed in liver or other normal tissues. Moreover, the OCC-OCT micelles showed higher tumor-targeting efficiency which led to higher accumulation of micelles in the tumors than OCC micelles. The result provided the decisive evidence that the designed OCC-OCT micelles were available for the tumor-specific drug delivery. This high tumor-targeting ability of micelles might be due to a combination of an EPR effect and receptor mediated the uptake of micelles. Li et al (2013) studied the *in vivo* targeting efficiency of syp-1-PEG-liposomes (S-P-LS) to melanoma by delivery of liposomal 1,1′-dioctadecyl-3,3,3,3′-tetramethyl indotricarbocyanine iodide (DiR) to nude mice bearing MDA-MB-435 tumor in NIRF imaging. Attachment of the peptide to the liposome surface resulted in remarkable improvements in tumor targeting of fluorophore-encapsulating liposomes. The accumulation of S-P-LS/DiR in the tumor site was significantly more than that of lyp-1-phosphatase-PEG-liposomes (L-P-LS) / DiR at 24 h post-injection.

4. Conclusion

By consulting relevant literature, it could be concluded that some biocompatible materials could be used as carrier of

targeting preparation, for instance, folic acid, chitosan, transferrin, oxide-carbon nanotubes, PAMAM-PEG-PAMAM, linear-dendritic copolymers, etc. Through linking these carriers with antitumor agents, they could target specific spots to non-tumor cells to cure diseases. As a result, active targeting preparation could improve safety, decrease toxicity, prolong action time, enhance bioavailability, and increase the compliance of patients. These evaluation methods indicated significant means and provided sufficient proof for the development of antitumor agents.

5. Prospect

Active targeting carrier system mediated by receptor is a promising therapy method to solve human serious diseases. There are numerous researches showed that drugs were prepared into microballoons, nanoparticles, liposomes, and so on, and then linked corresponding ligands as active targeting system, however they are still in the experimental or preclinical research stage so far. How to increase the stability, control drug release rate, extend the half-life, reduce the side effects of active targeting preparations, and improve pharmacokinetics and tissue distribution (Tang et al, 2012) along with applying it for clinical practice are urgent and deliberative problems. Some receptors are overexpressed on lesion location, but still have a lower expression in normal tissues. How to avoid targeted drug delivery causing damage and side effects to normal cells is also an urgent question. In addition, the combination of the receptor and the ligand exists saturation problems. In spite that there are many key technologies to be solved for the active targeting system, it shows a huge superiority in terms of specific targeting. Thus seeking more efficient and scientific evaluation methods would be an important orientation for the development of the active targeting preparations, which would certainly expedite its progress. In a word, researchers need to choose bio-degradable and biocompatible materials as vector, conduct the necessary decoration to target specific spots, and at the same time explore the perfect evaluations of active targeting agents.

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