Review

Application of sialic acid/polysialic acid in the drug delivery systems

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ABSTRACT

The properties of modified biomaterial are gaining more and more importance in drug delivery systems. Sialic acid (SA) and polysialic acid (PSA) serve as endogenous substances, which are non-immunogenic and biodegradable. At the same time, SA modification of the drugs/carriers can enhance the uptake of tumor cell and retention in brain; PSA modification can reduce the immunogenicity of the proteins or polypeptides and increase circulation time of the modified drugs/carriers in the blood, thus achieving active targeting effect. These properties offer a variety of opportunities for applications in drug delivery systems. This article summarizes the biological functions of SA and PSA and presents the technologies of SA/PSA modified small molecule drugs, proteins and carriers in drug delivery systems.

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1. Introduction

Nature sialic acid (SA) is constituted structurally by nine-carbons 3-deoxy-ulosonic acids. This saccharide moiety attached to carbohydrate chains of glycolipids and glycoproteins plays a crucial role in many important biological events [3]. Interestingly, it has been found that SA is a well-known ligand for selectin, which is known to have a close relationship with tumor metastases. Owing to its modulation of cell–cell interactions among leukocytes, platelets, endothelial cells and tumor cells, selectin is responsible for tumor metastasis [2–5]. SA serves as endogenous substances that can specifically bind to selectin [6–8]. Hence, the application of SA-modified carriers in cancer targeted therapy has a certain significance [9].

Polysialic acid (PSA) is a homopolymer of sialic acid in α-2,8 or α-2,9 linkages or a mixture of α-2,8 and α-2,9. PSA constituted of α-2,8 bond is non-immunogenic and biodegradable
and then reduces the immunogenicity of the protein polypeptide. Likewise PSA shows the properties of escaping from phagocytes and presenting long circulation time in vivo [10,11]. This review summarizes the applications of SA/PSA in targeting carriers construction and briefly discusses their prospects in drug delivery systems.

2. Sialic acid, polysialic acid and derivatives

2.1. Sialic acid

In 1957, Blix [1] isolated a substance from the submandibular gland mucin via a hydrolysis method, which produced a purple color reaction with Bial’s reagent. He named the substance as sialic acid (SA). Afterward, people got perfect crystalline SA from colostrum and bird’s nest. With the progress of biological evolution, the amount of SA in vivo also increased, such as fish, amphibians, reptiles, birds and other vertebrates. In mammals, SA mainly distributed over the cerebrospinal fluid and mucus.

SA belongs to a family of neuraminic acid (5-amido-3,5-dideoxy-β-glycero-β-galacto-nonulosonic acid). In this family, there are three main SA derivatives, which are N-acetyl neuraminic acid (Neu5Ac), N-acetyl neuraminic acid hydroxylalkyl (Neu5Gc) and 3-deoxy-β-glycero-β-galacto-nonyl ketose (KDN) (Fig. 1). The other SA derivatives are further derived from these.

Each SA carries a unit negative charge, which generates repulsion or attraction between SA and plasma protein. Furthermore, the charge will modulate membrane surface charge density, while the carboxyl groups of SA can regulate its own pH. The surface charge density and pH coordinately regulate the transmembrane transport capacity of cell membrane [12].

SA spreads widely over mammalian cell surface, wherein the surfaces of red blood cells and endothelial cells are highly sialylated [13]. It has been found that after treated by sialidase, the lifetime of erythrocytes dropped from the initial 120 days to just a few hours [14]. In addition, many pathogens employ SA to camouflage themselves and mask their epitopes, which inhibits complement activation pathway to reduce immunogenicity and thus protect the pathogens from the attacks of host immune system [15–17].

2.2. Polysialic acid

Polysialic acid (PSA) was first found in E. coli (Escherichia coli) K-1 and K-235 by Barry [18]. Subsequent studies have discovered that PSA was one of the ingredients of bacterial capsular materials, such as Neisseria meningitidis B, Salmonella toucra 048 and Citrobacter freundii 05, etc. The structure of PSA was shown in Fig. 2.

In vertebrates, PSA can attach to the neural adhesion molecule (NCAM), with its fragments connecting to the asparagine at Ig5 of NCAM [19]. PSA can attenuate NCAM–NCAM interaction and facilitate cell migration and nerve regeneration. Accordingly, Polysialylated NCAM exists abundantly in the embryo brain. But for the brain tissue of adults, PSA only appears in the hippocampus and olfactory bulb with continuous growth ability [20].

Polycondensation of SA occurs between hydroxyl groups at its 2 and 8 positions and makes the constructed PSA more stable [21]. The study also found that PSA showed excellent water solubility, low viscosity, good biocompatibility and degradation in vivo [22].

PSA has been found in various tumors including small cell and non-small cell lung carcinomas, rhabdomyosarcoma, multiple myeloma, neuroblastoma, and Wilms tumor etc [20]. Study [23] found patients with extensive rhabdomyosarcoma showed a high level of PSA-NCAM, while patients had a lesser amount after chemotherapy. They concluded that the presence of PSA on the tumor cells reduced adhesion of NCAM, and enhanced cell motility, thus allowing the tumor cells that expressed PSA to deviate from the primary tumor and format metastases. In another study [24], small cell lung carcinoma cells expressing different amounts of PSA were isolated from H69 cell line. After subcutaneous inoculation with nude mice, tumor cells that express PSA produced more intracutaneous metastasis than tumor cells poorly expressing PSA. Suzuki [20] et al. reported among 44 patients with astrocytoma examined, 30 cases were NCAM-positive, of which nine cases PSA were detected, interestingly the nine cases had strong diffusion ability, revealing that PSA closely correlated with tumor invasion. Therefore, PSA plays an important role in tumor invasion and recurrence.

2.3. Ganglioside

Ganglioside is widely used as a SA derivative, which is quite rich in the brain. It cannot only promotes nerve cell differentiation, growth and synaptic formation, but also takes part in regulation of neural plasticity and functional recovery after brain injury. Among gangliosides, monosialylganglioside (GM1) have drawn the most interest by far. GM1 is mainly distributed over the central nervous system, which promotes the neuronal proliferation, neurotroph, anti-excitatory nerve repair and other multiple effects. GM1 can be used clinically for the treatment of central nervous system injury diseases, such as stroke, traumatic brain injury,
Sialic acid/polysialic acid in the drug delivery systems

In the history of drug delivery systems (DDS), carrier and drug molecules PEGylated (PEGylation) technology is regarded as a milestone. PEGylation nanoparticles can effectively reduce the macrophage uptake in vitro, prolong the circulation half-life in vivo and decrease the accumulation of nanoparticles in liver and spleen. PEGylation can also improve the accumulation of nanoparticles in tumor tissue by exerting the enhanced permeability and retention (EPR) effect that is the basis for delivering the macromolecular drugs to the site of solid tumors selectively [26]. However, the PEG layer brings the following three problems. 1) Cellular uptake blocked phenomenon. The “cloud” of hydrophilic steric barrier plays a crucial role in prolonging the residence time but it can dramatically suppress their interaction with cells [27]. 2) Accelerated blood clearance phenomenon. When repeated injection, PEGylated carriers abundantly produce anti-PEG IgM and consequently enhance blood clearance because of anti-PEG IgM mediating complement activation under certain conditions. The phenomenon is called “accelerated blood clearance (ABC)” [28]. 3) Security. PEG is difficult to degrade in the body and accumulates in lysosomes, which can induce toxicity and even a small amount of PEG oxidation products in vivo is also harmful [29]. So ABC phenomenon and the accumulation of PEG may bring fairly serious consequences to the body.

SA/PSA may successfully solve the above problems [30]. As an endogenous substances, SA can specifically bind to selectin, which is highly expressed on tumor and endothelial cell surface [4,6,12,31-34]. SA can transport the drug into the cell by receptor-mediated endocytosis, which proves to be of high specificity and strong affinity and increases the efficiency of drug transportation and thus solves the problem of cellular uptake blocked [35]. At the same time, PSA on the surfaces of drugs/carriers will provide a “water cloudy” barrier, mainly owing to its high hydrophilicity and chain flexibility. The “cloud” protects modified drugs/carriers from interacting with plasma proteins or macrophages, which causes less RES uptake and prolongs the circulation half-life [10,11]. What is more, antibody induced by 2-8 linked, shows a low level in the blood, which indicates weak affinity and would not enhance modified drugs/carriers clearance from the blood circulation. Meanwhile, degradation products of SA/PSA are CO2 and water that is non-toxic. Therefore, using SA/PSA as a non-immunogenic and biodegradable new material, has the potential of reducing or eliminating PEGylation preparation inducing ABC phenomenon.

3.1. The modification of drug/carrier

3.1.1. The modification of small molecule drug

When modified by SA/PSA many excellent properties of SA/PSA can be transferred to the coupling small molecules and endow the conjugates with targeting, biocompatible and nontoxic characteristics.

SA was covalently conjugated with PEG and DOX through a critic acid spacer. Stability, spontaneous and esterase-stimulated drug release was evaluated. More than 40% of DOX was released from the conjugate in the presence of esterase enzyme, whereas it was stable at pH 7.4. SA-PEG-modified doxorubicin (DOX), PEG-DOX and free DOX were incubated with A2780 ovarian cancer cells, respectively. It is found that SA-conjugated DOX enhanced cytotoxicity when compared with non-targeted prodrugs and free DOX [6].

The potential of polysialic acid (PSA) for low molecular weight anti-cancer drugs also has been explored. A PSA-epirubic (PSA-Epi) conjugate was synthesized and it was compared against three other Epi conjugates, named: N-(2-hydroxypropyl) methacrylamide copolymers (HPMA), poly(ethylene glycol) (PEG) and polyglutamic acid (PGA). In vitro biological evaluation, which was conducted in the breast cancer cell line MCF-7 and in the anthracycline resistant MCF-7/DX, both showed that the PSA-Epi conjugate possessed the highest activity. In vivo evaluation showed that all conjugates had a significantly longer retention than free Epi [12].

This is the first study that PSA was used as a carrier to conjugate with a low molecular drug. In this case, the study
illustrated the synthesis, characterization and biological evaluation of the PSA-Epi. Meanwhile, the authors also carried out a systematic comparison with those of the most common drug carriers, aiming at obtaining some insight into the impact of the polymeric carrier on the activity of an anticancer drug conjugate.

3.1.2. The modification of protein
PSA is non-immunogenic, biodegradable, and can maintain activity of the modifiers. Studies have pointed out that PSA-asparaginase was non-immunogenic [11], and the activity of the asparaginase almost remained, while after PEG modification, its activity declined seriously [10]. Moreover, the PSA modified protein/peptide eliminated immunogenicity and antigenicity. At the same time, it also did not seem to influence their connection with the respective receptors [11]. PSA is a human endogenous substance, which can be completely degraded non-toxic SA by neuraminidase. However PEG is a synthetic polymer, which generates ketone and aldehyde groups by cytochrome P450 enzymes at a very low rate, and its high/low molecular weight forms tend to accumulate in the tissues [36]. When PEG was long-term injected, it would cause accumulated toxicity. In this point of view, Gregoriadis claimed that PSA is the best alternative to PEG [37]. PSA can improve the pharmacokinetic properties of the drug and reduce toxicity [38,39]. Currently a PSA modification industrialization technology platform named PolyXen/C210 Technology (Lipoxen PLC, London, UK) is commercially available, which has been applied to insulin (SuliXen/C210, clinical stage II), alfa2b-interferon (InferoXen/C210), granulocyte colony-stimulating factor (StimuXen/C228) and erythropoietin (ErepoXen/C210).

3.1.3. The modification of nanocarrier
To date, there are few reports on PSA-modified nanocarrier, Bader et al. synthesized its derivatives in the preparation of drug-loaded micelles. Carboxyl group of PSA reacted with n-decylamine to produce PSA–decylamine derivative which may self-assemble into micelles thus load insoluble drugs [40]. The schematic was shown in Fig. 3. 3 years later, the same laboratory alternated the hydrophobic end ODA with poly-caprolactone (PCL). They found that the new derivative presented more stability and less cytotoxicity in vitro [41].

The first work applying GM1 modification to nanocarriers was introduced by Gabizon and Papahadjopoulos [42]. After intravenous injection in mice, it demonstrated that GM1-modified liposomes reduced a 3.4-fold decrease in reticulo-endothelial system (RES) uptake compared with conventional liposomes (PC:Chol = 10:5, w/w). Afterward, Liu et al. [43] proved that GM1-modified liposomes could escape RES uptake. After GM1 liposomes intravenously administered in Balb/c mice, these liposomes were rapid uptake by the spleen instead of liver Kupffer cells. The reason may be due to the GM1 on the liposomes, which prolonged liposomes residence time and then offered more chances to reach the spleen area.

To make a comparison of the long circulating effects promoted by PEG and GM1, Maruyama et al. took DOX as a model drug and prepared GM1 and PEG-modified liposomes. After 6 h, intravenously injection in DBA/2 mice, the drug concentrations in the bloodstream was 2.3-fold and 2.9-fold when loaded with GM1 and PEG compared with conventional liposomes, respectively. Meanwhile, GM1 and PEG-modified liposome Blood/ERS uptake ratios were increased by 4.6-fold and 7.3-fold, respectively [44].

Neu5Ac-PA (Fig. 4) is a sialic acid derivative. The effects of Neu5Ac-PA modified liposomes on the blood circulation and tissue distribution was investigated compared with liposomes composed of GM1. When liposomes were intravenously administered in rats, the liver and spleen uptakes of liposomes containing Neu5Ac-PA were significantly reduced compared with those ones of liposomes containing GM1. The plasma concentration of liposomes containing Neu5Ac-PA was significantly greater than that of GM1 at all times and was about as 10.3-fold as liposomes containing GM1 at 24 h [45].

GM1 and Neu5Ac-PA are both sialic acid derivatives and they have been used to modify nanocarrier. Studies found that the modified nanocarrier presented a longer residence time and reduced uptake of RES. PSA modified nanocarriers have been study little, but we believed that PSA can offer more chances to receive the ideal residence time.

3.2. Targeting applications

3.2.1. Tumor targeting
SA can be served as a targeting ligand for selectin. Selectin is highly expressed on tumor vascular endothelial cells.

Fig. 3 – The preparation of polysialic acid decylamine derivatives and their micelles.

Fig. 4 – The structure of Neu5Ac-PA.
uptake and induced apoptosis. In vitro targeting effects on human cervical carcinoma cells (HeLa).

People can make use of SA binding to selectin, which is overexpressed on the surfaces of tumor cells. Afterward, SA modified drugs/carriers are delivered to tumor cells, thus achieving targeted therapeutic effects. Zheng et al. prepared modified density in the drug delivery systems. At the same time, SA/PSA is non-immunogenic, biocompatible and biodegradable, which can reduce or eliminate ABC phenomenon of PEGylation preparation to some extent. Hence, it is of great significance to find SA/PSA appropriate modification sites, change drugs/carriers linkage and optimize SA/PSA modified density in the drug delivery systems.

SA can be applied for cancer treatment mainly in two aspects. On the one hand, selectin serves as a target receptor. People can make use of SA binding to selectin, which is overexpressed on the surfaces of tumor cells. Afterward, SA modified drugs/carriers are delivered to tumor cells, thus achieving targeted therapeutic effects. Zheng et al. prepared the selenium nanoparticles modified by SA to investigate the targeting effects on human cervical carcinoma cells (HeLa). In vitro showed that, compared with the general selenium nanoparticles, SA modification could enhance cancer cell uptake and induced apoptosis.

On the other hand, SA can be applied to inhibit tumor metastasis. As is known to all, several cytokines produced by tumor cells induce endothelial cells to express adhesion molecules, such as selectin which mediates the adhesion of malignant tumor cells to endothelium. Selectin targeting can inhibit cancer cell adhesion by competing with ligand-bearing, and deliver anti-cancer drugs to tumor cells via activated endothelial cells. Therefore, this kind of receptor could prevent the adhesion and thereby inhibit metastasis. SA has been applied for inhibiting tumor cells adhesion with endothelial cells. It is known that E-selectin overexpressed on tumor cells mediated tumor cells adhesion. Authors found SA-modified liposomes can inhibit tumor cells adhesion with endothelial cells by competing with E-selectin.

SA seems to be an useful model in vitro for studies on site-specific interactions and acts as an inhibitor in cancer therapy.

3.2.2. Brain targeting
Studies have shown that the SA receptors exist in the brain parenchymal, so brain targeting applications of SA were explored. Bondioli and colleagues synthesized SA-PLGA and prepared the double covered (SA and glycopeptides) nanoparticles, after intravenous injection in rats. They found that therapeutical efficacy of loaded loperamide persisted for more than 24 h, which presented a significant difference compared with unmodified nanoparticles. The study suggested that the modified nanoparticles passed through the blood brain barrier with the help of the glycopeptides on the surfaces. Then owing to SA on the nanoparticle’s surfaces, the modified nanoparticles could interact with brain SA-specific receptors in the brain parenchymal, thus brain residence time was extended and loperamide gained an increased activity.

3.2.3. Lymphocyte targeting
Sunamoto et al. demonstrated that SA involved in the activation of lymphocytes, especially T cells. Meanwhile the SA has been applied to the preparation of liposome vaccine. Modified tumor surface antigen (TSAP) was incorporated in liposome. After BALB mice were intravenously administrated by liposomal vaccine, T lymphocytes were effectively activated and tumor growth was largely inhibited.

In the following research, TSAP was substituted with ganglioside (GT1b or QG1b) in liposome preparation, which presented a more effective stimulation of T lymphocytes. The authors suggested that the concentration of ganglioside on the liposome surface was also crucial, demonstrating both the number of SA groups and the position of the linkage on the liposome play an important role in the activation of the T lymphocytes. But the pathway that activated lymphocytes mechanisms needs to be further explored.

4. Conclusions
SA/PSA has shown specific advantages of tumor targeting, cancer inhibition and stealth properties in the drug delivery systems. At the same time, SA/PSA is non-immunogenic, bio-compatible and biodegradable, which can reduce or eliminate ABC phenomenon of PEGylation preparation to some extent. However, SA receptors lie in other normal cells, tissues and organs that will bring undesired problems. Hence, it is of great significance to find SA/PSA appropriate modification sites, change drugs/carriers linkage and optimize SA/PSA modified density in the drug delivery systems.

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References


