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EXPERT OPINION

Heparosan, a promising 'naturally good' polymeric conjugating vehicle for delivery of injectable therapeutics

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Many therapeutics have issues with delivery due to nonoptimal pharmacokinetics and/or detrimental side effects due to their nonhuman nature. Injectable biologic drugs are one class that often needs assistance. The pharma industry has employed a variety of delivery strategies and this Editorial focuses on drug–polymer conjugates, in particular those utilizing a newly introduced system using a natural carbohydrate called heparosan. This molecule, the biosynthetic precursor to the well-known drug heparin, appears tolerated due to its 'self' nature as well as exhibits intrinsically favorable behavior in the bloodstream and tissues. The polysaccharide is stable in the extracellular spaces of mammals, but degraded by lysosomal enzymes following entry into the cell. Heparosan manufacture utilizes a novel synchronized, stoichiometrically controlled reaction employing a sugar-polymerizing enzyme in an aqueous buffer system that results in a quasi-monodisperse product. Heparosan is predicted to serve as a conjugating vehicle to extend the plasma half-life of biologics without liabilities of polydispersity, immunogenicity and/or unwanted accumulation in the body that are observed for other types of polymer such as PEG, hydroxyethyl starch, or poly(sialic acid).

Keywords: biologics, drug conjugates, heparosan, PEG, vehicle

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1. Introduction

Polypeptides or nucleic acids with therapeutic activity, often termed biologics, are becoming an ever increasingly useful type of modern medicine, but may have problems due to short-half plasma life and/or negative effects with the body's systems. Covalent modification with a poly(ethylene glycol) (PEG) vehicle, called PEGylation, was one of the early attempts to rectify these issues with success resulting in multiple FDA-approved drugs [1]. PEGylation can shield the drug cargo molecule's surface from antibodies, regulatory enzymes and/or clearance receptors. PEGylation can also increase the hydrodynamic size of the cargo preventing rapid elimination through renal filtration, which is especially important for therapeutics < ~ 60 – 70 kDa.

However, PEG has some liabilities as a conjugating vehicle, including lack of a safe degradation pathway, accumulation in some tissues, emerging immunogenicity and potential triggering of the complement system [2]. Therefore, other polymers have been experimentally employed as substitutes for PEG. Some of the promising candidates have been carbohydrates due to their perceived improved safety, including modified hydroxyethyl starch (HES) and poly(sialic acid) (PSA; **Table 1**) [3]. In a similar vein, genetic fusion of stretches of hydrophilic amino acid residues to protein cargo has also been employed, but this approach is applicable to a more limited subset of drugs (i.e., only human proteins).

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Table 1. Characteristics of drug delivery systems.

Vehicle	Quasi-monodisperse?	Biodegradable?	Not immunogenic?
PEG	Yes	No	Sometimes
Heparosan	Yes	Yes	Yes
HES	No	Yes	Yes
PSA	No	Yes	Sometimes
Fusion	Yes	Yes	No*

The various vehicles have different production methods, purity and biological behavior in the body. The ideal delivery agent should have a 'Yes' response in all critical categories below.

* In theory, antibody production against any nonhuman polypeptide sequence is a possibility.

HES: Hydroxyethyl starch; PSA: Poly(sialic acid); Fusion: Fusion peptide composed of hydrophilic amino acids added by molecular genetic means to the cargo protein; quasi-monodisperse (i.e., very narrow size distributions approaching the ideal polydispersity value of 1).

In addition to the 'biological' issues surrounding the drug's behavior in the body, the polymer should also be amenable to facilitate manufacture and quality control. Therefore, the polymer size distribution (polydispersity) should be narrow such that the final drug will have uniform effectiveness. In addition, the efficiency of coupling to the drug should be as high as possible. PEG, with a longer history of use and simpler chemical synthesis, has addressed the issues of polydispersity and activation, but the HES and PSA carbohydrate systems are still somewhat lacking in these regard.

2. A new drug delivery vehicle – theory and experiment

Heparosan ([-4-*N*-acetylglucosamine-α1,4-glucuronic acid-β 1-]$_n$) is a natural polysaccharide related to heparin, one of the most widely used drugs in the pharmacopeia. From a chemophysical standpoint, the heparosan chain is very hydrophilic due its two hydroxyl groups on every monosaccharide unit and a negative carboxylate group on every other monosaccharide unit (**Figure 1**). Heparosan should be biocompatible in the human body because it is the endogenous natural precursor in the heparin/heparan sulfate (HS) biosynthetic pathway [4]. Stretches of heparosan exist in the HS chains found on virtually every human cell thus it is not perceived as a foreign molecule. Certain pathogenic bacteria even exploit the 'self' nature of heparosan by using a heparosan coating or capsule to evade the immune system during infection. In the pre-genomic era, the difficulty in raising antibodies to heparosan for use as 'typing sera' is exemplified by the need to use either a capsule-specific bacteriophage (a virus) or bacterial heparin-degrading enzymes to make these microbial identifications.

In contrast to HS and heparin, the heparosan molecule is neither decorated with sulfate groups nor epimerized at glucuronic acid residues thus is relatively biologically inactive with respect to coagulation (i.e., clotting factors not activated), modulation of proliferation (i.e., growth factors do not bind), inflammation (i.e., cytokines do not interact) and a plethora of other activities [5]. Furthermore, enzymes that degrade (heparanase) or receptors that clear HS from the bloodstream (hyaluronan receptor for endocytosis or stabilin) do not recognize heparosan because the sulfate groups essential for activity are absent from this polymer [6,7]. In other words, heparosan reads as 'a hole in the sugar code' that is ignored by the HS recognition systems. Therefore, heparosan is stable in the extracellular spaces where many therapeutic drugs act. Over time, bulk cellular fluid uptake or pinocytosis will internalize heparosan, but once the polymer arrives at the lysosome, the normal degradation pathway for HS/heparin removes sugars from the nonreducing end in a sequential fashion.

The theoretical predictions for the behavior of heparosan in the body have been borne out by various experimental tests in rodents and primates (U.S. Patent Application 20100036001, Feb 2010). The molecule has 0.5- to 8-day half-life in bloodstream depending on its molecular weight and the route of injection. The polymer chain length is stable in the plasma. No accumulation in tissues was observed. Over time, the metabolites of the probe linker with short sugar chain are excreted in urine and feces. The polymer does not cross the blood–brain barrier, which may be important for ensuring the safety of some therapeutics. No detrimental toxicological effects were observed with acute doses of 100 mg/kg in a rodent model.

A variety of biologics have been modified with heparosan with retention of activity and improvement of pharmacokinetics (schematically depicted in **Figure 1**; *data not shown*). As observed in the case of PEG–drug conjugates, the nature of the cargo molecule can potentially affect the behavior of heparosan–drug conjugates. In some conjugates, the drug is cleared or inactivated by efficient mechanisms (e.g., receptor-based or protease-mediated) that shorten the observed half-life more than the prediction based solely on the heparosan vehicle alone while in other conjugates, the cargo assumes the longer half-life of the vehicle.

Most of the typical chemistries used to modify molecules with PEG are amenable to use with heparosan. For example, reactive heparosan polymers with a single amine-reactive (e.g., aldehyde), sulfhydryl-reactive (e.g., maleimide, iodoacetyl) or carbonyl-reactive (e.g., amine) functionality at the reducing terminus have been conjugated to proteins and to aptamers. Based on testing in parallel, the coupling

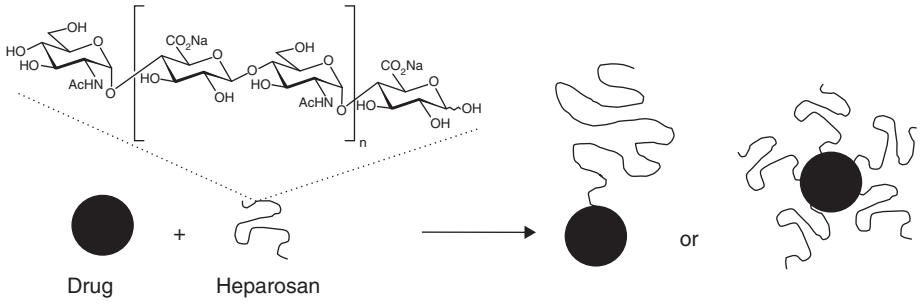

Figure 1. Heparosan modification of drug cargo. Heparosan (curved lines), a polymer composed of [-4-*N*-acetylglucosamine-α 1,4-glucuronic acid-β1-] repeats, is found in the heparan sulfate chains of mammals. This polymer in an unsulfated form can be attached to drug cargo (sphere) in a variety of formats to create conjugates with enhanced therapeutic action. Polysaccharide chains of different lengths, where *n* can range from 2 to ~11,000, can be reacted at either a single site (left) or at multiple sites (right), as desired.

efficiencies of heparosan-based reagents are comparable to PEG-based reagents; typically, the limiting factor in the formation of the conjugate is the intrinsic stability and properties of the drug cargo itself.

From a manufacturing perspective, mammalian HS or heparin is not the ideal starting material for heparosan because chemical transformation by desulfation is problematic and will always result in damaged backbone chains and/or residual sulfate groups. In addition, animal-derived materials are not perceived favorably by drug regulatory agencies due to the potential risk of contamination by adventitious agents (e.g., prions, virus, etc.). The human enzymes that form heparosan by polymerizing the uridine diphospho-sugar (UDP-sugar) precursors in the HS biosynthetic pathway are also nonideal; they are weakly expressed in recombinant systems and are poor catalysts *in vitro*. Fortunately, certain pathogenic bacteria possess very useful enzymes that produce heparosan [8]. One such enzyme, PmHS1 from *Pasteurella multocida*, is very active and stable in recombinant form.

PmHS1 can be utilized *in vitro* to synthesize quasi-monodisperse (i.e., very narrow size distributions approaching the ideal polydispersity value of '1') polymer preparations [9] with homogenous reactive end groups for coupling to biologic targets. The narrow size distribution is achieved by synchronizing the polymerization reaction using a primer, a short heparosan fragment, which allows the normal slow-chain initiation step of biosynthesis to be bypassed. Therefore, all polymers are rapidly extended by PmHS1 in a virtually parallel fashion thus all final chains have a very similar length. No post-polymerization purification for size control is required. The primer also contributes the unique reactive group(s) that helps assure that every polymer chain can be activated for drug coupling. In addition, the primer position in the heparosan chain at the reducing terminus does not interfere with lysosomal degradation allowing the heparosan chain to be digested to a tiny stub containing the linker site used for drug attachment; this stub is readily excreted in the urine or feces.

The chain size or molecular weight of any particular heparosan preparation is controlled by manipulating the stoichiometric ratio of the primer to the UDP-sugar precursor. Basically, for a given amount of UDP-sugars, a low concentration of primer yields longer chains while, on the other hand, a high primer concentration yields shorter chains (of course, the former case has fewer moles of product formed than the latter). Heparosan molecules in the range of ~10 – 4500 kDa (or ~50 – 22,500 monosaccharide units) have been synthesized thus potentially accessing a wider useful size range than possible for PSA, HES or PEG.

The heparosan chemoenzymatic process has been run at the 150-g level, while retaining the same polydispersity (~1.02 – 1.1, depending on chain size) as observed in the milligram level syntheses thus scaling to the kilogram production by this method is predicted. The polymer uniformity facilitates production and quality control aspects essential for drug approval.

3. Expert opinion

Heparosan meets the criteria for a desirable drug delivery vehicle on multiple fronts. From an intrinsic point of view, heparosan has a ~ 500 million year safety profile; all animals from hydras to human synthesize and display HS (with its endogenous stretches of heparosan) on their cell surface. In the simplest embodiment of modification of proteins, the geometry of attachment to cargo is also identical to the natural proteoglycans (HS post-translationally modified glycoproteins) where the reducing end of the heparosan chain is attached to the polypeptide chain.

Furthermore, metazoan cells intracellularly metabolize heparosan along with HS and heparin; the chain is degraded from the nonreducing end. Therefore, in the simplest embodiment of the heparosan-based technology, after lysosomal processing only a short stub composed of the synthetic linker (the attachment site to cargo) and 1 – 3 monosaccharides that is excreted

from the body in a similar fashion to the pathways for many small-molecular-weight drugs, hormones, and hydrophobic molecules. This scenario is a major improvement over PEG where the lack of a natural degradation and excretion pathway contributes to accumulation of the unnatural polymer in tissues.

From a business perspective, the intellectual property portfolio is also desirable. The production catalyst, the synchronized stoichiometrically controlled polymer synthesis, and the drug delivery concept are all relatively new patents or pending patents. Currently, heparosan is more expensive to prepare than PEG, which has been in production for decades, but even at this early stage, the material cost will probably not add > $1 – 2 US per dose if used in a comparable fashion and amounts as PEG-based drugs. In summary, heparosan has ideal intrinsic and economic characteristics for a delivery vehicle.

Declaration of interest

PL DeAngelis is Chief Scientist for Caisson Biotech, LLC and has a financial interest in the heparosan-based technologies. This work was supported in part by the Oklahoma Center for Advancement of Science & Technology, National Institutes of Health, and Emergent Technologies, Inc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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