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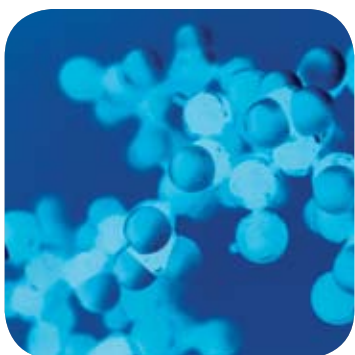


SMALL MOLECULES

BIOLOGICS

The Benefits and Challenges of PEGylating Small Molecules

Timothy Riley and Jennifer Riggs-Sauthier



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Polyethylene glycol (PEG) conjugation is a highly effective technical and commercial strategy to develop macromolecules. A properly designed PEGylated drug exhibits increased half life, greater bioavailability, and reduced clearance. The authors explain the benefits and process of PEGylation and how it may be applied to small molecules.

A versatile technology based on repeating units of polyethylene glycol (PEG), known as PEGylation, was first described in the literature in 1977 (1). PEG is a water-soluble, amphiphilic, nontoxic, and nonimmunogenic compound. It is safely cleared from the body and is currently a component of seven approved macromolecular drugs administered parenterally. Although the primary use of PEGylation has been to improve the physicochemical properties of large molecules, it may also be used with small molecules, provided certain challenges are met.

Small molecules have few sites to which PEGs can be attached without compromising their functionality. In addition, small molecules generally are delivered orally, and formulators believed that PEGylation would compromise oral bioavailability. These challenges have heretofore prevented the technology from being tried successfully on small molecules. A large-PEG prodrug approach to small-molecule PEGylation was unsuccessful. The strategy of permanently attaching a small PEG to a small molecule is untested and counterintuitive because low molecular weights are generally favored. These challenges in PEGylating small molecules have been overcome, however.

PEGs can be designed with various pharmacokinetic-altering architectures. They can be synthesized as linear, branched, or forked structures with functional groups at one or more termini to enable several conjugation strategies. Linkers covalently attach the molecule to the parent drug directly or indirectly. The choice of linkers enables PEG to be placed in various positions on the molecule. Placement can fine tune a drug's pharmacokinetic properties and maintain its efficacy. Overall, PEGylated molecules demonstrate enhanced solubility and stability, on the shelf and *in vivo*, and an improved safety profile.

Conjugation bonds can be stable or releasable to create entirely new compounds or novel prodrugs. Stable PEG linkages create new pharmaceutical entities with respect to such pharmacokinetic parameters as circulating half life, clearance, absorption, and bioavailability. PEGs may harm the

Timothy Riley, PhD,* is vice-president of PEGylation research, and **Jennifer Riggs-Sauthier** is director of science and technology at Nektar Therapeutics, 490 Discovery Dr., Huntsville, AL 35806, tel. 800.457.1806, fax 256.533.4805, triley@nektar.com.

*To whom all correspondence should be addressed.

Table I: Potential benefits of PEG technology.

Large molecular-weight prodrug PEGs (e.g., PEG–irinotecan and PEG–docetaxel)	Small molecular-weight PEG technology (e.g., PEG–naloxol and PEG–diphenhydramine)
Increase circulating half-life	Modify biodistribution
Modify biodistribution	Decrease transport
Improve safety profile	Improve oral bioavailability
Enhance water solubility	Modify metabolism

Note: PEG is polyethylene glycol.

pharmacodynamic properties of target binding because of steric hindrance. The releasable attachments used in PEG prodrugs enable controlled drug release through the choice of architecture, attachment sites, and linker molecules.

PEGylation imparts valuable, and in some cases crucial, pharmacokinetic properties to macromolecules, whether stable molecules or prodrugs, and has emerged as the dominant strategy for improving macromolecular drugs. PEGylation is associated with numerous clinical benefits in these drugs, including increased efficacy, decreased side effects, and lower frequency of dosing (see Table I). Table II shows the current, macromolecular PEG landscape. The technology is applicable to many therapeutic areas (e.g., oncology, metabolic diseases, and infectious diseases) and to various macromolecular classes (e.g., cytokines, antibodies, enzymes, and aptamers).

PEGylating small molecules

Is small-molecule PEGylation technically and commercially feasible? If the benefits of macromolecular PEGylation are well understood, so too are the challenges. The principal challenges of PEGylating macromolecules are identifying the specific sites of PEGylation and characterizing the final

product created when a 5000–40,000-dalton PEG molecule is attached to a 5000–50,000-dalton drug molecule. Properly designed, a PEGylated drug exhibits increased half life, greater bioavailability, and reduced clearance, which more than compensate for its reduced target binding. But what challenges are involved in PEGylating a 500-dalton drug that must have oral bioavailability? The feasibility of this process seems dubious, but it has proven possible.

Choice of molecule. The small-molecule universe encompasses a vast number of compounds, some of which are approved (and available by prescription or over the counter), some of which are in clinical trials, and some of which are in the early stages of discovery research. Many of these drugs possess poor physicochemical properties and demonstrate suboptimal efficacy or pharmacokinetics, making them potential candidates for PEGylation. No inherent technical constraints apparently exist (for experienced medicinal and PEGylation chemists) that limit the molecules that can be PEGylated. The first challenge is to choose compounds whose performance, clinical efficacy, and market potential are substantially limited by their oral bioavailability, solubility, half-life, or immediate clearance by first-pass metabolism. PEGylation can be used to exclude drugs from certain physiological compartments, such as the central nervous system (CNS), by impeding passage across the blood–brain barrier. This modification can reduce a drug’s side effects.

Next, the chemistry and structure-activity relationships (SAR) of the molecule must be considered and understood. Though it is hypothetically possible to PEGylate all small-molecule drugs, small molecules lack macromolecules’ multiplicity of attachment sites. The availability of these

Table II: PEGylated macromolecular drugs.

Commercial name	Drug name	Parent drug	Molecular weight (daltons)	PEG size (daltons)	Indication	Class	Year approved
Adagen	Pegadamasase	Adenosine deaminase	~90,000	5000	Severe combined immunodeficiency disease	Enzyme	1990
Oncaspar	Pegaspargase	Asparaginase	133,000–141,000	5000	Leukemia	Enzyme	1994
PEG-intron	Peginterferon-alpha2B	IFN-alpha2B	19,271	12,000	Hepatitis C	Cytokine	2000
PEGasys	Peginterferon-alpha2A	IFN-alpha2A	20,000	40,000	Hepatitis C	Cytokine	2001
Neulasta	Pegfilgrastim	G-CSF	19,000	20,000	Neutropenia	Cytokine	2002
Somavert	Pegvisomant	GH antagonist	21,998	4–5 x 5000	Acromegaly	Hormone	2003
Macugen	Pegaptanib	Anti-VEGF aptamer	10,000	40,000	Age-related macular degeneration	Nucleic acid	2004

nucleophilic sites and the feasibility of engineering them in must be considered. A key component to this decision is whether these sites, whether natural or engineered, will impede target binding to the point of negating the pharmacokinetic benefits of PEGylation. This decision requires not just an extensive knowledge of a candidate drug's SAR, but also an experience-based understanding of the effect of PEGylation: a combination of the art and science that frequently perfects the drug.

Multiple PEG platforms. Although PEG technology is conceptually simple, its execution is not. Multiple platforms are required to ensure the widest availability of small-molecule choices and the greatest variety of drug enhancements. Nektar Therapeutics (San Carlos, CA) employs two basic platforms based on the molecular weight of its PEGs. Both platforms confer the pharmacokinetic benefits of PEG but are employed based on a drug's specific characteristics such as its route of administration. The large molecular-weight platform involves PEGs whose molecular weights range from 1000 to more than 60,000 daltons. This platform involves a prodrug approach with the goal of increasing the circulating half life and extended exposure of drugs that are administered parenterally. The small molecular-weight platform involves PEGs that have a much lower molecular weight. This platform is exceptionally useful in creating or increasing oral bioavailability and decreasing penetration of specific barriers.

In addition to molecular weight, other important parameters must be considered when choosing PEGs. These include PEGs' architecture, which can be linear, branched, or forked (branched PEGs impart a greater degree of steric hindrance to enzymatic degradation) and the number of binding sites required (which can be manipulated through the choice of architecture and methoxy caps). The selection and design of the optimum PEG from the permutations available is ultimately dictated by the profile of the parent drug and the enhancements required to optimize it.

Table III summarizes current advances in PEG technology. Using these advances, small molecules can be successfully PEGylated, but challenges exist. These challenges are as follows:

- Proximity of the PEG chain to the target binding site can reduce drug activity significantly
- The scarcity of potential PEG conjugation sites on a small molecule can require sites to be engineered in
- The optimum conjugation position to choose is not always immediately obvious without substantial expertise in medicinal chemistry, PEGylation, and SAR
- Many active small molecules require organic environments, in contrast to aqueous-based proteins
- A fine balance must be struck between enhancing a drug's properties and increasing its molecular weight past the point of oral availability.

Several noteworthy examples of PEGylated small molecules show that these challenges can be overcome.

Table III: Advances in PEGylation technology.

Early PEGylation	Advanced PEGylation
Few small PEGs	Custom PEGs <ul style="list-style-type: none"> • Large range of molecular weights • Multiple functionalities
Multiple PEGs per drug	Single PEG per drug
Nonspecific PEGylation	Site-selective and -specific PEGylation <ul style="list-style-type: none"> • High bioactivity
Variable product purity	High product purity <ul style="list-style-type: none"> • Reproducible • Easier, validated analytics Prodrugs with adjustable release rates

Note: PEG is polyethylene glycol.

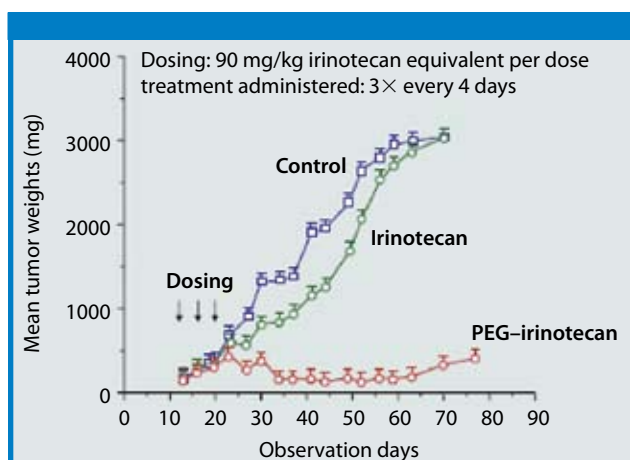


Figure 1: The effects of irinotecan and PEG-irinotecan on the growth of irinotecan-resistant human colon tumor (HT29) in a mouse xenograft model.

Examples of PEGylated small molecules in development

Small-molecule drugs in development can be coupled to large-molecular-weight PEGs as prodrugs. The potential benefits of using large-molecular-weight PEGs as prodrugs are:

- Increased circulating half life
- Modified biodistribution
- Improved safety
- Enhanced water solubility.

A molecule that would particularly benefit from this approach is irinotecan. Irinotecan is a \$1-billion oncolytic drug with suboptimal pharmacokinetics that treats colorectal cancer and other solid tumors. Irinotecan is cleared from the body within a few hours, and its short half life necessitates high doses to achieve therapeutic drug levels. The drug consequently causes many side effects, including neutropenia and severe diarrhea. Dose reductions for patients who cannot tolerate the side effects compromise the drug's therapeutic efficacy.

The goal of PEGylation is to increase the half life and exposure profiles of irinotecan to improve its efficacy and in-

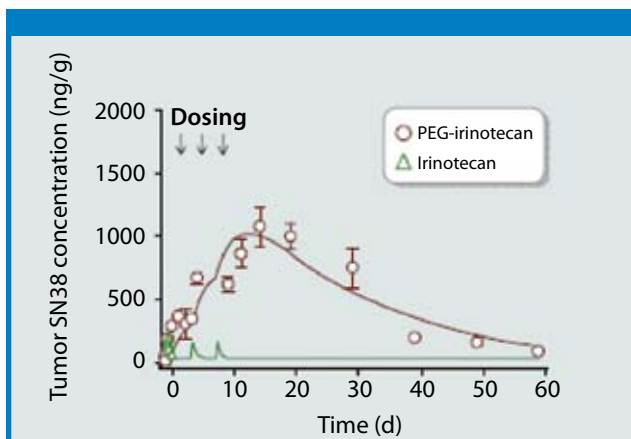


Figure 2: Concentration of tumor SN38 in mice with HT29 tumors.

crease the maximum tolerated dose (MTD). These changes ultimately could improve care, outcomes, and patients' quality of life. The strategy to achieve these benefits uses a large-molecular-weight PEG with a linkage to irinotecan that is gradually cleaved to release the parent compound.

Preclinical comparisons of PEG-irinotecan with irinotecan demonstrated the former's superior efficacy in three mouse xenograft models (irinotecan-resistant colon cancer, breast cancer, and lung cancer). PEG-irinotecan produced a 2–3-log increase in exposure to irinotecan's active metabolite (SN-38) in colorectal tumors in mice, and reduced neutropenia and diarrhea and achieved a higher MTD in rats and dogs (see Figures 1–3). Animal data also show a more sustained level of SN-38 than that achieved with native irinotecan as a consequence of PEG-irinotecan's slower release and longer half life.

A similar effect was seen in humans. In Phase I clinical trials, PEG-irinotecan demonstrated reduced neutropenia in the active-dose range in addition to some preliminary antitumor activity. PEG-irinotecan is currently in Phase II clinical trials.

In addition to irinotecan, the large molecular weight PEG prodrug approach is being applied to docetaxel, a \$2.2 billion drug used to treat solid tumors. This drug produces dose-limiting neutropenia. Preclinical studies show antitumor activity in xenograft models of prostate, breast, and lung cancers as well as superior efficacy in taxane-resistant cell lines. PEG-docetaxel is currently in development.

Small-molecule drugs can also be coupled to small-molecular-weight PEGs for CNS exclusion. The potential benefits of small molecular weight PEG technology are:

- Modified biodistribution
- Decreased transport
- Improved oral bioavailability
- Modified metabolism.

An example of the use of this technology is the “retasking” of the opiate antagonist naloxol to treat opioid-induced constipation and bowel dysfunction. Opioids exert their effect by binding mu receptors in the CNS to provide analgesia. Mu receptors are also present in the gastrointestinal

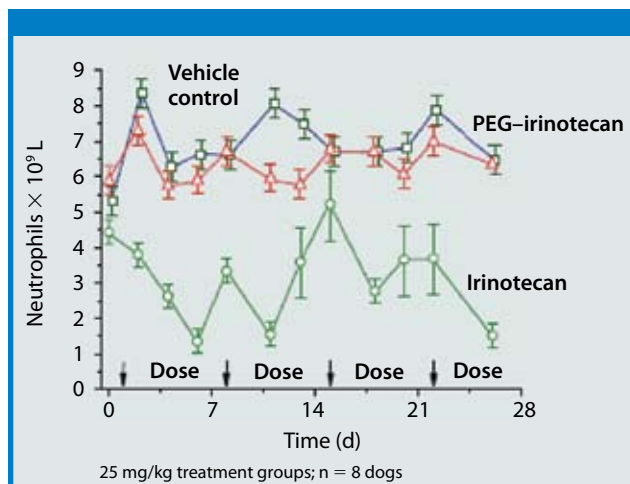


Figure 3: The effect of irinotecan and PEG-irinotecan on neutropenia in animals.

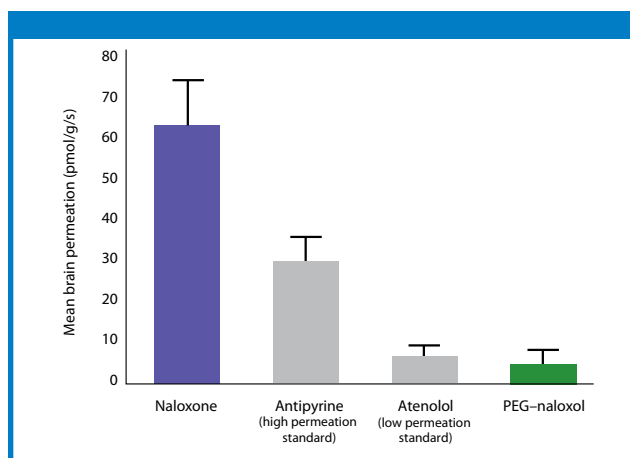


Figure 4: Permeation of PEG-naloxol, naloxone, and reference standards in rats.

(GI) tract. Opioid binding in the GI tract can cause severe constipation and bowel dysfunction, which is a significant problem for patients who require continuous use of opioids to control intractable pain. It is estimated that 200 million opioid prescriptions are filled each year in the US and that more than 40% of patients will experience opioid-induced constipation and bowel dysfunction.

Naloxol and naloxone are well known antagonists of opioids and are used to treat drug overdose. Conjugating naloxol to a small-molecular-weight PEG such as PEG-naloxol prevents the drug molecule from crossing the blood–brain barrier and interfering with analgesia. Yet the technique allows naloxol to bind to the mu receptors in the GI tract to mitigate the undesirable effects of opioids. PEGylation has the additional benefit of increasing the oral availability of the molecule.

Preclinical studies of PEG-naloxol in rats demonstrate reduced permeation of the rat brain, compared with naloxone, improved GI transit, and maintenance of pain relief (see Figures 4–5). Phase I clinical-trial data of PEG-naloxol

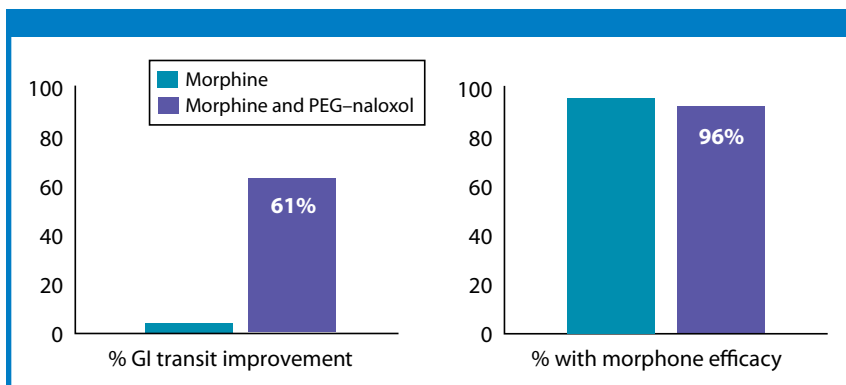


Figure 5: The effect of PEG-naloxol on gastrointestinal (GI) transit time and central morphine efficacy.

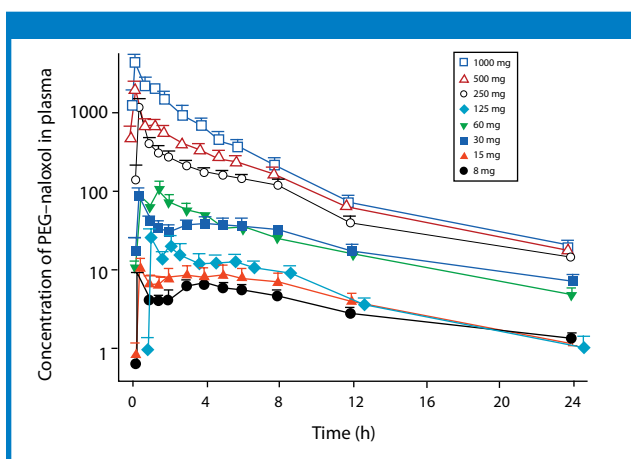


Figure 6: Concentration of PEG-naloxol in plasma over time.

indicate that the compound provided a 10-fold increase in oral bioavailability over that of naloxone, was rapidly absorbed in plasma, provided a clinically relevant improvement in transit time through the gut (as measured by the hydrogen breath test), and demonstrated minimal reversal of CNS efficacy (as measured by pupillometry) (see Figure 6). Moreover, the data show an extended half life of 10 h. PEG-naloxol is currently in Phase II development.

Another application of PEGylated CNS-exclusion technology involves using small-molecular-weight PEGs on diphenhydramine to treat allergic rhinitis. The allergic-rhinitis market is a \$6.7-billion segment with a large unmet market need. The most effective antihistamines make patients drowsy, and those that are nonsedating are not strong enough to meet the needs of all patients. Preclinical data show that PEGylating diphenhydramine dramatically reduces the concentration of the drug in the CNS compared with the native molecule, while retaining the antihistamine activity. The molecule is currently in preclinical studies.

Commercial considerations

Extensive expertise with PEGylated macromolecules has led to the conclusion that no inherent technical or commercial constraints limit the manufacture and scale-up of small-molecule PEGs. The chemistries of these molecules are different (i.e., organic as opposed to aqueous), however, and PEGs must sometimes be built into a small molecule during synthesis. This procedure requires specific expertise. Cost is not expected to significantly affect the commercial feasibility of PEGylated small molecules because costs are well within commercial parameters.

Shelf life and stability could actually improve over those of the parent compounds because PEG can be a stabilizing molecule. From a regulatory point of view, the US Food and Drug Administration considers PEG small molecules as new chemical entities (NCEs) subject to the same standards as nonPEGylated NCEs. The technology and molecules can be protected by patents. For example, Nektar has 55 issued patents and 490 applications worldwide that cover polymer structures, polymer linkers, composition of matter, manufacturing, and mechanism and delivery modalities. Thus, patent protection of non-PEGylated drugs is not expected to affect PEGylated drugs' commercial prospects.

Conclusion

PEGylating small molecules is a feasible strategy for enhancing and optimizing current compounds. The technology can be effectively transferred from macromolecules to small molecules to provide a similar range of clinically important benefits such as greater efficacy, reduced side effects, a reduced dosing schedule, and increased patient compliance. The universe of compounds that could be PEGylated is large, but extensive knowledge of structure-activity relationships and PEGylation properties is crucial to tapping this resource. Given the slow rate of new-chemical-entity discovery that currently afflicts the pharmaceutical industry, PEGylating small molecules appears to offer a technically feasible and cost-effective route to the creation of improved drugs.

Reference

1. A. Abuchowski *et al.*, "Effect of Covalent Attachment of Polyethylene Glycol on Immunogenicity and Circulating Life of Bovine Liver Catalase," *J. Biol. Chem.* **252** (11), 3582–3586 (1977).