EFFECT OF PEGYLATION ON PHARMACEUTICALS

J. Milton Harris & Robert B. Chess‡*

Protein and peptide drugs hold great promise as therapeutic agents. However, many are degraded by proteolytic enzymes, can be rapidly cleared by the kidneys, generate neutralizing antibodies and have a short circulating half-life. Pegylation, the process by which polyethylene glycol chains are attached to protein and peptide drugs, can overcome these and other shortcomings. By increasing the molecular mass of proteins and peptides and shielding them from proteolytic enzymes, pegylation improves pharmacokinetics. This article will review how PEGylation can result in drugs that are often more effective and safer, and which show improved patient convenience and compliance.

HALF-LIFE
The amount of time it takes for one-half of a drug dose to be lost through biological processes.

SHELF LIFE
The amount of time a stored drug retains its activity.

**Nektar Therapeutics, 490 Discovery Drive, Huntsville, Alabama 35806, USA. ‡Nektar Therapeutics, 150 Industrial Road, San Carlos, California 94070, USA.*
e-mails: jmharris@al.nektar.com; robert_chess/inhale@inhale.com
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The biotechnology revolution has produced novel peptides and proteins (henceforth referred to as polypeptides) that have become important new drugs. More than 80 polypeptide drugs are marketed in the United States, and 350 more are undergoing clinical trials. About a third of drug candidates in clinical trials today are polypeptides. In 2000, sales of polypeptide drugs were estimated at US $15 billion worldwide[1].

Recombinant DNA techniques using *Escherichia coli* and other organisms also allow polypeptide drugs to be produced in large quantities. Despite these tremendous advances, polypeptide drugs possess several shortcomings that limit their usefulness. These disadvantages include their susceptibility to destruction by proteolytic enzymes, short circulating HALF-LIFE, short SHELF LIFE, low solubility, rapid kidney clearance and their propensity to generate neutralizing antibodies[2]. In addition, most polypeptide drugs must be delivered by injection, either subcutaneously or intravenously[2].

Scientists have searched for years for ways to overcome the problems associated with polypeptides as drugs. Various researchers have attempted to improve the clinical properties of polypeptides by altering amino-acid sequences to reduce degradation by enzymes and antigenic side effects, by fusing them to immunoglobulins or albumin to improve half-life, and by incorporating them into drug-delivery vehicles such as LIPOSOMES[3–5]. Although sometimes successful, these methods have limitations, as illustrated by studies of liposomes. Liposomes not only deliver drugs to diseased tissues, such as tumours, but also rapidly enter the liver, spleen, kidneys and RETICULOENDOTHELIAL SYSTEMS, and leak drugs while in circulation[6]. In addition, liposomes activate COMPLEMENT, which causes pseudoallergic reactions that damage heart and liver cells[7]. Pegylation is an alternative method that overcomes these deficiencies[8,9] by attaching polyethylene glycol (PEG) chains to polypeptides or other candidate molecules. In fact, liposomes are now pegylated to improve the delivery of encapsulated drugs, such as the anticancer agent doxorubicin[10] (BOX 1).

The FDA has approved PEG for use as a vehicle or base in foods, cosmetics and pharmaceuticals, including injectable, topical, rectal and nasal formulations. PEG shows little toxicity, and is eliminated from the body intact by either the kidneys (for PEGs < 30 kDa) or in the faeces (for PEGs > 20 kDa)[11]. PEG lacks immunogenicity[12], and antibodies to PEG are generated in rabbits only if PEG is combined with highly immunogenic proteins[13]. No one has ever reported the generation of antibodies to PEG under routine clinical administration of pegylated proteins. However, under extreme experimental conditions, antibodies to PEG have been generated in animals as a result of the injection of PEG–protein conjugates[14].

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Box 1 | **Pegylated liposomes**

Liposomes are pegylated to prolong their blood circulation time. Compared with classical liposomes, pegylated counterparts show increased half-life, decreased plasma clearance, and a shift in distribution in favour of diseased tissues. PEG is incorporated into the lipid bilayer of the liposome, forming a hydrated shell that protects it from destruction by proteins. For the antitumour drug doxorubicin, peglyation of the liposome brings an eightfold increase in plasma half-life of the liposome compared to an unmodified liposome[18]. Pegylated liposomes are also less extensively taken up by the reticuloendothelial system and are less likely to leak drug while in circulation[10].

First-generation pegylation processes

In the late 1970s, Frank Davis and his colleagues at Rutgers University pioneered the first chemical steps of pegylation that enabled the protection of proteins from destruction during drug delivery[15]. Their studies generated the unexpected finding that pegylation also improves the PHARMACOKINETIC and PHARMACODYNAMIC properties of polypeptide drugs by increasing water solubility, reducing renal clearance and limiting toxicity[16,17]. Pegylation reduces kidney clearance simply by making the molecules larger, and, as the kidneys filter substances basically according to size, larger molecules clear more slowly. The PEG is not degraded prior to elimination[18].

PEG is formed by a process of linking repeating units of ethylene glycol to form polymers with linear or branched shapes of different molecular masses (FIG. 1). These PEG structures are then chemically attached to the drug of choice in a process called pegylation. Studies of PEG in solution reveal that each ethylene glycol subunit is tightly associated with two or three water molecules. This binding of water molecules makes pegylated compounds function as though they are five to ten times larger than a corresponding soluble protein of similar molecular mass, as confirmed by size-exclusion chromatography and gel electrophoresis[19]. The PEG polymer, along with the associated water molecules, acts like a shield to protect the attached drug from enzyme degradation, rapid renal clearance and interactions with cell surface proteins, thereby limiting adverse immunological effects. Pegylated drugs are also more stable over a range of pH and temperature changes[20] compared with their unpegylated counterparts. Consequently, pegylation confers on drugs a number of properties that are likely to result in a number of clinical benefits, such as sustained blood levels that enhance effectiveness, fewer adverse reactions, longer shelf life and improved patient convenience[21]. However, pegylation can produce a decrease in the *in vitro* activity of proteins, but generally this negative effect is offset in biological systems by an increased half-life. Pegylation can influence the binding affinity of therapeutic proteins to cellular receptors, which results in changes in the bioactivity of polypeptides[18].

To maximize the pharmacological benefits of pegylation, a stable bond is formed between the PEG polymer and polypeptide drug of choice. In general, a PEG polymer is first chemically activated in order to react with a polypeptide drug[22]. A variety of chemical modifications are used to prepare an active PEG derivative with a functional group — such as active carbonate, active ester, aldehyde, or tresylate, as illustrated in FIG. 2 — suitable for coupling to a given target molecule. The activated PEG derivative is then covalently linked to a reactive group on the polypeptide drug. The most common reactive sites on polypeptides for attaching PEG polymers are the α or ε amino groups of lysine or the N-terminal amino-acid groups of other amino acids[23]. Changes in the size, structure and molecular mass of PEG polymers can affect the biological activity of the attached drug. In general, pegylation of a polypeptide lowers its renal clearance, increases its half-life and improves its biological activity[18]. The careful selection of pegylation chemistries and reaction conditions yield pegylated polypeptides with different therapeutic properties. For example, pegylation of granulocyte colony-stimulating factor (G-CSF) through an amine linkage increases the liquid-phase stability of G-CSF five times compared with pegylation through an amide bond[24].

The first-generation pegylation methods were fraught with difficulties. With first-generation pegylation, the PEG polymer was generally attached to the ε amino groups of lysines. This resulted in the modification of multiple lysines, and gave mixtures of PEG isomers with different molecular masses[25]. The existence of these isomers makes it difficult to reproduce drug batches, and can contribute to the antigenicity of the drug and poor clinical outcomes. In addition, first-generation methods mainly used linear PEG polymers with molecular masses of 12 kDa or less. Unstable bonds between the drug and PEG were also sometimes used, which leads to degradation of the PEG–drug conjugate during manufacturing and injection[26]. An additional problem was that early pegylation was performed with methoxy–PEG (m–PEG), which was contaminated with PEG DIOL and which resulted in the crosslinking of proteins to form inactive aggregates. Diol contamination can reach up to 10–15% (REF 27).

Despite these limitations, several first-generation pegylated drugs received regulatory approval. Still in use today are pegademase (Adagen), a pegylated form of the enzyme adenosine deaminase for the treatment of severe combined immunodeficiency disease (SCID), and pegaspargase (Oncaspar), a pegylated form of the enzyme asparaginase for the treatment of leukaemia.

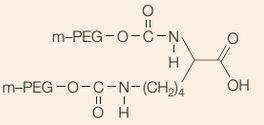
Linear PEG-OH	$H-(OCH_2CH_2)_n-OH$
Linear m–PEG-OH	$CH_3-(OCH_2CH_2)_n-OH$
Branched m–PEG_2	

Figure 1 | **Structural formulae of polyethylene glycol (PEG) molecules.** m–PEG, monomethoxy–PEG.

LIPOSOME
Phospholipid capsules that protect enclosed drugs from degradation.

RETICULOENDOTHELIAL SYSTEM
A community of phagocytic cells of the body, located primarily in the spleen, liver and lymph nodes, that protect against infection.

COMPLEMENT
A complex series of blood proteins whose actions augment the work of antibodies to destroy bacteria, produce inflammation and regulate immune reactions.

PHARMACOKINETICS
The movement of drugs throughout the body, including their absorption, distribution, metabolism and excretion, and the mathematical models that describe these actions.

PHARMACODYNAMICS
Changes in measurable clinical parameters related to a drug, such as increase in antitumour activity, decrease in nausea, or decrease in viral load.

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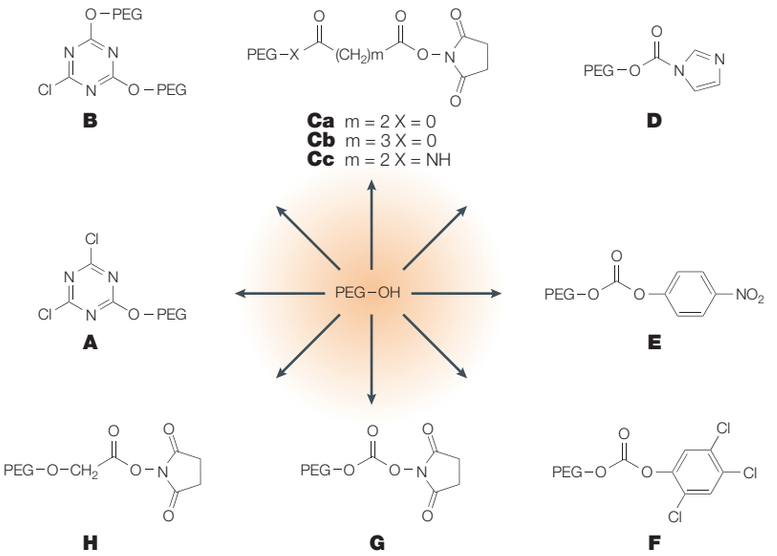

Figure 2 | **Method for the activation of PEG molecules. A** | Cyanuric chloride method. **B** | A variation on the cyanuric chloride method. **Ca** | Polyethylene glycol (PEG)–succinimidyl succinate method. **Cb** | Substitution of the succinate residue by glutarate. **Cc** | Substitution of the aliphatic ester in **Ca** by an amide bond. **D** | Imidazoyl formate method. **E** and **F** | Variations using phenylcarbonates of PEG. **G** | Succinimidyl carbonates of PEG. **H** | Succinimidyl active ester of PEG.

Second-generation pegylation processes

Second-generation pegylation chemistry strives to avoid the pitfalls associated with mixtures of isomers, diol contamination, unstable bonds and low-molecular mass m–PEG. Researchers have created an array of chemistries to improve PEG derivatives and their linkages to drugs. For example, using PEG–propionaldehyde instead of PEG–acetaldehyde prevents the formation of impurities in the PEG (by aldol condensation in this case)[28] (FIG. 3). Generating carboxylic acid intermediates of PEG permits up to 97% of diol impurities to be removed by ion-exchange chromatography before PEG attachment to polypeptide drugs[20]. An overall goal of second-generation pegylation methods is to create larger PEG polymers to improve the pharmacokinetic and pharmacodynamic effects seen with lower molecular mass PEGs. In some cases, the changes are dramatic, such as the pegylation of interleukin-6 (IL-6), which increases the half-life of IL-6 100-fold, which in turn results in a 500-fold rise in its thrombopoietic potency[18]. TABLE 1 summarizes the influence of pegylation on the pharmacokinetic and pharmacodynamic properties of several therapeutic polypeptides.

Pegylating site-specifically can minimize the loss of biological activity and reduce immunogenicity. For instance, because there are far fewer cysteine residues than lysine groups on polypeptides, the THIOL groups of cysteine are ideal for specific modifications. Moreover, cysteines can be added to polypeptides precisely where they are desired by genetic engineering[29]. The preparation of a highly active, long-circulating and stable conjugate of interferon-β (IFN-β) illustrates how such structural manipulation can lead to the development of a pegylated drug. In this case, a two-step method with m–PEG orthopyridyl disulfide (OPSS) was used[30]. In the folded structure of native IFN-β, the free cysteine residue at position 17 is proximal to the surface, but is hidden[31]. Consequently, a PEG derivative of high molecular mass cannot be directly attached to this residue. Instead, one active group of the smaller, low-molecular-mass (2 kDa) difunctional reagent di-OPSS–PEG is first attached to the hidden cysteine of IFNβ, followed by coupling of a high-molecular-mass m–PEG thiol to the other active group of OPSS–PEG. Although many proteins might not benefit from site-specific pegylation, in others, such as antibody fragments, it is crucial that the PEG is attached at a site distant from the binding site.

Other advances in the chemistries for manipulating PEG derivatives include the incorporation of degradable linkages to release drugs at targeted sites as well as the synthesis and use of HETEROBIFUNCTIONAL PEGs. One method (of the many under investigation) for releasing drugs from PEG employs a para- or ortho-disulfide of benzyl urethane[32]. When subjected to mild reducing conditions, such as inside the endosomes of cells, the drug breaks free. Heterobifunctional PEGs contain dissimilar terminal groups, which are advantageous for applications in immunoassays, biosensors and probes to link macromolecules to surfaces, as well as for the targeting of drugs, liposomes or viruses to specific tissues[33].

Another improvement in second-generation PEG polymers is the use of branched structures, in contrast to the solely linear structures found in first-generation PEGs[20]. Branched PEGs of greatly increased molecular masses — up to 60 kDa or more, compared with the 12 kDa or less found in first-generation PEGs — have been prepared. A branched PEG 'acts' as if it were much larger than a corresponding linear PEG of the same molecular mass[34]. Branched PEGs are also better at cloaking the attached polypeptide drug from the immune system and proteolytic enzymes, thereby reducing its antigenicity and likelihood of destruction[35]. The transition from first-generation to second-generation chemistries is taking place at a rapid pace, and the examples mentioned in this section are just a few of the novel chemistries reported (BOX 2). Further details are reviewed elsewhere[2,18].

Early clinical PEG conjugates

The FDA has approved several pegylated polypeptides as therapeutics and more are undergoing clinical investigation. In 1990, pegademase (Adagen) received approval for the treatment of severe combined immunodeficiency (SCID), a disease associated with an inherited deficiency of adenosine deaminase[36]. Before the availability of pegademase, SCID patients were transfused with red blood cells containing adenosine deaminase. However, blood transfusions raised the risks of iron overload and transfusion-associated viral infections[37,38]. Because pegademase carries about 1,800 times more adenosine deaminase activity per millilitre than red blood cells, the drug achieves higher blood levels of the missing enzyme[39].

DIOL
PEG with two terminal hydroxyl groups that lead to crosslinking and loss of activity.

THIOL
An–SH group.

HETEROBIFUNCTIONAL
PEGs with two different terminal groups, making them capable of performing different functions.

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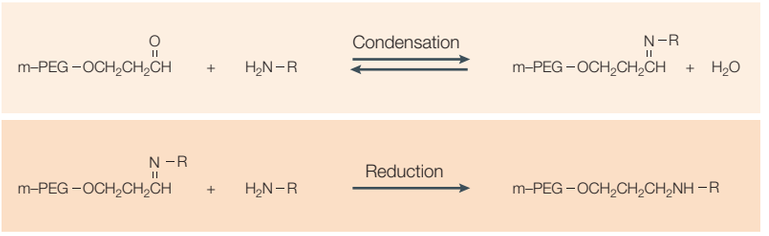

Figure 3 | **Reductive amination using PEG–propionaldehyde.** In the first reaction, a methoxy–polyethylene glycol (m–PEG) aldehyde is covalently linked to an amino group on the protein. This occurs predominantly on the N terminus of the protein. The linkage formed in the first step, a so-called Schiffs-base linkage, can be reversed by hydrolysis. This linkage, however, is rapidly stabilized to a stable, non-hydrolyzable amine linkage in the second step by sodium borohydride reduction.

Pegaspargase (Oncaspar), contains the pegylated enzyme L-asparaginase, used clinically in combination with chemotherapy for the treatment of acute lymphocytic leukaemia, acute lymphoblastic leukaemia and chronic myelogenous leukaemia. Leukaemic cells cannot synthesize asparagine, and depend on outside sources of this amino acid; asparaginase, therefore, kills leukaemic cells by rapidly depleting them of asparagine[40]. Unpegylated asparaginase causes allergic reactions and generates neutralizing antibodies that shorten its half-life[41]; pegylation, however, extends the half-life from the 20 hours observed for unpegylated asparaginase to 357 hours for pegaspargase. In addition, pegaspargase reduces adverse immune responses[43].

In 2001, peginterferon α2b (PegIntron) became available as a once-a-week treatment for hepatitis C. Chronic hepatitis C virus (HCV) infection is the leading cause of liver cirrhosis and liver cancer[43], and the principal reason for liver transplants in the United States. An estimated 2.7 million Americans are chronically ill with the virus, and about 35,000 new infections arise each year. Hepatitis C causes 8,000–10,000 deaths yearly, and the number of deaths is estimated to increase to 38,000 by 2010 (REF. 19). The molecular mass of IFN-α2b is 19 kDa, whereas the molecular mass of PegIntron is 31 kDa. Structural and biological characterization of pegylated recombinant IFN-α2b indicates that the principal pegylation site (about 50% of pegylation) is His34, although pegylation also occurs at lysines and the N terminus[44].

Clinical studies show PegIntron to be superior to unpegylated IFN-α2b (Intron-A). PegIntron gives significantly higher virological responses, and allows the reduction of dosages from three times a week to once weekly. PegIntron has a sevenfold lower clearance rate and a fivefold greater *in vivo* half-life than Intron A. A standard three-times-weekly administration of Intron-A causes peaks and troughs in blood levels of interferon. In comparison, once-weekly administration of PegIntron produces more constant and longer-lasting blood levels of interferon, which results in the better suppression of viral replication[45].

In a pivotal Phase III monotherapy trial of 1,219 chronic hepatitis C adult patients, subjects received PegIntron once weekly at doses of 0.5, 1.0 or 1.5 μg per kilogram body weight, or a standard dose of Intron A given three times a week. All patients had chronic hepatitis C with compensated liver disease and were positive for hepatitis C viral RNA. The treatments lasted 48 weeks, and the efficacy endpoint was the sustained elimination of detectable hepatitis C viral RNA. At the end of therapy, 33, 41 and 49% of the respective PegIntron treatment groups showed detectable loss of viral RNA, compared with 24% of Intron A recipients. The investigators concluded that all three PegIntron doses were as safe as, but superior to, Intron-A[46].

Second-generation clinical PEG conjugates

Using the new chemistries described above, second-generation clinical PEG conjugates started to appear in the early 1990s. A competing treatment for chronic hepatitis C utilizes IFN-α2a coupled to PEG. The first formulation in 1999 used a first-generation linear PEG of 5 kDa. In the first clinical trials, this pegylated drug was administered to patients with chronic hepatitis C once weekly and compared with its unpegylated counterpart administered three times a week. The pegylated IFN-α2a produced no clinical advantages[47]. A second-generation, branched PEG of 40 kDa was then coupled to IFN-α2a[48]. This version (Pegasys) was subjected to

Table 1 | **Influence of pegylation on pharmacokinetics and pharmacodynamics***

Pharmacokinetic effect	Pharmacodynamic effect
Interferon-α2a	
Sustained absorption	*In vivo* antiviral activity increased 12- to 135-times
Increased half-life (from 3–8 h to 65 h)	Antitumour activity increased 18-fold
Decreased volume of distribution (from 31–73 l to 8–12 l)	Improved sustained response to chronic hepatitis C
Decreased systemic clearance (from 6.6–29.2 to 0.06–0.10 l/h)	
Interleukin-6	
Increased half-life (from 2.1–206 min)	Thrombopoietic potency increased 500-times
Tumour necrosis factor	
Increased half-life (from 3 to 45–136 min)	Antitumour potency increased 4-to 100-times

*Influence of pegylation on pharmacokinetics and pharmacodynamics of some therapeutic proteins, compared with corresponding native proteins (adapted from REF. 18).

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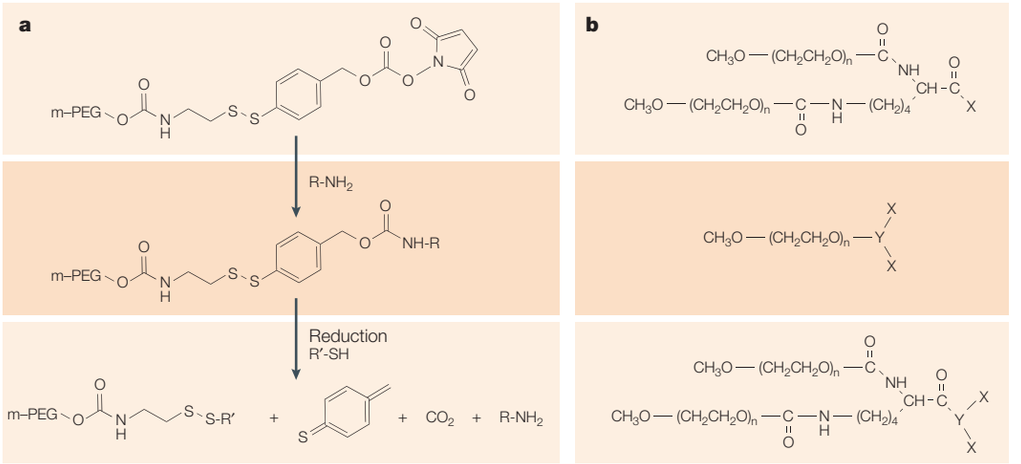
Box 2 | **Special chemistries for pegylation**

In most cases, a stable bond is desired between the polyethylene glycol (PEG) polymer and polypeptide drug. However, in some cases, it might be desirable for a drug to be released from its PEG polymer when it reaches a target site to improve its action. Zalipsky and co-workers[31] fashioned a releasable PEG that uses a para- or ortho-disulfide of benzyl urethane, as shown in the figure (part a). Inside the endosomal compartment of a cell, the mild reducing conditions release the original polypeptide.

Branched PEGs stabilize polypeptides and can be made in different ways. Two linear PEGs can be linked to α or ε amino groups of lysine, as shown in the figure (part b, top panel). This type of PEG acts much larger than a linear PEG of the same molecular mass. Branched PEGs can also have a forked structure, carrying two proximal reactive groups at one end of a single PEG chain (part b, middle panel) or at the end of the branched PEG of part b, top panel (part b, bottom panel).

m–PEG, methoxy–PEG.



clinical investigation by Hoffmann-La Roche. Once-weekly injections of Pegasys produce nearly constant blood concentrations of IFN-α2a (FIG. 4), and renal clearance is reduced 100-fold relative to unpegylated IFN-α2a. Pegylation increases the half-life of IFN-α2a from 9 to 77 hours[49].

A Phase III trial of 531 patients with hepatitis C compared Pegasys administered once weekly with unpegylated IFN-α2a administered three times a week for 48 weeks. Undetectable levels of hepatitis C viral RNA occurred more in the Pegasys group (68%) than in the other group (28%). Compliance was also better in the Pegasys group, with 84% of patients completing the study, compared with 60% of patients receiving the unpegylated drug. The reduction of dosing injections for Pegasys can result in improved patient compliance. The investigators concluded that "a regimen of peginterferon α2a given once weekly is more effective than a regimen of interferon α2a given three times weekly"[50].

Another randomized study compared Pegasys with unpegylated IFN-α2a in 271 patients with chronic hepatitis C and liver cirrhosis. Two doses of Pegasys (90 or 180 μg injected once weekly) were compared with unpegylated IFN-α2a injected three times a week. At the end of 72 weeks, hepatitis C viral RNA was undetectable in 8, 15 and 30% of the patients treated with unpegylated IFN-α2a, 90 μg of Pegasys and 180 μg of Pegasys, respectively. In addition, liver biopsy samples showed histological improvements of 31, 44 and 54% for the same respective drugs and doses[51].

Before the development of pegylated interferons, the optimal treatment for chronic hepatitis C combined interferon and the antiviral agent ribavirin, which results in enhanced clinical outcomes over interferon alone[52–54]. Studies were undertaken to assess whether combining pegylated interferons with ribavirin also offers clinical advantages. A small pilot study of Pegasys plus ribavirin in twenty patients with chronic hepatitis C found that sustained virological and biochemical responses occur in nine out of twenty patients. The genotype of hepatitis C appears to affect the outcome: just five of sixteen patients infected with genotype 1 achieved sustained responses, whereas all four patients infected with other genotypes responded[55].

A Phase III study investigated the response of 1,530 patients with hepatitis C, who received PEG-Intron plus ribavirin or Intron A plus ribavirin for 48 weeks. A 54% sustained virological response was observed in those receiving PegIntron plus ribavirin, compared with 47% in those receiving Intron A plus ribavirin. This response rate can be improved when the HCV genotype is taken into account. The response rate for patients with HCV genotypes 2 and 3 reached 80% on both treatments, but only 42% for HCV genotype 1 (REF. 56). Another trial compared Pegasys plus ribavirin with IFN-α2b plus ribavirin in 1,121 patients with chronic hepatitis C. A significantly higher proportion of patients who received Pegasys plus ribavirin showed sustained elimination of detectable virus than patients who received IFN-α2b plus ribavirin (56 versus 44%,

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ACROMEGALY
A disease characterized by abnormal enlargement of the skull, jaw, hands and feet, which is caused by excessive secretion of growth hormone by the pituary gland.

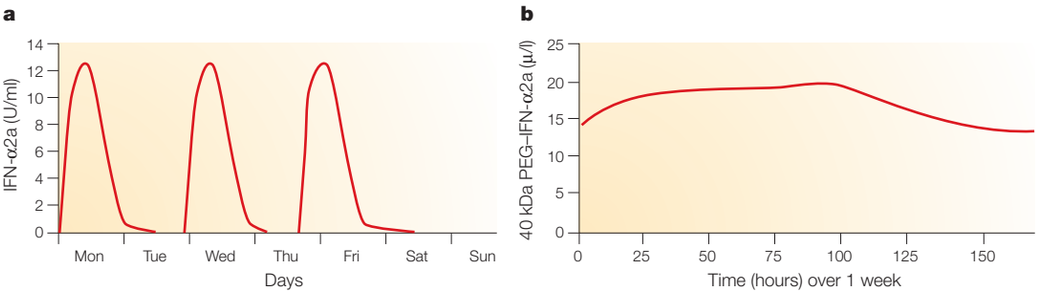

Figure 4 | **Pharmacokinetic profiles for interferon (IFN)-α2a and 40 kDa polyethylene glycol (PEG)–IFN-α2a.** These graphs represent blood levels in humans resulting from subcutaneous injection of **a** | IFN-α2a and **b** | branched PEG 40 (kDa) IFN-α2a. IFN-α2a is injected every other day and its short lifetime in circulation leads to pulsed blood concentrations levels which cycle below efficacious levels. The branched PEG 40 (kDa) IFN-α2a has a long circulating lifetime due to the presence of the PEG, and the once-weekly injection leads to near constant blood concentrations above the therapeutic level over the one-week period.

respectively). However, the proportions of patients with HCV genotype 1 who sustained virologic response were only 46 and 35%, respectively[57].

The competing pegylated forms of IFN-α (PegIntron and Pegasys) show superior efficacy compared with their unpegylated counterparts. Our knowledge of pegylation chemistry predicts that the higher-molecular-mass branched formulation of Pegasys should show a superior pharmacokinetic profile. However, only head-to-head clinical trials can confirm whether one drug is superior to the other under different clinical circumstances. In the treatment of a chronic illness such as hepatitis C, clinical efficacy will be determined by the therapy that induces the highest, sustained blood levels of the drug with the fewest injections. Pegasys was approved by the FDA for the treatment of chronic hepatitis C in October 2002, and the combination Pegasys and ribavirin therapy was approved in December 2002.

Several other pegylated polypeptides are undergoing clinical trials. A pegylated form of human growth hormone antagonist called pegvisomant (Somavert) is being developed for the treatment of ACROMEGALY. Patients with acromegaly experience extremely high serum levels of insulin-like growth factor-1 (IGF1) that contributes to soft-tissue enlargement. A Phase III trial of pegvisomant showed that daily treatment normalized levels of IGF1 and improved soft-tissue enlargement in 131 patients[58,59]. Pegvisomant has been approved in Europe, and is awaiting FDA approval in the US.

Box 3 | **Pegylated nanoparticles for brain delivery**

The blood–brain barrier (BBB) is formed by special endothelial cells sealed with tight junctions. This unique membrane blocks many compounds that might be of therapeutic value in the treatment of neurological or psychiatric disorders. Injecting drugs directly into the brain or disrupting the BBB carries high risks for patients.

Polymer nanoparticles, such as n-hexadecylcyanoacrylate (PHDCA), show promise as a way to transport drugs across the BBB. Animal studies show that PEG–PHDCA penetrates into the brain to a significantly greater extent than PHDCA alone. PEG–PHDCA distributes into deep areas of the brain, including the striatum, hippocampus, and hypothalamus. Furthermore, this movement occurs without damage to the BBB or other brain structures. The method seems promising for the development of drug carriers for brain delivery[74].

The cytokine tumour necrosis factor-α (TNF-α) plays a key role in mediating inflammation. A cloned soluble recombinant form of a natural inhibitor of TNF-α — sTNF receptor type I (TNF-RI) — has been attached to a high-molecular-mass PEG to improve its pharmacokinetics, and preclinical studies in rodent models of rheumatoid arthritis (RA) and Crohn's disease demonstrate its potential efficacy[60]. A 12-week trial of 194 patients with RA compared two doses of PEG-TNF-RI with a placebo. Patients receiving the drug reported improvement in physical function, pain, general health, vitality, social function and mental health in a dose-dependent manner[61]. A competing pegylated anti-TNF-α antibody fragment (CDP870) also has encouraging clinical results[62].

Other pegylated drugs have received FDA approval for the treatment of different types of tumours or related clinical problems. Pegfilgrastim (Neulasta), which was approved in 2002, is a pegylated form of the earlier drug filgrastim (Neupogen). Both contain recombinant methionyl human G-CSF, which is known as filgrastim. The drugs stimulate the production of the infection-fighting white blood cells (neutrophils) that are depleted by cancer chemotherapy. Whereas filgrastim requires daily injections for about 14 days, pegfilgrastim requires one injection per chemotherapy cycle. Data from two pivotal Phase III trials in breast-cancer patients indicated that a single dose of pegfilgrastim provides as much protection from infection as 11 daily injections of filgrastim[63,64].

Doxil — a pegylated liposomal formulation of doxorubicin — was approved in 1995 for the treatment of Kaposi's sarcoma. Doxil is also proving successful in treating metastatic ovarian cancer. When compared with topotecan, ovarian-cancer patients survived longer on Doxil and suffered fewer complications of neutropenia and anaemia. Doxil requires a single infusion once monthly, whereas topotecan requires daily 30-minute infusions for five days every three weeks[65]. Doxil is also proving effective against breast cancer[66].

PEG-based hydrogels

PEG can be chemically crosslinked to form polymer networks that swell and form gels. These swollen, jelly-like materials are called hydrogels, and are well

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suited for a range of medical applications. The biocompatibility of hydrogels makes them ideal for wound-healing applications[67].

In 2000, the FDA approved surgical sealant FocalSeal to prevent air leaks in the lungs following the removal of lung tumours and other chest surgeries. FocaSeal uses a PEG that is applied as a liquid, and then transformed into a waterproof hydrogel seal by irradiation. The sealant protects wound sites from leaking during tissue healing, and then naturally degrades and dissolves. Clinical trials show that 93–100% of surgery patients treated with FocalSeal remain free of air leaks, compared with 20–30% in patients who do not receive the treatment[68,69].

SprayGel, another biodegradable hydrogel, prevents post-operative adhesion formation[70]. Following surgery, internal wounds often develop adhesions — a type of scar tissue — that cause severe pain, and which are a leading cause of small-bowel obstructions and infertility in women. SprayGel is sprayed onto the wound site and acts as a protective barrier during healing. This material also degrades and dissolves at a programmed rate. Other PEG-based hydrogels under development deliver encapsulated drugs as implants. Degradable linkages between hydrogels and incorporated drugs allow drugs to be slowly and specifically released in the body[67].

Novel applications of pegylation

These are just a few of the biomedical applications of pegylation either approved by the FDA or undergoing investigation. Although proteins and peptides have been the main targets for pegylation, other molecules, including small-molecule drugs, cofactors, oligonucleotides, lipids, saccharides and biomaterials, can be pegylated as well[18]. Other candidates include pegylated insulin with a lengthened circulation time and reduced immunogenicity[71]; pegylated antibody fragments for immunotherapy or tumor targeting[72]; and pegylated superoxide dismutase for the treatment of ischaemia/reperfusion injury or burns[73]. The benefits of pegylated catalase, uricase, honeybee venom, haemoglobin, pyrrolidone and dextran are also under investigation[9]. Other researchers are designing pegylated nanoparticles to cross the blood–brain barrier[74] (BOX 3), or using pegylated DNA-containing liposomes with tethered antibodies to provide targeted gene therapy[75].

Conclusion

Pegylation has taken 20 years to emerge as a viable pharmaceutical tool. During this time there have been important advances in the chemistry of pegylation, in the generation of biomolecule therapeutics and in understanding PEG–biomolecule conjugates. Pegylation is now established as the method of choice for improving the pharmacokinetics and pharmacodynamics of protein pharmaceuticals. Applications of PEG-based hydrogels and PEG-modified liposomes have become increasingly important. New frontiers for the technology are now emerging, for example, in small-molecule modification, and it is certain that pegylation will play an increasingly important role in pharmaceutical science and technology.

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