LEADING ARTICLE

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Development of Pegylated Interferons for the Treatment of Chronic Hepatitis C

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Abstract

The chemical attachment of poly(ethylene glycol) [PEG] to therapeutic proteins produces several benefits, including enhanced plasma half-life, lower toxicity, and increased drug stability and solubility. In certain instances, pegylation of a protein can increase its therapeutic efficacy by reducing the ability of the immune system to detect and mount an attack on the compound.

A PEG-protein conjugate is formed by first activating the PEG moiety so that it will react with, and couple to, the protein. PEG moieties vary considerably in molecular weight and conformation, with the early moieties (monofunctional PEGs; mPEGs) being linear with molecular weights of 12kD or less, and later moieties being of increased molecular weights. PEG2, a recent innovation in PEG technology, involves the coupling of a 30kD (or less) mPEG to lysine that is further reacted to form a branched structure that behaves like a linear mPEG of much larger molecular weight. These compounds are pH and temperature stable, and this factor along with the large molecular weight may account for the restricted volume of distribution seen with drugs utilising these reagents.

Three PEG-protein conjugates are currently approved for clinical use in the US, with more under clinical development. Pegademase is used in the treatment of severe combined immunodeficiency disease, pegaspargase for the treatment of various leukaemias, and pegylated interferon-α for chronic hepatitis C virus infections. As illustrated in the case of the 2 pegylated interferon-αs, all pegylated proteins are not equal. The choice of PEG reagent and coupling chemistry is critical to the properties of the PEG-protein conjugate, with the molecular weight of the moiety affecting its rate and route of clearance from the body, and coupling chemistry affecting the strength of the covalent attachment of PEG to therapeutic protein.

1. Introduction to Protein Pegylation: Properties of Poly(ethylene glycol) (PEG) Moieties

Pegylation was developed in the 1970s by Abuchowski and colleagues[1] to enhance the delivery of therapeutic proteins. The changes in pharmacokinetics and pharmacodynamics produced by pegylation have prompted research on the use of PEG-protein conjugates in several therapeutic areas, including oncology and infection.

Both protein and nonprotein molecules can be pegylated, although research efforts have focused primarily on PEG-protein conjugates. Pegylation, by increasing the molecular weight (MW) of the protein, typically reduces its excretion, permitting a decrease in dosage frequency. Thermal and pH stability is enhanced, helping to increase the termi-

nal half-life of the protein. By forming a 'shell' around the protein, pegylation can also decrease the immmunogenicity of the protein an protect it from proteolytic degredation. Drug toxicity is reduced, presumably as a result of the reduction in plasma concentration fluctuations induced by multiple daily administrateions.

The specific characteristics of poly(ethylene glycol) [PEG] moieties relevant to pharmaceutical applications are:

- water solubility
- high mobility in solution
- lack of toxicity
- lack of immunogenicity
- ready clearance from the body
- altered distribution in the body.[2,3]

Studies of PEG moieties in solution have shown that each ethylene oxide unit is tightly associated with 2 to 3 water molecules. This hydration, coupled with the rapid motion of the PEG molecule in aqueous medium, gives the PEG molecule the solubility properties of a molecule 5 to 10 times as large as a soluble protein of comparable MW. This size effect is readily revealed by several techniques such as size exclusion chromatography and gel electrophoresis.

The lack of toxicity of PEG has been established through many years of experience in the food, cosmetic and pharmaceutical industries.[4] The observed low immunogenicity of PEG is supported by several studies (reviewed by Harris[2,3]). A point of interest is the recent demonstration of the generation of anti-PEG antibodies under extreme conditions in rabbits.[5] This immune response, however, is not considered to be clinically significant, as there are no known examples of antibodies against PEG being generated from injection of therapeutic proteins into humans. The clearance of PEG moieties of differing MWs has been studied. Results show that the PEG moiety is cleared, without structural change, with clearance being proportional to the MW. Below a MW of about 20kD, the molecule is cleared via the kidney and excreted in the urine. Higher MW PEGs are cleared more slowly through the kidney, with liver clearance increasing as the PEG MW increases.[6]

Many properties of the native polymer are transferred to PEG-protein conjugates, which therefore exhibit a variety of desirable characteristics, some of which are mentioned above. All of these effects are dependent on the MW of the attached PEG moiety. The effect of PEG moiety attachment on solubility can be dramatic and is frequently observed with small molecule pharmaceuticals. The shielding ability of a covalently attached PEG moiety can lead to reduction or elimination of protein immunogenicity.[1,7] Unfortunately, insufficient knowledge of protein structure or the inability to selectively place the PEG moiety may hinder attempts to eliminate protein immunogenicity. The general approach is to attach the PEG moiety to some of the available lysines on the protein surface and hope that the desired result is achieved. Similarly, the shielding effect of the PEG moiety leads to reduced sensitivity to proteolysis for PEG proteins, and this effect is especially strong when high MW, branched PEG moieties are used.[8] Attachment of PEG moieties to a small protein will lead to a larger PEG-protein conjugate with a reduced rate of renal clearance. In sum, these properties enhance the circulation half-lives of proteins. PEG proteins also exhibit altered distribution in the body,[9] and when injected subcutaneously exhibit absorption into the bloodstream that is dependent on the MW of the PEG moieties. PEG proteins have enhanced stability that leads to a longer shelf life.

2. Chemistry for Pegylation

To couple a PEG moiety to a protein it is first necessary to activate the polymer by converting the hydroxyl terminus to some functional group capable of reacting with the functional groups found on the surface of proteins. The most common method has been to activate the PEG moiety with functional groups suitable for reaction with lysine and N-terminal amino groups. The PEG used is typically monofunctional PEG (mPEG) because this polymer resists cross-linking and aggregation. One early example of this approach is the reaction of mPEG tresylate with alkaline phosphatase [fig. 1].[10]

Several lysine residues are typically substituted

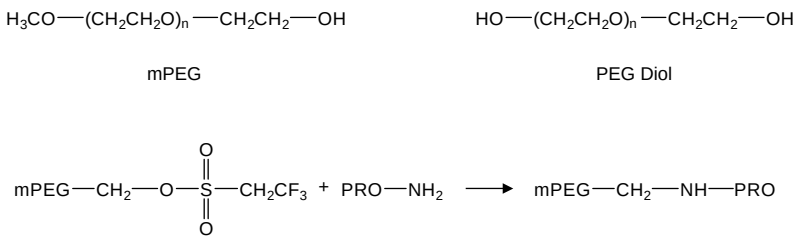


Fig. 1. Reaction of mPEG tresylate with alkaline phosphatase (represented as PRO-NH2).

during these reactions and each molecule of the protein can have a different pattern of lysine substitution. PEG moieties are polydisperse, which means that there is a distribution of MWs about the mean. The usual measure of polydispersity is the ratio of weight average MW to number average MW (Mw/Mn), with a larger ratio indicating a broader distribution. Compared with many polymers, PEG moieties have an unusually narrow polydispersity that is typically less than 1.05, and frequently close to 1.01; higher MWs typically have broader distributions.

The heterogeneity in lysine substitution and in PEG MWs is of some importance for PEG protein pharmaceuticals, and it is generally necessary to demonstrate that the pattern for a particular pharmaceutical can be measured and reproduced. It is possible in some instances, however, to reduce or eliminate heterogeneity in the position of substitution.

A major problem faced in PEG chemistry is that mPEG-OH is often contaminated with PEG diol (HO-PEG-OH). Some manufacturers produce low-diol mPEG, but some of this impurity is always present. Diol content can range as high as 10 to 15%.[11] Activation of the diol leads to a difunctional contaminant that is associated with cross-linking and aggregation. Diol arises from the presence of water contamination during the base-catalysed polymerisation of ethylene oxide to form mPEG, and given that there is a lower concentration of initiator (methoxide) in preparation of high MW mPEGs, water contamination and diol formation is a more serious problem in this case. Also, because the diol chain can grow at each end, the contaminating diol typically has a higher average MW than the desired mPEG.

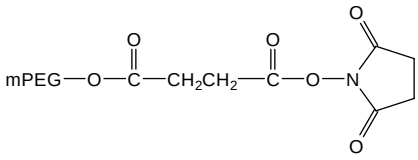
One characteristic of pegylation chemistry is that the diol and the resulting difunctional PEG are not removed. Consequently, one must be prepared to accept some protein cross-linking in the final product or make the effort to remove cross-linked product. An alternative is now available to purify the activated PEG reagent and remove the difunctional material. This route has the advantage of minimising the loss of expensive protein.

2.1 Monofunctional PEG (mPEG)

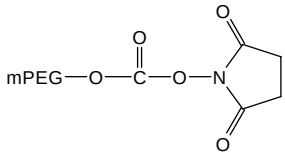
A wide range of PEG derivatives has been used for protein pegylation. Until recently these reagents were linear PEGs with MWs of 12kD or less. Examples of 2 PEG reagents are mPEG succinimidyl succinate (SS-PEG) and mPEG succinimidyl carbonate (SC-PEG) [fig. 2]. Although these reagents are widely used, including use in US Food and Drug Administration (FDA)-approved PEG-protein products, they are associated with several disadvantages including weak linkages between the PEG moiety and protein, potential unwanted side reactions, contamination, and restriction to low MW PEGs.

2.1.1 mPEG Succinimidyl Succinate

Activation of acids as succinimidyl esters has long been a popular chemistry, and SS-PEG has



SS-PEG



SC-PEG

Fig. 2. Examples of two PEG reagents, mPEG succinimidyl succinate (SS-PEG) and succinimidyl carbonate (SC-PEG).

been used for many years.[12,13] The polymer is synthesised by reacting mPEG with succinic anhydride, followed by activation of the carboxylic acid as the succinimidyl ester. Contained in the structure of this polymer is a backbone that includes an ester linkage. This linkage remains after the mPEG is coupled to the protein (via a strong amide linkage) and is highly susceptible to hydrolysis after the polymer has been attached to the protein (fig. 3).

2.1.2 mPEG Succinimidyl Carbonate

SC-PEG is another widely used activated mPEG.[14] An advantage of this reagent relative to SS-PEG is that it does not contain a degradable ester linkage that can lead to the hydrolytic removal of the PEG moiety from the PEG-protein conjugate. The linkage formed between SC-PEG and a lysine residue is a urethane linkage (fig. 4). Urethanes formed with lysine groups are hydrolytically stable. It has recently been observed, however, that SC-PEG couples at a low pH to the histidine moieties of interferon (IFN)-α to form a hydrolytically unstable linkage.[15] This weak linkage can be used as an advantage in the preparation of a controlled release formulation with a mechanism similar to a prodrug, or it can be a disadvantage if stability during purification or storage is a concern. Another problem with SC-PEG is that it can undergo a Lossen rearrangement (the reaction of hydroxamic acids with water-soluble carbodiimides) during coupling to a protein.[16] This rearrangement can leave a chemical tag on the protein, resulting in protein-protein dimers.

The other problems typical of PEG chemistry are also found with SC-PEG. In particular, there is no mechanism for removing difunctional SC-PEG formed from the PEG diol contaminant. Since diol contamination is more prevalent with high MW mPEG, SC-PEG chemistry is restricted from a practical standpoint to low MW mPEGs such as mPEG 5000 and 12 000. This is a serious limitation, as there is now a great deal of interest in using higher MW mPEGs where it is only necessary to attach a single PEG moiety to a protein to achieve the desired effect of pegylation.

2.1.3 mPEG Propionaldehyde

More advanced forms of pegylation chemistry have been designed to avoid the problems of diol contamination, restriction to low MW mPEG, unstable linkages and lack of selectivity in substitution. One example is mPEG-propionaldehyde, which is easier to prepare and use than PEG-acetaldehyde

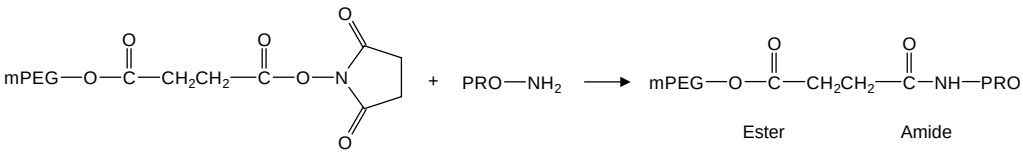


Fig. 3. Reaction of mPEG-succinimidyl succinate with a protein (represented as PRO-NH_2).

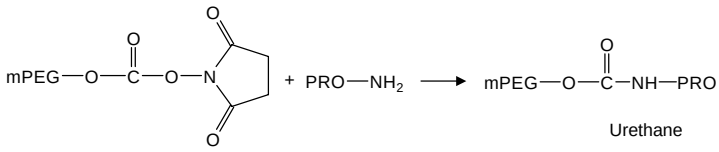


Fig. 4. The linkage formed between succinimidyl carbonate (SC) and a protein lysine is a urethane linkage.

because the acetaldehyde is very susceptible to dimerisation via aldol condensation. A key property of mPEG-propionaldehyde, as disclosed by Kinstler et al.[17] in work on pegylation of granulocyte colony-stimulating factor (G-CSF), is that the aldehyde is highly selective for the N-terminus of G-CSF. Although complete selectivity is not observed, the extensive heterogeneity normally seen with lysine chemistry is greatly reduced. Coupling of aldehyde proceeds through a Schiff base that is reduced *in situ* to give a stable secondary amine linkage (fig. 5).

Kinstler et al.[17] utilised mPEG 20 000 for coupling to G-CSF. Because of the size of G-CSF (18kD), this coupling resulted in an approximate doubling of the absolute MW of the molecule, and the heavy hydration and rapid motion of PEG resulted in an effective MW that is at least twice that of the native protein. It is expected that G-CSF with a single mPEG of 20kD would exhibit improved pharmacokinetic properties and enhanced efficacy relative to the native molecule. Clinical trials will ultimately determine the effects of pegylation on G-CSF. High MW PEG-propionaldehyde has also been used for pegylation of the tumour necrosis factor receptor for the treatment of rheumatoid arthritis.[18] In all these instances it was necessary to have a robust purification procedure to remove small amounts of contamination produced from PEG-diol-associated chemistry.

2.1.4 mPEG Vinylsulfone

Total selectivity in protein pegylation can be achieved by the introduction of a cysteine into the amino acid sequence of a protein by site-directed mutagenesis. In this way it is possible to position the mPEG in a location on the protein remote from the active or binding site. For example, mPEG vinylsulfone can be used to give selective conjugation with a cysteine group, with no lysine modification.[19] Another derivative that is useful for selective thiol pegylation is mPEG maleimide. Generally, this derivative is more reactive and easier to use than the mPEG vinylsulfone. Similarly, thiol pegylation can also be accomplished with orthopyridyldisulfide (OPSS) mPEG (OPSS-PEG). El Tayar et al.[20] recently prepared a highly active pegylated IFNβ using this derivative. In this case the available thiol was located in a sterically

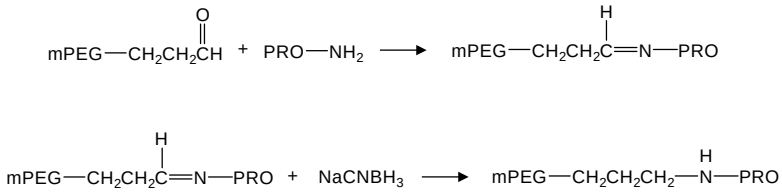


Fig. 5. Coupling of propionaldehyde proceeds through a Schiff base that is reduced *in situ* to give a stable secondary amine linkage in the resulting mPEG-protein conjugate.

crowded region and little modification could be achieved with mPEG maleimide or mPEG vinylsulfone of high MW. The approach ultimately adopted was to couple a low MW di-OPSS-PEG (MW 2000) to the IFN and then couple a high MW PEG thiol to the remaining, terminal OPSS group.

2.1.5 mPEG Carboxylic Acid

The final hurdle to overcome with pegylation chemistry is removal of the difunctional derivative formed from diol contamination. This can be accomplished by preparing a mPEG carboxylic acid and purifying this acid by ion exchange chromatography.[21] By using this route it is possible to remove the di-acid formed from the diol. Also, we have found that there is some improvement in the polydispersity of the mPEG carboxylic acids because low MW PEG acids bind more tightly to the ion-exchange column than do the high MW PEG acids.

The first mPEG carboxylic acid that was produced was the succinate discussed earlier (see section 2.1.1). The ester linkage present in this molecule, however, leads to hydrolysis and degradation in an ion exchange column. Carboxymethylated PEG has been known for some time. Unfortunately, the succinimidyl ester of this compound is so reactive that it is difficult to use. To correct these problems, Harris and Kozlowski[21] prepared mPEG propionic acid, and then converted this compound into the succinimidyl active ester (SPA). This SPA-PEG compound has ideal reactivity and can be prepared with a diacid content of less than 1% for MW 20 000 PEG. Note that the starting mPEG for this product had about 5% of diol, but ion exchange chromatography of the intermediate acid reduced the difunctional material to less than 1%. SPA-PEG has been used for pegylation of human growth hormone receptor antagonist for the treatment of acromegaly. Clinical trials are complete and the PEG-protein conjugate is under regulatory review for marketing approval.[22-24]

2.2 PEG2

Another approach to preparing a pure, high MW, monofunctional PEG moiety is to couple crude mPEG-benzotriazole carbonate (BTC), of a MW up to 30kD, to lysine. This yields a high MW PEG acid with no hydrolytically degradable linkages. The crude PEG2 acid is contaminated with unreacted mPEG-BTC, 'PEG1', in which only a single mPEG is coupled to lysine, and the 'PEG3' that is formed from the reaction of activated PEG diol (or BTC-PEG-BTC) with 2 lysines followed by the coupling of 2 mPEG-BTC molecules to the remaining 2 amino groups (thus PEG3 is a diacid). During aqueous work-up, the unreacted mPEG-BTC is converted back to mPEG-OH, greatly simplifying subsequent ion exchange chromatography. Thus the reaction mixture contains a diacid, a zwitterion, a neutral mPEG and the desired monoacid. Careful ion exchange chromatography permits large-scale production of the desired pure PEG2-CO_2H. Activation of the succinimidyl ester (PEG2-NHS) is straightforward (fig. 6).

The PEG2 compound turns out to be a very exciting protein pegylation reagent that can be prepared in monofunctional form up to MW 60kD. Experiments have shown that PEG2, attached to a protein, 'acts' much larger than a corresponding linear mPEG of the same MW. This is due to the branching that occurs with the PEG2 moiety. Branched chain PEG-protein conjugates have increased pH and thermal stability compared with linear PEG conjugates.[8] Furthermore, the branched

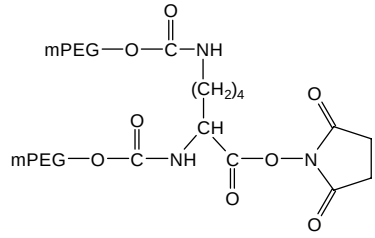


Fig. 6. PEG2-NHS is a pure, high molecular weight (MW), monofunctional PEG moiety created by coupling mPEG-benzotriazole carbonate of MW up to 30 kD to lysine.

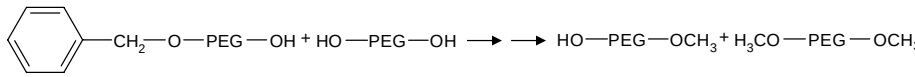


Fig. 7. Benzyloxy-PEG, containing diol impurity, is methylated and then hydrogenated to remove the benzyl group. Thus, the diol is converted to the inert dimethyl ether.

structure of the PEG moiety may contribute to the restricted volume of distribution observed with PEG molecules. The availability of the very large PEG2-NHS means that only a single point of attachment is required to achieve the desired pegylation effect.[25] In addition, the pure PEG2-NHS can be converted into a range of other pure, monofunctional PEG derivatives including aldehyde and maleimide compounds that previously were always contaminated with difunctional material.

Another approach to the solution of the diol problem has recently been demonstrated by Bentley et al.[26] In this work, a crude PEG benzyloxy, containing diol impurity, was methylated and then hydrogenated to remove the benzyl group. Thus, the diol was converted to the inert dimethyl ether, which could be removed after activation and protein attachment (fig. 7).

3. Pharmaceutical PEGs

3.1 Pegademase

In light of the properties described above, several PEG-protein conjugates are been approved for clinical use by the US FDA or are currently under clinical development. Pegademase was approved in 1990 for use in severe combined immunodeficiency disease (SCID), a disease associated with an inherited deficiency of adenosine deaminase (ADA).[27] Before the availability of pegademase, patients with SCID were treated with partial exchange transfusions of red blood cells that contain ADA. These transfusions placed patients at risk for iron overload and transfusion-related viral infections.[28,29] Pegademase has approximately 1800-fold more ADA activity per millilitre than red blood cells; therefore, the drug produces higher concentrations of ADA activity than partial exchange transfusion.[30]

3.2 Pegaspargase

Pegaspargase is available for the treatment of acute lymphocytic leukaemia, acute lymphoblastic leukaemia and chronic myelogenous leukaemia. The native asparaginase compound is associated with a high incidence of allergic reactions and the development of neutralising antibodies that shorten its half-life, making it difficult to maintain effective plasma concentrations.[31] Pegaspargase reduces the tendency of the enzyme to induce an immune response, thus allowing patients with hypersensitivity to the native enzyme to tolerate pegaspargase without further incident.[32]

3.3 Other Therapeutic PEG Conjugates in Development

There are several important PEG proteins in advanced stage clinical trials, and it is expected that some of these proteins will receive approval within the next year. Included in this list of probable approvals are PEG-growth hormone receptor antagonist for treatment of acromegaly,[33] free radical scavengers, blood derivatives, antineoplastic agents, cardiovascular agents and antigens.[28]

Further evidence of the suitability of PEG-protein conjugates for human therapeutics is given by the FDA approval of 2 other PEG-containing products: stealth PEG-liposomes for delivery of doxorubicin for the treatment of Kaposi's sarcoma

(pegylated liposomal doxorubicin; Doxil®[1]) and PEG-lactide-glycolide-acrylate (FocalSeal®) used as a sealant in lung surgery.

3.4 Pegylated Interferon-α

Chronic hepatitis C virus (HCV) infection is a rapidly emerging disease that is the leading cause of liver cirrhosis and hepatocellular carcinoma.[34] The protein IFNα has been used extensively to treat HCV; however, monotherapy with IFN is associated with a sustained response in only a small percentage of patients.[34,35] This poor response is probably the result of multiple host (e.g. viral load) and viral factors (e.g. HCV genotype) as well as the inadequate pharmacokinetics of IFN. In particular, serum concentrations of IFN fall below the limit of detection within 24 hours of a single subcutaneous dose. Thus, even though the current treatment regimen calls for subcutaneous injection of IFN 3 times weekly, the virus has extended periods during which it is not exposed to a sustained therapeutic concentration of the drug. The addition of ribavirin to IFN in the treatment of HCV has increased the number of patients achieving a sustained virological response (38 to 43%).[35,36] This increase in response, however, is accompanied by a higher incidence of adverse events, some of which lead to discontinuation of therapy.

Pegylation of IFN improves the pharmacokinetics and, as a result, the pharmacodynamics of the drug, most likely leading to an increase in response. In 1999, clinical trials were conducted to evaluate the efficacy of IFNα-2a modified by attachment of mPEG of MW 5kD in patients with chronic HCV. Once weekly administration of PEG(5kD) IFNα-2a at doses of 15, 45, 90 and 135μg did not show improved efficacy over unmodified IFNα-2a 3 million international units (MIU) 3 times weekly.[37] More recently, clinical trials have evaluated a pegylated IFNα-2a prepared by coupling a branched PEG of MW 40kD to IFNα-2a.[38] The results of these trials are highly encouraging.[39,40] Additionally, a linear pegylated IFNα-2b of 12kD is currently approved in the European Union and the US for the treatment of HCV.

Further developement of pegylated IFNα-2a has involved examination of various pegylation chemistries. A detailed report of these studies will be published elsewhere.[41] After investigation, the moiety chosen for coupling with IFNα-2a was PEG2-NHS 40 000. Since the protein has a MW of about 19kD, the conjugate is a much larger molecule. Preclinical studies and modelling indicated that this conjugate, PEG(40kD) IFNα-2a would have desirable pharmacokinetics and activity. In particular, it was predicted that the steady-state blood concentration would be essentially constant (fig. 8). In practice, the desired result was achieved, and nearly constant blood concentrations are attained with once weekly subcutaneous injections.[42] Pegylation with PEG2-NHS 40 000 reduces renal clearance 100-fold relative to native IFNα-2a, and gives an elimination half-life of 77 *vs* 9 hours. PEG(40kD) IFNα-2a exhibits sustained absorption and a restricted volume of distribution when administered subcutaneously. This allows for a more convenient administration schedule (once weekly *vs* 3 times weekly for unmodified IFNα-2a).

Clinical results are now available for PEG (40kD) IFNα-2a. An open-label, parallel dose, global phase III trial randomised 531 patients to receive either PEG(40kD) IFNα-2a 180μg once weekly for 48 weeks (n = 267) or unmodified IFNα-2a 6 MIU 3 times weekly for 12 weeks followed by 3 MIU 3 times weekly for 36 weeks (n = 264). Results showed that PEG(40kD) IFNα-2a is associated with a greater virological response at week 48 (69 *vs* 28%, p = 0.001) and sustained virological response at week 72 (39 *vs* 19%, p = 0.001) compared with unmodified IFNα-2a.[40] Additionally, an open-label randomised, parallel dose study was performed in 271 patients with chronic HCV and biopsy-proven cirrhosis or bridging fibrosis. Patients were randomised to receive 48 weeks of treatment with IFNα-2a 3 MIU 3 times weekly

1 Use of tradenames is for product identification only and does not imply endorsement.

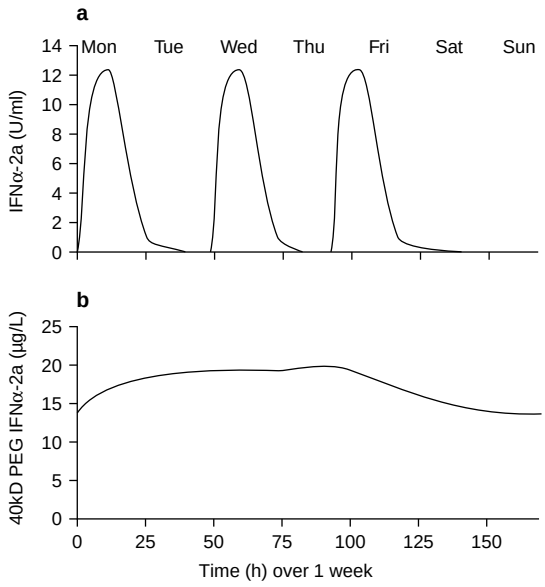


Fig. 8. Pharmacokinetic profiles for interferon (IFN)-α-2a and PEG(40kD)-IFNα-2a.

(n = 88) or PEG(40kD) IFNα-2a 90μg (n = 96) or 180μg (n = 87) once weekly followed by a 24-week treatment-free period. 44% of patients who were treated with 180μg PEG(40kD) IFNα-2a had a virological response at the end of 48 weeks' treatment (p = 0.001 *vs* IFNα-2a), and 30% achieved a sustained virological response at 72 weeks (p = 0.001 *vs* IFNα-2a). Among the 88 patients who received IFNα-2a 3 MIU 3 times weekly, only 14% achieved a virological response at 48 weeks and 8% had a sustained virological response at 72 weeks.[39]

It is well known that combination therapy with IFN and the antiviral agent ribavirin leads to enhanced efficacy compared with IFN alone.[34-36] Thus, it is of interest to combine PEG(40kD) IFNα-2a with ribavirin in the hope that improved results will be seen. Clinical trials to address this point are currently under way and the results of a small pilot study on PEG(40kD) IFNα-2a plus ribavirin in 20 patients have been announced.[43] Sustained virological and biochemical responses were observed in 9 of 20 patients. Five of 16 patients infected with HCV genotype 1 achieved a sustained virological response, as well as all 4 patients infected with other HCV genotypes.

A pegylated form of IFNα-2b has also been approved by the FDA.[44,45] Pegylation technology for preparation of a linear 12kD form of pegylated IFNα-2b with mPEG-SC 12 000 has been patented (PEG interferon-α-2b).[15] A special feature of this work, as noted above, is that pegylation at low pH (around pH 5) leads to extensive coupling to histidine via a hydrolytically unstable linkage.

Results from a phase III clinical trial comparing pegylated IFNα-2b once weekly with unmodified IFNα-2b 3 times weekly[45] showed that at the optimal dose (1.0 μg/kg), pegylated IFNα-2b gave sustained HCV clearance in 25% of 297 patients, whereas 12% of 303 patients who received native IFNα-2b showed sustained viral clearance ($p < 0.001$).

Results were recently reported for a phase III trial involving 1530 patients who were randomised to receive either pegylated IFNα-2b 1.5 μg/kg once weekly plus ribavirin 800 mg/day for 48 weeks (PEGα-2b 1.5/R); pegylated IFNα-2b 1.5 μg/kg once weekly plus ribavirin 1000 to 1200 mg/day for 4 weeks followed by pegylated IFNα-2b 0.5 μg/kg once weekly plus ribavirin 1000 to 1200 mg/day for 44 weeks (PEGα-2b 0.5/R); or IFNα-2b 3 MIU 3 times weekly plus ribavirin 1000 to 1200 mg/day for 48 weeks. A 54% sustained virological response was found overall in the pegylated IFNα-2b 1.5/R group compared with 47% in the IFNα-2b/ribavirin group (p = 0.01 *vs* interferon-α-2b + ribavirin), whereas 42% (p = 0.02 *vs* IFNα-2b/ribavirin) and 82% of patients in the PEGα-2b 1.5/R group with HCV genotype 1 and non-1, respectively, achieved a sustained virological response.[46] It is important to note, however, the difference between the highest response rate with pegylated IFNα-2b and IFNα-2b/ribavirin was only 7%.

Based on available data, it is obvious that both pegylated IFNα-2b and PEG(40kD) IFNα-2a have

superior efficacy when compared with unmodified IFNα-2b and IFNα-2a, respectively. The higher MW, branched pegylated IFNα-2a may have a better pharmacokinetic and pharmacodynamic profile than the lower MW pegylated IFNα-2b, offering a true once weekly administration regimen with the ability to maintain adequate serum IFN concentrations and possibly achieving improved sustained responses even in more difficult-to-treat patients. Confirmation of this supposition will require a head-to-head clinical trial between PEG(40kD) IFNα-2a and pegylated IFNα-2b. Clearly, pegylation of therapeutic proteins in general is valuable, and pegylation of IFN promises to increase treatment success in a growing population of patients diagnosed with HCV infection.

Acknowledgements

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References

1. Abuchowski A, van Es T, Palczuk NC, et al. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J Biol Chem 1977; 252: 3578-81
2. Harris JM, Zalipsky S, editors. Poly(ethylene glycol): chemistry and biological applications. Washington, DC: American Chemical Society, 1997
3. Harris JM. Polyethylene glycol chemistry. Biotechnical and biomedical applications. New York: Plenum, 1992
4. Working PK, Newman MS, Johnson J, et al. Safety of poly(ethylene glycol) and poly(ethylene glycol) derivatives. In: Harris JM, Zalipsky S, editors. Poly(ethylene glycol): chemistry and biological applications. Washington, DC: American Chemical Society, 1997: 45-59
5. Cheng TL, Wu PY, Wu MF, et al. Accelerated clearance of polyethylene glycol-modified proteins by anti-polyethylene glycol IgM. Bioconjug Chem 1999; 10: 520-8
6. Yamaoka T, Tabata Y, Ikada Y. Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice. J Pharm Sci 1994; 83: 601-6
7. Katre NV. Immunogenicity of recombinant IL-2 modified by covalent attachment of polyethylene glycol. J Immunol 1990; 144: 209-13
8. Monfardini C, Schiavon O, Caliceti P, et al. A branched monomethoxypoly(ethylene glycol) for protein modification. Bioconjug Chem 1995; 6: 62-9
9. Caliceti P, Schiavon O, Veronese FM. Biopharmaceutical properties of uricase conjugated to neutral and amphiphilic polymers. Bioconjug Chem 1999; 10: 638-46
10. Yoshinaga Y, Harris JM. Effects of coupling chemistry on activity of a polyethylene glycol-modified enzyme. J Bioact Comp Polym 1989; 4: 17-24
11. Dust JM, Fang ZH, Harris JM. Proton NMR characterization of polyethylene glycols and derivatives. Macromolecules 1990; 23: 3742-6
12. Abuchowski A, Kazo GM, Verhoest CR, et al. Cancer therapy with chemically modified enzymes. I. Antitumor properties of polyethylene glycol-asparaginase conjugates. Cancer Biochem Biophys 1984; 7: 175-86
13. Harris JM. Synthesis of polyethylene glycol derivatives. J Macromol Sci Rev Macromol Chem Phys 1985; C25: 325-73
14. Zalipsky S, Seltzer R, Menon-Rudolph S. Evaluation of a new reagent for covalent attachment of polyethylene glycol to proteins. Biotechnol Appl Biochem 1992; 15: 100-14
15. Gilbert CW, Park-Cho M. Interferon polymer conjugates. US Patent 5,951,974, Sep. 14, 1999
16. Zalipsky S. Alkyl succinimidyl carbonates undergo Lossen rearrangement in basic buffers. Chem Commun 1998; 69-70
17. Kinstler OB, Brems DN, Lauren SL, et al. Characterization and stability of N-terminally PEGylated rhG-CSF. Pharm Res 1996; 13 (7): 996-1002
18. Edwards CK. Pegylated recombinant human soluble tumor necrosis factor receptor type I (rHu-sTNF-RI): novel high-affinity TNF receptor designed for chronic inflammatory diseases. Ann Rheum Dis 1999; 58 Suppl. 1: 173-81
19. Morpurgo M, Veronese FM, Kachensky D, et al. Preparation and characterization of poly(ethylene glycol) vinylsulfone. Bioconjug Chem 1996; 7: 363-8
20. El Tayar N, Roberts MJ, Harris JM, et al. Polyol-IFN-beta conjugates, WO 99/55377
21. Harris JM, Kozlowski A. Polyethylene glycol and related polymers monosubstituted with propionic or butanoic acids and functional derivatives thereof for biotechnical applications. US Patent 5,672,662, Sep 30; 1997
22. Thorner MO, Strasburger CJ, Wu Z, et al. Growth hormone (GH) receptor blockade with a PEG-modified GH (B2036-PEG) lowers serum insulin-like growth factor-I but does not acutely stimulate serum GH. J Clin Endocrinol Metab 1999 Jun; 84 (6): 2098-103
23. Goffin V, Bernichtein S, Carriere O, et al. The human growth hormone antagonist B2036 does not interact with the prolactin receptor. Endocrinology 1999 Aug; 140 (8): 3853-6
24. Ross RJ, Leung KC, Maamra M, et al. Binding and functional studies with the growth hormone receptor antagonist, B2036-PEG (pegvisomant), reveal effects of pegylation and evidence that it binds to a receptor dimer. J Clin Endocrinol Metab 2001 Apr; 86 (4): 1716-23
25. Chapman, AP, Antoniw P, Spitali M, et al. Therapeutic antibody fragments with prolonged in vivo half-lives. Nat Biotechnol 1999; 17: 780-3
26. Bentley MD, Harris JM, Kozlowski A. Heterobifunctional poly(ethylene glycol) derivatives and methods for their preparation. PCT US99/23536: Oct 8; 1999
27. Hershfield MS. Biochemistry and immunology of poly(ethylene glycol)-modified adenosine deaminase (PEG-ADA). In: Harris JM, Zalipsky S, editors. Poly(ethylene glycol): chemistry and biological applications. Washington, DC: American Chemical Society, 1997: 145-54
28. Burnham NL. Polymers for delivering peptides and proteins. Am J Hosp Pharm 1994; 51: 210-8
29. Hillman BC, Sorensen RU. Management options: SCIDS with adenosine deaminase deficiency. Ann Allergy 1994; 72: 395-404

30. Hershfield MS. PEG-ADA replacement therapy for adenosine deaminase deficiency: an update after 8.5 years. Clin Immunol Immunopathol 1995; 76: S228-32
31. Keating MJ, Holmes R, Lerner S, et al. L-asparaginase and PEG asparaginase – past, present, and future. Leuk Lymphoma 1993; 10 Suppl.: 153-7
32. Holle LM. Pegaspargase: an alternative. Ann Pharmacother 1997; 3: 616-24
33. Olson K, Gehant R, Mukku V, et al. Preparation and characterization of poly(ethylene glycol)ylated human growth hormone antagonist. In: Harris JM, Zalipsky S, editors. Poly (ethylene glycol): chemistry and biological applications. Washington, DC: American Chemical Society, 1997: 170-81
34. Liang JT, Rehermann B, Seeff LB, et al. Pathogenesis, natural history, treatment and prevention of hepatitis C. Ann Intern Med 2000; 132: 296-305
35. McHutchison JG, Gordon SC, Schiff ER, et al, and the Hepatitis Interventional Therapy Group. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med 1998: 339; 1485-92
36. Poynard T, Marcellin P, Lee S, et al. Randomised trial of interferon alfa-2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alfa-2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998; 352: 1426-32
37. O'Brien C, Pockros P, Reddy R, et al. A double-blind, multicenter, randomized, parallel dose-comparison study of six regimens of 5kD, linear peginterferon alfa-2a compared with Roferon-A in patients with chronic hepatitis C. Antiviral Therapy 1999; 4 Suppl. 4: 15
38. Bailon P, Spence C, Schaffer CA, et al. Pharmacological properties of five polyethylene glycol conjugates of interferon alfa-2a. Antiviral Therapy 1999; 4 Suppl. 4: 27
39. Heathcote EJ, Shiffman ML, Cooksley GE, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000; 343: 1673-80
40. Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000; 343: 1666-72
41. Bailon P, Palleroni A, Schaffer CA, et al. Rational design of a potent, long-lasting form of interferon: a 40 kDa branched polyethylene glycol-conjugated interferon alpha-2a for the treatment of hepatitis C. Bioconjug Chem 2001 Mar-Apr; 12 (2): 195-202
42. Algranati NE, Sy S, Modi MW. A branched methoxy 40 kDa polyethylene glycol (PEG) moiety optimizes the pharmacokinetics (PK) of peginterferon α-2a (PEG-IFN) and may explain its enhanced efficacy in chronic Hepatitis C (CHC). Hepatology 1999; 30 (4 Pt 2): 190A
43. Sulkowski MS, Reindollar R, Yu J. Pegylated interferon alfa-2a (PEGASYS™) and ribavirin combination therapy for chronic hepatitis C: a phase II open-label study. Gastroenterology 2000; 118 Suppl. 2: 950
44. Glue P, Fang JW, Rouzier-Panis R, et al. Pegylated interferon-alpha2b; pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. Clin Pharmacol Ther 2000 Nov; 68 (5): 556-67
45. Trepo C, Lindsay K, Niederau C, et al. Pegylated interferon alfa-2b (PEG-Intron) monotherapy is superior to interferon alfa-2b (Intron A) for the treatment of chronic hepatitis C [abstract GS2/07]. J Hepatology 2000; 32: 29
46. Manns MP, McHutchison JG, Gordon S, et al. Peginterferon alfa-2b plus ribavirin compared to interferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C: 24 week treatment analysis of a multicenter, multinational phase III randomized controlled trial. Hepatology 2000; 32 (4): 297A

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