

# Nanomedicines in the treatment of chronic hepatitis C – focus on pegylated interferon alpha-2a

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**Abstract:** Nanotechnology is the application of nanotechnology within medicine. An illustration of this is the use of pegylation as a means of modifying naturally occurring proteins which may have clinical applications, in order to improve the pharmacodynamics of the protein resulting in an effective medication. An example of this is pegylated interferon. The purpose of this review is to examine the chemistry, clinical pharmacology, pharmacokinetics, pharmacodynamics, and clinical studies with 40 kDa pegylated interferon to illustrate the general principles of pegylated biological proteins. The use in clinical practice is reviewed along with the evidence for both efficacy, safety, and advantages over standard interferon.

**Keywords:** nanomedicines, nanotechnology, pegylation, pegylated interferon, 40 kDa pegylated interferon alpha-2a, hepatitis C

## Introduction

Nanostructures measure between 1 and 100 nm and because of their size confer novel properties. These have applications in disease diagnosis, treatment, and prevention. Nanomedicine is defined as the application of nanotechnology within medicine, that is, the monitoring, repair, construction, and control of human biological systems at the molecular level using nanodevices and nanostructures. Nanoparticles and nanocapsules may be effective delivery systems for drug and gene therapy, and can alter the distribution of drugs and other therapeutic agents within the patient's body (Bogunia-Kubik and Sugisaka 2002; Editorial 2004). Until now nanotechnology has been used to modify existing drugs to improve their pharmacokinetic properties.

## Pegylated modification of interferon alpha

Once administered, protein drugs such as interferon alpha are susceptible to enzyme degradation and rapid clearance. In order to be effective they need to be administered frequently to provide consistent serum concentrations within the therapeutic range. This frequent dosing is both inconvenient for patients and can lead to wide fluctuations in blood levels. This may reduce efficacy and potentially lead to an increase in adverse reactions caused by intermittent high peak concentrations (Reddy et al 2002).

Attaching a large inert molecule to a protein is a well-established method for decreasing the protein's clearance. Since the late 1970s, the molecule of choice for this process has been polyethylene glycol (PEG). When interferon, a biologically active antiviral agent is combined with PEG ("pegylated"), its half-life is increased and the rate of drug clearance is decreased. Biological activity is maintained (Reddy et al 2002).

The purpose of this review is to examine pegylation as an example of a clinical application of nanotechnology and nanomedicine. Here we look at the chemistry, clinical pharmacology, pharmacodynamics and clinical studies with the 40 kDa

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pegylated interferon (peginterferon) alpha-2a, to illustrate the general principles of pegylated biological proteins. Other long-acting type 1 interferons have been created and one other peginterferon (12 kDa pegylated alpha-2b) is currently licensed for clinical use. It is not the purpose of this review to examine the similarities and differences between these two drugs and we will therefore restrict our discussion to the 40 kDa peginterferon alpha-2a.

## Chemistry and potential advantages of pegylation

The molecular weight of the PEG moiety, its structure, the number and location of peg moieties attached to the protein, and the chemical method of attachment determine the physicochemical and pharmacological properties of the resulting protein conjugate. For a circulating pegylated protein, the optimum mass of a PEG molecule required to prolong renal and cellular clearance of molecules is estimated to be 40–60 kDa for once-weekly dosing (Yamaoka et al 1994; Fung et al 1997; Heathcote et al 1999). Smaller pegylated proteins are filtered freely in the glomerulus (Delgado et al 1992; Yamaoka et al 1994), and therefore extend protein half-life to a lesser extent. However, the optimum size of the PEG molecule needed to extend a protein half-life cannot be derived from mathematical models and needs to be determined empirically. This is because the overall half-life of a pegylated protein is determined by a complex variety of factors including absorption from injection sites and tissue distribution.

PEG chains may be attached at a single or multiple sites on the protein molecule. It is preferable for there to be a single attachment site as excessive pegylation will decrease the activity of the drug (Fung et al 1997; Olsen et al 1997). Excessive pegylation will also produce a protein with many positional isomers that may have different biological properties. There are various methods available to join the mono-functional PEG chains (ie, those with a single attachment site) to proteins. Most involve coupling the PEG molecule either to amines located on the N-terminus or to lysine residues on the protein. These form stable covalent bonds. Alternatively, PEG derivatives can be synthesized that are reactive towards other nucleophiles on the protein such as sulfhydryl groups. The PEG molecule can be stably conjugated to the protein or coupled with a hydrolyzable bond that allows slow release of the active interferon moiety (Reddy et al 2002).

In the development of the 40 kDa peginterferon alpha-2a, studies in rats initially tested a wide range of PEG moieties for attachment to interferon alpha-2a (Fung 1997). These

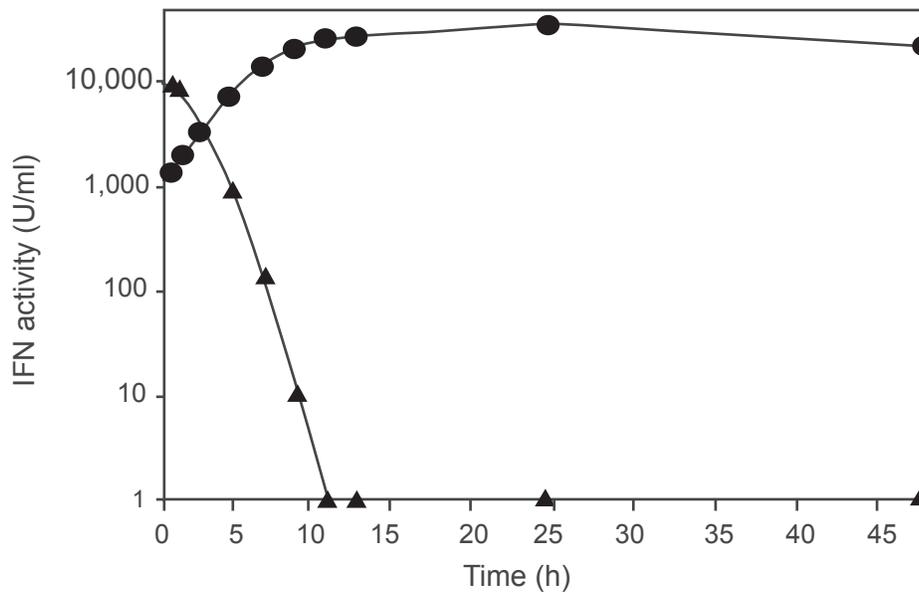
studies showed that conjugates of interferon alpha-2a with PEGs of various molecular weights and structures improved the absorption, distribution, and elimination characteristics of the protein (Bailon et al 1999, 2001). Different PEG conjugates exhibited marked differences in pharmacokinetic properties. Peginterferon alpha-2a (40 kDa) conferred enhanced pharmacological properties of standard interferon alpha-2a in terms of antiviral activity, terminal half-life, and mean plasma residence time. Administration of 40 kDa peginterferon alpha-2a produced sustained plasma concentrations compared with standard interferon alpha-2a (Bailon et al 2001). An additional benefit of the pegylated molecule was a reduction in immunogenicity (Figure 1). That is, standard interferon alpha-2a elicited immunogenic responses in mice whereas pegylated molecules were less immunogenic. Smaller PEG interferons (such as 5 kDa linear peginterferon alpha-2a) have reduced immunogenicity and 40 kDa peginterferon alpha-2a has been shown not to elicit an antibody response (Bailon et al 2001).

## Kinetics of 40 kDa peginterferon alpha-2a

Two studies have examined the pharmacokinetic properties of single dose sub-cutaneous 40 kDa peginterferon alpha-2a in healthy volunteers.

In a study by Xu and colleagues, subjects were administered either interferon alpha-2a (3 million international units, MIU) or 40 kDa peginterferon alpha-2a (45 µg, 135 µg, or 270 µg), followed by administration of interferon alpha-2a (18 MIU) (Xu et al 1998). The pharmacokinetics of peginterferon alpha-2a (40 kDa) were linear in this dose range. In comparison with standard interferon alpha-2a, the pegylated form had sustained absorption, reduced systemic clearance, and increased serum half-life.

Similar pharmacokinetic results were reported by Algranati and colleagues, who assessed subcutaneous administration of 40 kDa peginterferon alpha-2a (180 µg) in 20 healthy volunteers. Substantial serum levels of 40 kDa peginterferon alpha-2a were reached 3–8 hours after dosing and the maximal serum concentration of the drug was reached about 80 hours post injection, reflecting the sustained absorption of the drug. The mean absorption time was markedly sustained for 40 kDa peginterferon alpha-2a compared with published data for interferon, indicating that sustained concentrations of peginterferon would be present throughout a 1-week dosing interval. This was assuming a first-order absorption process (Algranati et al 1999).



**Figure 1** Mean serum activity of standard IFN alpha-2a (▲) and 40 kDa peginterferon alpha-2a (●) after subcutaneous administration in rats. Reprinted with permission from Bailon P, Palleroni A, Schaffer CA, et al. 2001. 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. *Bioconj Chem*, 12:195–202. Copyright © 2001 American Chemical Society.

During multiple dosing in chronic hepatitis C patients treated once weekly over 48 weeks, 40 kDa peginterferon alpha-2a had sustained absorption and reduced apparent total body clearance (Modi et al 2000). During steady state concentrations, reached after 5–8 weeks of treatment, the peak to trough ratio of 40 kDa peginterferon alpha-2a was constant, indicating a uniform concentration throughout the dosing interval (Modi et al 2000). The pharmacokinetic properties are similar both in healthy volunteers and in patients with and without cirrhosis (Heathcote et al 1999).

In summary, clinical studies in both healthy volunteers and patients with a variety of different stages of liver disease have shown similar results to the initial animal studies and indicate that pegylated interferons have enhanced pharmacokinetic properties.

### Antiviral activity

The value of enhanced pharmacokinetic properties needs to be shown in clinical studies of efficacy. In patients receiving treatment for chronic hepatitis C infection (HCV), the response to therapy is usually analyzed by determining the proportion of patients who have no detectable virus in their circulation 6 months after completing therapy. This is known as sustained virological response (SVR). Long term follow up studies have shown that patients who achieve an SVR have an extremely low probability of relapse and are probably cured. It is now

well established that different strains of HCV (known as genotypes) respond differently to therapy – patients infected with genotype type 1 HCV have much reduced response rates compared with patients infected with genotypes 2 and 3. It is now recognized that combining ribavirin with interferon-based treatment regimes significantly increases the number of patients who achieve a sustained virological response. The results from various clinical trials demonstrate that treatment with 40 kDa peginterferon alpha-2a provides significant improvement over standard interferon alpha-2a therapy.

### Efficacy alone and in combination with ribavirin

It has been shown that combination therapy in the form of interferon and ribavirin markedly improves therapy results compared with monotherapy with interferon alone (Friedrich-Rust et al 2005). The development of peginterferon has led to a further improvement of virological response rates especially for genotype 1-infected patients. Results from both phase 2 and phase 3 trials have shown once weekly administration of 40 kDa peginterferon alpha-2a to be more effective than three times a week administration of standard interferon alpha-2a (Zeuzem et al 2000; Reddy et al 2001).

This result has been shown to be the case in all genotype cohorts of hepatitis C patients. Patients with genotype non-1 tend to respond better than genotype 1. But a marked benefit has

been shown in genotype 1 treated with pegylated interferon. Genotype 4 usually exhibits a poorer response to treatment than other genotypes, in trials using pegylated interferon the response rates have been better (Sherman et al 2000).

In the case of patients with bridging fibrosis and cirrhosis, which are notoriously difficult to treat, pegylated interferon has shown better outcomes in terms of virological, biochemical, and histological responses. Meta-analysis of all peginterferon alpha-2a monotherapy trials confirms that the response rates to 40 kDa peginterferon alpha-2a are much higher than to standard interferon alpha-2a both in patients with difficult-to-treat disease because of genotype or with complications such as cirrhosis (Pockros et al 2000).

In combination with ribavirin, peginterferon alpha-2a has been shown to be superior to peginterferon alone and standard interferon alpha-2b plus ribavirin, in interferon-naive patients (Fried et al 2001). In a large randomized control trial involving two thirds patients with genotype 1 infection, patients treated with both 40 kDa peginterferon alpha-2a and ribavirin had better virological responses than other treatment regimes (Fried et al 2001).

## Tolerability

Several studies have compared peginterferon with standard interferon in patients with hepatitis C, both with and without complications such as fibrosis and cirrhosis. In these studies peginterferon alpha-2a has been shown to be tolerated as well as if not better than standard interferon alpha-2a (Heathcote et al 2000; Zeuzem et al 2000; Reddy et al 2001) The frequency of adverse events are also reported as similar with peginterferon and standard therapy interferon alpha-2a.

Side-effects reported with peginterferon are typical of those reported for interferon therapy and include: depression, pyrexia, rigors, nausea, vomiting, impaired concentration, and alopecia (Zeuzem et al 2000; Reddy et al 2001). Psychiatric events such as severe depression, psychosis, and personality disorder have been reported as similar in patients taking standard interferon and peginterferon (Heathcote et al 2000; Zeuzem et al 2000) In the general chronic hepatitis C population similar findings have been reported.

Another well-established group of adverse effects of interferon treatment are hematological abnormalities, particularly in those patients with cirrhosis. In studies comparing standard interferon with peginterferon, the incidence of neutropenia is reported as similar (Heathcote et al 2000; Zeuzem et al 2000) Thrombocytopenia, although uncommon, has been reported as higher in one study in those patients with

cirrhosis treated with 40 kDa peginterferon alpha-2a rather than those treated with standard interferon alpha-2a. In the same study the patients with chronic hepatitis C and not cirrhosis had similar rates of thrombocytopenia (Heathcote et al 2000). Anemia has been reported with both therapies and in one study one patients the discontinuation of therapy with peginterferon due to anemia (Zeuzem et al 2000).

Using quality of life questionnaires, results have been better in patients treated with 40 kDa peginterferon alpha-2a compared with standard interferon alpha-2a. An analysis of patients participating in a randomized control trial revealed that those receiving 40 kDa peginterferon alpha-2a experienced significantly better quality of life and less fatigue than those treated with standard interferon alpha-2a as early as 2 weeks into therapy with the differences persisting at 12 weeks (Rasenack et al 2000). At this time significantly more patients treated with peginterferon alpha-2a rated themselves as feeling "better or much better" than 1 year earlier. Similar results have been reported for patients with cirrhosis (Cooksley et al 2000).

Based on these results, it is clear that patients with hepatitis C with and without cirrhosis may safely be treated with peginterferon alpha-2a. By adding the PEG molecules to interferon additional adverse events are not experienced, and quality of life is generally better in those treated with pegylated interferon.

## Disadvantages of peginterferon

The addition of PEG moieties to type I interferon has significantly enhanced the pharmacokinetic and pharmacodynamic properties of the molecule. However production of peginterferon is not a trivial process and has required substantial investment in manufacturing technology which has resulted in a substantial increase in production costs. Furthermore the peginterferon is less active on a mole for mole basis when compared with unmodified interferon. In the clinic this problem is easily overcome by using larger quantities of drug, but the use of large amounts of an expensively produced therapeutic inevitably significantly increases unit costs. Thus the chief disadvantage of the peginterferon is the unavoidable increase in drug prices leading to a significant increase in drug costs. Although the use of the more expensive peginterferons is cost effective there is a net increase in health care expenditure that is burdensome to many.

## Health economic data

The value of any new technology needs to be assessed in both clinical and economic terms and pegylated interferon has been

assessed in this way. A 10-year projection from 1998 estimates that increases in chronic hepatitis C related diseases will occur as follows: cirrhosis (61%), decompensated cirrhosis (279%), hepatocellular carcinoma (68%), liver transplantation (528%), and liver-related deaths (223%) (Davis et al 1998). Approximately 170 million people worldwide are infected with hepatitis C (WHO 1999). Approximately 100 000 new cases occur every year in the United States (CDC 1998; EASL 1999; Alter et al 1999). Presently hepatitis C-related liver disease accounts for 8000 to 10 000 deaths per year in the United States (CDC 1998). Patients with develop hepatitis C are at risk of serious long-term sequelae including cirrhosis, hepatocellular carcinoma, liver transplantation, and death from liver disease-related causes.

Health economic models have been used to assess the long-term effects and cost effectiveness of peginterferon therapy in hepatitis C patients. They have shown that in terms of QALY (quality adjusted life years), peginterferon alpha-2a is cost effective in the management of hepatitis C (Annemans et al 2004).

## Conclusion

Standard interferon is absorbed rapidly and then quickly eliminated from the body, peak levels occur within hours of subcutaneous administration of the drug, and decline rapidly to be undetectable at 24 hours. This means patients experience sub-therapeutic levels of the drug for the majority of the time.

Using techniques evolved through nanomedicine, the protein interferon has been altered by way of attaching a PEG moiety. This adaptation improves the pharmacokinetic properties of the protein. This includes increased solubility, increased resistance to enzyme degradation, reduced immunogenicity, and reduced renal clearance. Peginterferon alpha-2a (40 kDa) exhibits sustained absorption, less variability in peak and trough concentrations, a restricted volume of distribution, and reduced renal clearance compared with standard interferon.

Pegylation results in a drug that can be administered once a week producing a sustained virological response throughout the whole week. The trials that have been performed to compare standard and peginterferon have shown better efficacy in combination with ribavirin than standard interferon. These results are probably due to the reasons summarized. Pegylation does not seem to result in more adverse events or side-effects than standard interferon and patients have reported feeling better while taking peginterferon than standard interferon.

Over the next 10 years it is evident that hepatitis C will become a global health care problem, inducing end-stage liver disease in many millions of individuals. It is therefore important in both in clinical and economic terms to establish the best possible ways of treating the disease, and preventing long-term complications. Combination therapy involving interferon and ribavirin have been shown to be effective in the treatment of the disease. Peginterferon has advantages over standard interferon, and is an effective method of administering interferon.

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