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Review

PEGylated drugs in rheumatology—why develop them and do they work?

Thomas McDonnell[1], Yiannis Ioannou[1] and Anisur Rahman[1]

Abstract

Lack of efficacy and drug-related adverse effects are important reasons for the discontinuation of treatment in patients with rheumatic diseases. The development of new biologic therapies seeks to address these problems by specifically targeting the pathogenic mechanisms of disease. Most current biologics are proteins (particularly antibodies and enzymes) administered parenterally. It is important to optimize properties such as serum half-life, immunogenicity and solubility. Companies have thus begun to modify the drugs by conjugate chemistry, binding inert molecules such as polyethylene glycol (PEG) to biologic molecules to improve their pharmacodynamic properties. The use of PEG to alter these properties has to be weighed against the negative aspects of PEGylation, such as decreased activity and heterogeneity. This review focuses on the currently available PEGylated drugs used in rheumatological diseases, their efficacy, drawbacks and the current clinical trial evidence supporting their use.

Key words: PEGylation, gout, rheumatoid arthritis, clinical trials.

REVIEW

Introduction

PEGylation refers to the covalent binding of polyethylene glycol (PEG) molecules to proteins. The large PEG groups alter the physical properties of the molecule, such as solubility, thermal stability and immunogenicity. These changes may be utilized to render biologically active proteins suitable for therapeutic use through modification of their pharmacokinetic and pharmacodynamic properties, such as prolongation of half-life *in vivo* or inhibition of degradation by proteases.

In rheumatology, PEGylation is highly relevant to the new generation of biologic drugs, most of which are proteins. Examples include antibodies such as anti-TNF drugs, used in inflammatory arthritis, and enzymes such as uricase, used in gout. This review describes the challenges that have been encountered in developing these PEGylated biologic agents and the evidence regarding their efficacy in treating rheumatological diseases.

[1]Centre for Rheumatology Research, Division of Medicine, University College London, London, UK.

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Correspondence to: Anisur Rahman, Centre for Rheumatology Research, Division of Medicine, University College London, Room 412 Rayne Institute, 5 University Street, London WC1E 6JF, UK.
E-mail: anisur.rahman@ucl.ac.uk

Advantages and disadvantages of PEGylation

PEG molecules bind to amino acids in the target protein by means of chemical linkers [1]. The number, sites of attachment and molecular weights of the PEG molecules can be varied in order to optimize the biologic properties of the PEGylated product, notably half-life and immunogenicity. PEG itself has low immunogenicity, low antigenicity and low toxicity [1, 2]. PEGylation prolongs the *in vivo* half-life of protein-based drugs by several mechanisms [3–6]. The large PEG groups increase the hydrodynamic volume of the molecule such that it is less likely to be excreted by the kidney due to low levels of permeability through the renal basement membrane [2, 7]. The hydrodynamic volume of the PEGylated molecule increases sharply with the molecular weight of the PEG attached. A 10 kDa PEG has a similar hydrodynamic volume to a protein of 65.4 kDa, but a 40 kDa PEG has a hydrodynamic volume approaching that of a protein of 670 kDa [8]. PEG groups protect the protein from proteolysis and from the immune system, avoiding formation of immune complexes and degradation [2, 9]. Repulsion between PEG groups on separate molecules reduces aggregation and improves thermal stability [8, 10]. PEG dissolves at high concentrations in both water and organic solvents, so PEGylation improves the solubility of proteins in both [8, 10]. PEG also has very low toxicity, having been shown to be harmless to animals at concentrations as high as 16 g/kg (1000-fold higher than the normal therapeutic dose of protein–PEG conjugates in humans) [7].

One of the main problems with PEGylation is the heterogeneity of the products produced. Since most proteins contain a number of possible attachment sites for PEG molecules, the product of a chemical reaction between protein and PEG is typically a mixture of isomers containing different numbers of PEG groups (monoPEGylated, diPEGylated, etc.) or in which PEG has been attached at different places. These isomers may vary in biologic activity so it is difficult to control or predict the properties of the mixture. A number of techniques can be used to address this problem. Some PEGylation methods target specific amino acids such as histidine, cysteine or disulphide bridges, limiting the potential sites of PEGylation on any protein. Restriction of the PEG:protein ratio used in the reaction and variation of temperature conditions can drive the reaction in favour of producing only monoPEGylated products. MonoPEGylated products can be separated from those containing larger numbers of PEG by size-exclusion chromatography, though it is very difficult to separate different monoPEGylated isomers from each other. However, it is possible that using a mixture of such monoPEGylated isomers therapeutically could have advantages. For example, antibodies against one isomer might not bind to others, thus reducing their effect on the efficacy of the drug as a whole. In fact, antibodies to PEG itself have been estimated to occur in 8–25% of the population [11, 12], probably due to the presence of PEG in normal household items such as hand creams.

Another issue with the efficacy of PEGylated drugs is that the PEG groups may hamper access of the substrate to the active site of the molecule, thus reducing the binding affinity and biologic activity of the molecule. Affinity can also be reduced where the binding of PEG causes conformational changes and disruption of the pattern of electrostatic charges on the surface of the molecule [9]. Depending on the method of PEGylation and the weight of bound PEG, activity retained in the PEGylated product may vary widely, from 7% to 98% [1]. One study showed that binding of 40 kDa of PEG to IFNα-2 a (19 kDa) reduced its activity to 7% [2] of the native protein. Conversely, others have noted that use of bifunctional PEG (i.e. able to bind two target molecules creating dimers) can give highly active conjugates with little variability in size [1]. The position at which the PEG is attached can also be critical in determining the effects on activity. For example, filgrastim is recombinant human granulocyte colony-stimulating factor used to treat neutropenia. UnPEGylated filgrastim has a short serum half-life, necessitating daily injections. Random PEGylation can be used to increase the half-life, but at the cost of reduced activity. However, site-specific PEGylation of filgrastim at the N-terminal amino group enabled better retention of biologic activity; this monoPEGylated variant is marketed as Neulasta [13]. More recently a new variant of PEGylated filgrastim, in which a single PEG is added enzymatically at glutamine 135, was shown to have 50% better activity in a bioassay than Neulasta [14] (although it was still not as active as unPEGylated filgrastim).

In some PEG–drug conjugates, the PEG is released *in vivo* due to the use of a linker, which is either hydrolytically unstable or susceptible to enzymatic cleavage. Loss of the PEG might lead to toxicity or a shortened half-life and this problem led to abandonment of one drug (Pegamotecan, a PEGylated camptothecin) due to a lack of efficacy [2]. On the other hand, gradual loss of PEG can also be seen as an advantage if the PEGylated form is seen as a pro-drug of reduced activity from which the fully active form of the protein is released by hydrolysis of the linker and loss of PEG. PEG-intron (PEGylated IFNα-2 b) is an example of such a pro-drug. PEGylation at His-34 is unstable because of the susceptibility of the linker at that site to hydrolysis [2, 15].

In one study using PEGylated TNF-binding protein, administration of the conjugate (but not free PEG or free protein) induced the unexpected side effect of vacuolation of the renal cortical tubular epithelium after 3 months [8]. The interaction between PEG and protein can have significant effects on viscosity. Thus Kerwin *et al.* [16] showed that a mixture of PEG with soluble TNF receptor 1 (sTNF-R1) had a viscosity up to five times higher than that of PEG alone or sTNF-R1 alone, depending on the pH. Viscosity was highest at pH $>$ 5.2. A highly viscous product would not be ideal for therapeutics. Having described the potential advantages and disadvantages of PEGylation, we will now outline the ways in which both have affected the development of PEGylated agents for two rheumatological conditions: gout and RA.

PEGylated uricase in gout

Uricase (also referred to as urate oxidase) is an enzyme, thought to have been lost in evolution in humans, that metabolizes uric acid to allantoin [4–6]. Uricase has been investigated as a treatment for patients who do not respond fully to xanthine oxidase inhibitors such as allopurinol.

Initially uricase purified from *Aspergillus flavus* and pigs suffered the problems of a short half-life and the great possibility of an immunogenic reaction, curtailing its progression into clinical trials [6]. More recently, clinical trials have been carried out on PEGylated uricase [4–6, 17, 18].

An initial pre-clinical study [4] assessed intra-peritoneal administration of recombinant chimeric pig–baboon uricase to uricase double-negative homozygote mice. Unmodified recombinant uricase was ineffective. It disappeared from the circulation 4–24 h after injection and reduction of serum urate from 10.2 mg/dl at baseline to 6.3 mg/dl at 4 h was transient, returning to normal in 24 h. The transient nature of this effect could not be solved by repeated injections of unmodified uricase because anti-uricase antibodies appeared after the second injection and increased after subsequent injections. The results with PEGylated enzyme were much better. Repeated injections (up to 10) did not result in any serum anti-uricase antibodies, and when the injections were given at 5-day intervals the presence of serum uricase and reductions in both serum urate and urinary uric acid were maintained between injections [4]. Use of a recombinant uricase

combining sequence features of the pig and baboon enzymes was advantageous. Baboon uricase is less active than porcine uricase but more similar to the human enzyme (reducing immunogenicity). This PEGylated pig–baboon uricase, later designated pegloticase, was then taken forward into clinical trials.

Phase 1 trials of single doses of s.c. ($n = 13$) [17] and i.v. ($n = 24$) [6] PEGylated uricase were carried out in patients with hyperuricaemia and clinical gout who did not respond to standard oral urate-lowering agents. Both routes of administration produced rapid decreases in plasma uric acid, with the effect being maintained for 21 days in 11/13 patients given s.c. drug and 24/24 given i.v. drug. However, the latter route gave lower uric acid and higher plasma uricase levels, whereas localized pain at the injection site and poor absorption were problems with s.c. injection. The PEGylated uricase was immunogenic, with anti-PEG uricase or anti-PEG antibodies developing in 5/13 patients given s.c. drug and 9/24 patients given i.v. drug. Although development of these antibodies caused more rapid clearance of PEGylated uricase, it did not cause severe allergic reactions and the i.v. drug was well tolerated. It was taken forward into a longer (12–14 week) phase II trial [5] in which 41 patients with refractory hyperuricaemia were randomized to one of four doses: 4 mg every 2 weeks, 8 mg every 2 weeks, 8 mg every 4 weeks or 12 mg every 4 weeks.

Although there was evidence of efficacy, it is striking that 15 of the 41 patients withdrew from the study, with infusion reactions being the reason in 12 of these. The mean urate level remained <6 mg/dl in all four treatment groups and the primary end point (plasma urate <6 mg/dl for at least 80% of the study period) was achieved by 50–88% of the patients in different groups. The best response was in the 8 mg every 2 weeks group, where 88% achieved this end point. On the other hand, 88% of all patients reported at least one flare of gout during the study period and 76% of patients developed anti-pegloticase antibodies. The presence of these antibodies reduced efficacy and increased clearance rates while antibody-negative patients showed a 100% response rate [5]. The authors reported that anti-pegloticase-positive patients who were treated every 2 weeks were more likely to respond than those who got the drug every 4 weeks (80 *vs.* 33%) [5]. Overall this trial established 8 mg/infusion as the likely optimal dose, with a possible benefit of fortnightly rather than monthly administration, and the efficacy of this approach was tested in two replicate double-blind, randomized, placebo-controlled studies reported in 2011 [18].

In these replicate trials all subjects received fortnightly infusions that were either 8 mg pegloticase every infusion, 8 mg pegloticase alternating with placebo (i.e. the active drug was being given monthly) or placebo every infusion. Responders were defined as having plasma uric acid <6 mg/dl for at least 80% of the time in months 3 and 6. Of the 225 patients enrolled, all had a decrease in plasma uric acid after the first pegloticase infusion, but this response was not maintained in all subjects such that the overall response rate was 42% in the fortnightly pegloticase group, 35% in the monthly pegloticase group and 0 in the placebo group. There was a reduction in flares of gout in the fortnightly pegloticase group compared with placebo between months 4 and 6, but not at earlier time points. However, flares of gout did occur in ~80% of subjects across all three groups. Although patients were given prophylactic hydrocortisone, fexofenadine and acetaminophen, infusion reactions remained more common in the fortnightly (26%) and monthly pegloticase (42%) groups than the placebo group (5%). Withdrawals due to infusion reactions were less frequent, however, than in the previous open study (10% for the fortnightly group and 13% for monthly) [5]. Although seven subjects died, this was not thought to be related to the drug. Three deaths occurred in the placebo group and the patients who died had co-morbidities such as cardiovascular disease.

As in the previous studies, pegloticase proved to be immunogenic, with 134/150 subjects who received the drug developing anti-pegloticase antibodies. In these phase III trials, the authors showed that the titre of anti-pegloticase was important. Those who developed high titre antibodies ($>1:2430$) almost invariably lost their response to the drug, whereas 52/82 patients who had lower titre anti-pegloticase retained their response. Furthermore, a higher titre was associated with increased risk of infusion reactions, which were reported in 31/52 patients with high titre anti-pegloticase and 16/84 with low titre anti-pegloticase.

In summary, PEGylation has facilitated the development of a new drug for patients with refractory gout. The relatively low response rates in the phase III trials must be viewed with the knowledge that these were patients who were already very difficult to treat. PEGylation of uricase did fulfil the aims of increasing the half-life and allowing infrequent dosing—even monthly doses are appreciably better than placebo—but there is a high frequency of antibodies to pegloticase and higher antibody levels are related to poorer clinical outcomes. However, the particular issue of s.c. injection site reactions that necessitated i.v. use of this drug may be specific to pegloticase, as it is thought to be related to localized release of hydrogen peroxide when urate is oxidized.

PEGylated therapies in RA

TNF-α inhibitors have been used as an alternative treatment for patients with RA who fail to respond to standard DMARDs. There are currently four non-PEGylated TNF inhibitors on the market, all of which have been shown to be effective in patients with RA [19]. Certolizumab pegol (CZP), the first PEGylated TNF inhibitor, has recently been introduced into clinical practice. No head-to-head trials of the five different anti-TNF agents have been carried out, but meta-analyses suggest that all have similar efficacy and safety characteristics [19, 20].

An early attempt to treat RA with PEGylated TNF receptor utilized a conjugate [TNF binding protein (TNFbp)] in which two molecules of the extracellular domain of TNF-RI were conjugated with PEG [21]. Although this construct

was effective at inhibiting the biologic effects of TNF, e.g. in a murine model of multiple sclerosis [21], a phase I/II clinical trial in patients with active RA was not successful [22]. There were 33 patients in the study and doses of TNFbp ranging from 30 to 100 μg/kg were given intravenously at baseline, 3 and 6 weeks. Although there was some reduction in the swollen and tender joint counts at 21 days, the development of antibodies to the drug occurred in 88% of subjects that received it. Furthermore, the presence of these antibodies increased clearance and reduced the half-life of the drug. These effects were magnified after repeated doses, so this molecule was considered non-viable as a long-term treatment for RA in the clinic [22]. After further studies to identify soluble TNF-R-based drugs with better pharmacotherapeutic properties than TNFbp (including low immunogenicity), a PEGylated monomeric form of sTNF-R1 (PEGsTNF-R1 or pegsunercept) was selected for further evaluation.

PEGsTNF-R1 was initially designed as an anti-angiogenic factor in RA [23]. It did reduce swelling and general pain, showing a good effect [23–25] when initially tested in an RA model in rats, and showed good activity when used as a monotherapy and in combination therapy with other drugs such as dexamethasone and indomethacin [26] or with IL-1 receptor antagonists; however, it showed little effect on neovascularization in animal models and induced an IgM response in several experiments [23–25]. Other studies in primates have shown no antigenic response in multiple or single dosing studies [26]. It is currently being taken forward to phase II clinical trials. This drug remains a promising route of research, but it is hard to judge the effect it will have in the clinic when it is so early in the process of development. In contrast, CZP has shown more long-term success and is already licensed for the treatment of RA.

CZP is an Fc-free anti-TNF-α humanized Fab fragment bound to 40 kDa PEG. PEGylation has increased its half-life to ~14 days [11, 20, 27]. The drug has gone through several clinical trials [11, 20, 27, 28] that have shown promising results for long-term care.

No preclinical data in animals have been published regarding this drug. One small-scale phase II study initially showed both a low antibody response in humans when the drug is given in high doses, extended half-life in comparison with most TNF-α inhibitors (~14 days) and clinical improvement that compared favourably with etanercept and infliximab [29]. A number of phase III trials have followed.

The RA Prevention of Structural Damage (RAPID) 1 [27] and RAPID 2 [30] studies were both multi-centre, randomized, double-blind, placebo-controlled trials that tested the efficacy of CZP given in combination with MTX in patients with RA not controlled by MTX alone. In the RAPID 1 study there were three groups: 400 mg CZP every 2 weeks ($n = 390$), 400 mg CZP at weeks 0, 2 and 4 followed by 200 mg CZP every 2 weeks ($n = 393$) and placebo every 2 weeks ($n = 199$). Patients in all three groups continued MTX at the same dose that they were taking at study entry. The protocol for RAPID 2 was very similar, although the trial was shorter (24 weeks) and smaller (total number of subjects = 619).

There were clear differences between the CZP and placebo groups in both RAPID trials. For example, in RAPID 1 the percentage of patients completing 52 weeks treatment was 70.3% in the 400 mg CZP group, 64.9% in the 200 mg CZP group and 21.6% in the placebo group [27]. In fact, 62.8% of the placebo group had withdrawn due to lack of efficacy by week 16. After just 1 week of treatment significantly more patients in the treatment groups achieved ACR20 responses than with placebo (22.9% for 200 mg, 22.3% for 400 mg and 5.6% for placebo) [27]. Differences between the CZP and placebo groups remained significant for the entire follow-up period [27]. ACR50 and ACR70 responses were also better in the CZP group compared with the placebo group. Secondary outcomes were better in the treatment groups than in the placebo group regarding the slowing of structural damage (using Sharp radiological scores), improved physical function, quality of life and general patient well-being [20, 27]. Most adverse effects were mild or moderate and withdrawal due to adverse events was rare—3.3, 5.6 and 7.0 per 100 patient-years in the placebo, 200 and 400 mg groups, respectively [27]. There were no drug-related deaths. Very similar results were seen in RAPID 2 [30], with the additional finding from an open-label extension period that clinical and radiological benefits were sustained for up to 3 years and only two patients withdrew from CZP due to lack of efficacy [30]. Antibodies to CZP were seen in 6.4% of patients who received it in RAPID 1 and 5.1% in RAPID 2, which is in keeping with the naturally circulating anti-PEG levels in the population [12]. The number of antibody-positive patients was too low to detect any effect on clinical response.

Evidence for efficacy of CZP given subcutaneously as monotherapy comes from the Efficacy and Safety of CZP—4 Weekly Dosage in RA (FAST4WARD) study, in which patients who had failed one or more DMARDs were randomized to either CZP 400 mg ($n = 111$) or placebo ($n = 109$) every 4 weeks for a period of 24 weeks. The results showed better outcomes for patients on CZP as opposed to the placebo group [11]. Significantly fewer patients in the CZP group than the placebo group withdrew due to lack of efficacy (21.6% *vs* 68.8%, $P < 0.001$). At week 24, ACR20, 50 and 70 response rates were all significantly better in the treatment group compared with the placebo group—45.5% *vs* 9.3%, 22.7% *vs* 3.7% and 5.5% *vs* 0% for ACR20, 50 and 70, respectively. In the treatment group, 8.1% were positive for anti-CZP antibodies, which reduced the effect on ACR20 at week 24 by ~5%—this is a common effect of neutralizing antibodies in biologic therapeutics [3, 11, 20]. There were also improvements in HAQ Disability Index (HAQ-DI) values, pain and fatigue in the treatment groups [11]. Most adverse effects were mild or moderate. There were no deaths.

Further studies (reported in abstract form only) extend the possible range of patients in whom CZP may be effective. The RA Evaluation in Subjects Receiving TNF Inhibitor CZP (REALISTIC) study [31] includes >1000

subjects, of whom 38% had already received some form of TNF inhibition therapy, i.e. this study looks at the potential use of CZP in 'anti-TNF failure' patients. Subjects were randomized to receive either CZP 200 mg fortnightly or placebo. Whereas 216 subjects received CZP monotherapy, 635 received CZP with a DMARD and 212 received placebo (with or without a DMARD). At 12 weeks the ACR20 response was achieved in 51% of CZP-treated patients compared with 26% of placebo-treated patients. Importantly, this level of response to CZP was achieved regardless of whether patients were on DMARDs, which DMARDs were given or whether they had previously failed other TNF inhibition treatments. Preliminary results of the CZP in the Treatment of RA Remission Induction and Maintenance in Patients With Low Disease Activity (CERTAIN) study [30] show that even patients with low or moderate disease activity may benefit from CZP in combination with DMARDs. Of 194 such patients recruited and randomized to CZP (400 mg at weeks 0, 2 and 4 followed by 200 mg every fortnight) or placebo, only 37% of CZP patients had moderate or high activity at 24 weeks compared with 70% of the placebo group. At both 20 and 24 weeks, more than twice as many patients on CZP as on placebo were in remission from RA.

In summary, there is clear trial evidence that CZP is effective in treating patients with DMARD-non-responsive RA both as monotherapy and in combination and emerging evidence for its use in patients with low to moderate disease activity and those who have failed other TNF inhibitors, although there is nothing to suggest that it is more effective after one TNF failure than other anti-TNFs. The efficacy and safety seen are comparable to those for the other TNF-α inhibitors [20, 27]. A unique feature of CZP is the lack of an Fc component (which also necessitates the use of PEGylation or some other means of increasing the size of the Fab), and some have argued that this would be advantageous in preventing this drug from crossing the placenta [20]. However, there are as yet no convincing data to guide us in using any form of TNF inhibition during pregnancy.

Conclusion

PEGylated biologics in rheumatology: the future or a distraction

In the development of PEGylated uricase and CZP the potential drawbacks of PEGylation (such as heterogeneity, reduced activity and immunogenicity) did not apply or have been circumvented. Different PEGylated agents produce different levels of antibody response. Most patients treated with pegloticase develop anti-drug antibodies (which can affect efficacy), whereas very few patients treated with CZP develop anti-CZP antibodies. PEGylation increased the half-life of both agents, allowing fortnightly or monthly doses to be effective.

The nature of the molecule to be PEGylated is critically important. In the case of pegloticase, a recombinant artificial uricase with optimal properties was designed. In the case of CZP, an Fc-free Fab was used. Pegloticase has relatively poor efficacy data compared with CZP, but may be used in patients for whom there is currently no other effective drug. In contrast, CZP would have failed had it not been as effective in trials as the other TNF inhibitors available. There is therefore reason to believe that PEGylated drugs will find a role both for small groups of refractory patients and in broadening the range of available agents for wider groups of patients.

In conclusion, successful trials of PEGylated agents in gout and RA have shown that the potential gains from PEGylation can be realized whereas the potential drawbacks can be circumvented. PEGylation of relatively small molecules (as in both these examples) may be especially important in the future.

Rheumatology key messages

- PEGylation is a chemical method of modifying biologic molecules to increase half-life *in vivo*, enhancing their therapeutic utility.
- Potential disadvantages of PEGylation include reduced activity and immunogenicity.
- CZP for RA and pegloticase for gout are in clinical use after being tested in trials.

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