Immunogenicity of Polyethylene Glycol (PEG)

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Abstract: Covalent attachment of polyethylene glycol (PEG) to active proteins (PEGylation technology) successfully generated several FDA-approved compounds, including four blockbusters, which are considered non immunogenic. However, PEG antibodies and intolerance to the infusion were reported in some patients with gout treated with pegloticase, a PEG-uricase recently approved by the FDA (Food and Drug Administration, USA). Therefore, we reviewed the literature concerning immunogenicity of PEG alone or covalently attached to proteins. We consulted databases (Scirus, Pub Med, Cochrane Library, and Science Direct) and the references of selected articles and main journals publishing articles on the subject. Animal studies clearly showed that PEG-uricases and some other PEGylated proteins might elicit antibody formation against PEG. This anti-PEG response can accelerate the clearance of PEGylated proteins. Of major importance is the recent finding of a 22%-25% occurrence of PEG antibodies in healthy blood donors. PEG antibodies may limit therapeutic efficacy and/or reduce tolerance of PEG-asparaginase (PEG-ASNase) in patients with acute lymphoblastic leukemia and of pegloticase in patients with chronic gout, but apparently do not impair hyposensitization of allergic patients with mPEG-modified ragweed extract and honey bee venom or the response to PEG-interferon in patients with hepatitis C. In conclusion, pre-existing or newly developed PEG antibodies may limit therapeutic efficacy and/or reduce tolerance of PEGylated proteins in some patients. The immunogenicity of PEGylated therapeutic agents in clinical use or development deserves to be re-examined by investigating PEG antibodies.

Keywords: Gout, immunogenicity, PEGylated proteins, PEGylation, polyethylene glycol, uricase.

INTRODUCTION

Pegloticase (Krystexxa®, Savient Pharmaceuticals, USA) a polyethylene glycol (PEG) conjugate of a porcine-like uricase, was recently approved by the FDA (Food and Drug Administration, USA) for the treatment of chronic gout, refractory or intolerant to conventional therapy [1]. Placebo-controlled, 6-month clinical trials showed that pegloticase, at doses of 8 mg every 2 weeks, induced a significant decrease in plasma uric acid, associated with the dissolution of tophi in 40% of patients at final visit (45% in patients treated for 25 weeks) [2-5]. However, 58% of the patients were non-responders (according to the criteria of plasma urate <360 μM for 80% of the time), which correlated with the formation of anti-pegloticase antibodies and intolerance to the infusion (see also [6]). This was a surprising result because PEG-protein conjugates are widely regarded as immunologically safe [7, 8]. Moreover,PEGylation technology successfully generated several FDA-approved compounds which are considered non immunogenic, including four blockbusters (PegIntron®, a PEGylated form of interferon-α2b; Pegasys®, a PEGylated form of interferon-α2a, both for the treatment of hepatitis C; Neulasta®, a PEGylated form of granulocyte colony stimulating factor for the treatment of chemotherapy-induced neutropenia; and Mircera®, a PEGylated epoietin-β for the treatment of anemia associated with chronic renal failure) [7]. Therefore, we reviewed the literature concerning immunogenicity of PEG alone or covalently attached to proteins.

METHODS

Documentary Sources and Study Selection

Studies on immunogenicity of PEG published from 1977 to today. We consulted databases (Scirus, PubMed, Cochrane Library, Science Direct) using “immunogenicity”, “PEG”, “PEGylated” and “anti-PEG” as key-words. We also consulted the references of the selected articles and main journals publishing articles on the subject.

RESULTS

Early Studies of PEGylated Protein Immunogenicity

PEGylation was first described in the 1970s by Abuchowsky, Davies et al. [9, 10] in two key studies investigating immunogenicity of PEGylated bovine serum albumin and PEGylated bovine liver catalase. Their rational was elegant and convincing:

« Good immunogens typically have a rigid, complex surface structure to which antibodies can be made. We rationalized that the covalent attachment of a linear, flexible, uncharged hydrophilic polymer to available but nonessential groups on an enzyme might provide a shell around the enzyme that covers antigenic determinants and, by presenting a flexible, unbranched, hydrophilic surface for inspection by the immune processes, prevent recognition of the interior enzyme as a foreign substance against which an immune response would be provoked. At the same time, the shell would be permeable to the smaller substrates so that enzymatic activity could continue. »
Rabbit antisera against native and all tested PEGylated proteins, prepared and tested by immunoprecipitation, showed reducing immunogenicity with the increase in % PEGylation [10]. This was confirmed by the observation that, unless native catalase, PEGcatalase clearance remained unchanged in the long term [10]. Finally, the authors mention that they also tested repetitive injections of PEG-uricase with absence of apparent immunological effects and extended blood circulating life [10].

Following the above pioneering studies, the procedure of PEGylation was expanded and developed tremendously to prolongate half-life of active protein and peptide biopharmaceuticals [7, 8]. In most of these studies, immunogenicity was tested against the active protein. In particular, this was the case with the preclinical, proof of concept study of pegloticase, where immunogenicity was tested with anti-uricase antibodies [10, 12].

**PEG Antibodies in Animal Models**

PEG is generally considered to be non-immunogenic by itself, but the s.c. administration of 5900 kD PEG to mice was able to elicit a weak and transitory immune response [13] A stronger anti-PEG immune response was found with PEGylated proteins, particularly ovalbumin [13]. The hapten inhibition in the PEG anti-PEG system by PEG 0.3 kD indicated that the antigenic determinant of PEG may be a sequence of 6-7 -CH2CH2O-units [13].

The haptenic character of PEG depends on its molecular weight, the immunogenicity of the anchoring protein and the presence of adjuvants [13-15]. Tsuji et al. [16] investigated immunogenicity of a purified uricase from *Candida utilis*, which was modified to varying degrees with monomethoxypolyethylene glycol of different molecular weights. As increasing amounts of PEG were attached to uricase, it showed decreasing ability to elicit anti-uricase antibody production in rabbits. When sufficient polymers were attached, the modified uricase was devoid of the capacity to combine *in vivo* and *in vitro* with anti-uricase antibodies, while it was still able to react with antibodies to PEG-uricase conjugate. Antibodies against PEG-uricase conjugates also reacted with PEG modified superoxide dismutase. The authors conclude that the coupling of PEG to uricase resulted in the loss of original antigenicity and immunogenicity, but in the appearance of new antigenicity and immunogenicity which never showed any cross-reactions against the native uricase [16].

In mice receiving multiple injections of beta-glucuronidase-PEG, IgG antibodies against the native protein and PEG (protein independent) are produced, with similar affinities against the native protein and the conjugate [17, 18]. Anti-beta-glucuronidase IgM antibodies are also produced which exhibit high affinity against the conjugate, but did not recognize the native protein. The anti-polymer antibodies could also recognize other PEG conjugates.

PEG antibodies were also described in serum from rabbits, obtained after weekly injections of small liposomes containing 20% PEG- phosphatidyl ethanolamine [19]. Wang *et al.* [20] found that, irrespective of the presence or absence PEG-coating, a single first dose of liposomes is capable of inducing a strong anti-PEG IgM response. A good correspondence was observed between the amount of IgM associating with both PEGylated and conventional liposomes, concomitant complement activation triggered by those liposomes and the magnitude of accelerated blood clearance against those liposomes [20]. Conversely, development of antibodies against PEG-methioninase did not result in any immunological reaction or decreased activity in monkeys [21], although no attempt was done to discriminate between PEG and methioninase antibodies.

**PEG Antibodies in Humans**

In 1984, Richter and Akerblom [14] reported the presence of naturally occurring PEG antibodies in 0.2% of healthy blood donors and in 3.3% of untreated allergic patients. During hyposensitization with methoxy PEG(mPEG)-modified ragweed extract and honey bee venom, respectively, PEG antibodies of the IgM isotype were detected in 50% of the patients directly after the first treatment course [14]. After 2 years of treatment the percentage of patients with such titers declined to 28.5%. The anti-PEG response was considered to be of no clinical significance [14].

Recently, Garratty *et al.* [22, 23] detected a high occurrence (22%-25%) of PEG antibodies in 350 healthy blood donors and identified both IgG and IgM. In patients with acute lymphoblastic leukemia treated with PEG-asparaginase (PEG-ASNase), the presence of anti-PEG was very closely associated with rapid clearance of PEG-ASNase [22, 23].

Ganson *et al.* [6] investigated immunogenicity of subcutaneous injections of PEG-uricase to 13 patients with gout. In five subjects, plasma uricase activity could not be detected beyond ten days after injection; this was associated with the appearance of relatively low-titer IgM and IgG antibodies against PEG itself rather than the uricase protein.

Hamad *et al.* [24] reported that highly concentrated near-monodisperse endotoxin-free PEGs can generate complement activation products in human serum on a time scale of minutes. Tillmann *et al.* [25] found a high frequency of PEG antibodies in patients with hepatitis C. These PEG antibodies did not lead to impaired response to PEG-interferon.

**DISCUSSION**

In contrast to the general assumption that PEG is non-immunogenic, animal studies clearly showed that some PEGylated proteins can elicit antibody formation against PEG [13, 16, 19]. This anti-PEG response can accelerate the clearance of PEGylated proteins [17, 18, 20]. In humans, PEG antibodies can be produced and may limit therapeutic efficacy and/or reduce tolerance of PEG-ASNase in patients with acute lymphoblastic leukemia [22] and of pegloticase in patients with chronic gout [2-5], but apparently do not impair hyposensitization of allergic patients with mPEG-modified ragweed extract and honey bee venom [14] or the response to PEG-interferon in patients with hepatitis C [25, 26].

The binding epitope of anti-PEG has been shown to be 4-7 repeat ethoxy units [13, 23]. On the other hand, cyanuric chloride activated mPEG has one extra active chloride group that can absorb other amino targets in immunological
reactions [27]. Other linkers, such as succinimidyl carbonate or propionate, do not have this kind of active group.

Of major importance is the recent finding of a 22%-25% occurrence of PEG antibodies in 350 healthy blood donors [22, 23], whereas a very low 0.2% occurrence was reported by Richter and Akerblom [14] in 1984. Armstrong [22, 23], whereas a very low 0.2% occurrence was reported occurrence of PEG antibodies in 350 healthy blood donors

Because of the medical importance of gout and the relative success of pegloticase, research efforts should be dedicated to investigate the immunogenicity of other PEG-uricases in development, such as pegseticase [28] and to reduce uricase immunogenicity. Freitas da Silva et al. [29] have obtained a recombinant uricase from Candida sp. pegylated with mPEG-pNP or mPEG-CN. When injected repeatedly in mice for 21 days, the uricase did not induce detectable antibody response. A different approach was used by Tan et al. [30] by encapsulating an uricase from Candida in lipid vesicles. Finally, due to a nonsense codon inserted into the uricase gene, this enzyme is produced as a truncated, 10 aminoacids long, inactive fragment in humans and apes [31, 32]. Therefore, another approach can be to “reactivate” human uricase, by eliminating nonsense mutations [11].

In conclusion, pre-existing or newly developed PEG antibodies may limit therapeutic efficacy and/or reduce tolerance of PEGylated proteins in some patients. The immunogenicity of PEGylated therapeutic agents in clinical use or development deserves to be re-examined by investigating PEG antibodies.

CONFLICT OF INTEREST

None.

ABBREVIATIONS

FDA = Food and Drug Administration
PEG = polyethylene glycol

REFERENCES


