



PEGylated Proteins: How Much Does Molecular Weight Matter?

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Abstract

Polyethylene glycols (PEGs) are inert polymers of repeating ethylene oxide subunits. Attaching PEGs to therapeutic proteins may reduce the protein's immunogenicity and antigenicity, improve solubility and stability, slow protein degradation, and increase the half-life ($t_{1/2}$). This usually results in less frequent administration, improved quality of life and convenience, and potentially better adherence and lower costs. The advantages and disadvantages of PEGylated proteins differ according to the structure of the PEG moiety, particularly its molecular weight. The larger the PEG molecular weight, the longer the $t_{1/2}$ and time to steady state. PEGs have low toxicity and undergo minimal metabolism. The PEG moiety usually undergoes renal elimination and is excreted in urine, but with greater molecular weights, renal elimination declines and biliary excretion increases. Because PEG molecules are not broken down, there is potential for PEGs to accumulate in the cytoplasm, forming vacuoles, mostly in macrophages, although this does not affect their function. The risk of vacuolation increases with molecular weights > 30 kDa. However, even high molecular weight PEGs are used at doses markedly lower than the European Medicines Agency safety threshold for paediatric use. People can develop antibodies to PEGs, and this may increase the overall clearance of the PEGylated protein if antibody levels are sufficiently high (> 500 ng/mL according to one modelling study). In conclusion, it is important for physicians to understand how PEG molecular weight and architecture can impact stability, immunogenicity, glomerular filtration and cellular uptake, to better understand the overall safety, efficacy and pharmacological profile of PEGylated proteins.

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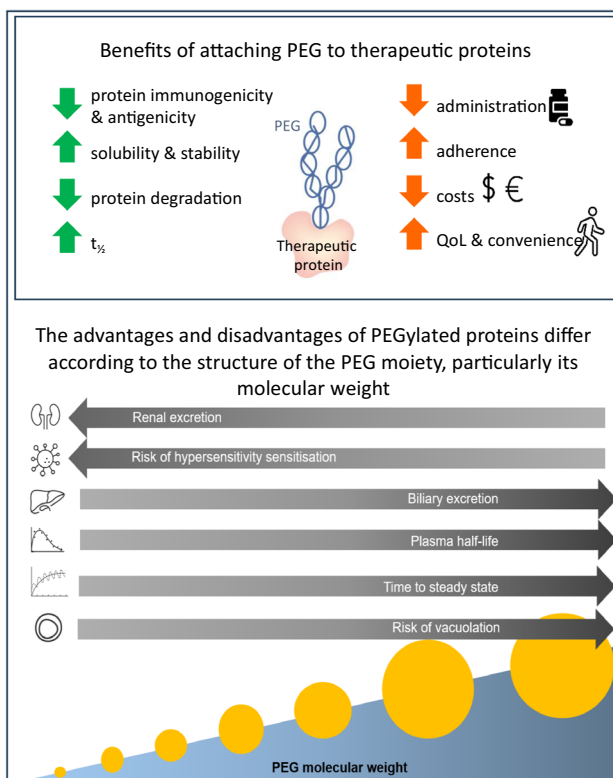
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Graphical Abstract

Clinical Pharmacokinetics

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PEG polyethylene glycol, $t_{1/2}$ terminal elimination half-life.



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Plain Language Summary

The effectiveness and safety of proteins used to treat medical conditions are affected by the immune response they may generate. Attaching a polymer to the protein can protect it from the immune system and slow down its removal from the body (elimination). A common polymer used for this purpose is polyethylene glycol (PEG). ‘PEGylation’ changes the physicochemical properties of the protein but does not affect the protein structure or biological activity. Different lengths and configurations of PEG polymer chains are used to PEGylate proteins. This article describes how the PEG size (molecular weight) affects the properties of the therapeutic protein. The higher the molecular weight, the slower the process of elimination, so products containing large PEGs are retained in the body for longer and require less frequent dosing. This is a major advantage for treatments taken over long periods (e.g. clotting factors for haemophilia), significantly improving the convenience of treatment and patient quality of life. Small PEGs are eliminated by the kidneys in urine, but larger PEGs are

eliminated through bile and eventually faeces. There is a risk that larger PEGs may accumulate in cells (a process called vacuolation), although this does not generally affect the normal functioning of the cell. Animal studies suggested that problems could arise if large PEGs formed vacuoles in the choroid plexi in the brain. However, the European Medicines Agency has set safety standards to limit this possibility, and even the largest PEGs in current use fall well within these safety standards.

Key Points

PEGylation (the attachment of polyethylene glycols [PEGs] to therapeutic proteins) reduces the immunogenicity and antigenicity of proteins; it also improves protein solubility and stability, slows degradation and increases half-life, resulting in less frequent administration, improved quality of life and convenience, and potentially better adherence and lower costs.

Paradoxically, and only with some therapeutic proteins, an immune response against PEGs may be elicited, which may interfere with the therapeutic response. In these cases, PEG acts as an hapten and the therapeutic protein as a carrier.

In some conditions, PEGs also have the potential to accumulate in cell cytoplasm in the form of vacuoles, although the pathological impact of this phenomenon is controversial and probably negligible in many instances.

The molecular weight and architecture of PEGylated proteins can have substantial impacts on their stability, immunogenicity, glomerular filtration, vacuolisation and cellular uptake; understanding these characteristics is important to fully evaluate the safety, efficacy and pharmacological profiles of PEGylated proteins.

was in 1990, when pegademase bovine (Adagen®) received regulatory approval for the treatment of severe combined immunodeficiency disease [2, 3]. Since then, many other PEGylated proteins have become available in a range of indications including inherited haemophilias, haematological malignancies, anaemia or neutropenia, paroxysmal nocturnal haemoglobinuria, autoimmune and inflammatory conditions (e.g. multiple sclerosis, Crohn's disease and rheumatoid arthritis), growth disorders (e.g. growth hormone deficiency or acromegaly), infections (e.g. hepatitis B and C virus), age-related macular degeneration, opioid-induced constipation, gout and Fabry disease [4, 5]. More recently, PEGylated drug delivery nanocarriers have been developed, including for use in mRNA vaccines—used to prevent severe acute respiratory syndrome coronavirus 2 disease [4].

While there is no doubt that PEGylation offers advantages in terms of drug delivery profiles, there are also potential disadvantages. Moreover, the nature of the advantages and disadvantages differ according to the structure of the PEG moiety, particularly its molecular weight. The aim of this article is to describe the impact of PEGylation and the nature of the PEGs themselves, specifically their molecular weight, on the characteristics of therapeutic proteins, including their immunogenicity and pharmacokinetic profile, to aid physicians in making informed decisions about the use of individual PEGylated proteins. This article will focus on the use of PEGylation to extend the half-lives of therapeutic proteins, rather than the use of PEGs to extend the $t_{1/2}$ of chemical entities, or in nanocarriers such as liposomes and micelles to enhance the delivery of drugs or RNA therapeutics.

1 Introduction

The development of therapeutic proteins has revolutionised medicine, from the initial use of animal-derived insulin in the 1920s to the current era of monoclonal antibodies and recombinant proteins for enzyme replacement, thrombolysis, hormones, growth factors, clotting factors and chemokines [1]. However, therapeutic proteins are capable of eliciting an immune response that can affect both the efficacy and safety of the protein [1].

In the 1960s, Professor Frank Davis of Rutgers University in the USA suggested that conjugating a protein to a hydrophilic polymer could help the protein to evade the immune system, enhancing its pharmacokinetic half-life ($t_{1/2}$) [2]. Thus began the use of polyethylene glycols (PEGs) as conjugates, and the PEGylation of human proteins for therapeutic use [2]. The first approval of a PEGylated therapeutic protein

2 What Are PEGylated Proteins?

PEGs are inert, thermally stable polymers with a simple structure of repeating ethylene oxide subunits (Fig. 1) [6–8]. The terminal end of the PEG chain can attach to various functional groups on bioactive molecules [9].

Adding PEGs to a protein does not markedly affect the protein structure but it can affect its physicochemical properties [7]. Water molecules readily attach to PEG chains, with two to three water molecules binding to each ethylene oxide subunit [7], thus PEGs are used to improve the water solubility of hydrophobic drugs [9]. PEGylation can also affect the thermal stability of proteins, but the impact is dependent on the characteristics of the protein and not on the size of the PEG [7].

Fig. 1 Key features of polyethylene glycols [8, 10, 11]. PEG polyethylene glycol

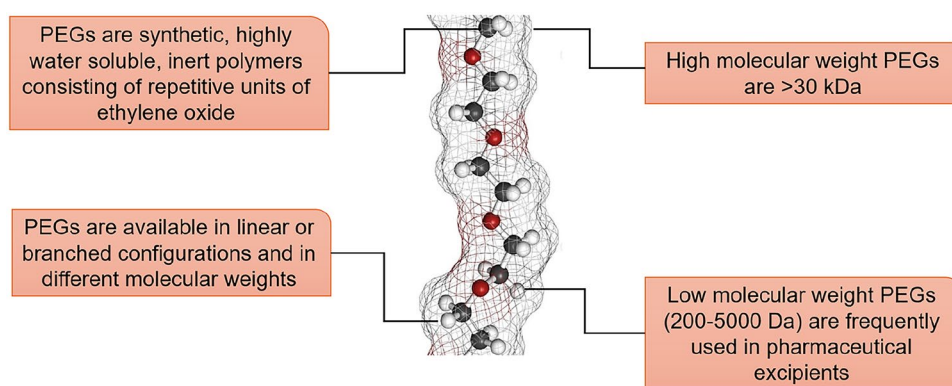
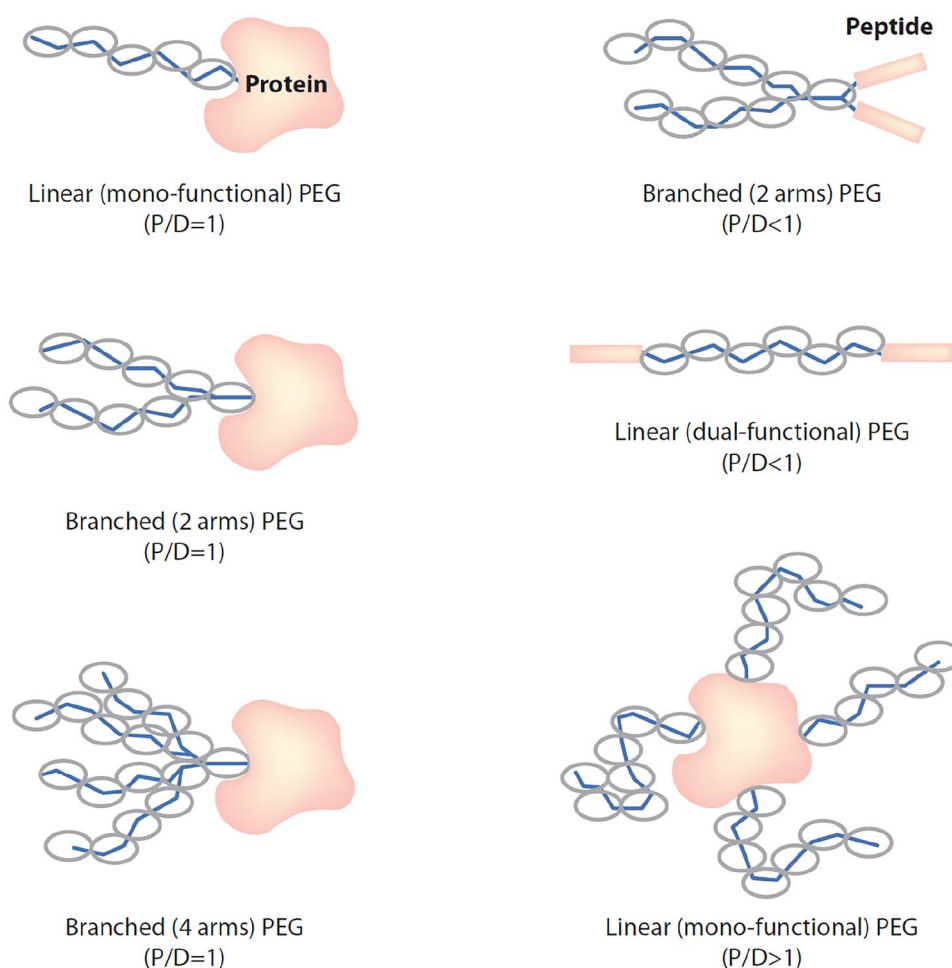


Fig. 2 Schematic diagram of linear and branched PEG chains, and the various structures of available PEGylated peptides and proteins [4, 12]. Adapted from Fig. 2 in Gao Y, et al. PEGylated therapeutics in the clinic. *Bioeng Transl Med.* 2024;9:e10600 (<https://doi.org/10.1002/btm2.10600>) [4], by redrawing the images in panel C, under a CC-BY 4.0 license (creativecommons.org/licenses/by-nc/4.0/). The circles represent bound water molecules. *P/D* PEG/drug ratio, *PEG* polyethylene glycol

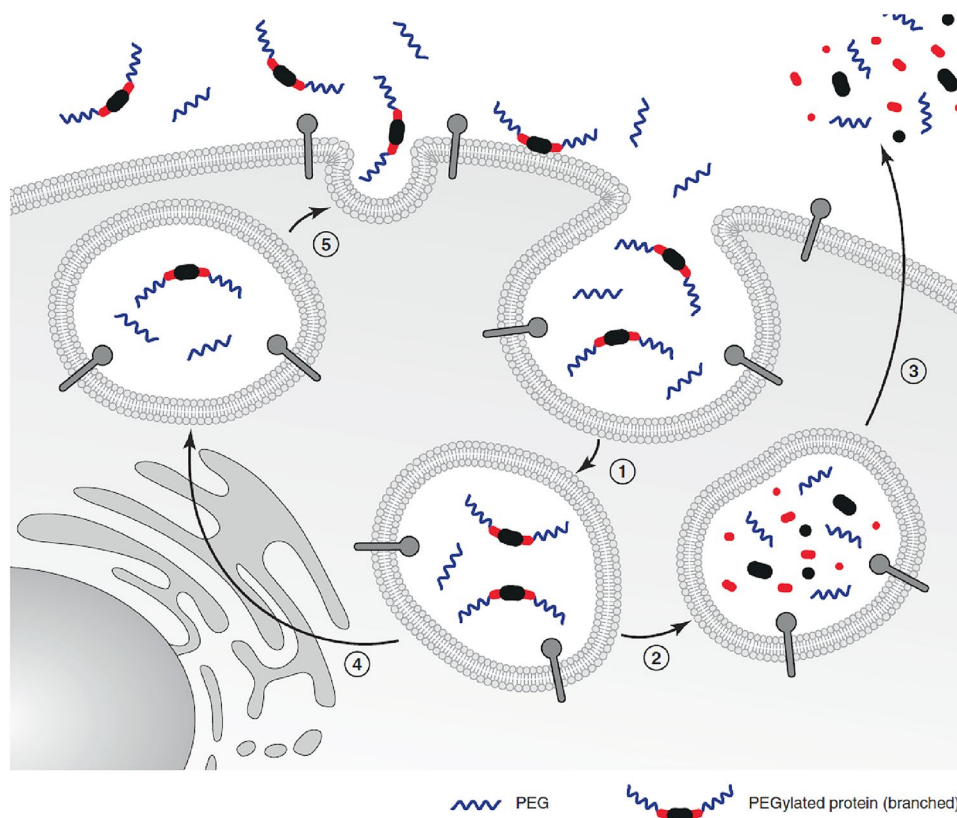


PEGs differ in chain length, structure and molecular weight [9–11]. Small molecular weight PEGs (< 3500 Da) are frequently used as pharmaceutical excipients or as components of liposomes/micelles [3, 10], whereas high molecular weight PEGs (> 5000 Da) are typically used to conjugate therapeutic proteins [10]. These high molecular weight PEGs may have a linear or branched structure and may attach to the payload in different conformations (Fig. 2)

[4, 12, 13]. As a result, the ratio of PEG to payload may be 1:1, or it may be < 1 or > 1 [4], but in most cases, PEG contributes more to the overall molecular weight than the protein does [5].

Most PEGylated proteins are administered subcutaneously and are absorbed more slowly than their non-PEGylated counterparts [10], but intravenous, intramuscular and intravitreal administration may also be used [4]. Once

Fig. 3 Cellular uptake of PEGylated proteins by pinocytosis (non-receptor-mediated) and intracellular processing [10]. Reprinted from *Drug Discovery Today*, volume 19, issue 10, Baumann A, et al. Pharmacokinetics, metabolism and distribution of PEGs and PEGylated proteins: quo vadis?, pp. 1623-1631. Copyright (2014), with permission from Elsevier. (1) The PEGylated protein undergoes pinocytosis, and (2) is transported to the endosomes and then lysosomes. (3) Within the acidic environment of the lysosome, the PEGylated protein is metabolised, and the metabolic products removed from the cell. (4) and (5) The PEGylated protein can also be removed from the cell without undergoing metabolism. PEG polyethylene glycol



in the circulation, PEGylated proteins may enter cells by either pinocytosis or receptor-mediated endocytosis (if the PEG does not prevent the protein from binding to its receptor) [10]. After cellular uptake, the protein is cleaved from the PEG moiety, which is usually released from the cell by exocytosis or by cell turnover/death (Fig. 3) [10].

While PEGs can elicit an immune response, they are for the most part biologically inert, resulting in low toxicity and minimal metabolism [10, 14]. Most of the adverse effects reported in clinical trials with PEGylated products are related to the pharmaceutical protein rather than the PEG moiety [14], as PEGs themselves undergo minimal metabolism (unlike their protein payloads) [10]. Instead, the PEG moiety usually undergoes renal elimination and is excreted in urine [10].

3 What Are the Advantages of PEGylated Proteins?

PEGylated proteins may have a number of advantages over their unmodified forms, including reduced immunogenicity and antigenicity, greater solubility, improved stability, slower protein degradation and longer residence time [13, 15]. The improved solubility is mediated by the hydrophilicity of the PEG moiety [9], but the other effects are mediated

by the PEG chains shielding the protein from immune cells, proteolytic enzymes and antibodies [15]. PEG conjugation reduces the immunogenicity of therapeutic proteins primarily through its bulky and hydrophilic nature, which enhances the hydrodynamic size of the protein and shields it from recognition by the immune system, cellular receptors or proteases. This shielding effect decreases renal, enzymatic and cellular clearance, resulting in prolonged circulation half-lives in the bloodstream. Consequently, the likelihood of immune system activation against the therapeutic protein is diminished, thereby enhancing its therapeutic efficacy and safety [16]. The size and shape of the PEG affects its ability to shield the protein, with a more marked reduction in the immunogenicity of proteins attached to large branched PEGs compared with small linear PEGs [17]. Longer residence time arises from slower absorption and prolonged $t_{1/2}$ compared with non-PEGylated forms, so less frequent dosing is generally required for PEGylated proteins than for the unmodified proteins [10, 13]. This is particularly advantageous for treatments that need to be administered periodically throughout life, such as clotting factors for patients with haemophilia. In order to prevent bleeding in patients with haemophilia A or B, prophylactic factor VIII (FVIII) and IX (FIX) levels need to be maintained above a trough level of 1% [18]. However, the $t_{1/2}$ of FVIII concentrate is between 8 and 12 h [19], and that of FIX concentrate is

between 18 and 24 h, requiring frequent administrations [18]. In contrast, PEGylated FVIII concentrates have a $t_{1/2}$ that is 1.3- to 1.6-fold higher than the $t_{1/2}$ of native FVIII [20], and maintain trough levels of FVIII at > 1% for about 6.5 days after a 50 U/kg dose. Similarly, the $t_{1/2}$ of PEGylated FIX concentrate is more than 5-fold higher than native FIX concentrate, and trough levels are maintained at > 1% for about 22 days after 50 U/kg [18]. Therefore, PEGylation not only reduces the frequency of prophylactic doses but may also help to maintain higher circulating levels of FVIII and FIX compared with non-PEGylated factor concentrates, which may reduce the severity of any breakthrough bleeding. A patient with severe haemophilia A may need infusions of FVIII concentrate every second day to maintain levels > 1%, but could maintain these levels with a PEGylated FVIII infusion twice a week or every 3–5 days, and a patient with severe haemophilia B who needs two infusions per week of FIX concentrate could maintain levels > 1% with an infusion of PEGylated FIX every 1–3 weeks [18].

Such reductions in infusion requirements represent a considerable improvement in quality of life and convenience for patients and their families [21], with fewer clinic or home-care nurse visits, and a reduced need for central venous access devices with the accompanying risks of infection and thrombosis [18, 19]. This could also translate into cost savings for the healthcare system, as well as less time off work or school for patients and their families [19]. Patients may be more willing to start prophylaxis earlier if the regimen is less burdensome, and this (along with improvements in adherence) could translate into better outcomes, such as less joint damage and the avoidance of expensive orthopaedic intervention [18, 19].

Such benefits of improved convenience and adherence with PEGylated proteins have been noted in other indications, including cancer [22]. Data in patients with hepatitis C indicate a better sustained virological response in those receiving PEGylated interferon compared with standard interferon treatment [23], an effect that may be at least partially attributable to improved adherence [24].

4 What Are the Disadvantages of PEGylated Proteins?

One disadvantage of PEGylated proteins is the potential for vacuolation (i.e. the formation of multiple round vacuoles in the cytoplasm caused by sequestration of the PEG moiety within lysosomes), which is primarily seen in macrophages but potentially affects other cell types [25]. While the protein payloads of the PEGylated moiety are broken down by lysosomes, the PEG molecule is not. Therefore, there is potential for PEG to accumulate in the cytoplasm, forming vacuoles.

Not all PEGylated proteins cause vacuolation, with an increased risk at molecular weights > 30 kDa [11]. Vacuolation appears to be minimal for PEGs of < 5 kDa [11], presumably because they are renally eliminated. In addition, the pattern of vacuolation differs according to the structural features of the PEG. For example, PEGs $\geq$ 30 kDa may be associated with vacuolation in parenchymal organs, whereas smaller PEGs (15–20 kDa) tend to be associated with vacuolation in renal tubule epithelial cells [25]. Other factors affecting the site and extent of vacuolation are PEG complexity (e.g. linear versus branched structure), dose administered, dosing frequency, duration of treatment, balance of receptor-mediated uptake versus pinocytosis, immunogenicity and turnover kinetics of affected cells [25].

Generally, the presence of vacuolation is not associated with adverse effects on organ function [11], and vacuoles containing PEG in macrophages have no significant effect on their functionality [26]. In addition, vacuolation is reversible once treatment with the PEGylated protein has been stopped, although the time for full reversal is variable [11].

Another potential disadvantage of PEGylated proteins is the formation of antibodies, although not all PEGylated biopharmaceuticals are associated with anti-PEG antibody development [11]. The immunogenicity of PEGs is directly related to the immunogenic potential of the conjugated protein, as PEGs are generally not immunogenic on their own [11]. With recombinant proteins designed to replace an endogenous protein, the likelihood of anti-PEG antibody development is greater in patients with more marked protein deficiency [5]. For example, patients with haemophilia who lack FVIII activity show a high incidence of anti-FVIII antibody development compared with patients who have some residual FVIII activity [5]. This is because B and T cells against the missing protein were not negatively selected in the individuals with residual endogenous protein production [5]. In addition, the anti-PEG antibody response increases when there is more dissimilarity between the endogenous and the recombinant protein [5]. For this reason, anti-PEG antibodies are more frequent with non-human therapeutic proteins.

To summarise, PEGylation strongly reduces the production of anti-drug antibodies (ADAs), but in some circumstances PEGs can act as a hapten, with the therapeutic protein as a carrier, causing the production of anti-PEG (rather than anti-drug) antibodies [27]. This response is largely dependent on the patient and the type of protein [27]. Clinical studies suggest that, even if these two opposite immunological effects of PEG occur, there is still a strong advantage in terms of immune response in using a PEGylated protein [28–30].

However, a more recent concern about the use and the efficacy of PEGylated proteins arises from the observation that anti-PEG antibodies have been noted in individuals who

have never received PEGylated proteins, presumably because these people were exposed to PEG through other products (e.g. as an excipient in vaccines, other pharmaceuticals or personal care products such as cosmetics or toothpaste, or as nanoparticle carriers) [11, 27]. From many studies it could be concluded that positive rates of pre-existing anti-PEG antibodies seem to be increasing over 40 years from less than 1% to 24–97.5%, with large differences among populations [31]. The positive rate and concentration of anti-PEG immunoglobulin G (IgG) are higher than those of IgM [28]. These observations partially undermine the idea that PEG is immunologically inert and raise questions about the potential effect of pre-existing anti-PEG antibodies on therapeutic responses. Pre-existing anti-PEG antibodies were suggested as a reason for poor efficacy and induction of hypersensitivity reactions in response to first-dose PEGylated therapeutics. However, contradictory outcomes about their impacts have been reported after the first administration of different kinds of PEGylated therapeutics [24]. Anti-PEG antibodies may or may not affect treatment efficacy or the pharmacokinetic profile of the PEGylated protein, but regulators require manufacturers to investigate and report on antibody formation and its potential clinical impact [11]. If anti-PEG antibodies form immune complexes with the PEGylated protein, blood clearance of the biopharmaceutical compound may be increased owing to complement activation and phagocytosis by macrophages [5]. While pharmacokinetic modelling suggests that accelerated blood clearance of PEGylated proteins only occurs when anti-PEG antibody levels exceed 500 ng/mL [32], additional clinical studies are required to confirm

that this value is a valid parameter to be used in clinical practice.

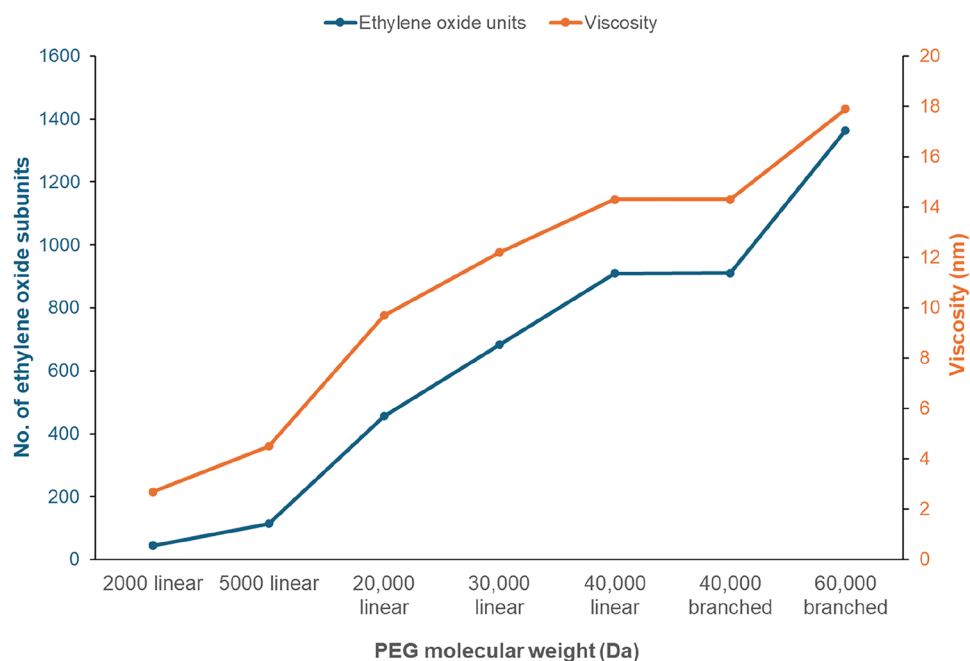
PEGylated proteins may also be associated with hypersensitivity reactions caused by complement activation and/or anti-PEG antibodies [9].

5 PEG Structure Affects Biopharmaceutical Characteristics

The pharmacokinetic profile of a PEGylated protein is markedly influenced by the structure and molecular weight of the PEG moiety [10]. Linear PEGs typically have a molecular weight of between 2 and 40 kDa, but branched PEGs are usually larger [5]. The molecular weight is directly related to the number of ethylene oxide subunits present, which also affects the viscosity of the molecules (Fig. 4) [5]. PEGs of < 6 kDa are able to pass through glomerular basement membrane pores and undergo unrestricted renal ultrafiltration [33]. PEGs of 8–30 kDa are slightly too big for unrestricted passage through these pores, but because the polymers are highly flexible and deformable, they are able to ‘snake’ their way gradually through the renal glomeruli (leading to slower elimination) [33]. Renal elimination is dramatically slower for PEGs of > 30 kDa [33], and these larger PEGs primarily undergo biliary excretion [34]. It is likely that Kupffer cells play a pivotal role in the biliary excretion of PEGs [35].

Animal models have demonstrated that the tissue distribution, area under the concentration time curve (AUC) and $t_{1/2}$ differ according to PEG molecular weight [36, 37], with

Fig. 4 The relationship between the molecular weight of PEG moieties, the number of ethylene oxide subunits and the viscosity of the PEG [5]. PEG polyethylene glycol



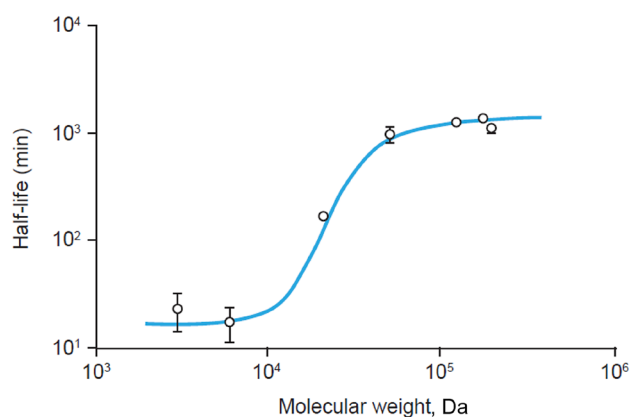


Fig. 5 The relationship between the molecular weight of PEG and its plasma half-life in a murine model [37]. In this murine study, the PEG half-life increased from 18 min to 1 day as the molecular weight increased from 3 to 190 kDa. Reprinted from *Journal of Pharmaceutical Science*, volume 83, issue 4, Yamaoka T, et al. Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice, pp. 601-606, Copyright (1994), with permission from Elsevier. PEG polyethylene glycol

a $t_{1/2}$ of 18 min for a 3 kDa PEG and 24 h for a 190 kDa PEG (Fig. 5) [37].

However, low molecular weight PEGs may also prolong the $t_{1/2}$ of therapeutic proteins. For example, pegunigalsidase alfa (the PEGylated form of α -galactosidase A) for Fabry disease uses small 2 kDa PEG chains to link two enzyme subunits to form a homodimer [38]. Pegunigalsidase alfa has an elimination $t_{1/2}$ of about 80 h [39] compared with ≤ 2 h for non-pegylated agalsidase alfa [40]. This increase is greater than would be expected by PEGylation alone (based on the animal model described above) and likely results from both PEGylation and the dimerisation of the two enzyme subunits [38]. Despite the differences in $t_{1/2}$ between pegunigalsidase alfa and non-pegylated agalsidase alfa, the two products are both administered once every 2 weeks [40, 41] because the dosing frequency of agalsidase alfa is based on its $t_{1/2}$ in the lysosomal compartment of target cells rather than its $t_{1/2}$ in the systemic circulation [42]. However, *in vitro* and *in vivo* data suggest that the PEGylated enzyme has greater biodistribution, lower hepatic clearance and is less likely to bind pre-existing neutralising antibodies compared with agalsidase alfa or beta [38, 43]. This may translate into clinical differences, but there are currently limited comparative data in patients with Fabry disease. The BALANCE study, a phase III randomised head-to-head comparison of pegunigalsidase alfa with non-pegylated agalsidase beta, found that pegunigalsidase alfa was non-inferior to agalsidase beta for the primary endpoint of rate of change in renal function at 2 years, but was associated with a lower rate of infusion reactions [44].

Because PEG is slowly cleared from the circulation, plasma and tissue levels tend to rise until steady-state is reached, at which time there is no further increase or accumulation of PEG with further doses [45]. As with other pharmacokinetic parameters, time to reach steady-state increases with higher PEG molecular weight [45], which reflects the general rule that the steady-state depends on the $t_{1/2}$ of the product.

Molecular weight is not the only factor determining the pharmacological fate of PEGylated proteins. Depending on the structural conformation, PEGylation may prolong the circulating residence time of proteins by protecting them from enzymatic degradation or recognition by immune defences [46].

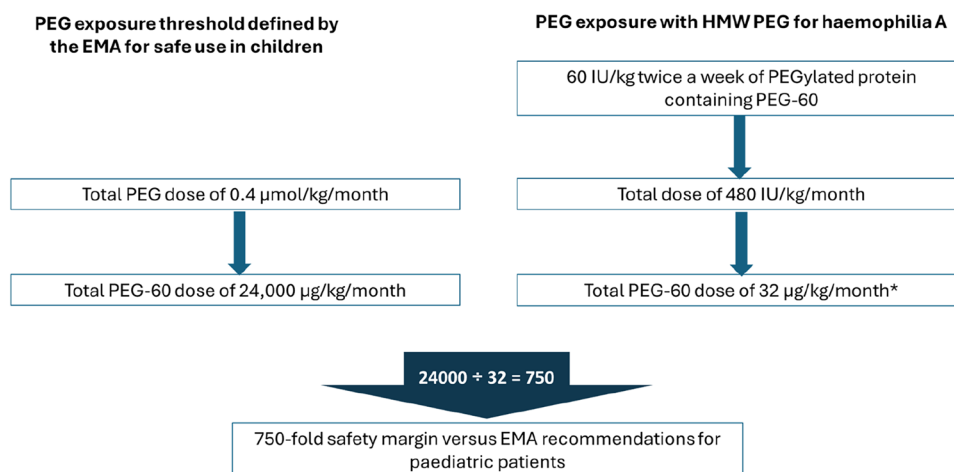
6 PEG Structure Affects Safety

As described above, vacuolation may develop as a result of PEG accumulation in lysosomes, although generally without adverse effects on organ function [11]. However, the development of vacuolation in choroid plexus ependymal cells (the key source of cerebrospinal fluid) in animal studies using PEGs of ≥ 40 kDa raised concerns about the potential for vacuolation at critical sites. As a result, the European Medicines Agency (EMA) developed guidance for the safety testing of PEGylated proteins in children [47]. At that time, only one of the five PEGylated products available in the European Union (EU) was approved for use in children [47], but since then a number of PEGylated products have been developed that may be of benefit to paediatric patients.

According to the EMA report, vacuolation in the choroid plexus developed when three conditions were fulfilled: (1) the animals were cynomolgus monkeys, (2) the PEG moiety had a molecular weight ≥ 40 kDa, and (3) the monthly PEG exposure was ≥ 0.4 $\mu\text{mol/kg/month}$ [47]. Based on these requirements, even a PEGylated product containing a large PEG moiety (e.g. 60 kDa) results in monthly exposure that is 750-fold lower than the EMA threshold for safety in children (Fig. 6) [48].

PEGs may induce different types of hypersensitivity reactions. Complement activation-related pseudoallergy (CARPA) has been reported with PEGylated proteins, and is linked to pre-existing anti-PEG antibodies [9]. However, most reports of type 1 (IgE-mediated) hypersensitivity involve PEGs administered orally as pharmaceutical excipients or bowel lavage solutions [49]. The risk of hypersensitivity reactions appears to be affected by the molecular weight of the PEG moiety, with low molecular weight PEGs more sensitising because they penetrate the skin and mucosa more readily [9, 49]. Other critical determinants of whether a PEG elicits a hypersensitivity reaction are dose and route of exposure [9, 49]. Perhaps unsurprisingly, the most common

Fig. 6 The expected monthly exposure to PEG using standard doses of damoctocog alfa pegol, which contains a PEG moiety of 60 kDa [48] compared with the standards defined for the safe use of PEG in children by the European Medicines Association [47]. *EMA* European Medicines Agency, *HMW* high molecular weight, *PEG* polyethylene glycol, *PEG-60* polyethylene glycol with a molecular weight of 60 kDa. *Damoctocog alfa pegol contains 0.036 µg of PEG per IU



products implicated in type 1 PEG-related hypersensitivity reactions are laxatives containing PEG 3350 or 4000 used as bowel preparations for colonoscopy [50], since these agents are delivered at very high doses to patients whose mucosal integrity may be compromised [49, 51]. If PEG hypersensitivity is suspected, skin prick testing is advised [51], noting that lower concentrations of high molecular weight PEGs will be required to elicit a response because of their multivalence [49]. PEG-containing products should be avoided in patients with known hypersensitivity if suitable alternatives are available. If the hypersensitivity is ambiguous or unconfirmed, the first dose of PEG-containing treatment should be administered in a monitored setting where anaphylaxis can be promptly managed [49]. A medical alert bracelet or adrenaline auto-injector may be advisable for patients with confirmed PEG anaphylaxis because of the ubiquity of PEGs and potential for accidental exposure [51].

7 Conclusions

PEGylation has proven to be a very successful technique for improving the stability and residence time of therapeutic proteins. The biopharmaceutical properties of a PEGylated protein depend on the physicochemical characteristics of both the PEG moiety and the protein itself. PEG characteristics, such as molecular weight and architecture, can affect the biological fate of the PEGylated protein by impacting stability, immunogenicity, glomerular filtration and cellular uptake. Therefore, PEG molecular weight does matter, and knowing how PEG characteristics affect biological behaviour will enable physicians to better understand the overall safety, efficacy and pharmacological profile of the PEGylated proteins they prescribe.

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Declarations

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Conflicts of Interest Diego Maria Michele Fornasari has no conflict of interest to declare in relation to this paper.

Author Contributions Diego Maria Michele Fornasari had sole responsibility for developing the concept of this article, providing editorial direction, reviewing all drafts and approving the draft for submission.

Availability of Data and Material Data sharing is not applicable to this article as no datasets were generated or analysed.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Code Availability Not applicable.

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