Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Polyethylene glycol: Properties, applications, and challenges

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Received: October 9, 2023; revised: November 12, 2023; accepted: November 20, 2023

Abstract

Polyethylene glycol (PEG) is a versatile polymer with overwhelming properties that enhance its abundant use in different applications. It is widely used in the food industry, various biomedical and pharmaceutical applications, and many daily used products such as cosmetics. In addition, PEGylation, the covalent binding of PEG molecules to nanocarriers, proteins, peptides, or drug molecules, has been designed as a brilliant approach to improve the pharmacokinetics and therapeutic efficacy of PEGylated therapeutics. The extensive application of PEGylation in the drug delivery field is reflected by the increasing number of commercially available PEGylated therapeutics approved through the last two decades and many others are under clinical trials for sooner approval. However, anti-PEG antibodies, both induced after the treatment with PEGylated therapeutics or naturally occurring in healthy individuals, were reported to negatively affect the pharmacokinetics and the therapeutic efficacy of the administered PEGylated therapeutics through the so-called accelerated blood clearance (ABC) phenomenon. Furthermore, hypersensitivity reactions (HSRs) have been reported in several cases following the administration of PEGylated therapeutics or other PEG-containing products. In addition, PEG nonbiodegradability, PEG degradation, and toxicity associated with polymerization reaction residues are remaining challenges. As a result, numerous polymers have been developed and investigated as potential alternatives for PEG in drug delivery and other medical and pharmaceutical applications.

Keywords:
Polyethylene glycol (PEG); Anti-PEG antibodies; ABC phenomenon; Hypersensitivity reactions; PEG alternatives

1. Introduction

Polyethylene glycol (PEG) is a synthetic, versatile, and highly hydrophilic polymer with different chain lengths and numerous functional end groups (1). It has broad-spectrum applications in biophysical, biochemical, and biological research and as an ingredient in the food industry, cosmetic products as well as medical therapy (2, 3). PEG has the general formula HO–$(CH_2CH_2O)_n$–H where n is the number of ethylene oxide units (**Fig. 1**). It is synthesized through ring open polymerization reaction of ethylene oxide in methanol or water, as a reaction initiator, to produce methoxy-PEG (mPEG) or diol PEG respectively (4). The resultant PEGs polymers vary in their chain length and thus the molecular weight (5). The free lone hydroxy group in mPEG and the two free hydroxy groups in diol-PEG are easy to be modified via different activation strategies to be reactive against other chemical groups (4). PEGs have various geometries including linear, branched, comb, and star-shaped (6). PEGs have a wide range of molecular weights (200- 35000 kDa) with different physicochemical properties. They are liquids or solids with low melting points based on their molecular weight. Those with low molecular weights are clear liquids (less than 400) while those with higher molecular weights (above 1000 kDa) are solids (7).

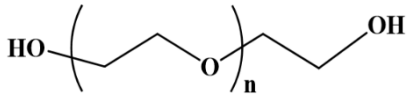

Figure 1. Chemical structure of polyethylene glycol

PEG and PEG derivatives with varying molecular weights are extensively used in various applications (8). They have been approved to be used in the food industry, cosmetic preparations, and numerous pharmaceutical applications (1). In the pharmaceutical field, PEGs are widely used as an excipient in many formulations such as oral pills, tablets and capsules, suppositories, ointment bases, aqueous solutions, laxatives, emulsions, and injectable formulations. Recently, they have been widely used in drug delivery applications through PEGylation, the conjugation of PEG molecules to drug molecules or nanocarriers, for therapeutic efficacy enhancement (9). In addition, PEGs are widely used in cosmetic products, skin disinfectants, and other household products (10). For topical applications, PEGs act as humectants, emulsifying agents, penetration enhancers, and skin conditioners (11). Thus, they are abundantly used in the manufacturing of skin lotions, toothpaste, shaving creams, face makeup products, lipsticks, bath products, face and hair care products, shampoos, soaps, and many other

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daily used household products (11). Furthermore, PEGs and many PEG derivatives have been approved by the US Food and Drug Administration (FDA) and Codex Alimentarius Commission to be used safely in food industry as emulsifiers (12). Accordingly, they are commonly used in bread, chocolates, and dessert productions (12).

However, the optimistic features and the abundant applications of PEGs and PEG derivatives are counteracted by PEG immunogenicity, PEG nonbiodegradability, and cellular vacuolization (10, 13). Several reports showed that PEGylated therapeutics and other PEG-containing products can stimulate the host immune response eliciting anti-PEG antibodies (14-16). This type of anti-PEG antibodies is commonly referred to as treatment-induced anti-PEG antibodies. In addition. Anti-PEG antibodies were reported in healthy volunteers without being treated with PEGylated therapeutics or any PEG-containing products and are known as naturally occurring or pre-existing anti-PEG antibodies (17, 18). Both types of anti-PEG antibodies, either induced following treatment with PEGylated therapeutics or naturally occurring in healthy individuals, are reported to negatively affect the safety and efficacy profiles of the administered PEGylated therapeutics (19, 20). These anti-PEG antibodies were found to induce a rapid blood clearance of the administered PEGylated therapeutics through the so-called accelerated blood clearance (ABC phenomenon). Moreover, hypersensitivity reactions (HSRs) that are reported with the administered PEGylated therapeutics have been strongly correlated to the circulating anti-PEG antibodies (21, 22). Additionally, PEG nonbiodegradability and cellular vacuolization represent critical issues to be considered in the clinical setting.

Table 1. Advantageous properties and challenges of polyethylene glycol

Advantages	Challenges
Low toxicity	PEG immunogenicity and antigenicity
Higher solubility in aqueous and organic media	PEG-related ABC phenomenon
Wide range of molar masses	PEG related CARPA
Low polydispersity index	Nonbiodegradability
High chain flexibility	Toxicity of polymerization residues
Approved by the FDA to be used in human	Cellular vacuolization

Therefore, in this review, we discuss the main advantageous properties of PEG and its applications. Moreover, the challenges that counteract its broad-spectrum applications in drug delivery and other pharmaceutical applications were reviewed.

2. Advantageous properties of PEG

For polymers to be used in the medical, and pharmaceutical fields, numerous structural and physicochemical features are to be considered. PEGs are reported to have optimistic properties that enable their widespread medical and pharmaceutical applications (**Table 1**).

2.1. Low toxicity

PEGs are Generally Recognized as Safe (GRAS) and have been approved by the FDA to be used in food industry and medical and biological uses (23). PEGs of molecular weights of 1-5 kDa are reported safe to be administered in 10% solutions to guinea pigs, monkeys, and rats (24). PEG toxicity is related to its physiological absorption, the latter depends on the PEG molecular weight. Accordingly, toxicity decreases with increasing molecular weight with limited systemic absorption. PEG with a molecular weight of less than 400 g/mol is easily absorbed through the gastrointestinal tract while less than 10% of PEG 330 g/mole can be absorbed (25).

In topical applications, PEG shows no toxicological effects with no dermatological irritation reactions and the LD50 value is higher than 10g/kg body weight (26). Only PEGs with molecular weight less than 3350 g/mole can penetrate the intact skin (27).

2.2. Higher solubility in water and many organic solvents.

PEGs are hydrophilic polymers, and their higher water solubility renders them ideal for biological applications. Therefore, PEGs are conjugated to hydrophobic molecules or nanocarriers to increase their water solubility (10). Moreover, PEGs are highly soluble in many organic solvents such as methanol, ethanol, and dichloromethane. Therefore, their end groups are easy to be modified and this modification is important to increase their applications (10). The higher solubility in both aqueous and organic media is assumed to be attributed to the important balance between the hydrophobic force resulting from the repeated ethylene units ($–CH_2–CH_2–$) and the hydrophilic interactions of oxygen in the oxirane units and that in the terminal groups (28).

2.3. PEG molecular weight

PEGs are synthesized with different chain lengths and a wide range of molecular weights. However, PEG molecules that are commonly used in different medical and pharmaceutical applications have a molecular weight range of 0.4 kDa to 50 kDa. Those with larger molecular weights (20-50 kDa) are commonly used to conjugate small molecules such as oligonucleotides, proteins, and peptides (29). This conjugation prolongs the circulation time of the rapidly cleared small molecules by increasing the overall size of the PEG-modified products and thus, decreasing their rapid loss through renal clearance (29). In contrast, PEG with low molecular weight (1-5 kDa) commonly conjugates larger molecules such as nanocarriers and antibodies. In this case, PEGylation protects the recognition and opsonization, and subsequent elimination by the reticuloendothelial system (RES), and decreases the degradation of biomolecules by the proteolytic enzymes (29). Moreover, PEG molecules have a molecular weight of 3-4 kDa have laxative

effects, and are commonly used in colonoscopies such as MoviPrep and GolYTELY (30).

2.4. Low polydispersity

The polydispersity index (PDI) is a crucial prerequisite parameter for polymers to be used in the medical and pharmaceutical fields. The PEGs have a PDI of 1.01 for PEGs of molecular weight less than 5 kDa and 1.1 for PEGs of molecular weight higher than 50 kDa (4). This low PDI is important for polymer homogeneity and *in vivo* reproducibility and decreased immunogenicity when PEGs are conjugated with nanocarriers or other therapeutics (31).

2.5. High chain flexibility

One important property of the PEG chain is its high flexibility and mobility. This results in an increased number of PEG chain conformations which is thermodynamically favorable (32, 33). Moreover, two molecules of water can be strongly attached to each ethylene glycol unit in the PEG chain increasing the overall hydrodynamic radius (34). Yang et al. found that PEGylation of single-chain antibody Fv (scFv) has a molecular weight of 20 kDa using branched PEG of molecular weight 30 kDa resulted in a PEGylated product of calculated molecular weight of about 65 kDa (20 for branched PEG and 25 for scFv) (35). However, this PEGylated product showed an apparent molecular weight of 670 kDa which represents about 10 folds higher than the calculated molecular weight. This increase in the apparent molecular weight of the PEGylated product is attributed to PEG chain flexibility and the ability of PEG to attract water molecules. Consequently, PEGylation could increase the apparent molecular weight to about 5-10 folds higher than the naked molecules (9, 36).

3. Applications of PEG

PEG has a long history of usage in the medical and pharmaceutical fields. PEG of molecular weight range 0.2-9.5 kDa has been approved by the FDA for human uses (37). Moreover, PEG of molecular weight up to 10 kDa has been used in cosmetics and many other pharmaceutical applications without any safety problems reported (8). Nowadays, PEG and its derivatives are widely used in many medical and pharmaceutical applications.

3.1. Medical applications

Owing to the higher water solubility, non-toxicity, biocompatibility, and low immunogenicity of PEG and its derivatives they are widely used in the medical field for wound treatments, equipment material in surgical operations, and many other applications (37).

PEGs of high molecular weight are used in organ transplantation as preservatives and restoring agents to prevent apoptosis (37, 38). Valuckaite et al. (38) found that PEG of a molecular weight of 15-20 kDa represents a potential adjuvant to histidine-tryptophan-ketoglutarate (HTK) solution in organ transplantation that could prolong the survival time of implant during organ harvest. Also, Tokunaga et al. showed that PEG could produce an immunosuppressive effect and donor antigens tolerability during organ transplantation (39). In blood banks, PEG is also used in pretransfusion tests, it shows higher sensitivity than the low ionic-strength solution in detecting antigens and antibodies in blood samples (40). In addition, PEG was found to repair the neuronal membrane and reduce lipid peroxidation in spinal cord injury *in vitro*. Luo et al. (41) reported that the addition of PEG immediately to spinal cord injury for 5 minutes resulted in the sealing of neuronal membrane, decreasing the oxidative stress and increasing the levels of glutathione at the site of injury and its surrounding segments in guinea pigs. The same authors, in a separate study, found that the addition of PEG-2000 immediately to spinal cord injury decreased the oxidative stress and calcium levels and was able to penetrate the cytoplasm of the injured cells and gather around the organelles. Also, it decreased mitochondrial permeability and prevents its apoptosis (41).

Furthermore, PEGs are used indwelling biomedical device coating to render their surfaces smooth, inert against the host immune system, and decrease biofouling such as bacterial adhesion (1). As a result, PEG-coated biomedical devices showed improved biocompatibility and limited thrombogenicity (42).

3.2. Pharmaceutical applications

In the pharmaceutical felid, PEG is used in many conventional formulations either as an additive or an active component. In nanotechnology, it found its use as a promising tool as a stealth coating polymer for nanocarriers and biomolecules such as proteins and peptides to decrease their blood clearance and reduce immunogenicity through the so-called PEGylation.

3.2.1. PEGylation

PEGylation means the covalent conjugation of PEG to biomolecules such as therapeutic proteins, or nanocarriers such as micelles, liposomes, lipid nanoparticles, dendrimers, and small molecules (43). The concept of PEGylation was first used by Abuchowsky et al. late in 1970 (44). They found that the conjugation of PEG to bovine serum albumin (BSA) or liver catalase enzyme prolonged their half-lives without inducing any immune responses in rabbits (45). Since then, PEGylation massively developed and broadly applied in the pharmaceutical field as a brilliant technique for drug delivery (46). PEGylating was basically designed to improve the pharmacokinetic properties of the PEGylated products and consequently improve their therapeutic efficacy (9, 31). PEGylation could increase the water solubility of the poorly water-soluble conjugate due to the hydrophilic properties of PEG molecules and prevent self-aggregations and hence, improve the physical stability (47). For nanocarriers, PEGylation provides a steric hindering effect which decreases the interactions of the PEGylated therapeutics with cells of the mononuclear phagocyte system (MPS) and blood proteins. This results in a prolonged circulation half-life and hence, improved therapeutic efficacy (37). For proteins and other biomolecules, PEGylation was designed to reduce immunogenicity, shield sensitive molecules away from the proteolytic enzymes, and thus decrease their rapid degradation (43). In addition, the attached PEGs chains are able to attract water shell that increases the overall hydrodynamic size of the PEGylated products and thus prevent the rapid loss by renal clearance (**Fig. 2**) (34). Consequently, PEGylated therapeutics show higher stability, and fewer adverse effects with enhanced pharmacokinetic properties when compared with their naked ones (31, 43). To date, numerous PEGylated proteins, peptides, and nanocarriers have been approved by the US FDA and are available for clinical applications (**Table 2**).

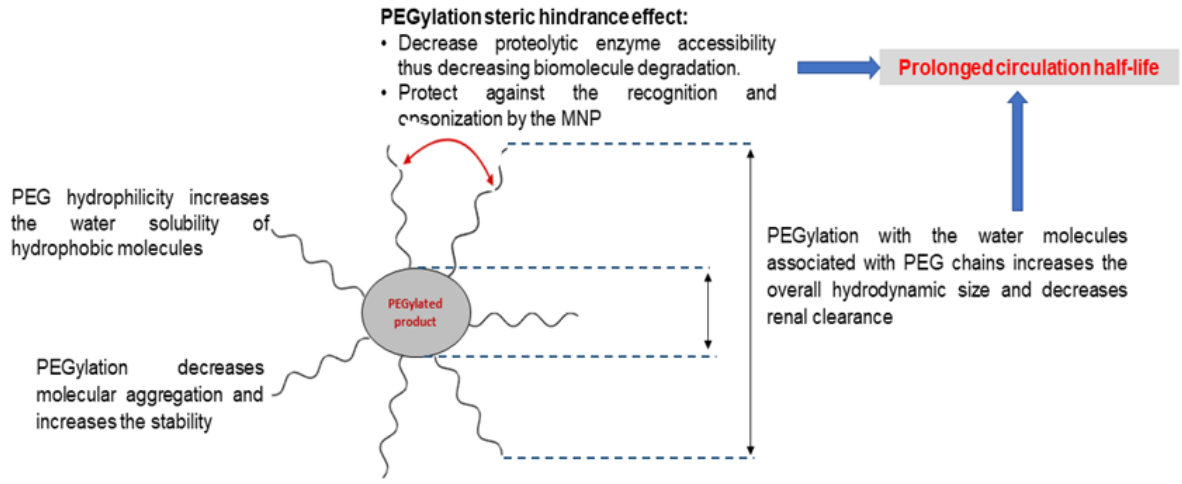

Figure 2: The impact of PEGylation on the water solubility, drug stability, and circulation half-life.

Table 2. Some of the FDA-approved PEGylated therapeutics according to the approval year.

Brand & generic name	Approval year & Producer	Component	uses	Ref.
Adagen®/ PEG-ademase bovine	1990/ Enzon	Adenosine deaminase	Severe combined immunodeficiency	(48)
Oncaspar®/ PEG-aspargase	1994/ Enzon	L-asparaginase	Acute lymphoblastic leukemia	(49)
Doxil ®/ Doxorubicin hydrochloride liposomes	1995/ OrthoSchering-Plough	Doxorubicin HCl	Ovarian cancer, Kaposi sarcoma, and breast cancer.	(50)
PEGasys®/ **PEG-interferon alfa-2a**	2001/ Hoffmann-La Roche	Interferon alfa-2a	Chronic hepatitis B & C	(51)
Neulasta®/ Pegfilgrastim	2002 / Amgen	Granulocyte colony-stimulating factor (G-CSF)	Chemotherapy-induced neutropenia	(52)
Cimzia/ Certolizumab pegol	2008/ UCB, Inc	Anti-TNFα	Rheumatoid arthritis	(53)
Sylatron / PEG-interferon α-2b	2011 / Merck	Interferon α-2b	Melanoma	(54)
Onivyde / Irinotecan liposome	2015/ Merrimack Pharma.	Irinotecan	Metastatic pancreatic adenocarcinoma	(55)
Rebinyn/ Coagulation factor IX	2017/ Novo Nordisk	Recombinant coagulation factor IX	Hemophilia B	(56)
Palynziq / Pegvaliase	2018 / BioMarin Pharma.	Phenylalanine ammonialyase	Phenylketonuria	(57)
Esperoct / Turoctocog alfa pegol	2019 / Novo Nordisk	Recombinant coagulation factor VIII	Hemophilia A	(58)
Ziextenzo / Pegfilgrastim-bmez	2019 / Sandoz	G-CSF	Chemotherapy-induced neutropenia	(59)
Spikevax / COVID-19 vaccine	2020 / Moderna	mRNA	COVID-19 vaccination	(60)
Comirnaty®/ BNT162b2	2021 / Pfizer-BioNTech	mRNA	COVID-19 vaccination	(61)
Skytrofa / Lonapegsomatropin	2021 / Ascendis Pharma	Somatropin	Pediatric patient with growth failure	(62)

4. Challenges of PEG applications

Despite the profound increase of PEG and PEGylated therapeutics applications in pharmaceutical research and even in clinical settings, however, this also raises the likelihood of potential adverse reactions occurrence. Unfortunately, the disadvantages of PEGs are hardly mentioned. Therefore, the potential adverse reactions related to the use of PEG and PEG derivatives will be discussed in the following sections (**Table 1**).

4.1. PEG immunogenicity and antigenicity

For a long time PEGs and PEG derivatives were considered inert molecules that cannot induce immune responses (63). However, a large mountain of evidence reported that the administration of some PEGylated therapeutics induced the production of specific antibodies against PEG molecules known as anti-PEG antibodies (64-67). These antibodies that are produced by the host immune response following the treatment with PEGylated therapeutics are known as treatment-induced anti-PEG antibodies.

The immunologic response against PEGylated products was first reported in 2000 by Dams et al. (68) They found that administration of PEGylated liposomes in rats or rhesus monkeys induced an immunological response that affect the second administered doses of PEGylated liposomes. The treatment-induced anti-PEG antibodies were detected not only following the administration of PEGylated liposomes but also different PEGylated products are reported to induce these antibodies such as PEGylated micelles (69), PEGylated protein (70), PEGylated, PEGylated adenovirus (71), and PEGylated lipid nanoparticles (14, 72, 73).

For anti-PEG antibody production by the PEGylated products, PEG is assumed to act as a hapten. A hapten is an inert molecule that is unable to induce antibody production unless conjugated with a larger molecule. Consequently, PEG as a hapten molecule, when conjugated with larger molecules such as proteins, peptides, or nanocarriers becomes immunogenic and is able to promote the immune response to produced ant-PEG antibodies production (74, 75).

Interestingly, the induction of anti-PEG antibodies by PEGylated liposomes and the magnitude of the ABC phenomenon were found to be strongly affected by the physicochemical properties of the administered formulations. Ishida and his group reported an inverse relationship between the lipid dose of the administered PEGylated liposomes and the levels of the induced anti-PEG antibodies (20). PEGylated liposomes with larger lipid doses (more than1 µmol phospholipids/kg) were associated with lower levels of anti-PEG antibodies (20). Moreover, the density of PEG chains on the surface of PEGylated nanocarriers was also found to inversely affect the levels of anti-PEG antibodies production (19). Higher PEG density is assumed to decrease the reactivity of PEG moiety toward the splenic B cells responsible for anti-PEG antibodies production (20).

In contrast, PEG hydrophobicity was reported to increase the immunogenic response against PEGylated formulations. Sherman et al. (75) reported that the levels of anti-PEG antibodies induced against PEGylated proteins significantly increased with increasing the hydrophobicity of the PEG terminal chains. They measured the anti-PEG antibodies levels in an animal model that received PEGylated proteins CH_3O-PEG (mPEG), HO-PEG, or t-butoxy-PEG (tBu-PEG) terminal chains. The immunogenic response was found to increase with increasing the hydrophobicity of PEG moiety. The order of immunogenicity against the administered PEGylated proteins was as follows Bu-PEG > mPEG > OH-PEG (75). Similarly, PEG chain length was reported to increase the immunogenic response against PEGylated proteins. Ishida and his group detected much higher anti-PEG antibodies against the intravenous administration of PEG_{20K}-OVA compared with those induced against intravenously administered PEG_{5k}-OVA (71).

Moreover, the time interval between the administered doses was found to influence the level of anti-PEG antibodies and the incidence of the ABC phenomenon. No ABC phenomenon was reported when the dosing interval between the first and the second dose of the administered PEGylated liposomes was less than 2 days or more than 28 days. The magnetite of ABC phenomenon was found to occur at a dose interval of 3-4 days between the first and the second dose (23).

On the other hand, anti-PEG antibodies were also reported in healthy blood donors without receiving any PEGylated therapeutics (76). These antibodies are commonly known as naturally occurring or pre-existing anti-PEG antibodies. In 1984, Richter and Akerblom were the first to detect pre-existing anti-PEG antibodies in 0.2% of healthy volunteers (77). Interestingly, a higher prevalence of the pre-existing anti-PEG antibodies was reported in later studies by using advanced and highly sensitive techniques for antibody detection. Recently, in 2019, Ehlinger et al. reported the incidence of pre-existing anti-PEG antibodies in 97.5% of healthy individuals (78). More recently, in 2021, Fang et al. (79) detected pre-existing anti-PEG antibodies in 65.3% of healthy blood donors. The wide range variation between the first report and the recent reports about the incidence of pre-existing anti-PEG antibodies may be attributed to the sensitivity of techniques used for antibody detection. However, it may indicate an actual increase in the incidence of pre-existing antibodies among healthy individuals.

Interestingly, the exact explanation for the incidence of these antibodies in healthy populations without being treated with PEGylated therapeutics remains unclear for a long time. There were assumptions that the PEG and PEG derivatives in many daily used products such as cosmetics, disinfectants, and other household products might play a role in the induction of the pre-existing anti-PEG antibodies (1, 10). These hypotheses were supported by many studies that reported a higher incidence of pre-existing anti-PEG antibodies in females than in males (79-81). PEGs and their derivatives are abundantly used in the manufacturing of a wide variety of cosmetics, beauty, and many other daily used products. These products are more extensively used by females than males and this might be the cause of the higher incidence of pre-existing anti-PEG antibodies in females compared with males. More recently, the role of the daily used PEG derivatives containing cosmetics on the induction of anti-PEG antibodies has been confirmed by Ibrahim et al. (82). They detected anti-PEG IgM antibodies in a mouse model after being topically treated with cosmetic products containing PEG derivatives. These results provide clear evidence of the role of these products in the induction of pre-existing anti-PEG antibodies among healthy individuals.

Importantly, despite PEG immunogenicity currently attracting more attention, however, many PEGylated therapeutics have been approved and reached the market with no linked serious immunogenic responses have been reported (83).

4.1.1. Anti-PEG antibodies induced accelerated blood clearance (ABC) phenomenon

Anti-PEG antibodies which are induced following the administration of some PEGylated therapeutics have been widely reported to negatively affect the pharmacokinetic behavior of the second administer dose through ABC (84, 85). The ABC phenomenon was first reported by Dams and his group (68). They found that the second dose of PEGylated liposomes administered in rats and monkeys was rapidly cleared from circulation when administered within 5-21 days from the first dose. Therefore, PEGylated liposomes lost their characteristic long circulation half-life when repeatedly administered in the same animal within a certain period (84). Later, a huge number of studies reported the induction of anti-PEG antibodies and subsequent ABC phenomenon for the second doses following the treatment with PEGylated products in animal models and clinical settings (19, 86-88). In addition to PEGylated liposomes, different PEGylated products have been reported to induce the ABC phenomenon via anti-PEG antibodies production. This includes PEGylated proteins (89), PEGylated polymeric nanoparticles (90), PEG-modified micelles (90), PEG-modified microemulsion (91),

PEGylated lipid nanoparticles (92) and PEGylated exosomes (93).

Furthermore, the ABC phenomenon has been reported with many of the FDA-approved PEGylated therapeutics. For example, one-third of the pediatric patient treated with PEG-asparaginase (FDA-approved PEGylated L-asparaginase) developed anti-PEG antibodies and subsequent rapid blood clearance and attenuated therapeutic efficacy of the subsequent dose of PEG-asparaginase (94). Similarly, pegintron (FDA-approved PEGylated interferon alpha 2a) and pegloticase (FDA-approved PEGylated uricase) were found to induce anti-PEG antibodies production in the treated patients. The treatment-induced antibodies were associated with the ABC phenomenon for the subsequently administered doses and thus decreased their therapeutic efficacy. (83, 86). Interestingly, the ABC phenomenon was also reported in healthy volunteers having pre-existing anti-PEG antibodies. Pegloticase showed a shorter circulation half-life with reduced therapeutic efficacy when administered in the presence of the pre-existing anti-PEG antibody (95). Also, the pre-existing anti-PEG antibodies significantly decreased the blood concentration and therapeutic efficacy of PEG-asparaginase without affecting the naked non-PEGylated enzyme (96, 97).

Indeed, the ABC phenomenon can be illustrated as follows, both treatment-induced and naturally occurring anti-PEG antibodies can selectively bind to the PEG moieties of the administered PEGylated therapeutics. This binding activates the complement system which induces opsonization of the PEGylated therapeutics via the C3 fragment. The opsonized products are rapidly uptaken by Kupffer cells and accumulated in the liver (**Fig. 3**) (19).

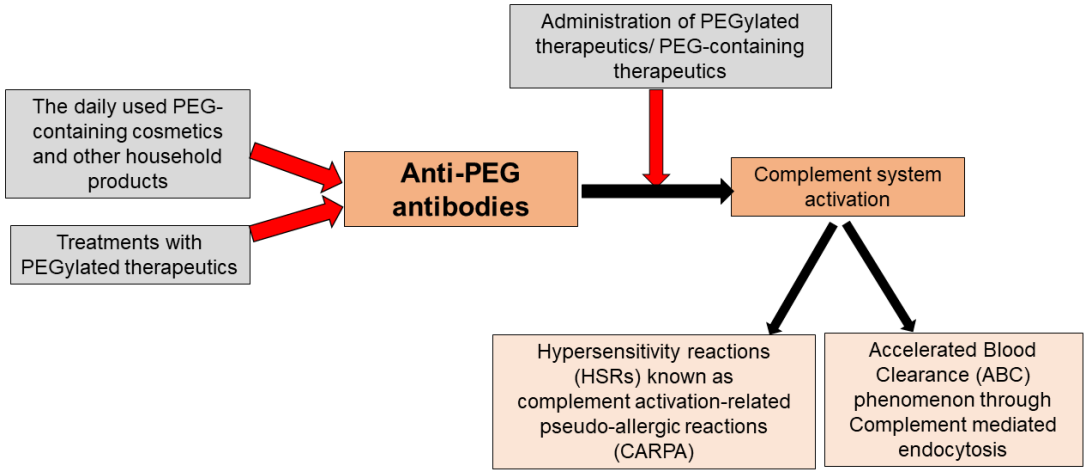

Figure 3. Anti-PEG antibodies induced accelerated blood clearance (ABC) phenomenon and hypersensitivity reactions

Table 3: Some of the reported cases of PEG-related HSRs

Source/exposure route	Gender/age	PEG included	Manifestations	Treatments	Ref.
Gaviscon double action tablet	Female/ 42	PEG 20000	Generalized urticaria and lip angioedema	Patients received anti-allergic medications (glucocorticosteroids and chlorpheniramine)	(113)
Gaviscon Double Action tablets	Male/20	PEG 20000	Periorbital swelling, nasal congestion, generalized urticaria, and dyspnea	Recovered in the intensive care unit	(113)
Depo-Provera®	Female/ 51	PEG 3350 and polysorbate 80)	Generalized pruritus, swelling (hands and feet), profuse vomiting, and severe hypotension	IM adrenaline (0.5 mg)	(113)
Depo Medrol (IM)	Female/ 52	PEG 3350	Flushing, pruritus (palms and feet), and light-headedness.	Two doses of adrenaline IM (0.5 mg), hydrocortisone, and chlorpheniramine	(113)
Moviprep oral	Female/70	PEG 3350	Plantar and groin pruritus, dyspnea, and light-headedness.	No treatment	(113)
Colyte® Oral solution	Male/39	PEG-3350	Loss of consciousness, dyspnea, severe respiratory distress	Recovered in the intensive care unit after IM administration of Epinephrine (0.3 mg)	(114)
Pfizer/BioNTech COVID-19 vaccine IM PEGylated nanoparticles	Female/52	PEG 2000	Cough, tachycardia, hypotension and loss of consciousness.	Two doses of IM adrenaline (0.5 mg), and IV intravenous hydrocortisone (200 mg) and chlorphenamine 10 mg	(111)
SonoVue® IV	Male/62	PEG 4000	Dyspnea, rash, sweating, loss of consciousness, and severe hypotension.	IV administration of epinephrine (100 µg) and fluid resuscitation	(115)
Transvaginal ultrasound gel.	Female/41	PEG 8000	Flushing, dyspnea, cough, generalized urticaria	IM epinephrine followed by IV diphenhydramine	(116)
Povidone–iodine gel	Male/33	PEG 400, PEG 4000, and PEG 6000	Immediate anaphylactic shock	IV corticosteroids and adrenaline	(117)

4.1.2. Anti-PEG antibodies induced hypersensitivity (HSRs)

The development of hypersensitivity reactions (HSR) toward the nanosized drug delivery systems is still challenging regarding their clinical applications (71). Even though PEGylation is considered a favorable approach to reducing immunogenicity, however, numerous studies reported the incidence of HSRs following the administration of some PEGylated therapeutics (98-100). PEGylated therapeutics have the ability to prime the host immune system by complement system activation inducing acute HSRs (99, 101). Such HSRs were reported with different PEGylated products such as PEGylated liposomes, PEGylated micelles, and PEGylated proteins (102, 103). These reactions are non-IgE mediated reactions that occur following the first exposure to the PEGylated therapeutics and involve complement system activation thus, they are well known as complement activation-related pseudo-allergic reactions (CARPA) (99, 104, 105). As a part of the immune system, complement system activation started when blood protein C3 is adsorbed on a certain surface, thus conformational changes occurred which trigger its hydrolysis in biochemical cascades into C3 and C5 fragments. The resultant fragments can label the foreign body by binding to its surface. Consequently, leukocytes, macrophages, and mast cells that have specific receptors for these complement fragments are activated to remove the labeled foreign bodies and release inflammatory mediators including histamine and proinflammatory cytokines (**Fig. 3**) (106).

The most common symptoms of PEG-induced HSRs include purities, flashing, urticaria, fever, and in severe cases, anaphylactic shock may result (8). Several reports showed that Doxil® can induce complement system activation leading to HSRs in animals and clinical studies (21, 107, 108). Furthermore, fetal HSRs were reported against PEGinenstide (PEGylated peptide) in 0.02% of the treated patients leading to its rapid withdrawal in 2013 after only one year from its approval late in 2011(109, 110). More recently, during the first day of the COVID-19 pandemic vaccination in the UK, there were two cases of anaphylaxis a few minutes following the IM administration of Pfizer/BioNTech COVID-19 messenger RNA (mRNA) PEGylated lipid nanoparticles vaccine (111).

Interestingly, PEG-induced HSRs are not restricted to only PEGylated therapeutics. However, many studies reported HSRs following the administration of pharmaceutical products containing PEG with different molecule weights. This includes the intravenous, intramuscular, oral, subcutaneous, and vaginal administration of PEG-containing products. **Table 3** summarizes some of the reported cases of PEG-induced HSRs with different dosage forms.

Moreover, PEG molecular weight was found to significantly influence the severity of the PEG-related HSRs. According to Hesselbach et al. (112) the response to the skin prick test showed that 1% PEG 8000 induced urticaria, cough, and big weal/fare response, while small weal/fare response resulted from 1% PEG-400 however, 1% PEG 3000 gave a negative result.

4.2. PEG non-biodegradability

One main disadvantage of PEG is its non-biodegradability (10). Reports have documented a slow degradation of PEG only by aldehyde dehydrogenase, alcoholic dehydrogenase, and cytochrome P-450 (118-120). Accordingly, it is advisable to use PEG molecules having low molecular weights. However, PEG of molecular weights less than 400 Da were reported to be toxic due to their oxidation into toxic metabolites (diacid and hydroxy acids) by alcohol and aldehyde degenerate (121). Therefore, PEG molecules with molecular weights above 400 Da are preferred as oxidative degeneration was reported to decrease by increasing the PEG molecular weights (121). Importantly, molecules with larger molecular weights may exceed the threshold of renal clearance which limits their complete elimination and thus increase their accumulation in the liver. A molecular weight of 20-60 kDa is reported as a limit for nonbiodegradable polymers (corresponding to albumin excretion limits) and polymers of higher molecular weight over the limit might circulate in the blood for a longer time and accumulate within the liver (120). It seems that PEG clearance depends basically on molecular weights. PEGs of molecular weight less than 20 kDa are easily secreted in urine. While those with higher molecular weights are slowly eliminated and their clearance is predominant through the liver (4).

As PEGs are nonbiodegradable polymers, they are not degraded by mammalian enzymes, therefore, after the uptake of the coated nanocarriers or molecules by the cells, in vivo accumulation of PEG may lead to impairments in cell functions in the long term (122).

4.3. PEG degradation

Stability represents a crucial property for polymers to be safely used in pharmaceutical applications to render the stability and efficacy of products during storage and treatments (4). Polymer instability can be brought by specific chemical reactions induced by water, oxygen, heat, radiation, and/or mechanical stress (10). PEG in solution or solid-state was reported to undergo significant degradation under heat (123). Scheirs et al. (124) reported a 10-fold decrease in the molecular weight of PEG (in solid state) after aging under heat (60 C) and air for 30 days (100 kDa to 10 kDa). The (infrared) IR spectra of the degradation products showed the formation of aldehydes, carboxylic acids, and alcohols. It seems that PEGs are sensitive to degradation due to the ether linkages in their structure. The ether bonds in PEG chain are more likely to be broken leading to faster degradation of PEG molecules in comparison with other polymers having carbon backbone such as polyvinylpyrrolidone (125). Therefore, stress and other degradation factors such as heat and oxygen should be considered during storage and drug formation. It is important to mention that stress degradation studies have been performed for only industrial PEGs with a molecular weight of range 50-4000 kDa. Moreover, high shear stress (up to 9 kPa) has been investigated which is significantly higher than the in vivo forces (about 1 kPa) (125, 126). However, the *in vivo* partial degradation of PEGylated therapeutics with prolonged circulation properties should not be excluded.

4.4. Toxicity of PEG polymerization residues

During PEG synthesis, it may contain polymerization residues such as ethylene oxide and formaldehyde. These compounds are classified by the International Agency for Research on Cancer (IARC) as carcinogenic substances in humans (group-1) (10). Accordingly, the European Pharmacopoeia limits the amount of ethylene oxide and formaldehyde in PEG indented for pharmaceutical applications to 1 and 3 ppm respectively. Consequently, PEG of pharmaceutical grade must be used in pharmaceutical applications.

4.5. Cellular vacuolization phenomenon.

Owing to the PEG's non-biodegradability, it is more likely to form microscopic cellular vacuolization (127). This phenomenon may be attributed to the accumulation of PEG of high molecular weight within the affected cells (128). Rudmann et al. (128) reported that after the IV administration of 10 mg/kg of PEG of the molecular weight of 40 kDa in rats for 3 months, macrophages vacuolization was observed in the lung, heart, spleen, choroid plexus. Also, vacuolization of the epithelial cells was observed in the kidney and the choroid plexus. The authors assumed that PEG vacuolization was an adaptive immune response to the administered PEG and not considered an adverse effect.

The US FDA biological license application reviews reported that 9 out of 11 of the commercially approved PEGylated proteins were associated with the formation of microscopic cellular vacuolization (129, 130). PEG cellular vacuolization was reported in non-clinical studies for PEGylated therapeutics such as Omontys, Cimazia, Krystexxa, somavert, and Macugen. The incidence of PEG vacuoles increased with increasing the cumulative dose of PEGylated therapeutics as in the case of PEGylated Cimzia which is chronically administered biweekly at a dose of 400 mg (131). PEG vacuoles were investigated in the splenic reticuloendothelial cells or phagocytic cells such as macrophages, and Kupffer cells in the liver (132). Accordingly, the European Medicines Agency (EMA) guidance recommended more caution when PEGylated therapeutics were administered to pediatric patients (130, 133).

5. Future perspectives

Despite the promising features of PEG and its abundant applications in food, medical, and pharmaceutical industries in addition to the daily used cosmetic and other household products, however, PEG's non-biodegradability, immunogenicity, and antigenicity are challenges. Furthermore, the manufacturing of PEGylated therapeutic requires an expensive clinically activated grade of PEG derivatives and thus increases the production cost (130, 134).

These large challenges associated with PEG and PEG derivatives strengthened the search for PEG alternatives. Numerous biodegradable polymers have been developed and tested in different studies as potential alternatives for PEGs. Romberg et al. (135) tested the use of poly amino acids such as Poly(hydroxyethyl-l-asparagine) (PHEA) and poly(hydroxyethyl-l-glutamine) (PHEG) for liposomes stealth coating in a rat model. They reported extended circulation life of the coated liposomes comparable to PEGylated liposomes. Moreover, unlike PEGs, the ploy amino acid polymers are susceptible to degradation by proteases.

On the other hand, PEG immunogenicity and antigenicity and the associated pharmacokinetics alteration and HSRs remain major obstacles regarding clinical applications. Therefore, special interest goes to the PEG derivatives and PEG alternatives with less or no immunogenic response.

Both linear and hyperbranched polyglycerol, a hydrophilic polymer that has structural similarity to PEG, have been employed for liposomes and hydrogel conjugation (136, 137). Abu Lila et al. (138) showed that, unlike PEGylated liposomes, the ABC phenomenon was not repeated upon the repeated administration of polyglycerol-coat liposomes encapsulating doxorubicin in an animal model (139). Later, the same group found the second dose of polyglycerol-modified lipoplex, unlike the PEGylated form, showed tumor accumulation equivalent to the first dose. They assumed that the polyglycerol-coated products could have improved the *in vivo* fate compared with PEGylated products which rapidly cleared from the circulation through the ABC phenomenon (138, 139). Also, lactose, which naturally occurs on the surface of the blood cells, has been used for coating nanocarriers with less risk of developing an immune response. Lactose-modified lipoplexes showed higher accumulation in tumor tissue compared with naked ones in a mice model. Moreover, higher drug accumulation in tumor tissue lasted for up to 24 h following the IV administration of lactose-modified lipoplexes which indicates no ABC phenomenon was induced (140). Accordingly, lactose may act as a promising alternative to PEG for nanocarrier delivery systems with less risk of stimulating the immune response (141).

Recently, more interest went to the uses of PEG derivatives polymers with less or no immunological response. This might be attributed to the long history of PEG applications in human use and the large investments in PEG that already exist in the markets. Accordingly, PEG-derived polymers may represent potential alternatives to PEG itself with fewer adverse reactions. The hyperbranched bottlebrush PEG, polyoligo (ethylene glycol) methyl ether methacrylate, with its high density of ethylene oxide units represents a promising PEG-derived polymer for stealth coating (142). Ashutosh et al. (143) reported that the bottlebrush-exendin-4 conjugate (peptide used for type 2 diabetes mellitus type 2) showed no reactivity toward the pre-existing anti-PEG antibodies with enhanced therapeutic efficacy compared with the naked ones in an animal model. In addition, Liu et al. (144) found that PEGylation of liposomes and nano-emulsion with branched PEG extended the circulation half-lives compared with those PEGylated with linear PEG molecules. Furthermore, they reported neither complement activation nor ABC phenomenon upon repeated injection of these formulations in an animal model. According to the authors, the branched PEG induced less immune response compared with the linear one, hence, fewer adverse effects are expected.

Conclusion

Polyethylene glycol is a synthetic polymer widely used in medical and pharmaceutical fields in addition to the daily used cosmetics and many other household products. However, PEG immunogenicity represents a remarkable obstacle regarding the safety and efficacy profiles of PEGylated therapeutics in clinical settings. Moreover, PEG nonbiodegradability and toxicity related to polymerization residues are still challenges. Accordingly, recent research focuses on the development of biodegradable PEG alternatives that lack immunogenicity and antigenicity.

Conflicts of interest

There are no conflicts of interest for any of the authors.

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