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Liposomes and PEGylated liposomes as drug delivery systems

Marwa Mohamed*, Eman Alaaeldin, Amal Hussein, Hatem A. Sarhan

Department of Pharmaceutics, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

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Abstract

Generally, it has been thought that PEG-conjugated nanocarriers are non-immunogenic. However, many reports have revealed that unexpected immune responses occur with such PEG-conjugated nanocarriers. The Most important one is the rapid clearance of PEGylated nanocarriers upon repeated administration which is called accelerated blood clearance phenomenon involving the production of antibodies against nanocarrier components, which reduces the safety and effectiveness of the encapsulated therapeutic agent. Such immunogenicity of PEGylated nanocarriers is a potential concern in the evaluation and clinic use of PEGylated therapeutics. Accordingly, screening of the immunogenicity of nanocarriers-based therapeutics is a prerequisite before their adoption into clinical settings to disclose any possible interactions with immune system. This review gives an overview of PEGylated liposomes, immunogenicity of PEG, explanation of accelerated blood clearance (ABC) phenomenon, its mechanism, various factors affecting it and side effects of PEGylated liposomes.

Key words

Accelerated blood clearance (ABC) phenomenon, anti-PEG IgM; PEGylated liposomes, polyethylene glycol (PEG), marginal zone (MZ)

1. Introduction

Nanotechnology is defined as the manipulation of matter with at least one dimension sized from 1 to 100 nanometers and has immense applications in drug delivery system. Nanotherapeutics have shown a great impact in different pharmaceutical and medical fields such as chemotherapy, tissue engineering and gene delivery. For instance, nanocarrier systems such as, micelles, nanoparticles and liposomes afford a good opportunity to deliver poor water soluble drugs (e.g. cytotoxic drugs), genes and targeting ligands directly to their target sites with a minimal toxicity to the surrounding normal cells and tissues [1]. Other nanocarrier based drug delivery systems such as polymer-drug conjugates and liposomes have enormous biomedical applications [2].

In the field of cancer therapy, nanotherapeutics have favorable properties such as optimal size; shape and the ability to apply surfaces modification make them very interesting candidates as drug delivery systems for highly hydrophobic chemotherapeutic agents (such as doxorubicin and oxaliplatin). Moreover, their ability to encapsulate these drugs and improve their solubility profiles gives them unique preference over conventional drug delivery systems. Surface modification with polymers and targeted ligands improved their intracellular uptake and extended their circulation time in vivo, making them superior to other treatment regimens [3]. Accordingly, nano-based formulations of cytotoxic agents enhance the toxicity profile as well as the therapeutic efficacy of anti-tumor drugs when compared to the conventional drug formulations [4].

2. Liposomes and drug delivery system with liposomes

Liposomes were discovered in the 1965 by Bangham [5]. liposomes were the first nano drug delivery systems that have been successfully translated into real-time clinical applications in 1995 [6]. Liposomes chiefly consist of phospholipids which self-assembled into spherical vesicles of one or more concentric lipid bilayers enclosing aqueous compartment within the lipid membrane [1]. The vesicles exhibit either single or multiple lipid bilayers arrangements. Clinically, liposomes are considered to be the most approved controlled drug delivery system known to date; liposomes are valued for their biological and technological advantages such as; biodegradability, biocompatibility, flexible structure, their ability to enhance solubility profiles and to decrease unwanted side effects of encapsulated drugs. Moreover, many researches demonstrated that liposomes have the capability of entrapping a diversity of hydrophilic and lipophilic drugs. Also, they are a versatile drug delivery system when chemically modified with specific surface ligands [1, 7]. There are several types of liposomes including, stimuli-triggered liposomes[8], nebulized liposomes [9], long circulating liposomes [10], elastic liposomes for topical [11], oral [12] and transdermal delivery [13] and covalent lipid-drug complexes.

In the last decade, there is a promising approach to develop conventional liposomes through applying several advanced approaches such as modification of liposomes bilayers with certain polymer (like stealth liposomes); enhance their elasticity (like transferosomes) and recently employing targeting ligands.

**** Correspondence: Marwa Mohamed***

Tel.: *+00201092416265****; Fax:*** *+20 862369075*

Email Address: *marwa_msm88@yahoo.com*

Liposomes applications involve treating gliomas, different types of cancers (e.g. breast cancer), heart diseases as well as managing infectious and inflammatory disorders [6] **(Table 1)**. In the field of cancer therapy, there are many liposomal formulations that have been approved for the treatment of cancer, involving DepoCyte® (Cytarabine liposomes) [14], DaunoXome® (Daunorubicin liposomes) [15], Marqibo® (Vincristine liposomes) [15] and Myocet® (Doxorubicin liposomes) [16]. For example, arsenic trioxide (ATO) encapsulated liposomes have a potential for targeting HPV-infected cells for the aim of treating human papillomavirus (HPV)-associated cancers. It was reported that ATO loaded liposomes have demonstrated a higher therapeutic efficacy against HPV than pure ATO through decreasing the levels of HPV-E6 oncogene and stimulating Hela cells apoptosis. Moreover, the study has shown that ATO liposomes have the ability to target HPV-infected cells and transforming them from cancer cells to healthy one with minimal toxicity [17].

Table 1: Commercially available liposomes-based pharmaceuticals

Trade name	Particle type	Active ingredient	Uses
Doxil® Caelyx® [18]	PEGylated liposomes	Doxorubicin	Ovarian cancer, Kaposi sarcoma, Multiple myeloma, Breast cancer
Ambisome® [19]	Non-PEGylated liposomes	Amphotericin B	Fungal infections
DaunoXome® [20]	Non-PEGylated liposomes	Daunorubicin	Kaposi sarcoma
Visudyne®[21]	Non-PEGylated liposomes	Verteporfin	Age-related macular degeneration

3. PEGylation of liposomes

PEGylation is a technique used to coat the surface of nanocarrier drug delivery systems by polymer such as polyethylene glycol (PEG), which is a hydrophilic linear, non-toxic and non-ionic ether diol with the molecular formula ($C_{2n}H_{4n+2}O_{n+1}$) [22]. PEGylation of liposomes can be performed by two methods; either *via* inclusion of PEG-lipids throughout liposomes preparation (pre-insertion method) or *via* mixing aqueous solution of PEG lipids with the preformed liposomes (post-insertion method) [23]. PEGylation of liposomes often produced by using certain PEG-phospholipid like, methoxy-PEG-distearoyl phosphoethanolamine with the molecular weight of 2000 (mPEG-$DSPE_{2000}$) which is considered to be the most utilized PEG lipid. MPEG-$DSPE_{2000}$ has the ability to interact with the lipid bilayer via hydrophobic interaction. PEGylated liposomes are called stealth liposomes (SLs) due to their stealth properties as they prevent the adsorption of plasma proteins and/or recognition by the immune system. Thereby, they are able to decrease the interactions of liposomes with the mononuclear phagocyte system (MPS), resulting in prolonged biological half-life of drug delivery system [16, 24]. PEG is an effective steric stabilizer due to its high hyrophilicity, electric neutrality and absence of functional groups [25]. Each ethylene glycol subunit attracts two or three water molecules. Thus, PEG forms a water shell around therapeutic drug molecules and makes a nonspecific steric hindrance barrier preventing their binding to serum proteins (opsonins) [26]. Moreover, PEG has the ability to prevent cellular as well as humoral immunogenicity [27].

PEGylation efficacy is highly related to both the length and the coating density of PEG on liposomes surfaces, as very short PEG (ex: PEG1K or less) lack the ability to prevent the interaction of PEGylated liposomes with serum proteins thereby cannot effectively prolong the circulation time. On the other side, very long PEG (ex: PEG5K or more) have a great adverse effect which is decreasing the cellular uptake and the endosomal escape of liposomes. Consequently, medium sized PEGs (about PEG2K) are the most suitable one [23]. When non-PEGylated liposomes are injected, opsonins are adsorbed into their surfaces initiating their ingestion by phagocytic cells in the liver and spleen, consequently a short circulation time is expected [28, 29]. Moreover, the interaction between PEGylated liposomes and macrophages relies on two factors; steric hindrance of PEGylated liposomes toward macrophages and interaction of plasma proteins and PEGylated liposomes. Increasing the density of PEG used for liposomes surface coating results in more brush like structure thus improve the steric barrier effect and prolong circulation time of PEGylated liposomes [23].

Another interesting type of PEGylated liposomes in which PEG-dendron-phospholipids have been used to produce more stable drug delivery system than stealth liposomes is called super stealth liposomes (SSls). The structure of dendron serves as a platform that allows many phospholipids to link to a single PEG chain. This structure improves the strength of interaction between mPEG-phospholipid and bilayers of phospholipid, thus producing more stable liposomes [16]. Moreover, in the field of cancer treatment, PEGylated liposomes therapeutics provided a promising platform as a drug delivery system. For example, it was demonstrated that single treatment of murine colorectal tumor model with oxaliplatin (l-OHP) encapsulated PEGylated liposomes improved the oxaliplatin tumor accumulation and altered the distribution pattern of the tested doses of PEGylated liposomes in the intratumor tissues. In addition to, the combination therapy with metronomic S-1dosing (which is multiple administrations of anticancer drugs at doses mainly under the maximum tolerated dose (MTD) without extended drug-free breaks) and l-OHP based PEGylated liposomes extensively improved the apoptotic effect of liposomes on tumor cell and increased intratumor accumulation of cytotoxic drugs. Therefore, some reports showed that this combination treatment enhanced the therapeutic profile of S-1 and 1-OHP

based PEGylated liposomes in the (C26) murine colorectal tumor model [30].

3.1. Importance of PEGylation

PEGylation is one of the most important techniques that have been clinically used for over 25 years to enhance the pharmacokinetic (PK) properties of drugs and to decrease the immunogenicity of nanotherapeutics [31]. Many pharmaceutical products based on PEGylated therapeutics have been reached to the market, also new PEGylated therapeutics are being produced to extend drug half-life [32]. PEGylated drug delivery systems are fundamental carrier in cancer treatment. Advantages of PEGylation include, increasing plasma half-life of a variety of therapeutics like, proteins, enzymes, small molecular drugs, nanoparticles and liposomes. This could be achieved via preventing their removal from blood stream by opsonins resulting in improving their therapeutic index. Using PEG of different chain length, shape, density and molecular weight for surface modification of nanocarrier showed a significant potential for development of advanced drug delivery system for cancer therapy [33].

PEGylated protein conjugates are commonly used as therapeutics. The most approved proteins conjugates by the Food and Drug Administration (FDA) are covalently linked to poly (ethylene glycol) (PEG). These PEGylated therapeutics have a prolonged circulation time in the bloodstream, resulting in less frequent dosing thus improving patients compliance [34]. Although polyethylene glycol (PEG) provide a great advantage for bioactive molecules in pharmaceutical and biological applications (through increasing protein stability, therapeutic efficacy and shelf life), the optimal location of PEG attachment onto proteins is still not well elucidated. Wilding et al reported that PEGylation efficiency, protein stability, and protein activity changed according to PEGylation site, hence further studies should be done for clinical validation [35, 36].

4. Pharmacokinetic of PEGylated liposomes

It was proposed that nanocarrier-based drugs (such as PEGylated liposomes and non-PEGylated liposomes) are mainly removed from the blood through phagocytosis by the elements of the reticuloendothelial system (RES). RES is placed basically in the liver (hepatic kupffer cells), spleen and bone marrow including, macrophage, monocytes as well as dendritic cells. As a result, factors which influence RES activity could influence the clearance, toxicity, and response of PEGylated liposomes [37]. Nanomedicine and liposomes after systemic administrations are removed from circulatory system rapidly by the aid of immune system mainly macrophages of the RES. Hence, the characteristic pharmacokinetics of nanocarrier systems affects their delivery [38]. For example, PEGylated liposomes encapsulating cytotoxic drugs are proven to enhance tumor delivery of them through modifying the pharmacokinetic and the distribution patterns of encapsulated drugs after intravenous injection. Chemotherapeutic agents are found to leak slowly from blood vessel and accumulate preferentially in tumor tissues resulting in an improved anticancer activity and diminished toxicity when compared to conventional cytotoxic drugs [37, 39, 40]. Consequently, nanocarrier drugs should be designed probably to prevent these clearance mechanisms and to avoid complement activation in order to increase the half-life of anticancer drugs in the blood stream [39].

5. Tumor accumulation of PEGylated liposomes via EPR effect

Neovascularization is essential for tumor progression, to ensure an adequate supply of oxygen and nutrients which support their growth. The imbalance of angiogenic regulators create an abnormal vascular network of tumor tissue that is highly disorganized and characterized by dilated, leaky blood vessels and exhibiting poor lymphatic drainage [30]. Such unique pathophysiologic properties of tumor vasculature provide nano-sized drug carriers with long circulation time. For example, PEGylated liposomes were leaked and retained specifically into tumor tissues rather than normal ones. Consequently, reduced drug toxicity is expected when using PEGylated liposomes containing anticancer drugs. This process is known as enhanced permeability and retention effect (EPR) and is extensively exploited to achieve passive targeting of chemotherapeutic agents [30, 41, 42]. EPR was described for the first time by Maeda and co-workers [43]. Also, it is demonstrated that EPR effect is the major mechanism for the selective accumulation of nanotherapeutics in tumors [44]. Nanocarrier therapies such as PEGylated liposomes have been extensively utilized as drug delivery system to achieve tumor tissues targeting via EPR effect. Surface modification with PEG was found to dramatically decrease the uptake of liposomes by MPS, extend liposomes half-life and increase tumor accumulation of PEGylated liposomes. It was clear that PEGylated liposomes do not distribute uniformly in solid tumors after passive accumulation because of the broad heterogeneity of EPR effects within tumor tissues [30].

On the other hand , tumor properties like tumor size, type of cancer and extent of tumor vasculature have a great effect on the extent of extravasation and tumor accumulation of PEGylated liposomes [45]. An ideal example is Doxil®, PEGylated doxorubicin liposomes, which has shown stealth properties and was demonstrated to increase the serum half-life of their loaded active. It was found that doxorubicin was released only in the tumor's interstitial fluid because of the considerable raised level of ammonia concentration which produced in tumor tissues due to the glutaminolysis (special metabolic pathway of tumor cells). After that, the liberated doxorubicin from liposomes is absorbed by the tumor cells and damage them [41].

6. Clinical applications of PEGylated liposomes

Liposomes loaded with chemotherapeutic drugs have become a promising drug delivery system for cancer therapy. The main road block to get efficacious cancer treatment is related to the resistance of cancer to anticancer drugs and this phenomenon is referred to as cancer multidrug resistance (MDR). Moreover,

nanocarriers such as liposomes encapsulating chemotherapeutic drugs are able to overcome different mechanisms responsible for MDR, enhancing the therapeutic efficacy toward multidrug resistant cancers. Besides that, PEGylated liposomes could specifically transport cytotoxic drugs into cancerous cells [46]. Doxil® is a typical example which is 100 nm doxorubicin loaded PEGylated liposomes (mPEG2000 was used as a surface coating) has proven to produce an efficient anticancer effect through EPR effect against wide range of cancers such as ovarian cancer, breast cancer and AIDS associated Kaposi's sarcoma [46].

Recently, Glutathione PEGylated liposomal doxorubicin based formulation (e.g. (2B3-101) or (G-Technology®) are emerging as interesting approach for treatment of brain cancer. It is based on Doxil® with an additional surface coating of glutathione for enhancement of safe drug delivery to the brain through the blood brain barrier (BBB) [47, 48]. In two separate experiments, an extensive suppression of brain tumor growth factor was detected by using 2B3-101 liposomes as measured by bioluminescence intensity. 2B3-101 when was administrated once weekly in a dose of 5 mg/kg has displayed higher anticancer effect compared to PEGylated liposomal doxorubicin and saline. Inconsiderable reduction of tumor was observed as about 2 from 9 animals which have been receiving 2B3-101 exhibited an entire tumor regression. However, when 2B3-101 injected twice times weekly in dose of 5 mg/kg had a substantial impact in suppressing brain tumor growth in comparison with doxorubicin based PEGylated liposomes and saline, and a full suppression was detected in only one animal treated with 2B3-101. Moreover, 2 times injection per week of 2B3-101 essentially increased the average survival time by 38.5% and 16.1 % in comparison with saline and PEGylated liposomal doxorubicin, respectively [47].

To sum up, these results showed that glutathione PEGylated liposomal doxorubicin improved the delivery of doxorubicin to brain tumors effectively and provided a superb promising opportunity to deliver the targeting ligands directly to the brain which holds the hope to defeat brain cancers [47]. A well-studied example for combination therapy is the co-delivery of oxaliplatin (1-OHP) containing liposomes and the molecules of RNAi against thymidylate synthase complexed to PEGylated based liposomes (PEGylated TS shRNA (EXPLAIN)-lipoplex) for solid tumor treatment. This combined treatment represents a promising approach to optimize the therapeutic efficacy of 1-OHP through targeted delivery of TS-shRNA to tumor tissues and to attenuate the immunogenic response induced by RNAi molecule. Accordingly, a substantial tumor growth inhibition displayed by TS-shRNA which essentially inhibit cell proliferation through gene silence when compared to single therapy either with l-OHP based liposomes or with TS-shRNA alone [49]. Other clinical applications of PEGylated liposomes include employing PEGylated ^{64}Cu-liposomes in clinical diagnostic positron emission tomography (PET) imaging and PEGylated ^{177}Lu-loaded liposomes for internal tumor radiotherapy technique [45].

7. Immunogenicity of PEG

PEG is commonly used for surface modification of nanocarrier system to sustain the biological half-life of drugs and prevent their clearance by RES because of its poor immunogenic and antigenic properties [50]. A number of studies showed that specific anti-PEG antibodies could be formed toward PEGylated formulations [27, 51, 52]. It was assumed that eliciting immunogenic response against PEG can take place in humans. Armstrong et al. demonstrated that there are high levels of anti-PEG antibodies, about 25 %, in healthy subjects. Moreover, Yang and Lai reported an even higher titers of PEG- antibodies of about 42 % in patients with no history of treatment with PEGylated products [52]. Also pre-existing anti-PEG antibodies may be represented in 56-72 % of people [44]. This could be explained by some researchers due to the common use of PEG products. In addition to, Yang and Lai have proposed the tentative mechanism that: the human body is exposed to various conditions like, ulcerations, abrasions and skin tears that may result in local inflammatory reactions and induction of immune response. The frequent use of PEG-coupled products (such as, soap, shampoo, toothpaste, lotion, detergent), PEG is delivered to inflammation sites and contacted with certain immune cells, which has the capability to stimulate anti-PEG antibodies formation. As a consequence, when human body is subjected to PEGylated therapeutics may further trigger an immunogenic response to PEG [52]. Finally, both together natural and induced anti-PEG antibodies represent an obstacle to PEGylated medicines in the clinical settings [35].

A study on PEGylated liposomes immunogenicity showed that when mice were injected with PEGylated liposomes encapsulating lipid-arrayed membrane-proximal external region (MPER) of HIV-1, an immune responses toward liposome components and MPER were detected. Injected mice considerably produced antibodies against PEG which represented in anti-PEG IgM, anti-PEG IgG and to MPLA. by a lesser extent [53]. Another example on PEG immunogenicity is PEGylated asparaginase which is a PEG-modified protein applied as a treatment for acute lymphoblastic leukemia. PEGylated asparaginase has the ability to abrogate the immunogenic response of asparaginase. It was reported that 32% of pediatric patients experienced anti-PEG antibodies when injected with PEGylated asparaginase for lymphoblastic leukemia [54].

8. Accelerated blood clearance phenomenon (ABC) phenomenon on PEGylated liposomes

Despite of the benefits of PEGylation, PEG has been identified as the cause of an unexpected immunogenic response known as the “accelerated blood clearance (ABC) phenomenon. In animal models, a second dose of PEGylated liposomes, injected within a time interval of 5 and 21 days, was cleared very rapidly from the blood circulation. This is referred to as accelerated blood clearance (ABC) phenomenon [27, 55, 56]. This phenomenon has a serious implications for the clinical use of liposomal

formulations, because multiple drug therapy of liposomes are applied frequently in clinical applications [57].

Moreover, Ishida et al. reported that repeated injections of PEGylated liposomes in the same animal in time interval dependent manner triggered anti–PEG IgM production which leads to blood clearance of subsequently injected PEGylated liposomes [57]. Also, some reports illustrated that the repeated injection of PEGylated-liposomes exhibited no change in the pharmacokinetic pattern of encapsulated drugs when the interval between two injected doses was 24h, 48h or 6 weeks [58, 59]. Dams et al. was the first one to illustrate that a second dose of empty PEGylated liposomes was rapidly eliminated when the time interval between two doses was 5 days [60].

Studies demonstrated that ABC effect has not been found to occur in patients receiving PEGylated liposomes containing doxorubicin (caelyx®). But empty PEGylated liposomes were able to trigger the ABC effect through anti-PEG IgM production [57, 59, 61]. While, Laverman et al. illustrated that repeated injections of doxorubicin PEGylated liposomes in murine animal model never elicited the ABC effect. This was attributed to the cytotoxic effect of doxorubicin to the cells of the RES which result in significant suppression in anti-PEG IgM amount produced therefore; reduced complement activation in rats sera was demonstrated [60]. It was assumed that chemotherapeutic agents liberated from liposomes accumulate in the spleen and hinder anti-PEG IgM production. As a result of the inhibition of B cell proliferation and/or damaging of B cells in the marginal zone and consequently abrogate the ABC phenomenon [59, 62].

9. Mechanism of ABC phenomenon

The tentative mechanism of ABC phenomenon was found to comprise two phases: the induction phase and the effectuation phase. The induction phase followed the initial dose of PEGylated liposomes in which the biological system is "primed" by stimulation of spleen cells to trigger anti-PEG IgM production. The effectuation phase occurs at day 3-7 after the initial dose in which a subsequent dose of PEGylated liposomes is rapidly taken up from the bloodstream by the Kupffer cells of liver MPS in coordination with anti-PEG IgM and complement system [28, 63, 64]. Besides that, the degree of the induction of the ABC effect is intensively linked to the magnitude of anti-PEG IgM production in response to an initial injected dose of PEGylated liposomes [65].

Generally, another acceptable mechanism for the ABC phenomenon as follows: Once first dose of PEGylated liposomes reach the spleen, they bind and crosslink to surface immunoglobulins on reactive B cells in the splenic marginal zone (MZ) and consequently trigger the production of an anti-PEG IgM that is independent of T-cell help. Repeated administration of PEGylated liposomes, if anti-PEG IgM, produced in response to the first dose, still exists in the blood circulation, it binds to the PEG on the liposomes, resulting in activation of the complement system, leading to opsonization by C3 fragments and enhanced uptake by Kupffer cells via complement receptor-mediated endocytosis. Thereby, the ABC phenomenon is expressed [28, 66, 67] as shown in **(Figure 1)**.

Dams et al. [60] reported that serum transfusions into normal rats from rats pretreated with PEGylated liposomes also elicited the enhanced blood clearance of a first dose of PEGylated liposomes. Also this phenomenon could be abolished when the rat serum was preheated at 56^0C for 30 min prior to transfusion, the temperature at which complement is made inactive and this illustrate that IgM-related complement activation is the rate determining step in the ABC phenomenon against PEGylated liposomes [60]. Ishida et al, showed that BALB/c nu/nu mice (T cell deficient mice) exhibited the ABC effect while BALB/c SCID mice (B cell and T cell deficient mice) did not experience this effect. Moreover, by the injection of PEGylated liposomes the anti-PEG IgM production increased in BALB/c nu/nu mice but never in BALB/c SCID mice. We can conclude from these data that the induction of the ABC effect is T-cell independent B cells response [68].

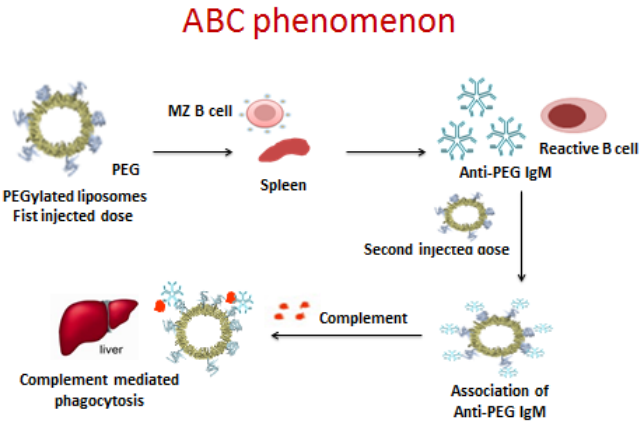

Figure 1: Representation of the sequence of events leading for induction of accelerated clearance of PEGylated liposomes.

Upon the injection of first dose of PEGylated liposomes, they bind and crosslink to surface immunoglobulins on reactive B cells in the splenic marginal zone and consequently induce the production of an anti-PEG IgM with independent T-cell manner. On administration of the second dose within 2 weeks, anti-PEG IgM, produced in response to the first dose, binds to the PEG on the liposomes, and subsequently activates the complement system, resulting in opsonization by C3 fragments and enhanced uptake by Kupffer cells via complement mediated phagocytosis.

10. Factors affecting ABC phenomenon

There are enormous factors that have an impact on the ABC phenomenon which include animal species, lipid dose, time interval, route of administration, encapsulated drug as well as physicochemical properties of prepared liposomes **(Figure 2)**.

10.1. Effect of animal species

Repeated intravenous injection of PEGylated liposomes into various types of animals models (rats, rabbits, mice, minipigs and guinea pigs) has been reported to trigger the ABC phenomenon [60, 63, 69]. However, the magnitude of the elicited ABC phenomenon varied with the animal species. It was reported that anti-PEG IgM production and the ABC phenomenon were not detected in mice treated with Doxil® (2 and 20 mg DXR/m^2), while in minipigs treated with Doxil® (2 mg DXR/m^2), anti-PEG IgM production and the ABC phenomenon were significantly increased [55]. Also, it has been shown that beagle dogs were more sensitive to doxorubicin PEG-modified liposomes than rodents in triggering ABC effect and to anti-PEG IgM production. This might be due to variations in the sensitivity of the immune system between dogs and rodents or due to differences in the pharmacokinetics of the initial dose of PEGylated liposomes [70]. Similar results were obtained when PEGylated liposomes of Topotecan administrated in mice, rats and beagle dogs. These data showed that PEGylated liposomes containing cytotoxic agents could elicit ABC response in these species of animal [55].

10.2. Effect of lipid dose

Ishida et al. demonstrated that there was a considerable inverse correlation between the extent of the ABC phenomenon and the amount of first injected dose of PEGylated liposomes [50]. Rats intravenously injected with a high dose of PEGylated liposomes (higher than 5 μmol phospholipid /kg), did not exhibit increased levels of anti-PEG IgM and the subsequent ABC phenomenon. On the other hand, the ABC phenomenon was significantly increased at phospholipid doses less than 1 μmol phospholipid/kg [50, 68]. The higher the lipid dose, the lower the ABC phenomenon [71, 72]. It was presumed that low concentrations of phospholipid could activate marginal zone B cells (MZ-B), and trigger anti-PEG IgM production, while higher initial doses of PEGylated liposomes could cause MZ-B to elicit immunological tolerance or anergy [59, 73].

10.3. Effects of time interval

Some studies showed that the induction and the magnitude of the ABC phenomenon exhibited a time interval dependent mechanism. It has been reported that the accelerated blood clearance of second injected dose of PEGylated liposomes was extensively high when the time interval between two injected doses of PEGylated liposomes was from 4 to 7 days. On the other side, some reports demonstrated that no alteration in the clearance of encapsulated drugs was observed with repeat injections of PEGylated liposomes when the interval between the initial and subsequent dose was less than 2 days or more than 4 weeks [74, 75]. This could be explained on the basis that the production of anti-PEG IgM occurred by 3–4 days after the initial dose [71, 76] and the IgM disappeared within its biological half-life of 3 weeks [77].

10.4. Effect of route of administration

It has been proven that the administration route of PEG-based nanocarriers have an essential impact on triggering the ABC phenomenon. A slow vascular infusion showed more intense ABC Effect rather than bolus injections with the same dose of PEGylated therapeutics. By slow infusion technique, a low dose of PEG-modified liposomes enter to the blood circulation, the immunogenic response against initial dose of PEGylated liposomes might be in excess in relation to subsequent injected dose. Therefore result in substantial accelerated blood clearance of the second dose of PEGylated liposomes. To the contrary, when a high dose of PEG-based liposomes entered the circulation via bolus intravenous IV injection, the amount of anti-PEG IgM might be not enough to neutralize such a large dose of PEGylated liposomes resulting in induction of the ABC phenomenon [78].

10.5. Effect of physicochemical properties of prepared liposomes

The physicochemical properties of PEGylated liposomes such as size, lipid composition, extent of PEGylation and surface charge, all affected the extent of the ABC phenomenon as well as its existence. For instance, using phospholipid characterized by long, saturated acyl chains, PEGylated lipid and membrane stabilizer (cholesterol) for preparation of liposomes altered the pharmacokinetic pattern of liposomes and their distribution in the tissues. Moreover, the insertion of negatively charged phospholipid such as phosphatidylserine for PEG-modified liposomes preparation made them rapidly eliminated from the blood circulation. However, a number of studies have illustrated that the size and charge of initially injected liposomes doesn't affect the blood circulation and hepatic clearance of subsequently injected dose [79, 80].

10.6. Effect of encapsulated drug

Injection of doxorubicin containing liposomes in rats caused a significant decrease in the Anti-PEG IgM amount produced and consequently abrogated the induction of complement system in the rat serum when pre-injected with such liposomes [62]. It was confirmed that liposomes containing anticancer drugs such as oxaliplatin and mitoxantrone essentially alleviates the induction of the ABC effect. It was assumed that repeated administration of chemotherapeutic based liposomal formulations don't induce ABC response. However, multiple injections of Topotecan PEGylated liposomal formulations in beagle dogs and wistar rats triggers ABC phenomenon largely. Reports suggested that the lipophlicity of Topotecan retain it inside the liposomes which lead to inadequate distribution of Topotecan to the B cells of the spleen resulting in stimulation of immunogenic response instead of inhibition of splenic B cells proliferation [78]. We can conclude that a caution should be taken to design a study involving animal selection for preclinical studies including PEGylated nanocarrier therapies even if containing immunosuppressant drugs like chemotherapy drugs.

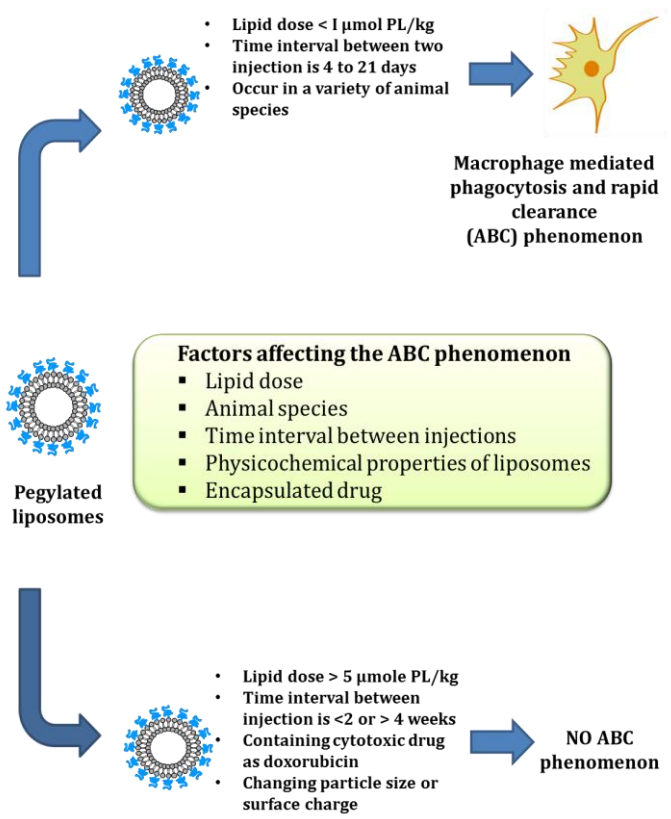

Figure 2: Factors affecting the accelerated blood clearance phenomenon

11. Side effects of PEGylated liposomes

It was demonstrated that nanocarrier drug delivery systems interact with the natural immune system, involving the mononuclear phagocytic system as well as the complement system in different degrees and these interactions with the innate immune system emerged important clinical effects. They can produce an infusion reaction referred to as complement activation-related pseudoallergy (CARPA) through the activation of complement system proteins which usually circulate in the blood stream [44]. CARPA cause various symptoms in most of body organs, including dyspnea, facial swelling, chest pain, flushing, skin rash, hypotension or hypertension and pain in the heart [81]. It was reported that there are many factors that trigger complement activation including multilamellar vesicles, large size liposomes and incorporation of high amount of cholesterol in the lipid bilayers [82].

However, PEGylated liposomes have the ability to enhance the therapeutic index of drugs, it should be illustrated that PEGylated liposomes might result in complement system activation causing pseudoallergic reactions or hyper sensitivity reactions [16]. A representative example is Doxil® which is the formulation of doxorubicin PEGylated liposomes that is often used for ovarian cancer and AIDS-related Kaposi's sarcoma treatment. Although, Doxil® has the ability to buffer the cardiac toxicity of free doxorubicin; Doxil® itself exhibited some serious side effects such as the hand–foot syndrome and has been implicated as the cause of acute infusion reactions in cancer patients. Results showed that Hypersensitivity reactions (HSR) have been demonstrated in about 25% of cancer patients in some studies, with an average result of 8% through all patients even if patients are pretreated with antihistaminic or corticosteroids therapies [18, 83]. These infusion reactions are suggested to be resulted from the activation of the complement system on first injection of PEGylated formulations without any memory of immune response which is dissimilar to type I allergy [18].

12. Conclusion

Nanomedicine based therapeutics such as PEGylated liposomes have a potential approach in chemotherapy treatment. Accordingly, an immunological response to PEGylated liposomes is a phenomenon that should be taken into account when expanding clinical uses of PEGylated liposomes. Since the ABC phenomenon might decrease the therapeutic effect of second and subsequent liposome doses. Consequently, clear understanding of the mechanisms underlying these responses, along with various techniques that can help in the avoidance of ABC phenomenon is essential for the development of PEGylated liposomes and other lipid-based nanocarriers in the future.

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