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Research paper

A frustrating problem: Accelerated blood clearance of PEGylated solid lipid nanoparticles following subcutaneous injection in rats

Yongxue Zhao, Chunling Wang, Long Wang, Qiang Yang, Wenya Tang, Zhennan She, Yihui Deng *

College of Pharmacy, Shenyang Pharmaceutical University, Shenyang, PR China

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ABSTRACT

Colloidal particles have preferential access to the lymphatic system following subcutaneous administration, achieving lymphatic targeting by drug accumulation in the regional lymph nodes. Moreover, the surface PEGylated colloidal particles have shown enhanced drainage into lymphatics and uptake by macrophages of the regional lymph nodes after subcutaneous injection. Nevertheless, it is reported that upon repeated intravenous injection, the PEG-specific IgM produced by the administration of the PEGylated colloidal particles markedly accelerates the clearance of subsequent doses of PEGylated particles. In this article, we report that the first subcutaneous injection of PEGylated solid lipid nanoparticles also induces the intravenously administered PEGylated particles to be cleared very rapidly from the circulation, and the "ABC index," a parameter for the intensity of accelerated blood clearance, for subcutaneous injection was equivalent to or even lower than that following the first intravenous injection. Moreover, the small quantities of distributed particles in the spleen after the first s.c. dose but the significantly higher elimination rate of the second i.v. dose, strongly suggest that, in addition to the spleen, the regional lymph nodes also play a promotive role in this phenomenon, although the exact lymphocytes causing this phenomenon remain unclear. Our observations may thus have important implications for considering combination therapy with PEGylated productions requiring different administration routes such as intravenous and subcutaneous injection, and great care is needed.

1. Introduction

The subcutaneous (s.c.) route of administration has potential utility in the treatment for lymph and lymph node resident diseases such as infection and tumor metastases [1–5] and has been most extensively investigated for the lymphatic targeting. Previous studies have shown that the colloidal particles have preferential access to the lymphatic system after s.c. injection [3,4], and the therapeutic drugs encapsulated can be targeted to the lymph nodes directly to produce sustained release, leading to a positive therapeutic effect [6,7].

The PEGylation technique has been applied for more than 30 years in the pharmaceutical field since the 1980s [8,9], and colloidal particles after PEGylation have been shown to markedly reduce recognition by the mononuclear phagocyte system (MPS) and produce a prolonged blood circulation time when given intravenously (i.v.) [10]. Doxil, PEGylated liposomal Doxorubicin, has shown superior activity against AIDS-related Kaposi's sarcoma in clinical situations [11]. Moreover, investigations involving the lymphatic targeting of colloidal particles have also shown that the degree of lymphatic transport may be increased by surface PEGylation [12,13]. The group led by Oussoren [14,15] and Hawley and his colleague [16–20] believe that a surface that is neither too hydrophobic nor too hydrophilic is needed for the lymphatic targeting of colloidal particles. Use of an appropriate hydrophilic agent might ensure that the particles could pass into the lymphatic capillaries from the interstitium, while a suitable hydrophobic agent could increase the possibility of retention in the lymph nodes after lymphatic absorption. Clearly, PEGylated colloidal particles have a bright future for lymphatic targeting when given by s.c. injection.

Nevertheless, Ishida et al. [21] and Dams et al. [22] recently reported that PEGylated liposomes lost their sustained circulation characteristics upon repeated i.v. administration after a certain time in the same animal [i.e., "accelerated blood clearance (ABC) phenomenon"]. Furthermore, it is worth noting that this phenomenon was also triggered following the administration of PEGylated cationic liposomes containing nucleic acids [23,24], polymeric nanoparticles [25,26], and polymeric micelles [27,28]. Further studies have suggested that the spleen plays a critical role in the induction phase [29], and the PEG-specific IgM produced after the first injection of PEGylated particles is thought to be responsible for this abnormal phenomenon [30]. Taking all of these observations into consideration, there is concern that after the initial s.c. injection, the PEGylated particles also have the potentiality to elicit

* Corresponding author. College of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China. Tel./fax: +86 024 23986316.
E-mail address: pharmdeng@gmail.com (Y. Deng).

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the PEG specified IgM, the production of anti-PEG IgM may have a major effect on the pharmacokinetic behavior of i.v. injected PEGylated particles, limited the possible treatment for combination lymphatic targeting with systemic application (for example, a combination of lymphatic visualization and systemic treatment or a combination of PEGylated cytokines and PEGylated particles, e.g., Doxil). Therefore, we were prompted by this eagerness to investigate exactly how the s.c. administration of PEGylated particles affects the pharmacokinetic behavior of the particles following i.v. injection.

In our previous study, we showed that the PEGylated solid lipid nanoparticles (SLNs) also induced the ABC phenomenon upon repeated intravenous injection [31]. Hence, in this article, we used PEGylated SLNs as model PEGylated particles, and encapsulated tocopheryl nicotinate as an indicator, to study the influence of different anatomical sites of injection and lipid doses after s.c. administration on the pharmacokinetic behavior of the particles given by i.v. injection. The results obtained indicated that, after s.c. administration, regardless of the injection sites and the lipid dose levels, the clearance of subsequently i.v. injected PEGylated SLNs was accelerated, exhibiting the characteristics of the classic ABC phenomenon. Moreover, in comparison with the initial intravenous injection, the subcutaneous route was more prone to induce a markedly accelerated clearance of the test dose. These results implied that, in addition to the spleen, the regional lymph nodes also played an important role in the induction of the ABC phenomenon. Our study strongly suggested that when considering combination therapy with PEGylated particles given by different administration routes, such as intravenous and subcutaneous injection, great care should be taken in choosing the administration regimen.

2. Materials and methods

2.1. Materials

Glycerin monostearate (GMS, Sigma-Aldrich, St. Louis, MO, USA) was used as the matrix for the SLNs. Injectable Soybean lecithin (S75) was purchased from LIPOID GmbH (Ludwigshafen, Germany), and N-(carbonyl-methoxypolyethyl-ene-glycol-2000)-1,2-distearoyl-sn-glycero-3-phos-phoethanolamine ($mPEG_{2000}$-DSPE) was obtained from Genzyme Corporation (Cambridge, MA, USA). The cleavable PEG-lipid derivative, $mPEG_{2000}$-cholesteryl hemisuccinate ($mPEG_{2000}$-CHEMS) was synthesized in our own laboratory [32]. Tocopheryl Nicotinate (TN) was supplied by Northeast Pharmaceutical Group Co., Ltd. (Shenyang, China). Ethanol was pharmaceutical grade, and all other reagents were chromatographic grade.

2.2. Animals

Male Wistar rats weighing 180–200 g were purchased from the Laboratory Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The animals had free access to water and rat chow. All animal care and experiments were carried out in accordance with the guidelines of the Animal Welfare Committee of Shenyang Pharmaceutical University.

2.3. Preparation of the PEGylated SLNs

The SLNs were made by the melt–emulsification process. Briefly, GMS, S75, $mPEG_{2000}$-DSPE (or $mPEG_{2000}$-CHEMS), TN (56/9/1/7.5, molar ratio) were mixed and dispersed in ethanol at 65 °C, and then the solvent was removed. The melted mixture was then dispersed in 5% glucose solution under mechanical agitation to achieve a final phospholipid (S75 and $mPEG_{2000}$-DSPE or $mPEG_{2000}$-CHEMS, 9/1, molar ratio) concentration of 5 μmol/mL. The warm primary emulsion was further treated using a laboratory ultrasonic cell pulverizer (JY92-II, Ningbo Scientz Biotechnology Co., Ltd., Zhejiang, China), for at least a 2-min cycle (200 w) and an additional 3-min cycle (400 w). Following sonication, to ensure homogeneity in size, the suspensions were then passed through polycarbonate membranes with a pore size of 0.22 μm. The mean particle size and *zeta* potential of the SLNs were determined in purified water at 23 °C by the dynamic light scattering method using a Submicron Particle Sizer (Nicomp 380™; Particle Sizing Systems, Inc., Santa Barbara CA, USA). For $mPEG_{2000}$-DSPE modified SLNs (PEG-SLNs), the mean particle size was 121.5 ± 7.8 nm (n = 6) and the *zeta* potential was −21.65 ± 2.9 mV (n = 6); for $mPEG_{2000}$-CHEM-S modified SLNs (PEG-CHS-SLNs), the mean particle size was 160 ± 5.7 nm (n = 4) and *zeta* potential was −11.52 ± 1.8 mV (n = 4).

2.4. Biodistribution and pharmacokinetic of PEGylated SLNs

Male Wistar rats were randomly divided into 17 groups. The rats were initially given s.c. injections either into the nape of the neck or into the right hind footpad, or i.v. injections via the tail vein, with different doses of PEGylated SLNs, and control animals were given i.v. injections of 5% glucose solution instead of PEGylated SLNs. Seven days later, for the second injection, PEGylated SLNs were intravenously injected at a dose of 5 μmol phospholipids/kg via the tail vein. The detailed scheme is presented in Table 1. At specified time points (0.083, 0.25, 0.50, 1.0, 2.0, and 4.0 h) following the second injection, blood samples were collected and centrifuged at 4000 rpm for 10 min to separate the plasma. After withdrawing the last blood sample, the rats were killed and livers, spleens, and the sites of s.c. injection were removed and the tissues were rinsed in ice-cold normal saline. The blood samples and tissue samples were stored at −20 °C until analysis.

The concentration of TN in plasma and tissue samples was assayed by high performance liquid chromatography (HPLC) using a P230 pump and a UV230 UV/Vis Detector (Dalian Elite Analytical Instruments Co., Ltd., Liaoning, China), operated at 264 nm and a Hypersil BDS C18 column (200 mm × 4.6 mm) filled with particles of diameter 5 μm. The mobile phase was methanol/isopropanol (80/20, *v/v*), the flow rate was 1 mL/min at 30 °C, and the limit of quantitation was 0.04 μg/mL. Samples were treated as follows. To 100 μL of plasma or homogenates (equivalent to 0.1 g tissue), 100 μL of methanol, 100 μL of internal standard (Tocopheryl acetate, 100 μg/mL), and 600 μL of *n*-hexane were added. The mixture was vortexed for 5 min and centrifuged at 10,000 rpm for 10 min, and then 500 μL of supernatant was collected and dried using a CentriVap Centrifugal Vacuum Concentrator (Labconco Corporation, USA). Following this, the dried material was mixed with 100 μL of mobile phase and vortexed for 1 min and then centrifuged at 10,000 rpm for 10 min, and the 20 μL of the supernatant was subjected to HPLC analysis.

2.5. Detection of anti-PEG IgM antibodies

It has been proposed that the anti-PEG IgM produced by the initial dose is responsible for the ABC phenomenon [30,33,34]. So, to examine the ability of PEGylated SLNs to generate anti-PEG IgM after s.c. or i.v. injection, serum samples were collected 7 days after a single dose of PEGylated SLNs. Anti-PEG IgM was then determined using a slightly modified version of the previously published procedure [35]. Briefly, 10 nmol $mPEG_{2000}$-DSPE in 50 μL of 100% ethanol was added to each well of a 96-well microplate (Corning Incorporated, New York, USA) and thoroughly air dried. Wells were then blocked with 100 μL of 1% bovine serum albumin in 50 mM Tris-buffered saline (pH 8.0) for 60 min. The wells were then washed five times with Tris-buffered saline containing 0.05%