Pharmacokinetic Properties of Single and Repeated Injection of Liposomal Platelet Substitute in a Rat Model of Red Blood Cell Transfusion-Induced Dilutional Thrombocytopenia

MAI HASHIMOTO,[1] KAZUAKI TAGUCHI,[2] SHIGERU OGAKI,[1] HIROSHI WATANABE,[1,3] MANABU KINOSHITA,[4] KAHOKO NISHIKAWA,[5] SHINJI TAKEOKA,[6] YASUO IKEDA,[6] MAKOTO HANDA,[7] MASAKI OTAGIRI,[1,2,8] TORU MARUYAMA[1,3]

[1]Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Chuo-ku, Kumamoto 862-0973, Japan
[2]Faculty of Pharmaceutical Sciences, Sojo University, Nishi-ku, Kumamoto 862-0082, Japan
[3]Center for Clinical Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan
[4]Department of Immunology and Microbiology, National Defense Medical College, Tokorozawa, Saitama 359-8513, Japan
[5]Department of Traumatology and Critical Care Medicine, National Defense Medical College, Tokorozawa, Saitama 359-8513, Japan
[6]Department of Life Science and Medical Bioscience, Graduate School of Advanced Science and Engineering, Waseda University, Shinjuku-ku, Tokyo 162-8480, Japan
[7]Center for Transfusion Medicine and Cell Therapy, Keio University, Shinjuku-ku, Tokyo 160-8582, Japan
[8]DDS Research Institute, Sojo University, 4-22-1 Ikeda, Nishi-ku, Kumamoto 862-0082, Japan

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ABSTRACT: A preclinical study of dodecapeptide (^{400}HHLGGAKQAGDV^{411}) (H12)-(adenosine diphosphate, ADP)-liposomes for use as a synthetic platelet (PLT) substitute under conditions of red blood cell (RBC) transfusion-induced dilutional thrombocytopenia is limited to pharmacological effect. In this study, the pharmacokinetics of H12-(ADP)-liposomes in RBC transfusion-induced dilutional thrombocytopenic rats were evaluated. As evidenced by the use of ^{14}C, ^{3}H double-radiolabeled H12-(ADP)-liposomes in which the encapsulated ADP and liposomal membrane were labeled with ^{14}C and ^{3}H, respectively, the H12-(ADP)-liposomes remained intact in the blood circulation for up to 3 h after injection, and were mainly distributed to the liver and spleen. The encapsulated ADP was mainly eliminated in the urine, whereas the outer membrane was mainly eliminated in the feces. These successive pharmacokinetic properties of the H12-(ADP)-liposomes in RBC transfusion-induced dilutional thrombocytopenic rats were similar to those in healthy rats, except for the shorter retention time in the circulation. When H12-(ADP)-liposomes were repeatedly injected into RBC transfusion-induced dilutional thrombocytopenic rats at intervals of 5 days at a dose of 10 mg lipids/kg, the second dose of injected H12-(ADP)-liposomes were rapidly cleared from the circulation, namely, via the accelerated blood clearance phenomenon. These novel pharmacokinetic findings provide useful information for the further development of H12-(ADP)-liposomes as a PLT substitute. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3968–3976, 2015

Keywords: liposome; adenosine-diphosphate; dodecapeptide; accelerated blood clearance phenomenon; transfusion; thrombocytopenia; platelet substitute; pegylation; clearance; pharmacokinetics

INTRODUCTION

Red blood cell (RBC) transfusions are first-line medication for the treatment of severely injured trauma patients to counter the effects of hemorrhagic shock. Nevertheless, potentially lethal coagulopathy and thrombocytopenia are frequently observed in hemorrhagic patients receiving massive RBC transfusions. As a result, a platelet (PLT) concentrate is also transfused for the treatment of coagulopathy or thrombocytopenia in such patients. A series of these emergency medical strategies effectively reduce mortality,[1] thus RBC and PLT transfusions are absolutely imperative therapeutic transfusion treatments for patients with massive hemorrhaging to maintain vital activity. However, blood transfusions, including RBC and PLT, can introduce a variety of complications, such as virus infections and allergic reactions. In addition, their short-term viability and the stringent storage conditions required have also become a serious issue, as this creates a very limited supply to be available in an emergency situation such as disasters and pandemics. Furthermore, the populations of several countries, including Japan, are rapidly aging, and there is a concern that a shortage of blood donors will become a serious problem in the near future. To overcome these problems, attempts have been made to develop several types of blood substitutes, such as RBC substitutes[2,3] or PLT substitutes.[4,5]

We developed adenosine diphosphate (ADP)-encapsulated liposomes, of which the surface of phospholipid vesicles is modified with polyethylene glycol (PEG). Furthermore, in order to enhance the hemostatic effect, this PEGylated

Abbreviations used: RBC, red blood cell; PLT, platelet; ADP, adenosine diphosphate; H12, dodecapeptide (^{400}HHLGGAKQAGDV^{411}); HbV, hemoglobin-vesicles; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DHSG, 1,5-dihexadecyl-*N*-succinyl-L-glutamate; PEG, polyethylene glycol; DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[monomethoxypoly(ethyleneglycol)]; WBC, white blood cells; $t_{1/2}$, half-life; CL, clearance; AUC, are under the concentration-time curve; ABC, accelerated blood clearance.

Correspondence to: Toru Maruyama (Telephone: +81-96-361-4150; Fax: +81-96-362-7690; E-mail: tomaru@gpo.kumamoto-u.ac.jp)

Mai Hashimoto and Kazuaki Taguchi contributed equally to this work.

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liposome-based PLT substitute bears a synthetic dodecapeptide (^{400}HHLGGAKQAGDV^{411}) (H12) on its surface, corresponding to the carboxy terminus of the fibrinogen γ-chain [H12-(ADP)-liposome]. H12 is a specific binding site of the ligand for activated glycoprotein IIb/IIIa, which is present on the PLT membranes.[6,7] In fact, previous *in vitro* [8,9] and *in vivo* studies[10,11] clearly showed that the modification of H12-(ADP)-liposomes such as H12 and encapsulation with ADP enables them to specifically interact with the glycoproteins IIb/IIIa on activated PLT and stimulate PLT aggregation. Furthermore, a pharmacokinetic study of H12-(ADP)-liposomes in healthy animals showed that they have an adequate circulation time in the blood to permit them to function as a PLT substitute and that they have acceptable biodegradable properties.[12] Taken together with these characteristics of H12-(ADP)-liposomes in preclinical trials, H12-(ADP)-liposomes are promising candidates for use as a PLT substitute in clinical applications.

In previous studies, we found that the pharmacokinetics properties of hemoglobin-vesicles (HbV), the liposomal characteristics of which are similar to those for H12-(ADP)-liposomes, were different between healthy and pathological conditions.[13,14] Thus, we previously investigated the effects of H12-(ADP)-liposomes in the case of an adaptation disease (thrombocytopenia) on their pharmacokinetic characteristics using a rat model of anticancer drug-induced thrombocytopenia. As expected, thrombocytopenic conditions were found to affect the pharmacokinetics of H12-(ADP)-liposomes.[15] As the changes in pharmacokinetic properties in such a pathological condition may cause the pharmacological effects to be reduced or unexpected adverse effects to be induced, clarifying the pharmacokinetics of H12-(ADP)-liposomes in animal models of an adaptation disease should provide useful information. However, such information regarding pharmacokinetics of H12-(ADP)-liposomes under conditions of an adaptation disease (thrombocytopenia) have been limited to the above-mentioned study, despite the fact that thrombocytopenia is clinically induced by not only anticancer drugs but also another factors such as hemodilution.

We herein further investigated the pharmacokinetic properties of H12-(ADP)-liposomes and their components using a rat model with RBC transfusion-induced dilutional thrombocytopenia, a disease that could be treated with H12-(ADP)-liposomes. For this purpose, we first created RBC transfusion-induced dilutional thrombocytopenia model rats by alternating blood withdrawal and isovolumic RBC transfusion. We then evaluated the pharmacokinetic properties after single and repeated injections of the H12-(ADP)-liposomes and their components in the RBC transfusion-induced dilutional thrombocytopenia model rats using ^{14}C-, ^{3}H-labeled H12-(ADP)-liposomes, in which the encapsulated ADP and membrane component (cholesterol) was labeled with ^{14}C and ^{3}H, respectively, and examined the differences in the findings for those obtained using healthy rats, anticancer drug-induced thrombocytopenic rats, and RBC transfusion-induced dilutional thrombocytopenic rats.

MATERIALS AND METHODS

Reagents

Cholesterol and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) were purchased from Nippon Fine Chemical (Osaka, Japan), and 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[monomethoxypoly(ethyleneglycol)] (DSPE-PEG, 5.1 kDa) was obtained from NOF (Tokyo, Japan). 1,5-Dihexadecyl-*N*-succinyl-L-glutamate (DHSG) and H12-PEG-Glu2C18 were synthesized as reported previously.[16] ^{14}C-, ^{3}H-abeled H12-(ADP)-liposomes were prepared, as described in a previous report.[12] The diameter and zeta potential of the H12-(ADP)-liposomes used in this study were in the range of 250 ± 50 nm and −10 ± 0.9 mV, respectively. Before being used in pharmacokinetic experiments, all of the radiolabeled samples were mixed with unlabeled H12-(ADP)-liposomes.

Animals

All animal experiments were reviewed and approved by the Animal Care and Use Committee of Kumamoto University. The care and handling of the animals were in accordance with NIH guidelines. All animals were maintained under conventional housing conditions, with food and water *ad libitum* in a temperature-controlled room with a 12-h dark/light cycle. All male Sprague–Dawley rats (200–240 g) were purchased from Kyudou Company (Kumamoto, Japan). The systemic blood volume of the rats was estimated to be 56 mL/kg.[13]

RBC Transfusion-Induced Dilutional Thrombocytopenic Rat Model

After anesthetizing Sprague–Dawley rats with pentobarbital, polyethylene catheters (PE 50 tubing, outer diameter equal to 0.965 mm, and inner diameter equal to 0.58 mm; Becton Dickinson and Company, Tokyo, Japan) containing saline and heparin were introduced into the right femoral artery for blood withdrawal, and into the left femoral vein for transfusion. Thereafter, 25% of the total blood volume (14 mL/kg) were drawn from right femoral artery at a rate of 1 mL/min. PLT-rich phase were separated by centrifugation at 100*g* for 15 min and discarded.[17,18] The remaining RBC that included few PLTs were mixed with saline containing 5% human serum albumin to adjust the volume to be the same as the withdrawn blood volume. Thirty minutes after the blood was withdrawn, a prepared RBC solution was transfused via the left femoral vein, and the next blood sample was then withdrawn. We repeated this blood withdrawal and RBC transfusion cycle for a total of four times (Fig. 1). After the final RBC transfusion, lactated Ringer's solution (8 mL/kg) was transfused via the left femoral vein, and blood samples for hematology analysis were then obtained. Hematology analyses were performed using a KX-21NV instrument (Sysmex, Kobe, Japan).

Pharmacokinetic Studies of a Single Injection of H12-(ADP)-Liposomes in RBC Transfusion-Induced Dilutional Thrombocytopenic Rats

Sixteen RBC transfusion-induced dilutional thrombocytopenic rats were given a single injection of ^{14}C-, ^{3}H-labeled H12-(ADP)-liposomes (10 mg lipids/kg). This dose is lowest recommended dosage required to exert a sufficient hematostatic effect in thrombocytopenic rats.[19] In all rat groups, four rats were selected to undergo a plasma concentration test and an excretion test. Under ether anesthesia, approximately 200 μL blood samples were collected from the tail veins at multiple time points after the injection of the ^{14}C-, ^{3}H-labeled H12-(ADP)-liposomes (10, 30 min, 1, 2, 3, 6, 12, and 24 h) and the plasma was separated by centrifugation (3000*g*, 5 min). Urine and feces were collected at fixed intervals in a metabolic cage until 7 days after