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Influence of the physicochemical properties of liposomes on the accelerated blood clearance phenomenon in rats

Xin Yu Wang, Tatsuhiro Ishida, Masako Ichihara, Hiroshi Kiwada*

Department of Pharmacokinetics and Biopharmaceutics, Graduate School of Pharmaceutical Sciences, The University of Tokushima, 1-78-1, Sho-machi, Tokushima 770-8505, Japan

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Abstract

We have recently reported that PEGylated liposomes (PL) are cleared rapidly from the blood circulation when they are administered twice in the same rat at certain intervals, even if the liposomes are sterically stabilized by a surface modification with PEG (referred to as the accelerated blood clearance (ABC) phenomenon, *J. Control. Release*, 88, 35–42 (2003)). Now we report on the influence of physicochemical properties (PEG-modification, size and surface charge) of either the first or the second dose of liposomes on the ABC phenomenon. When, for the first dose, conventional liposomes (CL; without a PEG coating) of 110-nm diameter were injected, only a very slight ABC phenomenon was observed, irrespective of the liposomal surface charge: both clearance rate and hepatic accumulation of the second injected PL were only slightly enhanced compared to those of a single dose of PL. Interestingly, when for the first injection small-size liposomes (60 nm) were used, either charged or PEG-modified, but not neutral, the ABC phenomenon was clearly manifest. Apparently, the induction of the ABC phenomenon is not only determined by the PEG coating but also by the size and surface charge of the first dose of liposomes. Also when for the second dose small-size PEGylated liposomes were used, the ABC phenomenon was observed after induction by a first injection of PL, whereas plasma kinetics and organ uptake of a second dose of negatively charged CL (NCL, 110 nm) or small-sized NCL (SNCL, 60 nm) were not altered. Apparently, the PEG coating on the second dose is essential for the liposomes to be susceptible to the ABC phenomenon. The results reported here suggest that the physicochemical properties of both the first and second dose of

Abbreviations: ABC, accelerated blood clearance; CHOL, cholesterol; ^{3}H-CHE, ^{3}H-cholesterylhexadecyl ether; CL, conventional liposomes; CLh, hepatic clearance; DCP, dicetylphosphate; DPPC, dipalmitoyl phsophatidylcholine; DPPG, dipalmitoyl phsophatidylglycerol; HEPC, hydrogenated egg phosphatidylcholine; HYNIC, hydrazinonicotinamide; NCL, negatively charged CL; $mPEG_{2000}$–DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy(polyethylene glycol)-2000]; MLV, multilamellar vesicle; MPS, mononuclear phagocyte system; PCL, positively charged CL; PEG, polyethylene glycol; PL, PEGylated liposomes; PS, phosphatidylserine; SA, stearylamine; SCL, small-sized CL; SNCL, small-sized NCL; SPCL, small-sized PCL.

* Corresponding author. Tel.: +81 88 633 7259; fax: +81 88 633 9506.
E-mail address: hkiwada@ph.tokushima-u.ac.jp (H. Kiwada).

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liposomes are important either for the induction of the phenomenon or for its expression. Our observations may have a considerable impact on the clinical application and engineering of liposomal formulations for use in multiple drug therapy.

Keywords: Polyethylene glycol (PEG); Accelerated blood clearance (ABC) phenomenon; Liposomes

1. Introduction

Liposomes have been extensively studied for use as a drug carrier system because they can reduce the toxicity of drugs associated with them by altering their pharmacokinetics [1]. In early studies, with conventional liposomes (CL), it was found that they readily interact with serum proteins after intravenous injection, resulting in massive uptake by cells of the mononuclear phagocyte system (MPS) [2,3]. Various approaches to overcome this problem have been reported. A real breakthrough in the field of liposomal drug carriers was the design of long-circulating liposomes. The most widely used way to produce such liposomes was the steric stabilization of the surface of liposomes by the incorporation of polyethylene glycol (PEG)-conjugated lipid (PEGylated liposomes (PL) or sterically stabilized (Stealth) liposomes) [1,4,5]. Such long-circulating liposomes are currently widely used in targeted drug delivery to tumors and inflammatory regions, and have shown impressive improvement of the therapeutic index of encapsulated drugs [6].

Although numerous studies have been reported on the biodistribution and pharmacokinetics of PL, only a limited amount of data is available on the effect of multiple injections on their pharmacokinetics. Recently, we and others reported that a single dose of intravenously injected PL trigger rapid clearance of a subsequently injected dose, given several days after the first injection (referred to as the "accelerated blood clearance (ABC) phenomenon") [7–11]. These reports indicate that the first dose of PL is likely to reduce the expected therapeutic efficacy of subsequent doses of PL when these PL were injected repeatedly within certain time intervals. Understanding the underlying mechanism of the induction of the ABC phenomenon is important in order to further improve the utility of PL as an in vivo carrier by preventing undesired alterations in pharmacokinetics and biodistribution of PL.

Dams et al. [9] showed that the accelerated blood clearance of a second dose of PL is mediated by soluble serum proteins (or factors) produced in response to the first injection. This was supported by our recent results showing that transfusion of serum, collected from rats pretreated 5 days before with PL, into naïve (non-treated) rats could indeed elicit enhanced clearance of PL in these rats [8]. Laverman et al. [10] distinguished two phases in the ABC phenomenon: the induction phase, following the first dose of PL during which the biological system is "primed" [may be reflected in the formation of the transfusable serum proteins (or factors)], and the effectuation phase, following the second dose, in which PL are rapidly cleared from the blood circulation, presumably due to enhanced uptake by Kupffer cells in the liver.

The effects of physicochemical properties of liposomes on the rate of blood clearance and the biodistribution of the liposomes have been extensively described [3,12]. For instance, the inclusion of phospholipids with long, saturated acyl chains or cholesterol (CHOL) into the liposomal lipid composition, or surface modification of liposomes with monosialo-ganglioside or PEGylated lipid led to an extended blood residence time [5,12–14]. It is noteworthy that the incorporation of negatively charged phospholipids, such as phosphatidylserine (PS), into PL enhanced their removal from the blood circulation [15], despite the PEG coating. The exact mechanism(s) causing the different biological behavior of differently composed liposomes are still not entirely clear, although it has been repeatedly proposed that the interaction with serum proteins and/or cells of the MPS is responsible for this phenomenon [2,3,16–26]. This led us to the assumption that the production of serum proteins (or factors) in response to a first dose of injected liposomes may be affected by the physicochemical properties of the liposomes and that the degree of interaction with Kupffer cells and/or the produced