Repeated injections of PEGylated liposomal topotecan induces accelerated blood clearance phenomenon in rats

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\begin{abstract}
The “accelerated blood clearance (ABC) phenomenon” of PEGylated liposomes following multiple injections has been reported recently. This immunogenicity poses a problem for research into liposomes and hinders their clinical application. However, since doxorubicin liposomes and mitoxantrone liposomes have been reported to fail to induce the ABC phenomenon, some people believe that cytotoxic drugs loaded liposomes will not produce this ABC phenomenon under multiple-dosing regimens. Nevertheless, in the present study, we report that a first injection of the PEGylated liposomal topotecan (a cell cycle-specific drug for the S phase) still produced a strong ABC phenomenon. Likewise, when the first dose of “empty” PEGylated liposomes or topotecan liposomes was increased, the ABC phenomenon of the subsequent dose was accordingly attenuated. Unlike doxorubicin and mitoxantrone, the blood clearance rate of topotecan was dramatically rapid, and the hepatic and splenic accumulations of topotecan liposomes were anomalous because of the ABC phenomenon. These findings may present new challenges to the clinical application of formulations of cytotoxic drugs loaded liposomes that require repeated administrations.
\end{abstract}

1. Introduction

Liposomes are an attractive drug delivery system for a diverse array of therapeutic agents due to their ability to enhance the stability and improve the pharmacokinetic properties of the encapsulated drug, reducing its toxicity and improving its in vitro and in vivo activity (Gabizon et al., 1982; Forssen and Tokes, 1983; Hong et al., 2000; Rahman et al., 1980). The appearance of long-circulating or sterically stabilized liposomes (SLs), such as polyethylene glycol (PEG)-modified liposomes (PEGylated liposomes), has opened up a new ear of liposome research. The grafting of PEG to the surface of a liposomal carrier substantially increases the physical stability and the circulation time of the liposomes because PEGylated liposomes can avoid clearance by the Reticuloendothelial System (RES) due to the water shell and steric barrier formed by PEG, thereby allowing the full advantage of the EPR effect and producing higher levels of drug accumulation within the tumor sites (Gabizon et al., 1994; Huang et al., 1992; Wu et al., 1993), accordingly resulting in superior therapeutic antitumor activity (Gabizon, 1992; Boman et al., 1994). Generally, PEGylated liposomes without encapsulated or surface coupled proteins are believed to have a lower immunogenicity or none at all (Alving, 1992; Harding et al., 1997). However, we and others (Ishida et al., 2003a,b, 2004, 2005; Ishida and Kiwada, 2008; Wang et al., 2007; Xu et al., 2010) recently found that repeated injections of PEGylated liposomes in the same animals at certain intervals induced significant immune responses (i.e., so called the “accelerated blood clearance (ABC) phenomenon”). An initial dose of PEGylated liposomes greatly reduced the circulation time and increased the hepatic and splenic accumulations of a subsequent dose. This immune response presents a tremendous challenge to liposomal research and their clinical use, since multiple injections of liposomes are very common in clinical settings. Nevertheless, it is worth noting that for PEGylated liposomal doxorubicin (Doxil/Caelyx), such a phenomenon has not been reported to occur in patients (Ishida et al., 2006a). Laverman et al. (2001) demonstrated that in a murine model, repeated administrations of Caelyx never induce the ABC phenomenon, which may be due to the toxicity of doxorubicin to the RES. Ishida et al. (2006a) proposed that the ABC phenomenon of PEGylated liposomal doxorubicin was abrogated resulted from the reduced production of anti-PEG IgM caused by the interference of doxorubicin with the proliferation of B cells. Cui et al. (2008) also found that an initial injection of PEGylated liposomal mitoxantrone failed to induce the ABC phenomenon. Taken together these observations may suggest that repeated injections of cytotoxic drug loaded liposomes do not induce the ABC phenomenon. However, it is important to note that recent researches were mainly focused on the ABC phenomenon induced by non-cell cycle specific drugs loaded liposomes, such as doxorubicin and mitoxantrone. Bally...
et al. (1990) have shown that the blood residence time of liposomes encapsulated with doxorubicin was significantly longer than that for identically prepared empty liposomes due to the doxorubicin-induced inhibition of RES activity (this inhibition, or “RES blockade,” is considered to be cytotoxic to phagocytic cells responsible for clearance). Also a study by Tardi et al. (2000) demonstrated similar plasma elimination rates for topotecan-loaded liposomes and mock-loaded or empty carriers, indicating that topotecan did not inhibit the population of nondiving RES cells. As stated above, the RES and B cells blockade induced by cytotoxic drugs is believed to be responsible for preventing the ABC phenomenon, suggesting that for cell cycle specific drugs, such as topotecan, repeated injections of PEGylated liposomes may still induce accelerated blood clearance in the same animals.

Thus, in the present study, we examined the effect of the encapsulated cell cycle specific drug-topotecan in PEGylated liposomes on the induction of the ABC phenomenon. The results may have important implications for the evaluation and therapeutic use of PEGylated liposomal (cytotoxic) drug formulations in humans.

2. Materials and methods

2.1. Materials

Hydrogenated soy phosphatidylcholine (HSPC) was purchased from Lucas Meyer (Düsseldorf, Germany). Topotecan HCI (TPT, purity 98.0% by HPLC) was supplied by Chengdu Tianyuan Natural Product Co. Ltd. (Chengdu, China). Cholesterol (CH) was provided by Nanjing Xinpai Pharmaceutical Co. Ltd. (Nanjing, China). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol) – 2000] (mPEG2000-DSPE) was obtained from Shanghai Nanjing Xinbai Pharmaceutical Co. Ltd. (Nanjing, China). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol) – 2000] (mPEG2000-DSPE) was obtained from Shanghai Yare Biotech, Inc. (Shanghai, China). Sephadex G-50 (medium) was supplied by Sigma Chemical Company (St. Louis, MO). Anion and cation exchange resins were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade.

2.2. Animals

Male Wistar rats weighing 210–240 g were purchased from the Laboratory Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Upon arrival, all rats were housed in microisolation cages and given free access to food and water. All experiments were carried out in accordance with the guidelines of the Animal Welfare Committee of Shenyang Pharmaceutical University.

2.3. Preparation of liposomes

Liposomes consisted of HSPC/CH/mPEG2000-DSPE (3/1/1, wt/wt/wt) were prepared by the modified ethanol injection method. Briefly, the lipid mixture was dissolved in ethanol and subsequently the solvent was removed at 65 °C. The resulting dry lipid film was hydrated at 65 °C in ammonium sulfate solution (200 mM (NH₄)₂SO₄, pH 5.5) under mechanical agitation to achieve a final lipid concentration of 50 mg/ml. Then, the resulting multilamellar vesicles (MLV) were sonicated using a JY92-2D Vibra-cell probe sonicator (Ningbo Xinzhi Biological Technology Co. Ltd., China) equipped with a tapered tip, for at least a two-minute cycle (200 w) and an additional six-minute cycle (400 w). In order to remove large particles, after sonication, the suspensions were passed through 0.8, 0.45 and 0.22 μm filter membranes at 25 °C. The mean diameters and zeta potentials of the liposomes were determined in purified water at 23 °C using a NICOMP 380 HPL submicron particle analyzer (Particle Sizing System, CA, USA). The zeta potential (V) was calculated from the electrophoretic mobility (m²/V S) using the Smoluchowski equation.

2.4. Drug loading and determination of encapsulation efficiency (EE)

TPT was encapsulated into liposomes using an ammonium sulfate gradient. The external buffer of the liposomes was exchanged by passage through an ion exchange resin column equilibrated with purified water, and the eluted liposomes were diluted with histidine buffer (100 mM, pH 5.5) to yield a final phospholipid concentration of 4.4 mg/ml. Subsequently, the liposomal dispersion and TPT solution (2 mg/ml) were mixed at a drug to lipid ratio of 1/10 (wt/wt), then incubated for 10 min at 50 °C.

After loading, untrapped TPT was removed with a Sephadex G-50 mini-column (10 × 70 mm) equilibrated with 5% glucose solution. The concentration of TPT in liposomal samples was assessed photometrically at 430 nm (Uvikon 756MC, The Third Analysis Instrument Factory of Shanghai, China) after solubilization of liposomes with 70% (v/v) ethanol containing 0.1 mol/l NaOH. The encapsulation efficiency (EE) was calculated as the percentage of TPT remaining in the liposomes following elution.

2.5. Determination of phospholipids by HPLC–ELSD

HPLC–ELSD analysis was carried out using an HPLC Elite instrument (Dalian, China) equipped with a P230 pump and a Rheodyne manual injector with a 20 μl sample loop. The analytical column (200 × 4.6 mm, 5μm) was packed with a silica normal-phase (Hanson Science & Technology Co., Ltd.). The mobile phase was methanol-acetic acid (50:1, v/v, pH was adjusted to 5.0 with triethylamine). The flow rate of the eluent was 1.0 ml/min. A Sedex (S.E.D.E.R.E., Alfortville, France) model 75 ELSD was used: the pressure of the nebulizer gas (nitrogen) was maintained at 2.0 bar and the drift tube temperature was set at 40 °C. Liposomes were lysed with methanol, and HSPC was determined by the HPLC–ELSD method as described above.

2.6. Pharmacokinetics and biodistribution of PEGylated liposomes

Male Wistar rats were randomly divided into 11 groups. The initial injection used PEGylated liposomes, with or without TPT, at a dose of 1, 3, 5, 10 or 20 μmol phospholipids/kg, and injection was via the tail vein. Control animals received an injection of glucose instead of liposomes. The interval between the two injections was 7 days. For the second injection, TPT-labeled test liposomes were injected intravenously at a dose of 5 μmol phospholipids/kg via the tail vein. At 0.0167, 0.083, 0.25, 0.5, 1.0, 2.0 and 4.0 h following i.v. injection, blood samples were obtained via eye puncture. The injection schemes for liposomes are presented in Table 1. After withdrawing the last blood sample at 4 h, the livers and spleens were excised, rinsed in ice-cold normal saline, and snap frozen. Blood samples were centrifuged at 2125 g for 10 min to separate the plasma. The plasma and tissue samples were stored at −20 °C until analysis.

2.7. Analytical procedure

The concentrations of TPT in plasma and tissue samples were assayed by a spectrofluorometric method. Before analysis, the plasma samples were treated as follows. To 100 μl plasma, 0.5 ml ethanol was added, and the mixture was then vortexed for 30 s, and 2 ml BaCl₂ solution (0.12 M) was added. The resulting mixture was vortexed for 30 s again, then 2.1 ml ZnSO₄ solution (0.12 M) was added after 5 min, the mixture was then vortexed for 1 min, and the supernatant was obtained by centrifuging the mixture at 5312.5 g for 10 min. Tissues were first homogenized using a Tissue Tearor equipped with a 80-mm probe (IKA WORKS GUANGZHOU,
Table 1

The injection protocols for liposomes.

<table>
<thead>
<tr>
<th>Group</th>
<th>First injection (empty liposomes or TPT liposomes)</th>
<th>Second injection (TPT liposomes)</th>
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<tbody>
<tr>
<td>A</td>
<td>Glucose injection</td>
<td>TPT-PEG-L</td>
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<tr>
<td>B</td>
<td>PEG-L (1)</td>
<td>TPT-PEG-L</td>
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<tr>
<td>C</td>
<td>TPT-PEG-L (1)</td>
<td>TPT-PEG-L</td>
</tr>
<tr>
<td>D</td>
<td>PEG-L (3)</td>
<td>TPT-PEG-L</td>
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<tr>
<td>E</td>
<td>TPT-PEG-L (3)</td>
<td>TPT-PEG-L</td>
</tr>
<tr>
<td>F</td>
<td>PEG-L (5)</td>
<td>TPT-PEG-L</td>
</tr>
<tr>
<td>G</td>
<td>TPT-PEG-L (5)</td>
<td>TPT-PEG-L</td>
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<tr>
<td>H</td>
<td>PEG-L (10)</td>
<td>TPT-PEG-L</td>
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<tr>
<td>I</td>
<td>TPT-PEG-L (10)</td>
<td>TPT-PEG-L</td>
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<td>J</td>
<td>PEG-L (20)</td>
<td>TPT-PEG-L</td>
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<tr>
<td>K</td>
<td>TPT-PEG-L (20)</td>
<td>TPT-PEG-L</td>
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PEG-L (1), PEG-L (3), PEG-L (5), PEG-L (10), and PEG-L (20) represent initial-dose empty PEGylated liposomes at lipid doses of 1, 3, 5, 10, and 20 μmol phospholipids/kg, respectively. TPT-PEG-L (1), TPT-PEG-L (3), TPT-PEG-L (5), TPT-PEG-L (10), and TPT-PEG-L (20) represent initial-dose TPT-containing PEGylated liposomes at lipid doses of 1, 3, 5, 10, and 20 μmol phospholipids/kg, respectively.

2.8. Quantification of IgM

Serum samples were collected on day 7 after a single dose of empty PEGylated liposomes or liposomal TPT at different doses, and the serum collected before the first injection was used as a control. Quantification of IgM was performed using a slightly modified procedure based on the published method (Ishida et al., 2007). Briefly, 50 μl of mPEG2000-DSPE ethanol solution (0.2 mmol/l) was added to each well of a 96-well microplate (Corning Inc., NY, USA) and thoroughly air dried. Then 100 μl of the blocking solution (50 mM Tris, 1% BSA, pH 8.0) was added to each well and the plate was incubated for 30 min. After incubation, the wells were washed five times with Tris-buffered saline containing 0.05% Tween 20. Then 100 μl of serum samples in the sample diluent (1% BSA, 0.05% Tween 20, 50 mM Tris, pH 8.0) were added to the wells, followed by incubation for 30 min and washing five times as described above. Horseradish peroxidase (HRP)-conjugated antibody (100 μl, 1 μg/ml, Goat anti-rat IgM IgG-HRP conjugate, Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) was added to each well. After incubation for 30 min, the wells were washed five times as described above. Washing was carried out by the addition of 100 μl of 0.05% Tween 20, 1% BSA, 50 mM Tris, pH 8.0. After incubation for 5 min, the reaction was stopped by adding 100 μl of 2 N H2SO4, and the absorbance was measured at 490 nm using a Microplate reader (Bio-Rad Laboratories Ltd., Hertfordshire, UK). All incubations were performed at 37 °C.

2.9. Statistical analysis

Statistical comparisons were performed using a One-Way ANOVA assay or univariate test with SPSS 16.0 software. P-values lower than 0.05 were considered statistically significant.

3. Results

3.1. The characteristics of the liposomes

It has been reported that the in vivo characteristics of liposomal drugs may be influenced by many factors, such as liposome size, zeta potential and drug-loading efficiency (Drummond et al., 1999). Therefore, the liposomes need to be fully characterized. In the present study, liposomal TPT was prepared by a transmembrane ammonium sulfate gradient. The mean particle size of the TPT liposomes was 134.4 ± 4.0 nm. Zeta potential measurement demonstrated that all the liposomes were negatively charged, with a zeta potential of ~10 mV. Also for all formulations, the percentage encapsulation was >90% and the drug-to-lipid ratio was 1:10 (wt/wt). Details with regard to the pharmaceutical properties of the prepared liposomes were summarized in Table 2, and the particle size and size distribution were described in Fig. 1.

3.2. Effect of TPT in the initial dose of PEGylated liposomes on inducing the ABC phenomenon of a second injection

The effects of TPT in an initial injection of PEGylated liposomes on inducing the ABC phenomenon of a second dose were investigated. Rats were given with PEGylated liposomes, with or without TPT, at a dose of 1 μmol phospholipids/kg, corresponding to 0.08 mg/kg TPT (Referred to the clinical dose of TPT (Armstrong, 2004)). Then according to the recommended scheduling of TPT liposomes by Inex Pharmaceuticals Corporation, the second (test) dose of drug-labeled PEGylated liposomes (5 μmol phospholipids/kg) was given on day 7 after the first injection (Madden and Sepmele, 2006). As shown in Fig. 2, the control group (group A) showed a long-circulating time as its main characteristic. However, to our surprise, unlike other cytotoxic drugs such as doxorubicin and mitoxantrone, repeated injections of TPT liposomes induced an obvious ABC phenomenon. Compared with the control group, the plasma concentration of TPT decreased significantly (P < 0.001) after repeated injections of PEGylated liposomes with or without TPT (groups B and C, Fig. 2). The plasma clearance rate (Cl) of liposomal TPT (group C) showed a sharp increase from 0.009 ± 0.001 to 0.305 ± 0.013 (P < 0.001).

3.3. Effect of the phospholipid dose and TPT dose in the first injection of PEGylated liposomes on the pharmacokinetics and distribution of a subsequent dose of PEGylated liposomes

The effect of the phospholipid dose and TPT dose in an initial injection of PEGylated liposomes on inducing the ABC phenomenon of a subsequent dose was also studied. Rats were pre-treated with PEGylated liposomes with or without TPT, and the injected TPT dose was 0.08, 0.24, 0.40, 0.80, and 1.60 mg/kg, corresponding to 1, 3, 5, 10, and 20 μmol phospholipids/kg, respectively. As shown in Fig. 3A, when rats received a relatively high (10 or 20 μmol phospholipids/kg) pre-dose of “empty” PEGylated liposomes, within 15 min the plasma concentration of TPT was significantly higher than the low (1, 3, or 5 μmol phospholipids/kg) pre-dose of “empty” PEGylated liposomes (P < 0.05, the Cl of groups B, D, F, H and J was 0.299 ± 0.011, 0.229 ± 0.009, 0.185 ± 0.007, 0.168 ± 0.004, and 0.074 ± 0.009 separately). In addition, when the TPT dose in pre-dose PEGylated liposomes increased, the plasma concentration of TPT of the second dose was increased correspondingly, especially within 1 h (Fig. 3B), indicating that increased phospholipids dose and TPT dose of the first injection could induce reduced ABC phenomenon.

Also biodistribution of the second dose at 4 h was investigated. However, to our surprise, the control group (group A) showed no...
decrease in the hepatic accumulation of the test dose compared with group B

And the splenic accumulation of the control group (group A) was significantly higher than group B (P < 0.05, Fig. 4B). In addition, there were no significant differences in the hepatic accumulation of the test dose among group B which rats were pretreated with different doses of PEGylated liposomes, with or without encapsulated TPT (P > 0.05). And as can be seen in Fig. 4B, when rats received a relatively low pre-dose of “empty” PEGylated liposomes (1 μmol phospholipids/kg, group B) or TPT loaded PEGylated liposomes (0.08 mg TPT/kg, group C), the splenic accumulations of the drug were correspondingly lower than in the other nine groups (P < 0.05).

3.4. IgM determination in rats following intravenous injection of empty PEGylated liposomes or liposomal TPT

We also determined the effect of the encapsulated TPT on the IgM production. Anti-PEG IgM concentration in serum was assessed on day 7 after a single injection of empty PEGylated liposomes or liposomal TPT at different doses. As depicted in Fig. 5, the serum anti-PEG IgM level of the control group was significantly lower than the other ten groups (P < 0.05), indicating that the induction of the ABC phenomenon accompanied with the increase of serum IgM. However, no significant decrease in IgM was observed after the injection of liposomal TPT, even though the TPT dose was up to 1.60 mg/kg. And there was no clear reverse relation between the anti-PEG IgM level and the phospholipid dose or TPT dose, indicating that the amount of anti-PEG IgM antibody did not completely correlate with the level of the ABC phenomenon.

4. Discussion

In this study, we investigated the effect of the encapsulated cell cycle specific drug-topotecan in PEGylated liposomes on the induction of the ABC phenomenon. The results we obtained demonstrated that, in contrast to non-cell cycle specific drugs such as doxorubicin and mitoxantrone, pre-dosing with TPT PEGylated liposomes...
liposomes can still induce a strong ABC phenomenon of the second dose. Furthermore, we also observed that an increased dose of phospholipid or TPT in the pre-dose liposomes causes an obvious reduction in the enhanced blood clearance effect. At present, the mechanism of the ABC phenomenon is generally considered to be as follows: the initial dose of PEGylated liposomes stimulates B cells in the splenic marginal zone to produce anti-PEG IgM, this serum factor selectively binds to the PEG presented on the surface of the second dose of liposomes, then activates the complement system, and subsequently induces the ABC phenomenon (Ishida et al., 2003b, 2006b,c). And for cytotoxic drugs loaded liposomes, as is stated above, B cells blockade in the splenic marginal zone induced by cytotoxic drugs is reported to be responsible for abrogating the ABC phenomenon. Therefore, the concentration of drugs in the splenic marginal zone, the exposure time of drugs to the B cells, and the effect time of drugs on the B cells in the splenic marginal zone may all have marked effects on B cell proliferation, accordingly leading to different degrees of the ABC phenomenon. This notion is supported by the observation that injection of PEGylated liposomal doxorubicin (1 μmol phospholipids/kg corresponding to 0.16 mg/kg doxorubicin) prevents the induction of the ABC phenomenon while injection of free doxorubicin (0.16 mg/kg) plus empty PEGylated liposomes (1 μmol phospholipids/kg) does not impair the ABC phenomenon (Ishida et al., 2006a). TPT is a cell-cycle specific drug (Abraham et al., 2004) which can only inhibit the population of B lympholeukocyte in the splenic marginal zone occupying in S phase. Furthermore, retention of TPT intraliposomes is not as satisfactory as that of anthracyclines due to its lipophilicity (Drummond et al., 2010). Our previous studies showed that at 24 h after injection, the amount of drugs remaining in the circulation was less than 10% and greater than 40% for liposomal TPT and liposomal doxorubicin, respectively. Therefore, based on the factors described above, we propose that when rats received an injection of TPT liposomes, only a fraction of the drugs can accumulate in the spleen due to drug leakage in the circulation, and these drugs can merely inhibit the population of B lympholeukocyte of S phase in the splenic marginal zone. Accordingly, the effect of drugs on the B cells in the splenic marginal zone was reduced...
dramatically, thereby resulting in that a first injection of PEGylated liposomes containing encapsulated TPT can still induce a strong ABC phenomenon of the second dose. In the present study, we also found that when rats were pre-treated with different doses of PEGylated liposomes with or without encapsulated TPT (1, 3, 5, 10 or 20 μmol phospholipids/kg, corresponding to 0.08, 0.24, 0.40, 0.80 and 1.60 mg/kg TPT), differences of the ABC phenomenon of a subsequent dose were only exhibited during the first 1 h (except first dose of liposomal TPT at 1.60 mg/kg). After 1 h, the plasma concentration of TPT (group B~J) decreased sharply and the drug levels in plasma of group B~J were similar to each other. This observation may be explained by the serum factor proposed by Dams et al. (2000), i.e., anti-PEG IgM reported later (Ishida et al., 2006b, c; Koide et al., 2010). First injection of PEGylated liposomes, with or without TPT, in rats elicits the production of anti-PEG IgM, which selectively binds to the surface of PEGylated liposomes injected subsequently, leading to a more rapid drug leakage from liposomes in the circulation. Thereby the plasma drug levels of group B~J decreased dramatically as a result of both the ABC phenomenon and a more rapid drug leakage caused by the considerable amount of anti-PEG IgM.

We also investigated the hepatic and splenic accumulation of group A~K. Interestingly, our hepatic and splenic accumulation test demonstrated that the control group (group A) showed no decrease in the hepatic accumulation of the test dose compared with group B~G (Fig. 4A). And the splenic accumulation of the control group (group A) was significantly higher than that of group B~K (P < 0.05, Fig. 4B). In addition, the effects of the phospholipids and TPT doses on the hepatic and splenic accumulation were contradictory to the previous studies which proposed that a higher lipid dose reduced the accumulation of liposomes in liver (Ishida et al., 2005, 2006a; Xu et al., 2010). In our study, it was clear that the higher the lipid and TPT dose, the larger the splenic accumulation (statistical significance could be demonstrated between group K and group B~I, P < 0.05), and this may also be explained by the drug leakage from liposomes induced by anti-PEG IgM. As described by Hiroshi Kiwada (Ishida et al., 2006a), when rats received a pre-administration of PEGylated liposomes at a lower dose, more anti-PEG IgM bound to liposomes of the second dose. Accordingly the drug leakage of the second injection was more rapid in the circulation, resulting in more “empty liposomes” accumulating in the liver/spleen. Therefore, even though a considerable amount of liposomes accumulated in the liver or spleen, only a little of the drug remained in the liposomes and, accordingly, the hepatic and splenic accumulation of the drug was lower than in the groups where rats received a higher dose of first injection. This suggests that using liposomes labeled with phospholipids is more reasonable than that labeled with a drug to investigate the hepatic and splenic accumulation of liposomes when drug retention is poor.

In general, the studies described here indicate that repeated injections of PEGylated liposomal TPT in rats can still induce a strong ABC phenomenon, suggesting that cytotoxic drugs loaded liposomes may induce different extent of the ABC phenomenon due to their roles in different stages of cell proliferation. In addition, the cytotoxic drug dose, dosage schedule, and drug retention characteristics intraliposomes may all have effects on the strength of ABC level, which may have important implications and need further investigation.

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References


Fig. 5. Anti-PEG IgM response following a single intravenous injection of empty PEGylated liposomes or liposomal TPT at a dose of 1, 3, 5, 10 or 20 μmol phospholipids/kg, corresponding to 0.08, 0.24, 0.40, 0.80 and 1.60 mg/kg TPT, respectively. Each value represents the mean ± S.D. of three separate experiments. P values apply to differences between the control and treated groups. *P < 0.05, **P < 0.01, and ***P < 0.001.


