phenomenon was also reported on various kinds of polymeric micelles [11,12].

TI antigens like PEGylated liposome can activate B cells and induce IgM antibodies production at the early stage of immunization [13,14]. On the other hand, at high doses, they lack the activation ability on B cells, and immune tolerance against PEGylated liposome was developed [15]. The ABC phenomenon is therefore suppressed by high-dose PEGylated liposome [15–19]. In this study, Lactosome with high dose is examined on the immune tolerance and the Lactosome ABC phenomenon.

2. Materials and methods

2.1. Materials

All regents and solvents were purchased commercially and used without further purifications. Poly(sarcosine)$_{64}$-*block*-poly(L-lactic acid)$_{30}$ (poly(Sar)$_{64}$-*block*-PLLA$_{30}$) and indocyanin green (ICG) labeled PLLA 30mer (ICG-PLLA$_{30}$) were synthesized as previously reported [5,20,21]. For the preparation of Lactosome, poly(Sar)$_{64}$-*block*-PLLA$_{30}$ (1, 5, 10, 15, 25, 35 mg) in test tube was dissolved into chloroform, and then evaporated to form polymer film on the test tube. Water was added to the tube so as concentration of the amphiphilic polymer to be 1 mg/mL (1–10 mg) or 5 mg/mL (15–35 mg). The dispersion was heated at 85 °C for 20 min. After freeze-drying of the solution, saline (1 mL) was added, and the solution was filtered with using syringe filter (pore size: 0.20 μm) just before administration. For the preparation of ICG-Lactosome, ICG-PLLA$_{30}$ (0.5–1 nmol) was additionally mixed into chloroform solution on polymer film formation step. Molar number of ICG-PLLA$_{30}$ was determined using UV absorbance of ICG-Sulfo-OSu at 795 nm. Subsequent protocol is the same with above.

2.2. Instruments

Hydrodynamic diameter of Lactosome was measured by dynamic light scattering (DLS) on a Nano-ZS (Malvern, United Kingdom). Near infrared fluorescence (NIRF) images were taken by Clairvivo OPT (Shimadzu Corp. Japan, ex. 785 nm/em. 845 nm). The pseudo images were constructed from the photon counts. ELISA assay for anti-Lactosome antibody was performed using Multiskan Spectrum Microplate Photometer (Thermo Scientific, USA).

2.3. Preparation of tumor-bearing mice

BALB/c nu/nu mice (Body weight: 20–22 g) were purchased from Japan SLC, Inc. (Japan). SUIT-2/pEF/luc cells (5×10^5 cells) were dissolved in phosphate-buffered saline (PBS, 20 μL), and subcutaneously inoculated into right femoral region of 7-week-old male nude mice [5]. The mice were used for *in vivo* experiments after 2 weeks from the tumor transplantation.

2.4. In vivo near-infrared fluorescence (NIRF) imaging

On experiment, which evaluate effect of first Lactosome dosage amount to the Lactosome ABC phenomenon, tumor bearing mice were divided into 7 groups (n = 3). To the mice of each group, Lactosome of 35 nm in diameter was intravenously injected (5, 25, 50 mg/kg/100 μL, 150, 250, 350 mg/kg/200 μL). To the control mice (Lactosome 0 mg/kg), saline (100 μL) was injected. After 7 days from the first Lactosome dosage, ICG-Lactosome (diameter: 35 nm) was intravenously injected (100 μL, 5 mg/kg) to the mice. Injected ICG amount was set to be 5 nmol/kg. NIRF images were taken at 5 min and 24 h after the administration. During the imaging process, the mice were held on the imaging stage under anesthetized condition with 2.5% of isoflurane gas in air flow (1.5 L/min).

On experiment, which evaluate effect of second Lactosome dosage amount to the Lactosome ABC phenomenon, tumor bearing were divided into 6 groups (n = 3). To the all mice except control group ones, Lactosome of 35 nm in diameter was intravenously injected (100 μL, 5 mg/kg). To the mice of control group (Lactosome 0 mg/kg), saline (100 μL), was injected. At 7 days after the first Lactosome administration, ICG-Lactosome (diameter: 35 nm) was intravenously injected (5, 25, 50 mg/kg/100 μL, 150, 350 mg/kg/200 μL) to the mice. Injected ICG amount was set to be 5 nmol/kg on all groups. NIRF images were taken in the same method with above.

2.5. Preparation of serum

On experiment, which evaluate effect of first Lactosome dosage amount to the Lactosome ABC phenomenon, mice blood was collected after 7 days from the first Lactosome (diameter: 35 nm, injection volume: 100 μL (5, 25, 50 mg/kg), 200 μL (100, 150, 200, 250 mg/kg)) dosage. Collected blood from inferior vena cava under anesthesia condition was transferred into Microstrainer® tube (BD Corp. USA). Blood serum was separated by centrifugation (10 min, 3000 rpm) and saved at −40 °C.

On experiment, which evaluate effect of second Lactosome dosage amount to the Lactosome ABC phenomenon, mice were occurred ABC phenomenon to be injected Lactosome (5 mg/kg). To the mice, Lactosome (diameter: 35 nm, injection volume: 100 μL (5, 25, 50 mg/kg), 200 μL (150, 350 mg/kg)) was injected at 7 days after first injection. The mice blood were collected at 7 days after second administration, and treated in the same way with above.

2.6. Enzyme-linked immunosorbent assay (ELISA) experiment

Lactosome (0.5 μg) in 50 μL distilled water was added to 96-well plates and air dried completely for 1 day. Then, 150 μL of blocking buffer (2% BSA/PBS) was added and incubated for 2 h. The wells were washed four times with washing buffer (PBS-T: 0.05% Tween 20 in PBS). Mice serum were added to the wells and incubated for 2 h. After the incubation, the wells were washed four times using PBS-T. Peroxidase conjugated goat-anti-mouse IgM in 0.1% BSA/PBS (50 μL, Southern Biotech, USA) was added as the secondary antibody. After incubation of the solution for 2 h, and then the wells were washed again four times with PBS-T. *o*-Phenylenediamine (0.5 mg/mL, Sigma, St. Louis, MO), which was dissolved in 0.0003% H_2O_2-0.1 M citrate phosphate buffer (pH 5.0), was added to the microplate. 10 min after the *o*-Phenylenediamine addition, optical density (OD) was determined from UV absorbance at 490/reference at 620 nm.

2.7. Ethics

All of our *in vivo* animal experiments were approved by the Animal Research Committee of Kyoto University. Animals were treated humanely.

3. Results

3.1. Preparation of Lactosome and ICG-Lactosome

Lactosome, which is composed of poly(Sar)$_{64}$-*block*-PLLA$_{30}$ amphiphilic polydepsipepitde, was prepared by the film rehydration method. ICG-Lactosome was prepared by the same method with Lactosome but with an appropriate amount of ICG-labeled poly(L-lactic acid) 30mer (ICG-PLLA$_{30}$) in addition to the amphiphilic polydepsipeptide on polymer film preparation (Fig. 1). Lactosome showed no toxicity up to 2000 mg/kg (Fig. S1) and blood-half time of ICG-Lactosome was calculated to be 17.8 h in mice (Fig. S2).

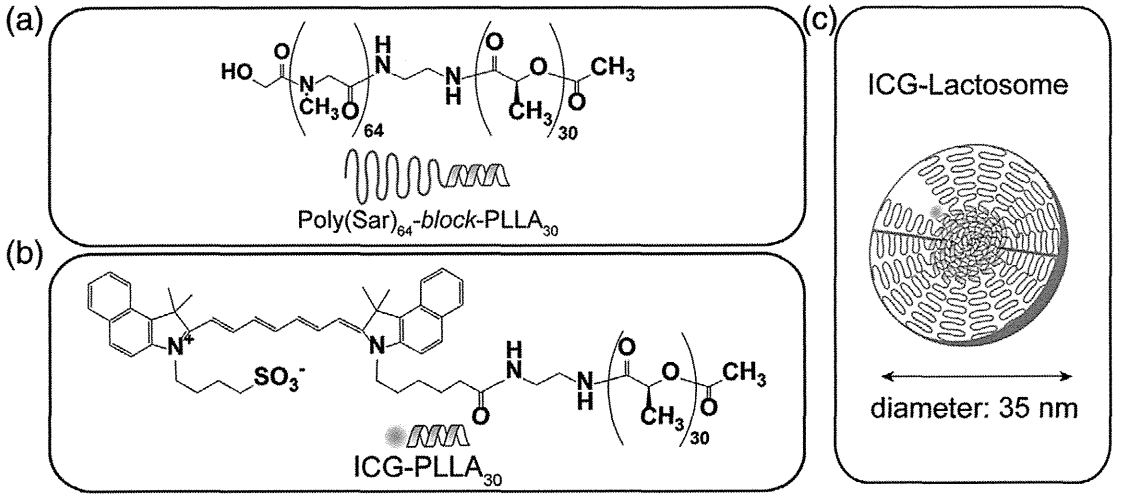

Fig. 1. Chemical structure of (a) poly(Sar)$_{64}$-*block*-PLLA$_{30}$ (b) ICG-PLLA$_{30}$. (c) Illustration of polymeric micelle of ICG-Lactosome.

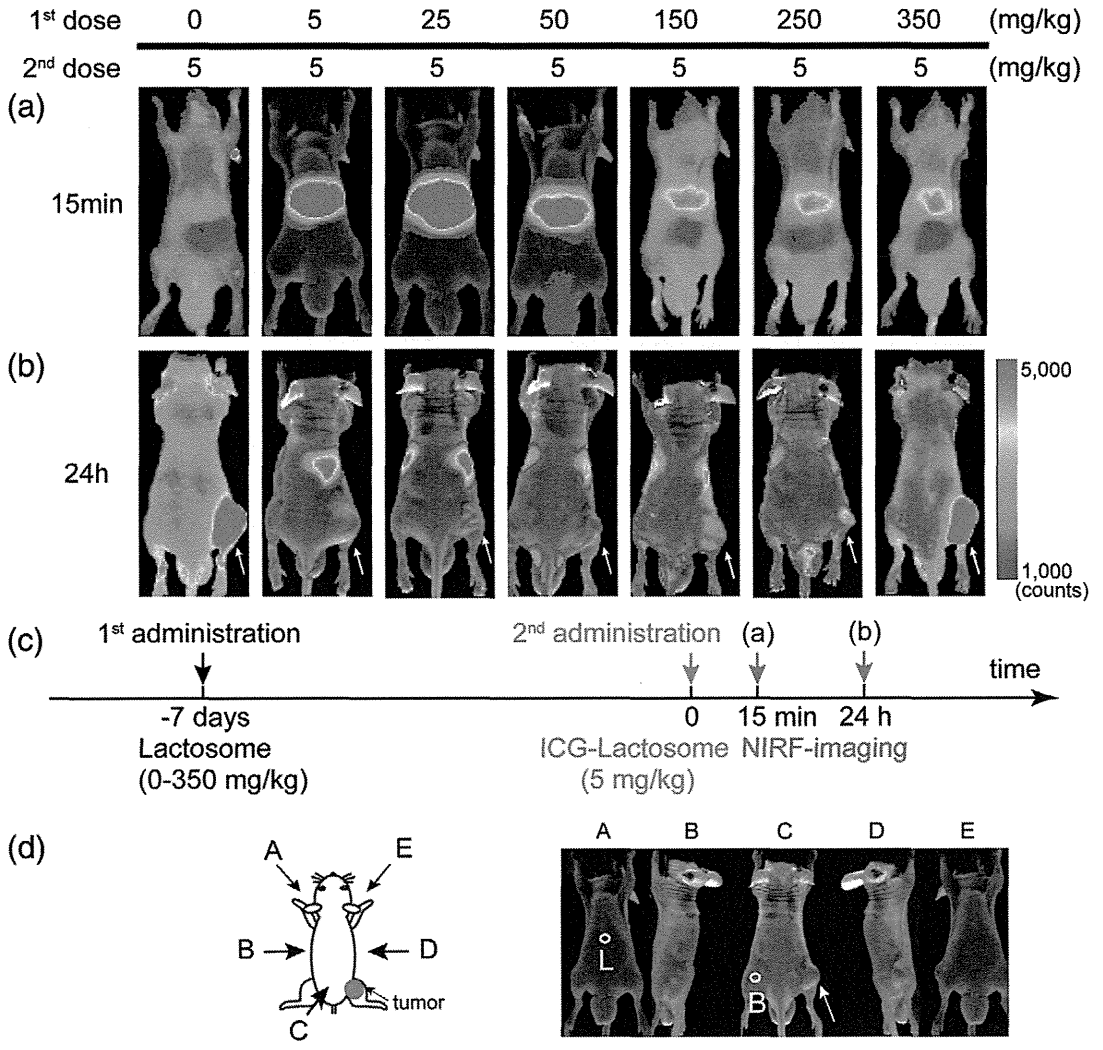

Fig. 2. The effect of the first Lactosome dose on the Lactosome ABC phenomenon. NIRF images of mice at (a) 15 min (view from A in Fig. 2d) and (b) 24 h (view from C in Fig. 2d) after ICG-Lactosome of 2nd dose. Lactosome (5, 25, 50 mg/kg/100 μL, 150, 250, 350 mg/kg/200 μL) were injected to the mice 7 days before the ICG-Lactosome administration. SUIT-2/pEF/luc cells were transplanted at the right femoral region of mice, which tumor sites are indicated by white arrows. The fluorescein signal ranges were set to be the same for all the images from max count 5000 to min count 1000. (c) Time schedule for the NIRF imaging. Black and green arrows indicate the injection time points of Lactosome and ICG-Lactosome, respectively. NIRF imaging was performed at red arrows. (d) NIRF images were taken with using Shimadzu Clairvivo OPT, which can take five images from different directions (A–E) with one time shot. The white circles indicate positions of ROI (L: liver, B: Background).

3.2. Effect of the first Lactosome dose on the ABC phenomenon

Tumor bearing mice were divided into seven groups and Lactosome was pre-administrated at different doses of 5, 25, 50, 150, 250, and 350 mg/kg. As a control group, only saline was administrated. At 7 days after the first Lactosome administration, ICG-Lactosome (5 mg/kg) was injected, and NIRF images were taken at 15 min and 24 h after the second injection (Fig. 2a and b). The Lactosome ABC phenomenon, which is indicated by accumulation of NIRF signal at liver instead of spreading over the whole body, was observed with the groups of the first Lactosome doses in the range of 5–50 mg/kg. With increasing the amount of Lactosome pre-dose over 150 mg/kg, the NIRF signals were found to spread over the whole body via blood stream, followed by accumulation in the transplanted tumor regions in the NIRF images at 24 h.

The region of interest (ROI) analyses at liver were carried out with taking the fluorescence intensity at the healthy left femoral region as background using NIRF images, which were taken 15 min after ICG-Lactosome administration (Figs. 2a, d, and S3). The signal intensity ratios at liver against background (L/B) are summarized in Table 1 with those at the single Lactosome dose (corresponding to the case of 1st dose of 0 mg/kg), $(L/B)_0$. Occurrence of the Lactosome ABC phenomenon can be evaluated by the numerical values of $(L/B)/(L/B)_0$ taking more than the critical value of 2.0 according to Table 1.

Since the Lactosome ABC phenomenon is well related with production of anti-Lactosome IgM induced by the first Lactosome administration irrespective of ICG labeling [6], the levels of anti-Lactosome IgM at 7 days after the dosing in the range of 5–250 mg/kg were evaluated by ELISA (Fig. 3). The group of the Lactosome dose of 5 mg/kg showed the highest IgM production. As the Lactosome dose was increased, the amount of anti-Lactosome IgM was inversely decreased.

3.3. The dose dependence of Lactosome pharmacokinetics at the second administration

With using mice immunized with the Lactosome pre-dose of 5 mg/kg (Fig. 2), ICG-Lactosome (5–350 mg/kg with keeping ICG concentrations the same for all the groups) was administered to evaluate the dose dependence of the pharmacokinetics. NIRF images were taken at 15 min and 24 h after the administration (Figs. 4a, b, and S4). When the second Lactosome dose was as low as 5 or 25 mg/kg, the Lactosome ABC phenomena were observed. On the other hand, with the second Lactosome doses over 50 mg/kg, ICG-Lactosome spread over the whole body, and ICG signal was detected from the transplanted tumor region at 24 h after the Lactosome second administration.

Table 2 shows the results of the ROI analyses on NIRF imaging at 15 min after ICG-Lactosome administration (Figs. 4a and S4). When the second Lactosome doses were 5 and 25 mg/kg, $(L/B)/(L/B)_0$ became 10.8 and 6.32, respectively, indicating occurrence of the ABC phenomenon. Whereas, $(L/B)/(L/B)_0$ decreased below 2.0 in the cases that the second doses were over 50 mg/kg.

Table 1
The effect of first Lactosome dose on Lactosome biodistribution at second administration.

Lactosome administration (mg/kg)	(L/B)	$(L/B)/(L/B)_0$[a]	ABC phenomenon[b]
0	1.03 ± 0.08	–	–
5	11.1 ± 5.5	10.8 ± 5.4	Yes
25	8.67 ± 6.42	8.40 ± 6.22	Yes
50	6.57 ± 5.09	6.36 ± 4.93	Yes
150	1.80 ± 0.53	1.74 ± 0.52	No
250	1.15 ± 0.17	1.12 ± 0.17	No
350	1.47 ± 0.22	1.43 ± 0.22	No

[a] The $(L/B)_0$ value was determined to be 1.03 from NIRF images of mice at 15 min after single administration of ICG-Lactosome (Lactosome pre-dosage of 0 mg).
[b] The $(L/B)/(L/B)_0$ values taking more than 2.0 are indication of occurrence of the ABC phenomenon.

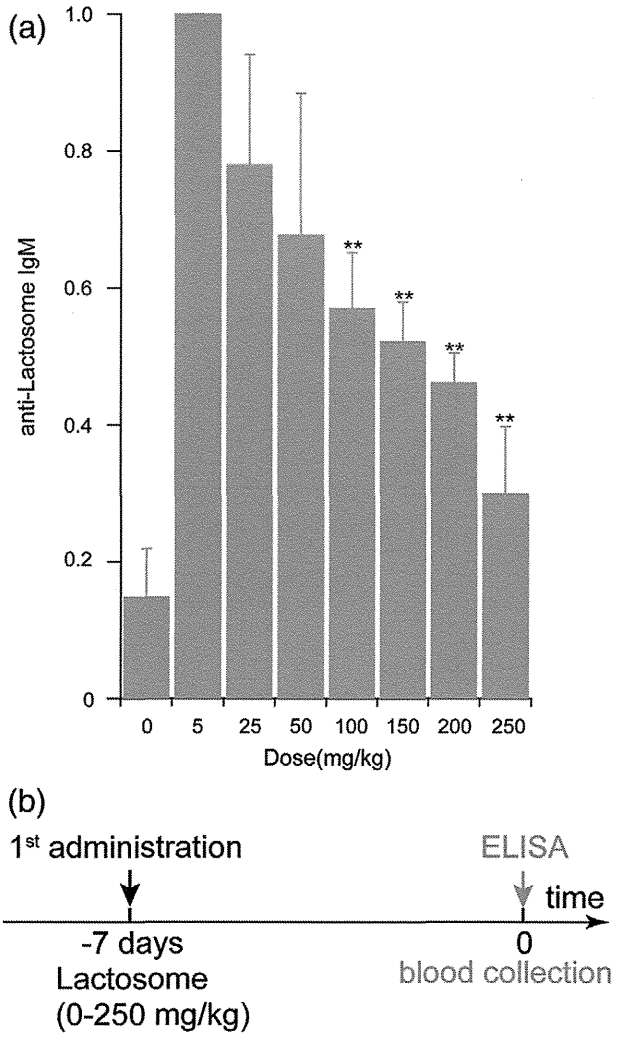

Fig. 3. (a) The anti-Lactosome IgM level at 7 days after the Lactosome administration with varying the amounts (5, 25, 50 mg/kg/100 μL, 100, 150, 200, 250 mg/kg/200 μL). Each value was normalized with taking the IgM production with 5 mg/kg dose as a reference. (b) Time schedule for the experiment. Black arrow indicates the injection time point of Lactosome. The serums were collected at 7 days after the injection (the green arrow) and subjected to ELISA. ** represents significant difference from 5 mg/kg dose groups with $p < 0.01$.

The second dose dependence of anti-Lactosome IgM productions was evaluated by ELISA. At 7 days after the Lactosome second administration with varying the doses from 5 to 350 mg/body, mice serums were collected to evaluate the anti-Lactosome IgM level. At any doses, anti-Lactosome IgM was found to maintain the high level (Fig. 5).

4. Discussion

The first Lactosome dose dependence of the ABC phenomenon shows a critical value of 50 mg/kg dose. When the first Lactosome dose was below 50 mg/kg, the ICG-Lactosome at the second administration was entrapped in liver (Fig. 2) with high $(L/B)/(L/B)_0$ values (Table 1), indicating occurrence of the Lactosome ABC phenomenon as previously reported [6]. On the other hand, when the first Lactosome dose was over 150 mg/kg, the ICG-Lactosome at the second administration spread over the whole body followed by the accumulation in the tumor region by the EPR effect (Fig. 2) with low $(L/B)/(L/B)_0$ values below 2.0 (Table 1). These results suggest that tolerance to immunogenic Lactosome was developed with high dose similarly to the report that high-dose PEGylated liposome evaded from the ABC phenomenon [22]. However, the anti-Lactosome IgM levels in serums decreased just

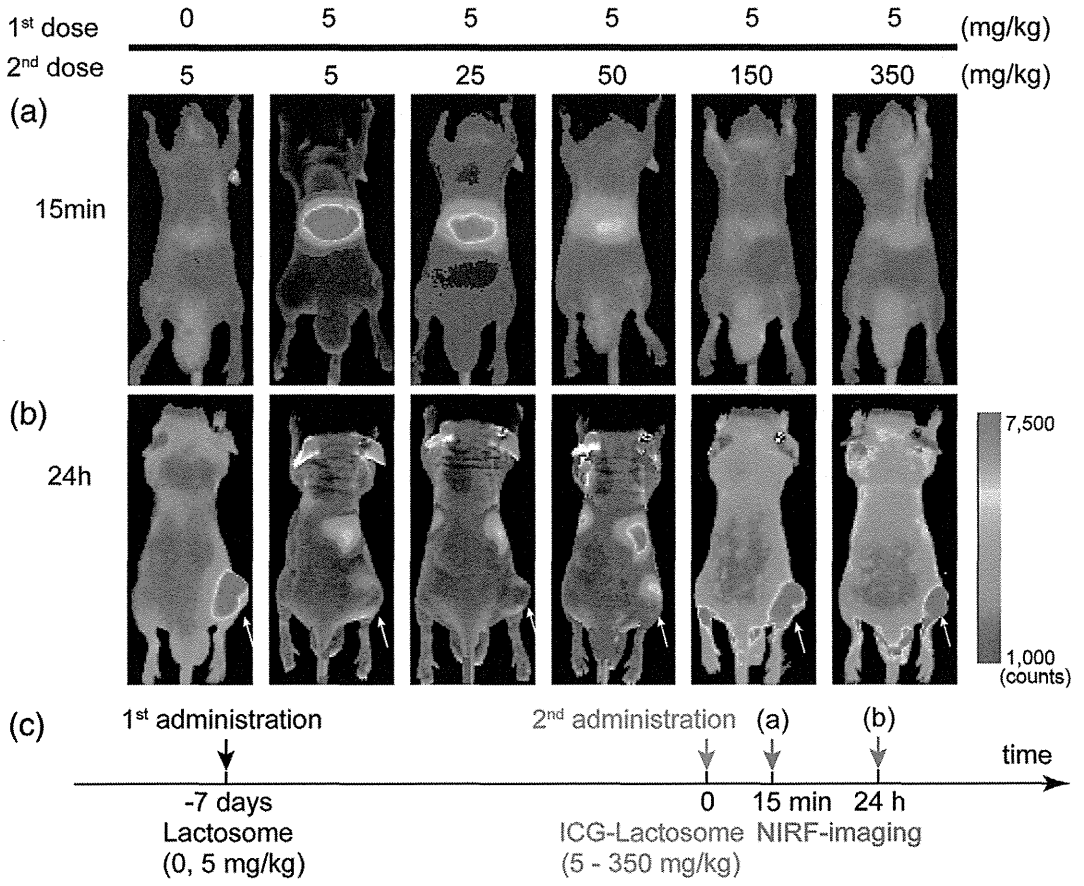

Fig. 4. The second dose dependence of the Lactosome pharmacokinetics. The second Lactosome doses were varied from 5 to 350 mg/kg (5, 25, 50 mg/kg/100 μL, 150, 350 mg/kg/200 μL). The injected ICG amounts were kept the same to be 5 nmol/kg for all the groups. NIRF images of tumor-transplanted mice at 15 min (a) and 24 h (b) after ICG-Lactosome administration. The white arrows indicate the tumor region. Fluorescence ranges were set to be the same for all the images from max count of 7500 to min count of 1000. (c) Time schedule for the NIRF imaging. The black and green arrows indicate the injection time points of Lactosome and ICG-Lactosome to the tumor-transplanted mice, respectively. NIRF imagings were performed at the time points of red arrows.

monotonously with increasing the first Lactosome doses without showing any critical value (Fig. 3), meaning that the development of immune tolerance is insufficient with these doses even though the anti-Lactosome IgM level diminished with a large amount of Lactosome dose. In order to analyze the immune response more quantitatively, Lactosome doses at the second administration were varied.

When the ICG-Lactosome dose over 50 mg/kg was administered to the immunized mice, the NIRF signals spread over the whole body followed by accumulation in the tumor region (Fig. 4). However, anti-Lactosome IgM in serum kept high level irrespective of the second doses in the range from 5 to 350 mg/kg (Fig. 5). The high doses therefore did not develop the immune tolerance. The ABC phenomenon was still there, but anti-Lactosme IgM should be consumed with excessive doses of over 50 mg/kg at the second Lactosome administration, leading ICG-Lactosome free from binding of anti-Lactosme IgM.

Table 2
The effect of second Lactosome dose on its biodistribution.

Lactosome administration (mg/kg)	(L/B)	$(L/B)/(L/B)_0$[a]	ABC phenomenon[b]
0	1.03 ± 0.08	–	–
5	11.1 ± 5.5	10.8 ± 5.4	Yes
25	6.52 ± 1.85	6.32 ± 1.80	Yes
50	1.65 ± 0.67	1.60 ± 0.64	No
150	0.91 ± 0.01	0.88 ± 0.01	No
350	0.91 ± 0.06	0.88 ± 0.06	No

[a] The $(L/B)_0$ value was determined to be 1.03 from NIRF images of mice at 15 min after single administration of ICG-Lactosome (Lactosome pre-dosage of 0 mg).
[b] The $(L/B)/(L/B)_0$ values taking more than 2.0 are indication for occurrence of the ABC phenomenon.

The concentration of anti-Lactosome IgM in serum at 5 days after the first Lactosome administration was roughly determined to be 49 μg/mL by the Mouse IgM ELISA quantitation set (Table S1). With using a total blood pool of a nude mouse and the molecular weight of IgM of ca. 2.0 mL and 970 kDa, respectively [23], the total amount of anti-Lactosome IgM in blood is calculated to be ca. 5 nmol/kg. Under the assumption that Lactosome is consisted of ca. 200 amphiphilic polydepsipeptide, Lactosome of 50 mg/kg corresponds to 35 nmol/kg. The Lactosome dose over 50 mg/kg is thus far excessive to the amount of anti-Lactosome IgM. In other words, anti-Lactosome IgM does not work sufficiently against the high dose of Lactosome.

Taken together, all the data presented here can be interpretable as follows. With increasing the first Lactosome dose, the anti-Lactosome IgM level decreases gradually due to partial immune tolerance. When the anti-Lactosome IgM level becomes lower than the amount required for opsonization of Lactosome of 5 mg/kg, the ABC phenomenon disappears at the second administration of the ICG-Lactosome of 5 mg/kg. On the other hand, even mice, which produce the high level of anti-Lactosome IgM with Lactosome pre-dose of 5 mg/kg, do not show the ABC phenomenon when the ICG-Lactosome is administered together with excessive amount of Lactosome, which should consume anti-Lactosome IgM produced in immunized mice.

It is considered that immune tolerance is induced by frequent administration of low dose antigen or administration of high dose antigen [24,25]. As far as TI antigens are concerned, PEGylated

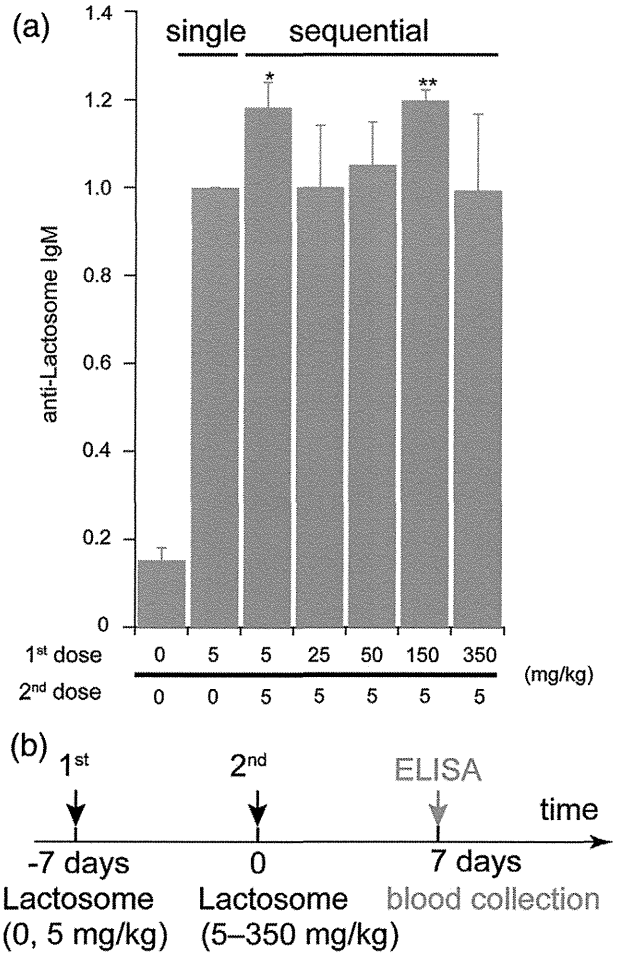

Fig. 5. (a) The effect of second Lactosome dose on the produced anti-Lactosome IgM level. Each value was normalized by anti-Lactosome IgM production level when 5 mg/kg of Lactosome was singly administrated to the mice. (b) Time schedule for the experiments. The black arrows indicate the injection time points of Lactosome. The serums were collected at 7 days after the second Lactosome administration (5, 25, 50 mg/kg/100 μL, 150, 250, 350 mg/kg/200 μL), which is indicated by the green arrow. * and ** represent significant difference from the groups of single administration of 5 mg/kg with $p < 0.05$ and $p < 0.01$, respectively.

liposome was shown to induce immune tolerance with administration of high dose PEGylated liposome [15–19]. Similarly, TI antigen of Lactosome is shown here to induce partial immune tolerance at higher doses of first administration. In this case, the immune tolerance is usually interpretable as B cell's anergy or apoptosis [23]. In order to get more information on it, the regular dose of Lactosome was administered to the partially immunized mice with a high dose at first administration (Fig. S5 and Table S2). The NIRF imaging using ICG-labeled Lactosome clearly shows the occurrence of the ABC phenomenon upon the second regular dose. It is therefore suggested that B cell's apoptosis is a more plausible explanation than the establishment of negative signaling to B cell against the immunogenic Lactosome.

Once anti-Lactosome IgM level was raised by Lactosome administration, the production could not be suppressed by following Lactosome administration with high doses. Lactosome is found to be one of strong TI-2 antigens, which may be ascribed to the long clearance time from the blood stream and also to the repetitive and regular epitope presentation on the surface of nanoparticles. Lactosome, therefore, effectively activates B1 to generate IgM with the first administration of Lactosome, which may lead to keep the memory effect [26,27]. The repeated exposure to Lactosome may induce the strong immune response of B cells, but which details remain to be studied.

5. Conclusion

T-cell independent immune response is known to be tolerated by high-dose antigens. In this study, the Lactosome ABC phenomenon, which is caused by production of anti-Lactosome IgM, could be suppressed by high-dose Lactosome at either first or second administration, and transplanted tumor could be imaged. In terms of the anti-Lactosome IgM level, the high dose at the first administration develops a partial immune tolerance, but at the second administration does not. Importantly, any acute toxicity with Lactosome of high dose was not observed. Therefore, high-dose Lactosome is a considerable approach to evade from the Lactosome ABC phenomenon, which makes it possible to use Lactosome for multiple imaging.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2013.03.024.

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トランスレーショナルリサーチへの薬剤師の新たなかかわり

治験薬GMP基準の特殊無菌製剤室の設置

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2002年京都大学薬学部卒業，2004年京都大学大学院薬学研究科修士課程修了，2007年京都大学大学院薬学研究科博士後期課程修了．博士(薬学)取得，2007年より現職．

1　はじめに

我が国のライフサイエンス分野の基礎研究成果は国際的にも高い評価を受けている．しかしながら，臨床研究への橋渡しを支援する体制の基盤が十分整備されていないため，基礎研究成果が医療・製薬等の臨床現場に届いておらず，国民に成果が還元されていないと指摘されている．第3期科学技術基本計画に基づき，2007年度より基礎研究成果を効率的に臨床へ橋渡しするための基盤整備を目的とした文部科学省橋渡し研究支援推進プログラムが開始された(http://www.tr.mext.go.jp)．医療としての実用化が見込まれる有望な基礎研究の成果を効率的に開発し実用化するためには，シーズの開発戦略策定や，薬事法に基づく試験物製造のような橋渡し研究の支援を行う機関を整備し，トランスレーショナルリサーチ※1を推進していくことが重要である．

2　治験薬good manufacturing practice (GMP)に基づいた試験薬製造の重要性

当院は，文部科学省橋渡し研究推進プログラムの橋渡し研究支援推進機関として採択され，創薬・新規医療開発のアカデミア拠点形成を目指している．基礎研究の成果を基に早期探索的臨床研究を実施するに当たり，試験薬の供給は障壁の一つとなる．特に，細胞製剤をはじめとする革新的な試験物を用いる場合，研究者が自ら製造する必要が生じる．治験を実施する際に製薬企業等で製造される治験薬の製造管理および品質管理については，「治験薬の製造管理，品質管理等に関する基準(治験薬GMP)」[1]が定められている．他方，臨床試験で用いる試験薬を院内で製造する場合の製造管理および品質管理については，明確な基準はない．しかし，被験者の安全と試験結果の信頼性を確保するためには，適切な製造管理と品質管理の下で製造された高品質な試験薬が必要と考えられる．

2008年7月9日に，マイクロドーズ臨床試験を含めた早期探索的臨床試験など，開発早期に用いる試験薬(治験薬)への治験薬GMP適用の観点から，開発段階に応じて柔軟に運用できるよう治験薬GMPが改正された．したがって，臨床試験の試験薬を院内で製造する場合にも，被験者保護と臨床試験の信頼性確保のために，治験薬GMPレベルでの品質保証が望ましいと考えられる．

3　治験薬GMP基準を満たした特殊無菌製剤室の設置

1．製造施設の構造設備の整備(図1)

既存の無菌製剤室とは独立して，資材置き場，無塵衣の脱着を行う前室，器具の洗浄・滅菌を行う洗

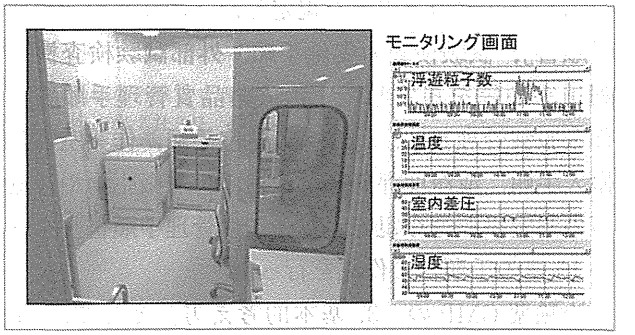

図1　治験薬GMP基準の特殊無菌製剤室の構造設備

※1　基礎研究の成果を基に，研究者が新たな疾病治療法や診断法などの開発を目的として，ヒトへの臨床応用を行う研究のことをいう．

浄室，環境モニタリング装置を設置した無菌室からなる特殊無菌製剤室(1ユニット)を新たに配置した．無菌室には環境モニタリング装置を有するクリーンベンチを設置した．また，洗浄室および前室にはRO精製水を供給する流しを設置した．治験薬製造施設の基準として「治験薬の製造施設の構造設備基準」が定められており，この基準に従って評価したところ，幾つかの逸脱点が確認されたが，おおむね基準を満たしていた．

治験薬GMPでは，開発初期から必ずしもすべての構造設備の要求を満たすことを求めていない．すなわち，開発に伴う段階的な状況やリスクを考慮して，適切だと判断される要件については柔軟に運用することとされている．特に製造施設の構造設備については，治験薬GMP「3.4 治験薬の製造施設の構造設備については，…」において，治験薬の製造スケール等，開発と共に大きく変更されることが必然であり，「開発段階に応じたより適切な管理が求められる」とされている．また，「医薬品の製造販売承認の要件及び医薬品の製造業許可の要件として求められる製造所の構造設備を認識した上で，必要な対応を図ること」と述べられている．[1] したがって，本施設においても「治験薬の製造施設の構造設備基準」をすべて満たすものではないが，初期の段階においては個々の試験薬製造に適したバリデーション，ベリフィケーション，試験薬の安定性試験を実施することで，早期探索的臨床試験に応じた適切な管理のもと製造された試験薬の提供が可能であると考える．

2. 手順書・記録書の整備(図2)

治験薬GMPに基づいて，原料や治験薬の保管管理，製造工程や構造設備の管理，治験薬の製造指図を示した文書の運用等を規定した「治験薬製造管理手順書」，試験検査の実施手順，外部試験検査機関利用手順等を規定した「治験薬品質管理手順書」，作業服装管理基準，作業室入退室手順，作業室物品搬入手順，清浄度評価基準，表面付着微生物や浮遊菌の測定手順等を規定した「衛生管理手順書」を含む15冊の手順書の作成を行った．

治験薬GMPの「3. 基本的考え方」として，[1] 治験薬の製造管理および品質管理に係るすべての記録について，後日の確認が取れるように保管すること，開発段階におけるすべての変更を管理し，文書化し，記録として保存することが挙げられている．今回，治験薬GMPで求められる手順書を作成し，製造管理，品質管理の各段階においてすべての情報に関する文書を作成することにより，確実に記録を残すことができる体制を構築した．このことにより，臨床試験で得られた成果や試験薬の安全性に関する信頼性が担保されると考えられる．さらにアカデミアで実施した臨床試験の後，治験の実施等，承認を目指した開発のために臨床試験の成果を企業にライセンスアウトする．治験薬GMPに従って製造管理，品質管理，変更等に関する記録が保管されていることで，知財権だけでなく製造に関するノウハウを含めて導出が可能となった．したがって，アカデミアにおける臨床試験成果が，論文作成の情報となるだけでなく，今後の開発において有用な資料になり得る．

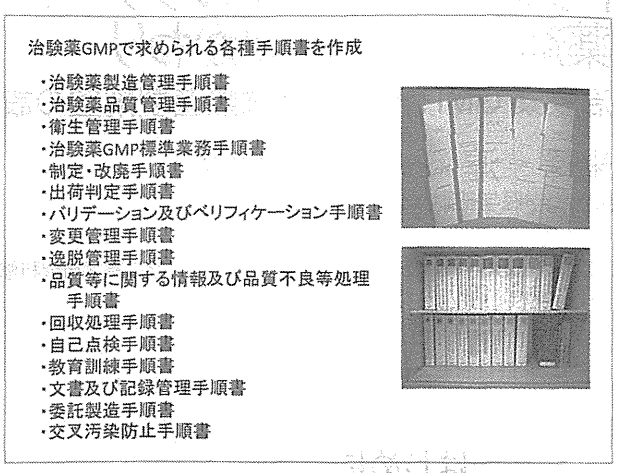

図2 治験薬GMP基準の手順書および記録書

3. 管理体制の確立(図3)

担当人員について，治験薬GMP標準業務手順書に治験薬製造，品質管理に携わる責任者および担当者を任命するための手順を規定し，治験薬GMPで要求される人員配置を行った．なお，本施設においては治験薬の品質管理に係る部門(治験薬品質部門)の担当者が，製剤部門責任者を除く責任者を兼務することにより，4名の人員配置を行った．製剤部門責任者のもと治験薬ごとに，治験薬製造部門と治験薬品質部門を互いに独立させて設置した．

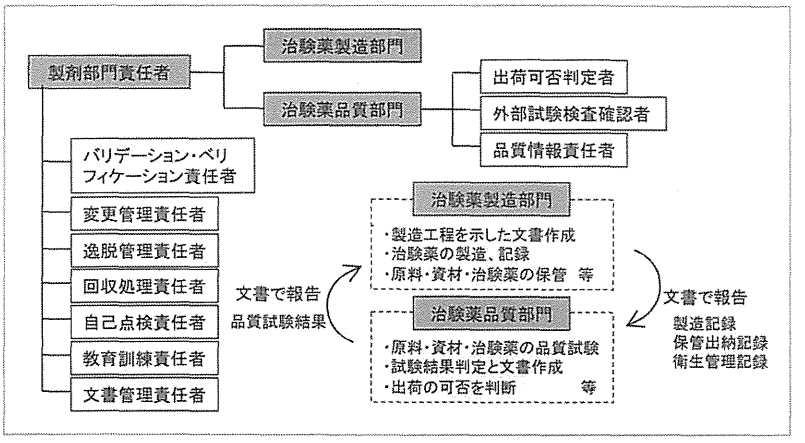

図3 治験薬GMP基準の管理体制

4 治験薬GMP基準の院内製造試験薬を用いた臨床試験

本施設において，治験薬GMP基準を満たして製造を行った試験薬を用いた臨床試験が，第3項先進医療(高度医療)「生体内吸収性高分子担体を用いた塩基性線維芽細胞増殖因子による血管新生療法　慢性閉塞性動脈硬化症又はバージャー病」として承認された.[※2] なお本臨床試験は，院内製剤を用いた初めての高度医療である.

他にも，「急性高度難聴に対する内耳IGF 1投与治療のランダム化対照臨床試験(UMIN 000004366)」を実施している．本臨床試験では，当院においてDDS器材を製造して他施設への配布を行い，多施設で臨床試験を行っている．他施設への院内製剤の交付にあたり，厚生労働省へ「スーパー特区における臨床研究を目的とする未承認医薬品・医療機器の提供に係る特別個別相談」を実施し，薬事法に抵触しないよう助言をいただき，交付を開始した.

以上のように，適切な管理を行いながら製剤を製造し，先進医療や多施設臨床試験などを行っている.

※2 本臨床試験に関するホームページ　http://www.mhlw.go.jp/topics/bukyoku/isei/sensiniryo/kikan02.html，http://www.kyoto-u.ac.jp/ja/news_data/h/h1/news6/2010/100901_1.htm

5 おわりに

トランスレーショナルリサーチの推進のためには，基礎研究者，プロジェクトマネージャー，医師，治験コーディネーター，生物統計家，製薬企業など種々の専門家が協力して行うことが最も大切である．現在進行中の臨床試験も，探索医療センターを中心として，多くのメンバーが参画し研究を遂行している.

早期探索的臨床試験を実施するに当たり，試験薬の供給は障壁の一つになる．そのため薬剤師が専門を生かし，チームの一員として院内製剤製造を行うことにより，トランスレーショナルリサーチの推進に寄与することができる．今回，当院薬剤部に治験薬GMP基準の臨床試験用院内無菌製剤室を設置したことにより，高品質な試験薬の提供が可能となり，被験者の安全と試験の信頼性を確保した質の高い臨床試験に貢献できたものと考える.[2]

参考文献

1) 治験薬の製造管理，品質管理等に関する基準(治験薬GMP)，2008年7月9日，薬食発第0709002号.
2) 岡真千子ほか，医療薬学，38，423-434(2012).