The nanoparticulation of α-galactosylceramide by octaarginine-modified liposome enhances α-galactosylceramide mediated antitumor effects in an experimental lung metastasis model

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ABSTRACT SUMMARY

Alpha-galactosylceramide (αGC) is predicted to have clinical applications as a new class adjuvant. However, the administration of soluble αGC did not result in significant clinical benefits. That is, the delivery of administered αGC to antigen presenting cells (APCs) is inefficient. An efficient delivery system is needed to achieve efficient αGC therapy by systemic injection. Here, we incorporated αGC into stearylated octaarginine-modified liposomes (R8-Lip). We reported herein show that the nanoparticulation of αGC significantly enhanced the presentation of αGC on the CD1d of APCs, the production of IFN-γ and expansion of invariant natural killer T (NKT) cells, leading to effective anti-metastatic effect in a highly malignant lung metastasis model, after intravenous administration.

INTRODUCTION

αGC, a synthetic glycolipid, is a unique adjuvant that enables the activation of both specific and non-specific immune responses. αGC activates innate and adaptive immunity via the production of a large amounts of INF-γ, which would be expected to show antitumor activity independent of the specific class of tumor. αGC is presented by CD1d molecules to NKT cells. Activated NKT cells then induce strong antitumor immunity. Owing to its unique and strong antitumor ability, many pre-clinical and clinical studies of αGC as KRN7000 had been initiated. Although an intravenous administration of soluble αGC did not result in measurable clinical benefits, the intravenous injection of αGC-loaded dendritic cells led to immune responses. In other words, while αGC has few effects in in vivo direct treatments, it appears to be effective in ex vivo cell therapies. These results clearly suggest that soluble αGC was not taken up efficiently by APCs. Hence, to induce a sufficient immune response by the systemic administration of αGC, it will be necessary to control the disposition and cellular uptake of αGC with the delivery systems in use.

In a previous study, we reported on the potential of stearylated octaarginine-modified liposomes (R8-Lip) as a vaccine delivery system. Although we have generally demonstrated the utility of R8-Lip as a vaccine delivery system for the in vitro targeting of DCs and subcutaneous immunization, an application of R8-Lip for systemic immunization, the issue of whether it can be used in systemic immunization, namely intravenous administration has not been addressed. Moreover, very few studies have been devoted to examining adjuvant effects of αGC by incorporating αGC into delivery systems.

In this study, we incorporated αGC into R8-Lip and investigated the potential of R8-Lip as a delivery system for αGC therapy via a systemic treatment.

EXPERIMENTAL METHODS

Preparation of Liposome: Liposomes were prepare by hydration method, and were composed of egg phosphatidylcholine (EPC), cholesterol (Chol) and STR-R8 (70:30:5 molar ratio). N-(Carboxylmethoxypolyethylene glycol 2000)-1, 2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) was included in some cases. The liposome suspension was extruded through polycarbonate membrane filters for sizing of liposomes.

αGC presentation on CD1d in vitro: JAWSII cells were pulsed with αGC/R8-Lip or soluble αGC. Soluble αGC is a DMSO solution after dissolving αGC at 80 °C. After 24 hours, the cells were stained with anti-mouse alpha GalCer:CD1d Complex PE. The fluorescence of stained cells was measured by FACS Calibur.

Anti-tumor effect in lung metastatic tumor models: On day 0, 2×10³ cells of B16-F10-luc2 were intravenously injected into C57BL/6 mice. On day 4, soluble αGC and αGC/R8/PEG-Lip were intravenously injected at a dose of 5 μg αGC. Soluble αGC was dissolved in PBS by using 5.6% sucrose, 0.75% L-histidine and 0.5% Tween20 with heating at 80 °C for several minutes. On day 19, the mice were sacrificed, and the lungs were collected. For the quantification of tumor metastasis, the luciferase activities were then measured.

αGC presentation on CD1d and population of NKT cells in vivo: Soluble αGC and αGC/R8/PEG-Lip were intravenously injected at a dose of 5 μg αGC. After 15 hours, splenocyte cells were stained by anti-mouse alpha GalCer:CD1d Complex PE and Alexa Fluor 647 anti-mouse I-A/I-E for αGC presentation on CD1d. After 3 days, splenocyte cells were stained by PE/Cy7 anti-mouse CD19, FITC anti-mouse CD3 and mouse CD1d Tetramer-SA-PE for examining the population of NKT cells. The fluorescence of stained cells was measured by FACS Calibur. NKT cell population was identified as CD19/CD3⁺/CD1d tetramer⁺ cells.

RESULTS AND DISCUSSION

We first investigated the efficiencies of the antigen presentation of αGC on CD1d by αGC/R8-Lip in JAWSII cells, a cell line of mouse DCs. The diameter and zeta potential of the αGC/R8-Lip particles were 98 nm and 40
mV, respectively. The αGC presentations induced by the αGC/R8-Lip increased in a dose-dependent manner and were significantly higher than the corresponding values for soluble αGC (Figure 1a).

We next examined the production of IFN-γ after the intravenous administration of αGC/R8-Lip in mice. Unexpectedly, the concentration of IFN-γ after the αGC/R8-Lip treatment was similar to that of soluble αGC (Figure 1b). This result indicates the biodistribution needs to be optimized, in addition to the efficient internalization to APCs to promote immune responses in the case of systemic administration. The spleen is a major target tissue for immunization via intravenous administration. We modified polyethylene glycol (PEG) on the surface of the particles and controlled the size, in an attempt to improve the action of αGC/R8-Lip. As a result of having optimized PEG modification and size control, the αGC/R8/2%PEG-Lip that was modified with 2 mol% of PEG and was sized by the 800-nm filter showed the highest promotion effect (Figure 1b). Consequently, we used the αGC/R8/2%PEG-Lip sized by passage through a 800-nm filter as the optimized αGC/R8-Lip (αGC/R8/PEG-Lip) in subsequent experiments. We also examined the tissue distribution of αGC/R8-Lip and αGC/R8/PEG-Lip. As a result, although αGC/R8-Lip accumulated in the liver in a time-dependent manner, the accumulation of αGC/R8/PEG-Lip in the liver was suppressed and it rapidly accumulated in the spleen.

We then investigated the antitumor effect of αGC/R8/PEG-Lip against lung metastasis mediated by a highly malignant melanoma, B16-F10 cells. In addition to the macroscopic observations, the amount of lung metastasis was quantitatively examined by measuring luciferase activities in the lung. As shown in Figure 2a, αGC/R8/PEG-Lip treatment significantly inhibited lung metastasis in comparison with non-treatment and the treatment with soluble αGC.

We examined the issue of whether αGC presentation on CD1d and expansion of NKT cells were promoted by an in vivo αGC/R8/PEG-Lip treatment. The αGC/R8/PEG-Lip treated group significantly increased the number of cells stained by anti-MHC class II and anti-αGC/CD1d, although the soluble αGC treated group failed (Figure 2b). As shown in Figure 2c, NKT populations in αGC/R8/PEG-Lip treated groups were drastically higher than the corresponding values for the non treated group and the soluble αGC treated groups. The results confirm that the nanopaticulated αGC, αGC/R8/PEG-Lip in this study, can also activate NKT cells at general manner of αGC-mediated immune responses, and can enhance each process of the αGC-mediated immune responses.

![Figure 1: (a) Antitumor effects in a lung metastasis model. (b) αGC presentation on CD1d in APCs. (c) Population of NKT cells. Value are the mean (n=3, *P<0.05, **P<0.01). Comparisons between multiple treatments were made by one-way analysis of variance, followed by the Tukey-Kramer test.](image1)

CONCLUSION
The findings reported herein show that αGC/R8/PEG-Lip significantly enhanced the presentation of αGC on the CD1d of APCs, the production of IFN-γ and expansion of NKT cells, leading to effective anti-metastatic effect in a lung metastasis model, after intravenous administration. Hence, we conclude that R8-Lip can function as a potent delivery system for αGC therapy via systemic treatment.

REFERENCES

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