論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Antigen presenting cells-targeted liposome by Levilactobacillus brevis surface layer protein coating
著者(和文)	陳政霖
Author(English)	Zheng Lin Tan
出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第12261号, 授与年月日:2022年9月22日, 学位の種別:課程博士, 審査員:山本 直之,八木 透,林 宣宏,折原 芳波,小倉 俊一郎
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第12261号, Conferred date:2022/9/22, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学院　系 ライフエンジニアリング　コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Philosophy)
学生氏名： Student's Name	Tan Zheng Lin	指導教員（主）： Academic Supervisor(main)	山本　直之
		指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Drug delivery to intestinal antigen presenting cells (APCs) is a challenging task due to the harsh and diverse environment in mammalian gastrointestinal (GI) tract, short retention time in intestines, mucosal and endothelial barriers which prevent the drugs from accessing Peyer's patches where APCs resides. To deliver drugs to intestinal APCs, the drugs should be encapsulated in carriers which are stable against environment in GI tract and possess APCs-targeting capability. In this study, a targeted ligand drug delivery system to target intestinal APCs was developed through coating of carriers with SlpB from *Levilactobacillus brevis*.

SlpB from *Lv. brevis* was extracted with 5 M lithium chloride solution and coated on drug carriers. The adsorption capacity of SlpB on all drug carriers evaluated in this study was 400 mg g^{-1} [SlpB LP^{-1}]. The adsorption curve was compared to the concentration of SlpB required to achieve maximum stability of SlpB-coated liposome (SlpB-LP), and the liposome coated with SlpB at maximum coating capacity showed maximum stability. Formation of 12.9 nm-thick layer of SlpB on liposome was observed with microscopy, and analysis with electrophoretic mobility showed that SlpB reduces ζ potential of anionic liposomes. Improvement of colloid stability of liposome via SlpB-coating due to increase in absolute ζ potential was confirmed with narrower size distribution of SlpB-LP.

SlpB-coating enhance stability of liposome against pH ranging from pH 2 - 9. Robustness of SlpB-adsorption on the surface of liposome under various pH was confirmed, and the result suggests that SlpB-adsorption was stable. Furthermore, stability against 0.5 - 3.0% gall solution which can emulsify liposome, and stability against simulated gastric fluid and simulated intestinal fluid which contains pepsin and pancreatin were also improved by SlpB-coating. The result suggests that SlpB has improved stability of liposomes under all gut-mimicking environments.

Furthermore, endocytosis of SlpB-coated carriers was evaluated with dendritic cell (DC) and macrophage (MΦ). I have found that SlpB-coating has significantly enhanced endocytosis of carriers into DC and MΦ. The effect of size of liposome on endocytosis was negligible. Investigation of receptors which binds to SlpB suggests that SlpB binds to DC-SIGN and Mincle which are both C-type lectins, through glycan chain.

In vivo study suggests that SlpB could improve stability of liposome in GI tract by 5.4- and 6.1-fold at 1 h and 3 h after oral administration. SlpB has facilitated enrichment of LP in Peyer's patches and blood, while no unspecific absorption into intestine and low retention in liver was detected. Enrichment of liposome in Peyer's patches is correlated to bioavailability, and SlpB has improved bioavailability by 427.6-fold. Unlike SlpA, no unspecific absorption of SlpB-LP to intestine and mucosal layer was detected. The route of SlpB-LP delivery to intestinal APCs was through transcytosis by M cells and specific endocytosis by $CD23^{+}$ APCs, which consist of follicular DC and MΦ. Improved antigen presentation at interfollicular region and germinal centre were also confirmed.

Moreover, SlpB also exhibit adjuvant effect which might improve therapeutic effect of drug. Production of IL-6, IL-10,

IL-12 and IL-17 increased significantly when DC was co-stimulated with ovalbumin and SlpB or lipopolysaccharide and SlpB. Evaluation with α-galactosylceramide-loaded liposome ($^{\alpha GC}$LP) showed increase in expression of anti-tumour cytokines both *in vitro* and *in vivo*. For instance, SlpB-$^{\alpha GC}$LP has increased production of IL-12 and decreased production of IL-10, while upregulated expression of IL-6, IL-10, TNF-α in DC. On the other hand, *in situ* expression of IL-4 and IL-5 were downregulated, while expression of IL-12, IFN-γ and TNF-α were upregulated in Peyer's patches of mice administered with SlpB-$^{\alpha GC}$LP compared to $^{\alpha GC}$LP. The results have suggested that oral delivery of SlpB-LP could enhance therapeutic effect through improved stability of liposome, specific uptake of drug carriers by APCs, and induction of adjuvant effect, which has stimulated injection-like effect.

To investigate the mechanism of SlpB-binding to APCs, presence of glycan chain in SlpB was investigated. N-glycan structure was revealed in SlpB by treatment with N-glycosidase. The function of sugar chain in DC-interaction was confirmed with competitive assay with D-glucose, D-galactose and D-mannose. Fragments of trypsinised SlpB which are responsible for DC-binding were identified, and the result suggests that 4 fragments with highest hydrophilicity were responsible in DC-binding.

In conclusion, coating of SlpB from *Lv. brevis* has improved stability of liposome, functionalised to enhance transcytosis through M cells, enhanced endocytosis by APCs via specific binding with the receptors and potentiated the therapeutic of drugs.