

Development of Inhalable Sustained-Release Formulation of Glucagon

OSatomi Onoue¹, Kazuki Kuriyama¹, Atsushi Uchida¹, Takahiro Mizumoto², George Nemoto³, and Shizuo Yamada¹

¹ Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

² ILS Inc., 1-2-1, Kubogaoka, Moriya, Ibaraki 302-0104, Japan; ³ American Peptide Company, 777 East Evelyn Ave. Sunnyvale, CA, 94086, U.S.A.



1. Introduction

Recently, glucagon (GLG)-replacement therapy for patients with pancreatotomy has attracted attention since the lack of pancreatic hormones including GLG sometimes causes hepatic dysfunctions and metabolic disorders. In spite of the therapeutic potential, owing to poor oral bioavailability, the clinical dosage form of GLG is currently limited to injection only, leading to limited clinical compliance. The present study aimed to develop novel inhalable sustained-release formulation of GLG without cytotoxic fibril formation for chronic GLG-replacement therapy.

2. GLG-loaded PLGA nanospheres

2.1. Preparation of PLGA nanospheres

◆ Emulsion solvent diffusion method in oil

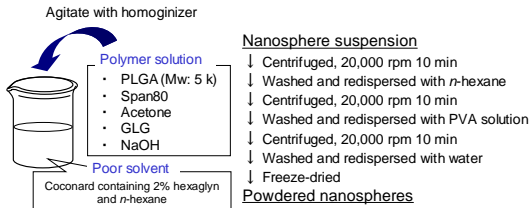


Table 1 Recovery of the GLG/NS

	GLG conc. in polymer solution (mg/mL)		
	1	5	10
Nanosphere recovery (%)	58	42	35
Drug content (%)	0.62	7.6	13

Higher drug content was observed in GLG/NS prepared with higher concentration of GLG solution.

3. Amyloidgenic properties of GLG

3.1. Characterization of GLG fibrils

◆ Preparation of GLG fibrils

✓ Concentration, 10 mg/mL; Temperature, 37°C; Aging time, 24 h.

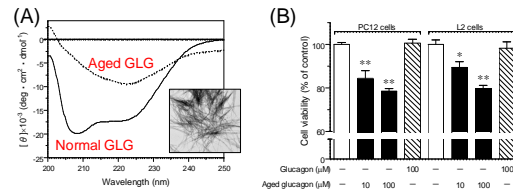


Fig.1 (A) TEM image and CD spectra of GLG. (B) Cytotoxicity of GLG fibril. Mean±SE (n=4). *P<0.05 vs control, **P<0.01 vs. control.

Treatment of GLG fibrils with β-sheet rich conformation resulted in concentration-dependent decrease of cell viability.

3.2. Formation of GLG fibrils in GLG/NS

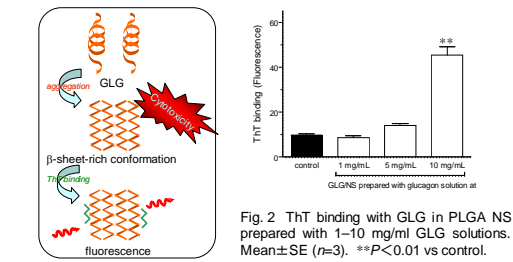


Fig. 2 ThT binding with GLG in PLGA NS prepared with 1–10 mg/ml GLG solutions. Mean±SE (n=3). **P<0.01 vs control.

Structural change of GLG in nanospheres was caused during preparation of GLG/NS prepared with 10 mg/mL.

4. Respirable formulation of GLG/NS

4.1. Properties of GLG/NS

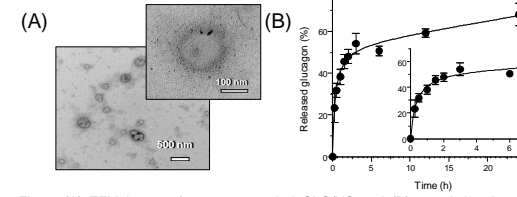


Fig. 3 (A) TEM image of water-suspended GLG/NS and (B) cumulative *in vitro* release of GLG from the GLG/NS in PBS (pH7.4). Mean±SE (n=3).

The drug release behavior exhibited biphasic pattern with initial burst and slow diffusion.

4.2. Properties of GLG/NS

◆ A mixture of GLG/NS and erythritol (1:1) was milled with A-O JET MILL. Particles were mixed with 5-fold amount of carriers (Respiptose SV-003), providing GLG/NS-RP.

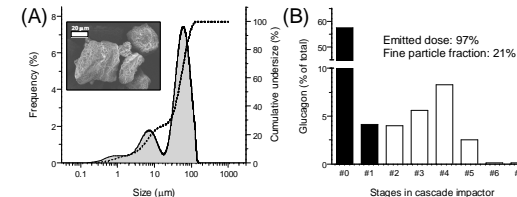


Fig. 4 Appearance and *in vitro* inhalation properties of GLG/NS-RP. (A) Particle size distribution of aerosolized GLG/NS-RP, and (B) Cascade impactor analysis of GLG/NS-RP.

GLG/NS-DPI had high dispersibility and optimal size for delivery to the respiratory organs.

5. *In vivo* experiments

5.1. Hyperglycemic effect and pharmacokinetic behavior

◆ Condition

- ✓ Animal Male wistar rats (300±33 g)
- ✓ Dose GLG 200 µg/kg body weight
- ✓ Control GLG-unloaded PLGA-RP

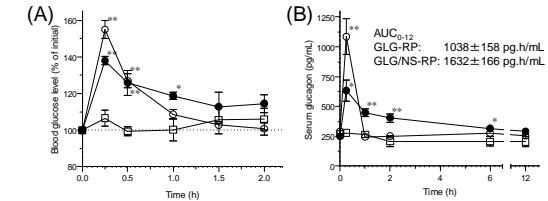


Fig. 5 (A) Blood glucose level and (B) serum GLG concentration after intratracheal administration of GLG formulations. □, control; ○, GLG-RP; and ●, GLG/NS-RP. Mean±SE (n=4). *P<0.05, **P<0.01 vs control group.

After inhalation of GLG/NS, GLG was released in a sustained release manner, leading to enhanced hyperglycemic effects.

6. Summary

1. Inhalable long-acting formulation of GLG was developed using a PLGA-based NS strategy, and it had high inhalation performance with a fine particle fraction of 20.5%.
2. Intratracheal administration of the GLG/NS-RP resulted in prolonged hyperglycemic responses, compared with those with GLG-RP.
3. New inhalable GLG-loaded PLGA formulation could be an efficacious dosage form with improved compliance as an alternative to the injection form of glucagon currently used.