รายงานวิจัยฉบับสมบูรณ์

โครงการ **การพัฒนาระบบนำส่งยาต้านไวรัสไข้หวัดนก (zanamivir) ในรูปแบบไลโปโซมและประเมินการดูดซึมยาทางลำไส้โดยใช้เซลล์ Caco-2**

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สัญญาเลขที่ TRG5080004

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ผู้วิจัย

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สังกัด

กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
และสมาคมผู้วิจัยและผลิตเภสัชภัณฑ์

กิตติกรรมประกาศ

ผู้ดำเนินงานวิจัยขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) และสมาคมผู้วิจัยและผลิตเภสัชภัณฑ์ (PREMA) ที่ให้ทุนสนับสนุนงานวิจัยชิ้นนี้จนแล้วเสร็จ ซึ่งงานวิจัยในรูปแบบการพัฒนาเภสัชภัณฑ์เป็นงานวิจัยที่ต้องศึกษาต่อเนื่องเพราะเป็นงานวิจัยในระยะยาว ดังนั้นการขอทุนวิจัยเพื่อพัฒนารูปแบบงานวิจัยนี้จะขอได้ค่อนข้างยากจากแหล่งทุนทั่วไป ซึ่งทางผู้ดำเนินงานวิจัยมีความซาบซึ้งที่ สกว. และ PREMA ให้โอกาสในการศึกษาครั้งนี้เป็นอย่างมาก

นอกจากนี้ผู้ดำเนินงานวิจัยขอขอบคุณศ.ดร. ณรงค์ สาริสุต อาจารย์ที่ปรึกษา ซึ่งท่านได้ให้คำแนะนำที่เป็นประโยชน์ตลอดระยะเวลาดำเนินงานวิจัย ซึ่งยังผลให้งานวิจัยชิ้นนี้ประสบความสำเร็จ

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Abstract

Project Code (รหัสโครงการ): TRG5080004

Project Title (ชื่อโครงการ): Development of novel zanamivir-liposomal formulations for avian influenza treatment and evaluation of intestinal absorption using human intestinal Caco-2 cell line

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The liposomes loading zanamivir was developed and investigated in the intestinal Caco-2 monolayer in order to study whether liposomes are able to enhance the ability of zanamivir to be absorbed across biological membranes. By using reverse-phase evaporation method, liposomal formulation composed of Phospholipon® 90 G : cholesterol at a 7:3 molar ratio provided the highest entrapment efficiency of zanamivir at 31.17%. The result revealed that the cationic and anionic surfactants (stearylamine and dicetylphosphate) in the liposomal formulation did not enhance the entrapment efficiency of zanamivir. The reduction of liposome size did not statistically influence the entrapment efficiency of zanamivir into liposomes. The transport study suggested that the prepared liposomes increased the transport of zanamivir through Caco-2 cells by 3.27 folds. The reduction of liposome size enhanced the permeation of zanamivir across Caco-2 monolayer by 1.90 folds. The study concluded that liposomes with entrapped zanamivir were able to increase the permeability of zanamivir across Caco-2 monolayer, presumably increasing delivery of the drug via gastrointestinal tract. The future work will be the study on the permeation and pharmacokinetic study of liposomes with the entrapment of zanamivir *in vivo*.

Keywords: liposomes, zanamivir, Caco-2, transport study, absorption

Abstract

Project Code (รหัสโครงการ): TRG5080004

Project Title (ชื่อโครงการ): การพัฒนาระบบนำส่งยาต้านไวรัสไข้หวัดนก (zanamivir)ในรูปแบบไลโปโซมและประเมินการดูดซึมยาทางลำไส้โดยใช้เซลล์ Caco-2

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ไลโปโซมบรรจุยา zanamivir ได้รับการพัฒนาเพื่อศึกษาว่าไลโปโซมมีความสามารถเพิ่มการดูดซึมยา zanamivir ผ่านทางผนังลำไส้ได้หรือไม่โดยใช้ Caco-2 monolayer ในการศึกษา ไลโปโซมถูกเตรียมโดยวิธี reverse-phase evaporation พบว่าสูตรของไลโปโซมที่ประกอบด้วย Phospholipon® 90 G และ cholesterol ที่สัดส่วน 7:3 โมลาร์ สามารถบรรจุ zanamivir ได้สูงสุดที่ 31.17 เปอร์เซนต์ ผลการทดลองยังพบว่า สารลดแรงตึงผิวทั้งชนิดประจุ บวก และ ลบ (stearylamine and dicetylphosphate) ที่ผสมในสูตรของไลโปโซม ไม่สามารถเพิ่มประสิทธิภาพการบรรจุยา zanamivir ได้ นอกจากนี้การลดขนาดของไลโปโซมไม่มีผลอย่างมีนัยสำคัญต่อการเพิ่มหรือลดประสิทธิภาพการบรรจุยา zanamivir ในไลโปโซม การศึกษาการดูดซึมยาพบว่าไลโปโซมที่เตรียมได้สามารถเพิ่มการซึมผ่านของยา zanamivir ในเซลล์ Caco-2 ได้ 3.27 เท่า และการลดขนาดไลโปโซมสามารถเพิ่มการซึมผ่านของยาได้ 1.90 เท่า เมื่อเปรียบเทียบกับไลโปโซมที่ไม่ได้ถูกลดขนาด การศึกษานี้สรุปได้ว่า ไลโปโซมที่บรรจุยา zanamivir สามารถเพิ่มการซึมผ่านของยาใน Caco-2 monolayer ได้ และงานวิจัยที่จะดำเนินการต่อในอนาคตคือการศึกษาเภสัชจลนศาสตร์ของไลโปโซมที่เตรียมได้ในสัตว์ทดลอง

Keywords: ไลโปโซม, ซานามีเวีย, Caco-2, การศึกษาการซึมผ่าน, การดูดซึม

1. Introduction

Each year, influenza viruses continue to cause major health problems and economic loss worldwide [1,11]. Especially, H5N1 viruses, virulent avian influenza, lead to an unacceptable number of deaths and serious concerns about global flu outbreak [2-4]. Neuraminidase inhibitors (zanamivir and oseltamivir) have been indicated to provide activity against influenza viruses [4,5,11]. Influenza viruses carry two surface glycoproteins, a hemagglutinin and a neuraminidase, which involve in the productive process for new virion [6]. Hemagglutinin binds cell surface by recognizing cellular sialic acid receptor [10]. Neuraminidase cleaves the terminal sialic acid residues and releases progeny virus from the surface of infected cell [16].

The World Health Organization (WHO) recommends oseltamivir and zanamivir for H5N1 avian flu treatment. Oseltamivir (Tamiflu®), the first orally active neuraminidase inhibitor, reduces secondary lower respiratory tract complications, bronchitis and pneumonia [5,12-14]. However, several studies reported neuraminidase mutations, which conferred to resistance of influenza viruses to neuraminidase inhibitors [1,3,5,7-8].

Tai and co-workers suggested that zanamivir has higher sensitivity to the Arg292Lys mutation in A/Victoria/3/75 strain (H3N2) than oseltamivir carboxylate by 1,100-fold, comparing to the wild type *in vitro* [9]. The resistance of influenza A viruses have been seriously concerned when a research team found that 18 percent of 50 children treated with oseltamivir had neuraminidase mutations in H3N2 viruses [1]. The identified mutations were Arg292Lys, Glu119Val and Asn294Ser, which reduced oseltamivir sensitivity about 10^4-10^5-fold, 500-fold and 300-fold, respectively [1]. A study revealed that the infection of the His274Tyr mutant influenza virus was low [17]. In contrast, Herlocher et al. evidenced that His274Tyr and Glu119Val neuraminidase mutant viruses could be transmitted in ferrets and the transmission of His274Tyr and Glu119Val viruses retained their genotypic characteristics associated with resistance [18]. The research team of Ives advised that the ferrets infected with His274Tyr mutant virus showed decrease in pathogenicity compared to wild type virus [17]. Contrarily, the isolation of oseltamivir-resistant influenza A (H5N1) variants from two patients, who died after treatment with

oseltamivir, contained His274Tyr mutation [8]. Especially, in one case oseltamivir was used as the early initiation treatment [8]. The same study showed the evidence to prove that the presence of influenza A (H5N1) with His274Tyr after full course of oseltamivir treatment associated with a poor outcome [8]. Le and collaborators also isolated oselamivir-resistant H5N1 virus with His274Tyr mutation from a Vietnamese patient [3].

Zanamivir (Relenza®), the first neuraminidase inhibitor, has been launched to the market only in a dry powder form for inhalation due to its poor oral bioavailability (~2%) [6,7,26], and consequently it should be used with caution in patients with chronic obstructive pulmonary diseases and asthma [6]. Pharmacokinetic study of zanamivir revealed that a median of 10 to 20% of the oral inhaled administration was systemically absorbed leading to low serum concentration [26]. Zanamivir has been reported to have activity against His274Tyr mutant neuraminidase [21]. However, the administration of zanamivir by oral inhalation provides drugs abundantly to the upper respiratory tract [15,19], while the virulent H5N1 associates with infection of the lower respiratory tract [4,20]. In addition, the replication of H5N1 is in lower respiratory tract and extrapulmonary site (such as gastrointestinal tract) [4,22]. Therefore, the development of zanamivir's formulation for oral administration is a remarkable milestone to treat oseltamivir-resistant influenza viruses. By using Chem Draw programme, zanamivir has the Log P value of -5.56. The negative LogP indicates that compound has greater solubility in water and lower hydrophobicity [23]. Moreover, the drug has a short plasma half-life (~2 h) and low protein binding (less than 10% of systemically circulating zanamivir) [27]. As a result, not only the improvement of zanamivir's absorption via gastrointestinal tract should be considered, but the retainment of zanamivir blood concentration should also be concerned.

Liposomes are low *in vivo* toxic and biodegradable and hence they are highly beneficial as drug carriers [24]. In addition, liposomes can be designed to deliver hydrophilic, lipophilic and amphipathic drugs due to their unique properties [24]. Encapsulation of active ingredients into liposomes is recommended to improve oral bioavailability by enhancing the ability of drug to be absorbed across biological membranes [25].

The objective of this study was to develop liposomes loading zanamivir and the prepared liposomes was evaluated for the intestinal absorption by using human intestinal Caco-2 cell model.

2. Materials and method

2.1 Chemicals

HPLC grade acetonitrile for the mobile phase was purchased from Mallinckrodt Chemicals (New Jersey, USA). Ultrapure water was obtained with UHQ Ultrapure Water from ELGA (Bucks, UK). Hank's balance salt solution (HBSS) was from Sigma-Aldrich (Steinheim, Germany). Phospholipon® 90 G (PL90G) was a generous gift from PHOSPHOLIPID GmbH (Cologne, Germany). Cholesterol (CHO) was received from Fluka (Tokyo, Japan). 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were obtained from Genzyme Pharmaceuticals (Liestal, Switzerland).

2.2 Synthesis of zanamivir

Zanamivir was synthesized by using modified method of Chandler and co-workers [28]. The identity of zanamivir was investigated by melting point, ^{1}H nuclear magnetic resonances (NMRs) and mass spectrometry.

2.3 Validation of HPLC method for the determination of zanamivir

The chromatographic resolution was performed using 98% (v/v) ultrapure water and 2% (v/v) acetonitrile as mobile phase with flow rate of 0.5 ml/min, BDS Hypersil Cyano column (length, 250 mm; inner diameter, 4.6 mm; particle size, 5 µm), and UV detection at 230 nm. The bioanalytical guidance of Food and Drug Administration of the United States of America (2001) was used throughout the studies [29]. The parameters validated for method development were sensitivity, linearity, accuracy, precision and stability.

2.4 Formulation of liposomes containing zanamivir

2.4.1 Preparation of liposomes

Liposomes loading zanamivir (ZVL) were prepared in HBSS by the reverse-phase evaporation method. Various types of liposomal formulations were studied. The continuation of evaporation was performed to ensure complete removal of the organic solvent. Subsequently, the evaporated liposomes were shaken and sonicated. The size of prepared liposomes was reduced by LiposoFast^{TM}.

2.4.2 Characterization of liposomes containing zanamivir

2.4.2.1 Particle size and entrapment efficiency

The determination of particle size was performed by sub-micron particle analyzer. All determinations were carried out in triplicate. The amount of drug entrapped was determined by using the HPLC system as mentioned before. All examinations was carried out in triplicate. The % drug entrapment was calculated.

% entrapment = (Mass of drug in liposomes/ Mass of drug used in the formulation) x 100

2.4.3 Transport studies of liposomes loading zanamivir

2.4.3.1 Cytotoxicity test

Caco-2 cells were seeded on 12-well plates at a density of 220,000 cells/cm^2 and allowed to attach for one week. ZVL and zanamivir solution (ZV) prepared in HBSS were added to the cells and incubated for 3 hr.

- Trypan blue exclusion assay

After incubation with the test solutions, Caco-2 cells were trypsinised and stained with the trypan blue (0.4%) and the number of viable and dead cells was counted. The percentage of viable cells was calculated as the following formulation:

% cell viability = (number viable cells/ number total cells) x 100

- MTT assay

For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazoliumbromide (MTT) assay, ZVL and ZV were removed and washed twice with phosphate buffer saline. The MTT solution was added to the cells and incubated for 3 h. The lysis buffer containing 10%

sodium dodecyl sulfate in 0.01 M HCl was added to solubilize the cells and formed formazan crystals. The plate was kept overnight in at 37°C and the amount of formazan will be quantified by measuring absorbance at 580 nm. The control group was treated in the same manner as test groups.

2.4.3.2 Permeation of prepared liposomes across Caco-2 monolayer

Caco-2 cells were seeded at a density of 220,000 cells/cm^2 onto 12-well Transwell® plate with an insert area and polycarbonate membrane of 3.0 µm. Cells were fed every other day and maintained at 37°C and 5% CO_2 humidified atmosphere for 21 days. The transepithelial electrical resistance (TEER) values were measured using a millicell®-ERS equipped with "chopstick' electrodes. The inserts with TEER values above 500 ohm.cm^2 were used in the study. Lucifer yellow was employed as a maker for the transport study. The drug solution and liposomal formations were added either into the apical or into the basolateral compartment with respect to the cell layer to study transport from apical to basolateral and bi-directional transport from basolateral to apical side. Permeation of drug across the Caco-2 cells was monitored by sampling solution in both compartments at 30, 60, 90, 120 and 180 min. Zanamivir in sampling solutions from cell culture was monitored by the validated HPLC method.

3. Results and discussion

3.1 Synthesis of zanamivir

Compound appeared as colorless powder with mp > 240 °C (decomp.), ^{1}H NMR δ_H (D_2O) 5.53 (1 H, d, 3-H), 4.50-4.38 (2 H, 2 dd, 4- and 6-H), 4.21 (1 H, dd, 5-H), 4.00-3.88 (2 H**,** dd and ddd, 9-H^a and 8-H**),** 3.70-3.62 (2 H, 2 dd, 9-H^b and 7-H), 2.05 (3 H, s, Ac); m/z: 333 $(M+1)^+$. These results confirmed the identity of the compound as zanamivir.

3.2 Validation of HPLC method for the determination of zanamivir

The reversed stationary phase C18, C8 and C4 columns were initially employed in this study; however, the components in HBSS were interfered with zanamivir peak. The BDS Cyano column could solve the interference problem and gave the best resolution of

zanamivir peak eluted at 6.67 min (Figure 1). The lower limit of quantification (LLOQ) for this method was 0.1 µg/ml. The linearity of the standard curve was carried out at a concentration range from 0.1 to 50 µg/ml. However, when the calibration range 0.1-50 µg/ml was performed, the linearity was not obtained. The best linear calibration was found when three calibration curves were constructed with three concentration ranges of 0.1-0.6, 0.6-5 and 5-50 µg/ml. The coefficient of determination for each standard curve was higher than 0.99, indicating that the equations for the quantification of zanamivir concentration in HBSS were defined (Table 1).

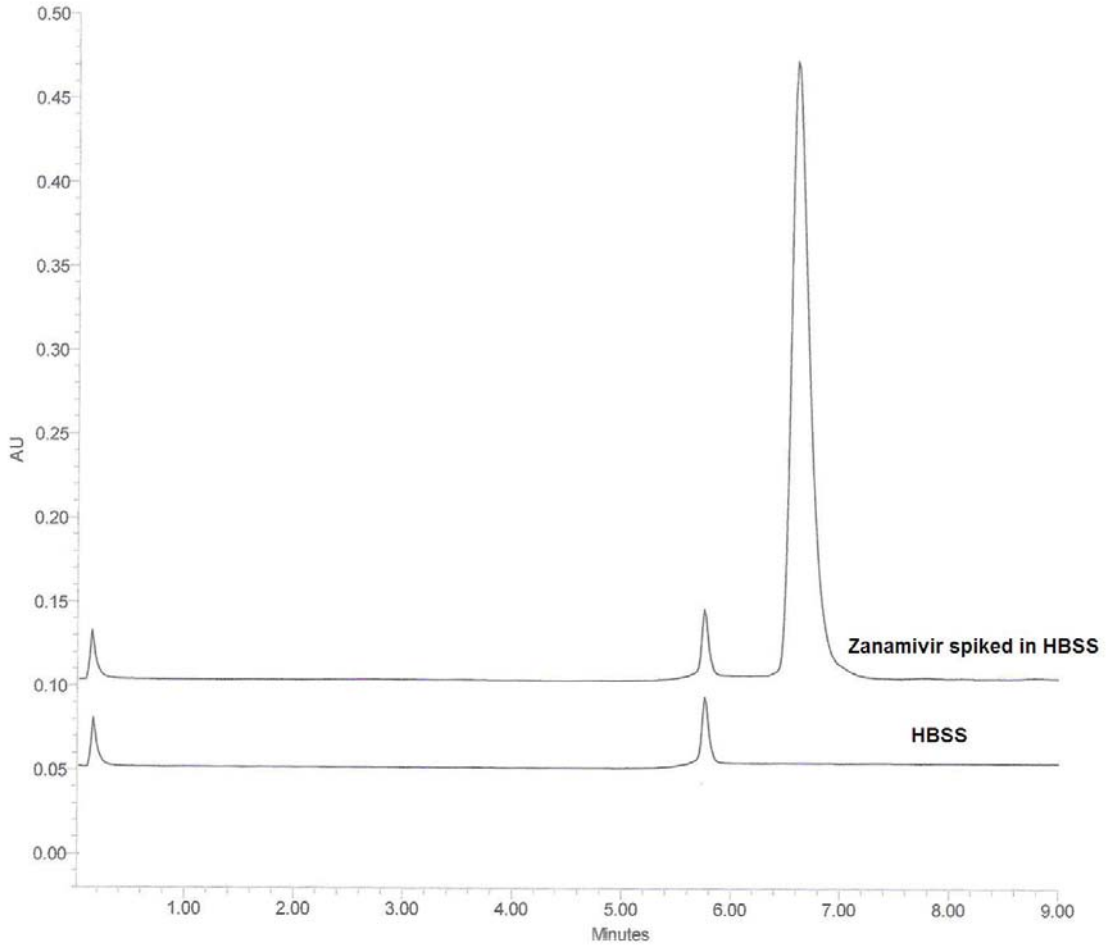


Figure 1 HPLC chromatogram of HBSS and zanamivir spiked in HBSS

Table 1 Linear regression analysis of calibration curves for the quantitation of zanamivir in HBSS

Transport medium	Calibration curve	Concentration range (μg/ml)	Slope	Intercept	Coefficient of determination
HBSS	A	0.1-0.6μg/ml	2490.9	23.7	0.9953
	B	0.6-5 μg/ml	1912.3	-98.88	0.9954
	C	5-50 μg/ml	1626.2	-127.9	0.9999

The accuracy, analyzed by using three calibration curves of 0.1-0.6 0.6-5 and 5-50 μg/ml in HBSS, summarized that the coefficient of variation was lower than the limit of FDA (15%). The results proved that the mean test results were close to the true concentrations of analyte (Table 2). The recovery of oseltamivir in terms of mean±C.V. for standard curves A (0.1-0.6 μg/ml), B (0.6-5 μg/ml) and C (5-50 μg/ml) in HBSS was 96.53±3.15, 98.92±1.13 and 99.52±1.72, respectively (Table 2).

Table 2 The determination of accuracy

Transport medium	Calibration curve	Concentrations (μg/ml)		S.D.	(%) Recovery	Mean (% recovery) ± C.V.
		Added	Recovered			
HBSS	A	0.1	0.09	0.01	92.46	96.53 ± 3.15
		0.3	0.29	0.02	97.39	
		0.6	0.60	0.01	99.75	
	B	0.6	0.59	0.02	98.26	98.92 ± 1.13
		3	2.93	0.06	97.78	
		5	5.04	0.08	100.74	
	C	5	4.87	0.09	97.43	99.52 ± 1.72
		25	25.41	0.89	101.62	
		50	49.75	0.71	99.49	

The values of coefficient of variation for repeatability or within-day precision were less than 7%, while those of reproducibility or between-day precision were lower than 10% (Table 3 and 4).

Table 3 Within-day precision

Transport medium	Calibration curve	Concentration (μg/ml)	Mean response ± S.D.	C.V. (%)
HBSS	A	0.1	246 ± 16.51	6.71
		0.3	752 ± 29.80	3.96
		0.6	1563 ± 30.44	1.95
	B	0.6	959 ± 13.49	1.41
		3	5450 ± 65.12	1.19
		5	9409 ± 138.28	1.47
	C	5	8090 ± 107.00	1.32
		25	40482 ± 367.50	0.91
		50	81412 ± 748.09	0.92

Table 4 Between-day precision

Transport medium	Calibration curve	Concentration (µg/ml)	Mean response ± S.D.	C.V. (%)
HBSS	A	0.1	253 ± 23.27	9.21
		0.3	755 ± 31.19	4.13
		0.6	1473 ± 90.89	6.17
	B	0.6	996 ± 31.79	3.19
		3	5430 ± 64.24	1.18
		5	9455 ± 258.36	2.73
	C	5	8123 ± 197.54	2.43
		25	40149 ± 824.59	2.05
		50	80772 ± 1362.18	1.69

The stability of four aliquots at two levels of drug concentration (0.1 and 50 µg/ml) during the three freeze and thaw cycles was evaluated. The recovery for each concentration with respect to the baseline for 0.1 and 50 µg/ml in HBSS was 98.40 and 101.31, respectively (Table 5). The results indicated that there was no effect to the drug by three freeze thaw cycles. In addition, low and high zanamivir concentrations at 0.1 and 50 µg/ml prepared in HBSS were evaluated after thawing at room temperature and kept at this temperature for 48 h. The short-term temperature stability for 48 h at room temperature was based on the duration that samples were maintained for the intended study. Four replicates were determined for each concentration. The recovery of the short-term temperature stability at concentrations of 0.1 and 50 µg/ml was 100.37 and 100.57, respectively. The results demonstrated that zanamivir was stable in HBSS at room temperature for at least 48 h (Table 6). The analytical methodology developed in this study was to facilitate the quantification of zanamivir in transport study using Caco-2 cell line.

Table 5 Freeze and thaw stability

Transport medium	Concentration (μg/ml)	% Recovery at basal level (Mean ± S.D.)	% Recovery after third cycle (Mean ± S.D.)	Overall recovery compared to basal level (%)
HBSS	0.1	100.68 ± 7.59	99.07 ± 3.09	98.40
	50	98.44 ± 2.37	99.73 ± 1.74	101.31

Table 6 Short-term temperature stability (48 h)

Transport medium	Concentration (μg/ml)	% Recovery at 0 h (Mean ± S.D.)	% Recovery after 48 h (Mean ± S.D.)	Overall recovery compared to 0 h (%)
HBSS	0.1	99.27 ± 4.91	99.64 ± 4.74	100.37
	50	99.68 ± 3.02	100.25 ± 2.25	100.57

3.3 Formulation of liposomes containing zanamivir

3.3.1 Preparation and characterization of liposomes

Various liposome formulations containing zanamivir were prepared by altering types and proportions of phospholipids. The highest entrapment efficiency of liposome-entrapped zanamivir was 31.17 %, which was found in the liposomal formulation composed of PL90G:CHO at a 7:3 molar ratio. Therefore, the molar ratio of PL90G:CHO at 7:3 was used for the following studies. As zanamivir was dissolved in HBSS, at pH about 7 of HBSS zanamivir was ionized to form zwitterionic molecule. The effect of charged liposomes on the % entrapment of zanamivir by mixing the cationic and anionic surfactants (stearylamine and dicetylphosphate) in liposomal formulation was studied. The result revealed that the charged liposomes did not increase the entrapment efficiency of zanamivir. In addition, the reduction of liposome size did not statistically influence the % entrapment of zanamivir into liposomes (Table 9).

Table 7 Characterization of liposomal formulations

Formulation	% Zanamivir Entrapment Efficiency	Mean Diameter (nm)
DSPC:Chol (3 µmol : 7 µmol)	1.34 ± 0.37	1640
DSPC:Chol (5 µmol : 5 µmol)	1.76 ± 0.21	1320
DSPC:Chol (7 µmol : 3 µmol)	2.63 ± 0.43	1660
DOPE:Chol (3 µmol : 7 µmol)	3.15 ± 0.97	1390
DOPE:Chol (5 µmol : 5 µmol)	2.83 ± 1.05	1480
DOPE:Chol (7 µmol : 3 µmol)	6.44 ± 1.17	1100
PL90G:Chol (3 µmol : 7 µmol)	10.69 ± 2.57	1290
PL90G:Chol (5 µmol : 5 µmol)	14.82 ± 3.03	1360
PL90G:Chol (7 µmol : 3 µmol)	31.17 ± 4.63	1450

Table 8 The effect of cationic and anionic surfactants on the liposome characteristics

Formulation	% Zanamivir Entrapment Efficiency	Mean Diameter
PL90G:Chol (7 µmol : 3 µmol)	31.17 ± 4.63	1450
PL90G:Chol:DP (6.5 µmol : 2.5 µmol : 1 µmol)	16.32 ± 3.08	1310
PL90G:Chol:SA (6.5 µmol : 2.5 µmol : 1 µmol)	22.73 ± 3.76	1210

Table 9 The effect of size reduction on liposome characteristics

Formulation	Size	% Zanamivir Entrapment Efficiency	Mean Diameter (nm ± S.D.)
PL90G:Chol (7 µmol : 3 µmol)	Normal	31.17 ± 4.63	1450

	Reduced	29.84 ± 3.97	290

3.3.2 Transport studies of liposomes loading zanamivir

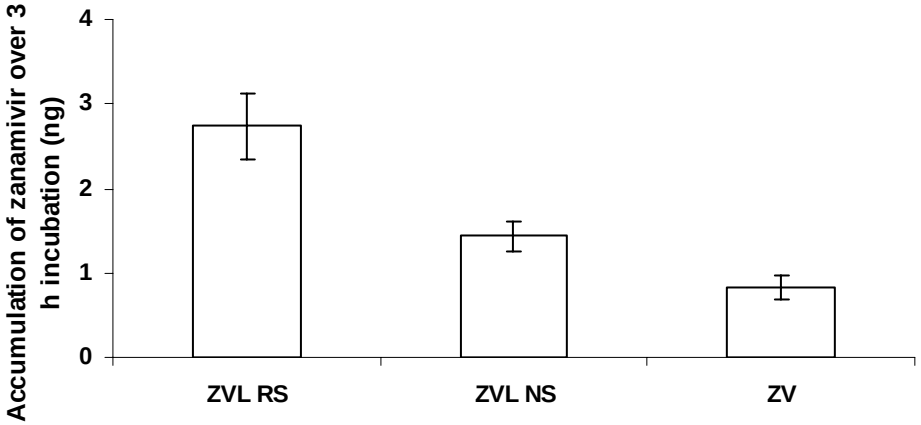
3.3.2.1 Cytotoxicity test

The viability of cells, treated with ZVL and ZV, was not statistically different with the control using trypan blue exclusion method ($P > 0.05$, two-tailed test, Student's *t*-test). In the MTT assay, no statistic difference on the mitochondrial activity of the cells after incubated with ZVL and ZV compared to the control ($P > 0.05$, two-tailed test, Student's *t*-test). The results presumed that the ZVL and ZV had no cytotoxic affect to the cells.

3.3.2.2 Permeation of prepared liposomes across Caco-2 monolayer

The transport study from both apical to basolateral (A to B) and from basolateral to apical side (B to A) suggested that The ZVL with size reduction increased the transport of zanamivir through Caco-2 cells by 3.27 and 3.07 folds compared to the ZV for A to B and B to A, respectively (Figure 2). ZVL without size reduction was able to permeate through Caco-2 monolayer 1.72 and 1.58 folds higher than ZV over three hour incubation period for A to B and B to A, respectively (Figure 2). The reduction of liposome size enhanced the permeation of zanamivir by 1.90 and 1.94 folds for A to B and B to A, respectively (Figure 2).

A) apical to basolateral



B) basolateral to apical

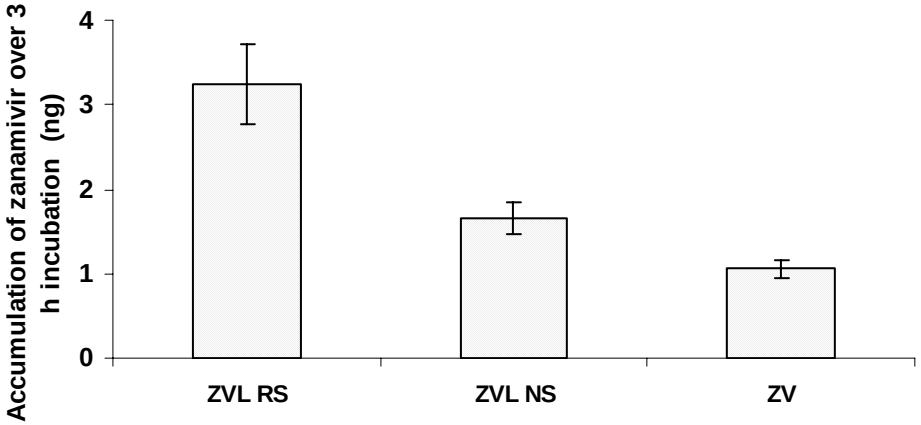


Figure 2 Accumulation of zanamivir across Caco-2 monolayer over 3 h incubation period A) apical to basolateral and B) basolateral to apical (ZVL RS = zanamivir loading zanamivir with size reduction, ZVL NS = zanamivir loading zanamivir without size reduction, ZV = zanamivir solution)

The results supported the works of O'Hagan and Desai et al., who explained the correlation of intestinal uptake and particle size [30,31]. The smaller particles can be crossed at sites of intestinal uptake more than larger particles as small particles can be

transported by various mechanisms such as persorption, endocytosis and phagocytosis [30,31]. Although liposomes could increase the permeation of zanamivir in Caco-2 model, the delivery mechanisms have not been elucidated. The transport may occur in various mechanisms [32]:1 endocytosis of the liposomes followed by the release of drug 2. fusion of liposomal membrane with the plasma membrane and release of the content in the cytoplasm 3. collision and possibly absorption of the liposome. Even though, Caco-2 model is commonly used as an in vitro tool to study absorption in man, it is necessary to assess the transport of ZVL in the animal model. Therefore, our future work will be the study on the permeation and pharmacokinetic study of ZVL *in vivo*.

4. Conclusion

The study revealed that liposomes with entrapped zanamivir were able to increase the permeability of zanamivir across Caco-2 monolayer, presumably increasing delivery of the drug via gastrointestinal tract. In addition, the validated HPLC method was able to indirectly apply to determine zanamivir in other biochemical assays using HBSS as a medium. The developed HPLC system used low amount of organic solvent; as a result, it had not only low direct environmental toxicity but also low risk to analyst health.

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การนำผลงานไปใช้ประโยชน์

การศึกษานี้มีประโยชน์ในการศึกษาพัฒนาระบบนำส่งในรูปแบบไลโปโซมสำหรับยาที่มี polarity สูง โดยใช้ยา zanamivir เป็นแม่แบบ ซึ่งงานวิจัยนี้มีโอกาสนำไปใช้ประโยชน์ในเชิงพาณิชย์ แต่อย่างไรก็ตามควรมีการศึกษาเพิ่มเติมในสัตว์ทดลอง และควรมีการศึกษาความคงตัว และเนื่องจากเป็นการพัฒนารูปแบบนำส่งยา ดังนั้นรูปแบบการนำส่งยาที่ได้ต้องมีการดำเนินงานวิจัยเพิ่มเติมเหมือนกับการพัฒนายาใหม่ซึ่งต้องใช้เวลาในการดำเนินงานวิจัยอีกพอสมควร

ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

ขณะนี้กำลังรวบรวมเพื่อจัดส่งตีพิมพ์ในวารสารวิชาการนานาชาติ