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Liposomal β-Glucan: Preparation, Characterization and Anticancer Activities

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Abstract

Context: An assortment of 1,3-β-glucans have been explored for their structural and pharmacological capabilities. The immunomodulatory effects of 1,3-β-glucans have served to fight cancer.

Objectives: The aim of this work was the investigation of the physiocochemical characterizations of 1,3-β-D-glucan encapsulation of liposomes and their effects on human lung carcinoma epithelial cell line A549.

Materials and Methods: β-glucan was encapsulated into liposomes composed of different lipids and cholesterol [PC, PC:Chol (6:1), DDAB:DPPC:Chol (4:2:1), DCP:DPPC:Chol (4:2:1), DPPC:Chol (6:1), DSPC:Chol (6:1), DSPC]. The liposomal formulations were prepared by the hydration-dehydration method. The mean diameter of liposomes and the polydispersity index were determined by photon correlation spectroscopy (PCS) with the use of Submicron Particle Sizer. The hemocompatibility of liposome on human erythrocytes was evaluated. The MTT assay was also performed to evaluate the A549 cells viability.

Results: The mean particle size of vesicles loaded with β-glucan varied from 130.20 ± 10.5 to 180.10 ± 11.3 nm. The polydispersity index ranged from 0.66 ± 0.0004 to 0.90 ± 0.08. The encapsulation efficiencies in DDAB:DPPC:Chol, DCP:DPPC:Chol and DPPC:Chol formulations were 17.20 ± 0.08%, 23.60 ± 0.20% and 18.23 ± 0.06%, respectively. The hemolysis rates did not exceed the negative control value by more than 2% and 8% at 10 and 24 h, respectively. These three formulations showed higher growth suppression of A549 cell compared to free β-glucan. This growth suppression of A549 cell by 1 μM doxorubicin was further promoted by liposomal β-glucan.

Conclusion: Liposomal 1,3-β-D-glucan might potentially enhance antitumor activity.

Keywords: β-glucan encapsulated liposome; Stability of liposome; Hemocompatibility; Cytotoxicity

Introduction

Glucans are structurally distinct polysaccharides consisting of D-glucose units linked by (1→3)-β and (1→6)-β glycosidic linkages [1]. Glucans demonstrated to control colon cancer cell proliferation via direct interaction of the glucan with the colon cancer cells and their apoptosis induction [2]. Glucan extracted from mushroom *Agaricus blazei* stimulated apoptosis or programmed cell death in human ovarian cancer HRA cells [3]. β-glucans in particular those extracted from fungi or yeasts have shown anticarcinogenic effects both *in vivo* and *in vitro* [4,5]. Barley β-glucan administration in mice enhanced the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models [6]. Mushroom extract, *Antrodia camphorate* showed promising therapeutic potential due to its cytotoxic effects against human pancreatic cancer B×PC-3 cell line which include inhibiting cell migration and inducing mitochondria-mediated apoptosis [7]. Mushroom β-glucan and resveratrol lowered the number of metastases in Lewis lung cancer model [8]. As an immunomodulating agent, β-glucan acts through the activation of innate immune cells such as macrophages, dendritic cells, granulocytes, and natural killer cells. This activation triggers the responses of adaptive immune cells such as CD4(+) or CD8(+) T cells and B cells, resulting in the inhibition of tumor growth and metastasis [9]. β-D-glucan regulates breast cancer-relevant gene expression and may be useful for inhibiting endocrine-resistant breast cancer cell proliferation [10]. Beta glucan has the potential to be used as a complementary or adjuvant therapy for improving quality of life in breast cancer patients in combination with cancer therapies [11]. Irradiated β-D-glucan exhibited three human cancer cell lines including Colo-205, T47D and MCF7 growth inhibition through enhancing antioxidant and antiproliferative activities [12].

Liposomes can entrap drugs inside their aqueous interior and/or into their bilayer [13]. These vesicles entrap in their interior volume part of the water phase and consequently can capture and segregate polar molecules. Moreover, because of the physicochemical properties of their constituents, they can also dissolve hydrophobic molecules in their bilayers. The properties of liposomes in addition to the general properties of surfactants, those make them useful for different applications are the structural thermodynamic and kinetic stability on dilution and ability to entrap both water soluble and insoluble substances and deliver them into desired environments. The liposomes are widely used for entrapment of therapeutic agents including a wide variety of drug molecules, especially anticancer drugs, and biomaterials. In addition, liposomal formulations are often proposed for the improvement of the therapeutic action of

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drugs especially for those used against cancer in order to reduce the toxicity and increase the efficacy and the pharmacokinetic profile. An ideal liposomal delivery system should be stable, long circulating, accumulate at a target site and release its drug in a controlled manner. In the current study, liposome was formulated with different lipids and cholesterol and entrapped β-glucans. Incorporation of cholesterol in liposome formulation was shown to enhance retention of entrapped solutes and reduce the serum-induced instability caused by binding of serum proteins to liposome membrane [14]. With these in mind, we encapsulated 1,3-β-glucan into liposome to examine their physicochemical properties, hemocompatibilty and anticancer activity of these formulations.

Materials and Methods

Chemicals

Didecyldimethylammonium bromide (DDAB), L-α-Phosphatidylcholine (PC), dicetyl phosphate (DCP), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide], and lactate dehydrogenase (LDH) assay kits, cholesterol and Triton X-100 were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1, 2-Distearoyl-sn-glycero-3- phosphocholine (DSPC) were obtained from Northern Lipids (Vancouver, BC, Canada). All other chemicals were purchased from Sigma-Aldrich.

Normal human pooled plasma

A pool of normal citrated human plasma from a minimum of 20 healthy individuals was obtained from Precision-Biologic (Dartmouth, NS, Canada).

Liposome preparation

β-glucan was encapsulated into liposomes composed of different lipids and cholesterol [PC, PC:Chol (6:1), DDAB:DPPC:Chol (4:2:1), DCP:DPPC:Chol (4:2:1), DPPC:Chol (6:1), DSPC:Chol (6:1), DSPC]. The liposomal formulations were prepared by the hydration-dehydration method [15,16]. Briefly, the lipids were dissolved in the chloroform: methanol solution (2:1 v/v). A Rotary evaporator (Rotavapor, BÜCHI Labortechnik AG) was used to evaporate the organic solven [17]. Once a thin dry lipid layer was formed β-Glucan solution (100 µg/ml) was added, which was followed by a series of sonication using the Sonic Dismembrator (Fisher Scientific FS20H, Ottawa, Canada) [18]. Excess unencapsulated drug was removed following three rounds of PBS wash at 18,300 × g for 15 min at 4°C. The pellet was then resuspended in PBS and encapsulation efficiency of β-glucan was determined using the Fungitec G test (Seikagaku Co., Japan) according to the manufacturer's protocol. Encapsulation efficiency was determined as the percentage of β-glucan incorporated into liposomes relative to initial total amount of β-glucan in solution. We must point out that this level of Triton X-100 (0.2%) has no effect on the performance of the assay and that this is the only experiment where we used a detergent. For the Doxorubicin liposomal sample, hydration of the dry lipid thin film was achieved with PBS containing 1 µM Doxorubicin. The flask was mechanically shaken for 15 min at 45°C.

Particle size of liposomes

The mean diameter of liposomes and the polydispersity index were determined by photon correlation spectroscopy (PCS) with the use of Submicron Particle Sizer, Model 270 (Nicomp, Santa Barbara, CA, USA). All liposomes were analyzed and Gaussian distribution was chosen based on our facility standard. Polydispersity index of 0.0 represents a homogeneous particles population while 1.0 indicates a heterogeneous size distribution in the liposome preparations.

Stability of liposomes loaded with β-glucan

The stability of liposomes loaded with β-glucan was evaluated in PBS and in normal human pooled plasma as we previously described [19]. Briefly, appropriate amount of liposomes was suspended in PBS (pH=7.4) and incubated at 4°C or 37°C with a mild agitation (100 rpm). Likewise, to mimic the physiological conditions, normal human pooled plasma was supplemented with liposomes and incubated at 37°C with a mild agitation. After incubation periods of 0, 0.25, 0.5, 1, 3, 6, 12, 24 and 48 h, samples were removed and centrifuged (18,300 × g for 15 min at 4°C) to remove the leaked β-glucan. The concentrations of β-glucan were determined by Fungitec G test as described above. The stability of Liposomes was evaluated by determining the amount of β-glucan released over a 48 h study period. β-glucan release was expressed as percentage of the concentration of the entrapped β-glucan measured by Fungitec G test.

Erythrocyte hemolysis assay

An erythrocyte hemolysis assay was performed according to the method outlined by [20,21]. Whole, heparinized human erythrocytes (provided by Dr. Onuska, St Joseph's Hospital, Sudbury ON) were pooled and centrifuged at 700 × g for 10 min at 4°C. The erythrocytes were washed two times, using centrifugation at the above mentioned specifications, with ten times their volume of an ice cold buffer (147 mM NaCl, 6 mM glucose, and 20 mM of Tris-HCl (pH 7.2). After washing, the erythrocytes were re-suspended in twenty times their volume of fresh, ice cold buffer. This suspension was stored at 4°C and was used within ≤ 2 weeks of preparation [20]. To perform the hemolysis assay, a 3% erythrocyte solution was prepared by suspending the appropriate volume of erythrocytes into a hemolysis buffer solution (74 mM NaCl; 147 mM sucrose; 6 mM glucose; 20 mM Tris-HCl (pH 7.2). Erythrocytes were incubated in a shaking water bath, under gentle agitation, at 37°C with liposomes in concentrations ranging from 50-500 µg/mL for up to 24 hrs. After 4, 10 and 24 hour incubation, samples were removed and centrifuged at 1000 × g for 10 min at 4°C. The supernatant was removed and was centrifuged at 15 000 × g for 15 minutes at 4°C. The higher centrifugation force was required to ensure that the liposomes were completely removed from the suspension. This did not result in the sedimentation of hemoglobin [22]. The supernatant was removed and the amount of hemoglobin release was measured spectrophotometrically at 540 nm (reference at 625 nm) [23]. Controls included 3% erythrocytes (representing 0% hemolysis), and red blood cells treated with 1% Triton X-100 (representing 100% red blood cell lysis). A blank consisting of hemolysis buffer alone was also included. The hemolysis buffer blank was subtracted from all measurements, and the % hemolysis was calculated from the following equation:

$$\% \text{ Hemolysis} = (Abs._{Sample} - Abs._{Control\ 0\%}) / (Abs._{100\%\ Lysis}) \times 100$$

Materials with a hemolysis ratio less than 5% were regarded as hemocompatible [24].

In vitro cytotoxicity assays

The cytotoxicity of the β-glucan containing liposomes was evaluated using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The human lung carcinoma epithelial cell line A549 (provided by Dr. Azghani, University of Texas), was cultured in DMEM (Hyclone) supplemented with 10% FBS. Cells

were maintained using standard cell culture procedures, through incubation in a humidified atmosphere at 37°C, 5% CO_2. Cells were passaged at sub-confluency, and all cells used were of late passage. At sub-confluency, the cells were seeded into 96-well plates at a density of 5 × 10^3 cells/well. In all instances, cells were allowed to adhere overnight, prior to treatment. Next, the cultures were incubated with 5, 10, 15, 20 and 40 mg/ml of liposomal β-glucan for 24 h. The nanoparticles were removed by careful washing with PBS. Then, 200 µl of MTT solution (0.5 mg/ml in PBS) was added and incubated for another 4 h at 37°C. The reaction product was solubilized in 200 µl DMSO and absorbance (Abs) was measured at 570 nm. For all studies, negative controls included untreated cells (media alone) and media containing 50% D-PBS (HyClone) (this would accommodate the maximum amount of PBS that could be released from the empty liposome formulations. H_2O_2 was chosen as a positive control, as it has been shown that H_2O_2 can induce apoptosis in the A549 cell line [25]. The cell viability was calculated using the following formula: cell viability=(Absorbance of sample/Absorbance of negative control) × 100. The viability of untreated controls was normalized to 100%. Cytotoxicity=100- % cell viability. All studies are expressed as the mean of a minimum of three separate experiments, ± the SEM in triplicate.

Data analysis

The data are expressed as means ± SEM of three independent experiments in triplicates. Comparisons were made by paired Student's *t*-test and $P \leq 0.05$ was considered significant. For multiple comparisons within and between groups, ANOVA with the two-tailed Dunnett's post test analysis was used.

Results

Particle size, encapsulation efficiency and polydispersity index of liposomes

The mean particle size of liposomes with different lipid composition are varied from 125.90 ± 15.01 to 180.10 ± 11.3 and the encapsulation efficiency was varied from 2.23 ± 0.03 to 23.60 ± 0.2 (Table 1). The encapsulation efficiency of DCP: DPPC: Chol (4:2:1), DPPC: Chol (6:1) and DDAB: DPPC: Chol (4:2:1) were significantly higher (p<0.0001) than other groups. The polydispersity index of the liposomes indicates heterogeneous in nature. The liposomal β-glucan encapsulation efficiency (%EE) of liposome formulations was in the following order: DCP: DPPC: Chol (4:2:1)>DPPC: Chol (6:1)>DDAB: DPPC: Chol (4:2:1)>PC: Chol (6:1)>DSPC>PC>DSPC: Chol (6:1).

Liposome Lipid Composition	Size (nm) Mean ± SEM	EE (%) Mean ± SEM	Polydispersity index (PI)
PC	130.2 ± 10.5	3.63 ± 0.05	0.85 ± 0.009
PC: Chol (6:1)	157.00 ± 9.12	6.10 ± 0.01	0.80 ± 0.03
DDAB:DPPC:Chol (4:2:1)	144.10 ± 11.15	17.20 ± 0.08****	0.78 ± 0.007
DCP:DPPC:Chol (4:2:1)	160.30 ± 13.35	23.60 ± 0.2****	0.77 ± 0.005
DPPC:Chol (6:1)	179.20 ± 12.72	18.35 ± 0.06****	0.89 ± 0.004
DSPC:Chol (6:1)	125.90 ± 15.01	2.23 ± 0.03	0.66 ± 0.004
DSPC	180.10 ± 11.3	4.70 ± 0.03	0.90 ± 0.08

Note: PC: L-α-Phosphatidylcholine; Chol: Cholesterol; DDAB: Didecyldimethylammonium bromide; DPPC: 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine; DCP: dicetyl phosphate; DSPC: 1, 2-Distearoyl-sn-glycero-3-phosphocholine.

Table 1: Liposomal β-Glucan: particle size, encapsulation efficiency and polydispersity index: Particle size, polydispersity index (P.I.) and encapsulation efficiency of β-Glucan into liposomes. Particle size and polydispersity index (P.I.) of liposomes were determined by photon correlation spectroscopy with the use of Submicron Particle Sizer. All liposomes preparations were analyzed and Gaussian distributions were recorded. P.I., gives the measurement of homogeneity of dispersion, ranging from 0.0 (homogenous) to 1.0 (heterogeneous). Encapsulation efficiency was determined as the percentage of β-Glucan incorporated into liposomes relative to initial total amount of drug in solution. Results are given as means ± S.E.M. of three separated experiments in triplicate. ****P<0.0001.

Stability of liposomes loaded with β-glucan

The stability studies of liposomes loaded with β-glucan were performed in PBS and plasma at 4°C (storage temperature) and 37°C (body temperature). The study was performed for a period of 48 h at intervals of 0, 1, 3, 6, 12, 24 and 48 h. It was evident from the stability data that liposomal β-glucan in PBS and plasma at 4°C was more stable compared to that stored at 37°C. In PBS at 4°C the highest retention was more than 95% for DPPC: CHOL formulation and at 37°C the highest retention was more than 95% for PC: CHOL formulation. However, in plasma the DPPC: CHOL formulation has highest retention of more than 80% at 4°C and 70% at 37°C. Among all, DPPC: CHOL is the only formulation has significantly higher (p<0.0001) retention in comparison to other formulations (Figure 1).

Hemocompatibility of liposomes loaded with β-glucan

In order to investigate the toxicity of different liposomal β-glucan formulations, the liposomes with different concentrations were incubated with human erythrocytes for 4, 10 and 24 h. The amount of hemoglobin released was measured spectrometrically and results are indicated in Figure 2A. After 4 h of incubation, no significant hemolysis was observed with different liposomal β-glucan formulations, the hemolytic activities were less than 1%. After 10 h of incubation, PC: CHOL is the only formulation at different concentrations showed significantly hemolysis (p<0.0001) than 1% and all the remaining formulations were less than 1%. Finally after 24 h of incubation, all the liposomal β-glucan formulations exhibited hemolysis range of 8-10% with increasing concentrations of 0-40 µg/mL. Remarkably, DCP: DPPC: CHOL formulation did not follow this pattern as the data showed decreased hemolysis at 24 hr for 10 µg/mL. The general pattern for all liposomal formulations was that the % of hemolysis increased over time, and with increasing concentrations.

Cytotoxicity of liposomes

The A549 cell cultures were incubated with 5, 10, 15, 20 and 40 mg/ml of liposomal β-glucan of different formulations for 24 h (Figure 3A) and also tested in the presence of Doxorubicin (Figure 2B). The liposomal β-glucan PC is the only formulation have obtained highest viability of 80% and DPPC: CHOL formulation showed significantly higher (p<0.0001) growth suppression compared with PC and free β-glucan groups. All the other liposomal β-glucan formulations have obtained viability in between the range of 40-80%. The empty liposomal formulations did not show any toxicity and all cells remain viable. In the presence of Doxorubicin (Figure 3B), the highest values of 70% were obtained for liposomal β-glucan PC formulation and in least 30% for liposomal β-glucan DPPC: CHOL formulation (p<0.0001). In between the range of 30-70% all other formulations in the presence of Doxorubicin has obtained viability. However, the all the cells remain viable and did not show any toxicity for empty liposomal formulations. In A549 cells, the half maximal inhibitory concentration (IC_{50}) value of free -glucan was 13.94 µg. The IC_{50} values of PC, PC: Chol (6:1), DDAB: DPPC: Chol (4:2:1), DCP: DPPC: Chol (4:2:1), DPPC: Chol (6:1), DSPC: Chol (6:1), DSPC liposome were 12.01, 11.71, 12.34, 13.26, 11.07, 9.516, and 10.86, respectively (Table 2).

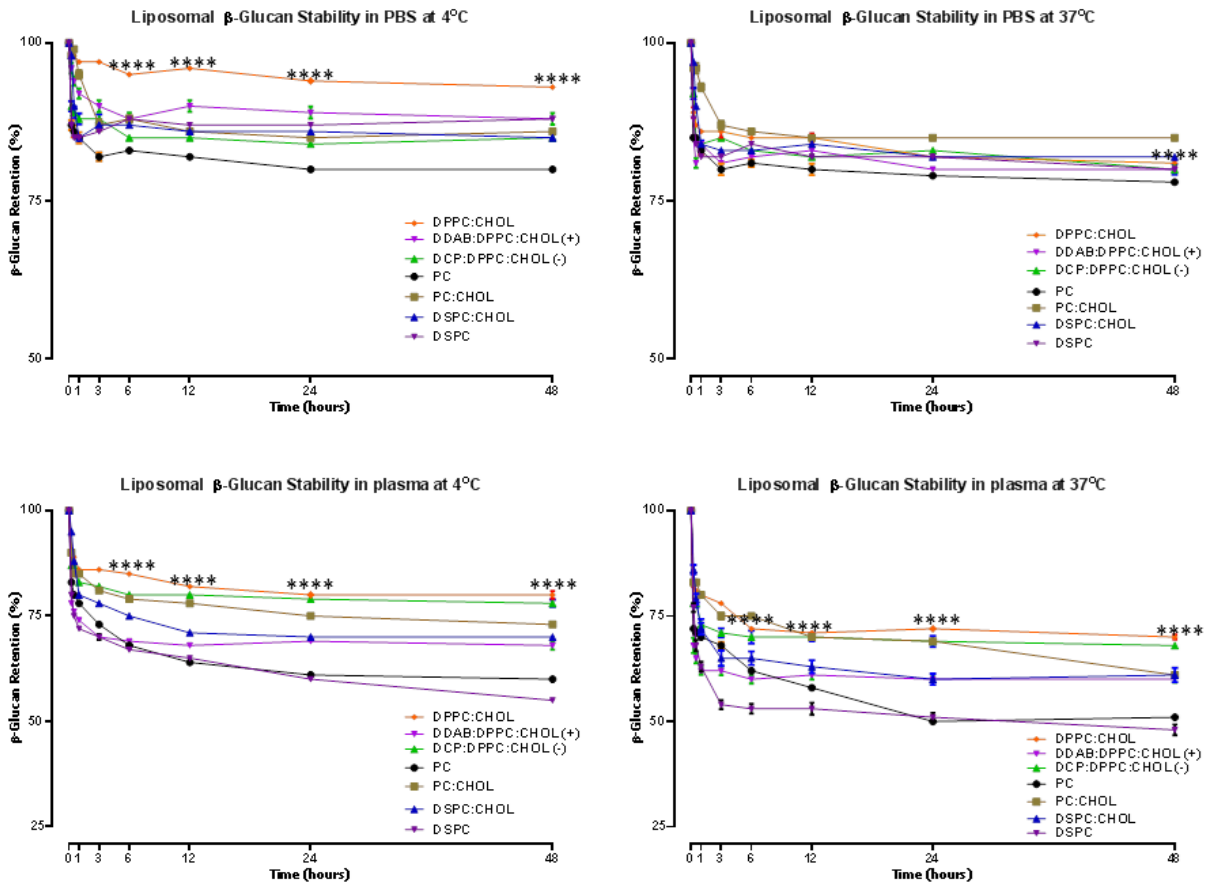

Figure 1: Stability of liposomal β-Glucan formulations in PBS at 4°C, 37°C and plasma with a mild agitation. At times indicated, samples were centrifuged at 18,300 g for 15 min and β-Glucan concentrations in the supernatants were determined by Fungitec G test. Results are given as means ± S.E.M. of three separated experiments in triplicate. ****P<0.0001.

Lipsome lipid composition	IC_{50} (µg)*	$R^{2\#}$
β-Glucan	13.94	0.959
PC	12.01	0.987
PC:Chol (6:1)	11.71	0.990
DDAB:DPPC:Chol (4:2:1)	12.34	0.983
DCP:DPPC:Chol (4:2:1)	13.26	0.976
DPPC:Chol (6:1)	11.07	0.988
DSPC:Chol (6:1)	9.516	0.940
DSPC	10.86	0.96

Note: * The concentration of β-Glucan necessary to produce 50% inhibition of cell growth. # R^2=the square of the correlation coefficient.

PC: L-α-Phosphatidylcholine; Chol: Cholesterol; DDAB: Didecyldimethylammonium bromide; DPPC: 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine; DCP: dicetyl phosphate; DSPC: 1, 2-Distearoyl-sn-glycero-3- phosphocholine.

Table 2: Liposomal β-Glucan: The IC_{50} values were calculated using sigmoidal concentration-response curve-fitting methods (GraphPad Prizm 6 software) and the logit-log equation. In all cases, best fits were obtained with the coefficient of correlation R^2 ranging from 0.94 to 0.99.

Discussion

The efficiency of liposomal drug delivery depends on physicochemical characteristics such as size, composition, loading efficiency and stability of liposomes. In the current study, we prepared 5 different kinds of liposomes with different lipids and cholesterol where concentration of cholesterol was optimized one-sixth of lipids. The use of high relative cholesterol amount in the lipid fraction of liposomes increased the drug entrapment percentage, due to the stabilizing effect of this steroid into the lipid bilayers [26]. Since it is known that the presence of cholesterol in the phospholipid bilayer can generally improve the stability of liposomes [27], liposomes containing DPPC: Chol molar ratios of 8:1 and 4:1 has been reduced the leakage but still more than 50% of 5(6)-carboxy-fluorescein (CF) was leaked within the first hour of cross-linking. On the other hand, 90% of CF was leaked from the pure DPPC liposomes within the first hour [28].

Since the glucan retention (%) is temperature dependent, the DPPC: Chol (6:1) in PBS showed more stable at 4°C while PC: Chol (6:1) showed more stable at 37°C. On the other hand DPPC: Chol (6:1) showed more stable in plasma at both 4°C and 37°C. With increasing cholesterol content the liposomes become less responsive to temperature. The acceleration of release due to melting of the bilayer was less visible when increasing the cholesterol concentration (Ullrich et al., 2013). In the case of isotonic conditions, higher concentrations of cholesterol (33 and 50 mol %) prevent the liposomes from releasing significant amounts of CF even above 60°C. The liposomes with less than 20 mol % of cholesterol released almost all the dye within a few minutes after reaching the phase transition temperature. The liposomes

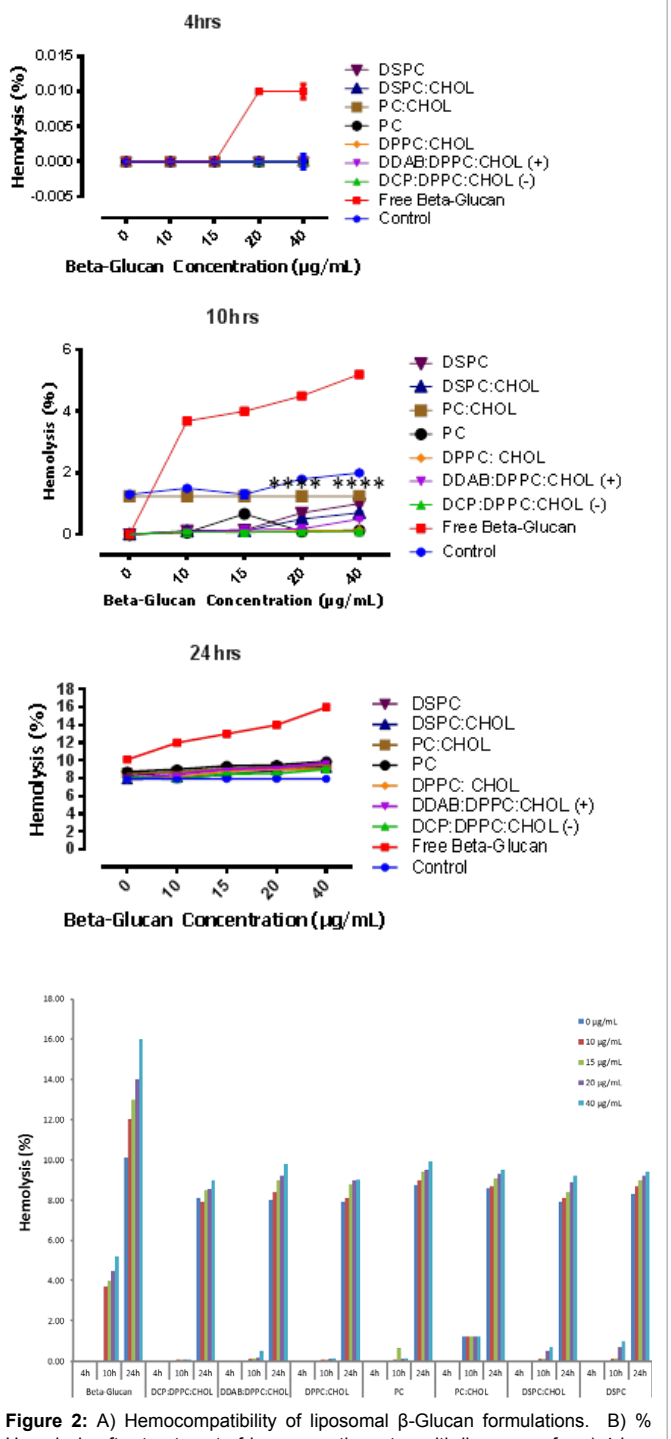

Figure 2: A) Hemocompatibility of liposomal β-Glucan formulations. B) % Hemolysis after treatment of human erythrocytes with liposomes for a) 4 hrs; b) 10 hrs; and c) 24 hrs, the amount of hemoglobin released was determined, and expressed as a percent hemolysis ± SEM relative to untreated red blood cells. Results are given as means ± S.E.M. of three separated experiments in triplicate. ****P<0.0001.

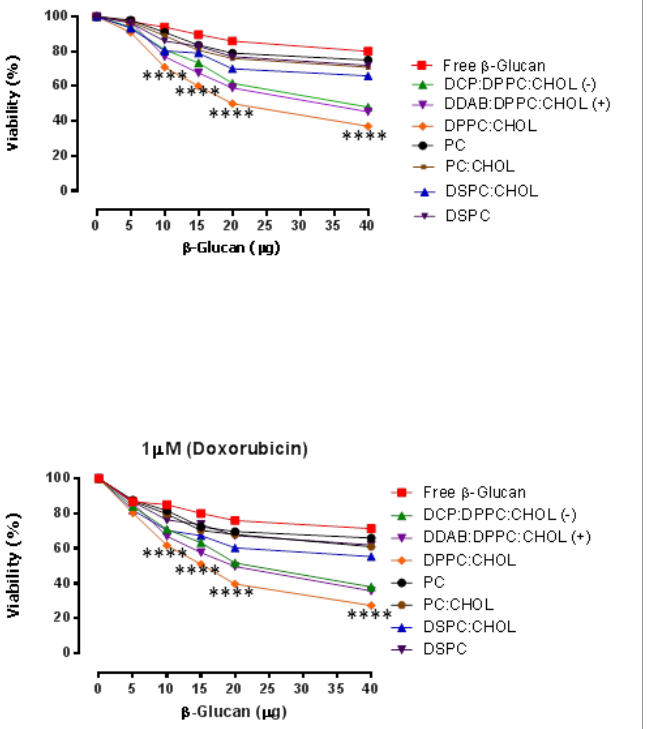

Figure 3: A. Effect of liposomal β-Glucan formulations on cell viability. A549 cell viability after 24 hours treatment with various concentrations of liposomal β-Glucan in absence. B. In the presence of 1µM Doxorubicin. Results are expressed as a percentage of PBS-treated cells. Concentrations are relative to the amount of encapsulated component in the liposomal β-Glucan formulation. NOTE: The empty liposomal formulations did not show any toxicity and all cells remain viable. ****P<0.0001.

with a molar ratio DPPC: Chol=2:1 and 1:1 survived heating to 40°C in the diluted buffer and some acceleration of release occurred around 55°C [28].

In pharmaceutical applications, drug encapsulation efficiency and *in vivo* behavior are highly dependent on liposome size [29]. The range of average size of liposomes was 130.20-180.10 nm which can easily transport to the blood stream through intestinal wall. Liposomes were revealed good hemotolerance in relation to major cellular components of the blood. As a confirmation to RBC counting, hemolysis rate was determined in the presence of the formations. The hemolysis rate did not exceed the negative control value by more than 2% at 10 h. However, the hemolysis rate was around 8% at 24 h. Therefore, the liposomes may be considered non-hemolytic [30,31]. The membrane bilayer became more polar in the presence of DPPC lipids and cholesterol, resulting in increased efficiency of incorporation of the hydrophilic drug [32]. In addition, an increase in the content of edge activator may have led to formation of pores in the bilayer [33].

1,3-β-glucans have been used in combinatorial therapies with antibodies in implanted human tumor xenografts from melanoma, epidermoid carcinoma, breast carcinoma, metastatic lymphoma and daudi lymphoma. The mice had higher survival rates in the presence of 1,3-β-glucans as compared to treatment with antibodies alone [34]. Chen et al. [35] showed that β-D-glucans from the mushroom *Fomes fomentarius* has a direct antiproliferative effect *in vitro* on human gastric cancer cells in a dose- and time dependent manner. Xie et al. [36] showed that *Ganoderma lucidum* glucan extract inhibits proliferation of SW480 human colorectal cancer cells. These examples demonstrate the versatility of 1,3-β-glucans in cancer inhibition and thus these polysaccharides can be modified to suit the desired application. We found significantly higher growth suppression of

human lung carcinoma epithelial cell line A549 with DPPC: Chol=6:1, DDAB:DPPC:Chol (+)=4:2:1 and DCP:DPPC:Chol (-)=4:2:1 liposome which was encapsulated with β-glucan. Our results showed dose dependent growth suppression. The mechanism could be involved in its inhibitory activity on tumor growth by stimulating the release of tumor necrosis factor TNF-α from monocytes/macrophages [37]. 1,3- β-glucans is highly dependent on the activation of T-lymphocytes-mediated adaptive immunity while down-regulate suppressive immune activity via Glucocorticoid-induced TNF receptor ligand interaction, leading to a more efficient defense mechanism against tumor development [38].

Doxorubicin was also encapsulated with β-glucan in liposome as it is one of the main chemotherapeutic agents [39]. It is effective against many types of cancer, including leukemia's, lymphomas, breast and ovarian cancers, etc.), than any other drug [40,41]. The main advantage of the liposomal doxorubicin is the significant reduced dose-dependent cardiotoxicity compared to the conventional form [42]. We found higher growth suppression of A549 cells with doxorubicin when it was delivered with liposomal β-glucans. Doxorubicin affects the uncontrollable division of cells, by inhibiting the activity of topoisomerase II, which prevents the replication of cellular DNA and RNA [43], thus stopping cancer proliferation. However, doxorubicin has high cardiotoxicity, causing damage to the myocardium. Early effects of doxorubicin administration include acute left ventricular dysfunction and arrhythmias and, with repeated use, dilated cardiomyopathy was observed that can eventually lead to drug-induced congestive heart failure and is fatal in approximately 50% of cases [44,45]. Other acute side effects of doxorubicin include neutropenia, nausea, arrhythmias, vomiting and alopecia [46]. Encapsulation of doxorubicin and other anti-tumor drugs in liposomes and micelles (especially polymeric micelles) alters the pharmacokinetics and distribution of those agents and thus reduces their cytotoxicity [47-49].

Conclusion

In conclusion, prepared liposome was hemotolerant, liposome formulation with cholesterol was stable in plasma at both 4 and 37°C and application of liposomal β-glucans was increased the anticancer activity of doxorubicin. Liposomal β-glucans can be an adjunct approach to treat cancer.

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References

1. Saraswat-Ohri S, Vashishta A, Vetvicka V, Descroix K, Jamois F, et al. (2011) Biological properties of (1>3)-beta-D-glucan-based synthetic oligosaccharides. J Med Food 14: 369-376.
2. Lavi I, Friesem D, Geresh S, Hadar Y, Schwartz B (2006) An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and proapoptotic effects on HT-29 colon cancer cells. Cancer Lett 244: 61-70.
3. Kobayashi H, Yoshida R, Kanada Y, Fukuda Y, Yagyu T, et al. (2005) Suppressing effects of daily oral supplementation of beta-glucan extracted from *Agaricus blazei* Murill on spontaneous and peritoneal disseminated metastasis in mouse model. J Cancer Res Clin Oncol 131: 527-538.
4. Yoon TJ, Kim TJ, Lee H, Shin KS, Yun YP, et al. (2008) Anti-tumor metastatic activity of beta-glucan purified from mutated *Saccharomyces cerevisiae*. Int Immunopharmacol 8: 36-42.
5. Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, et al. (2007) Orally administered marine (1-->3)-beta-D-glucan Phycarine stimulates both humoral and cellular immunity. Int J Biol Macromol 40: 291-298.
6. Hong F, Yan J, Baran JT, Allendorf DJ, Hansen RD, et al. (2004) Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J Immunol 173**:** 797-806.
7. Lee CI, Wu CC, Hsieh SL, Lee CL, Chang YP, et al. (2014). Anticancer effects on human pancreatic cancer cells of triterpenoids, polysaccharides and 1,3-beta-D-glucan derived from the fruiting body of *Antrodia camphorata*. Food Funct 5: 3224-3232.
8. Vetvicka V, Vetvickova J (2014) Natural immunomodulators and their stimulation of immune reaction: True or false? Anticancer Res 34: 2275-2282.
9. Yoon TJ, Koppula S, Lee KH (2013) The effects of beta-glucans on cancer metastasis. Anticancer Agents Med Chem 13: 699-708.
10. Jafaar ZM, Litchfield LM, Ivanova MM, Radde BN, Al-Rayyan N, et al. (2014). β-D-glucan inhibits endocrine-resistant breast cancer cell proliferation and alters gene expression. Int J Oncol 44**:** 1365-1375.
11. Ostadrahimi A, Esfahani A, Asghari Jafarabadi M, Eivazi Ziaei J, Movassaghpourakbari A, et al. (2014). Effect of Beta glucan on quality of life in women with breast cancer undergoing chemotherapy: a randomized double-blind placebo-controlled clinical trial. Adv Pharm Bull 4: 471-477.
12. Shah A, Ahmad M, Ashwar BA, Gani A, Masoodi FA, et al. (2015) Effect of gamma-irradiation on structure and nutraceutical potential of beta-d-glucan from barley (*Hordeum vulgare*). Int J Biol Macromol 72: 1168-1175.
13. Papachristos A, Pippa N, Ioannidis K, Sivolapenko G, Demetzos C (2014) Liposomal forms of anticancer agents beyond anthracyclines: Present and future perspectives. J Liposome Res 22: 1-8.
14. Yan EC, Eisenthal KB (2000) Effect of cholesterol on molecular transport of organic cations across liposome bilayers probed by second harmonic generation. Biophys J 79: 898-903.
15. Jia Y, Joly H, Omri A (2008) Liposomes as a carrier for gentamicin delivery: Development and evaluation of the physicochemical properties. Int J Pharm 359**:** 254-263.
16. Pumerantz A, Muppidi K, Agnihotri S, Guerra C, Venketaraman V, et al. (2011) Preparation of liposomal vancomycin and intracellular killing of meticillin-resistant *Staphylococcus aureus* (MRSA). Int J Antimicrobial Agents. 37: 140-144.
17. Alipour M, Suntres ZE, Halwani M, Azghani AO, Omri A (2009) Activity and interactions of liposomal antibiotics in presence of polyanions and sputum of patients with cystic fibrosis. PLoS ONE 4: e5724.
18. Alipour M, Suntres ZE, Omri A (2009) Importance of DNase and alginate lyase for enhancing free and liposome encapsulated aminoglycoside activity against Pseudomonas aeruginosa. J Antimicrob Chemother 64: 317-325.
19. Mugabe C, Azghani AO, Omri A (2005) Liposome-mediated gentamicin delivery: development and activity against resistant strains of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. J Antimicrob Chemother 55: 269-271.
20. Parnham MJ, Wetzig H (1993) Toxicity screening of liposomes. Chem Physics lipids 64: 263-274.
21. Yoshihara E, Nakae T (1986) Cytolytic activity of liposomes containing stearylamine. Biochim Biophys Acta 854: 93-101.
22. Patel GB, Omri A, Deschatelets L, Sprott GD (2002) Safety of archaeosome adjuvants evaluated in a mouse model. J Liposome Res 12: 353-372.
23. Papazafiri P, Avlonitis N, Angelou P, Calogeropoulou T, Koufaki M, et al. (2005) Structure-activity relationships of antineoplastic ring-substituted ether phospholipid derivatives. Cancer Chemother Pharmacol 56: 261-270.
24. Lin JJ, Lin WC, Dong RX, Hsu SH (2012) The cellular responses and antibacterial activities of silver nanoparticles stabilized by different polymers. Nanotechnology 23: 065-102.
25. Geiser T, Ishigaki M, Van Leer C, Matthay MA, Broaddus VC (2004). H(2)O(2) inhibits alveolar epithelial wound repair *in vitro* by induction of apoptosis. Am J Physiol Lung Cell Mol Physiol. 287**:** L448-453.
26. Lopez-Pinto JM, Gonzalez-Rodriguez ML, Rabasco AM (2005) Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. Int J Pharm 298: 1-12.

27. Liang X, Mao G, Ng KY (2004) Mechanical properties and stability measurement of cholesterol-containing liposome on mica by atomic force microscopy. J Colloid Interface Sci 278**:** 53-62.

28. Ullrich M, Hanus J, Dohnal J, Stepanek F (2013) Encapsulation stability and temperature-dependent release kinetics from hydrogel-immobilised liposomes. J Colloid Interface Sci 394: 380-385.

29. Demetzos C, Pippa N (2014) Advanced drug delivery nano systems (aDDnSs): A mini-review. Drug Deliv 21**:** 250-257.

30. Kuznetsova NR, Sevrin C, Lespineux D, Bovin NV, Vodovozova EL, et al. (2012) Hemocompatibility of liposomes loaded with lipophilic prodrugs of methotrexate and melphalan in the lipid bilayer. J Controlled Release 160: 394-400.

31. ASTM International (2009) Standard practice for assessment of hemolytic properties of materials. ASTM International, West Conshohocken, PA, USA.

32. Patzelt A, Richter H, Buettemeyer R, Huber HJ, Blume-Peytavi U, et al. (2008) Differential stripping demonstrates a significant reduction of the hair follicle reservoir in vitro compared to *in vivo*. Eur J Pharm Biopharm 70: 234-238.

33. Badran M, Shazly G, El-Badry M (2012) Effect of terpene liposomes on the transdermal delivery of hydrophobic model drug, nimesulide: Characterization, stability and *in vitro* skin permeation. Afr J Pharm Pharmacol 6**:** 3018-3026.

34. Cheung NK, Modak S, Vickers A, Knuckles B (2002) Orally administered beta-glucans enhance anti-tumor effects of monoclonal antibodies. Cancer Immunol Immunother 51**:** 557-564.

35. Chen W, Zhao Z, Chen SF, Li YQ (2008) Optimization for the production of exopolysaccharide from *Fomes fomentarius* in submerged culture and its antitumor effect *in vitro*. Bioresource Technol 99**:** 3187-3194.

36. Xie JT, Wang CZ, Wicks S, Yin JJ, Kong J, et al. (2006). *Ganoderma lucidum* extract inhibits proliferation of SW 480 human colorectal cancer cells. Exp Oncol 28: 25-29.

37. Majtan J, KoganG, Kovacova E, Bilikova K, Simuth J (2005) Stimulation of TNF-alpha release by fungal cell wall polysaccharides. Z Naturforsch C 60: 921-926.

38. Tian J, Ma J, Ma K, Ma B, Tang X, et al. (2012). Up-regulation of GITRL on dendritic cells by WGP improves anti-tumor immunity in murine Lewis lung carcinoma. PLoS ONE 7**:** e46936.

39. Barenholz Y (2012) Doxil® the first FDA-approved nano-drug: lessons learned. J Controlled Release 160**:** 117-134.

40. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56: 185-229.

41. Skeel RT (1999) Handbook of cancer chemotherapy. (8thedn), Lippincott Williams and Wilkins, Philadelphia.

42. Peng X, Chen B, Lim CC, Sawyer DB (2005) The cardiotoxicology of anthracycline chemotherapeutics: translating molecular mechanism into preventative medicine. Mol Intern 5: 163-171.

43. Gewirtz DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol 57**:** 727-741.

44. Chatterjee K, Zhang J, Honbo N, Karliner JS (2010) Doxorubicin cardiomyopathy. Cardiology 115**:**155-162.

45. Chlebowski RT (1979) Adriamycin (doxorubicin) cardiotoxicity: A review. Western J Med 131**:** 364-368.

46. Shi Y, Moon M, Dawood S, McManus B, Liu PP (2011) Mechanisms and management of doxorubicin cardiotoxicity. Herz 36: 296-305.

47. Allen TM, Hansen CB, Lopes De Menezes DE (1995) Pharmacokinetics of long-circulating liposomes. Adv Drug Deliv Rev 16**:** 267-284.

48. Sutton D, Nasongkla N, Blanco E, Gao J (2007) Functionalized micellar systems for cancer targeted drug delivery. Pharm Res 24: 1029-1046.

49. Wang S, Mi JB, Li YZ, Chang WB, Ci YX, et al. (2003) Pharmacokinetics and tissue distribution of iv injection of polyphase liposome-encapsulated cisplatin (KM-1) in rats. Acta Pharmacol Sin 24: 589-592.