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A simple and effective approach for the treatment of dyslipidemia using anionic nanoliposomes

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ABSTRACT

The present study was undertaken to evaluate the anti-dyslipidemic effects of nanoliposomes with different phospholipid compositions. Three sets of liposomal formulations (20 Mm; 100 nM in size) were prepared with low (SPC), medium (POPC) and high (HSPC) phase transition temperature values with and without cholesterol and anionic phosphatidyl glycerol (HSPC/DSPG; POPC/DMPG; SPC/EPG). The liposomal preparations were characterized for their size and zeta potential (dynamic light scattering), *J774A.1* macrophages uptake (flow cytometry) and lipid-modifying effects (tyloxapol-induced hyperlipidemic mouse model). Anionic formulations displayed the highest rate of uptake by macrophages. Among them, HSPC/DSPG and SPC/EPG liposomes had the best lipid-modifying activity. These two formulations exerted favorable impact on all lipid profile parameters by reducing LDL-C (by up to 76% [HSPC/DSPG] and 86% [SPC/EPG]), total cholesterol (by up to 52% [HSPC/DSPG] and 68% [SPC/EPG]), triglycerides (by up to 88% [HSPC/DSPG] and 73% [SPC/EPG]), apoB (by up to 44% [HSPC/DSPG] and 35% [SPC/EPG]) and elevating HDL-C (by up to 85% [HSPC/DSPG] and 75% [SPC/EPG]) concentrations. Atherogenic indices were also effectively reduced following HSPC/DSPG (by up to 69%) and SPC/EPG (by up to 79%) injections. Empty, cholesterol-free nanoliposomal formulations containing 75% anionic phospholipid (PG) might serve as effective and rapid acting anti-dyslipidemic agents. Further research is warranted to confirm the observed anti-dyslipidemic effects of anionic nanoliposomes in diet-induced hyperlipidemic models, and also to evaluate the potential protective effects in regressing atheromatous lesions.

1. Introduction

Coronary artery disease (CAD) is the leading cause of mortality worldwide. Annually, CAD imposes significant financial burden and casualties on healthcare systems. Therefore, the current focus of many health policy-making authorities is to find effective ways for the optimal prevention and management of CAD. Among the most important modifiable risk factors for CAD is dyslipidemia, which is characterized by elevated levels of low-density lipoprotein cholesterol (LDL-C) and decreased concentrations of high-density lipoprotein cholesterol (HDL-C). Findings of several landmark trials have confirmed the beneficial impact of lowering LDL-C levels on both primary and secondary prevention of cardiovascular disease (CVD) [1–4]. Most of the international guidelines recommend reduction of LDL-C to <70 mg/dL as an optimal target for primary prevention of patients at very high risk of CAD [5–9]. However, statins – as the most potent LDL-lowering class of drugs – are able to reduce serum LDL-C by up to 30–50% in most cases [10]. Besides, such a reduction would be achieved with aggressive therapy which may itself predispose the patient to adverse events such as myopathies and hepatotoxicity. Combination of low HDL-C and elevated triglycerides, known as atherogenic dyslipidemia, is another dyslipidemic phenotype that is frequently seen in patients with diabetes mellitus and metabolic syndrome, and is also a definite contributor to atherosclerotic CVD [11,12]. In addition to their inadequate potency for decreasing serum LDL-C to the optimal level, statins have also limited effect on serum HDL-C and triglycerides concentrations [13]. Owing to these limitations, there

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has been an increasing attempt to find novel lipid-modifying agents with improved efficacy and safety [14–22].

Liposomes are artificial phospholipid bilayers that have been used as carriers to enhance potency and reduce toxicity of drugs. These constructs have generated great interest for biomedical purposes owing to their biocompatibility, biodegradability, safety and lack of immunogenicity [23]. Nanoliposomes are known to have a short half-life in the circulation. Biodistribution studies have shown that nanoliposomes are efficiently taken up by the hepatic tissue within a few hours of intravenous injection [24]. This uptake has been shown to be performed through receptor mediated endocytosis, a process which normally occurs for the clearance of LDL from the circulation (Fig. S1) [25]. On the other hand, there has been evidence indicating the coalescence of liposomes containing 75–100% anionic phospholipids with LDL and the uptake of resulting complexes via either LDL receptors or macrophages [26–31]. In addition, liposomes have been shown to undergo vast lipid exchange with plasma lipoproteins, an interaction that promotes reverse cholesterol transport from peripheral tissues to liver [32–34]. In spite of these promising mechanisms, the potential of liposomes as anti-dyslipidemic agents has not been well clarified. To this end, the present study aimed to evaluate the impact of intravenous administration of different phospholipid liposomes on serum lipid profile.

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.07.045.

2. Materials and methods

2.1. Chemicals

Hydrogenated soy phosphatidylcholine (HSPC), soy phosphatidylcholine (SPC), Egg phosphatidylglycerol (EPG), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dimiristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-distearoyl-*sn*-glycero-3-phosphoglycerol (DSPG) were purchased from Lipoid GmbH (Germany). The fluorescent dye 1,1-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was from Invitrogen (USA). Tyloxapol and simvastatin were from Sigma (Germany) and Sami Saz Pharmaceutical Company (Iran), respectively. Alamar Blue was purchased from Biosource (International Inc., USA).

2.2. Preparation of liposomes

Conventional liposomes were prepared from different phospholipids (HSPC, SPC, POPC, DMPG, EPG or DMPG) by the method of dried lipid-film hydration plus extrusion. Phospholipids were different in their liquid crystalline transition temperature (T_m) and charge. Briefly, three sets of liposomal formulations (Table 1) were prepared with low (soy phosphatidylcholine, SPC; $T_m < 0\,^{\circ}C$), medium (palmitoyl-oleoyl-phosphatidylcholine, POPC; $T_m = 0\,^{\circ}C$) and high (hydrogenated soy phosphatidylcholine, HSPC; $T_m = 55\,^{\circ}C$) T_m values with (HSPC/chol; POPC/chol; SPC/chol) and without cholesterol (HSPC; POPC; SPC) and anionic phosphatidyl glycerol (HSPC/DSPG; POPC/DMPG; SPC/EPG) at final lipid concentration of 20 mM. The anionic formulations were cholesterol-free and contained an anionic phospholipid amounting to 75% of total phospholipid content. Fluorescence labeling of liposomes was performed using DiI (~0.2 mol% of phospholipid). After vacuum and freeze drying, the obtained thin lipid film was hydrated with histidine (10 mM)/sucrose (10%) buffer (pH 6.5). The resultant multilamellar dispersions were reduced in size lamellarity by vortexing, sonication and extrusion through 200 nm (5 cycles) and 100 nm (11 cycles) polycarbonate membranes using a thermobarrel extruder (Lipex Biomembranes Inc., Canada) [35].

2.3. Characterization of the liposomes

The particle diameter of each sample together with its polydispersity index was measured in triplicate using Dynamic Light Scattering Instrument (Nano-ZS; Malvern, UK). The zeta potential of liposomes was determined on the same machine using the zeta potential mode as the average of 20 measurements [36].

2.4. Phospholipid assay

The phospholipid concentration of formulations was determined according to Bartlette test [37]. Briefly, liposomal formulations (80 ± 50 nmol of phosphate containing lipid) were added into disposable borosilicate glass tubes. Then, 0.4 mL of 10 N H_2SO_4 was added to each tube. In the fume hood, the sample was digested at 195–210 °C for 60 min using a hot-plate apparatus. The tubes were then cooled for about 10 min at room temperature. Afterwards, 0.1 mL of 10% H_2O_2 was added to each sample and tubes were again heated for 10 min at 190–210 °C. After cooling the tubes for about 10 min, 4.7 mL of molybdate reagent and 0.5 mL of 10% ascorbic acid were added to each tube and vortexed immediately for 10 s. Samples were then heated at 100 °C for 10–20 min, quickly cooled and finally were measured for optical density at 800 nm. Phospholipid concentration of samples was calculated according to the phosphate standard curve.

2.5. Liposome uptake by macrophages

J774.A1 macrophages were seeded in 12-well tissue culture plates at 3.0 × 105 cells/well in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). Cells were incubated with different liposomal preparations (final phospholipid concentration in each well was set at 0.1 mM). After 3, 10 and 24 h of incubation, cells were washed with cold phosphate buffered saline (PBS) and then lysed using 1% Triton X-100. Flow cytometric analyses were performed on the obtained lysate by means of a Partec® instrument (Münster,

Table 1
Particle size distribution, zeta potential and polydispersity of liposomal formulations.

Formulation	Molar ratio	Z-average (d nm)	PDI	Zeta potential (mV)
HSPC	–	129.6 ± 2.76	0.175 ± 0.11	−10.2 ± 4.92
HSPC/Chol	5/1	121.7 ± 26.52	0.172 ± 0.12	−9.76 ± 8.19
HSPC/DSPG	1/3	108 ± 1.01	0.34 ± 0.01	−50.9 ± 14.50
SPC	–	81.64 ± 3.57	0.295 ± 0.04	−10.1 ± 0.57
SPC/Chol	5/1	82.40 ± 1.75	0.26 ± 0.03	−11.30 ± 1.70
SPC/EPG	1/3	95.31 ± 35.77	0.20 ± 0.02	−49.7 ± 15.8
POPC	–	119.30 ± 15.56	0.18 ± 0.02	−10.02 ± 2.65
POPC/Chol	5/1	121.98 ± 31.28	0.17 ± 0.10	−9.56 ± 0.11
POPC/DMPG	1/3	98.97 ± 7.96	0.25 ± 0.17	−49.55 ± 3.23

PDI, polydispersity index. Values are expressed as mean ± SD, $n = 3$.

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Germany). For each sample, mean fluorescence intensity together with the percentage of positively stained events was determined.

2.6. In vivo hypolipidemic experiments

Male albino mice (20–30 g) were obtained from the animal house of the School of Pharmacy, Mashhad University of Medical Sciences (Mashhad, Iran), and used for in vivo experiments. The animals were housed in constant room temperature (23–25 °C), kept in plastic cages (47 cm × 34 cm × 18 cm) with sawdust and had free access to food and water. Mice were starved for ~6 h prior to experiment. For the screening tests, animals were randomly divided into 11 groups of four mice each. Hyperlipidemia was induced in all groups via injection of a single dose of tyloxapol (Triton WR-1339) at a dose of 300 mg/kg mouse weight. Simvastatin (80 mg/kg) and saline were used as positive and negative control, respectively. Liposomal formulations were passed through a 0.22 μ intravenously injected through tail vein at a dose of 200 μmol phospholipid/kg. Blood collection was performed 1 h post-injection via cardiac puncture, following anesthesia with ketamine/xylazine combination. Dose-dependent experiments were conducted with the same protocol using 3 doses of 200 μmol/kg, 100 μmol/kg and 50 μmol/kg.

2.7. Lipoprotein measurements

After being allowed to clot at room temperature, collected blood was centrifuged at 14,000 rpm for 10 min to obtain serum. A complete lipid profile comprising LDL-C, HDL-C, total cholesterol and triglycerides, together with concentrations of apolipoproteins A (apoA) and B (apoB) were determined in serum samples. Lipoprotein measurements were performed based on the routine enzymatic methods using commercial kits (Biosystems, Spain). Atherogenic index was calculated using 2 routinely used equations i.e. LDL/HDL (AI_1) and log (triglycerides/HDL-C) (AI_2).

2.8. Cytotoxicity assessment

Cytotoxicity of nanoliposomes was assessed using Alamar Blue®. *J774.A1* cells were seeded in 96-well plates, allowed to adhere and grow for an overnight at 37 °C and 5% CO_2, and then used in the cytotoxicity experiment. A total of three doses (5, 10 and 15 μL of the 20 mM liposomal preparation); and three different time points (3, 12 and 24 h) were used to determine cytotoxicity of nanoliposomes. Alamar Blue® solution was directly added to the medium resulting in a final concentration of 10% in each well. After 4 h of incubation with Alamar Blue®, the absorbance was read at 545 nm using a microplate reader according to the manufacturer leaflet.

2.9. Statistical analysis

Statistical analyses were performed using SPSS software (version 13.0; SPSS Inc., Chicago, IL). Values were expressed as mean ± SEM. Between-group comparisons of serum LDL-C and macrophage uptake measures were performed using one-way analysis of variance (ANOVA) with Dunnett's test for post hoc multiple comparisons. A two-sided *p*-value of <0.05 was considered as statistically significant.

3. Results

3.1. Characterization of prepared liposomes

Prepared liposomal formulations had a diameter around 100 nm. Overall, the formulations were very homogenous and had a polydispersity index of <0.3. Zeta potential of prepared liposomes ranged between −9.56 (for HSPC/chol) to −50.90 (for HSPC/DSPG) (Table 1).

3.2. Macrophage uptake assay

Percentage of positively stained events along with the mean fluorescence intensity was used as markers of liposome uptake by *J774.A1* macrophages. After 3 and 10 h of incubation, high uptake rates were only observed for the HSPC/DSPG formulation. However, at 24 h time point, all anionic preparations (HSPC/DSPG, SPC/EPG and POPC/DMPG) were significantly greater taken up by macrophages compared to the control treatment (buffer solution) ($p < 0.001$). Higher uptake of anionic formulations was consistent using either the mean fluorescence intensity or percentage of positive events as marker (Figs. 1 and S2).

Supplementary Fig. S2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.07.045.

3.3. In vivo lipid-lowering activity

Prepared formulations were initially screened for their anti-dyslipidemic activity. Among the 9 tested formulations, the greatest effects on serum LDL-C and total cholesterol (Figs. S3 and S4) were observed from HSPC/DSPG and SPC/EPG, both having a net negative charge. The anti-dyslipidemic activity of these two formulations was higher than that of other formulations. There were no significant differences in the anti-dyslipidemic activity of these two formulations with simvastatin ($p > 0.05$). Based on the screening tests, these 2 formulations were selected and further evaluated for their dose-dependent anti-dyslipidemic effects.

Supplementary Figs. S3 and S4 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.07.045.

3.4. Dose-dependent experiments

3.4.1. LDL-C

SPC/EPG and HSPC/DSPG formulations showed a marked LDL-lowering capacity at all tested doses: high (H), medium (M) and low (L). The LDL-lowering activity of all tested liposomal formulations was significantly greater compared to the control group (injected with normal saline) and ranged between 64% [HSPC/DSPG (M)] to 86% [SPC/EPG (L)] [$p = 0.002$ for HSPC/DSPG (M) and $p < 0.001$ for the remaining formulations) (Fig. 2).

As low-doses of liposomal preparations showed efficacy in the in vivo model, they were further tested for LDL-C lowering activity at 12 h and 24 h post-injection, as well. The results indicated that reduced LDL-C levels following liposome injection are sustained at 12 h ($p < 0.001$) and 24 h ($p < 0.05$) time points, with no significant difference compared to those achieved at the 1 h time point post-injection (Fig. 2).

3.4.2. Total cholesterol

All doses of tested liposomal formulations reduced serum total cholesterol levels. The magnitude of effects among the liposomal formulations reached statistical significance for HSPC/DSPG (H) (52%; $p < 0.05$) and SPC/EPG (L) (68%; $p < 0.01$) (Fig. 2).

3.4.3. HDL-C

Promising elevations of serum HDL-C was observed following administration of liposomes. The magnitude of HDL-C-elevating effects among the liposomal formulations reached statistical significance for HSPC/DSPG (L) (85%; $p < 0.05$) and SPC/EPG (H) (75%;

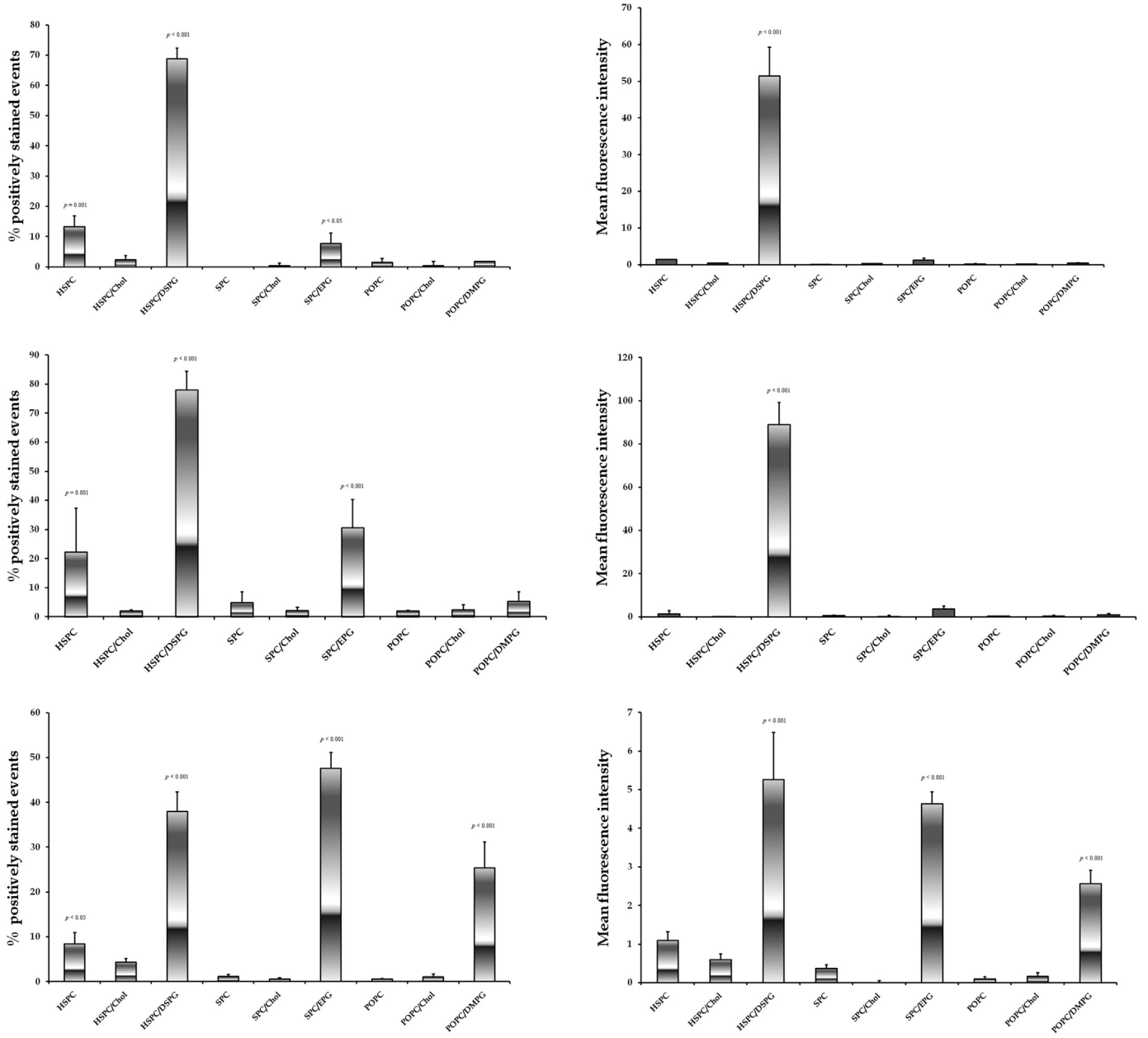

Fig. 1. Comparison of nanoliposomal formulations in terms of uptake rate by *J774.A1* macrophages following 3 (upper row), 10 (middle row) and 24 h (lower row) of incubation. The results are shown as % positively stained events (left) and mean fluorescence intensity (right) according to flow cytometric data. Values are expressed as mean ± SEM, $n = 3$.

$p < 0.05$) (Fig. 2). These two formulations had also a greater HDL-elevating effect compared with simvastatin.

3.4.4. Triglycerides

Significant reductions in serum triglycerides were observed following administration of nanoliposomes, being significant for all tested formulations [$p < 0.01$ for HSPC/DSPG (H) and HSPC/DSPG (M), and $p < 0.05$ for the rest of formulations] except SPC/EPG (M) ($p > 0.05$) (Fig. 2). The magnitude of triglyceride-reducing effect ranged between 52% [SPC/EPG (M)] to 88% [HSPC/DSPG (H)].

3.4.5. Atherogenic indexes

The overall impact of tested formulations on the cardiovascular risk was assessed by calculating atherogenic indexes AI_1 and AI_2. All tested formulations reduced AI_1 in a significant manner ($p < 0.001$) ranging between 65% [HSPC/DSPG (H)] to 79% [SPC/EPG (H)] (Fig. 3). With respect to AI_2, the efficacy of formulations reached statistical significance for HSPC/DSPG (H) (69%; $p < 0.05$) and HSPC/DSPG (L) (61%; $p < 0.05$) (Fig. 3).

3.4.6. Apolipoproteins

The impact of HSPC/DSPG and SPC/EPG formulations on serum apoA levels was tested at the most effective anti-dyslipidemic dose i.e. 50 μmol/kg. None of the formulations did significantly alter serum apoA ($p > 0.05$). In contrast, serum apoB levels were significantly reduced by both formulations ($p < 0.05$). The magnitude of reduction in apoB was slightly greater with the SPC/EPG (44%) compared with HSPC/DSPG (35%) formulation. Changes in serum apolipoproteins and the ratio of apoA/apoB are summarized in Fig. 3.

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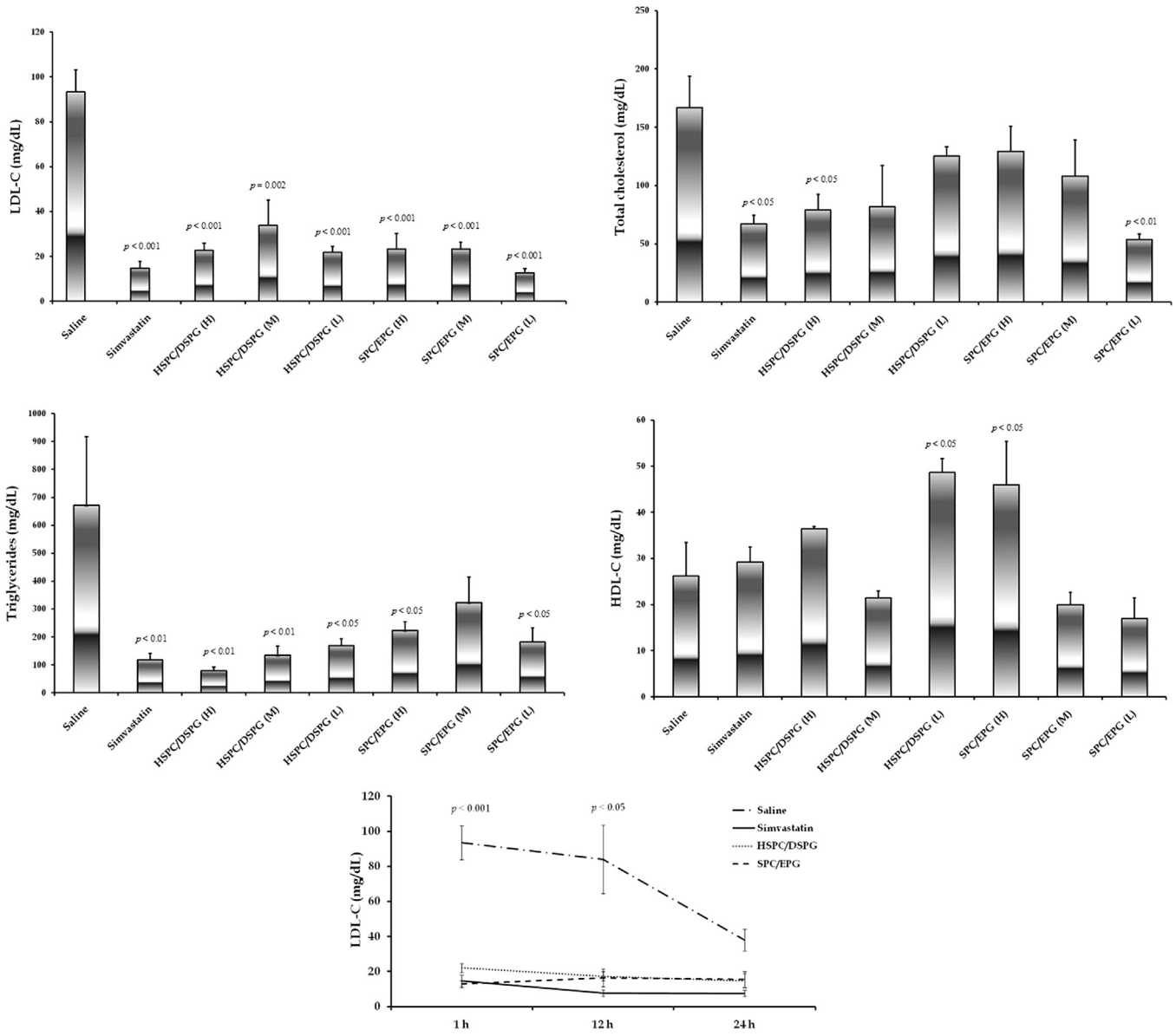

Fig. 2. In vivo effects of different doses of anionic nanoliposomes on serum LDL-C, total cholesterol, HDL-C and triglyceride concentrations. H: high concentration (200 μmol/kg); M: medium concentration (100 μmol/kg); L: low concentration (50 μmol/kg). Values are expressed as mean ± SEM. The last figure shows changes in serum LDL-C concentrations at different time points following injection of anionic nanoliposomes at a dose of 50 μmol/kg. *p*-Values refer to the statistical comparison of liposomal preparations and simvastatin with saline. No significant difference was found between liposomal preparations and simvastatin.

3.4.7. Cytotoxicity assays

Cytotoxicity of the HSPC/DSPG and SPC/EPG formulations was dose- and time-dependently evaluated using Alamar Blue® assay. Several doses (5, 10 and 15 μL) were used, and cell viability was determined after 3, 12 and 24 h of incubation. Overall, the viability of cultured macrophages was almost completely maintained in the presence of both tested liposomal preparations regardless of dose and duration of incubation ($p > 0.05$). The results of cytotoxicity assays are summarized in Fig. 4.

4. Discussion

The present study was designed to determine the effect of nanoliposomes with different phospholipid compositions, T_m and charge on serum levels of lipoproteins. In the in vitro uptake assays, liposomes containing anionic phospholipids (DSPG, EPG and DMPG) demonstrated a higher rate of uptake by macrophages. This finding is expected since liposomes containing high (≥75%) contents of PG have been previously shown to interact and coalesce with LDL particles. The resulting particles can then bind to LDL receptor via their apolipoprotein B-100 component [26,27]. Greenspan et al. demonstrated that such liposome/LDL complexes, upon formation, are phagocytosed by macrophages [28]. In addition, an electrostatic interaction has also been shown between liposomes containing 50% anionic phospholipids and cationic residues in apo B-100 [29,30]. Several lines of evidence have also indicated that anionic phospholipids are actively taken up by cells, in particular by macrophages, via SRBI scavenger receptors or CD36 [31]. According to Stamler et al., intravenous administration of phosphatidylinositol vesicles causes a significant induction of reverse cholesterol transport (RCT) and a striking increase in free cholesterol clearance from plasma. This phenomenon was found to be due to the increase in the surface potential of lipoproteins, in particular HDL, which is secondary to the incorporation of anionic phospholipids into the lipoproteins [38]. Moreover, it has been shown that atherosclerotic plaques are an important target site for anionic liposomes [39,40]. It has been

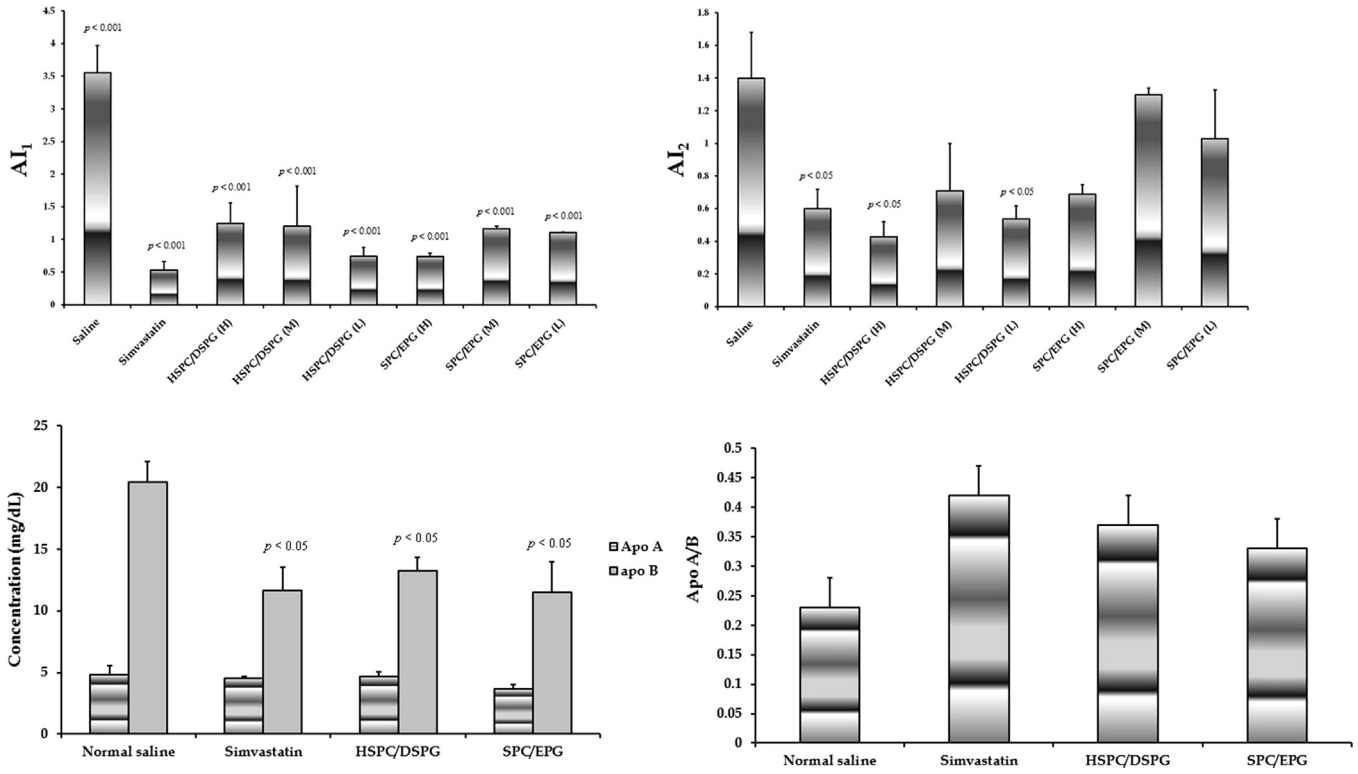

Fig. 3. In vivo effects of different doses of anionic nanoliposomes on AI_1 (calculated as LDL-C/HDL-C), AI_2 [calculated as log (triglycerides/HDL-C)], apoA apoB, and apoA/apoB. AI: atherogenic index; H: high concentration (200 μmol/kg); M: medium concentration (100 μmol/kg); L: low concentration (50 μmol/kg). Values are expressed as mean ± SEM.

recently shown that anionic liposomes composed of DMPC/DMPG (7:3 ratio) are taken up by macrophage-rich areas of vulnerable atherosclerotic plaques in *APOE*$^{-/-}$ mice via clathrin-mediated endocytosis [40]. In addition to the intrinsic therapeutic capacity, the affinity of anionic liposomes to macrophage rich regions of atheroma makes them ideal vehicles for targeted delivery of anti-atherosclerotic and imaging agents because no sign of uptake has been observed in non-diseased regions of vascular wall near to the advanced atheromas [39].

Following internalization, liposomes will be targeted to lysosomes where they will be further metabolized [41,42]. The accumulation of nanoliposome/LDL complexes in the hepatic tissue and their degradation by lysosomes represent an elimination route very similar to that naturally occurs for LDL. Therefore, the liposome-based anti-dyslipidemic approach described here is virtually a mimic of the body's natural mechanism.

In the in vivo assays, SPC/EPG and HSPC/DSPG formulations exhibited high LDL-lowering activity which was elicited as early as 1 h post-injection and sustained for at least 24 h. The effects of these two formulations were even comparable to those of simvastatin, used as the standard anti-dyslipidemic compound. It is interesting to note that most of the currently available lipid-lowering drugs such as fibrates, bile acid sequestrants and niacin cannot reach the same efficacy on LDL-C as statins [43].

An important finding of the current study was the remarkable effect of HSPC/DSPG and SPC/EPG nanoliposomes on serum HDL-C concentrations. Although the inverse association between HDL-C levels and CVD risk has been reported by numerous observational

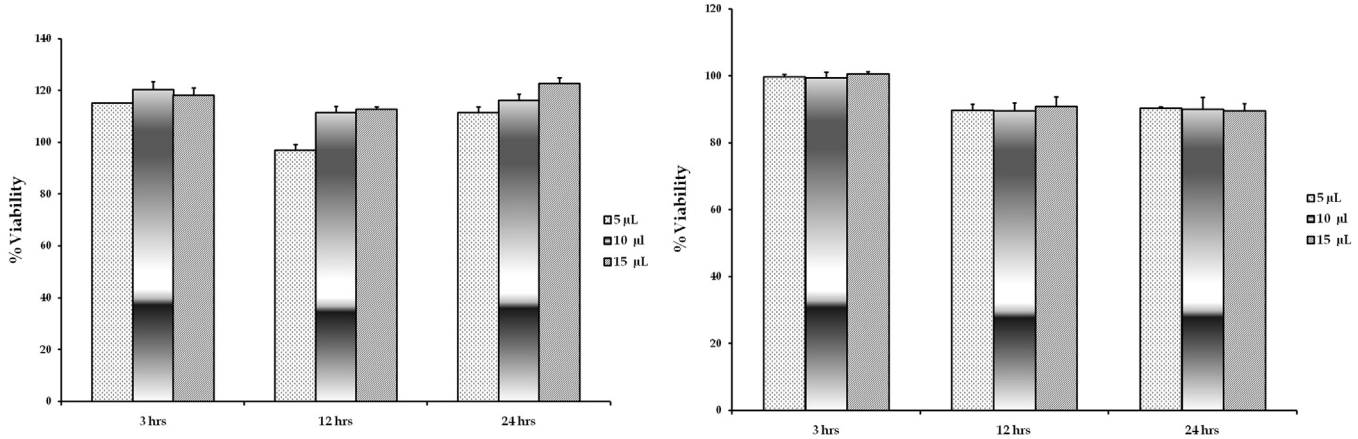

Fig. 4. Dose- and time-dependent evaluation of the viability of *J774.A1* macrophages in the presence of HSPC/DSPG (left) and SPC/EPG (right) nanoliposomes. Values are expressed as mean ± SEM.

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studies, large outcome trials with HDL-raising therapies have failed to confirm any positive impact of HDL elevation in reducing CV events [44,45]. Instead, it has been proposed that it is the functionality of HDL, rather than simply HDL-C level, that determines CVD risk [46–48]. Apart from increasing HDL-C concentration, liposomes may potentially enhance the function of HDL via remodeling and shuttling processes, both of which depleting cholesterol content of HDL and thus making it a better acceptor of subendothelial cholesterol [34]. Hypertriglyceridemia is another condition that accounts for a substantial part of residual CV risk in statin-treated subjects, even those at target LDL-C levels [49–51]. Triglyceride-rich lipoprotein metabolism results in the formation of remnant particles with potent atherogenic and pro-inflammatory properties [52,53]. Statins have only moderate effects on HDL-C and triglycerides, which is usually considered as a drawback for this class of drugs [13]. Hence, anionic liposomes may be regarded as potential anti-dyslipidemic agents that could simultaneously affect both LDL-C and HDL-C. Boosting HDL-C along with reduction of serum triglycerides may pose a possible benefit of anionic liposomes for the management of metabolic syndrome and related disorders including non-alcoholic fatty liver disease for which hypertriglyceridemia and reduced HDL-C are important risk factors.

The overall cardioprotective effect of a formulation could be assessed by calculating the AI. In the present study, promising impact of HSPC/DSPG formulation was observed on both AI_1 and AI_2. These effects were greater than those exerted by SPC/EPG. The higher activity of HSPC/DSPG liposomes is likely to be due to their higher T_m value which makes the resulting liposome/LDL complexes more stable. Consequently, such complexes would be longer available and accessible to the kupffer cells in the liver, and hence more efficiently taken up.

5. Conclusion

In summary, the findings of this pilot study implied that empty, cholesterol-free nanoliposomal formulations comprising HSPC/DSPG (3:1) or SPC/EPG (3:1) can serve as effective, rapid-acting, inexpensive, biocompatible and biodegradable anti-dyslipidemic agents. The lipid-lowering efficacy of both formulations was comparable, though HSPC/DSPG showed a better activity in reducing atherogenic index. Future characterization of the magnitude of LDL-lowering and other lipid-modifying effects of these formulations in high-fat diet-induced hyperlipidemia as well as genetic models of hyperlipidemia (particularly heterozygous familial hyperlipidemia [54]) is warranted. Finally, the potential benefit of HSPC/DSPG and SPC/EPG nanoliposomes in regressing atheromatous lesions deserves to be evaluated.

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Conflict of interest

The authors have no competing interests to declare.

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