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Lipid based noninvasive vesicular formulation of cytarabine: Nanodeformable liposomes

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ABSTRACT

Leukemia is the common cause of death and worldwide incidence of this disease is increasing. Chemotherapy is the first choice for leukemia treatment, but the major limitations of standard therapy are its side effects and poor patient compliances. Therefore it is imperative to look for a therapeutic system with lesser side effects urgently to address the underlying causes of poor treatment outcomes. In such a scenario transdermal route for delivery of chemotherapeutic drugs could be a better alternative to provide sustained drug level, enhanced activity, self administration and better patient compliances. The present work is focus on the design of nanolipid based transdermal carrier, deformable liposomes bearing cytarabine as a model drug for effective delivery of drug with enhanced transdermal flux. Developed nanocarriers were characterized for their size, morphology, entrapment efficiency, skin penetration and irritation. It could be concluded that nanodeformable liposomes accentuated transdermal flux of cytarabine and could provide a new strategy for leukemia.

1. Introduction

Treatment strategy of leukemia is a multidisciplinary effort. The modalities of treatment include radiotherapy, chemotherapy, immunotherapy and symptomatic and supportive therapy (Syam et al., 2010). Chemotherapy is the major form of treatment for leukemia. Chemotherapeutic treatments for both acute and chronic leukemia are effective at the early stages of the disease. However, the major limitations of standard chemotherapy in the clinical setting are its side effects, such as cardiac and renal toxicities, severe myelosuppression, and patient incompliance due to IV administration, leading to poor survival outcomes. Therefore it is imperative to look for a novel therapeutic system with lesser side effects urgently to address the underlying causes of poor treatment outcomes associated with conventional therapy (Rastogi et al., 2014). In such a scenario transdermal route for delivering chemotherapeutic drugs could be the better alternative that could provide sustained drug level, enhanced activity, self administration and better patient compliances (Attianese et al., 2011).

Although transdermal delivery of bioactives can offer many advantages including avoidance of first pass metabolism, lower fluctuation in plasma drug level and good patient compliance (Unchio et al., 2014), a major obstacle in transdermal drug delivery is the low penetration rate of drug molecule through the skin (Vanden Bergh et al., 1999). The stratum corneum (SC) provides the principal barrier to percutaneously applied molecules (Cevc, 2004). Most of the anticancer drugs are administered as IV infusion (water soluble), which also poses a challenge for transdermal penetration due to its hydrophilic nature (Raj et al., 2016). There has been a wide interest in exploring new techniques to increase the absorption of such molecules through the skin. Transdermal delivery of bioactives by lipid vesicles has evoked a considerable attention (Elsayed et al., 2007). Mezei and Gulasekharam, 1980 first reported the efficacy of liposomes for skin delivery. Recently it became evident that liposomes are of little or no value carriers as transdermal delivery. They do not penetrate to deeper layers, but rather remain confined to the upper layer of the SC (Touito et al., 2000). The properties of vesicles are modulated by changing the chemical composition resulting in an altered structure and physical state of vesicles. Depending on the composition, bilayers of vesicles are in either a liquid crystalline or gel state. Several studies investigated whether the physical state of vesicles is essential for their mode of action (Vanden Bergh et al., 1999). Intensive research over the past 25 years has led to ultradeformable (elastic or flexible) liposomes that have been termed as transfersomes (Cevc and Blume, 1992). Deformable liposomes were reported to penetrate into the skin along with therapeutic entity, while conventional liposomes were reported to have a localized effect or rarely transdermal penetration. Deformable liposomes are based on phospholipid and a single chain surfactant (an edge activator i.e., sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60 and Tween 80). Edge activator having a high radius of curvature destabilizes lipid bilayers of the vesicles and enhanced deformability of bilayers (Cevc, 1996, 2003). Because deformable liposomes have surfactant, they have better hydration and rheology, which are responsible for their skin penetrability (Ahad et al., 2012). Elastic liposomes self-modify

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their form and passing through the pore much lower than their own diameter. Shape variation is only possible because the surfactant moves all the way through the bilayer into the zones of major tension. The amount of lipid determines the extension of transfersome penetration (Cevc and Vierl, 2010; Ascenso et al., 2014). Similar mechanisms are proposed for the action of transfersome as liposomes. First, vesicles act as carrier and intact carrier enters the SC carrying drug molecule. Second, vesicles act as penetration enhancers and modify SC intercellular lipid lamellae and thus facilitate the penetration of free drug molecules into and across the SC (Elsayed et al., 2007; Bavarsad et al., 2012). Additionally elastic liposomes can effectively protect the drug against undesired skin clearance into cutaneous blood vessels and are capable of retaining the drug long enough on, in and below the skin barrier (Cevc et al., 2008). Several studies accounted that deformable liposomes were able to penetrate into intact skin (Paul et al., 1995, 1998; Guo et al., 2000a,b; Cevc et al., 1998; Cevc and Blume, 2001, 2003, 2004; Jain et al., 2005, 2006; Garg et al., 2006; Song and Kim, 2006; Dubey et al., 2006; Mishra et al., 2007; Liu et al., 2013; Maheshwari et al., 2012). The present work focuses on the design of nanolipid based transdermal carrier, deformable liposomes bearing cytarabine as a water soluble model drug for effective delivery with lower side effect and better patient compliance. Developed nanocarriers were characterized for their size, morphology, entrapment efficiency, skin penetration and in vivo skin irritation effect.

2. Methods

2.1. Materials

Soya phosphatidylcholine (SPC) and sodium deoxy cholate (SDC) were purchased from Sigma Aldrich (St. Louis, MO USA). Triton X-100 and Nile red were purchased from HiMedia (Mumbai, India). Cytarabine was purchased from TCI chemical (Chennai, India). All solvents were of analytical grade and double distilled water was used wherever required.

2.2. Preparation and characterization of cytarabine nanodeformable liposomes

Nanodeformable liposomes were prepared by an extrusion method (Dubey et al., 2006) with slight modification. Briefly, SPC (1 wt.%) and SDC (at different concentrations) were taken in a clean dry round bottom flask and dissolved in ethanol. Solvents were removed by a rotary evaporation method above the lipid transition temperature (Rotary Evaporator, Ultra Lab, New Delhi). Traces of ethanol from the deposited lipid film were removed under vacuum overnight. Lipid film was then hydrated with PBS pH 7.4 containing cytarabine by rotation (at 60 rpm, 1 h) above the lipid transition temperature. Carriers were then sonicated by a bath sonicator (Loba Life, Mumbai, India) and extruded manually through a 200 nm polycarbonate membrane filter (Millipore USA). Similarly Nile red loaded nanodeformable liposomes were prepared. Cytarabine nanodeformable liposomal formulations were optimized using a factorial design. A three factor three level simple factorial design was applied to study the effect of independent variables on dependent variables as depicted in Table 1. According to experimental design 29 formulations were run as shown in Table 2. Conventional liposomes were also prepared by the above-described method using 1% SPC and 0.3% cholesterol.

Table 1
Variables in factorial design for preparation of cytarabine nanodeformable liposomes.

Factor	Level used		
	Low (−1)	Medium (0)	High (+1)
Independent variables			
X_1 = sodium deoxycholate (wt.%)	0.16	0.18	0.20
X_2 = cytarabine (wt.%)	0.10	0.20	0.30
X_3 = sonication time (in min)	5	10	15
Dependent variables			
Y_1 = vesicle size (nm)			
Y_2 = polydispersity index (PDI)			
Y_3 = entrapment efficiency (EE) %			

2.3. Particle size and zeta potential

The average size of vesicles and zeta potential was measured by a light scattering method using a Malvern Zetasizer (Malvern, UK) at 25 ± 1 °C. Refractive index was set to 1.33 and samples appropriately diluted with PBS pH 7.4 before measurement. Experiments were carried out in triplicate.

2.4. Vesicular shape

Transmission electron microscopy (TEM; Philips CM12 Electron Microscope, Netherlands) was used to visualize the shape of developed nanodeformable liposomes. Samples were dried on copper grid and negatively stained with uranyl acetate. After 1 h grids were viewed under a microscope at an accelerating voltage of 200 kV.

2.5. Entrapment efficiency

Entrapment efficiency was determined using a size exclusion method. Nanodeformable liposomes and liposomes were centrifuged using a Sephadex G-100 minicolumn. The separation was repeated three times using a fresh syringe in order to remove traces of free drug. The purified nanodeformable liposomes and liposomes were lysed in 1% w/w Triton X-100. The final clear solution was analyzed for cytarabine at 280 nm using HPLC (Bhatnagar et al., 2012).

Table 2
Responses observed in factorial design of cytarabine nanodeformable liposomes.

Run	Independent variables			Dependent variables		
	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	−1	−1	−1	178 ± 8	0.21 ± 0.02	46.32 ± 4.56
2	0	−1	−1	136 ± 9	0.20 ± 0.01	45.71 ± 3.22
3	+1	−1	−1	128 ± 4	0.18 ± 0.03	42.36 ± 7.48
4	−1	0	−1	148 ± 10	0.15 ± 0.04	48.92 ± 4.65
5	0	0	−1	145 ± 11	0.15 ± 0.05	51.84 ± 2.28
6	+1	0	−1	139 ± 12	0.12 ± 0.02	50.27 ± 3.42
7	−1	+1	−1	184 ± 2	0.26 ± 0.05	33.83 ± 1.44
8	0	+1	−1	167 ± 6	0.25 ± 0.02	33.65 ± 5.32
9	+1	+1	−1	158 ± 9	0.24 ± 0.03	30.76 ± 8.58
10	−1	−1	0	137 ± 8	0.12 ± 0.03	48.52 ± 3.62
11	0	−1	0	132 ± 10	0.10 ± 0.05	45.32 ± 7.23
12	+1	−1	0	128 ± 4	0.11 ± 0.04	34.28 ± 2.19
13	−1	0	0	141 ± 8	0.13 ± 0.02	51.15 ± 2.57
14	0	0	0	135 ± 11	0.12 ± 0.01	48.76 ± 4.72
15	+1	0	0	95 ± 10	0.08 ± 0.05	40.56 ± 8.15
16	−1	+1	0	205 ± 9	0.24 ± 0.04	34.82 ± 6.49
17	0	+1	0	174 ± 3	0.18 ± 0.02	33.37 ± 1.45
18	+1	+1	0	168 ± 6	0.18 ± 0.01	36.68 ± 5.32
19	−1	−1	+1	150 ± 5	0.12 ± 0.08	50.35 ± 2.76
20	0	−1	+1	143 ± 6	0.11 ± 0.03	50.45 ± 1.45
21	+1	−1	+1	120 ± 12	0.10 ± 0.04	46.56 ± 2.81
22	−1	0	+1	136 ± 8	0.12 ± 0.03	53.62 ± 3.66
23	0	0	+1	114 ± 7	0.08 ± 0.04	55.26 ± 4.72
24	+1	0	+1	78 ± 5	0.09 ± 0.06	39.53 ± 1.56
25	−1	+1	+1	158 ± 5	0.16 ± 0.03	33.32 ± 3.27
26	0	+1	+1	172 ± 9	0.17 ± 0.02	31.22 ± 7.46
27	+1	+1	+1	185 ± 6	0.18 ± 0.05	28.87 ± 5.23