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Lung surfactant as a drug delivery system

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Abstract

Lung surfactant is a complex mixture of mainly phospholipids and proteins. The composition leads to a unique spreading effect of the surfactant as well as spontaneous vesicle formation, which may be favourable characteristics of a drug delivery system for pulmonary delivery.

The aim of study was to investigate the potential use of the surfactant extract, HL10 (LeoPharma, DK) as a drug delivery system. Studies involved incorporation of hydrophilic- and amphipathic model drugs (sucrose and acylated peptides) into HL10 and elucidation of the influence of surfactant proteins on the HL10 behaviour.

Results showed that HL10 vesicles did not retain sucrose indicating formation of leaky vesicles. Studying the influence of surfactant proteins on release from DPPC-liposomes showed tendencies toward a protein-induced release. Hence, the surfactant proteins may influence the membrane lipid packing and characteristics resulting in leakiness of the membranes. Incorporation of acylated peptides into HL10 depended on the chain length rendering a successful incorporation of the peptide acylated with C_{14}-acyl chains.

This study suggests that HL10 may be a promising drug delivery system for the pulmonary delivery of amphipathic drug substances, e.g. therapeutically active acylated peptides (e.g. acylated insulin).

Keywords: Drug delivery; Lung surfactant; HL10

1. Introduction

Lung surfactant is a complex mixture of lipids and proteins consisting of 78–90% phospholipid, 5–10% protein and 4–10% neutral lipid (Hawgood, 1991; Notter, 2000). Phosphatidylcholine (PC) accounts for 70–80% of total lipid and is obviously the most abundant component of lung surfactant. About 50–70% of the PC correspond to dipalmitoylphosphatidylcholine (DPPC) but also other saturated and unsaturated PC's are present as well as other phospholipid classes (Rooney et al., 1994; Notter, 2000). The neutral lipids are mainly accounted for by cholesterol. Four known surfactant proteins exist, i.e. SP-A, SP-B, SP-C, SP-D (Rooney et al., 1994; Notter, 2000).

LeoPharma, Denmark, has produced an exogenous lung surfactant product, HL10, which is extracted from pig lungs and differs from the above mentioned composition containing only hydrophobic surfactant proteins, SP-B and SP-C. Besides this, the HL10 content of cholesterol is lower, i.e. approx. 2% compared to 10% (Hawgood, 1991; Notter, 2000).

Lung surfactant is produced by the alveolar type II cells and it is secreted as phospholipid aggregates at the surface of the alveolar membrane. At the air–water interface lung surfactant adsorb from the aggregates and form a film, which acts to moderate surface tension during breathing. The different components of lung surfactant interact to determine the overall behaviour of the surfactant. The effect of lowering surface tension is mainly performed by the disaturated DPPC while the fluid phospholipids, the neutral lipids and the surfactant proteins greatly enhance the film respreading as well as increase adsorption of phospholipids at the air–water interface (Rooney et al., 1994; Notter, 2000).

Due to the general spreading effect of lung surfactant resulting in effective distribution of surfactant at the lung surface, combined with the ability of lung surfactant to form lamellar structures, i.e. liposomes (Larsson, 2002), it was hypothesized that HL10 may be a potential delivery system for drugs with limited pulmonary bioavailability and/or for drugs with targets in the lungs. Hence, drug molecules incorpo-

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rated into or associated with HL10 liposomes may have optimal possibilities of being exposed to and absorbed into the lung epithelium, which may enable increased drug concentrations at the target site as well as decreased systemic side effects.

Hence the aim of this study was to investigate the use of HL10 as a drug delivery system for drugs with different characteristics by elucidating the potential of HL10 to incorporate small water-soluble molecules, e.g. sucrose, and to bind amphipathic molecules, e.g. acylated peptides.

2. Methods

HL10 liposome suspensions (1–80% w/w) were produced by mixing HL10 with HEPES-buffer including isotonic ^{14}C-sucrose concentrations. Non-encapsulated sucrose was separated from the liposomes by ultracentrifugation or by elution through a Sephadex 50 column. For determining the percentage of encapsulated sucrose the ^{14}C-radioactivity of the pellet and the lipid fraction, respectively, was measured by liquid scintillation counting.

Unilamellar 100 nm calcein-DPPC-liposomes were produced as earlier described (Davidsen et al., 2002). The influence of SP-B and SP-C was investigated by incubation of calcein-liposomes (400 μM) for up to 2 h in the concentrations of 0.5–10% of the lipid concentration. The degree of calcein release was determined by measuring the difference in calcein fluorescence (excitation wavelength: 492 nm, emission wavelength: 520 nm) before and after the addition of the proteins.

HL10-binding of the model peptide, HWAHPGGHHA-NH_2, and the model peptide acylated with C_8- and C_{14}-chains, respectively, was investigated by measuring the change in tryptophan fluorescence at 360 nm from the peptides caused by the addition of HL10-suspensions up to a lipid–peptide ratio of 105:1. The dissociation constants were estimated according to the method described by De Kroon et al. (1990). Corrections of fluorescence were made due to HL10-fluorescence, dilution- and inner-filter effects.

3. Results and discussion

It was investigated whether it was possible to incorporate water-soluble substances, i.e. sucrose (MW 342), into HL10 suspensions of varying lipid concentrations. The HL10 phase-diagram (Larsson, 2002) showed that HL10-concentrations above 45% in water resulted in appearance of only lamellar structures. Below 45% the lamellar structures were mixed with water. By ultracentrifugation of the ^{14}C-sucrose-containing HL10-suspensions revealing separation of pellet and supernatant suggested no clear indication of sucrose incorporation (Table 1). Subsequent, elution of sucrose-containing HL10-suspensions through a Sephadex column showed no co-elution of HL10 and sucrose (Fig. 1). However, sucrose from HL10-suspensions and sucrose from a lipid-free sucrose-solution was detected in the same elution fractions (Fig. 1) confirming the negative encapsulation results. Thus, it was concluded that HL10 may form

Table 1
Incorporation of sucrose into HL10 liposomes

HL10 concentration (%)	Percentage sucrose in supernatant	Percentage sucrose in pellet
1	87.1	12.9
	82.8	17.2
5	78.1	21.9
	57.6	42.4
20	80.6	19.4
	85.6	14.4
80	92.9	7.1
	91.4	8.6

The low and varying percent of sucrose in the HL10-pellet suggested no clear indication of sucrose-incorporation.

open structures, which do not incorporate sucrose. A potential hypothesis was that the bilayer penetrating SP-B and SP-C may influence the leakiness of the HL10-structures. The elucidate this hypothesis SP-B and SP-C mediated release of calcein from DPPC-liposomes was studied. As HL10 is mainly composed of DPPC a potential interaction between SP's and liposome-membranes resulting in calcein-release may indicate an influence of SP's on the leakiness of HL10 structures. Only the highest concentration of SP-B seemed to have a weak effect on the release. The degree of release did not increase during the period (Table 2a) indicating no time-dependency. The effect of SP-C on release seemed more pronounced, corresponding to a maximum percentage release of 35–36% (Table 2b). The pronounced effect of SP-C was expected as it is supposed to integrate more effectively into the bilayer. The bilayer integration of SP-C has been shown to involve the hydrophobic region of the bilayer (Weaver and Whitsett, 1991) in contrast to SP-B, which is primarily located in the hydrophilic headgroup-region (Weaver and Whitsett, 1991). As the HL10 content of SP's is approximately up to 2% of the lipid content, the SP's may be able to create small discontinuities in the bilayers resulting in leaky vesicles, thereby preventing liposomal retention of small water-soluble molecules, like sucrose. Hence, the incorporation of larger water-soluble molecules, such as insulin, may result in a more effective incorporation. In addition, the lower cholesterol content of HL10 compared to other lung surfactant preparations (Hawgood, 1991; Notter, 2000) may cause formation of bilayers with a more loose lipid-packing favouring

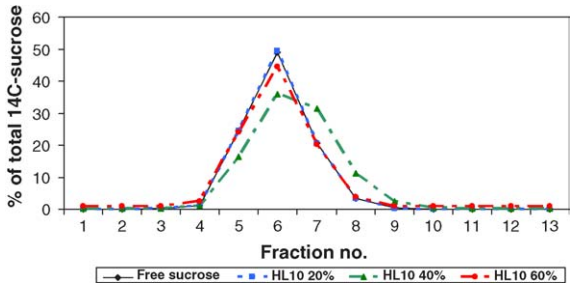

Fig. 1. Non-encapsulated sucrose was removed from HL10-liposomes by elution through a sephadex 50 column. A representative elution-sequence showed no co-elution of HL10 and sucrose indicating no sucrose-encapsulation.

Table 2
Calcein-release from DPPC-liposomes induced by SP-B (a) and SP-C (b), respectively

Incubation time	SP-B 0.5%	SP-B 1%	SP-B 2%	SP-B 10%
(a)				
5 *min*(%)	4.4	3.3	5.4	7.1 9.3
15 *min*(%)	4.9	5.1	6.6	6.2 5.9
45 *min*(%)	0	1.4	3.3	8.4 9.2
120 *min*(%)	0	0.8	2.5	7.4 8.1

Incubation time	SP-C 1%	SP-C 2%	SP-C 10%
(b)			
5 *min*(%)	0	12.7 9.7	21.8 18.5
15 *min*(%)	0	16.1 13.7	28.1 25.3
45 *min*(%)	0	19.0 18.2	29.8 27.6
120 *min*(%)	0	21.0 18.4	32.7 35.6

The observed release-% indicated a weak release-mediating effect of SP-B and a more pronounced effect of SP-C.

release of encapsulated compounds. This may seem obvious as it is well-known that cholesterol increase liposomal stability due to an increase in membrane packing (Shaw and Dingle, 1980).

Binding of acylated peptides to HL10 was studied by monitoring the changes in tryptophan fluorescence upon addition of HL10 to the peptide solution (De Kroon et al., 1990; Jacobs and White, 1986). A pronounced increase in fluorescence was observed when adding HL10 to the C_{14}-acylated peptide solution (Fig. 2). However, only a negligible fluorescence increase was observed due to the presence of the C_8-peptide and no increase by the addition of the non-acylated model peptide (Fig. 2). These results suggested incorporation of the C_{14}-peptide into HL10 as the increase in fluorescence results from an increase in the apolar surroundings of the peptide (De Kroon et al., 1990). Dissociation constant of the HL10-C_{14}-peptide association was estimated to 0.60 mM at 20 °C. These results confirmed earlier observed results. Pedersen et al. (2000) estimated the dissociation constant of the association between the C_{14}-peptide and DPPC-liposomes to 0.64 mM at 20 °C. This may seem probable as DPPC constitute the bulk of HL10. They also observed that the C_{14}-model peptide associated more effectively with DPPC-liposomes compared to the C_8- and C_2-acylated peptide (Pedersen et al., 2000). Hence, a chain-length depending lipid incorporation was suggested. It has been shown that the binding of myristic acid was too weak to fully anchor a protein to a cell membrane (Peitzsch and McLaughlin, 1993). Thus, increasing the chain length to C_{16} may render increased membrane-association (Ali et al., 2000). At 20 °C DPPC and HL10 are in gel-phase, however the peptide interaction with DPPC was shown to increase above the phase transition temperature (T_m) (Pedersen et al., 2000). Hence, the dissociation constant of the C_{14}-peptide association to DPPC-liposomes decreased to 0.28 mM in the fluid phase indicating a stronger association to the membranes. Hence, the association of C_{14}-peptide to HL10 is expected to increase above the T_m of HL10 (37 °C).

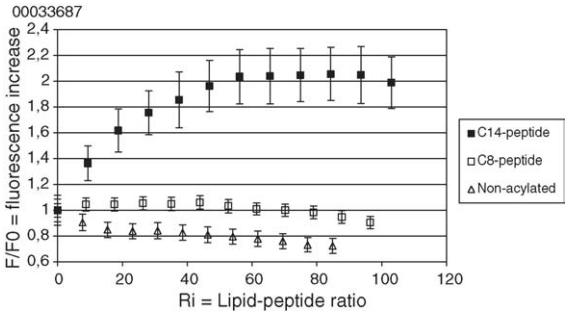

Fig. 2. Binding of acylated-modelpeptides to HL10. Increase in fluorescence by HL10 addition indicated a marked binding of C_{14}-peptide compared to C_8- and non-acylated peptide ($n = 3$, mean ± S.D.).

In conclusion this study suggests that HL10 may be a promising drug delivery system for pulmonary delivery of amphipathic drugs such as therapeutically active acylated drugs, e.g. acylated insulin, which is a long acting insulin analog (Kurtzhals et al., 1996). Besides this, insulin uptake by type II cells in vitro showed enhanced absorption in the presence of lung lavage fluid compared to buffer indicating an absorption-enhancing effect of lung lavage fluid (Mitra et al., 2001).

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