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Note

Pulmonary administration of IgG loaded liposomes for passive immunoprophylaxy

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Abstract

Local passive immunoprophylaxy has been used in pulmonary infectious diseases successfully. However, the short immunoglobulins half-life in the lungs limits the duration of their action. The aim of the present study was to evaluate the efficiency of human polyvalent intravenous immunoglobulins (IVIG) when protected after encapsulation within EPC: DPPG liposomes by dehydration/rehydration. Two IVIG concentrations were chosen: 10 and 1 mg/ml for further studies in mice infected by influenza A. For the highest concentration (10 mg/ml), IVIG loaded liposomes did not significantly differ from IVIG/unloaded liposomes mixture with around 45% association yield. For the lowest concentration (1 mg/ml), two thirds of the IVIG associated were found inside the vesicles. In vivo, IVIG administered intranasally at 10 mg/ml (500 μg per mouse) 4 days before the infection led to 100% survival whatever the formulation. When administered at a lower dose (1 mg/ml—50 μg per mouse) 2 days before the challenge, loaded liposomes were found less efficient than free IVIG while unloaded liposomes showed a slight aspecific immunoprotection. Gastrointestinal clearance must be responsible for a major loss of liposomes compared to IVIG solution because of a higher viscosity of the formulation. Discrepancies with the literature are discussed.

Keywords: Passive immunoprophylaxy; IVIG; Liposomes; Influenza A

In the beginning of the 20th century, the use of antibody constituted the first specific treatment for infectious diseases. Immunotherapy was thereafter supplanted by antibiotics and vaccination. Nevertheless, immunotherapy has recently regained interest, especially in the field of warfare agents (Casadevall, 2002).

Several studies (Mazanec et al., 1992; Ramisse et al., 1996, 1998; Weltzin and Monath, 1999) showed that pulmonary administrations of immunoglobulins (Ig) can cure and protect animals and humans against viral and bacterial pneumonia, with reduced doses compared to intravenous administrations. However, the duration of the action still needs to be improved, in order to decrease the administration rates. The best way to increase the Ig half-life in the lungs is likely to protect them by encapsulation in an appropriate controlled release system (Zeng et al., 1995).

Both liposomes (Liu et al., 1993; Wong et al., 1994) and microparticles (Armstrong et al., 1996; Bot et al., 2000) have already been proposed for pulmonary administrations of various molecules including Ig, with two possible goals: local or systemic delivery. Some toxicity has been reported with the use of polymers, even biodegradable ones, and more precisely

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inflammation (Armstrong et al., 1996). Liposomes, on the contrary, might be formulated with endogenous surfactant components, hence well tolerated and innocuous (Schreier et al., 1993), which makes them largely investigated for lung delivery of drugs.

We have chosen here liposomes to encapsulate human polyvalent intravenous Ig (IVIG) and evaluated the benefit of this new formulation in mice infected with influenza A. Wong et al. (1994) previously showed encouraging data with a similar model, and especially that negatively charged liposomes must be preferred over cationic ones. Indeed, if the presence of charged lipids improves both the stability and the encapsulation ability of vesicles (Vemuri and Rhodes, 1995), the IgG delivered to the lungs using negatively charged liposomes versus positive ones were two times more efficiently retained, even 48 hours post-liposome administration.

Thus, the aim of this work was to prolong the IVIG residence time in the lower respiratory tract by the use of negatively charged liposomes and to evaluate their prophylactic efficiency in mice infected by influenza A virus.

EPC:DPPG (molar ratio 7:1) liposomes were obtained after lipid film hydration by TRIS buffer and extrusion. IVIG were encapsulated by the rehydration method after a 24 h freeze-drying. It's an appropriate method for fragile drugs like proteins allowing high entrapment efficiency in mild and solvent-free conditions (Kirby and Gregoriadis, 1984).

The liposomes size and zeta potential were measured by quasielastic light scattering and electrophoretic mobility determination, respectively. The IVIG to liposomes association yield was determined after ultracentrifugation (90,000*g* at 4 °C during 1 h 30 min) and IVIG assay in the supernatant according to the Lowry method (applied to bicinchoninic acid).

$$\%\,\text{association} = \frac{[\text{Ig}]_{\text{total}} - [\text{Ig}]_{\text{supernatant}}}{[\text{Ig}]_{\text{total}}}$$

Two IVIG concentrations were chosen: 10 and 1 mg/ml in the total liposomal suspension. The association yield obtained when IVIG were simply mixed with unloaded liposomes was determined as control.

As seen in Table 1, in the presence of a high Ig concentration (10 mg/ml), both liposome size and P.I. increased whereas zeta potential was reduced. This could be the consequence of IVIG adsorption on the liposome surface, as confirmed by the high association yield found when IVIG were mixed with unloaded liposomes: 41.5%. For Ig concentration 10 times less (1 mg/ml), the liposome size did not change compared with the unloaded batch and the surface charge only slightly decreased. This could be correlated with a lower surface adsorption rate than previously (14.5% versus 41.5% for the mixture Ig + unloaded liposomes).

Nevertheless, the rather high association yield found (38.5%) for 1 mg/ml IVIG loaded liposomes suggests that around two thirds of the Ig associated were encapsulated inside the vesicles. In conclusion, the comportment of IVIG during the liposome preparation appeared to vary along with the concentration, with some destabilisation or fusion effect at high Ig concentrations (10 mg/ml). Further studies are now in progress to investigate this point.

The overall formulations have been investigated in vivo, in mice infected by influenza virus. Female mice BALB/c 6 weeks old, specific pathogen-free, were infected by influenza A Scotland H3N2 through nasal

Table 1
Characteristics of unloaded and IVIG loaded liposomes, compared to the mixture IVIG + unloaded liposomes

IVIG concentration	Liposome batches	Size ± S.D. (nm)	Polydispersity index (P.I.)	Zeta potential ± S.D. (mV)	% Association
10 mg/ml	Unloaded liposomes	312 ± 128	0.556	−67.5 ± 0.3	–
	IVIG loaded liposomes	466 ± 205	0.843	−26.9 ± 0.2	47.5
	IVIG + unloaded liposomes	448 ± 194	0.742	−20.4 ± 0.4	41.5
1 mg/ml	Unloaded liposomes	278 ± 108	0.383	−60.0 ± 0.1	–
	IVIG loaded liposomes	276 ± 111	0.489	−55.9 ± 0.4	38.5 ± 3.5
	IVIG + unloaded liposomes	254 ± 105	0.524	−52.2 ± 1.5	14.5 ± 2.1

Unloaded liposomes were obtained by the rehydration method in the same condition as IVIG loaded liposomes.

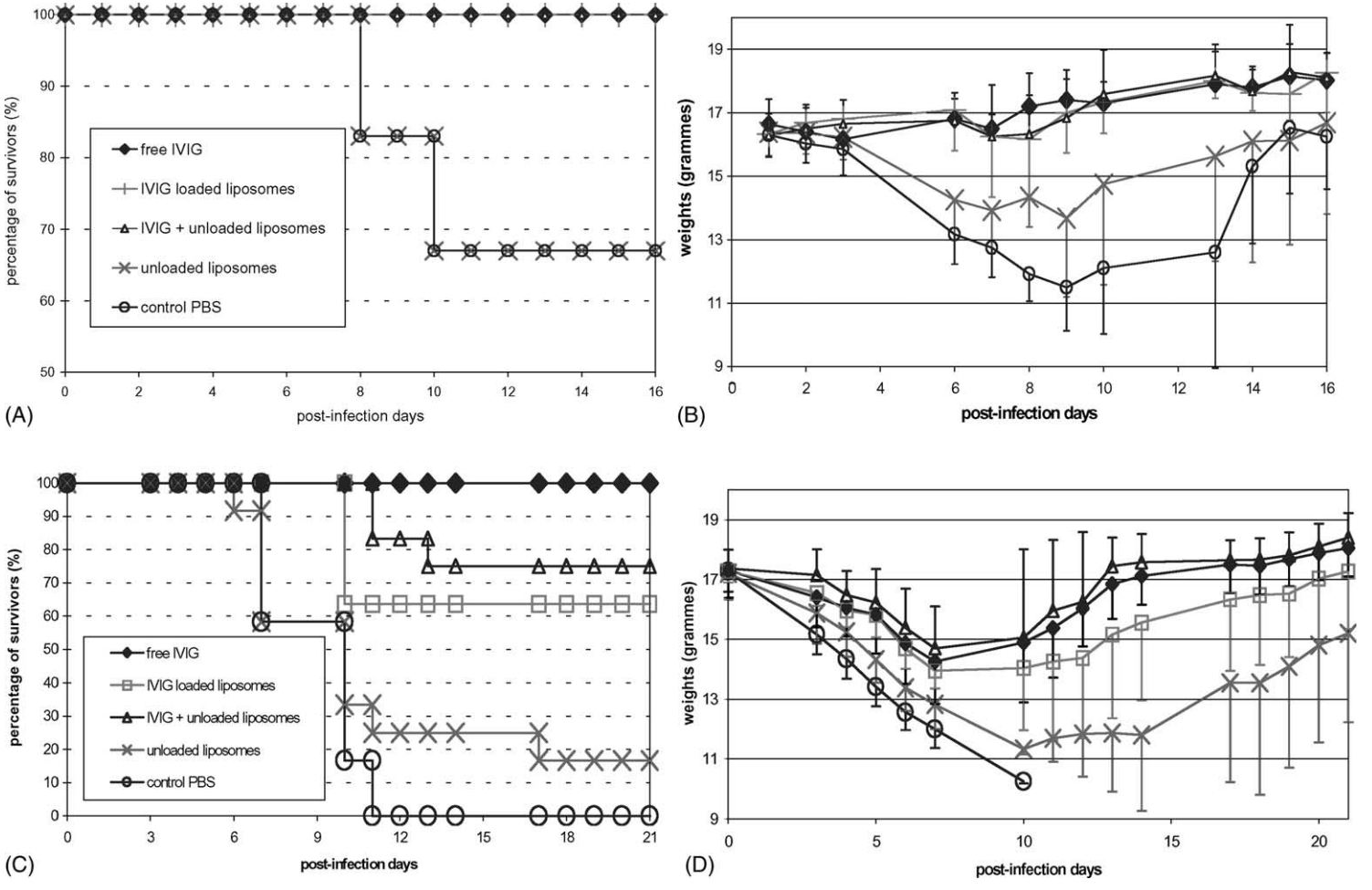

Fig. 1. Mice mortality (A, C) and weights (B, D) as a function of time following administration of IVIG formulations at 10 mg/ml (A, B) and 1 mg/ml (C, D).

instillation of 50 μl of a viral suspension leading to 100% mortality in 14 days.

Wong et al. (1994) previously showed that the intranasal and intratracheal administration routes were equally effective for the delivery of IgG loaded liposomes, with 90% of the IgG administered being localised in the lungs 2 h after the administration. Subsequently, we chose the intranasal administration route because of its practical advantages.

In a first experiment, mice received 4 days before the infection 50 μl of 10 mg/ml IVIG preparations (Ig solution, Ig loaded liposomes, Ig solution mixed with unloaded liposomes). PBS buffer and unloaded liposomes were used as controls. The animals ($n = 6$) weight and death rate have been followed along time as disease indicators.

As seen in Fig 1A and B, no difference could be observed between IVIG free and encapsulated within liposomes or mixed with liposomes, neither in mortality nor in weight curves. It might be due to the fact that the IVIG dose (500 μg) was very high in respect to the virus infected dose and led to 100% protection in every case. Hence, in a second experiment, we decreased the IVIG dose to 50 μg per mouse. In this case, mice ($n = 10$) received intranasally 50 μl of 1 mg/ml IVIG preparations 2 days before the infection. As shown in Fig. 1C and D, IVIG solution led to a total protection whereas IVIG associated to liposomes (loaded into or mixed with) only protected about 60 or 70% mice, respectively, even if no significant differences could be evidenced on weight curves. In the same time, unloaded liposomes showed a tendency to increase the resistance of mice to the infection (20% survival).

The efficient delivery of drugs to the respiratory tract is hindered by several barriers. These obstacles are first the geometry of the airways, but also clearance mechanisms due to the presence of surfactant, enzymes and alveolar macrophages (Smith, 1997).

Actually, previous study using labelled liposomes administered intranasally in mice showed that only 50% liposomes remained in the lungs after 24 h, the other 50% being principally cleared in the gastrointestinal tract (Vidgren et al., 1995). In our case, because of a difference in the samples viscosity free IVIG might have been more efficiently retained than liposomes which could have been eliminated by mucociliary escalator and finally swallowed.

Furthermore, macrophages have been shown to phagocyte liposomes and especially negatively charged ones, hence leading to the stimulation of the immune response (De Haan et al., 1995). The uptake of unloaded liposomes by alveolar macrophages in our study could explain the aspecific immuno-stimulation we reported in vivo.

IVIG have to be delivered in the alveolar spaces to neutralise influenza virus before it infects the alveolar cells (Sidwell, 1999). All the possible liposomes clearance mechanisms will lead to lower Ig concentration at the target site, hence leading to lower efficiencies. As already mentioned, Wong et al. (1994) obtained positive results in a similar model, but they used a specific anti-influenza IgG. IVIG are polyclonal with a comparatively low titre of anti-influenza Ig (Ramisse et al., 1998). That's probably why the material loss in our case significantly affected the efficiency of Ig loaded liposomes because of a lower dose.

In conclusion, the intranasal route was found to be unappropriate for the efficient delivery of liposomal formulations low concentrated in Ig, and their efficiency will have to be investigated by the endotracheal route, minimizing the clearance effects while being non traumatic for the mice. In the same time, physico-chemical studies will be performed to elucidate the role of Ig concentration on vesicles fusion, in relation to the encapsulation procedure by the rehydration method.

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