

Physicochemical characteristics of a liposomal ciclosporin A formulation pre and post nebulisation in a customized eFlow[®] electronic nebulizer

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Summary

PARI has developed an aqueous 0.4% liposomal ciclosporin A formulation. Ciclosporin A loaded liposomes were prepared by high pressure homogenisation and subsequently lyophilised using a sugar as lyoprotectant. It was our objective to investigate potential effects on particle size and structure of the liposomes caused by lyophilisation, reconstitution and nebulisation. Photon correlation spectroscopy showed a vesicle size of about 50nm for the lyophilised and reconstituted formulation, and was comparable to non-lyophilised formulations. After aerosolisation by a customised eFlow[®] electronic nebuliser the particle size increased up to 110 nm. Cryo-TEM revealed small vesicles with a unilamellar membrane for the lyophilised, reconstituted and aerosolised product. Data show, that the characteristics of CsA-liposomes are only slightly affected by lyophilisation, reconstitution and aerosolisation via a customised eFlow[®] electronic nebulizer.

Introduction

Ciclosporin A (CsA) is an immunosuppressive drug to reduce rejection of transplanted organs, such as the lung. Since systemic drug administration is associated with severe side effects as obvious from information in the leaflet, Physician Desk Reference and general clinical literature other routes of administration for drug targeting to the site where the drug is needed would be most desirable. Reports on positive therapeutic effects upon nebulisation of CsA dissolved in ethanol and propylenglycol [1,2,3] indicated that topical administration via nebulisation into the airways of lung transplanted patients may be advantageous. However, administration of CsA dissolved in organic solvents was irritating and poorly tolerated, and required pre-treatment with nebulised Lidocaine being an anaesthetic. In addition, nebulisation lasted up to one hour and was burdensome for the patients [3]. Thus, the development of a well tolerable inhalation formulation being deliverable into the lungs in a much shorter period of time was most desirable. Hence, various CsA inhalation formulations were developed with the objective to design a formulation having the capability to penetrate from the airways into the lung tissue to prevent or slow down the induction of Bronchiolitis obliterans (BO) being associated with host allograft rejection.

In addition to the characterisation of formulations regarding their physicochemical properties and aerosolisation performance it is necessary to investigate these formulations with respect to their cell tolerability and permeation characteristics through epithelial cells. Various formulations were tested in Calu-3 cells [4] in comparison with CsA dissolved in propylenglycol (PG). Not surprisingly, a Calu-3 cell damage was found when PG was administered in concentrations > 10% [5]. It was found, that an aqueous 0.4% liposomal ciclosporin A (L-CsA) formulation showed good cell tolerability and was non toxic when tested for 6 months in rats. In addition, a slow release feature could be observed for L-CsA compared to CsA dissolved in propylenglycol upon incubation of human lung cell homogenates [6]. Furthermore, a perfused rabbit lung model was selected to investigate the distribution profiles in the perfusate, bronchoalveolar lavage and lung tissue upon nebulisation of L-CsA compared to a commercial infusion solution.

L-CsA can be favourable nebulised via a customised eFlow[®] electronic nebuliser [7] generating an aerosol mist of defined droplet sizes via a perforated vibrating membrane principle [8]. Based on breath simulation tests L-CsA could be nebulised via a customised eFlow[®] electronic nebulizer very efficiently achieving a respirable fraction < 5 µm of 87.4 % and < 3.3µm of 58.9 % with an output rate of 345 mg/min [7]. Data from a deposition and pharmacokinetic study in single and double lung transplanted patients showed a total lung deposition efficacy of 40%, whereby 50% of the deposited drug was found in the periphery of the lung [9].

It is known from the literature, that shelf life stability of liposomal formulations is an issue. In order to improve and prolong the shelf life of L-CsA we have developed a lyophilised formulation to achieve a satisfactory long-term stability. The sensitivity of liposomes on shear stress as it may occur during the nebulisation process is also a known phenomenon [10] and was therefore investigated, too. It was our objective to assess if the size and drug load of the liposomes may be affected due to the lyophilisation

process, the subsequent dissolution with a diluent and by nebulisation. Thus, this study was conducted to investigate whether lyophilisation, redispersion and nebulisation may affect the structure, particle size, and integrity of CsA loaded unilamellar liposomes.

Materials and Methods

Ciclosporin A loaded liposomes were prepared by high pressure homogenisation using a Microfluidics 110EH followed by sterile filtration of the liquid formulation. The formulation was lyophilised using a sugar as lyoprotectant to preserve the liposomal structure. Aerosolisation was performed utilising a customized eFlow[®] 30XL electronic nebulizer configuration. This configuration is fitted with an atomising membrane generating droplets of about 3 μm . The size of the liposomes was measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 HS. The structure of the liposomes was characterised by cryo transmission electron microscopy (Cryo-TEM) utilising a Philips-CM120.



Fig. 1: Appearance of customised eFlow[®] electronic nebulizer configurations. The base unit is shown in the left side of the picture and the handset configurations at the right side of the picture. Extension of the mixing chamber from about 48 ml (L) to 94 ml (XL) increases the delivered dose from 69.8% to 76.4% when using an atomising head class 30 and reduces in parallel the expelled fraction from 9.3% to 4.3% [7].

Results

Size and structure of liposomes before nebulisation using Cryo-TEM visualisation

Cryo-TEM showed that the untreated liposomal formulation (Fig 2 left hand side) contains mainly very small vesicles with an unilamellar membrane in a size range of 10 to 30 nm. Also larger spherical unilamellar vesicles with a diameter of ~ 60 to 120 nm occur regularly. Only very few multilamellar vesicles can be seen.

After lyophilisation and reconstitution (Fig 2 right hand side) a similar image regarding vesicle size and shape was obtained. The reconstituted sample still consists of mainly unilamellar vesicles sized 10 to 90 nm. However, the number of very small vesicles (<20 nm) seems to be decreased, and the average size of vesicles marginally increased.

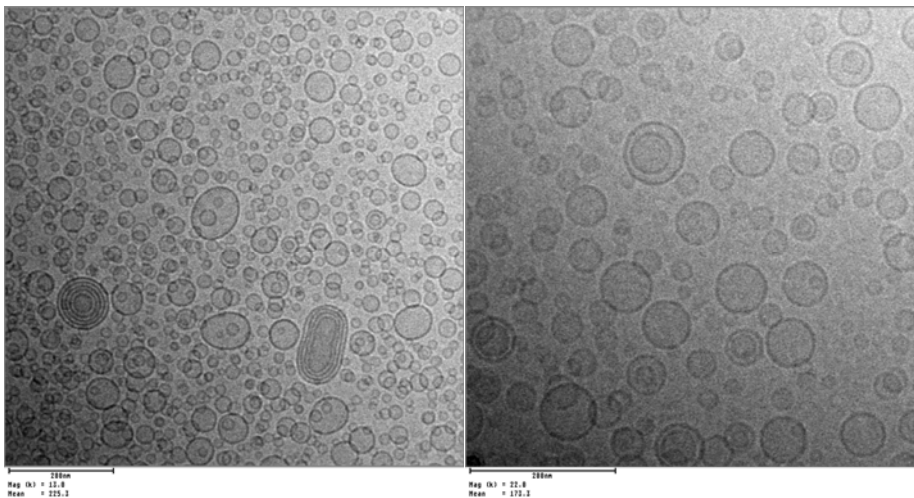


Fig 2 left: Cryo-TEM of liposomal CsA before nebulisation.

The visualisation on the left depicts the native formulation.

The visualisation on the right depicts the lyophilised and reconstituted sample.

Size bar is 200 nm.

Size and structure of liposomes after nebulisation using Cryo-TEM visualisation

The appearance of the nebulised L-CsA formulation is similar to the formulation prior to nebulisation as shown in fig.3 left. The majority of vesicles is in a size range from about 10 to 30 nm accompanied by larger unilamellar vesicles in a size range of about 80 to 150 nm being partially shaped like tubes or having sporadically an unilamellar biconcave discoidal (flattened) appearance. After nebulisation, the percentage of vesicles in the size range from about 80 to 150 nm seems to be slightly increased compared to non nebulised L-CsA formulations.

The appearance of the dissolved L-CsA lyophilisate after nebulisation is similar to a non lyophilised liquid formulation after nebulisation as shown in fig 3 right. Predominant are unilamellar vesicles sized 10 to 30 nm, a few larger unilamellar vesicles are also visible. Similar to the non-lyophilised formulations deformed vesicles (tube shape, biconcave discoid, invaginations) can be seen after nebulisation.

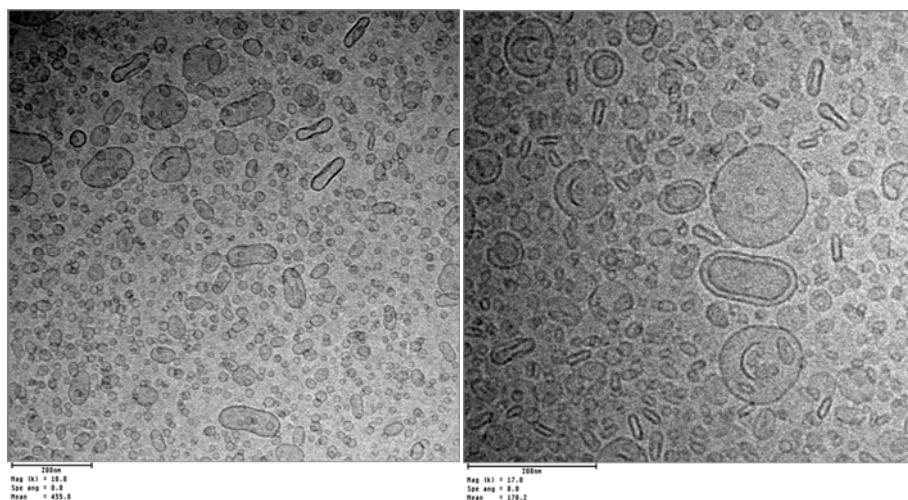


Fig 3 left: Cryo TEM of liposomal CsA after nebulisation.

The visualisation on the left depicts the native formulation after nebulisation.

The visualisation on the right depicts the lyophilised and reconstituted sample after nebulisation.

Size bar is 200 nm.

Size of liposomes measured by photon correlation spectroscopy (PCS)

PCS measurements of the untreated liposomal formulation resulted in a mean z-Average of 51.2 nm and a size distribution expressed as polydispersity index (PDI) of 0.276.

Lyophilisation and reconstitution of the same formulation containing an additional sugar as lyoprotectant caused a marginal mean size increase to 55.9 nm and a particle size distribution similar to the untreated liposomal formulation.

Nebulisation of a liquid non-lyophilised formulation resulted in an increased vesicle size having a mean z-Average of 107.3 nm and a broader particle size distribution with a PDI of 0.39. A similar increase in vesicle size could be observed after nebulisation of the dissolved lyophilisate exhibiting a mean z-Average of 101.55 nm and a PDI of 0.357, respectively. The vesicles of both formulations remained in a nano-range below 110 nm after nebulisation with a customised eFlow 30XL. It can be seen from table 1, that the vesicle size of the liposomes was nearly unaffected by the lyophilisation process. However, nebulisation caused a slight increase in particle size and particle size distribution for both, the non-lyophilised liquid and for the dissolved lyophilised formulation.

| | | z-Average | PDI |
|---|---------------------|------------------|------------|
| Liposomal CsA formulation | Before Nebulisation | 51.2 | 0.276 |
| | After Nebulisation | 107.25 | 0.393 |
| Liposomal CsA formulation + lyoprotectant, lyophilised and reconstituted | Before Nebulisation | 55.9 | 0.271 |
| | After Nebulisation | 101.55 | 0.357 |

Tab 1: Size of liposomes measured by photon correlation spectroscopy before and after nebulisation. Both formulations were nebulised in duplicate, each via an eFlow 30XL and nebulisation data represent the mean of two measurements, each.

Discussion and Conclusion

Characterisation of liposomal CsA formulations by Cryo transmission electron microscopy is a valuable tool to get a deeper insight into the shape, configuration, size and distribution pattern of liposomal vesicles. Cryo-TEM is helpful to better understand results from size measurements by photon correlation spectroscopy being the method of choice for routine analysis of vesicular colloidal systems. Liquid non-lyophilised L-CsA formulations prepared by high pressure homogenisation consist mainly of small unilamellar vesicles (SUV) in a size range of about 10-30 nm accompanied by a smaller quantity of SUV in a range from 50 to 100 nm. These results are in accordance with PCS-measurements showing a mean z-Average of the liquid L-CsA formulation of about 51.2 nm. The relatively high polydispersity index (PDI) of 0.276 can be explained by the abundance of very small besides larger vesicles. Our results are in agreement with the literature indicating that very small vesicles are representative for liposomal formulations prepared by high pressure homogenisation. Cryo-TEM data also demonstrate that the lyophilisation process has no clear impact on the liposome shape, size and size distribution, since vesicle images prior and after lyophilisation are more or less the same with a slight trend towards larger vesicles. This marginal increase is also reflected by PCS measurements showing a small increase in the mean vesicle size of less than 10 %, which is not associated with a change in the particle size distribution. We conclude from our investigations, that shape, appearance, size and size distribution of the liposomal vesicles is only very slightly affected by the selected lyophilisation process or the subsequent dissolution step when preparing an inhalation formulation from the lyophilisate.

However, nebulisation of liposomal CsA is causing an increase in the vesicle size of up to ~110 nm and an increased polydispersity index. This change is associated with some deformation in the shape and form of these vesicles. The effect can be observed irrespective a liquid or reconstituted L-CsA formulation is nebulised. At present we do not know, if the vesicle size increase or the deformation is due to shear stress caused by the nebulisation extrusion process via the vibrating membrane or due to the collection of the aerosol and the subsequent sample preparation. Nonetheless, vesicles are in the nano size range and no drug leakage out of the liposomes could be detected in another subset of experiments.

Deposition and pharmacokinetic data have demonstrated that L-CsA can be successfully nebulised and deposited to the target site in the lungs of transplanted patients [9]. Furthermore, safety studies in rats and preclinical data in Calu-3 cells, human lung homogenates and perfused rabbit lungs support our expectation; L-CsA will reach the target cells in the lungs. We hope, clinical studies will show, that bronchiolitis obliterans in lung- and stem cell transplanted patients can be treated successfully with inhaled L-CsA delivered via a customized eFlow[®] electronic nebuliser.

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