International Journal of Pharmaceutics, 37 (1987) 171–173
Elsevier

IJP 01253

Multilamellar or multivesicular vesicles?

H. Talsma [1], H. Jousma [2], K. Nicolay [3] and D.J.A. Crommelin [1]

[1] *Department of Pharmaceutics, Subfaculty of Pharmacy, State University of Utrecht, Utrecht (The Netherlands),*
[2] *Department of Pharmaceutical Technology, Center for Bio-Pharmaceutical Sciences, State University of Leiden (The Netherlands) and* [3] *Institute of Molecular Biology and Medical Biotechnology, State University of Utrecht (The Netherlands)*

(Received 17 November 1986)
(Accepted 28 December 1986)

Key words: Multilamellar vesicle; Multivesicular vesicle; Liposome characterization

Recently attention in liposome preparation technology has been focused on the preparation of liposomes with a large number of bilayers (Kim et al., 1985; Lenk et al., 1983, Pidgeon et al., 1986). These liposomes offer the possibility to encapsulate large amounts of hydrophobic drugs. All methods used to prepare these vesicles are modifications of the method used to produce reverse-phase evaporation vesicles (REV), as described first by Szoka and Papahadjopoulos (1978). The names for these multi-layered vesicles are: stable plurilamellar vesicles (SPLV) (Lenk et al., 1983), multilayered REV (MLV-REV) (Pidgeon et al., 1986) and multilamellar vesicles (Kim et al., 1985). In order to avoid confusion we will refer to the vesicles prepared by Bangham et al. (1965) as multilamellar vesicles (MLV) and to those multilayered vesicles prepared by emulsion techniques as REV-MLV.

Gruner et al. (1985) compared the physical characteristics of MLV and SPLV. They found that these vesicles had different properties for stability, entrapment efficiency and biological effects, even if they were made from the same materials and appeared quite similar in the electron microscope.

Here we report on our efforts to elucidate the structure of REV-MLV. To this end we measured the number of bilayers with two independent techniques, ^{31}P-NMR and small-angle X-ray diffraction. The composition of the bilayers was soybean phosphatidylcholine (Phospholipon 100, Nattermann Gmbh, Köln, F.R.G.)/ cholesterylhemisuccinate/ cholesterol (Sigma, St. Louis, MO, U.S.A.) 10:2:10 (molar ratio). The preparation procedure used was described by Pidgeon et al. (1986). Briefly 200 μmol of lipid was dissolved in 7.0 ml of freshly distilled diethylether, 0.3 ml Tris buffer [1] was added and the two-phase mixture was sonicated in a bath sonicator to form an emulsion. After mixing the ether was evaporated by slightly heating the dispersion with a gentle stream of nitrogen passing over it. During evaporation the dispersion was held in constant motion. After several minutes a gel is formed and the process is stopped. The final liposomal dispersion is formed by adding 7.7 ml of Tris buffer to the gel while shaking gently.

The time to stop the evaporation process should be properly chosen. When the evaporation is stopped too late, too much water has evaporated and a lipid film is formed along the glass wall of

Correspondence: H. Talsma, Department of Pharmaceutics, Subfaculty of Pharmacy, State University of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands.

[1] Buffer: 10 mM tris-(hydroxymethyl)aminomethane + 0.8% sodium chloride adjusted to pH 7.4 with diluted hydrochloric acid.

the vessel. Then the preparation process results in a mixture of MLV and REV-MLV preparation techniques (see also Pidgeon et al. 1986). To establish the number of bilayers in the prepared vesicles we used ^{31}P-NMR and small angle X-ray scattering (SAXS). The NMR-method gives information about the fraction of surface area that forms the outer surface of the liposomes, from which the number of bilayers can be calculated. From the recorded SAXS-curves direct information about the number of bilayers can be obtained. We used the procedures as described by Jousma et al. (1987). Particle size determination was done with dynamic light scattering using a Malvern PCS100SM spectrometer equipped with a type 7027 particle analyzer processor (Malvern Ltd, Malvern, U.K.) and a 100-mW helium/neon laser (NEC Corp., Tokyo, Japan). The mean particle size of 5 batches of the prepared vesicles ranged between 1.8 and 5.2 μm. The results of the NMR experiments indicated that only between 15 and 24% of the phospholipid molecules were exposed to the external medium. If the vesicles were MLV this should indicate that the vesicles contained an average of 2–3 bilayers around a relatively large aqueous core. From analysis of the SAXS-spectra it was found that the majority of vesicles was unilamellar. This discrepancy between NMR and SAXS analysis was surprising, because earlier investigations (Jousma et al., 1987) showed a good agreement between the results of these analytical techniques for MLV with a small number of bilayers. A possible explanation for this discrepancy is that the vesicles were in fact multivesicular vesicles. Fig. 1 shows schematically the classical MLV and multivesicular vesicles.

Kim et al. (1983) reported the existence and preparation of multivesicular vesicles. Although their preparation method differs somewhat from that described for SPLV and MLV-REV the basic processes involved are the same. The major difference between the REV-method of Szoka and Papahadjopoulos (1978) and the newer methods is that the lipid : water ratio at the start of the preparation procedure is much higher. One might speculate on what parameters are critical to form multivesicular vesicles instead of multilamellar vesicles. The emulsion method combined with a high lipid : water ratio seems to be necessary. Also certain steps in the preparation procedure (e.g. stop of the evaporation process) are critical. It is possible that variation of these parameters produces vesicles with different arrangements of bilayers in the vesicles.

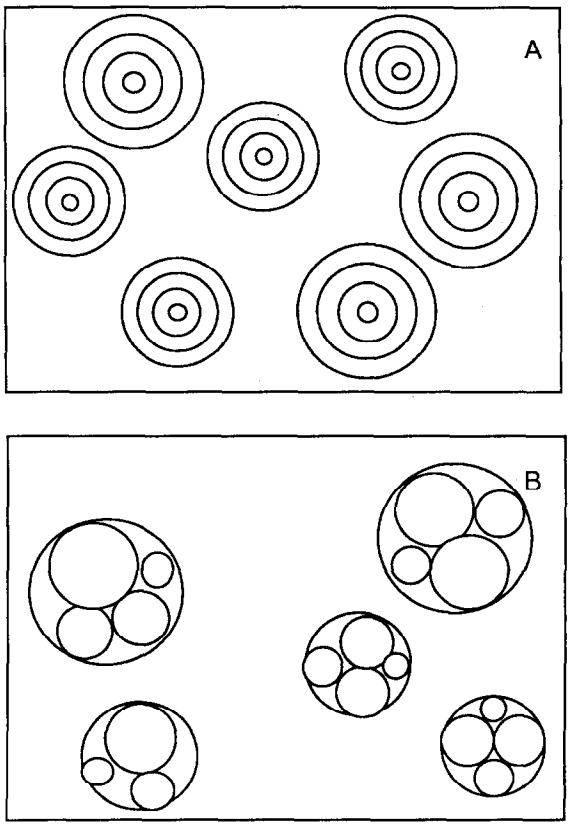

Fig. 1. Types of multilamellar vesicles. a: Classical multilamellar vesicles. b: Multivesicular vesicles.

It is well established that the therapeutic action of antibiotics incorporated in SPLV is better than in MLV (Dees et al., 1985). The MLV-REV vesicles might also behave therapeutically and pharmacokinetically different from MLV. Therefore a correct characterization of the type of vesicles used is of utmost importance. This communication emphasises the potential of the combination of SAXS and ^{31}P-NMR analysis to elucidate the inner structure of the vesicles and to draw conclusions about the structure of the vesicle under investigation.

Acknowledgements

We thank P. Everts for his help with the experimental work and Nattermann Gmbh for the gift of Phospholipon 100.

References

Bangham, A.D., Standish, M.M. and Watkins, J.C., Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.,* 13 (1965) 238–252.

Dees, C., Fountain, M.W., Taylor, J.R. and Schultz, R.D., Enhanced intraphagocyte killing of *Brucella abortus* in bovine mononuclear cells by liposomes containing gentamicine. *Vet. Immunol. Immunopathol.,* 8 (1985) 171–182.

Gruner, S.M., Lenk, R.P., Janoff, A.S. and Ostro, M.J., Novel multilayered lipid vesicles: comparison of physical characteristics of multilamellar liposomes and stable plurilamellar vesicles. *Biochemistry,* 24 (1985) 2833–2842.

Jousma, H., Talsma, H., Spies, F., Joosten, J.G.H., Junginger, H.E. and Crommelin, D.J.A., Characterization of liposomes. The influence of extrusion of multilamellar vesicles through polycarbonate membranes on particle size, particle size distribution and number of bilayers. *Int. J. Pharm.,* 35 (1987) 263–279.

Kim, S., Turker, M.S., Chi, E.Y., Sela, S. and Martin, G.M., Preparation of multivesicular liposomes. *Biochim. Biophys. Acta,* 728 (1983) 339–348.

Kim, S., Jacobs, R.E. and White, S.H., Preparation of multilamellar vesicles of defined size-distribution by solvent-spherule evaporation, *Biochim. Biophys. Acta,* 812 (1985) 793–801.

Lenk, R.P., Fountain, M.W., Janoff, A.S., Ostro, M.J. and Popescu, M.C., European Patent Office EP-921453-A, South Africa Patent ZA8302197-A and Japan Patent JS9500952-W (1983).

Pidgeon, C., Hunt, A.H. and Dittrich, K., Formation of multilayered vesicles from water/organic-solvent (w/o) emulsions: theory and practice. *Pharm. Res.,* 3 (1986) 23–34.

Szoka, F.C. and Papahadjopoulos, D., Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation, Proc. Natl. Acad. Sci. U.S.A., 75 (1978) 4194–4198.