ELSEVIER

International Journal of Pharmaceutics 176 (1999) 203–207

international journal of pharmaceutics

# In vitro binding of HSA, IgG, and HDL on liposomes of different composition and its correlation with the BLOOD/RES ratio of liposomes

Z. Panagi [a], K. Avgoustakis [b,*], G. Evangelatos [a], D.S. Ithakissios [a,b]

[a] *Institute of Radioisotopes and Radiodiagnostic Products, NCSR 'Demokritos', Aghia Paraskevi Attikis, Athens 153 10, Greece*
[b] *Pharmaceutical Technology Laboratory, Department of Pharmacy, University of Patras, Rion 261 10, Greece*

Received 26 March 1998; received in revised form 24 September 1998; accepted 6 October 1998

**Abstract**

The in vitro binding of the serum proteins human serum albumin (HSA), human serum immunoglobulin (IgG) or human serum high density lipoprotein (HDL) on unilamellar liposomes of different lipid composition was studied. HDL bound on liposomes at higher amounts than IgG and IgG at higher amounts than HSA. The protein binding on liposomes decreased when bovine brain monosialganglioside ($GM_1$) or poly(ethyleneglycol)-distearoylphosphatidylethanolamine (DSPE-PEG) was included in liposome membrane. With all three proteins, an inverse relationship was found between the amount of protein bound on liposomes after 1 h liposome–protein incubation in vitro and the BLOOD/RES ratio of the same liposomes 2 min after i.v. administration in mice (Panagi et al., Drug Dev. Ind. Pharm., 22 (1996) 217–224). The potential value of this in vitro–in vivo correlation, provided it is extended to additional liposome compositions, is that it may provide an in vitro method to screen liposomes in terms of blood clearance rates. 

*Keywords:* BLOOD/RES ratio; Liposomes; Protein binding; Serum proteins

Liposomes injected intravenously are rapidly eliminated from blood by phagocytic cells of RES. This severely limits liposome potential as drug carriers in controlled drug delivery and drug targeting applications. The clearance of liposomes from blood is believed to involve serum proteins which bind on liposome surface (Chonn et al., 1992). Recently, we found that a correlation existed between the rate of protein-induced carboxyfluorescein leakage from liposomes in vitro and the BLOOD/RES ratio of the same liposomes in vivo (Panagi et al., 1998). We report in this communication data indicating that a correlation

* Corresponding author. Tel.: +30 61 997726; fax: +30 61 997728.

0378-5173/99/$ - see front matter 
*PII* S0378-5173(98)00315-9

Table 1
Amount of protein bound on liposomes ($\mu$g protein/mg lipid $\pm$ S.D.) after 1 h incubation

| Liposome composition | HSA | IgG | HDL |
|---|---|---|---|
| DMPC:DMPG (7:3) | $16.12 \pm 0.49$ | $27.82 \pm 4.18$ | $58.54 \pm 10.07$ |
| DMPC:DMPG:$GM_1$ (7:3:0.25) | $5.58 \pm 0.75$ | $12.13 \pm 3.16$ | $40.90 \pm 2.83$ |
| DSPC:CH (2:1) | $2.47 \pm 0.65$ | $22.67 \pm 4.20$ | $42.31 \pm 11.34$ |
| DSPC:CH:$GM_1$ (2:1:0.33) | $1.25 \pm 0.48$ | 0 | $11.14 \pm 0.83$ |
| DSPC:CH:DSPE-PEG (2:1:0.33) | $0.40 \pm 0.14$ | $12.88 \pm 6.01$ | $29.47 \pm 1.25$ |

might also exist between the amount of specific serum proteins bound on liposomes in vitro and the BLOOD/RES ratio of these liposomes in vivo. The binding of HSA (human serum albumin), IgG (human serum immunoglobulin) and HDL (human serum high density lipoprotein) was determined. HSA was selected because it has been found to bind on liposomes in vivo, whereas IgG and HDL were included in the study because they have been implicated in liposome clearance from blood via opsonization (IgG) or disintegration (HDL) procedures (Woodle and Lasic, 1992).

The following materials were used in this study: distearoylphosphatidylcholine (DSPC), distearoylphosphatidylglycerol (DSPG), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG), cholesterol (CH), bovine brain monosialganglioside ($GM_1$), human serum albumin (HSA), human serum immunoglobulin (IgG), and human serum high density lipoprotein (HDL) which were obtained from Sigma (St. Louis, MO, USA). Also, poly(ethyleneglycol)-distearoylphosphatidylethanolamine (DSPE-PEG), molecular weight ca. 2691, which was purchased from Genzyme (Haverhill, Suffolk, England) and Biogel A-15 m 200–400 mesh chromatographic gel which was obtained from Serva (Athens, Greece).

Large unilamellar vesicles (LUVs) in the range of 100 nm were prepared by extrusion of multilamellar vesicles through polycarbonate filters (100 nm), as described previously (Panagi et al., 1996). The liposome compositions studied were DSPC:CH (2:1), DSPC:CH:$GM_1$ (2:1:0.33), DSPC:CH:DSPE-PEG (2:1:0.33), DMPC:DMPG (7:3) and DMPC:DMPG:$GM_1$ (7:3:0.25). The values in parentheses represent the lipid molar ratio in each composition. These compositions were selected to be studied because they have been used or proposed for drug delivery applications, and because it was expected, based on literature data, to exhibit wide differences in protein binding and biodistribution properties. Thus, the addition of $GM_1$ or DSPE-PEG in liposome composition has been shown to result in a significant increase of liposome residence time in systemic circulation (Gabizon and Papahadjopoulos, 1988; Allen et al., 1991). The amount of HSA, IgG, or HDL bound on liposomes after 1 h incubation in vitro was determined by incubating 1 ml mixtures of the liposomes (3.12 mg lipid/ml) and the protein (0.60 mg/ml) for 1 h at 37°C. It was observed that the amount of protein bound on liposomes did not change significantly with time after the first hour of incubation. The incubation medium was phosphate buffer pH 7.4. The 'spin-column' chromatography method (Chonn et al., 1991), using Biogel mini (1 ml) columns and veronal-buffered saline (10 mM sodium barbital, 154 mM NaCl, pH 7.4) as eluent, was applied in order to separate the liposomes from the non-bound on them protein fraction. The pooled liposome and the pooled protein fractions were analysed for protein using the Bradford protein assay (Bradford, 1976) and for phospholipid using a colorimetric phosphorus assay (Bartlett, 1959). The total lipid recovery from the mini-columns was 70–80%. Binding data were obtained from two batches of each liposome composition, performing three replicate measurements with each batch.

The amount of protein bound on liposomes after 1 h incubation is shown in Table 1. HDL bound on liposomes at higher amounts than IgG and IgG at higher amounts than HSA, both on a weight (Table 1) and on a molar basis. With all three proteins, the incorporation of monosialganglioside

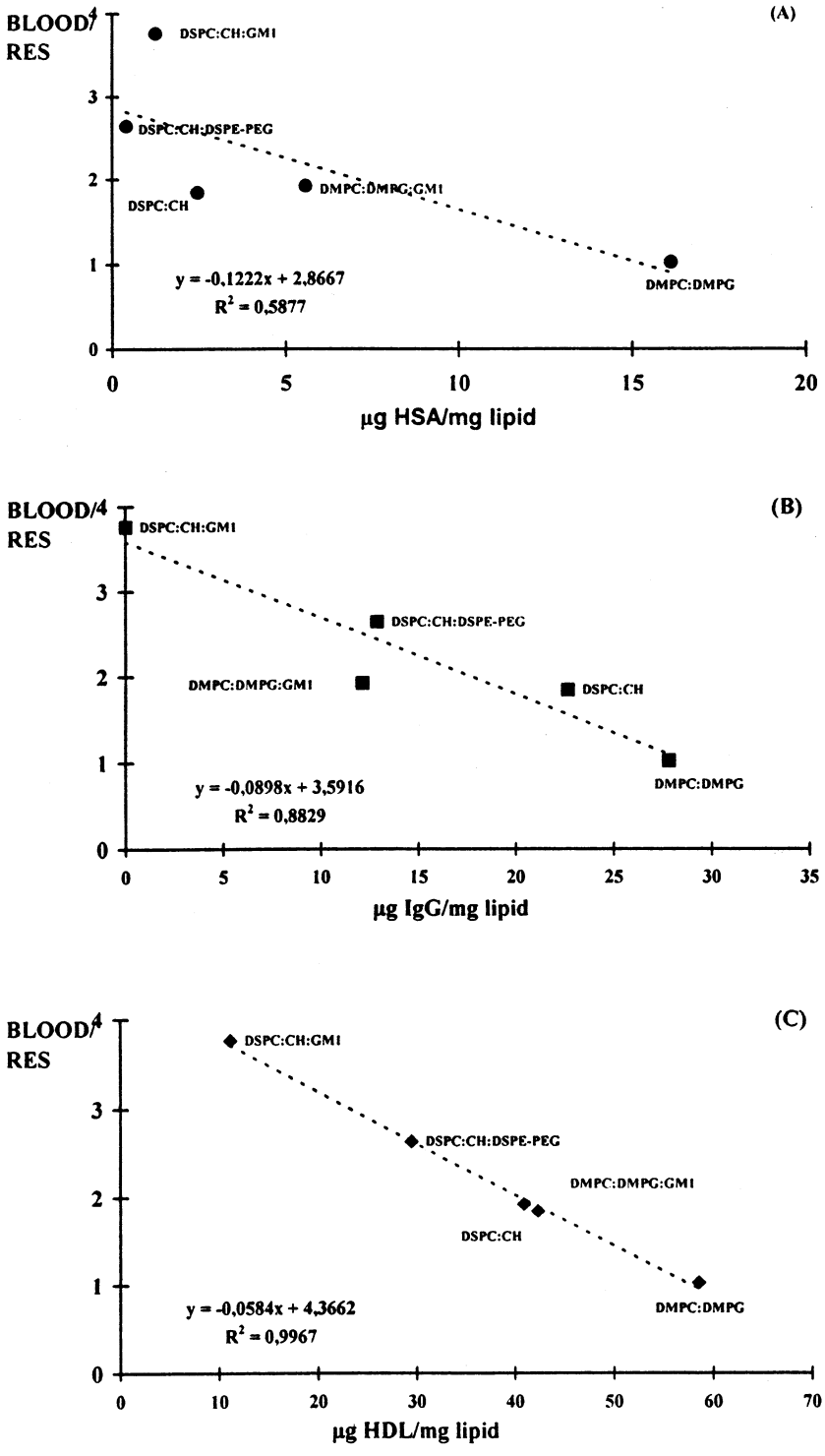


Fig. 1. Correlation of the amount of protein bound on liposomes after 1 h liposome–protein incubation in vitro with the BLOOD/RES ratio of these liposomes 2 min after i.v. administration in mice. (A) HSA, (B) IgG and (C) HDL.

$GM_1$ either in fluid DMPC:DMPG or in rigid DSPC:CH bilayers or the addition of the lipid derivative of polyethylene glycol DSPE-PEG in DSPC:CH bilayers reduced the amount of protein bound on the resulting DMPC:DMPG:$GM_1$ (7:3:0.25), DSPC:CH:$GM_1$ (2:1:0.33) and DSPC:CH:DSPE-PEG (2:1:0.33) liposomes.

The BLOOD/RES ratio represents the ratio of the fraction of liposome dose remaining in blood to the fraction of liposome dose captured in RES at a specific time after liposome administration, and is a measure of the ability of liposomes to remain in the systemic circulation avoiding RES uptake. Our group has recently reported that the BLOOD/RES ratios 2 min after i.v. administration in Swiss/De mice of the liposome compositions studied here were: 1.022 for DMPC:DMPG (7:3), 1.921 for DMPC:DMPG:$GM_1$ (7:3:0.25), 1.840 for DSPC:CH (2:1), 2.638 for DSPC:CH:DSPE-PEG (2:1:0.33) and 3.758 for DSPC:CH:$GM_1$ (2:1:0.33) liposomes (Panagi et al., 1996). Plotting these BLOOD/RES values against the amount of protein bound on the same liposome compositions after 1 h liposome–protein incubation in vitro, an inverse relationship was obtained with all three proteins studied. This relationship could satisfactorily be described by linear regression equations in the case of IgG and HDL (Fig. 1). The biodistribution data of liposomes 2 min after their i.v. administration were used due to their increased reliability as compared to the biodistribution data obtained at later times (Panagi et al., 1996). However, similar trends of the biodistribution data were observed at all times post administration studied (Panagi et al., 1996; Panagi, 1996). Thus, the time of BLOOD/RES ratio determination should not affect the validity of the inverse relationship between the liposome BLOOD/RES ratio and the in vitro protein binding on liposomes, provided that the time selected lies inside the time span of liposome circulation in blood.

In this work, data indicating that an inverse relationship probably exists between the in vitro binding of serum proteins HSA, IgG, and HDL on unilamellar vesicles and the BLOOD/RES biodistribution of the vesicles, were presented. The correlation was based on data for five liposome compositions specifically selected so that liposomes exhibiting a broad range of properties (in terms of rigidity, charge, and the presence in liposome membrane of specific components conferring to liposomes 'special' in vivo properties) were included in the study. As a result, vesicles with low (e.g. DSPC:CH:DSPE-PEG), medium (e.g. DSPC:CH), and high (e.g. DMPC:DMPG) rates of blood clearance (Gabizon and Papahadjopoulos, 1988; Allen et al., 1991; Panagi et al., 1996) were included in the study. Thus, liposome compositions with blood clearance rates covering essentially the whole span of possible clearance rates were studied, and, therefore, the obtained results could be considered representative for liposome compositions with similar physicochemical characteristics. The inverse relationship between the amount of protein bound on liposomes and the BLOOD/RES ratio of liposomes was observed with all three proteins studied, and this could not have happened by chance. As it might have been expected, the correlation was not equally satisfactory with all proteins, being better in the cases of IgG and HDL. This finding appears to be in good agreement with previously published data (review in Woodle and Lasic, 1992) indicating that the opsonization by immunoglobulins and the destabilization by HDL are the main in vivo processes leading to liposome clearance.

The reported findings could contribute significantly to liposome research. It is obvious, however, that additional studies with more liposome compositions performed by different laboratories are needed before the observed correlation is adapted as a tool to screen liposomes in terms of blood clearance rates.

## References

Allen, T.M., Hansen, C., Martin, F., Redemann, C., Yau-Young, A., 1991. Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives in vivo. Biochim. Biophys. Acta 1066, 29–36.

Bartlett, G.R., 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234, 466–468.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Chonn, A., Semple, S.C., Cullis, P.R., 1991. Separation of large unilamellar liposomes from blood components by a spin column procedure: towards identifying plasma proteins which mediate liposome clearance in vivo. Biochim. Biophys. Acta 1070, 215–222.

Chonn, A., Semple, S.C., Cullis, P.R., 1992. Association of blood proteins with large unilamellar liposomes in vivo. Relation to circulation lifetimes. J. Biol. Chem. 267, 18759–18765.

Gabizon, A., Papahadjopoulos, D., 1988. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc. Natl. Acad. Sci. USA 85, 6949–6953.

Panagi, Z., 1996. In vitro interaction of liposomes with serum proteins and its correlation with the BLOOD/RES distribution of radioactivity after i.v. administration of $^{99m}$Tc-liposomes. Ph.D Thesis, University of Patras, Greece.

Panagi, Z., Sawas-Dimopoulou, C., Avgoustakis, K., Ithakissios, D.S., 1996. Application of $^{99m}$Tc labeling for fast comparative screening of preformed liposomes in vivo. Drug Dev. Ind. Pharm. 22, 217–224.

Panagi, Z., Avgoustakis, K., Evangelatos, G., Ithakissios, D.S., 1998. Protein-induced CF release from liposomes in vitro and its correlation with the BLOOD/RES biodistribution of liposomes. Int. J. Pharm., 163, 103–114.

Woodle, M.C., Lasic, D.D., 1992. Sterically stabilized liposomes. Biochim. Biophys. Acta 1113, 171–199.