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Opsono-recognition of liposomes by tissue macrophages

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Abstract

On exposure to blood, liposomes of different morphology and lipid composition attract different arrays of proteins. The mode of interaction of blood proteins with liposomes vary in quantity and conformation. These differences may explain different observed patterns in blood clearance and tissue distribution of liposomes. In this paper, an attempt is made to assess the role of serum and its components in regulating liposome recognition by isolated phagocytes of rat liver and spleen.

Keywords: Liposome; Opsonin; Dysopsonin; Kupffer cell; Phagocyte; Reticuloendothelial system

1. Introduction

The exact mechanisms that govern the ways that liposomes are both detected as foreign particles in vivo and cleansed from the blood by phagocytic cells of the reticuloendothelial system are presently unsolved (Senior, 1987). Undoubtedly, the clearance rate of intravenously injected multilamellar liposomes from the blood by phagocytic cells of the liver and the spleen and their eventual intracellular degradation is dependent upon the vesicular lipid composition and cholesterol content (Patel et al., 1983; Dave and Patel, 1986; Derksen et al., 1988; Roerdink et al., 1989; Patel, 1992; Semple et al., 1996; Moghimi and Patel, 1998). For example, following intravenous administration, liposomes (100–400 nm) composed of egg PC, cholesterol, and DCP (mole ratio 7:0:1), otherwise termed as cholesterol-free vesicles, are cleared more readily from the blood than vesicles of the same size and fcomposition but containing 20 mol% cholesterol (cholesterol-poor liposomes), Patel et al., 1983. Furthermore, cholesterol-poor egg PC vesicles are cleared faster than their cholesterol-rich counterparts (vesicles with 46.6 mol% cholesterol). Cholesterol-free and cholesterol-poor egg PC vesicles are predominantly localized to the liver; only a small proportion of the injected dose is deposited in other

Abbreviations: DCP, dicetylphosphate; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; Egg PC, egg phosphatidylcholine; EGTA, ethylene glycol-bis(oxyethylenenitriol)tetraacetic acid; PS, phosphatidylserine.

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organs of the reticuloendothelial system (Patel et al., 1983). For cholesterol-rich vesicle, hepatic sequestration is rather poor when compared to cholesterol-free and -poor vesicles; such vesicles tend to localize more effectively in spleen and to some extend in bone marrow (Patel et al., 1983; Senior et al., 1985). Cholesterol can tighten the packing of phospholipids with low transition temperature, thus solidifying the liposomal membrane (Senior et al., 1985; Gregoriadis, 1994). Accordingly, such changes can diminish high density lipoprotein attack and complete liposome disintegration (Scherphof et al., 1978; Tall et al., 1986; Gregoriadis, 1994), and minimize susceptibility of the lipid interface to perturbation or penetration, either by opsonizing plasma proteins (e.g. components of complement system, pentraxins) or by Kupffer cell surface proteins serving to establish the initial contact between liposome and cell (Juliano and Lin, 1980; Moghimi and Patel, 1988; Roerdink et al., 1989). Because of prolonged residence in blood, cholesterol-rich liposomes are believed to encounter spleen more readily than cholesterol-free and cholesterol-poor vesicles and, hence, are filtered (Moghimi, 1995). The suggestion that the cholesterol content of liposomes has a pronounced effect on the rate of uptake by organs of the reticuloendothelial system is further supported by incorporation of 50 mol% cholesterol in multilamellar vesicles of DSPC/brain PS (Roerdink et al., 1989). Here, DSPC has a transition temperature well above the body temperature and brain PS exhibits a transition temperature of $\approx 10°C$. The incorporation of cholesterol in such vesicles is believed to abolish or diminish the phase boundaries, the sites that may allow proteins to penetrate a lipid bilayer, between solid DSPC and fluid PS domains. A recent study also showed that an increase in the membrane cholesterol content in DSPC vesicles was accompanied by a marked decrease in the total amount of plasma protein which bound to liposomes (Semple et al., 1996).

It is also interesting to note that multilamellar cholesterol-rich vesicles are substantially more resistant towards intracellular degradation in Kupffer cells than cholesterol-poor and cholesterol-free liposomes (Roerdink et al., 1989). Indeed, incubation of liposomes with lysosomal fractions prepared from rat liver also demonstrated a difference in susceptibility to lysosomal degradation: the cholesterol-free vesicles were much more sensitive to lysosomal esterases than cholesterol-containing liposomes (Roerdink et al., 1989).

2. An alternative hypothesis

Earlier Dave and Patel (1986) reported that liver and spleen respond differently to cholesterol-free and cholesterol-rich liposomes when injected to produce a reticuloendothelial blockade. This observation suggested that these organs handle the two types of liposomes differently. In order to explain the observed differences in organ distribution of intravenously injected multilamellar vesicles, a tentative hypothesis was put forward by us (Moghimi and Patel, 1988, 1989a,b, 1998). The scheme proposes that multilamellar liposomes of different phospholipid and cholesterol content attract different spectrum of plasma or serum proteins, the content and conformation of which may account for the observed differences in the rate and the site of vesicle clearance from the circulation and their subsequent metabolism. Some of these blood proteins could be those opsonins which exhibit affinity either specifically for Kupffer cells or for certain population of splenic scavenger cells or perhaps both. This assumption is based on the fact that macrophages constitute a highly heterogeneous population (Robinson et al., 1986; Gordon et al., 1992; Maruiwa et al., 1993; Moghimi, 1995). Accordingly, their specialized plasma membrane receptors and antigens vary greatly, depending on cell maturity and local modulation. Thus, such opsonins promote phagocytosis only in designated cells primarily by affecting the critical first step of attachment and are not generally regarded as being those factors which influence subsequent events in the process such as internalization and/or digestion of phagocytic substrates (Moghimi and Patel, 1998). In contrast to opsonins, a second group of naturally occuring substrates can inhibit phagocytic ingestion. Generally these agents (dysopsonins) act either by altering the

surface properties of the phagocyte or particle (or both), thereby interfering with or thwarting opsonization, or by altering the metabolic activity of the phagocyte (Moghimi and Patel, 1998). Thus, the amount and conformation of the adsorbed blood opsonic and dysopsonic molecules may regulate the rate of liposome clearance by different phagocytes of the reticuloendothelial system. For instance, dysopsonins could modulate the rate of liposome uptake by reducing the amount of liposome-bound opsonin and hence, prevent Kupffer cells from being destroyed by excessive binding and ingestion of liposomes and, in particular, they may prevent the massive accumulation of those vesicles which are more resistant towards lysosomal esterases (e.g. cholesterol-rich liposomes and vesicles made from diacyl phospholipids with a high transition temperature). This speculation is rather analogous to non-macrophage Hepatoma G2 cells (Bisgaier et al., 1989), where liposome uptake was shown to be readily mediated by apolipoprotein-E. Other apolipoproteins (A-IV and A-I) failed to increase cellular uptake of liposomes but modulated the receptor-mediated uptake of vesicles by reducing the amount of liposome-bound apolipoprotein-E.

3. In vitro evidence

Initially we studied the effect of rat serum on recognition and uptake of ^{125}I-poly(vinylpyrrolidone) incorporated multilamellar cholesterol-poor liposomes (composed of egg PC:cholesterol:DCP, mole ratio 7:2:1) and cholesterol-rich vesicles (mole ratio of 7:7:1) by freshly isolated rat liver and spleen phagocytes (Moghimi and Patel, 1993a). The purpose for the incorporation of DCP was to provide liposomes with a negative charge to prevent aggregation. We omitted cholesterol-free egg PC vesicles in the uptake process as they are unstable in serum (Moghimi and Patel, 1988). In a cell suspension assay we found that both liver and spleen phagocytes took up cholesterol-poor more than cholesterol-rich vesicles (250–350 nm) in the absence of fresh rat serum. But, inclusion of rat serum (25% v/v) had a variable effect on the uptake of liposomes depending on their cholesterol content and the source of phagocyte. For example, serum enhanced the uptake of cholesterol-poor vesicles by hepatic cells but suppressed that of cholesterol-rich liposomes in comparison to the results obtained in a serum-free media (a medium supplemented with serum albumin as an irrelevant protein). In contrast to hepatic phagocytes, serum stimulated the uptake of all cholesterol-containing vesicles by splenic phagocytes. Interestingly, serum enhanced the uptake of cholesterol-rich vesicles by spleen macrophages more than that of cholesterol-poor counterparts (Moghimi and Patel, 1988, 1989a,b).

In contrast to egg PC vesicle, multilamellar liposomes formed from saturated phospholipids such as DPPC and DSPC are more stable in serum. Serum suppressed the uptake of such vesicles (250–350 nm) by liver macrophages when compared to control incubations (serum albumin supplemented). When the rigidity of DPPC and DSPC vesicles was reduced by incorporation of cholesterol into the preparation (20 or 46.6 mol%), there was still no evidence of serum stimulating liposome uptake by liver phagocytes; suppression of liposome uptake was still evident (Moghimi and Patel, 1989a). In contrast to liver cells, serum failed to exert a suppressive effect on the recognition of cholesterol-free liposomes formed from saturated phospholipids by splenic scavengers, but the inclusion of cholesterol resulted in enhanced liposome uptake (Moghimi and Patel, 1989a); the higher the cholesterol content of liposomes the higher the uptake by spleen cells. Remarkably, these observations were comparable with those in vivo studies reported by us and others (Patel et al., 1983; Senior et al., 1985; Roerdink et al., 1989), and showed that phagocytes of liver and spleen respond and recognize liposomes differently in the presence of serum.

The complement system provides the first line of defense against foreign invaders and particles, ensuring their cytolytic and/or phagocytic clearance (Sim, 1993). A number of studies have indicated that liposomes of appropriate size and composed of certain net negatively charged phospholipids can activate the complement system in human, guinea pig, rat and mouse serum, and

depending on the lipid composition of the vesicles, C3b and iC3b are deposited on liposome surface (Chonn et al., 1991; Alving and Wassef, 1992; Devine et al., 1994). Liposomal activation of the classical pathway may occur when antibodies to liposomal phospholipids and cholesterol bind to the vesicles (Alving and Swartz, 1991; Alving and Wassef, 1992). Natural antibodies to phospholipids and cholesterol are widespread in all animal species, although specificities and titer show substantial interspecies variation. Liposomes can also activate complement through non-antibody-mediated mechanism via the classical and alternative pathways. Chonn et al. (1991) and Devine et al. (1994) have suggested that C3b and iC3b play an important role in the initial rapid phase of in vivo clearance of certain negatively charged and cholesterol-containing liposomes. They also suggest that the rate of clearance of liposomes may depend on their ability to activate complement system. Rat liver and spleen macrophages, indeed, express receptors for C3 fragments and immunoglobulins (Moghimi, 1995; Moghimi and Patel, 1998). To what extent the concept of complement activation and covalent attachment of C3 fragments on to liposome surface can explain the observed differences in serum mediated recognition and handling of cholesterol-poor and -rich egg PC liposome by phagocytes of the liver and the spleen?

Strategies which inactivate complement activity in serum, such as the heat treatment of serum at 55°C/30 min, removal of divalent cations from serum by dialysis or chelation with EGTA, and treatment of serum with ammonium hydroxide (which inactivates the complement components C4 and C3) and zymosan (which activates the alternative pathway to generate C3 and C5 convertase and hence, deplete C3 and to a lesser extent C5 and terminal components) demonstrated remarkable differences on the mode of liposome recognition by liver and spleen cells. For instance, heating of serum further enhanced liposome recognition by liver macrophages (Moghimi and Patel, 1989b). Similarly, dialysis of serum or its treatment with EGTA further enhanced the binding of cholesterol-poor vesicles to Kupffer cells (Moghimi and Patel, 1989b). This process was found to be due to removal of ionic calcium, or other divalent cations from serum, since upon the addition of calcium chloride, the opsonic activity of dialysed and EGTA-treated sera approached that of undialysed serum (Moghimi and Patel, 1990). On the contrary, elevation of serum calcium levels above the physiological concentration suppressed the opsonic activity on liposome recognition by liver phagocytes (Moghimi and Patel, 1990, 1996). Although these observations suggest a minimum role for complement-mediated liposome recognition by rat Kupffer cells, experiments with monoclonal antibodies against rat macrophage complement receptors (which are currently available) are still necessary to resolve this matter. In contrast to Kupffer cells, activation of complement system appears to play a role in liposome recognition by the splenic phagocytes (Moghimi and Patel, 1989b). However, an interesting observation was made with dialysed or EGTA-treated serum. These treatments resulted in partial loss of opsonic activity on liposomes recognition by splenic phagocytes. Since re-addition of serum dialysate or divalent cations (calcium and magnesium) to dialysed or chelated serum failed to reinstall the lost activity, we can not exclude the involvement of other serum components, which apparently undergo an irreversible damage upon dialysis of serum, that can mediate liposome recognition by certain splenic macrophage sub-populations (Moghimi and Patel, 1989b, 1990).

We also disregarded the involvement of the heat-labile fibronectin, which is known to stimulate particle recognition by macrophages (Rossi and Wallace, 1983; Kolb-Bachofen and Abel, 1991), in liposome clearance by Kupffer cells. For instance, complete depletion of fibronectin from serum had no effect on the opsonic activity of serum towards liposome uptake by Kupffer cells. Even biologically active fibronectin, which was purified from rat plasma, failed to enhance liposome recognition by Kupffer cells, both in the absence and the presence of its co-factor, heparin (Moghimi and Patel, 1989b). Furthermore, the inability of fibronectin to stimulate liposome uptake was not due to detrimental effects on fibronectin receptors during Kupffer cell isolation,

since isolated Kupffer cells could recognize and internalize plasma-opsonized or fibronectin-coated gold particles (an interaction which is initially mediated via a low affinity particle-galactose-specific receptor which recognizes a D-galactosyl group of attached plasma fibronectin, followed by a Kupffer cell plasma membrane integrin which recognizes the Arg-Gly-Asp containing peptide sequence on the carboxyl-terminal cell adhesion domain of surface bound fibronectin), Moghimi et al., 1993b.

Eventually, a heat-stable, calcium-sensitive protein was partially purified from rat serum that could mediate the uptake of cholesterol-poor egg PC vesicles by isolated Kupffer cells (Moghimi and Patel, 1996). Experiments are currently underway to determine whether this factor can stimulate liposome uptake via the Kupffer cell scavenger receptors, particularly a 95 kD receptor (de Rijke and Van Berkel, 1994) which recognizes oxidatively modified low density lipoproteins and many other ligands. We also demonstrated that purified factor had no effect on liposome recognition and uptake by the splenic phagocytes, thus confirming the involvement of different opsonins in liposome clearance by macrophages of liver and spleen. Here, I would like to stress that nonetheless, the different expression and functional state of opsonin receptors on macrophages may play an equally, if not more, important role in tissue specific recognition of the above-mentioned liposomal formulations.

In addition to the opsonic molecule, we also detected the presence of two other heat-stable factors in rat serum that could suppress liposome binding to Kupffer cells. These factors can be precipitated from serum in the range of 0–35% and 50–65% ammonium sulphate saturation (Moghimi and Patel, 1993b). The precipitated materials in the range of 50–65% saturation was at least one order of magnitude more potent than the 0–35% precipitated materials on a protein basis. These materials may represent serum dysopsonins and could play a regulatory role in the rate of liposome clearance by liver macrophages. Recently, other investigators also reported a suppressive role for serum and blood components on liposome uptake by Kupffer cells in a perfused rat liver system (Nicholas and Jones, 1991; Park and Huang, 1993; Liu et al., 1995). Again, these suppressive factors had no effect on liposome recognition by the splenic phagocytes.

4. Opsono-recognition of liposomes in a tumour model

Pathophysiological alterations in the blood opsonic activity and in macrophage function and responsiveness can affect the rate and the site of particle clearance from the blood (Graham and Saba, 1973; Ryder et al., 1975; Ellens et al., 1983; Luostarinen and Vorne, 1986; Moghimi et al., 1993a; Moghimi and Murray, 1996; Moghimi, 1997). For example, over two decades ago Ryder et al. (1975) demonstrated that the elevation of serum calcium levels above normal can inhibit opsono-recognition of lipid emulsions by Kupffer cells. A number of experimental and clinically encountered neoplasms have reported elevation in serum calcium levels above the physiological concentration (Gardner, 1969; Haskell et al., 1971; Gislason et al., 1987). Interestingly, upon chemotherapy or surgical resection of the tumour the calcium levels return to normal, while tumour recurrence is accompanied by a reappearance of hypercalcaemia. Saba and Antikatzides (1975) evaluated the functional state of the reticuloendothelial system during tumour growth and have revealed that the terminal stages of tumour growth and spread (a hypercalcemic serum state) were associated with pronounced serum opsonic dysfunction and associated depression in hepatic Kupffer cell clearance of particles from the blood. These findings, when placed in relationship to the temporal change of clacium levels with tumour growth, suggest a potential effect of serum calcium concentration on reticuloendothelial function. Since calcium can regulate opsono-recognition of multilamellar egg PC liposomes, we extended our studies to determine the role of calcium and serum opsonins on liposome recognition and clearance by macrophages of the liver and the spleen during the terminal stages of an experimentally induced non-metastatic tumour model (chondrosarcoma) in rats (Moghimi and Patel, 1996).

We demonstrated that the liver of chondrosarcoma-bearing animals manifest a decline in liposome clearance from the blood when compared to healthy animals of the same age. In contrast, an increase in splenic uptake of liposomes was encountered in tumour-bearing animals (Moghimi and Patel, 1996). The reticuolendothelial phagocytic and serum opsonic activity was then evaluated in an in vitro system. In a cell-suspension assay, serum of healthy animals equally enhanced liposomes recognition by freshly isolated hepatic phagocytes of both normal and tumour-bearing rats. In contrast, both cell populations manifested poor liposome recognition in the presence of serum pooled from tumour-bearing animals and the results were comparable to the corresponding liposome-cell interaction in the absence of serum. Interestingly, the opsonic activity of serum from tumour-bearing rats could only be demonstrated either by prior dialysis of serum against de-ionized water or by addition of EGTA. However, liver phagocytes of healthy animals recognized more liposome in the presence of dialysed or EGTA-chelated tumour-serum than that of liver cells derived from tumour transplanted rats (Moghimi and Patel, 1996). These observations may suggest the possibility of certain changes (e.g. receptor expression, receptor mobility) associated with liver macrophages that can affect opsono-recognition of liposomes in this cancer model. We also measured a significant increase in serum calcium concentration in all chondrosarcoma-bearing rats. When the concentration of calcium in the serum of normal animals was increased to the level that is encountered in tumour-bearing rats, a sharp drop in liposome recognition by liver phagocytes was observed. This drop in opsonic activity was not related to changes in the ionic strength of serum. These studies were also reproduced with the partially purified opsonic molecule, but liposome recognition by liver phagocytes of healthy animals was more enhanced in the presence of the opsonic factor from healthy rats than that of tumour-bearing animals (Moghimi and Patel, 1996).

In contrast to liver phagocytes, studies with isolated splenic phagocytes suggested that an increase in the opsonic activity of serum, but not the elevated calcium level, was responsible for hyperphagocytosis of liposomes by the splenic phagocytes of tumour-transplanted animals (Moghimi and Patel, 1996). Remarkably, these in vitro findings are in good agreement with the in vivo distribution pattern of intravenously injected liposomes and suggest that the alteration in macrophage clearance of liposomes during the terminal growth of cancer may be mediated in part by changes in the opsonic capacity of serum. Accordingly, some of these changes may have been induced by factors such as the elevated blood calcium levels. Although our conclusions are in close agreement with the views of Saba and Antikatzides (1975) it should be stressed that the nature of the opsonic molecule and test particles were different between the two studies. Gelatin-coated lipid emulsions were used as the phagocytic substrate by Saba and Antikatzides (1975) and the opsonic molecules were identified as fibronectin. The relationship of these observations to disturbances of the macrophage system during malignancy, which is known for its ability to alter the serum calcium concentration, warrants detailed investigation.

Finally, the work outlined in this essay was performed in collaboration with Dr H.M. Patel at the Biochemistry Department of Charing Cross Hospital, London. I would like to take this opportunity to thank Harish Patel for teaching me and initiating this stimulating research.

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