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Effect of opsonins on the uptake of magnetic starch microspheres by rat Kupffer cells

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The influence of various sera and proteins on the uptake of a superparamagnetic colloid (magnetic starch microspheres (MSM); particle size, 200 nm; crystal size, 10 nm) by the isolated and perfused rat liver has been studied. It is demonstrated that the capture of MSM is slightly reduced by the addition of rat blood to the protein-free perfusion medium but highly reduced by newborn calf serum (NCS). The SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analysis of proteins adsorbed on the nanoparticles incubated in NCS reveals major coating by albumin and IgG. The addition of bovine IgG to the perfusion fluid reduces the rate of MSM uptake in the same extent that NCS, whereas fetal calf serum that contains only traces of IgG weakly alters the MSM clearance. Finally, complemented and decomplemented NCS exhibit the same influence on the MSM hepatic extraction. It is concluded that although lectins are largely involved in the uptake of MSM administered in the absence of proteins, opsonins receptors are implicated when the perfusion medium contains relevant blood components.

Keywords: MRI contrast agents, superparamagnetic particles, opsonins, perfused liver.

INTRODUCTION

Superparamagnetic particles are promising agents for magnetic resonance imaging (MRI) contrast enhancement. They are made of ferrite cores (i.e., iron oxides) dispersed in biocompatible coating which prevents aggregation and may govern their biodistribution [1]. Magnetic starch microspheres (MSM) are such particles with an overall size of 200 ± 50 nm consisting of 10-nm magnetic crystals coated with starch [2]. After intravenous injection in animals, MSM are rapidly concentrated in the mononuclear phagocytotic system, mainly in the liver and spleen [3]. The mechanisms of recognition and clearance of these contrast agents are not yet fully understood. One remaining question is whether the recognition of MSM by Kupffer cells is mediated by opsonins.

As previously demonstrated in the isolated rat liver perfused with Krebs–Henseleit solution, the uptake of MSM by Kupffer cells is mediated by lectins [4]. However, *in vivo* recognition factors called opsonins are known to cover exogenous materials and to promote their binding to macrophage receptors. Numerous molecules show opsonic activity, e.g., fibronectin, immunoglobulins, and complement factors. The coating depends on the nature and on the size of the foreign body. This has been well documented for liposomes [5, 6] and polyacrylstarch microparticles [7, 8].

In the presence of blood components, other mechanisms than those involving lectins are, thus, expected to participate to the clearance of MSM by the liver, as Kupffer cells contain receptors for fibronectin, immunoglobulins (IgG and IgM), and factor C3 of the complement.

In this study, the influence of blood components from various origins on the hepatic MSM uptake was evaluated and the nature of some opsonins involved in the recognition of those colloidal contrast agents was determined by electrophoresis.

MATERIALS AND METHODS

Livers from male Wistar rats (±150 g) were isolated and perfused at 37°C with 3–4 ml min^{-1} g^{-1} of liver weight through the portal vein with 180 ml of a recirculating medium. Krebs–Henseleit solution was

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used for a control group (n = 10). For the other groups (n = 8 for each group), the perfusion fluid was supplemented with (i) 5% of rat blood from the liver donor, (ii) newborn calf serum (NCS) (Sigma, 021-06010) complemented and decomplemented by heating at 56°C for 30 min, and (iii) fetal calf serum (FCS) (Sigma, 011-06290). Two other groups of livers received 500 mg of bovine serum albumin and increasing doses (25 and 50 mg) of IgG, respectively.

MSM were added to the perfusion medium at a dose of 100 μg of iron per g of liver weight and their concentration in the perfusate was followed by proton relaxometry on aliquots of 0.3 ml at 0.47 T and 37°C on a spin analyzer (Minispec PC-120; Bruker, Karlsruhe, Germany). Knowing the r_2 relaxivity in these conditions (177 s^{-1} mM^{-1}), the concentration was calculated from the relaxation rate R_2 of the perfusion medium. ^{31}P–NMR spectra of the perfused livers were obtained on a spectrometer (AMX-300; Bruker, Karlsruhe, Germany) with the following conditions: number of averages = 300, flip angle = 60°, and repetition time = 1 s.

In order to identify the proteins adsorbed on the particles, the MSM were suspended in Krebs–Henseleit solution supplemented with NCS. The mixture was incubated at 37°C for 1 h. The particles were then concentrated and purified by magnetophoresis and washed with sodium dodecyl sulfate (SDS) 1%. SDS–PAGE (polyacrylamide gel electrophoresis) of proteins was performed on a LKB 2050 Midget electrophoresis unit.

RESULTS AND DISCUSSION

The concentration of MSM in the Krebs–Henseleit medium delivered to the isolated and perfused rat liver progressively decreases due to their uptake by Kupffer cells (A in Fig. 1). The observed half-life is 14 min.

The clearance of MSM by the liver is slightly reduced by rat blood (C in Fig. 1) but highly decreased in the presence of NCS (D in Fig. 1). This can be attributed either to an effect of the serum components on the phagocytic capability of the liver or to an alteration of the nanoparticles.

The first hypothesis of a direct influence of serum components on the liver was evaluated and rejected by the following experiments. First, no influence of the assayed sera on the hepatic metabolism was observed in the ^{31}P–NMR spectra which evolved identically to the controls for a period of 90 min. Second, the inhibiting effect of NCS could be counteracted by a subsequent addition of rat blood (results not shown).

In order to verify the assumption of a modification of the nanoparticles, electrophoresis was performed

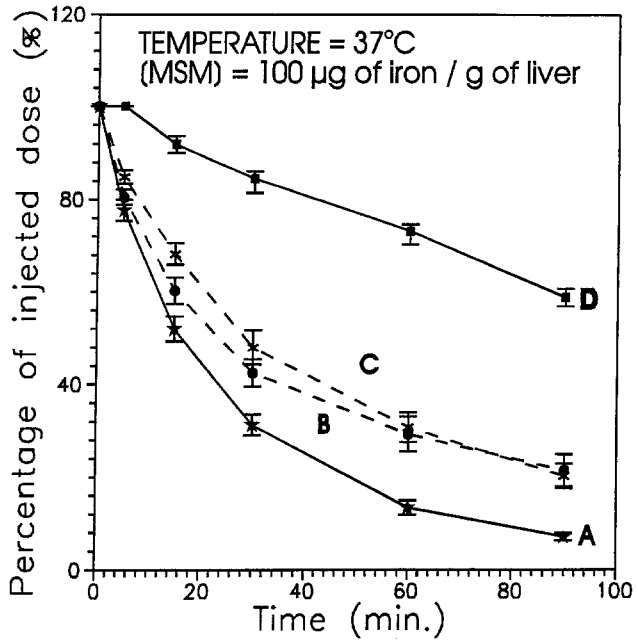

Fig. 1. Evolution of the MSM concentration in the perfusion fluid of isolated rat livers perfused with (A) Krebs–Henseleit solution, (B) fetal calf serum, (C) rat blood, and (D) newborn calf serum.

on MSM incubated in NCS. The electropherogram revealed two bands corresponding to albumin and IgG. These results give evidences for the opsonization of the nanoparticles by IgG. The weak albeit significant affinity of bovine IgG for rat Kupffer cells receptors could explain the large reduction of the MSM clearance induced by NCS with respect to rat blood. The experiment was repeated with FCS that is known to contain only traces of IgG (7.510^{-4} g L^{-1}) as compared to NCS (21 g IgG L^{-1}). The results obtained show but a weak influence of FCS on the MSM clearance (B in Figure 1) and, thus, confirm the predominant role of IgG.

On the other hand, bovine serum albumin (BSA) has a much smaller influence than bovine IgG, which, to the contrary, has a significant and dose-dependent effect (Fig. 2). Finally, the thermal denaturation of complement factors of NCS (56°C for 30 min) does not alter its effect on MSM clearance (data not shown).

CONCLUSIONS

Previously, we have demonstrated that the endocytosis of MSM by the isolated rat liver perfused with Krebs–Henseleit solution was mediated by Kupffer cells lectins [4]. We now could show that the addition of rat serum to the perfusion fluid promotes the

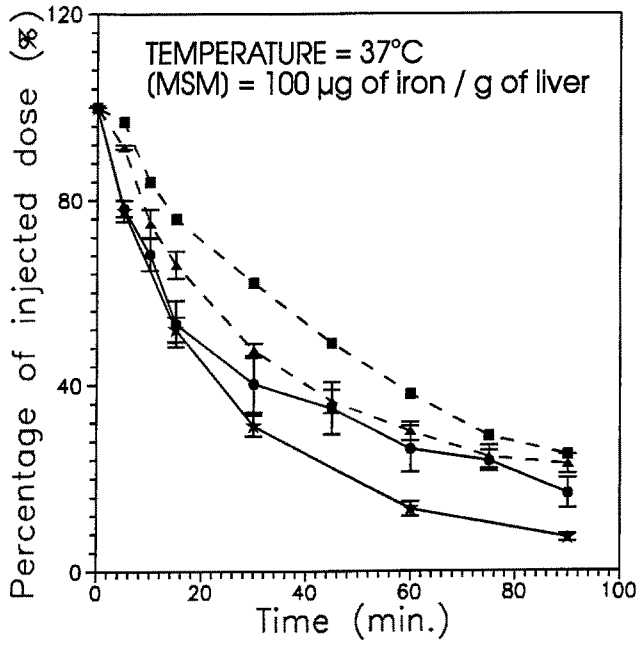

Fig. 2. Evolution of the MSM concentration in the perfusion fluid of isolated rat livers perfused with Krebs–Henseleit solution (★) and with Krebs–Henseleit solution supplemented with 500 mg of BSA (▲) or 25 mg (●) or 50 (■) of bovine IgG.

coating of the particles by IgG and albumin, leading to a shift to other receptors and to a decrease of the MSM clearance. Thus, the predominant role of IgG becomes clear. Harashima et al. [5] reported that opsonization decreased the extraction of small liposomes (0.2 µm) by the rat liver perfused *in situ* but increased the uptake of large liposomes (0.8 µm). The latter observation was supported by Artursson et al. [7, 8] who reported similar opsonization of polyacrylstarch and polyacrylmannan microparticles of about 2 µm, followed by an increase in uptake by macrophages in culture. The behavior of our colloidal system, which is characterized by a mean particle size of 0.2 µm, is thus in good agreement with the results reported by the first authors.

The binding of IgG to rat Kupffer cells receptors seems to be very specific because the coating of MSM by bovine IgG strongly prolongs the persistence of the particles in the perfusion medium. The process is reversed when nanoparticles coated with bovine IgG are opsonized with rat blood.

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