INVESTIGATIVE RADIOLOGY
Volume 29, Supplement 2, S223–S225
©1994, J.B. Lippincott Company

Evidence for Attachment of Magnetic Starch Microspheres to Kupffer Cells Receptors in Excised and Perfused Rat Liver

JEAN-MARIE COLET, MA, YVES VAN HAVERBEKE, PHD, AND ROBERT N. MULLER, PHD

Colet J-M, Haverbeke YV, Muller RN. Evidence for attachment of magnetic starch microspheres to Kupffer cells receptors in excised and perfused rat liver. Invest Radiol 1994;29: S223–S225.

KEY WORDS. **Magnetic resonance imaging contrast agents; perfused liver; receptors; magnetic particles.**

HEPATIC MAGNETIC RESONANCE imaging (MRI) contrast media fall in two categories of agents according to their cellular distribution[1]: parenchymal cells can be targeted by agents that are able to cross the sinusoidal wall (ie, low molecular weight paramagnetic compounds and very small particles (diameter < 30 nm) coated with arabinogalactan),[2,3] whereas the reticuloendothelial system specifically internalizes larger particles like iron oxide crystals coated by polysaccharides. After intravenous administration, these large magnetic particles are mainly distributed in the liver and the spleen because of their uptake by macrophages. Inside the cells, they are eventually enclosed and degraded in lysosomal vacuoles.[4]

Magnetic starch microspheres (MSM) are such agent made of iron oxide crystals coated with hydrolyzed starch.[5] Because of their relatively large size (200 ± 50 nm), MSM remain in the sinusoids of the rat liver, where they are exclusively phagocyted by fixed macrophages, the Kupffer cells.

Working on macrophages in culture, Fahlvik et al[6] have demonstrated that the uptake is inhibited at 4°C and is highly competed by degradable starch microspheres, suggesting a receptor-mediated mechanism for endocytosis. Kupffer cells are indeed specialized in this process, particularly for substances bearing carbohydrate moieties. Several lectins have been identified on plasma membrane of Kupffer cells, among which the galactose-terminated glycoproteins receptor,[7,8] the fucose receptor,[9] and a lectin for mannose.[10] The results previously obtained on macrophages in culture[6] and the nature of the MSM coating suggest a possible interaction of MSM with lectins at the Kupffer cells membrane. The purpose of this preliminary study is, therefore, to clarify the possible role of carbohydrate receptors in the uptake process of MSM by the isolated and perfused rat liver.

Materials and Methods

Wistar male rats weighing 120 to 150 grams were anesthetized with 0.2 g of urethane (U-2500, Sigma, St. Louis, MO) and injected with 500 U.I. of heparin (Novo Nordisk, Bagsvaerd, Denmark) into the vena cava.

The livers were isolated as described[11] and perfused at constant flow (3–4 mL per g of liver^{-1}min^{-1}) through the portal vein with 200 mL of a recirculating Krebs-Henseleit solution containing 5.5 mM of D(+)-glucose monohydrate (Merck, 8342, Darmstadt, Germany) and 5.5 mM of pyruvic acid sodium salt (Merck, 6619.0250).

Magnetic starch microspheres was used as the contrast agent in the experiments (MSM, Nycomed, Oslo, Norway). Because the transverse relaxation rate R2 of water protons is linearly related to the MSM content,[12] the dosage of superparamagnetic material in the perfusion fluid was achieved by relaxometric measurements (0.47 T, 37°C) on aliquots of perfusate on a spin analyzer Minispec-PC120 (Bruker, Karlsruhe, Germany). The transverse relaxivity of MSM is 177 s^{-1}mM^{-1} at 37°C.

Protocol 1

Eight rat livers were perfused at 37°C. After 10 minutes of stabilization, MSM were added to the perfusion medium at the dose of 40 μg of iron per gram of liver. The MSM concentration in the perfusion fluid is determined by relaxometry.

From the University of Mons-Hainaut, NMR Laboratory, Mons, Belgium.

Supported by the Action de Recherche Concertée Program 90/94-142 of the Communauté Française de Belgique.

Reprint requests: Robert N. Muller, NMR Laboratory, University of Mons-Hainaut, 7000 Mons, Belgium.

Protocol 2

Eight rat livers were submitted to the above mentioned protocol, but the perfusion temperature was set at 4°C. After 10 minutes at rest, MSM were administered at the dose used in the previous group, and the dosage was carried out the same way.

Protocol 3

Livers were perfused at 4°C in the presence of various specific antagonists likely to compete with MSM for the Kupffer cells receptors. One hundred milligrams of the antagonist was added to the perfusion medium and was allowed to circulate throughout the experiment. Magnetic starch microspheres were added to the perfusion fluid 20 minutes later, and its concentration was followed by relaxometry.

Soluble starch (Merck, 1253), D(+)-galactose (Merck, 4060), D(+)-fucose (ICN, 101485), D(−)-fructose (Sigma, F-0127), N-acetyl-glucosamine (Sigma, A-6825) and D(+)-mannose (Sigma, M-4625) were used. Eight livers were examined for each antagonist.

Results

At 37°C (Fig. 1A), the concentration of MSM progressively decreased in the perfusion fluid because of its hepatic uptake. A half-life of 17 minutes was observed.

The same experiment performed at 4°C to inhibit the internalization step of endocytosis by macrophages confirmed that the clearance was highly reduced (Fig. 1B). The half-life was then extended to 56 minutes.

These results support the view that the hepatic uptake of the MSM takes place via an endocytic mechanism that can be blocked by hypothermic perfusion.

This phenomenon is reversible, because the uptake is restored when the temperature is returned to 37°C (results not shown).

Although the clearance was highly reduced at low temperature, a small but significant fraction of particles still disappeared from the perfusion medium. This observation supports the hypothesis of an attachment of the MSM to receptors on the plasma membrane of Kupffer cells. To check this mechanism, the livers were perfused at 4°C with a solution containing soluble starch, a substance able to compete for the receptors potentially involved. As shown in Figure 1C, soluble starch considerably reduced the fraction of particles retained by the liver at low temperature.

A better description of the receptor involved was brought by perfusing with more specific antagonists of the carbohydrate receptors. The results shown in Figures 2B through 2F demonstrate that D(+)-galactose is the most efficient to reduce the attachment of MSM to lectins (Fig. 2B). A slow decrease of particle concentration indicates, however, that this sugar does not totally inhibit the process. D(+)-mannose (Fig. 2D) and D(+)-fucose (Fig. 2C) also demonstrated a significant, but weaker effect. Finally, N-acetyl-glucosamine (Fig. 2E) and D(−)-fructose (Fig. 2F) did not inhibit the MSM attachment.

Discussion

Initially proposed for reticuloendothelial MRI, particulate contrast media are now designed to target various other tissues. In this context, an extended plasma half-life may be a stringent requirement, and the uptake by resident macrophages, ie, Kupffer cells, should be avoided.

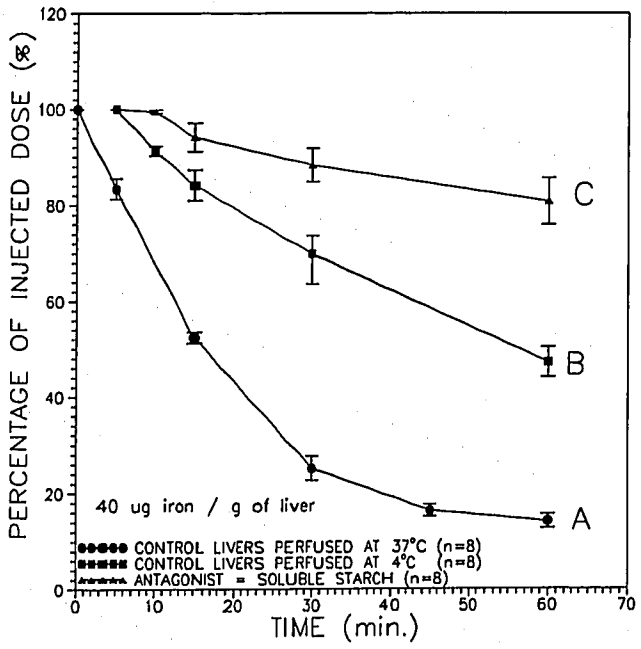

Fig. 1. Clearance of magnetic starch microspheres from the perfusion medium of rat livers perfused at 37°C (A), 4°C (B) and 4°C in the presence of soluble starch (C).

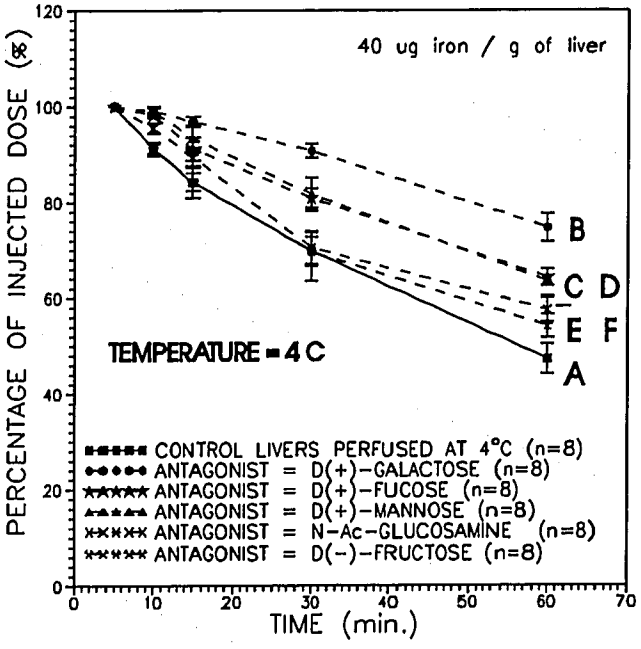

Fig. 2. Influence of specific antagonists on the clearance of magnetic starch microspheres from the perfusion medium of rat livers perfused at 4°C.

While the internalization of MSM can be inhibited in the isolated rat liver perfused at 4°C, thus confirming the involvement of an endocytic mechanism as previously hypothesized on macrophages in culture,[6] a small but significant fraction of particles is nevertheless retained by macrophages. An initial perfusion with soluble starch drastically reduces this uptake and demonstrates an attachment of MSM to cellular receptors.

Our inhibition experiments with various carbohydrates verify that MSM binding to Kupffer cells is caused by membrane lectins. The results give evidence for a major involvement of the receptor for galactose-terminated glycoproteins.

Binding of the particles to hepatocytes receptors also could be proposed to explain retention of MSM by the liver perfused at 4°C. However, several arguments can oppose this hypothesis. First of all, it has been shown that after its intravenous injection in rats, iron-59-radiolabelled MSM is only located in the nonparenchymal liver cell fraction.[13] This selectivity is attributable to the size of the particles, which are too large to cross the sinusoidal fenestrae. Smaller dextran-coated nanoparticles (10–70 nm) have been prepared and investigated in animal studies.[14] As expected, these particles of reduced size cross sinusoidal wall and reach the space of Disse where they are close to the hepatocytes. However, even in this case, the cellular distribution shows that the particles are only detected in the Kupffer cells. Definitive confirmation of selective retention of the particles in our protocol will be brought by electron microscopy of livers perfused at 4°C with MSM.

In the foregoing discussion, a possible interaction of MSM with biological macromolecules has been neglected, because in the described protocol, the livers were perfused at 4°C with a solution originally free of proteins. Opsonins released by the liver during the course of the experiment could however take part to the process. In this context, the influence of various sera on the hepatic uptake of MSM is currently investigated (J.M. Colet, et al. unpublished data, 1993).

Conclusions

The results of this study confirm the hypothesis of a receptor- mediated uptake of magnetic-starch-coated superparamagnetic particles by the liver. An attachment of the particles to the galactose-receptor has been demonstrated. Other lectins, still unidentified, contribute to this preliminary phase of the internalization.

From an experimental point of view, the potential of the isolated perfused rat liver model combined with relaxometric analysis has proven its efficiency for the study of hepatotropic MRI contrast media.

Acknowledgments

The authors thank Prof. P. Canioni (Marseille, France) and Dr. A.K. Fahlvik (Nycomed, Oslo) for valuable scientific advice.

References

1. Schuhmann-Giampieri G. Liver contrast media for magnetic resonance imaging. Interrelations between pharmacokinetics and imaging. Invest Radiol 1993;28:753–761.
2. de Haën C, Gozzini L. Soluble-type hepatobiliary contrast agents for MR imaging. J Magn Reson Imaging 1993;3:179–186.
3. Reimer P, Weissleder R, Lee AS, Wittenberg J, Brady TJ. Receptor imaging: application to MR imaging of liver cancer. Radiology 1990;177:729–734.
4. Weissleder R, Reimer P. Superparamagnetic iron oxides for MRI. Eur Radiol 1993;3:198–212.
5. Fahlvik AK, Holtz E, Leander P, Schroder U, Klaveness J. Magnetic starch microspheres: efficacy and elimination. Invest Radiol 1990; 25:113–120.
6. Fahlvik AK, Artursson P, Edman P. Magnetic starch microspheres: interactions of a microsphere MR contrast medium with macrophages in vitro. Intern J Pharm 1990;65:249–259.
7. Kolb-Bachofen V, Schlepper-Schäfer J, Vogell W. Electron microscopic evidence for an asialoglycoprotein receptor on Kupffer cells: localization of lectin-mediated endocytosis. Cell 1982;29:859–866.
8. Lentini A, Falasca L, Autuori F, Dini L. The simultaneous exposition of galactose and mannose-specific receptors on rat liver macrophages is developmentally regulated. Bioscience Reports 1992;12: 453–461.
9. Lehrman MA, Pizzo SV, Imber MJ, Hill RL. The binding of fucose-containing glycoproteins by hepatic lectins. J Biol Chem 1986;261: 7412–7439.
10. Wileman TE, Lennartz MR, Stahl PD. Identification of the macrophage mannose receptor as a 175 kDa membrane protein. Proc Natl Acad Sci 1986;83:2501–2505.
11. Delmas-Beauvieux MC, Gallis J-L, Rousse N, Clerc M, Canioni P. Phosphorus-31 nuclear magnetic resonance of isolated rat liver during hypothermic ischemia and subsequent normothermic perfusion. J Hepatology 1992;15:192–201.
12. Roch A, Bach-Gansmo T, Muller RN. In vitro relaxometric characterization of superparamagnetic contrast agents. Magma 1993;1: 83–88.
13. Fahlvik AK, Holtz E, Klaveness J. Relaxation efficacy of paramagnetic and superparamagnetic microspheres in liver and spleen. J Magn Reson Imaging 1990;8:363–369.
14. Pouliquen D, Le Jeune JJ, Perdrisot R, Ermias A, Jallet P. Iron oxide nanoparticles for use as an MRI contrast agent: pharmacokinetics and metabolism. J Magn Reson Imaging 1991;9:275–283.