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SHORT COMMUNICATION

Distribution of Liposome-Encapsulated Iodixanol in Rat Liver Cells

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ABSTRACT. Distribution of liposome-encapsulated [^{125}I]iodixanol in different types of liver cells following intravenous injection was studied in rats. The data showed that liposome-encapsulated [^{125}I]iodixanol was rapidly taken up by the liver; after 15 min, radioactivity corresponding to nearly 25% of the injected radioactivity could be recovered therein. After 4 hr, approximately 60% of the injected radioactivity was in the liver. One week after injection, nearly 30% of the encapsulated radioactivity could still be recovered in the liver. Liposome-encapsulated [^{125}I]iodixanol was taken up both by hepatocytes and the Kupffer cells. On a per cell basis, the uptake of liposome-encapsulated [^{125}I]iodixanol in Kupffer cells was more than 10-fold greater than that in hepatocytes, while the contribution of liver endothelial cells to uptake was negligible. Osmotic protection studies showed that iodixanol does not readily diffuse across lysosomal membranes, indicating that loss of iodixanol from the liver probably occurred by recycling rather than by diffusion across phagolysosomal and plasma membranes. BIOCHEM PHARMACOL **60**;4:553–559, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. iodixanol; hepatocytes; Kupffer cells; liposome; liver endothelial cells; phagocytosis; x-ray contrast medium

This study was performed to investigate in detail the cellular distribution of liposome-encapsulated [^{125}I]iodixanol, an exploratory x-ray contrast medium for liver imaging. Iodixanol is a 1.55-kDa water-soluble, hydrophilic, and non-ionic iodinated contrast agent for x-ray imaging. After intravenous administration, iodixanol is distributed into the extracellular fluid space and rapidly eliminated via the kidneys. After encapsulation into liposomes, the hydrophilic character of iodixanol is masked, changing the pattern of biodistribution in such a way that the liposomally encapsulated iodixanol will probably be specifically taken up by phagocytosis of the Kupffer cells of the liver. Since the phagocytic activity is predominantly found in normal hepatic tissue but not in tumors and lesions, liposomes containing x-ray contrast agents might be helpful tools in the diagnosis of liver tumors and lesions [1, 2]. The diagnostic potential of liposome-encapsulated iodixanol is mainly the detection of liver lesions.

Kupffer cells are the macrophages of the liver and are very active phagocytic cells directly exposed to the blood stream. Hepatocytes have, however, also been reported to phagocytose particles [3, 4], and one should therefore not exclude the possibility that liposomes could also be taken up by these cells. Furthermore, the formulation used in this study consists of a mixture of iodixanol encapsulated in liposomes and "free" non-encapsulated iodixanol. While encapsulated iodixanol would be taken up by phagocytes, non-encapsulated iodixanol is internalized by pinocytosis into many different cell types.§ Rat liver is very active in fluid-phase endocytosis, and this process may therefore contribute significantly to the uptake of blood constituents. Once internalized into the endocytic/phagocytic compartments, liposome-encapsulated [^{125}I]iodixanol may leave the cell either by recycling or by diffusion through the phagosomal/lysosomal membrane and the plasma membrane. The kinetics of these processes will probably vary in different cell types. The relative distribution of [^{125}I]iodixanol between different cell types in the liver could therefore vary over time following an intravenous injection.

While recycling from the endocytic pathway has been studied in detail, less is known about regurgitation from phagosomes. Although the importance of such a pathway for antigen presentation on MHC II class (major histocompatibility complex II) molecules is well established [5], much is yet to be learned about the kinetics of "retrophagocytosis", and the intracellular routes by which this occurs. Studies of phagocytosis using liposomes enclosing fluid-phase markers such as [^{125}I]iodixanol may therefore give valuable information about these processes.

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MATERIALS AND METHODS

Materials

ASOR* was prepared as described by Tolleshaug and Berg [6] and labeled by covalent attachment of a radioiodinated TC adduct according to Pittman *et al.* [7]. BSA, collagen (type IV), collagenase (type IV), dexamethasone, diaminobenzidine, EDTA, HEPES, and horseradish peroxidase were obtained from Sigma. Cell culture dishes (5.0 cm diameter) were obtained from Becton Dickinson. Dulbecco's modified Eagle's medium, gentamicin sulfate, L-glutamine, and Eagle's minimum essential medium were obtained from BioWhittaker. Human fibronectin was a gift from Dr. Bård Smedsrød, Institute for Medical Biology, University of Tromsø. Formulation of [^{125}I]iodixanol containing liposomes (made from phospholipids with encapsulated [^{125}I]iodixanol and non-encapsulated [^{125}I]iodixanol in the ratio of 1:1.4 (radioactive concentration 3.5 MBq/mL; 80 mg encapsulated iodine per mL); liposome size 200–400 nm; 50 mg hydrogenated phosphatidylcholine and 5 mg hydrogenated phosphatidylserine per mL), [^{125}I]iodixanol, iodixanol, heparin (5000 IU/mL), Nycodenz®, and TC were supplied by Nycomed Imaging A/S. The Micro BCA (bicinchoninic acid) Protein Assay Reagent Kit was obtained from Pierce. All additional chemicals were of analytical grade.

Animals

Male Wistar rats, weighing 180–200 g at the start of the study, were obtained from Møllegaard Breeding Center. The animals were kept three or four to a cage. They were housed in an animal room with a light/dark cycle of 12 hr, a temperature 24 ± 2°, and a relative humidity of 55 ± 10%. The animals were given water and food *ad lib*.

Uptake of Liposome-Encapsulated [^{125}I]Iodixanol

Fifteen rats were randomly allocated to 5 groups, with 3 rats in each group. The rats were anesthetized by i.p. injection of pentobarbital (50 mg/kg body weight). A dose of liposome-encapsulated [^{125}I]iodixanol (100 mg encapsulated I/kg body weight) was given by intravenous injection into the tail vein of 3 rats in each group. The injection rate was 1.2 mL per min. The volume of substance injected was approximately 250 μL, containing about 50×10^6 cpm. At various time points following injection (15 min, 1 hr, 4 hr, 24 hr, and 1 week), the rats were laparotomized and heparin-injected (300 μL heparin/rat i.v. injection, 5000 IU/mL). The portal vein was cannulated and a standard liver perfusion with collagenase was performed [8]. Immediately before collagenase perfusion, the smallest liver lobe was tied off, cut away, chilled on ice, and analyzed for total uptake in the liver. The liver cell suspension was separated into hepatocytes, endothelial cells, and Kupffer cells by a combination of differential centrifugation and centrifugal elutriation [9]. A fraction of the hepatocytes, Kupffer cells, and endothelial cells was purified by selective seeding [10], counted using an eye-piece graticule, and then scraped off the Petri dishes in PBS with 0.5% Triton X-100 and analyzed for radioactivity and protein. The rest of the Kupffer and endothelial cells was further purified by centrifugation through Nycodenz [11]. At this stage, aliquots of the cells were taken out for peroxidase staining [12] and counted in suspension. After counting, the cell suspensions were analyzed for radioactivity and protein.

Uptake of [^{125}I]Iodixanol

A dose of [^{125}I]iodixanol (100 mg I/kg body weight) was given by intravenous injection into the tail vein of 3 rats. The injection rate was 1.2 mL per min. The volume of substance injected was approximately 250 μL, containing about 10^7 cpm. Twenty-four hours following injection, the rats were laparotomized and heparin-injected (300 μL heparin/rat i.v. injection, 5000 IU/mL). The portal vein was cannulated and the liver perfused with calcium-free buffer for 10 min at a flow rate of 50 mL/min. Liver lobes were removed and analyzed for radioactivity.

Osmotic Protection Experiments

Osmotic protection experiments were performed according to Iveson with some modifications [13]. Rat liver lysosomes were isolated from rats injected with ^{125}I-TC–ASOR* 3 hr prior to killing in order to load lysosomes with ^{125}I-TC. The liver was homogenized in 2.5 vol. of ice-cold homogenization buffer (0.25 M sucrose containing 10 mM HEPES and 10 mM EDTA, pH 7.25). The homogenate was diluted fourfold and subjected to centrifugation for 2 min at 2000 *g* to remove nuclei. The postnuclear supernatant was subjected to differential centrifugation, and the fraction sedimenting between $1100 \times g$ (10 min) and $22{,}500 \times g$ (10 min) was gently resuspended in a volume of 0.25 M test solution (iodixanol, iohexol, or sucrose) equal to four times the original weight of the liver pulp. The suspension was placed in an incubation bath at 25°, and samples were withdrawn at 0, 30, and 60 min, layered on top of 10 mL of ice-cold homogenization buffer, and centrifuged for 10 min at $22{,}500 \times g$. Radioactivity was measured in pellet and supernatant. Osmolysis was calculated as radioactivity in the supernatant as a percentage of total.

Calculations and Statistics

The liver weight was taken as 4.1% of the body weight [14]. Total uptake was calculated and presented as mg iodine per gram liver. Uptake in isolated cells is presented as μg iodine per 10^6 cells or as ng iodine per cell protein. For calculation of the total uptake of radioactivity in the cell fractions, it was assumed that 1.0 g rat liver (wet weight) contains 1.9×10^7 Kupffer cells, 12.5×10^7 hepatocytes, and 3.5×10^7

* *Abbreviations:* ASOR, asialoorosomucoid; GPN, glycyl-phenylalanine 2-naphthylamide; and TC, tyramine cellobiose.

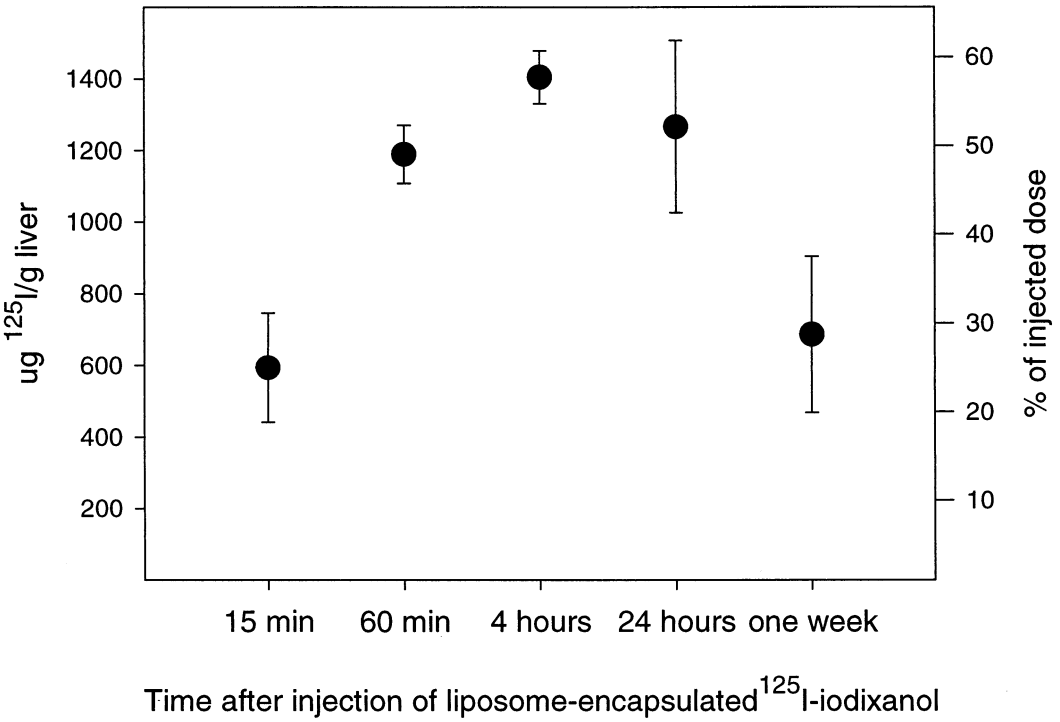

FIG. 1. Uptake of liposome-encapsulated [^{125}I]iodixanol in rat liver following intravenous injection. At various time points following intravenous injection, rats were laparotomized and the liver perfused with calcium-free buffer. The smallest liver lobe was removed and analyzed for total uptake in the liver. The ordinates show uptake expressed in μg ^{125}I/g liver (left) and the percentage of the injected dose recovered in the liver (right). The bars indicate one standard deviation (N = 3).

liver endothelial cells [15, 16]. Corrections for contamination by other cell types in the Kupffer fraction were made.

RESULTS

Uptake of [^{125}I]Iodixanol in Rat Liver

Since the formulation used in this study was composed of encapsulated [^{125}I]iodixanol as well as "free" [^{125}I]iodixanol outside of liposomes, we first wanted to determine the contribution of the non-encapsulated [^{125}I]iodixanol to the total uptake of radioactivity in the liver following intravenous injection. Rats were injected with a dose of [^{125}I]iodixanol comparable to the amount of "free" [^{125}I]iodixanol given in the liposome injection experiments; 24 hr following injection, liver samples were removed and analyzed for radioactivity. Uptake of [^{125}I]iodixanol was approximately 0.1% of the injected dose. The contribution of non-encapsulated [^{125}I]iodixanol to the uptake of radioactivity following intravenous injection of liposome-encapsulated [^{125}I]iodixanol is therefore negligible.

Uptake of Liposome-Encapsulated [^{125}I]Iodixanol in Rat Liver

Figure 1 shows the radioactivity in liver at various time points following injection of liposome-encapsulated [^{125}I]iodixanol. Liposome-encapsulated [^{125}I]iodixanol was rapidly taken up by the liver cells, and 15 min after injection, radioactivity corresponding to approx. 25% of the injected dose of encapsulated [^{125}I]iodixanol was recovered in the liver. A maximum of approximately 60% of the injected dose was recovered 4 hr after injection. The data also indicate that [^{125}I]iodixanol was retained in the liver for a substantial amount of time, since almost 30% of the injected dose of encapsulated [^{125}I]iodixanol remained in the liver one week after injection.

Cellular Distribution of Liposome-Encapsulated [^{125}I]Iodixanol

To investigate the cellular distribution of liposome-encapsulated [^{125}I]iodixanol in rat liver, various types of liver cells were isolated and analyzed for radioactivity as described in the Materials and Methods section. Figure 2 shows the per cell distribution of radioactivity in cultured hepatocytes, Kupffer cells, and liver endothelial cells at various time points following injection. On a per cell basis, the uptake of liposome-encapsulated [^{125}I]iodixanol by the Kupffer cells was much higher than that taken up by the other cell types at all time points. Four hours after injection, the radioactivity in the Kupffer cells was more than ten times that of the hepatocytes. Only negligible amounts of radioactivity were found in the liver endothelial cells at any time point after injection. Similar results were obtained using purified cell suspensions of hepatocytes, Kupffer cells, and liver endothelial cells (not shown).

The total cell-associated radioactivity in cultured hepa-

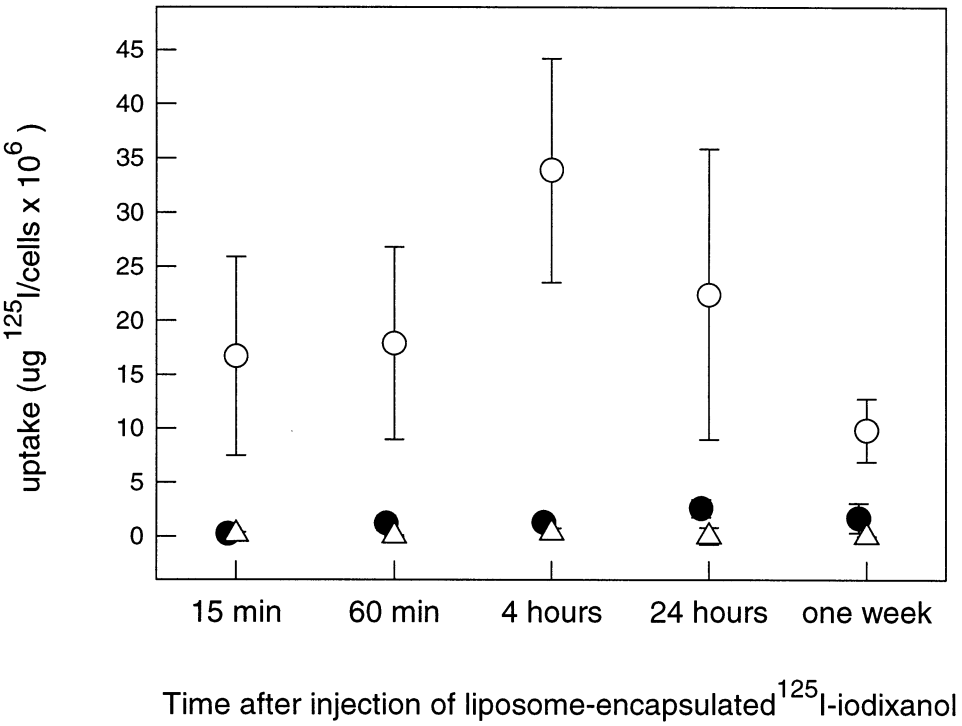

FIG. 2. Uptake of liposome-encapsulated [^{125}I]iodixanol by different cell types in rat liver. At various time points following intravenous injection, Kupffer cells (○), hepatocytes (●), and liver endothelial cells (△) were isolated and used as cultured cells. Cell-associated radioactivity was measured and expressed as μg ^{125}I/cells × 10^6. The bars indicate one standard deviation (N = 3).

tocytes, Kupffer cells, and liver endothelial cells at various time points following injection are shown in Fig. 3. While most of the radioactivity was recovered in the Kupffer cell fraction at early time points after injection, approximately equal amounts of radioactivity were recovered in the Kupffer and hepatocyte cell fractions at later time points, as the total level of radioactivity in the liver decreased. The data further indicate a different uptake kinetics in the Kupffer cells relative to the hepatocytes. While the initial uptake of liposome-encapsulated [^{125}I]iodixanol in the Kupffer cells was high, reaching a maximum level of cell-associated radioactivity 4 hrs after injection, radioactivity in the hepatocytes reached a maximum level after 24 hr.

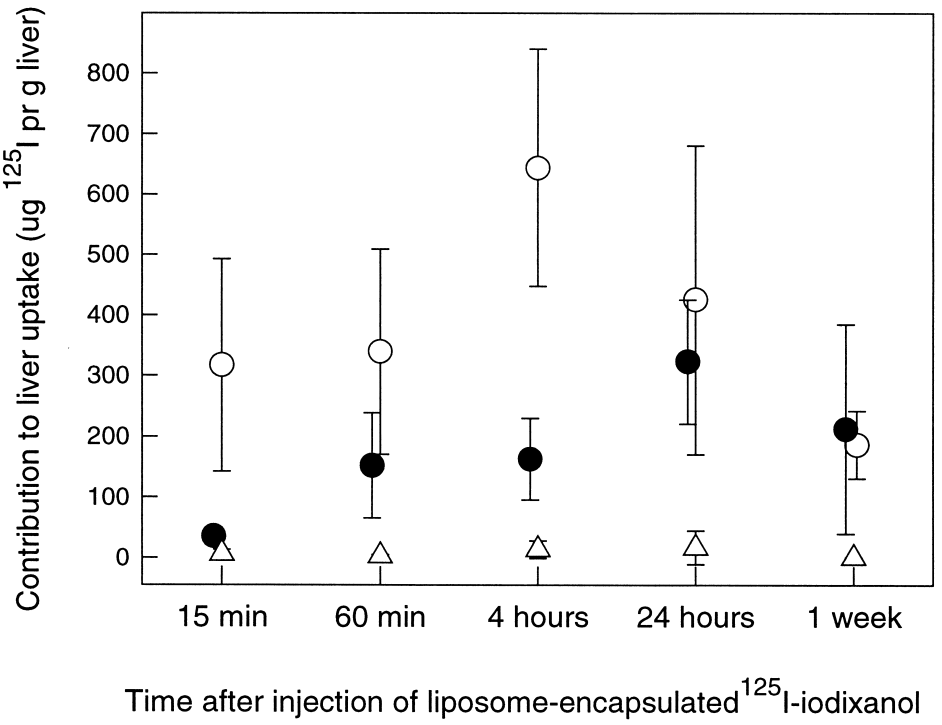

FIG. 3. Total contribution to uptake of liposome-encapsulated [^{125}I]iodixanol by different cell types in rat liver. At various time points following intravenous injection, Kupffer cells (○), hepatocytes (●), and liver endothelial cells (△) were isolated and used as cultured cells. Cell-associated radioactivity was measured and expressed as μg ^{125}I/g liver. Total contribution to uptake by the different cell types was calculated assuming that 1.0 g rat liver (wet weight) contains 1.9 × 10^7 Kupffer cells, 12.5 × 10^7 hepatocytes, and 3.5 × 10^7 liver endothelial cells. The bars indicate one standard deviation (N = 3).

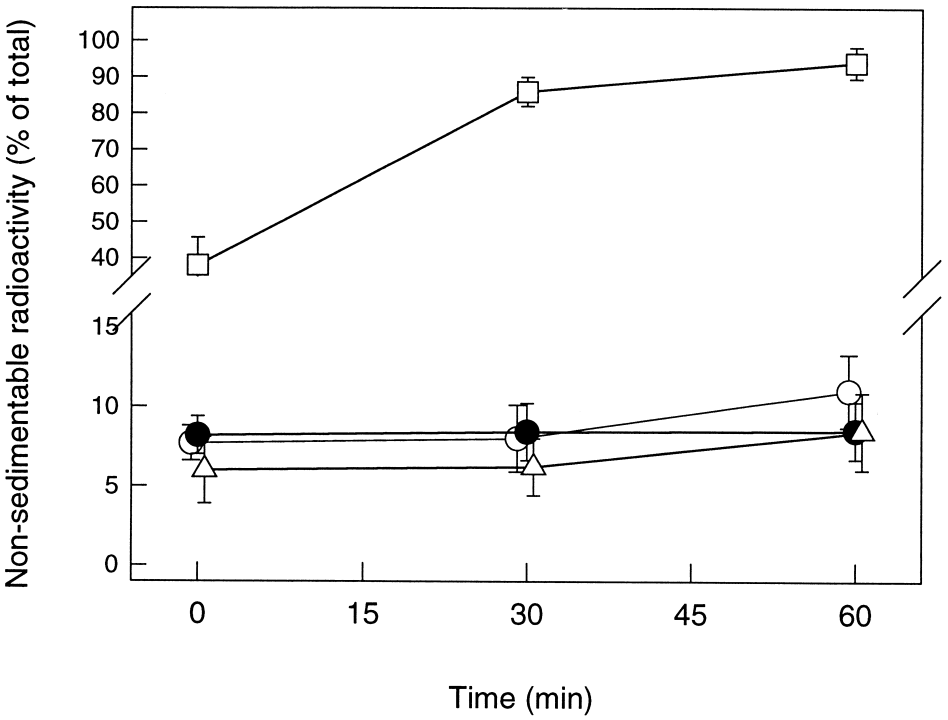

FIG. 4. Osmotic lysis during incubation with iodixanol. Rat liver lysosomes, isolated from rats injected with ^{125}I-TC–ASOR 3 hr prior to killing, were incubated in H_2O (□) or in the precence of 0.25 M iodixanol (●), iohexol (△), or sucrose (○), as described. At various times, samples were withdrawn and centrifuged for 10 min at 22,500 × g. Radioactivity was measured and osmolysis was calculated as radioactivity in the supernatant as a percent of total activity. The bars indicate one standard deviation (N = 3).

Osmotic Protection Experiments

To gain information on the ability of iodixanol to diffuse across lysosomal membranes, we performed osmotic protection experiments. Lysosomes, preloaded with ^{125}I-TC–ASOR, were isolated from rat liver and resuspended in 250 mM aqueous solution of solute (iodixanol, iohexol, or sucrose) as described. After incubation for various time intervals at 25°, the integrity of the lysosomes was assessed by pelleting them and measuring released radioactivity in the supernatants. As shown in Fig. 4, 250 mM iodixanol gave approximately the same osmotic protection as 250 mM sucrose. These results suggest that iodixanol does not readily diffuse across lysosomal/endosomal membranes.

DISCUSSION

The present data show that liposome-encapsulated [^{125}I]iodixanol is mainly taken up in the liver by Kupffer cells. Nevertheless, hepatocytes also play an important role in liver uptake, particularly at later time points after injection. One week after injection, the amount of iodixanol in liver was divided almost equally between Kupffer cells and hepatocytes. On a per cell basis, however, the uptake at all times tested much higher in the Kupffer cells was than in the hepatocytes and endothelial cells. The difference in uptake of liposome-encapsulated [^{125}I]iodixanol was even larger on a per protein basis, which merely reflects the difference in cell volume between the different cell types, since the protein content of cells is proportional to their cell volume. It is, however, the concentration of iodixanol in the different cell types that determines the level of imaging contrast. Therefore, the concentration of iodixanol per cell protein determines the usefulness of liposome-encapsulated iodixanol as an x-ray contrast agent.

The results presented show large variations between the three different animals in each group. Most of these variations probably reflect the difficulties in obtaining pure cell fractions. Both recovery and purity of the cells varied from experiment to experiment. This was especially the case with the Kupffer cells, which were difficult to isolate in high yield and to some extent were also contaminated by liver endothelial cells. Hepatocytes and liver endothelial cells were easier to purify. Nevertheless, since hepatocytes and liver endothelial cells contained little radioactivity, only a small contamination by Kupffer cells would cause a large overestimation of the uptake of iodixanol in these cells. These facts should especially be taken into consideration when comparing the uptake in hepatocytes and liver endothelial cells.

Approximately 60% of liposome-encapsulated [^{125}I]iodixanol found in liver lobes was recovered in the isolated cells. This discrepancy could have several explanations. First, the exact number of different liver cells per g liver is not known, and different reports vary in their estimates [15–17]. An overestimation of the number of isolated Kupffer cells would lead to an underestimation of uptake of iodixanol. Furthermore, iodixanol could to some degree have leaked out of the cells during the purification process. Moreover, the uptake of liposome-encapsulated iodixanol probably varied within the Kupffer cell population, and the methods used for purification of Kupffer cells may have selected such cells with low levels of iodixanol. The real uptake of liposome-encapsulated iodixanol in the Kupffer cell fraction was therefore probably higher than that observed.

It is likely that the difference in uptake of liposome-encapsulated iodixanol in Kupffer cells, hepatocytes, and liver endothelial cells reflects different mechanisms of uptake. The formulation used consisted of both [^{125}I]iodixanol contained in liposomes and of "free" [^{125}I]iodixanol outside liposomes. While most of the [^{125}I]iodixanol encapsulated in liposomes would be phagocytosed by the Kupffer cells, the bulk of radioactivity in liver endothelial cells probably comes from pinocytic uptake of non-encapsulated [^{125}I]iodixanol. There are, however, some indications that encapsulated [^{125}I]iodixanol may also be taken up by hepatocytes. The differences in uptake between hepatocytes and liver endothelial cells are not compatible with a mere pinocytic uptake of [^{125}I]iodixanol. Other studies on fluid-phase uptake in rat liver cells using various fluid-phase markers such as [^{3}H]raffinose, [^{125}I]-polyvinylpyrolidone, and [^{125}I]iodixanol showed that liver endothelial cells, on a per protein basis, have a much higher uptake than hepatocytes.* Moreover, intravenous injection of [^{125}I]iodixanol alone resulted in only a small liver uptake. Although the contribution of the individual cell types was not investigated in this experiment, it is reasonable to assume that on a per cell basis hepatocytes are the least active cell type in pinocytic uptake Since phagocytosis in hepatocytes has been reported by several groups [3], it is likely that the uptake of liposome-encapsulated [^{125}I]iodixanol in hepatocytes is not fluid-phase uptake of "free" [^{125}I]iodixanol, but mainly uptake of liposome-encapsulated [^{125}I]iodixanol.

Liposome-encapsulated [^{125}I]iodixanol was retained in the liver for at least one week following intravenous injection. From a clinical point of view, it is of course desirable that a compound used for x-ray imaging should not be retained in the body for a very long period of time. After uptake of liposome-encapsulated [^{125}I]iodixanol in the liver, the liposome membrane is probably rapidly degraded, leading to release of "free" [^{125}I]iodixanol into the phagosome. [^{125}I]Iodixanol could then either leave the cell by diffusion or be transported along the phagocytic/endocytic route and finally be regurgitated. Since iodixanol effectively protected isolated lysosomes against osmolysis, we favor the idea that iodixanol does not readily leave the cells by diffusion across lysosomal/endosomal membranes, but rather by recycling through the endocytic pathway.

The rate of recycling is known to vary along the endocytic pathway. More than 80% of internalized material from early endosomes may be transported back to the extracellular medium [18, 19]. There is much less recycling from late endosomes and lysosomes. The level at which the phagosomes containing liposome-encapsulated [^{125}I]iodixanol communicate with the endocytic pathway could therefore strongly influence the rate of regurgitation of [^{125}I]iodixanol from the cell. It is also possible that regurgitation or "retrophagocytosis" can occur directly from the phagosome, although little is known about this pathway.

Several studies have shown that chemical properties of the engulfed particle may determine how and to what degree the phagosome containing the particle communicates with the endosomal system [20–22]. The results of this study, therefore, do not rule out that the liposome composition somehow down-regulates the ability of phagosomes to transfer their content to the endosomal system. A more direct way to test this assumption would be to use liposome-containing fluorochromes instead of iodixanol. The transfer of phagosomal content to the endosomal system could then be visualized by fluorescence microscopy.

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References

1. Henze A, Freise J, Magerstedt P and Majewski A, Radioopaque liposomes for the improved visualisation of focal liver disease by computerized tomography. *Comput Med Imaging Graph* **13:** 455–462, 1989.
2. Passariello R, Pavone P, Patrizio G, Di Renzi P, Mastantuono M and Giuliani S, Liposomes loaded with nonionic contrast media. Hepatosplenic computed tomographic enhancement. *Invest Radiol* **25**(Suppl 1)**:** S92–94, 1990.
3. Ivancev K, Lunderquist A, McCuskey R, McCuskey P and Wretlind A, Experimental investigation of a new iodinated lipid emulsion for computed tomography of the liver. *Acta Radiol* **30:** 407–413, 1989.
4. Kanai M, Murata Y, Mabuchi Y, Kawahashi N, Tanaka M, Ogawa T, Doi M, Soji T and Herbert DC, *In vivo* uptake of lecithin-coated polystyrene beads by rat hepatocytes and sinusoidal endothelial cells. *Anat Rec* **244:** 175–181, 1996.
5. Berg T, Gjoen T and Bakke O, Physiological functions of endosomal proteolysis. *Biochem J* **307:** 313–326, 1995.
6. Tolleshaug H and Berg T, Investigation of the uptake of asialo-glycoproteins by isolated rat hepatocytes. Implications for its biological significance. *Hoppe Seylers Z Physiol Chem* **361:** 1155–1164, 1980.
7. Pittman RC, Carew TE, Glass CK, Green SR, Taylor CA Jr. and Attie AD, A radioiodinated, intracellularly trapped ligand for determining the sites of plasma protein degradation *in vivo*. *Biochem J* **212:** 791–800, 1983.
8. Seglen PO, Preparation of isolated rat liver cells. *Methods Cell Biol* **13:** 29–83, 1976.
9. Magnusson S and Berg T, Endocytosis of ricin by rat liver cells *in vivo* and *in vitro* is mainly mediated by mannose receptors on sinusoidal endothelial cells. *Biochem J* **291:** 749–755, 1993.
10. Smedsrod B, Pertoft H, Eggertsen G and Sundstrom C, Functional and morphological characterization of cultures of Kupffer cells and liver endothelial cells prepared by means of density separation in Percoll, and selective substrate adherence. *Cell Tissue Res* **241:** 639–649, 1985.
11. Magnusson S and Berg T, Extremely rapid endocytosis mediated by the mannose receptor of sinusoidal endothelial rat liver cells. *Biochem J* **257:** 651–656, 1989.
12. Knook DL and Sleyser EC, Preparation and characterization of Kupffer cells from rat and mouse liver. In: *Kupffer Cells and Other Liver Sinusiodal Cells* (Eds. Wiesse E and Knook DL), pp. 273–288. Elsevier/North Holland Biochemical Press, Amsterdam, 1977.
13. Iveson GP, Bird SJ and Lloyd JB, Passive diffusion of non-electrolytes across the lysosome membrane. *Biochem J* **261:** 451–456, 1989.
14. Blomhoff R, Helgerud P, Rasmussen M, Berg T and Norum KR, *In vivo* uptake of chylomicron [^{3}H]retinyl ester by rat liver:

* Kjeken R and Berg T, unpublished observations.

Evidence for retinol transfer from parenchymal to nonparenchymal cells. *Proc Natl Acad Sci USA* **79:** 7326–7330, 1982.
15. Munthe-Kaas AC, Berg T and Seljelid R, Distribution of lysosomal enzymes in different types of rat liver cells. *Exp Cell Res* **99:** 146–154, 1976.
16. Blomhoff R, Holte K, Naess L and Berg T, Newly administered [^{3}H]retinol is transferred from hepatocytes to stellate cells in liver for storage. *Exp Cell Res* **150:** 186–193, 1984.
17. Praaning-van Dalen DP, Brouwer A and Knook DL, Clearance capacity of rat liver Kupffer, endothelial, and parenchymal cells. *Gastroenterology* **81:** 1036–1044, 1981.
18. England IG, Naess L, Blomhoff R and Berg T, Uptake, intracellular transport and release of ^{125}I-poly(vinylpyrrolidone) and [^{14}C]-sucrose-asialofetuin in rat liver parenchymal cells. Effects of ammonia on the intracellular transport. *Biochem Pharmacol* **35:** 201–208, 1986.
19. Besterman JM, Airhart JA, Woodworth RC and Low RB, Exocytosis of pinocytosed fluid in cultured cells: Kinetic evidence for rapid turnover and compartmentation. *J Cell Biol* **91:** 716–727, 1981.
20. Wattiaux R, Jadot M, Dubois F and Wattiaux-De Coninck S, Phagocytosis by rat liver: Relationships between phagosomes and lysosomes. *Biochem Biophys Res Commun* **220:** 569–574, 1996.
21. Wattiaux R, Jadot M, Laurent N, Dubois F and Wattiaux-De Coninck S, Cationic lipids delay the transfer of plasmid DNA to lysosomes. *Biochem Biophys Res Commun* **227:** 448–454, 1996.
22. de Chastellier C, Lang T and Thilo L, Phagocytic processing of the macrophage endoparasite, *Mycobacterium avium*, in comparison to phagosomes which contain *Bacillus subtilis* or latex beads. *Eur J Cell Biol* **68:** 167–182, 1995.