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● *Original Contribution*

# LIPOSOMAL Gd-DTPA: EFFECT OF ENCAPSULATION ON ENHANCEMENT OF HEPATOMA MODEL BY MRI

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**Liposomes entrapping gadolinium-DTPA (Gd-DTPA) were synthesized from 60 mole percent egg phosphatidylcholine (EPC) and 40 mole percent cholesterol or EPC alone entrapping Gd-DTPA in diameters of 100 and 200 nm. Rats bearing Morris hepatoma in their flanks were imaged by MR pre- and post-contrast with free Gd-DTPA and liposomal Gd-DTPA for up to four hours after IV contrast. Comparison of images after free and liposomal Gd-DTPA showed dramatic differences in tumor and organ enhancement. Liposomal Gd-DTPA enhancement of tumor corresponded more closely to histologically proven vascularized portions of tumor than free Gd-DTPA. Hepatic enhancement was greater with liposomal than free Gd-DTPA and time course of liver, kidney and tumor enhancement was prolonged. The 100-nm EPC Gd-DTPA liposomes caused the greatest enhancement.**

**Gd-DTPA liposomes may be useful as liver and blood pool contrast agents. By varying lipid composition and vesicle size, patterns of enhancement may be selectively modified.**

*Keywords:* **Liposomes; MRI; Contrast agents.**

## INTRODUCTION

The vascular contrast agent gadolinium-DTPA (GD-DTPA) is not well suited to the enhancement of tumors within the liver and spleen because of its rapid equilibration with the extracellular space and subsequent renal clearance.[1,2,4,15] By utilizing the fact that lipid vesicles are cleared from the circulation in part by uptake into the cells of the reticuloendothelial system, we may use lipid vesicles loaded with Gd-DTPA, to deliver contrast agent to the Kupffer cells and hepatocytes of the liver.[3,9,12] Additionally, liposomes may be used to prolong the blood pool phase of Gd-DTPA for vascular or blood volume imaging.

In our previous study[14] we showed that Gd-DTPA entrapped in lipid vesicles increased the enhancement of liver and blood pool. Additionally, the in vitro enhancement caused by Gd-DTPA in 400-nm diameter vesicles was less than that caused by free Gd-DTPA. We consider that this is primarily a consequence of shielding of the paramagnetic nucleus from the bulk water by the intervening lipid bilayer and that the relaxivity of the entrapped Gd-DTPA may be improved by using lipid vesicles of smaller diameter. In this study we report results of the enhancement caused by Gd-DTPA encapsulated in vesicles of 100 and 200 nm in rats bearing a flank-implanted Morris hepatoma.

## MATERIALS AND METHODS

### *Vesicle Preparation*

Unilamellar vesicles composed of either egg phosphatidylcholine (EPC) alone, or with 40 mole percent cholesterol were prepared by extrusion of multilamellar lipid vesicles through polycarbonate filters.[8] Briefly, EPC and cholesterol were combined in chloroform solution, the solvent removed under nitrogen, then under reduced pressure and the combined lipids rehydrated in a solution of 0.67 M Gd-DTPA (sodium salt) pH 7 by vortexing at room temperature to form multilamellar vesicles. These multilamellar vesicles were subjected to five cycles of freeze-thawing followed by repeated extrusion through polycarbonate filters of defined pore size. Untrapped Gd-DTPA was removed by exhaustive

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dialysis. Details of the vesicle preparation and characterization in terms of trap volume and Gd-DTPA content are described elsewhere.[13]

*Tumor Model*

Fischer 344 rats were implanted with tumor cells from the Morris hepatoma cell line as previously described.[10,11]

*Imaging*

Phantoms and animals were imaged using a Siemens 1.5 T Magnetom whole-body scanner (Iselin, NJ). Animals were imaged with a $T_1$-weighted sequence with parameters, TE/TR 16/400 msec, 4 acquisitions, 256 × 256 matrix, 15-cm field of view and 2-mm slices. Five Fischer 344 rats bearing a flank-implanted Morris hepatoma were imaged pre- and post-contrast administration of either free Gd-DTPA (0.5 mM/kg) 2 rats, and 1 rat each with either 0.5 mM/kg Gd-DTPA entrapped in 100-nm EPC vesicles, 100-nm EPC/cholesterol (6:4 molar ratio), or 200-nm EPC/cholesterol (6:4 molar ratio) vesicles. Animals were lightly anesthetized with ether, then fully sedated with i.p. 80 mg/kg ketamine and 12 mg/kg xylazine. Animals were scanned in both transaxial and coronal planes, and the signal intensities of liver, kidney, tumor, muscle and other structures measured pre- and post-contrast for up to 4 hours. After scanning, the animals were sacrificed and studied histologically in order to correlate images with pathology.

*Histology*

Immediately after imaging, the rats were sacrificed by ether inhalation overdose and the liver and tumors removed. The tissues were fixed in 10% formalin for 48 hours then sectioned in the coronal plane in 2-mm sections. Representative sections of tumor and liver were photographed and then prepared using standard techniques with hematoxylin and eosin stains for histological examination.

## RESULTS

*Relaxivity of Vesicles*

The characterization of different size lipid vesicles with respect to their relaxivity has been described elsewhere.[13] Briefly, the relaxivity for entrapped Gd-DTPA is less than free Gd-DTPA and exhibits an approximately linear dependence upon the surface to volume ratio for vesicles of 70 to 400 nm diameter. Values for the relaxivities of vesicles used in this and previous studies are listed in Table 1, from which it is clear that Gd-DTPA in smaller vesicles exhibit a greater relaxivity.

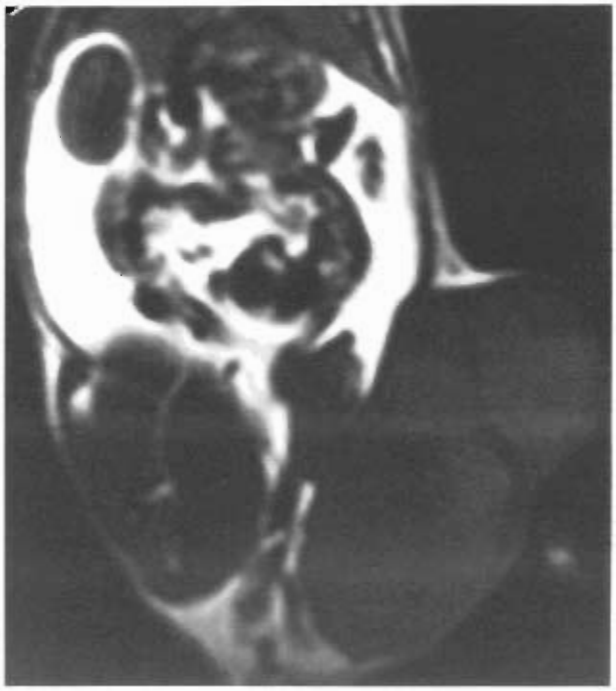
Table 1. Relaxivity of Gd-DTPA in lipid vesicles of different diameter

| | Relaxivity ($sec^{-1}$, $mM^{-1}$) |
|---|---|
| EPC/Cholesterol (6:4) Vesicles | |
| *Vesicle Diameter* (nm) | |
| Free Gd-DTPA | 2.79 |
| 70 | 1.60 |
| 100 | 1.05 |
| 200 | 1.51 |
| 400 | 0.42 |
| EPC Vesicles | |
| *Average Diameter* (nm) | |
| 100 | 1.40 |

## ANIMAL IMAGES

*Free Gd-DTPA*

Pre-contrast images (Fig. 1(A)) showed liver and tumor approximately isointense. The central necrotic portion of the flank tumor was marginally hyperintensive relative to the surrounding viable tumor mass. Both tumor and liver were hyperintensive relative to muscle. At 7 minutes post-contrast (Fig. 1(B)) after IV injection of 0.5 millimole/kg (mM/kg) free Gd-DTPA, there was a noticeable enhancement of liver vessels and muscle such that the liver/muscle signal ratio was approximately the same as in pre-contrast images. There was a significant darkening of the renal pelvis due to accumulation of high concentrations of Gd-DTPA and consequent signal loss via $T_2$ effects. The flank tumor showed moderately intense contrast enhancement of the peripheral portions, but the central necrotic region of the tumor (confirmed by histology) showed no enhancement. We noted that the contrast enhancement of the tumor periphery was relatively homogeneous. At 24 min post-contrast (Fig. 1(C)) there was noticeable washout of the peripheral portion of the flank tumor leaving a rind of enhancement at the interface between the central necrotic core and surrounding viable tissue. The renal pelvis remained dark, indicating continued high concentrations of Gd-DTPA within the central collecting system. Both liver and muscle tissues were decreased in intensity compared to immediate post-contrast images. The changes in signal intensity for various tissues with time post-contrast are given in Fig. 1(D). It is evident from this figure that the washout of the tumor periphery continues over the time-course of these measurements. Note also that with time the renal cortex showed



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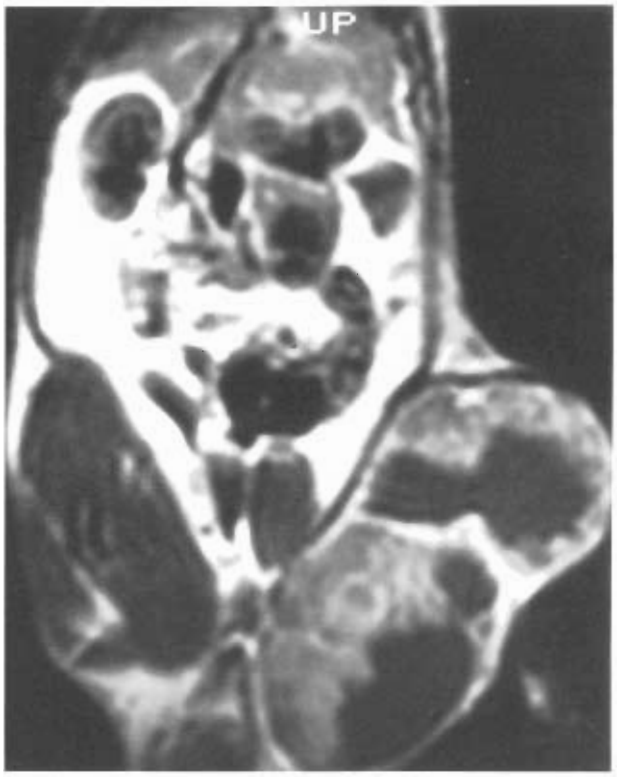


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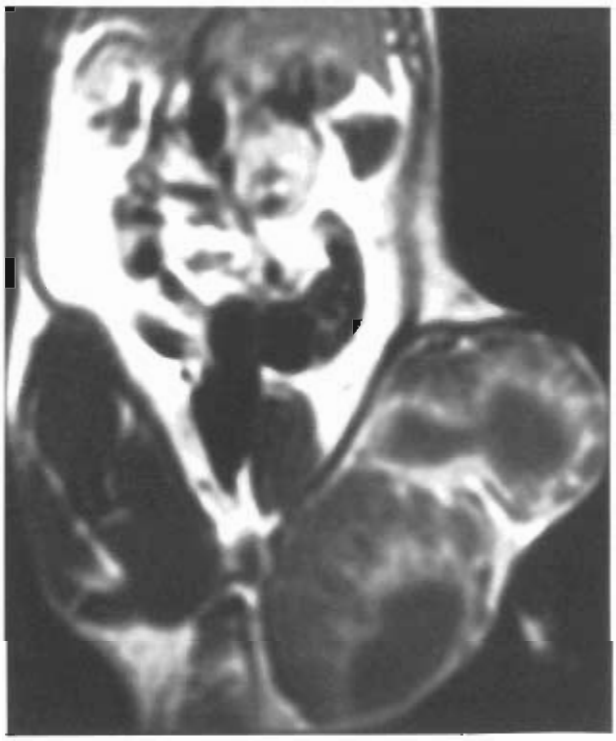

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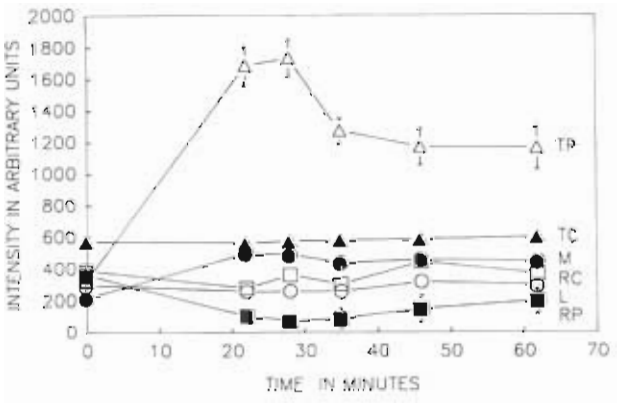



D

Fig. 1. (A) Male 350 g Fischer 344 rat with flank-implanted Morris hepatoma imaged using a $T_1$-weighted TE/TR 16/400 msec pulse sequence. Pre-contrast. (B) Post-contrast at 7 min after injection of 0.5 mM/kg IV free Gd-DTPA. (C) Post-contrast at 24 min after injection showing washout from tumor periphery. (D) Time-course for variation in signal intensity for various tissues post-contrast after 0.5 mM/kg free Gd-DTPA. These data are from a different animal than shown in Figs. 1(A)–(C). RP = renal pelvis (■); RC = renal cortex (□); L = liver (○); M = muscle (●); TC = tumor center (▲) and TP = tumor periphery (△).

greater enhancement, whereas the liver to muscle ratio was approximately constant.

*100-nm EPC Vesicles*

Pre-contrast images showed the flank tumor as having a homogeneous signal intensity slightly hypointense compared to muscle and approximately isointense with liver. As in the previous pre-contrast image, the central portion of the flank tumor was slightly hyperintensive compared to the peripheral portion of the tumor.

At 7 min post-contrast (Fig. 2(B)) after IV injection of 100-nm EPC vesicles with entrapped Gd-DTPA such that the total dose of Gd-DTPA was 0.5 mM/kg, there was noticeable enhancement of liver, both in terms of absolute intensity and also relative to muscle. Both renal cortex and pelvis showed enhancement, with renal pelvis hyperintense to cortex post-contrast. This is the reverse of pre-contrast images where cortex was hyperintensive relative to pelvis. The peripheral portion of the flank tumor was also enhanced. However, this was not a margin-like enhancement as seen with free Gd-DTPA, but rather small cords of areas of enhancement, reflecting areas of vascularized tissue as confirmed by histology. At longer time post-contrast, the enhancement of the liver was increased relative to muscle and there was a washout of the enhancement of the tumor periphery (Fig. 2(C)) although it should be noted that this washout was not so rapid as for free Gd-DTPA. Histology of the flank-implanted hepatoma is shown in Fig. 2(D). The changes in signal intensity for various tissues with time post-contrast is shown in Fig. 2(E). There was also sustained enhancement of liver and tumor periphery at up to 4 hours post-contrast.

*200 nm EPC/Cholesterol (6:4) Vesicles*

Pre-contrast images (Fig. 3(A)) showed liver hyperintense relative to muscle with flank tumor relatively homogeneous, hyperintense relative to muscle and approximately isointense with liver. The renal pelvis was darker than cortex.

At 7 min post-contrast (not shown) IV injection of 200-nm EPC/cholesterol Gd-DTPA vesicles renal pelvis was enhanced, but now with the pelvis hyperintense relative to cortex. The peripheral portions of the flank tumor were also enhanced compared to the central necrotic core, the regions of enhancement corresponding to vascularized portions of the tumor. Subsequent images showed the same pattern of contrast enhancement, with the overall observation that the degree of enhancement observed with the 200-nm vesicles was less than an equivalent dose delivered by 100-nm EPC vesicles. At 2 hours post-contrast (Fig. 3(B)), it was observed that the liver showed sustained enhancement. Note also the lacy pattern of intratumoral enhancement suggesting blood pool effects. The changes in signal intensity for various tissues with time post-contrast are given in Fig. 3(C).

Figure 4 shows the time-course of the signal intensity ratio of liver to muscle for each of free Gd-DTPA, 100-nm EPC and 200-nm EPC/cholesterol (6:4) vesicles. It is evident that for free Gd-DTPA there was little change in the liver-to-muscle signal ratio whereas this ratio was increased for both of the vesicle preparations with the 100-nm vesicle more effective than the 200-nm vesicles. Note that the enhancement of the liver was maintained for up to 1 hour post-contrast.

*100-nm EPC/Cholesterol (6:4) Vesicles*

The pre-contrast images showed tumor approximately isointense to liver. The post-contrast images showed a pattern of liver and tumoral enhancement between that of the liposomal Gd-DTPA in 100 nm EPC and 200 nm EPC/cholesterol vesicles. Post-contrast imaging was continued until 4 hours and 45 minutes post-contrast, and there was still an increase in signal intensity ratio of 2.27 (liver signal intensity post-contrast ÷ liver signal intensity pre-contrast).

## DISCUSSION

Due to rapid equilibration with the extravascular space and renal clearance, the Gd-DTPA accumulates in the collecting system of the kidney.[1,2,15] At concentrations greater than approximately 2 mM, the $T_2$ effects of $Gd^{+3}$ will dominate with concomitant signal loss,[5] as observed. The homogeneous enhancement of the tumor periphery (Fig. 1(B)) suggests that there was leakage of contrast from the vascular spaces within the perfused portion of the tumor into the extracellular spaces, thus resulting in a more homogeneous distribution of the Gd-DTPA within the viable subregions of the tumor. We note that this pattern of enhancement was different for the Gd-DTPA entrapped within lipid vesicles. In the latter case, enhancement appeared to be relatively confined to small cord-like structures which correlate with vascularized tissue as confirmed by histology. For free Gd-DTPA, enhancement of the tumor periphery showed washout over the time-course of the measurements leaving a rind of enhancement between central necrotic tissue and surrounding viable tissue. By comparison, the tumor washout with liposomal Gd-DTPA had a much longer time-course.

The liver to muscle ratios of enhancement caused by free Gd-DTPA showed little change while liposomal Gd-DTPA caused an increase in post-contrast

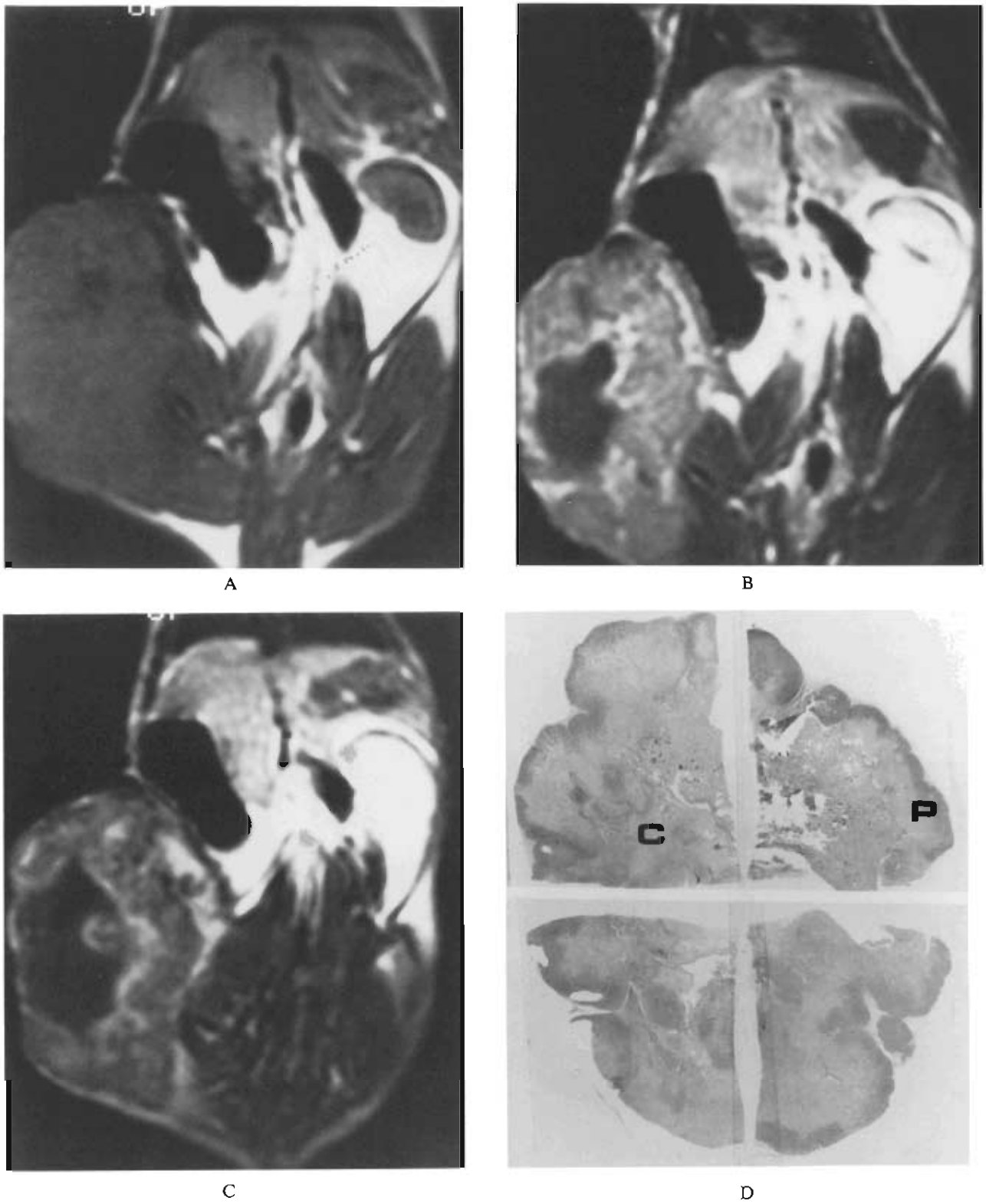
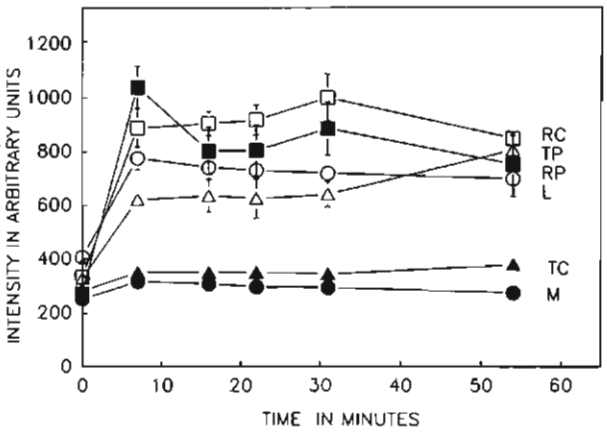



Fig. 2. (A) Male 340 g Fischer 344 rat with a flank-implanted Morris hepatoma imaged using a $T_1$-weighted TE/TR 16/400 msec pulse sequence. Pre-contrast. (B) Post-contrast at 6 min after IV injection of Gd-DTPA entrapped in 100-nm EPC vesicles. The total dose of Gd-DTPA was 0.5 mM/kg. (C) Post-contrast image at 28 min indicating washout from the periphery of the tumor. (D) Histology of flank tumor showing peripheral vascularized portion, P, and central necrotic nonvascularized core, C. (Figure continued on overleaf.)

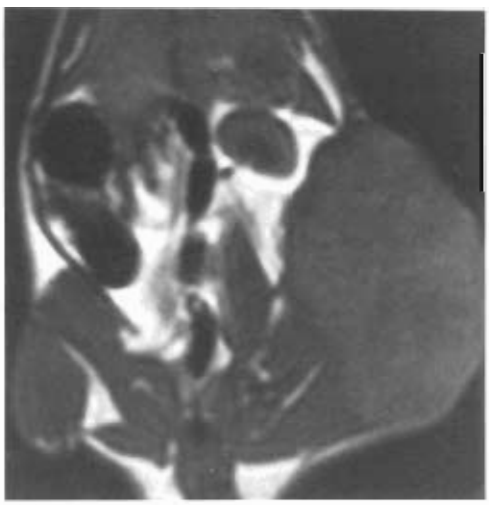



E

Fig. 2. (contd.) (E) Time-course for variation in signal intensity of various tissues post-contrast after IV injection of 0.5 mM/kg Gd-DTPA entrapped in 100-nm EPC vesicles. RP = renal pelvis (■); RC = renal cortex (□); L = liver (○); M = muscle (●); TC = tumor center (▲) and TP = tumor periphery (△).

liver to muscle signal ratios. In the case of free Gd-DTPA this likely reflects equilibration between intra- and extracellular fluid space with similar concentrations of free Gd-DTPA in both liver and muscle tissue. The preferential greater enhancement of liver caused by liposomal Gd-DTPA reflects in part a higher concentration of Gd-DTPA within the tissue due to uptake by the Kupffer cells and possibly by hepatocytes, although the greater blood volume within the liver compared to muscle may also contribute to the preferential enhancement.

The patterns of tumoral enhancement observed in this study may have clinical relevance. Recent human tumor oxygenation measurements in vivo have shown that the tumor center is generally most hypoxic.[6] Tumor oxygenation measurements have confirmed that hypoxia confers radiation resistance.[7] Conversely, well-oxygenated tumors tend to be sensitive to radiation therapy. The most important determinant of hypoxia is most likely perfusion. The contrast enhance-



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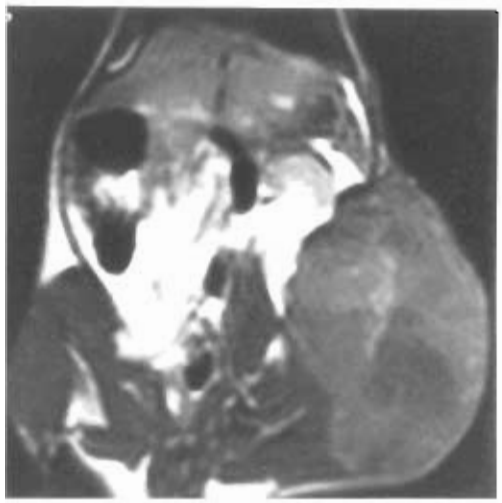

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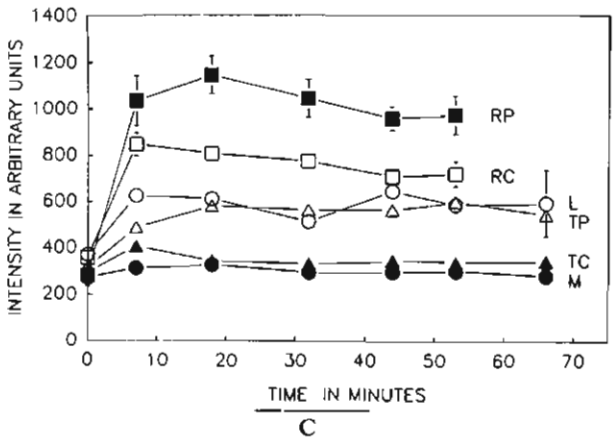


C

Fig. 3. (A) Male 350 g Fischer 344 rat with a flank-implanted Morris hepatoma imaged with a TE/TR 16/400 msec sequence. Pre-contrast. (B) Post-contrast at 120 min after IV injection of Gd-DTPA entrapped in 200 nm diameter EPC/cholesterol (6:4 molar ratio) vesicles. The total dose of Gd-DTPA was 0.5 mM/kg. (C) Time-course for variation in signal intensity of various tissues post-contrast after IV injection of 0.5 mM/kg Gd-DTPA entrapped in 200-nm diameter EPC/Cholesterol (6:4 molar ratio) vesicles. RP = renal pelvis (■); RC = renal cortex (□); L = liver (○); M = muscle (●); TC = tumor center (▲) and TP = tumor periphery (△).

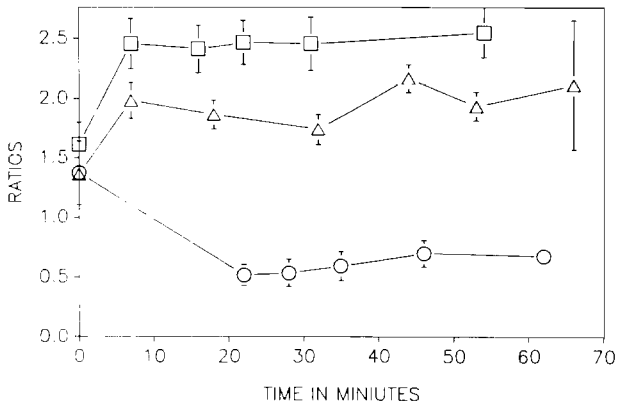


Fig. 4. Time-course for the change in liver to muscle intensity ratio for (○) free Gd-DTPA and Gd-DTPA in (□) 100-nm EPC and (△) 200-nm EPC/cholesterol (6:4) vesicles.

ment caused by liposomal Gd-DTPA corresponded to vascularized portions of the tumor. This suggests that liposomal Gd-DTPA might be used to evaluate tumor perfusion or blood pool which in turn should reflect oxygenation and could be important in tailoring or directing therapy. An effective blood pool contrast agent could also be useful for assessing blood flow or perfusion in brain, kidney, liver, or other organs.

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