Targeted Transarterial Therapy of Vx-2 Rabbit Liver Tumor with Yttrium-90 Labeled Ferromagnetic Particles Using an External Magnetic Field

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Abstract. *Background: Our goal was to study the efficacy of liver cancer embolization with magnetically targeted Yttrium-90 labeled ferromagnetic particles and establish the biodistribution profile of these particles. Materials and Methods: Of twenty rabbits, nine underwent transarterial radioembolization of implanted Vx-2 tumor with increasing ^{90}Y-MTC doses, three were treated with carrier particles alone, four remained untreated and four were sacrificed early to document biodistribution. At various intervals, animals were sacrificed and biodistribution, liver cancer viability and toxicity were measured. Results: There was a dose related degree of tumor necrosis, with greater than 90 Gy yielding 100% necrosis (baseline 50%). Blood radioactivity one hour post-radioembolization was less than 0.0275 μCi/g. No hematological toxicity was observed. Except for the non-targeted right liver lobe, organ radioactivity levels were within tolerance levels. Significant left (targeted) hepatic lobe necrosis was seen in subjects receiving high doses. Conclusion: Hepatic arterial radioembolization with ^{90}Y-MTC bolstered by external magnetic field has significant tumoricidal effect and a favorable biodistribution profile.*

Many loco-regional treatments, including selective internal radiation therapy (SIRT) with Yttrium-90 (^{90}Y) microsphere radioembolization, are currently available for unresectable liver neoplasms. Preliminary evidence suggests a survival benefit (1) in selected patients. A major concern however, is the risk of non-target embolization, especially lung, which can result in radiation pneumonitis and rarely death (2). ^{90}Y microspheres are particles with a diameter of 25±10 micrometers and thus embolize at the arteriolar level. They do so throughout the hepatic lobe targeted. There is an approximately 2:1 predilection for the tumor bed compared to the liver parenchyma, likely due to the tumor hypervascularity (1). In addition, the presence of hepatic artery-vein shunting in the tumor or cirrhotic parenchyma will result in increased lung radiation dose and possible pneumonitis. Such concerns continue to plague patients with unresectable liver neoplasms who do not respond to TACE and are candidates for ^{90}Y microsphere radioembolization.

The development of a targeting method that limits systemic biodistribution and increases tumor targeting and residence time of ^{90}Y, could therefore result in an improved tumoricidal effect and systemic toxicity profile as well as a lower risk for radiation-induced pneumonitis. To this end, ferromagnetic carrier particles were tested measuring 1-3 micrometers labeled with ^{90}Y, which can be sequestered in a specific anatomical region by the use of an externally applied static magnetic field. The purpose of our study was to examine the biodistribution profile and assess tumoricidal effect of these magnetically targeted carriers labeled with ^{90}Y in animal model of liver cancer (rabbit Vx-2).

Materials and Methods

IRB approval for this study was obtained.

Physical profile of agent and preparation of MTC-Y90 test material. ^{90}Y is a pure beta emitter (937 KeV) that decays to Zirconium-90 with a half-life of 64.2 hours. The emitted electrons have an average tissue penetration of 2.5 mm (effective max 10 mm) (2-5). For these experiments, the radioactive microparticles were prepared in a two-step process; a chelation step followed by irreversible adsorption onto the MTC microparticles. A solution of yttrium-90 chloride (Perkin Elmer, Billerica, MA, USA) was mixed with an ammonium acetate buffer solution of 2-*p*-aminobenzyl-DOTA, ABz-DOTA, (Macrocyclics, Dallas, TX, USA). The

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Table I. *Overview of the experimental design.*

Animal no.	Treatment group	Treatment type	Delivered dose μCi/Gy	Tumor necrosis	Outcome
1	Untreated control	None	None		Sacrificed post-op day 7
2				50%	
3				50%	
4					
5	MTC control	25 mg MTC (No ^{90}Y)	None	70%	
6				50%	
7				70%	
8	Intra-arterial radioembolization	^{90}Y chelated to 25 mg MTC	108/ 38		
9			124/ 44	85%	Sacrificed 24 h post-op
10			142/ 50	70%	Sacrificed post-op day 7
11			147/ 52	70%	
12			203/ 71	85%	
13			238/ 84	95%	
14			244/ 86	95%	Sacrificed 80 hrs post-op
15			250/ 88	100%	Sacrificed post-op day 7
16			266/ 94		Died post-op day 5
17			297/105	100%	Sacrificed post-op day 7
18			369/130	100%	
19			372/131	100%	
20	Peripheral *i.v.* infusion		380/134		Sacrificed 3 h after infusion

Four animals (1, 2, 3 and 4) were untreated and used to quantify the baseline necrosis of the VX2 tumor which was calculated to be 50%. Three animals (5, 6 and 7) were treated with bland MTC particle embolization (no ^{90}Y). Seven days post-treatment tumor necrosis was 50-70%, slightly higher compared to untreated controls. The remainder 13 animals were treated with increasing levels of radioactivity (38-134 Gy) but identical number of magnetic carrier particles (25 mg MTC). Animals 9 and 14 were sacrificed 24 and 80 h after treatment respectively and used to assess organ and bone radioactivity. Animal 20 was given a high dose of ^{90}Y-MTC via the right common femoral vein, sacrificed 3 h post-treatment and radioactivity in selected organs was measured (Table II). Animals 8, 10, 11, 12, 13, 15, 17, 18 and 19 were sacrificed 7 days post-treatment and histopathologic studies assessed a percent necrosis directly related to radiation dose (Figure 2).

chelation efficiency was tested by thin layer chromatography. The chelated yttrium-90 solution was then mixed with the MTC particles for 30 min. At the end of the quantitative binding step, the MTCs were suspended in slightly viscous aqueous based solution to 5 mg/mL allowing for the administration of the suspension.

Animal model. Twenty, 3 kg male white New Zealand rabbits were used as test subjects. The Vx-2 carcinoma cell line was selected for tumor model because a) it grows rapidly within the liver allowing the experiment to take place within a reasonable time period, and b) it mimics human hepatocellular carcinoma (HCC) in terms of blood supply by recruiting its blood supply mainly from the hepatic artery. The tumor's hypervascularity results in a classic "tumor blush" on angiography which makes it easy to identify.

Tumor implantation. Liver implantation of the rabbit Vx-2 tumor has been used extensively by this laboratory for evaluating liver cancer therapies (Geschwind *et al.*, 2002, Ko *et al.*, 2001, Geschwind *et al.*, 2000), as intra-arterial administration of therapeutic agents necessitates a larger animal tumor model for hepatic artery catheterization. Tumor characteristics and implantation were as follows: the Vx-2 tumor was first grown for 2 weeks on the hind leg of carrier rabbits. All animals were anesthetized with a mixture of *i.m.* administered acepromazine and ketamine hydrochloride. Intravenous access was gained *via* a marginal ear vein, and sodium pentothal was used to maintain anesthesia. The resultant Vx-2 tumor was excised from the carrier rabbits, minced and placed in Hanks' solution. Recipient animals were anesthetized as above and intubated with a 3mm endotracheal tube. Under sterile conditions, a midline abdominal incision was made in recipient animals and 0.1-0.2 mL of the minced Vx-2 tumor were directly implanted into the left lobe of the liver. The abdomen was closed and animals were allowed to recover for 7-14 days. During that period the tumors predictably grew to 1.5-3 cm in diameter.

Experimental procedure. After the 7-14 days recovery period, anesthesia was administered as described above and the animals were intubated with a 3 mm endotracheal tube. Groin surgical cut-down was performed and access was obtained into the right common femoral artery with a 3 French sheath (Cook, Inc., Bloomington, IN, USA) was placed. A 2 French JB1 cerebral catheter (Cook Inc., Bloomington, IN, USA) was then advanced into the celiac axis after which a diagnostic celiac arteriogram was performed revealing the tumor blush within the left lobe of the liver. A 0.014 in Transend guidewire (Boston Scientific MediTech, Natick, MA, USA) was used to help select the left hepatic artery allowing selective positioning of the catheter tip within a distance of 1.5-5 cm from the tumor. With the catheter in this location, 5 ml of ^{90}Y-MTC complex was infused under fluoroscopic guidance over 2.5 minutes at a flow rate of 2 mL/min in each animal. A small externally positioned magnet was used to create a localized magnetic field in the body. A 1.25 cm

Table II. *Assessment of radioactivity in selected organs and bone in three animals.*

Animal no.	Treatment type	Sacrificed	Organ	Mean activity (μCi/g)	Total activity (μCi)	Percent of total radioactivity
20	Peripheral *i.v.* infusion	3 h	Liver	1.287	111.9	66%
	380 mCi	post-op	Lungs	4.818	49.5	29%
			Spleen	4.353	5.6	3.3%
			Rt Kidney	0.171	1.7	1.0%
			Heart	0.035	0.25	0.15%
9	Lt hepatic artery	24 h	Lt Liver Lobe	0.694	47.6	67%
	^{90}Y-MTC	post-op	Rt Liver Lobe	0.627	20.3	29%
	124 μCi (44 Gy)		Spleen	1.350	1.20	1.7%
			Lung	0.1179	1.517	2.1%
			Bone	0.0124	0.030	0.04%
14	Lt hepatic artery	80 h	Lt Liver Lobe	0.526	19.40	34%
	^{90}Y-MTC	post-op	Rt Liver Lobe	0.850	34.22	59%
	244 μCi (86 Gy)		Spleen	2.317	2.69	2.2%
			Lung	0.112	1.244	4.7%
			Bone	0.014	0.042	0.07%

Animals 9 and 20 received 44 and 86 Gy of ^{90}Y-MTC in their left hepatic artery, respectively. Percent radioactivity in lungs, the value associated with pneumonitis – the main side effect of ^{90}Y hepatic trans-arterial embolization – was 2.1% and 4.7%. In humans, serious radiation pneumonitis is generally seen with pulmonary exposure of about 30 Gy [2]. For a high dose treatment of 150 Gy this translates to a maximum shunt rate of 20%. Significant exposure is recorded in the non-target liver lobe (right) in both animals. This is likely a result of back flow during radioembolization of the rabbit's small left hepatic artery. Radioactivity organs and bone (other than liver and lungs), even in the animal that received a peripheral high dose i.v. infusion of ^{90}Y-MTC (no. 20), was small enough to be of no clinical significance.

(diameter) by 4.5 cm (length) neodymium-iron-boron (NdFeB) cylindrical dipole magnet mounted on a flexible arm assembly was positioned distal to the end of the catheter tip on the skin surface over the tumor. Positioning was determined angiographically. The magnetic field strength at the pole of the magnet was 3 kgauss. The magnet was kept in place overlying the tumor location for the duration of the infusion and for an additional 15 minutes afterwards. The animals were then recovered. Four of twenty animals were not treated and used as controls to assess baseline tumor necrosis. Treatments consisted of intra-arterial administration of either 25 mg MTCs alone (n=3, used as controls) or MTC-Y90 given at one of three doses intended to deliver 50, 100 or 150 Gy along with 25 mg MTCs (n=9) as shown in Table I. One animal received a peripheral *i.v.* infusion of ^{90}Y-MTC. Table I shows the experimental design. In addition, for the pharmacokinetic studies, one animal (#20, Tables I and II) was given a peripheral *i.v.* infusion of 380 μCi ^{90}Y-MTC and euthanized 3 hours after the infusion. Two other animals were euthanized one at 24 hours (#9, Tables I and II) and the second at 80 hours (#14, Tables I and II) post-left hepatic artery ^{90}Y-MTC radioembolization. Liquid scintillation was used to record the absolute and relative radioactivity in selected organs and in bone. Results are shown in Table II. Venous blood samples were obtained within the first hour in 4 animals selected randomly and liquid scintillation was used to calculate the radioactivity circulating in blood (Table III). Hematology counts were obtained pre-procedure and 7 days post-procedure (just prior to euthanasia) in three animals (One untreated control, one medium and one high dose) (Table IV). Seventeen animals – twenty minus two early sacrifices and one premature death – received MRI scans to document MTC particle localization and were then euthanized on post-operative day number 7. The liver was explanted and the targeted (left) and non-targeted (right) lobes were separated. They were then placed in 10% formalin for expected paraffin embedding, sectioning and H&E staining. Slides were examined and percent necrosis was assessed by a pathologist.

Table III. *Blood radioactivity measured using liquid scintillation in venous samples taken within one hour after ^{90}Y-MTC left hepatic embolization.*

Animal no.	Radioactivity in blood (μCi/g)	Delivered dose μCi/G
8	0.0172	108/38
10	0.0274	142/50
11	0.0262	147/52
12	0.0190	203/71

Animals were chosen at random. The circulating activity in blood is miniscule compared to the total delivered activity suggesting that ^{90}Y-MTC embolizes and remains in the liver, and that the ^{90}Y-MTC complex shows no significant dissociation.

Results

Tumor necrosis. The Vx-2 tumor in the left lobe was easily identifiable at the time of pre-treatment angiography as a hypervascular blush (Figure 1). Histological examination of the untreated control animals documented a tumor necrosis of 50%, which is in keeping with previous reports on Vx-2 tumors implanted in rabbit livers. The MTC

Table IV. *Blood counts measured before and seven days post radioembolization in three animals. Though variations noted in all three animals, no clinically significant changes were observed between the untreated control (2), medium dose (13) and high dose (18) test subjects.*

Complete blood count	Animal no. 2 untreated control		Animal no. 13 medium dose (84 Gy)		Animal no. 18 high dose (130 Gy)	
	Pretreatment	Day 7	Pretreatment	Day 7	Pretreatment	Day 7
WBC (10^3/mL)	4.04	3.42	3	4.6	3.77	5
Neutrophils (absolute)	0.748	2.22	1.68	3.90	1.81	3.83
Neutrophils (%)	18.5	64.9	56	84.7	48.1	76.7
Lymphocytes (absolute)	3.06	0.930	1.290	0.556	0.994	0.710
Lymphocytes (%)	75.7	27.2	43	12.1	26.4	14.2
Monocytes (absolute)	0.215	0.249	0	0.133	0.615	0.394
Monocytes (%)	5.33	7.28	0	2.90	16.3	7.89
Eosinophils (absolute)	0.009	0.014	0.030	0.010	0.068	0.043
Eosinophils (%)	0.216	0.422	1	0.219	1.81	0.869
Basophils (absolute)	0.010	0.009	0	0.002	0.278	0.016
Basophils (%)	0.243	0.260	0	0.049	7.37	0.312
RBC (10^6/mL)	4.81	5.15	5.32	5.17	6.12	5.57
Hemoglobin(g/dL)	11.2	11.6	12.5	11.5	12.6	11.3
Hematocrit (%)	33	35.5	35.9	35.4	36.7	34.6
MCV (fL)	68.7	68.9	67	68.4	60	62.2
MCH (pg)	23.3	22.6	23.5	22.3	20.5	20.2
MCHC (g/dL)	34	32.7	34.8	32.6	34.2	32.5
RDW (%)	14.2	14.3		16.3	13.6	14.6
Platelets(10^3/mL)	439	416	286	359	351	630
MPV (fL)	5.97	9.62		8.91	6.24	9.13
PCT (%)	0.262	0.400		0.319	0.219	0.575
PDW	17.1	17.6		17.7	16.3	17.6

treated (no ^{90}Y) controls showed tumor necrosis of 50-70% which is likely due to the effects of bland embolization and resultant tumor ischemia. The nine animals treated with ^{90}Y-MTC radioembolization of the left hepatic artery showed a dose-related degree of tumor necrosis (Figure 2). Animals that received a total dose to the tumor of 50-75 Gy showed tumor necrosis of 70-85%, minimally greater than that from bland embolization. Radioembolization with doses between 75-100 Gy resulted in necrosis of 90-100%. Doses beyond 100 Gy resulted in complete (100%) tumor necrosis. Sample histopathologic slides are shown in Figures 3, 4 and 5.

MRI imaging of MTC particles. The animals that survived for 7 days after magnetic targeted delivery of MTC-Y90 were imaged with MR in order to assess treatment-related effects on tumors and other tissues. Prior to necropsy, MR scans were taken of a few representative animals as a means of identifying the location of MTC particles. Since the particles are comprised of 75% iron, they are visible on MR imaging because they create a very intense magnetic susceptibility artifact resulting in profound signal loss. Here, the effects of the MTCs on MR signal are easily seen in the tumor indicating successful delivery of the radioactive microspheres to the target tissue (Figure 6).

Biodistribution of radioactivity and toxicity. Liquid scintillation performed in selected organs and bone in the two animals (#9 and #14) sacrificed early, identified only minimal activity in non-target organs. The exception was the contralateral liver lobe which was found to have high activity relative to the target lobe, *i.e.* 29% and 59% respectively (Table II). This was thought to be a result of reflux of the ^{90}Y-MTC particles as the flow in the targeted left hepatic artery slowed during the embolization. The percent activity measured in the lungs of these two animals, was 2.1% and 4.7% which translated to 0.9 and 4.0 Gy respectively, well within the lung tolerance. Doses to other organs and bone were even less. The radioactivity circulating in blood within one hour of treatment was measured in four animals (#8, 10, 11 and 12) and found to range from 0.0172 to 0.0274 μCi/g (Table III).

With the exception of one animal (#16) that died on post-treatment day 5, no adverse clinical signs were observed. Comparison of complete blood counts before and seven days post-treatment in three animals – one untreated control, one medium dose (84 Gy) and one high dose (130 Gy) – is shown in Table IV. No clinically significant changes were noted. In the animals treated with high dose ^{90}Y-MTC radioembolization and whose histopathologic examination showed 100% tumor necrosis, there was extensive destruction

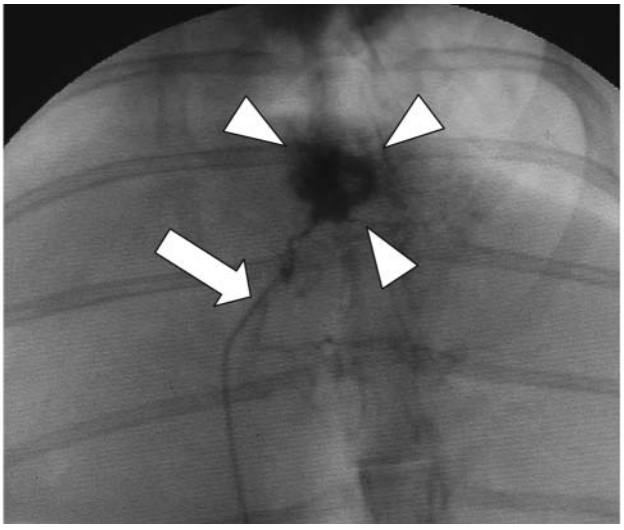

Figure 1. *Frontal view of a selective left hepatic arteriogram via a catheter (arrow) inserted through the rabbit's right common femoral artery shows the VX2 tumor "blush" in the left liver lobe (Arrowheads). The positive pole of a permanent magnet was placed externally to the animal as close to the "blush" as possible.*

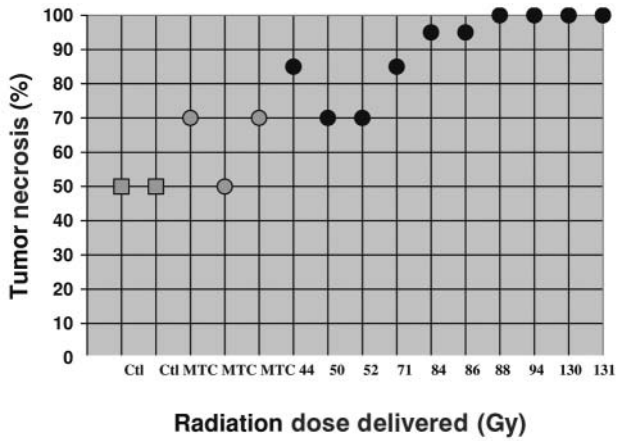

Figure 2. *Graph showing the percent tumor necrosis versus radiation dose delivered in the left hepatic artery as ^{90}Y-MTC. Untreated controls (gray squares) establish the baseline necrosis of VX2 tumor as 50%. Control animals treated with bland MTC particle embolization (gray circles) showed a slightly higher degree of necrosis between 50-70%. Animals treated with ^{90}Y-MTC (black circles) showed a dose-related degree of necrosis ranging from 70-100%.*

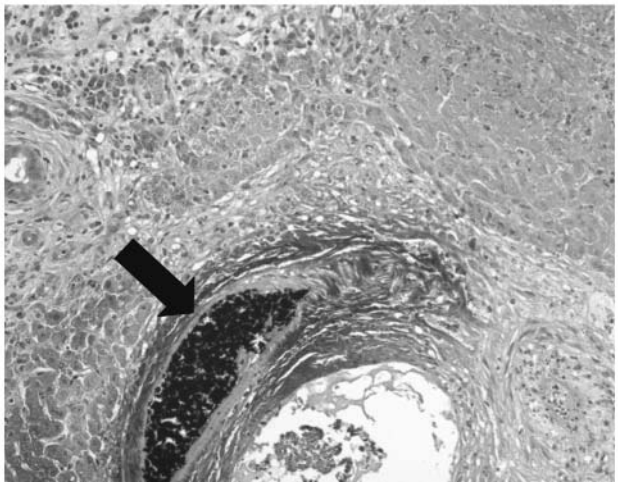

Figure 3. *Hematoxylin and Eosin stained sample of VX2 tumor in rabbit liver seven days post-radioembolization with ^{90}Y-MTC. The black MTC particles are seen embolized within an arteriole (arrow) which is surrounded by both viable and necrotic tumor cells.*

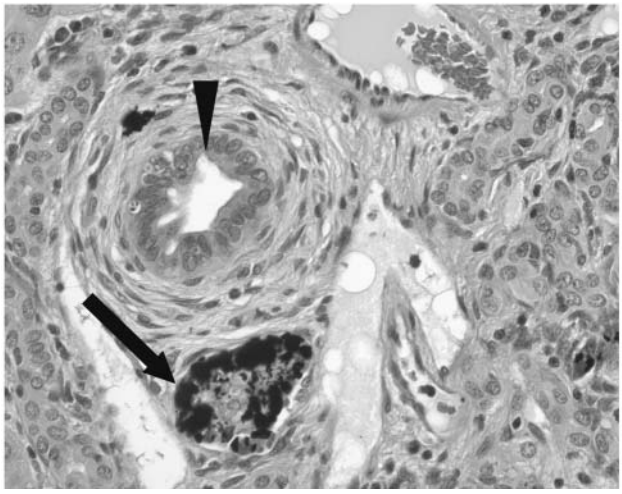

Figure 4. *Hematoxylin and eosin stained sample of VX2 tumor in rabbit liver seven days post-radioembolization with ^{90}Y-MTC. MTC particles are sequestered in macrophages (arrow) near a bile duct (arrowhead).*

of the targeted liver parenchyma. MRI showed the ^{90}Y-MTC particles to be confined to the targeted liver lobe (Figures 6).

Discussion

Results of our experiments using these new microspheres clearly show that this new delivery method can be quite effective, both from the standpoint of the satisfactory biodistribution profile and the dose-dependent efficacy on tumor cell kill. Here, we established an excellent correlation between radiation dose delivered to the tumor and actual tumor necrosis. Indeed, at the maximum dose exceeding 100 Gy, the entire tumor was destroyed by the radiation. This point alone proves that the microspheres that carried the Y90 were in fact delivered to the tumor and once there, destroyed the entire tumor, thereby establishing proof of this concept. The externally applied static magnetic field was therefore sufficient to sequester the magnetically targeted particles within the tumor allowing them to exert their radioactive

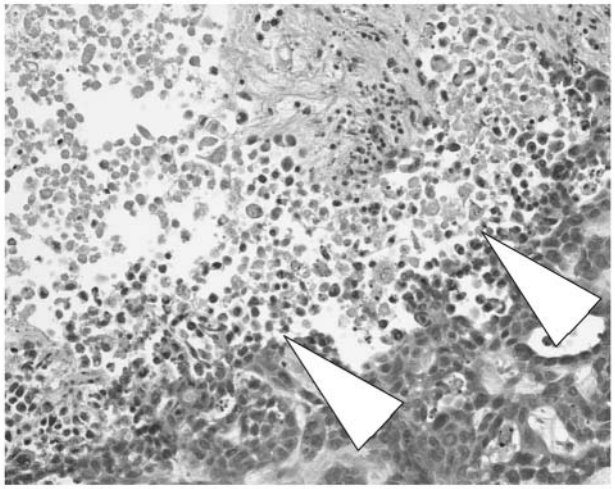

Figure 5. *Hematoxylin and eosin stained sample of VX2 tumor in rabbit liver seven days post-radioembolization with ^{90}Y-MTC. 64x magnification shows extensive necrosis (arrowheads).*

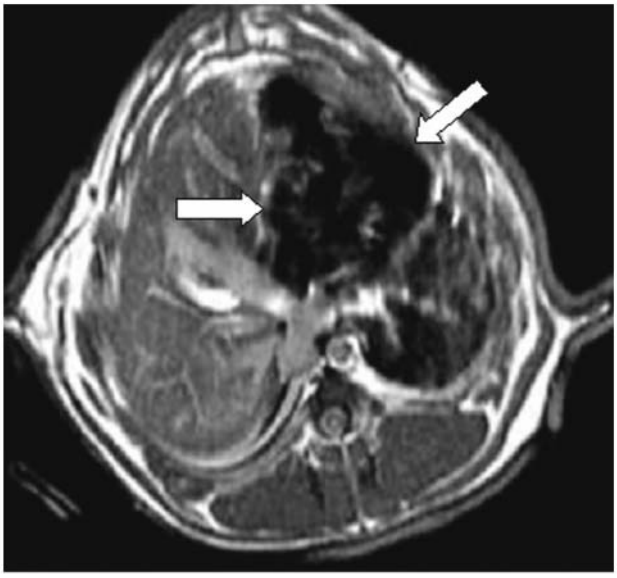

Figure 6. *Axial gadolinium enhanced T1-weighted image of the rabbit liver 7 days after radioembolization with ^{90}Y-MTC. Loss of signal (arrows) confirms the Yttrium-90 labeled, ferromagnetic MTC particles are confined in the left lobe. Histopathologic studies showed complete necrosis of that section of the liver which included the tumor.*

effects within the tumor. Although successful from this standpoint, safety issues remain as, at the highest radiation doses, significant destruction of adjacent liver parenchyma was also noted. It is interesting that despite such destruction, the animals did well clinically (except for one early death) and it is likely that with experience and better dosing, liver toxicity could be minimized further improving the safety profile of this delivery system. Another attractive and critically important aspect of this delivery system is its ability to be visualized on MR imaging. Because the MTCs are loaded with iron, they create a well-described artifact on MR imaging known as susceptibility artifact, which results in profound signal loss easily seen on MRI (Figure 6). This allows tracking of the microspheres into the tumor bed. This notion is important when contemplating delivery of toxic agents to tumors in order to kill them while preserving healthy tissues. Histopathologic analysis of multiple samples confirmed the MR imaging findings and revealed the presence of numerous microspheres located within the tumor.

The biodistribution profile, another goal of our study, was also quite favorable. Here, the majority of the radioactivity did not reach the systemic circulation after administration but rather stayed confined to the liver with the greatest amount localized to the tumor. Only small amounts of radioactivity were found in non-targeted organs. The miniscule activity found in circulating blood one hour after treatment and the MRI scans showing the particles localized within the liver, suggests that the vast majority of ^{90}Y-MTC particles embolized early and do not recirculate. These findings support the conclusion that externally sequestered ^{90}Y-MTC particles given intra-arterially for the treatment of hepatic malignancy results in a high degree of tumor necrosis and may be clinically useful in unresectable patients. Further experiments to establish the optimum dosage in humans (to minimize toxicity to the underlying liver parenchyma and maximize tumor necrosis) are needed. Whether this offers an added survival benefit over regular ^{90}Y-therasphere radioembolization in patients with unresectable disease will also need to be investigated.

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