A Correlative Study of CT, MR and Pathology in Rabbit Liver after Embolization by a China-Formulated Lipiodol Emulsion

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OBJECTIVE To explore the MR characteristics following lipiodol retention in rabbit liver and to evaluate the sensitivity of CT (CT value >400 HU) and MR in displaying the hepatic degeneration and necrosis following embolization.

METHODS Thirty-two rabbits were randomly divided into 3 groups. In the control group (n=8), 2 ml of normal saline was injected into the right branch of the portal vein. In the first experimental group(n=12), 4 ml of lipiodol emulsion was injected into the main portal vein. In the second experimental group (n=12), 2 ml of lipiodol emulsion was injected into the right branch of the portal vein. CT and MR images were obtained before and after surgery in each group. The histopathologic condition was determined for all liver tissue specimens.

RESULTS In the control group, CT and MR did not show any significant changes in the livers after surgery. After the operations in the experimental groups, the regional CT attenuation was 601±101 HU in the largest slice, which had no abnormal signals on T_1WI and T_2WI. In the first group, histologic examinations showed there were concentrated lipiodol droplets around the portal areas. In the second group, serious degeneration and necrosis in the right hepatic lobe occurred in 9 rabbits. T_1WI displayed homogenous or non-homogenous low signals and T_2WI mainly displayed a high signal. However, these pathologic changes did not appear on CT scanning due to high attenuation of the lipiodol.

CONCLUSION There were no remarkable hepatic changes on MR in rabbits following good retention of the formulated lipiodol emulsion mixture of lipiodol and urografin(CT value > 400 HU). MR displayed serious degeneration and necrosis of the liver following embolization.

KEYWORDS: lipiodol, rabbit liver, CT, MR, pathology.

In 1983, Nakakuma et al.[1] reported on the use of lipiodol for anticancer treatment by transcatheter arterial embolization (Lp-TAE). A series of investigations indicated that the use of Lp-TAE produced effective therapy for hepatic cancer.[2-4] Following those studies, Lp-TAE achieved satisfactory results for small hepatocellular carcinomas (< 3 cm) treated with percutaneous ethanol injection (PEI) into the liver tumor. Some investigators suggested that it could rise the ratio of tumor necrosis using a combination of Lp-TAE and PEI.[5] In a follow-up, CT could display both the size of the tumor and the lipiodol

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retention in the tumor and normal hepatic parenchyma tissues. Nevertheless, CT had low sensitivity in displaying the tumor vestige and relapse of the tumor. The main reasons were : 1) underdiagnosis because of a partial volume effect due to high attenuation of the lipiodol; 2) the distortion of special contrast enhancement due to high attenuation of the lipiodol. Imaeda et al.[2] indicated that if the CT value was above 365 HU in the region of the hepatic tumor by 3~4 weeks after Lp-TAE treatment, the necrosis of the tumor was more than 96%. The sensitivity and specificity were 89% and 73% individually.

As MR imaging techniques develop,[6] it is expected that MR will be able to visualize the tumor residuals or diminution of a tumor accurately after Lp-TAE treatment. However, the MR characteristics of the lipiodol are controversial. [7-9] This article is a report of embolization in rabbit liver using a formulated lipiodol emulsion. The MR signal clearly showed the rabbit liver while its CT attenuation was more than 400 HU. After embolization, the sensitivity of both CT and MR were evaluated regarding their ability to determine the degree of the hepatic degeneration and necrosis.

MATERIALS AND METHODS

Animals and surgical procedures

Thirty-two healthy rabbits, both male and female weighing 2.5~3.0 kg and were randomly divided into 3 groups.

The control group(8 rabbits)

Under general anesthesia (2.5% urethane, 4 ml/kg IV injection), we prepared and disinfected the abdominal skin of the rabbits and performed a median-incision laprotomy. Using blunt dissection, the main portal vein and branches were clearly exposed. Classically, the right branch is smaller than the left one. The left branch was closed using a vascular clamp and we put a No.7 nylon-casting-with a needle into the right branch of the portal vein. After that, the needle was drawn back and a syringe was connected. Then, we injected 2 ml normal saline and took off the vascular clamp and nylon casting. As soon as the surgeon had taken the nylon casting out, an assistant compressed the puncture point using gauze. After assurance there was no bleeding, we sutured the abdominal cavity and injected garamycin 80,000 U I.M. Four days later, the rabbits were examined by CT and MR. The tissue specimens that had been collected from the right and left hepatic lobes were fixed in 10% formalin and stained with H&E for examination.

The first experimental group (12 rabbits)

Initially all the rabbits were examinated by CT and MR under general anesthesia. The surgical procedure followed was similar to the control group, but the left branch was not closed by a vascular clamp and the puncture point was in the main portal vein where we injected 4 ml of lipiodol emulsion. Soon following the injection, part of the liver became mottled with yellow spots, and the rabbits were scanned by CT and MR. Then, tissue specimens were taken from the left and right liver lobes and fixed in 10% formalin followed by staining with both H&E and Sudan IV.

The second experimental group(12 rabbits)

As noted previously, CT and MR scans of the rabbits were conducted before surgery. The surgical procedure was almost same as in the control group except that we injected 2 ml lipiodol emulsion into the right portal vein followed by CT and MR scanning. Four days later, CT and MR scans of the rabbits were conducted again and tissue specimens from the right hepatic lobe were collected and fixed in 10% formalin followed by staining with both H&E and Sudan IV.

Preparation of the lipiodol emulsion

A 40% lipiodol injection fluid (batch number 931205) was produced by Huaihai Pharmaceutical Plant in Shanghai. We used a three-way tube between 2 syringes. One syringe contained 2 ml 40% lipiodol and the other contained 2 ml urografin (one volume of water plus 5 volumes 60% urografin; the solution with the same specific gravity as 40% lipidol).[2] The fluid was mixed completely by injections from the 2 syringes.

CT and MR examination

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CT Shimadizu 3000T, Matrix 340× 340. Slice thickness: 5 mm, no gap.

MR Siemens Magnetom 1.0T. SE sequence, Matrix 192×256. Head coil, Slice thickness: 5 mm, no gap. T_1WI (TR 500 ms/ TE 15 ms); T_2WI (TR 1600 ms/TE 15, 90 ms).

Statistical analysis

Statistical evaluation was performed using the *t* test to compare the T_2 value of the liver before and after the operation and relative analysis between the changes of CT value and the changes of T_2 value before and after operation on the liver. A P value<0.05 was considered to be statistically significant.

RESULTS

In the control group, there were no remarkable images in the right and left hepatic lobes on CT and MR. Except for some abdominalities due to the operations, histologies were normal.

In the first and second experimental groups, the liver mottled with yellow spots after being injected with the lipiodol emulsion into the portal vein. These spots appeared diffusely with high density in the left and right lobe (Fig.1). The regional CT value was from 425 to 801 HU (601± 101 HU) on the largest slice. There was no difference on MR images after treatment (Figs.2, 3). By changing the width and the level of the CT window, we found that the distribution of lipiodol was non-homogeneous. When we defined the size as 8 mm^2 in each area of interest, the difference was 431 HU between the highest and the lowest value in the same lobe. There was no evidence to indicate that the images were different in MR signal intensity both on T_1WI and T_2WI. Comparisons of CT attenuation and MR T_2 values before and after treatment are shown in Tables 1 and 2.

Measurement of MR T_2 values by the *t* test before and after the surgical procedures showed t =1.9< $t_{0.05\ (23)}$= 2.1, P >0.05, indicating no significant difference between them.

The correlation coefficient (r) was -0.30 between the changes of the CT value and the changes of MR T_2 value after treatment.

Table1. Comparison of the CT value before and after treatment in the region of interest.

Before (HU)				After (HU)			
65	69	62	67	478	705	552	469
68	63	64	69	614	617	547	533
67	67	62	62	492	590	596	705
62	64	64	62	626	482	481	801
64	63	65	62	686	596	766	712
64.8 ± 2.5 ($\bar{x}\pm s$)				601.0 ± 101.0 ($\bar{x}\pm s$)			

Table 2. Comparison of the T_2 value before and after treatment in the region of interest.

Before (msec)				After (msec)			
45.4	40.5	43.6	40.8	42.3	39.1	41.3	42.6
40.1	42.7	42.1	43.8	42.0	42.9	43.5	39.2
38.2	41.9	40.7	41.5	41.0	40.4	39.5	38.3
39.7	39.5	40.4	43.9	38.9	38.4	40.3	41.6
40.9	44.1	41.2	38.3	39.1	40.6	39.8	38.1
40.6	39.9	40.1	40.6	37.9	43.8	41.5	37.8
41.3 ± 1.9 ($\bar{x}\pm s$)				40.4 ± 1.8 ($\bar{x}\pm s$)			

The free degree was 24-2=22. Looking for the r limitation value table, $r_{0.05\ (22)}$= 0.40>|-0.30|, P>0.05, therefore we could conclude there was no significant correlation between the changes of CT values and changes in MR T_2 values after treatment.

No rabbits died from administration of lipiodol emulsion embolization.

Histological findings for the first experimental group showed the interlobe veinlet was dilated on H&E×40. H&E×200 displayed many infinitely small vacuoles in the hepatic cells, and also numerous bulbous oil droplets in the hepatic sinuses. Using Sudan IV staining, lipiodol became red in color with the surrounding of the portal area. We could see a great deal of red staining lipiodol in the hepatic sinuses and hepatic cells adjacent to portal areas (Fig.4).

In the second experimental group (4 days later), each rabbit liver still displayed diffusely high density in the right lobe on CT. But, there were many differences in MR images in 9 rabbits. On T_2WI, a high signal on the

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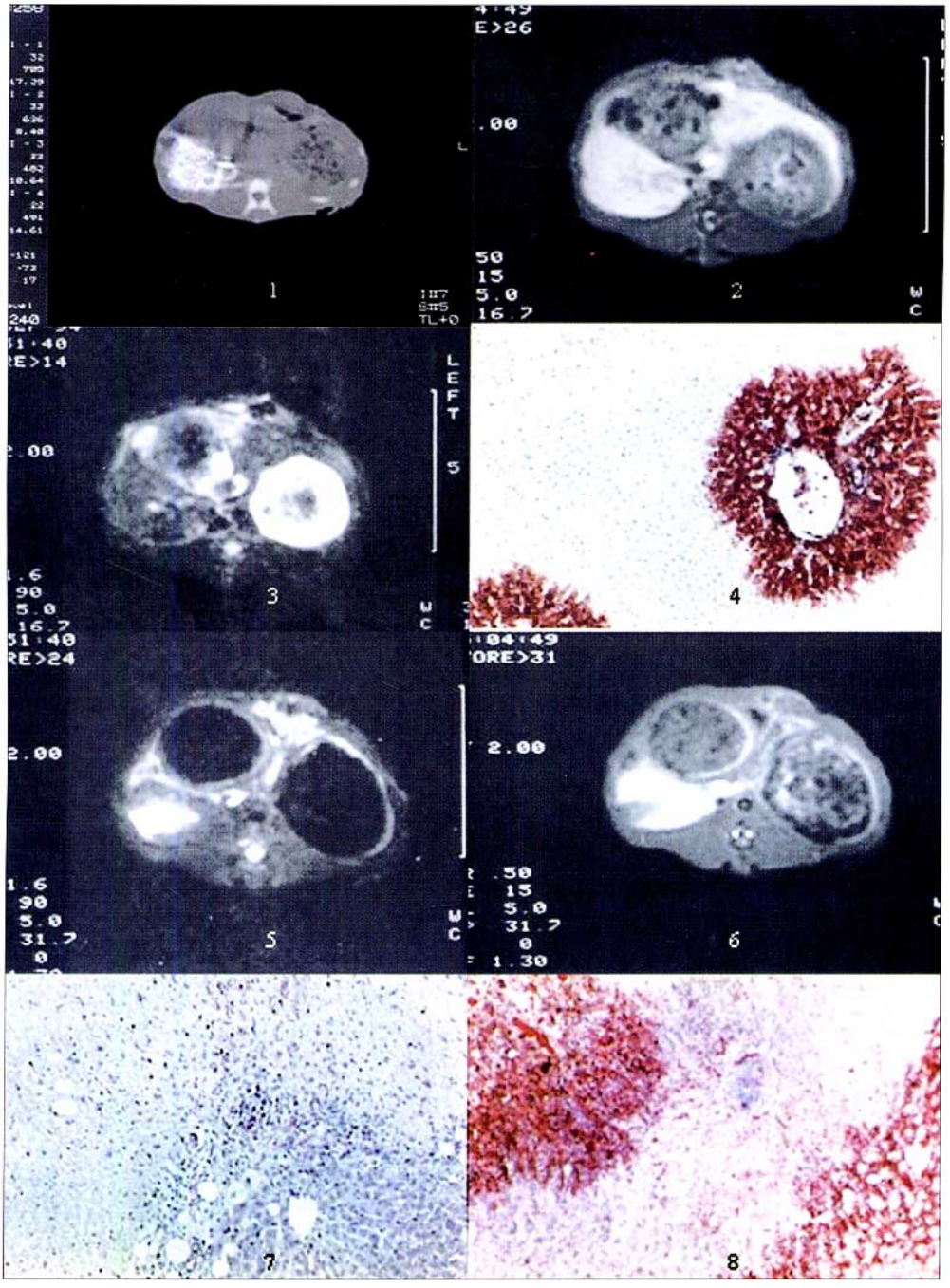

Fig.1. In the second experimental group immediately after the operation, CT showed a high density in the right hepatic lobe, but it was not homogenous. The CT value appeared in the left side.

Fig.2. The same animal as Fig.1, there was no abnormal signal on T_1WI (500 ms/15 ms).

Fig.3. The same animal as Fig.1, there was no abnormal signal on T_2WI (2000 ms/90 ms).

Fig.4. The first experimental group stained with Sudan IV. The red stained lipiodol concentrated in the hepatic sinus and cells surrounding the central portal area.

Fig.5. The same animal as Fig.1, 4 days later after operation, T_2WI visualized a cuneiform high signal.

Fig.6. The same animal as Fig.1, 4 days later after operation, T_1WI visualized a cuneiform low signal.

Fig.7. The same animal as Fig.1, 4 days later after operation, showing a large part of necrosis and serious degeneration in the middle of the right lobe.

Fig.8. The same animal as Fig.1, 4 days later after operation stained with Sudan IV. The red staining lipiodol was disorganized and discolored. Part of the lipiodol tended to be the central vein.

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right lobe appeared as patched or cuneiform (Fig.5). Among them, the right lobe visualized a low signal on T_1WI in 4 rabbits (Fig.6). In these cases, hepatic cells showed serious degeneration and focal necrosis. The volume of hepatic cells was enlarged, while the nuclei of the hepatic cells were deformed and displaced. The other 5 rabbits displayed mixed signals on T_1WI in the right lobe. Pathologically, the hepatic tissues had extensive necrosis combined with patch-like hemorrhages(Fig.7). Inflammatory cells had infiltrated at the margin of the necrotic tissues. There also were a variety of irregular vacuoles in the necrotic area. The Sundan IV-stained red tissues showed that lipiodol was disorganized and discolored. A part of the lipiodol tended to surround the central veins of the hepatic lobes (Fig.8). In the last 3 rabbits, there were no MR signal changes on T_1WI or T_2WI. Under microscopy, H&E staining showed the liver cells were either enlarged or discolored. We could also see scattered oil drops in the hepatic tissues, but no hepatic necrosis occurred. On Sudan IV staining, the red-stained lipiodol diffused into the surroundings with a margin that was no longer sharp.

DISSCUSION

MR characteristic of lipiodol emulsion

In 1986, Buckwalter et al.[7] reported the results of CT and MR imaging of 2 women with known carcinoma of the cervix several days after lymphangiography. The CT (9800 scanner, General Electric, Milwaukee) images of the pelvis clearly demonstrated the presence of opacified lymph nodes. Using MR (0.35T, Diasonics, Milpitas, CA), neither in vitro nor vivo with either T_1WI (500 ms/28 ms) or T_2WI (2,000 ms/56 ms) at the same anatomic levels, could nodules opacified with Ethiodol be distinguished from the adjacent fat. In 1996, Havard et al.[8] studied a patient after lymphangiography who suffered from testicle embryonic carcinoma. As expected, CT showed extensively enlarged nodes in the para-aortic area with very high attenuation due to their iodine content. Three weeks later, MR imaging was performed using a Siemens 1.5T Magnetom. T_1WI (650 ms/17 ms) and T_2WI (2,000 ms/25, 90 ms) were obtained. On T_1WI, they found isointensity, while on T_2WI and a proton density image they found hyperintensity in the enlarged nodules in the para-aortic region. In 2004, Jiang et al.[9] reported on MR characteristics of formulated and imported lipiodol in various ratios of lipiodol and urografin. They indicated that there was a marked difference in the MR signal in vitro between the formulated and imported lipiodol. Nevertheless, there was no evident influence on the MR signals observed for lipiodol retention in hepatic tumors after Lp-TAE.

In our study, we formed the emulsion using 40% lipiodol (made in the Haihai Pharmaceutical Plant in Shanghai) and urografin (embolization agent for hepatic carcinoma). The lipiodol emulsion was injected into the rabbit's liver through the portal vein. The regional CT value was above 400 HU in the largest slice.[2] The lipiodol emulsion distributed diffusely in the liver, and after treatment, we found no abnormal changes in the liver on both T_1WI and T_2WI images. The T_2 value was 41.3±1.9 msec before treatment, and 40.4±1.8 msec after. Comparison of these values using the t test, $P>0.05$, showed no significant difference. In experimental groups, there was no significant correlation between the changes of the CT values and changes of the T_2 values before and after treatment (correlation coefficient $r=(-0.3)<r_{0.05\ (22)}=0.4$). The lipiodol emulsion dispersed diffusely, but non-homogenously in the rabbit liver. The difference in the CT values was 431 HU between the highest and lowest in 8 mm^2 region of interest in the same hepatic lobe. MR images of both T_1WI and T_2WI, showed no difference. Four days later in the second experimental group, 9 rabbits had serious degeneration and necrosis in the right lobe because of embolization of the right branch of the portal vein. These areas displayed high signals mainly on T_2WI. Four of the rabbits were in low signal on T_1WI, while the other 5 displayed mixed signals on T_1WI. The lipiodol retention was still in the right lobe on CT scanning.

In summary, we found that the use of lipiodol has no influence on the MR signal in rabbit liver. The results differed from past reports in the literature.[7,8] Our explanation is as follows: 1) Lipiodol is a mixture of iod-

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inated ethyl esters of various fatty acids, principally linoleic acid, and so it does not have a single structural formula. There might be some differences in its formulation among the manufactures. 2) We explored in a CT range from 425-801 HU, the best range for hepatic carcinoma.[2] CT values for lipiodol in the lymphnodes have not been reported before.[7,8] 3) There also was variation from previous reports in the MR imaging parameters, magnetic strength and the object of study. 4) In other reports, the time between the MR examination and the lymphangiography, varied from a few days by Buckwalter to 3 weeks by Havard. It is impossible to know whether some pathologic changes had occurred in the enlarged lymph nodes. Comparing our results with those of Jiang et al.,[9] the MR findings were almost the same between a China formulated lipiodol emulsion and imported UFL (lipiodol ultra-fluide, Guerbet, France) in the normal hepatic tissue and tumor. The difference was that the CT value of rabbit liver (425~801 HU) was evidently higher than that reported by Jiang (254.4~468.8 HU). In addition, they supposed that the pattern of distribution and accumulation of lipiodol were responsible for the different signals between the liver and lymph nodes. These results should be studied further.[9]

Mechanism of lipiodol embolization

Miller et al.[10] found that an a mount of lipiodol accumulated in the dead and dying carcinoma cells, except a part of the lipiodol was found in new pathologic vessels of the hepatocelluar carcinoma. In a study by Han et al.,[4] they showed in hepatic carcinoma cells a great deal of lipiodol in the cytoplasm in the form of granulation, while there was a little lipiodol in the nucleus. In our first experimental group, the rabbit liver specimens were collected during 6 h following the injection of lipiodol. We observed concentrated lipiodol droplets in the cytoplasm of the hepatic cell using H&E×200 staining. There were a large amounts of lipiodol seen in the hepatic sinuses and hepatic cells in the portal area with Sudan IV×100 staining. In the second experimental group, the little oily drops coalesced to form bigger ones, the cytoplasm was diffuse and large, the nucleus was compressed and displaced and the volume of the hepatic cells was enlarged further. Finally, the hepatic cells became necrotic or were in the process of being necrotic. We are not aware of reports of this phenomena in the literature. The mechanism of these findings will be further explored in the future. It is suspected that in normal hepatocytes there was a structure in the membrane to carry lipiodol into the hepatic cells.

In this article, the lipiodol emulsion was injected into the hepatic portal vein of rabbits. We conclude that there was no influence on the MR signals at the dose of lipiodol used to almost totally kill hepatic carcinoma.[2] MR could visualize the hepatic pathologic changes of serious degeneration and necrosis while lipiodol was retained in the liver. The lipiodol appeared in the cytoplasma of the hepatic cells within 6 h after injection. These events result in the degeneration and necrosis of the hepatic cells.

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