

# Target distribution of magnetic albumin nanoparticles containing adriamycin in transplanted rat liver cancer model

Lian-Sheng Gong, Yang-De Zhang and Su Liu

Changsha, China

**BACKGROUND:** Liver cancer is one of the most common diseases around the world. The aim of this study was to verify the effect of magnetic field application on target distribution of nanoparticles in transplanted rat liver cancer model and to find out a new method for the treatment of malignant liver tumor.

**METHODS:** Seven days after the establishment of the model, the abdomen of the rat was exposed through a midline abdominal incision. A cannula was inserted into the gastroduodenal artery. In the experimental group (12 rats), the tumor tissue was exposed to the magnetic field for 30 minutes. Magnetic albumin nanoparticles containing adriamycin or at an equal dose of free adriamycin (0.5 mg/kg) were injected into the hepatic artery. After the magnetic field was removed, the rat was immediately sacrificed. An equal dose of nanoparticles in absence of the magnetic field served as control (12 rats). Tissues of tumor, nontargeted sites of the liver, heart, kidney, lung, spleen, stomach and small intestine were analyzed for  $\gamma$ -counts and examined histologically.

**RESULTS:** In the experimental group, the radioactivity of tumor tissue was 8.7 times that of liver tissue. In the control group, the radioactivity of tumor tissue was 2.8 times that of normal liver tissue. The radioactivity of the lung was reduced more significantly in the experimental group than in the control group. No significant difference in the kidney, heart, spleen, small intestine and stomach was observed between the experimental group and control group. And over 80% of the injected nanoparticles distributed in the liver.

**CONCLUSIONS:** In the presence of magnetic field, magnetic albumin nanoparticles may accumulate in tumor tis-

ues, of which the radioactivity can increase to 8.7 times that of normal liver. Even if the magnetic field is not applied, magnetic albumin nanoparticles in tumor tissues still increase to 2.8 times that of normal liver tissues. These findings indicate that normal organs in the presence of magnetic field are less exposed to chemotherapeutic drugs.

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**KEY WORDS:** transplanted liver cancer model; magnetic albumin nanoparticles; magnetic target therapy

## Introduction

Primary liver cancer is most common in the world<sup>[1-4]</sup> and the prognosis of liver cancer without treatment is poor, with a median survival time between 0.7 and 8.3 months.<sup>[5]</sup> Hepatic resection, however, remains the treatment of choice, with a rate of only 10%-45%.<sup>[6,7]</sup> Autopsy series have revealed that the liver next to lymph nodes serves as a site of metastasis from primary tumors. In particular, liver metastasis will appear in most of the patients with gastrointestinal cancer.<sup>[7]</sup> Surgical resection is only indicated for 5%-20% of patients with metastatic liver tumor.<sup>[8,9]</sup> Therefore, it is important to make their chemotherapy as effective as possible. Various treatment modalities including systemic chemotherapy<sup>[10]</sup> and continuous hepatic artery infusion<sup>[11,12]</sup> have been tried, but their results are unsatisfactory.

In this study we established a transplanted rat liver cancer model, by which magnetic albumin nanoparticles were injected into the hepatic artery. The tumor site of the liver was exposed to 4250 GS magnetic field, attempting to observe the target accumulation of magnetic albumin with the aid of magnetic field.

## Methods

### Animals and groups

Twenty-four healthy male Wistar rats weighing 150-250 g were supplied by the Experimental Animal Center

**Author Affiliations:** Department of Hepatobiliary and Enteric Surgery, Xiangya Hospital, Central South University, Changsha 410008, China (Gong LS, Zhang YD and Liu S)

**Corresponding Author:** Lian-Sheng Gong, MD, Department of Hepatobiliary and Enteric Surgery, Xiangya Hospital, Central South University, Changsha 410008, China (Tel: 86-731-4327036; Email: gongliansheng2000@yahoo.com.cn)

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of Hunan Medical University, Changsha, China. Those with Walker-256 ascitic hepatoma were purchased from the Center of Tumor Pharmacology, Shanghai Medical Industry Institute, Shanghai, China. The tumor derived from Walker-256 hepatoma rats was transplanted into the left lateral lobes of the liver of the healthy rats. Ten-12 days later, the rats bearing transplanted tumor were divided into two groups. In the control group (12 rats), the tumor sites of the liver were exposed to 4250 GS magnetic field; in the experimental group (12 rats), the magnetic field was not applied. Surgical procedures performed under the anesthesia induced by pentobarbital sodium (30 mg/kg ip).

### Animal model

Tumor breeding rats were sacrificed 1 week after inoculation of the tumor and 0.5 ml ascites was drawn out from the peritoneal cavity with a syringe and inoculated into the inguinal subcutis of the male rats of 6 weeks old, weighing 70-80 g. A week later, the solid tumors were taken out and minced into small cubes of 0.5-2 mm. After depilation and disinfection, the rats underwent a 2-mm subxiphoid midline incision at the abdomen. The left lateral lobe of the liver was gently retracted out of the abdominal cavity. After the integument of the liver was incised using a sharp blade, a pocket was formed with a pair of microsurgical forceps. Compression hemostasis was followed by gent placement of a tumor fragment into the pocket, which was buried in the hepatic parenchyma. The lateral lobe of the liver was gently moved to the peritoneal cavity and the abdominal wall was closed. Ten-20 days after inoculation of liver tumor the abdomen was opened through a midline incision. The gastroduodenal artery was dissected and its distant end was ligated. A cannula of 0.2 mm in external diameter was placed into the hepatic artery. At the same time, a 4250 GS magnet was put on the surface of the liver tumor.  $^{125}\text{I}$ -magnetic albumin nanoparticles containing adriamycin (8.7 mg/kg body weight) were administered through the cannula within more than 10 minutes. In the experimental group, the tumor area of the liver was exposed to magnetic field for 30 minutes af-

ter the administration. The animals were sacrificed after removal of the magnetic field. An equal dose of nanoparticles was given in the absence of magnetic field served as a control. The tissues from the tumor, normal hepatic area, heart, kidney, lung, spleen, stomach and small intestine were analyzed for  $\gamma$ -counts. Tumor and liver tissues were examined histologically.

### Statistical analysis

The data were presented as the standard error of the mean (SEM). Student's *t* test was used to assess the difference between the variables.

### Results

In the presence of magnetic field, the radioactivity of the liver tissue was increased more significantly than in the nontargeted area of the liver ( $P < 0.001$ ). The radioactivity of the tumor tissue was 8.7 times that of the nontargeted area of the liver. In the absence of magnetic field, however, the radioactivity of the tumor tissue was 2.8 times that of the nontargeted area of the liver (Table 1).

The radioactivity of the lung in the experimental group decreased more significantly than that in the control group ( $P < 0.05$ ). No differences were observed in other organs between the two groups (Table 2).

In both groups, the radioactivity of the heart, kidney, lung, spleen, stomach and small intestine was lower than that of the liver (included targeted and nontargeted areas). The radioactivity of the lung reduced more significantly in the experimental group than that in the control group. The radioactivity of other organs was not different between the two groups. The radioactive ratio of the lung, spleen, and stomach to targeted area of the liver tissue in the experimental group decreased more significantly than that in the control group (Table 3).

The total radioactive percentage of the lung and spleen was less in the experimental group than that in the control group. No statistical differences were found in other organs between the two groups. Over 70%-80%

**Table 1.**  $\gamma$ -counts of tumor and normal liver tissue (cpm/g moist weight tissue)

Group	<i>n</i>	Tumor tissue	Normal liver tissue	Tumor/normal liver tissue
Experimental	12	20855.5 ± 4916.2 * #	2411.6 ± 285.8	8.7 ± 2.1 *
Control	12	11237.3 ± 2365.9 #	4105.8 ± 983.6	2.8 ± 0.4

\* : compared to control group,  $P < 0.001$ ; # : compared to control group,  $P < 0.0001$ .

**Table 2.**  $\gamma$ -counts of other organs (cpm/g moist weight tissue)

Group	<i>n</i>	Kidney	Heart	Lung	Spleen	Small intestine	Stomach
Experimental	12	229.19 ± 49.46	166.49 ± 37.65	838.63 ± 87.93 *	572.30 ± 94.55	225.66 ± 46.92	479.56 ± 96.11
Control	12	167.93 ± 16.74	140.60 ± 56.19	1541.67 ± 39.73	1057.60 ± 216.36	229.28 ± 109.21	560.57 ± 63.28

\* : compared to control group,  $P < 0.0001$ .

**Table 3.** The radioactivity ratio of other organs to tumor tissue

Group	n	Kidney	Heart	Lung	Spleen	Small intestine	Stomach
Experimental	12	0.011±0.002	0.009±0.003	0.043±0.007 *	0.029±0.005 <sup>#</sup>	0.012±0.003	0.025±0.006 <sup>#</sup>
Control	12	0.015±0.001	0.012±0.004	0.143±0.014	0.098±0.021	0.019±0.009	0.052±0.008

\* : compared to control group,  $P < 0.005$ ; # : compared to control group,  $P < 0.05$ .

**Table 4.** The percentage of albumin nanoparticles injected in different organs or areas (%)

Group	n	Tumor	Normal liver	Kidney	Heart	Spleen	Lung	Stomach	Others	Total
Experimental	12	37.2±2.2	47.1±1.2	1.0±0.2	0.3±0.1	0.9±0.2 *	3.8±0.3 *	2.0±0.3	7.7±1.2	100
Control	12	20.2±2.4	62.6±2.2	0.7±0.1	0.2±0.1	1.5±0.3	5.2±0.2	2.8±0.4	6.8±0.1	100

\* : compared to control group,  $P < 0.05$ .

of the injected nanoparticles distributed in the liver tissue (Table 4).

Histological examination demonstrated a lot of nanoparticles in tumor and ambient tissue in the experimental group, and a small amount of nanoparticles in tumor tissue in the control group. There were less nanoparticles dispersed in the normal liver tissue.

## Discussion

It is known that 20%–25% of blood supply of the liver is from the hepatic artery and 75%–80% comes from the portal vein. But 90%–95% of blood supply to liver cancer is dependent on the liver artery.<sup>[13]</sup> Thereby Goldstein put forward a method of liver artery embolism to treat liver cancer.<sup>[14]</sup> The materials for embolism include gelatin, unrust steel loop, etc. Because these materials only embolize larger vessels but likely produce embolism syndrome and acute liver necrosis, patients fail to accept this therapy. Moreover, it is easy to form lateral circulation and cause tumor recurrence. To improve this method researchers have suggested microspheres,<sup>[15–21]</sup> microcapsules<sup>[22–24]</sup> and others for liver chemotherapy. These methods have produced good results in the treatment of advanced liver tumor.<sup>[25–28]</sup> For chemotherapy microspheres or microcapsules of about 10–200  $\mu\text{m}$  in diameter which enter arterioles and cause embolism are unable to extravasate the endothelium of the tumor tissue. It has been shown that magnetic albumin nanoparticles can transfer from the blood compartment to the extravascular target tissue. Using magnetic carrier, we has demonstrated a 10 to 100-fold increase in the therapeutic index of associated drug,<sup>[29]</sup> but this increase in the therapeutic index of chemotherapeutic agents has not been demonstrated by any other drug targeting device. Since magnetic albumin nanoparticles can be endocytosed by tumor, the drug can release from the carrier in the interstitial or/and intracellular space then exerts its pharmacological action. In our experiment, the radioactivity of tumor tissue was 8.7 times that of normal liver tissue. In

the control group, the radioactivity of tumor tissue was also 2.8 times that of normal tissue in the absence of magnetic field. It is indicated that nanoparticles themselves are selective to tumor tissue even at the absence of magnetic field. This result is similar to the distribution of other nanoparticles in tumor and non-tumor tissue. The reported density of vessels in the tumor region was 2–6 fold greater than that in normal liver,<sup>[30]</sup> which indicates why more nanoparticles distribute in the area of tumor than in normal tissue. Magnetic field causes a great increase of nanoparticles in the targeted area of tumor. The distribution of magnetic albumin nanoparticles in the rat with transplantable liver tumor is similar to that in normal rat, but much more nanoparticles gather in the tumor region and normal liver, and the amount of nanoparticles in the other organs is much less. In our previous study in normal rats, the radioactive ratio of the spleen, lung, and stomach to tumor tissue in the experimental group was lower than that in the control group.<sup>[31]</sup> It means that the dosage in the experimental group would have been decreased if the same concentration of chemotherapeutic agent were obtained in the tumor region of both groups. Therefore, the side effects of chemotherapeutic agents can be abated. Histologically a large amount of nanoparticles existed in tumor tissue and peripheral vessels, many of which were filled with nanoparticles. However, only single, non-aggregated nanoparticles were seen in normal liver and tumor tissue of the control group. We consider that the nanoparticles in the magnetic area are magnetized to aggregate and cause vascular embolism, but the nanoparticles in the non-magnetic area are not aggregated. The concentration of chemotherapeutic agent in tumor tissue increases greatly because of the target of nanoparticles in the presence of magnetic field.

## Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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