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ARTICLE

Effect of Intrahepatic Arterial Delivery of Sorafenib on Normal Liver Tissue of Rabbit: An Experimental Study

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ABSTRACT

Objective: To assess the safety, feasibility and eluting efficiency of intrahepatic arterial delivery of sorafenib on normal liver tissue of rabbit. **Methods:** 24 New Zealand rabbits were randomly divided into three groups: group I (Lipiodol-sorafenid), group II (Lipiodol) and group III (Sorafenib). Group I and II were treated by transcatheter selective hepatic arterial embolization with emulsions of lipiodol and sorafenib or with only lipiodol, while group III was given hepatic arterial infusion with sorafenib. Sorafenib concentration in plasma was determined by HPLC (high performance liquid chromatography) in 0 min, 20 min, 1h, 2h, 4h, 8h, 16h, 32h and 48h respectively. The breathing rate, heart rate, rectal temperature and body weight were measured, as well the blood routine test and the function of liver, kidney, and heart. Two animals of each group were respectively killed in the 3rd day, 1st, 3rd and 6th week after treatment. Histopathologic study was done to liver, heart, kinney, lung, brain, gall bladder and intestine. **Result:** ① The peak sorafenib concentration (Cmax)and AUC(Area under curve) in plasma in group I was 2.46±0.101μg/ml and 945.72 ± 52.3 μg/mL.min respectively, while in group III which was 3.78±0.180 ug/ml and 546.98±21.1μg/mL.min. Compared with group III, the Cmax and AUC of group I had a significant statistics difference ($p<0.05$). ② The breathing rate, heart rate, rectal temperature and AST/ALT,WBC,NEU% of group I and group III has a significant statistics difference($p<0.05$) in the 3rd day. ③ CK ,CK-MB, DB, Cr, BUN,RBC,PLT in plasma did not change in all group. ④ Local necrosis was seen in group I and group II in the 3rd day and 1st week, but they did not seem to be different. Group III showed no necrosis. Granulation tissue with bile duty, portal vein and microfossils hyperplasia were seen in local necrosis area in the 3rd week. No pathological changes were found in brain, heart, kidney, intestine and gallbladder. **Conclusion:** TAE with emulsions of lipiodol and sorafenib is feasible, safe and has some slow-release effect.

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1. Introduction

Transcatheter arterial chemoembolization (short for TACE) is a priority for treatment of advanced liver cancer that cannot be surgically excised. The clinical research has proved that TACE can effectively prolong the patient's long-term survival time. It is a standard treatment method for HCC of Phase B of BCLC. However, tumor recurrence and metastasis are important factors affecting TACE's long-term efficacy. Studies have confirmed that local tissue ischemia and hypoxia after TACE promotes up-regulation of VEGF/VEGFR expression, which promotes neovascularization and is one of the important promoters of tumor recurrence [1-4]. The emergence of novel multi-target molecular targeted drugs, such as Sorafenib, provides a new approach to the treatment of HCC. Combined with oral administration of Sorafenib after TACE can inhibit tumor growth and angiogenesis and prevent tumor recurrence. However, oral Sorafenib has many shortcomings such as long oral administration time, high cost, large side effects, and low response.

Regional local sustained high-concentration slow-release drug technology for treating a variety of diseases has been widely used in clinical, such as local infusion chemotherapy drugs inactivated tumors, giving pancreatic enzyme inhibitors using local indwelling catheter for pancreatitis, local perfusion of pro-angiogenic substances (such as gigaton) to treat femoral head necrosis, continuous direct thrombolysis using local indwelling catheter for treating thrombosis etc. Regional local qualitative delivery of drugs has unique advantages due to its minimally invasive, sustained release of high-concentration drugs and small side effects. Intravascular interventional techniques combined with local molecular targeted therapy have been reported in related experiments and clinical studies [13-15], and achieved good results. Therefore, the local delivery of Sorafenib using intravascular interventional technique is expected to significantly increase the local drug concentration, improve the therapeutic effect, shorten the treatment cycle, reduce the incidence of side effects, reduce the cost, make up for the deficiency of simple interventional therapy, and completely inactivate the tumor, reduce the recurrence rate and prolong the long-term survival time of patients. Based on the above analysis, we propose an experimental research on TACE combined with intravascular delivery of Sorafenib powder with a goal to evaluate the feasibility and safety of intravascular injection of Sorafenib for pharmacokinetics and intravascular injection of Sorafenib.

2. Material & Method

2.1 Material

2.1.1 Animal

Healthy New Zealand white rabbits, a total of 24, 14 females, 10 males, weighing 2.5-3.0 kg, were provided by the PLA General Hospital Experimental Animal Center. Animal experiments were approved by the PLA General Hospital Animal Management Committee.

2.1.2 Drugs

(1) Biological targeting preparation - sorafenib original powder. The tablets were provided free of charge by Bayer and purified by the Biochemical Laboratory of the Academy of Military Medical Sciences. The specific method is as follows. According to the manufacturer's recommendations and our preliminary experiments, the proposed dose is 20mg/Kg [Cancer Res, 2006, 66: 11851].

(2) 40% iodized oil (super liquefied lipiodol produced in France).

(3) Anesthetic with 3% sodium pentobarbital injection (5mg/Kg body weight).

2.1.3 Instruments & Equipment

(1) Digital subtraction angiography (DSA) devices, equipment specification: Philips INTEGRIS, JWYXZ ZI No. DSA07

(2) Super smooth guide wire: RADIFOCUS, TERUMO, Japan

(3) Angiography catheter: 4F Cobra Catheter, Cordis, Johnson & Johnson, USA

(4) 4F arterial sheath: 4F, 24cm long, TERUMO, Japan

(5) Percutaneous puncture suite: Micropunture system, COOK, USA

(6) Agilent 1200 High Performance Liquid Chromatography System 1100 (HPLC), Diode Array Detector

(7) 3F microcatheter (Progreat): TERUMO company, Japan

(8) Optical microscope, type IX51, Olympus, Japan

2.2 Method

2.2.1 Purification of Sorafenib Powder

(1) 20 tablets (4g) of Toluenesulfonic acid Sorafenib were ground to a powder, and 100 ml of methanol was added thereto, and the mixture was heated under reflux for 30 minutes, and decolorized by adding activated carbon. After filtration and concentration of the filtrate, obtain 1.6 g of a solid, which was dissolved by heating with 30 ml of

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ethanol, filtered when it was still hot, and precipitated to crystallize. Filter and drain to obtain 1.3 g of white crystals.

(2) Purification of Sorafenib structure and purity verification: comparison of the melting point, molecular structure and space structure of the purified substance and Sorafenib substance.

① Melting point: compare the melting point of the purified product with the original powder of Sorafenib, the melting point of the purified product is 226-229 ℃ , and the melting point of the original powder is 223-231℃ in the literature. The melting point of the purified product is within the melting point of the original powder, and the two have the same possibility of substance.

② Elemental analysis: measured value C H N (theoretical value C of 52.79%, H of 3.80%, N of 8.79%), there was a difference of three thousandths between the measured value and the theoretical value. Compare compounds for the same substance.

③ Nuclear magnetic spectrum (spatial structure, Figure 1)

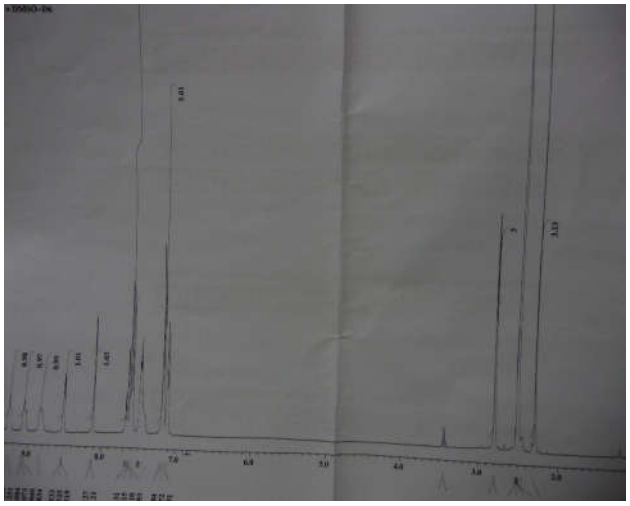

Figure 1. Nuclear magnetic spectrum of Sorafenib powder

Result analysis: 1H-NMR(DMSO-d6,400MHz): δ=2.29(s,3H,CH3); 2.79(d,J=4.8Hz, 3H,NCH3); 5.9(br,s,1H,SO3H); 7.14 (d,J=7.9Hz,2H,2’’’-H,6’’’-H); 7.17-7.22 (m,d,J=8.8Hz,3H,5-H,3’-H,5’-H); 7.44 (d,-J=2.0Hz,1H,3-H); 7.48 (d,J=8.0Hz,2H,3’’’-H,5’’’-H); 7.61 (d,J=8.8Hz,2H,2’-H,’-H); 7.63 (m,1H,5’’-H); 7.67 (m,1H,6’’-H); 8.14 (d,J=2.2Hz,1H,2’’-H); 8.53 (d,J=5.6Hz,1H,6-H); 8.88 (d,J=4.8Hz,1H,NHCH3); 9.1(br,s,1H,NHCO); 9.3 (br,s,1H,NHCO). The analysis by the above method showed that the substance purified by the tablets was Sorafenib.

Figure 2. Purified white Sorafenib powder

2.2.2 Experimental Design

(1)A total of 24 New Zealand white rabbits, 14 females and 10 males were randomly divided into 3 groups according to random number method, group I (iodine-Sorafenib emulsion group) and group II (simple iodized oil group). And group III (Sorafenib only), with 8 in each group. Hepatic artery embolization was performed in group I and group III, respectively, and group S was only treated with Sorafenib arterial infusion. The liver function changes of liver tissue before and after treatment were compared to evaluate the effect of Sorafenib on liver tissue. The drug concentration of iodized oil-Sorafenib emulsion group and Sorafenib group were compared to evaluate the drug loading characteristics of Sorafenib. Sorafenib-iodine was investigated. The change in physiological indexes and pathological examination of the oil group and the Sorafenib group before and after surgery were evaluated to evaluate the safety of Sorafenib.

(2) Hepatic artery catheterization technique: ① three groups of animals before routine experiment with venous blood to check blood routine, liver and kidney function. ② After the experimental rabbits were given intravenous anesthesia, they were inserted on the back of the rabbit plate in the supine position, and the right groin was prepared for skin and disinfection and drape. The local femoral artery was partially incision and separated, and the femoral artery was punctured under direct vision with a 21G micropuncture needle. The guided 4F soft sheath (product of COOK, USA) was inserted into the 4F arterial sheath. ③ Insert a 4F cobra catheter into the celiac artery under fluoroscopy for routine celiac angiography. The contrast agent for contrast was ultravist (370 mg I/ml). The parameters were set to: 4ml per second, a total of 16ml, a pressure of 300PSI. After angiography (DSA) to determine the anatomical details of the hepatic artery, the 3F microcatheter was super selectively inserted into the hepatic artery branch, and then iodized oil was injected according to the experimental needs. iodized oil - sorafenib emulsion, suspension of Sorafenib and phys-

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iological saline. Eight rats in group I were given a bio targeted preparation via a catheter-selective intrahepatic artery—sorafenib powder and iodized oil emulsifier, a total amount of 1.5-2 ml, containing 50-60 mg of Sorafenib powder. In group II, 8 rabbits were used, and iodized oil was administered via a ductal selective hepatic artery with a total amount of 1.5-2 ml. Eight animals were used in group III, and Sorafenib was administered via a transcatheter selective hepatic artery with Sorafenib 50-60 mg. The actual doses of Sorafenib in the survival group I and group III were: group I (53.04±3.209 for lipiodol-Sorafenib group) and group III (54.04±2.409 for Sorafenib group only). There was no statistical difference in the doses of Sorafenib used in the two groups (P=0.49>0.05).

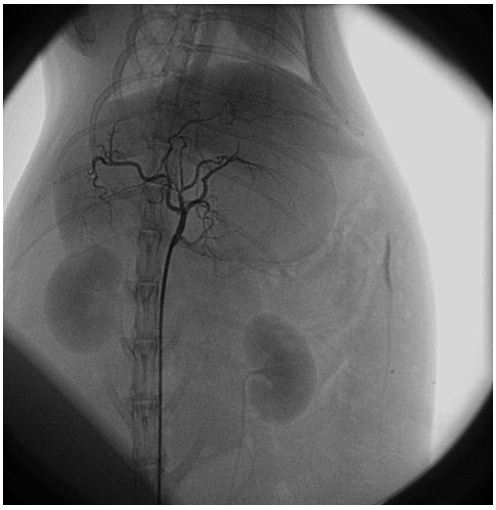

Figure 3. Rabbit celiac angiography

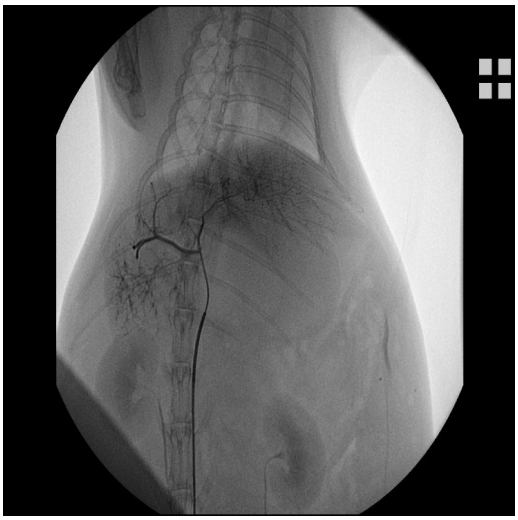

Figure 4. Iodine oil deposition after hepatic artery embolization

④ The catheter and catheter sheath were removed after operation, the right femoral artery was ligated, and the skin was sutured. After the operation, music injection of gentamicin of 20,000 units/Kg was performed for 3 days.

(3) Collection of specimens: Blood samples were collected from experimental animals at 0 hours (before administration) and 10 minutes, 20 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, 32 hours, and 48 hours after administration.

(4) Specimen detection: HPLC analysis was measured on rabbit plasma Sorafenib concentration, this part of the work was completed with assistance of the PLA General Hospital pharmacology laboratory. ① Instruments and reagents: Agilent 1200, high performance liquid chromatography. Sorafenib raw materials were provided by our laboratory; acetonitrile and methanol are chromatographically pure; triethylamine and phosphoric acid are of analytical grade. ② HPLC analysis conditions: phenomenex C18 column (5μ, 250mmmx4.6mmx5um); mobile phase: triethylamine phosphate buffer (ultra-pure water of 990ml plus triethylamine of 10ml, adjusted to pH 5.4 with phosphoric acid): acetonitrile = 50:50; flow rate : 1.0 m1/min; detection wavelength: 261 nm; column temperature: 250C. ③ Blood standard concentration determination and linear relationship test: standard solution preparation: precision weighing of 5mg of Sorafenib, placed in a 10ml volumetric flask, dissolved in 75% ethanol, the concentration of 0.5mg/ml; by pipetting from the above solution, it was precisely weighed 5 ml into a 10 ml volumetric flask and dissolved in 10 ml with 75% ethanol at a concentration of 0.25. The following three solutions were prepared in the same manner as above: 0.125 mg/ml, 0.05 mg/ml, and 0.025 mg/ml. Take the above five portions for 400 ul each.

Treatment of blood samples: centrifugation at 3000 r/min for 10 min. Take 100 ul of the supernatant, five portions each. The standard solution and the treated blood were mixed and made in a total of five parts, 500 ul each, and a methanol activated solid extraction cartridge (1x3) was equilibrated with 1 ml of water → 500 ul of the experimental serum was applied to the column, and the impurities were eluted with a 20% methanol solution → and again add 1 ml of methanol, collect the filtrate → dry naturally in a ventilated environment → dissolve the good residue with 200 ul of mobile phase (triethylamine phosphate buffer: acetonitrile = 50:50), centrifuge at 3000 r/min for 10 min → absorb 40 μl of the supernatant was subjected to HPLC analysis. The peak area (Y) was subjected to regression analysis for the corresponding content (X), Y = 0.201X + 0.0921, r = 0.9947. This equation shows that the concentration of plasma Sorafenib has a linear correlation with the peak area determined by the HPLC. The concentration of Sorafenib in the peripheral blood can be obtained from the peak area measured by the

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high-performance liquid phase, and the lowest drug concentration is 0.1 μg/ml.

(5) observation of other indexes after surgery: ① blood routine, liver and kidney functions. ② The experimental animals were sacrificed regularly (3 days, 1 week, 3 weeks, 6 weeks after operation), and the liver and kidney function indexes were taken before blooding. After the sacrifice, the experimental group and the control group were used for liver, heart and kidney diseases. The physical examination was fixed with 10% formalin, and the pathological changes were observed after paraffin sectioning.

2.2.3 Statistical Processing

Using CHISS statistical software, the measured data were expressed by x±s. The data comparison between groups was tested by group t test. When the variance between groups was not uniform or was not in line with the normal distribution, t' test was used.

3. Results

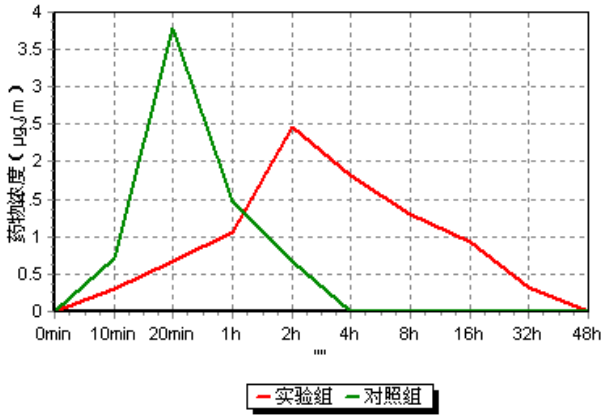
3.1 General Situation

19 survived and 5 died, including 2 in group I, 2 in group II, and 1 in group III. Pathological examinations were routinely performed after all animals died. Except for local necrosis of the liver embolization site, no clear pathological signs were found in other organs. It is considered that the inadequate experience in animal experiments or the possibility of animal intolerance in the early period. Surviving white rabbits had poor appetite, poor mental health, anorexia on the first 1-3 days after surgery, and gradually returned to normal on the 5th day after surgery. Four of the rabbits treated with III had symptoms of diarrhea in addition to the above symptoms, and the symptoms disappeared after 3 days. The other two groups did not show the above phenomenon.

Changes in the concentration of Sorafenib in the transcatheter (see Table 1): Changes in the concentration of Sorafenib of Group I (Iodine oil and Sorafenib emulsion) and Group III (pure Sorafenib group) were shown in the peripheral blood-time curve. The concentration trend of both groups decreased from high, and the concentration peaked at 2 hours after embolization in group I. After that, it decreased slowly for 32-48 hours. In group III (direct intra-arterial infusion of Sorafenib), the concentration peaked and then decreased rapidly. After 4 hours, the concentration of the bleeding drug could not be measured again. Group I (lipid-Sorafenib emulsion group) Cmax (maximum drug concentration, 2.46 μg/mL ± 0.101), AUC (area under the curve of drug time, 945.72 μg/mL.min ± 0.223) and Cmax of Group III (3.78 μg /mL). There was a statistically significant difference ($p<0.05$) between ±0.180) and AUC (546.98 μg/mL.min±21.1).

Table 1. Changes in Sorafenib Time – Blood Drug Concentration after administration in other ways than different than that of Group III (μg/ ml , x ±s)

Group	0 min	10 min	20 min	1h	2h	4h	8h	16h	32h	48h
I	0	0.30± 0.013	0.67± 0.022	1.06± 0.107	2.46± 0.101	1.82± 0.092	1.30± 0.211	0.93± 0.041	0.33± 0.01	0
III	0	0.72± 0.022	3.78± 0.180	1.48± 0.030	0.68± 0.100	0	0	0	0	0



实验組、对照組不同时间点外周血索拉非尼的药－时曲线图

Table 2. Changes in the liver and kidney function indexes before invention operation of the experiment and control groups

Index	Group	Pre-operation	Day 3	Week 1	Week 3	Week 6
ALT (U/L)	I	139.2±27.0	345.2±21.0	133.2±31.0	133.2±29.0	135.7±29.0
	II	140.2±35.7	398.2±40.7	143.2±38.6	139.2±33.2	144.3±36.7
AST (U/L)	I	83.7±35.9	254.7±25.4	86.3±40.9	82.8±32.2	83.4±34.8
	II	85.6±28.0	283.6±27.4	87.2±30.0	85.6±29.1	84.2±26.6
DB (umol/ L)	I	4.6±0.3	4.6±0.2	4.4±0.4	4.5±0.68	4.5±0.6
	II	4.0±0.9	4.1±0.8	3.9±1.0	3.9±0.9	4.1±0.9
ALB (g/L)	I	52.3±3.4	52.8±3.5	53.3±3.0	52.9±3.0	52.6±3.1
	II	50.1±2.9	49.8±1.9	50.3±1.9	51.0±7.6	51.1±8.5
Cr (umol/ L)	I	131.6±12.1	138.4±18.1	133.6±14.2	129.3±8.1	130.6±13.1
	II	140.5±10.7	141.5±20.4	140.3±10.8	141.0±8.4	141.5±23.7
BUN (mmol/ L)	I	10.0±1.3	9.8±1.2	9.6±3.3	10.3±1.6	10.5±1.8
	II	10.8±0.5	10.3±0.2	10.4±0.1	11.0±0.7	11.1±3.0
WBC (10^9/L)	I	4.1±0.9	5.0±0.9	4.0±0.9	4.2±0.1	4.2±0.2
	II	3.3±1.2	4.4±1.2	3.3±1.1	3.4±0.2	3.3±1.4
NEU%	I	39.5±9.4	57.8±9.2	38.5±8.3	38.8±10.3	39.7±8.4
	II	46±10.4	66±9.4	48±10.3	46±9.6	47±8.9
RBC (10^{12}/L)	I	2.3±0.3	2.2±0.8	2.2±0.9	2.3±0.7	2.3±0.9
	II	2.1±0.4	2.0±0.9	2.1±0.8	2.2±0.1	2.1±0.3
PLT (10^9/L)	I	270.2±33.2	269.2±30.2	272.2±34.5	274.2±40.3	269.1±28.2
	II	244.7±28.1	243.8±19.2	243.6±27.6	246.7±30.1	245.1±31.2

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The blood routine, liver and kidney function changes of the three groups of experimental animals before and after operation were shown in the table 2. The AST/ALT levels were significantly increased in the groups I and II 3 days after intervention operation ($p=0.02<0.05$), and the II group continued to recover 1 week after intervention ($p=0.24>0.05$). In group III (Sorafenib perfusion group only), there was no significant change in blood ALT/AST compared with the 3 days after intervention operation. There was no significant change in direct bilirubin between the experiment and control groups ($p=0.35>0.05$).

Pathological changes: (1) changes in liver tissues: liver tissue 3 days after intervention operation of Groups I, II, and 1 week after embolization were focally necrotic to varying degrees, showing hemorrhagic and coagulative necrosis, and the local liver structure completely disappeared (Figure 5, 6). Microscopically, there was no significant difference between the two groups in the extent of necrosis and necrosis. Three weeks after interventional embolization, the localized granulation tissue regeneration was observed in the embolized areas of group I and II. There were macrophage reaction of foreign bodies and neovascularization of capillaries, bile ducts and hepatocytes. Local fibrosis appeared after 6 weeks. Hyperplastic bile ducts, blood vessels, hepatocytes and fibrous tissues showed no manifestations of bile duct necrosis and cholestatic (Figure 7). In group III, there was no significant change in liver tissue after treatment. There was no abnormality in the structure of liver tissue in the unembedded area of the three groups. The central vein, bile duct, hepatocytes and micro-arteries were arranged normally. (2) The histopathological examination of the three groups of heart, kidney, lung, brain, intestine, gallbladder, etc. with no abnormal findings (Figure 8,9,10).

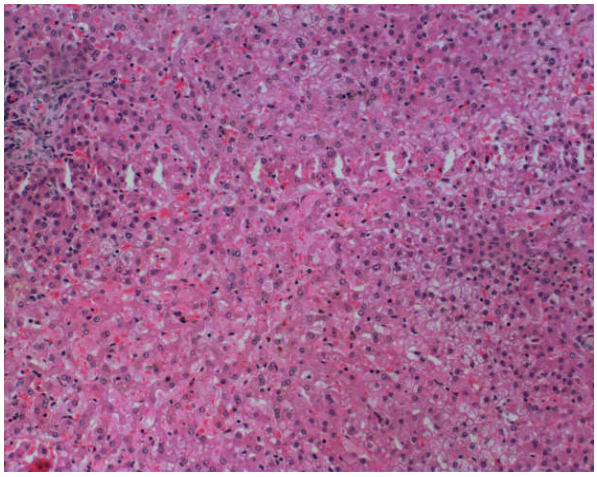

Figure 5. (x40): Normal liver

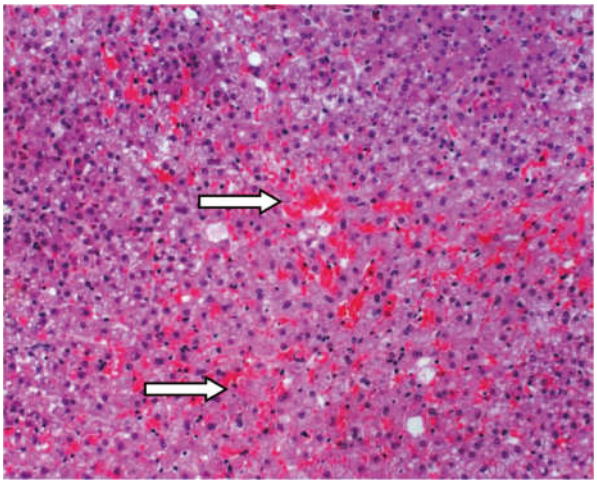

Figure 6. liver tissue (x40) 1 week after embolization: hemorrhagic necrosis, local hepatocyte necrosis surrounding the normal liver tissue (→)

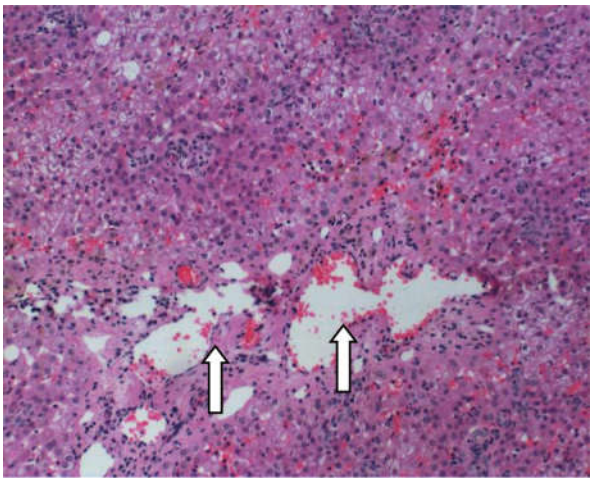

Figure 7. Liver tissue 3 weeks after embolization (x40 times): Local necrotic tissue and granulation tissue formation, visible hyperplastic vessels (↑).

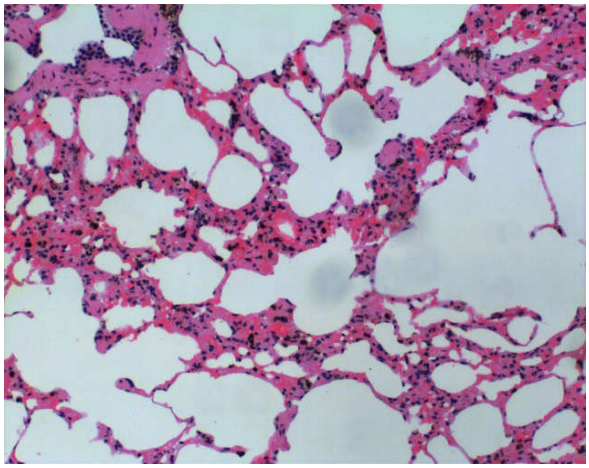

Figure 8. lung tissue, no abnormal manifestations

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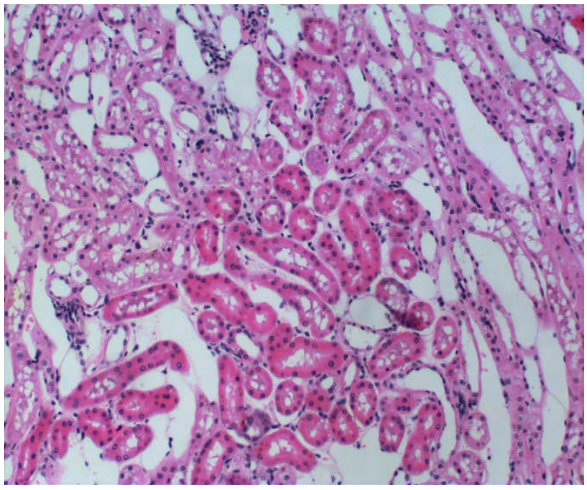

Figure 9. kidney tissues (x40), no abnormal manifestations

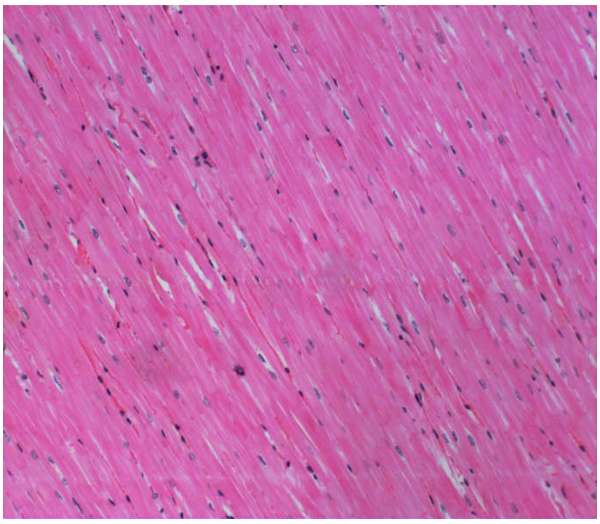

Figure 10. cardiac muscle tissue (x40): no abnormal manifestations

4. Discussion

TACE is currently one of the first measures to treat unresectable primary liver cancer. The principle of treatment is to completely inactivate the tumor through the synergistic effect of chemotherapy and embolization, and residual tumor recurrence and metastasis are one of the important factors affecting the long-term efficacy. Tumor recurrence and metastasis are associated with angiogenic factors such as VRGF and are the initiators of tumor recurrence and metastasis [1-5]. The emergence of molecular targeted agents provides a new means to control tumor recurrence and metastasis. Sorafenib is a multi-target multi-kinase inhibitor of the Raf/MEK/ERK signaling pathway that inhibits tumor growth and angiogenesis. Clinical studies have demonstrated that combined TACE and oral molecular targeted therapy can inhibit tumor growth and tumor angiogenesis. Our clinical application found that for long-term oral administration of Sorafenib in patients with primary liver cancer, angiography showed that the diameter of the hepatic artery was significantly thinner than that before administration. The above performance is related to the effect of Sorafenib on normal tissues. Therefore, the high selectivity of Sorafenib's target of action is relative. The TACE combined with local transcatheter delivery of the molecular targeted preparation not only makes the target of the targeted drug more selective, achieves the purpose of precise guidance, improves the tumor suppressing ability, but also reduces or avoids side effects caused by oral administration. In addition, the use of local intra-arterial delivery of Sorafenib also has the advantage of fully improving the bioavailability of Sorafenib. The bioavailability of conventional oral Sorafenib tablets is only 38-49%.

At present, domestic and foreign scholars have carried out beneficial explorations on TACE combined with molecular targeted drug therapy for HCC, laying a foundation for its clinical application[15~18]. The basic research on angiostatin gene fragments was performed by localized delivery of recombinant human endostatin injection (Endostar), fumagillin derivative (TNP-470), reaction stop (thalidomide) and other tumor angiogenesis inhibitors and angiogenesis by interventional techniques. Preliminary results showed that compared with the TACE group, the expression of VEGF, local tumor volume, VEGF and MVD around the tumor was significantly lower in the TACE combined with the local targeted drug group than in the control group. The combination therapy was more than a single technique. Significant inhibition of tumor growth. Foreign scholar Maataoui et al found that in animal models of liver cancer, TACE combined immunotherapy (OK-32) and anti-angiogenic therapy (TNP-470) can significantly delay tumor growth compared with TACE treatment alone but the combined anti-angiogenesis treatment effect is more significant. Antoine et al first performed percutaneous local radiofrequency ablation combined with oral multi-targeted drug Sorafenib in the treatment of renal cell carcinoma in mice. The results showed that the tumor tissue micro vessel density (MVD) was significantly reduced in the treatment group compared with the control group. Our experimental studies found that the side effects of the experimental animals after embolization with iodized oil-Sorafenib emulsion (group I) were not significantly different from those of simple iodized oil embolization (control group), and Sorafenib was directly perfused through the trans arterial artery. After (III), the side reaction was mild. In the control group, 4 of the experimental animals were

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directly perfused with Sorafenib, and there were 4 cases of recent diarrhea and other discomforts. Considering the relative sustained release of Sorafenib with iodized oil as a carrier, our pharmacokinetics was further verified. Our hypothesis is that the peak of drug concentration is reached in 20 minutes when Sorafenib is perfused alone, and the peak is reached 3 hours after intervention with lipiodol as a carrier, and there is a significant difference between the peak concentration and AUC. We consider the high drug concentration results in the production of Sorafenib toxicity.

Both combined TACE and transcatheter arterial delivery of Sorafenib formulations can exert synergistic anti-tumor effects [19-23]. There is synergy between anti-tumor and tumor angiogenesis between commonly used chemotherapeutic drugs and molecularly targeted drugs in TACE. Recent studies have shown that molecularly targeted drugs (such as endostatin, bevacizumab, Sorafenib, etc.) and chemotherapeutic drugs (such as doxorubicin, cisplatin, gemcitabine, etc.) have a certain synergy, providing basic theoretical support for its joint application. Therefore, the combination of previous academic and clinical research and our recent animal studies have shown that TACE combined with the local delivery of Sorafenib in the hepatic artery is operative and feasible. Local delivery of Sorafenib has significant advantages over systemic treatment.

The effect and deficiency of carrying Sorafenib powder with iodized oil as carrier [24-26]. Local routes of intra-arterial injection of molecularly targeted pharmaceutical agents are direct perfusion and indirect release of emulsions with lipiodol. Our studies found that the peripheral blood reached the peak concentration of the drug after 20 minutes of direct perfusion, and the concentration of the bleeding was not measured in the peripheral blood for 4 hours. The release of the iodized oil emulsion for 2 hours showed a peak drug concentration, and the peak value was significantly smaller than that of the direct perfusion drug, and lasted for 48 hours, indicating that the lipiodol emulsion can be used as a drug carrier to continuously release the molecular targeted agent, thereby continuing to inhibit tumor growth and angiogenesis. Compared with the literature reports, we used Solafenib and lipiodol as embolization of the hepatic artery to release the drug for a long time. Our analysis may be related to the dense emulsification of Sorafenib and lipiodol, both of which are insoluble in water, and can be formed more stable. Related to the emulsion. However, there are certain deficiencies in the use of lipiodol as a carrier. We found in the experiment that compared with the arterial infusion of Sorafenib, although the experimental group of lipiodol-Sorafenib emulsion can be relatively slow release after hepatic artery embolization, the drug concentration was not detected on the third day after the intervention, and the concentration of the drug at different times during the process of releasing showed some volatility. Our analysis may be related to the following factors: ① Iodine oil is a liquid terminal embolic agent, so its clearance is affected by high dynamic hepatic artery blood flow. In Kan et al., in the experiment of living arterial liver tumor, the arteriovenous shunt of ultra-liquefied lipiodol was observed under microscope, that is, the super-liquefied lipiodol was shunted to the small portal vein before entering the tumor vascular bed, and then the high-pressure hepatic artery blood flow was cleared up. Therefore, in order to prevent this from happening, it is often used to block arterial blood flow by combining other embolic substances, prolonging the time when the drug and super-liquefied lipiodol are cleared, super-liquefied lipiodol with chemotherapy drugs, embolization of portal vein, absorbable gelatin sponge Or polyvinyl alcohol embolization of the hepatic artery, this method is called “sandwich” therapy. The “sandwich” method is adopted to significantly increase the rate of tumor necrosis. We did not use the above embolic material in the experiment that is normal liver tissue, so its release rate should be significantly increased; ② Different from the liver tumor tissue structure, there are Kupffer cells in the normal liver, which enhances the clearance rate of lipiodol. The tumor tissue is unique. There is no Kufu’s cell in the tumor tissue, and the microvascular basement membrane is incomplete in the tumor. It consists only of a single layer of endothelial cells and a sheath lacking an elastic membrane. The blood vessels are fragile and easily broken with high permeability, and it is lacked of neurological conditions, enabling iodized oil and Sorafenib emulsion easier to enter into tumor tissues; ③ iodized oil itself as a carrier has instability and uncontrollable drug release. The main reason is that lipiodol as a carrier releases the drug through passive release rather than active release, so its release rate is first affected by various factors such as blood flow velocity. TACE combined with transcatheter arterial infusion of Sorafenib is safe. After interventional operative observation, it was found that there were different degrees of anorexia and other discomforts in the surviving rabbits. It is important to pay attention to that in 4 cases in the Sorafenib perfusion group alone, symptoms of diarrhea occurred for 3 days. However, there was no diarrhea in the iodized oil Sorafenib emulsion group and the simple lipiodol embolization group. We speculated that it may be related to the local perfusion of high concentration Sorafenib. Compared with preoperative, the basic physiological indexes of each group returned to normal

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one week after intervention. In the Surafenib emulsion group and the simple lipiodol group, the percentage of routine white blood cells and neutrality increased after 3 days, but the Sorafenib perfusion group did not change much, considering the post-embolization response. Regarding the effect of Sorafenib on liver function. The study showed that there was a transient increase in ALT/AST between the Sorafenib lipiodol emulsion group and the simple lipiodol group. There was a statistically significant difference between the two groups (P=0.002 <0.05). There was no significant difference in ALT/AST between the two groups (P=0.13>0.05). The ALT/AST was normal after intervention in the Sorafenib infusion group. There was no significant difference compared with preoperative. (P = 0.2 > 0.05). Five days after intervention, the ALT/AST of the simple lipiodol group returned to normal, and the ALT/AST of the Sorafenib lipiodol emulsion group was still at a high level, and the two were statistically different (P=0.04<0.05). This shows that the elevation of transaminase after liver intervention is related to the lipiodol used in the intervention, but not related to Sorafenib, but Sorafenib has a certain effect on the recovery of liver transaminase. Our analysis may be related to the characteristics of Sorafenib: ① Unlike chemotherapeutic drugs, Sorafenib is a molecularly targeted preparation with high target selectivity and does not directly cytotoxic to normal tissues, so it carries Sorafenib's lipiodol. It does not aggravate the toxicity to normal liver; ② The slower rate of ALT/AST recovery in the Sorafenib lipiodol emulsion group may be related to the involvement of Sorafenib in inhibiting liver microvascular regeneration. The mechanism of action may be due to tissue ischemia and hypoxia-induced VEGF expression after interventional embolization, while the sustained release effect of Sorafenib inhibits microvascular regeneration of liver tissue, resulting in slower recovery of liver function. ③ Because the expression of VEGF in normal liver tissue is very low, and Sorafenib drug metabolism is fast at local perfusion (up to 30 minutes, peripheral blood cannot be detected after 2 hours), so Sorafenib has a weaker inhibitory effect. When the hepatic artery was directly perfused on a normal liver, the changes in ALT/AST were not obvious. Our pathological examination further confirmed this phenomenon, and the liver pathology was normal after local perfusion of Sorafenib group. From a long-term perspective, ALT/AST in the lipiodol Sorafenib emulsion group and the simple lipiodol group returned to the preoperative level 1 week after intervention, and the liver pathology was compared between 1, 3, 6, and 12 weeks. Ischemic necrosis was the main manifestation in 1 week. The granulation tissue began to appear in the local necrosis area at 3 weeks after operation, and the fibrous regeneration nodules appeared in 6 and 12 weeks, which showed hyperplasia of bile duct, small blood vessel and portal vein. There was no significant difference in the extent, extent, and tissue proliferation of tissue necrosis. It shows that Sorafenib has little effect on the repair of tissue necrosis after liver embolization, and may also be related to the failure of the iodized oil as a carrier to fully exert sustained release drugs, and thus play a pharmacological role, needing to be further studied.

Shortcomings of this research: (1) due to the limitations of experiment time and conditions, sufficient radiology detection cannot be made; (2) Failure of detecting VEGF expression: the relationship between the drug concentration Sorafenib and blood VEGF cannot be studied; (3) the concentration of Sorafenib in tissue cannot be determined; (4) Due to the choice of normal animal as a model, the efficacy of local percutaneous delivery of Sorafenib on tumor cannot be completely evaluated, which needs further research and evaluation of local delivery of Sorafenib preparation on tumor.

5. Conclusions

The transcatheter delivery of Sorafenib Technique liver artery has high feasibility and safety, having significant injury of important organs such as liver. Iodipin-emulsified Sorafenib has certain sustained release, which can avoid adverse effect arising from transient concentration caused by perfusion. It still is affected by such factors as liver blood flow, removal of kuffer cells when released, and the sustained duration of release is still short.

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