Research Article

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Arterial infusion of rapamycin in the treatment of rabbit hepatocellular carcinoma to improve the effect of TACE

Abstract: Background: Hepatocellular carcinoma is one of the leading causes of cancer-related death. Hepatic transcatheter arterial chemo-embolization (TACE) is commonly used clinically for advanced hepatocellular carcinoma treatment. AIM: The aim of this study was to evaluate whether arterial infusion of rapamycin can improve the effect of TACE in treatment of rabbit hepatocellular carcinoma. Material and Methods: Eighteen healthy New Zealand white rabbits weighing 2.6 ± 0.3 kg were used in a standardised hepatocellular carcinoma model and randomly divided into three groups of 6 rabbits. Group A: the rabbits were treated with rapamycin and TACE by administering arterial perfusion of 2 mg/kg rapamycin + 1 mg/kg epirubicin, 0.2 mg/kg mitomycin, and lipiodol emulsion embolization. Group B: rapamycin was reduced to 1 mg/kg. And for Group C, the rabbits received only TACE treatment. 14 days post operation, CT scan and digital subtraction angiography (DSA) was performed to examine TACE efficacy. The rabbits were killed by air embolism and the expression of HIF-1a, VEGF, iNOS, and CD34 were measured in an immunohistochemical assay of thetumor tissue. Results: HIF-1a, VEGF and iNOS protein expression in Group A was significantly lower than that of Group B and Group C (P<0.05). The tumor MVD in group C was significantly higher than that of group A and group B (P<0.05); and the tumor MVD of group B was significantly higher than group A (P<0.05). Conclusion: Arterial infusion of rapamycin combined with TACE can improve treatment efficacy by decreasing HIF-1a, VEGF, iNOS and CD34 expression.

Keywords: hepatocellular carcinoma; therapeutic chemoembolization; rapamycin

1 Introduction

Hepatocellular carcinoma exhibits a high degree of malignancy and commonly occurs as an occult condition in its early stages [1]. Therefore, most patients are diagnosed at an advanced stage and have consequently missed the opportunity for timely surgical resection [2]. Patients unsuitable for surgery, and those who have experienced postoperative recurrence, are typically treated with hepatic transcatheter arterial chemo-embolization (TACE) therapy [3]. However, recurrence and metastasis rates after TACE are high [4-6]. The therapeutic goal of complete embolism may not occur despite TACE successfully embolizing the nourishing blood vessels of the tumor, as it cannot inhibit tumor angiogenesis. Tumor tissues are in an anoxic state after TACE, and this stimulates the overexpression of VEGF, HIF-1a, and iNOS, inducing tumor angiogenesis and proliferation [7]. Tumor angiogenesis is the basis for tumor recurrence and metastasis.

Rapamycin is a new type of large-ring lactone immunosuppressive agent. With effective immunosuppression, rapamycin can inhibit the growth of various cancer cells, such as liver [8], kidney, colon, breast [9], and

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ovarian cancers. Cell-based tests, and experiments involving animals given rapamycin orally, have revealed that this agent exhibits a remarkable inhibitory effect on tumor angiogenesis and induces apoptosis and autophagy of hepatoma cells. In the present study, arterial perfusion with rapamycin combined with TACE was administered to improve the therapeutic effect of TACE in treating hepatocellular carcinoma in rabbits.

2 Materials and methods

2.1 Modeling

To establish a rabbit hepatocellular carcinoma model, 18 healthy New Zealand white rabbits weighing 2.6 ± 0.3 kg were placed in the same cage, fed, and provided with unlimited access to drinking water. VX2 tumor was inoculated into the muscle tissue of the hind legs of the rabbits to create a tumor with a block length of 1–2 cm after 2 weeks. The tumor-bearing rabbits were intraperitoneally injected with 5% pentobarbital. Under aseptic conditions, the tumor was peeled from the thigh muscle tissue, and the necrotic tissue capsule was removed. White fresh tumor tissue was cut, placed in a sterile petri dish containing 1640 fluid, and sectioned into 1 mm^3 blocks using eye shears. The rabbits were given general anesthesia and fixed in a supine position on the experimental platform. Fur on the upper abdomen was removed, and the area was disinfected. Gelatin was aspirated with an 18 G puncture needle and a sponge bar until a tube length of 2 cm was reached. The gelatin was then cut into 2-4 tumor blocks measuring 1 mm^3 [10]. The left lobe of the liver was punctured under the guidance of CT. The lump and gelatin sponge strips were pushed into the liver parenchyma by

using a flat-head thimble. The needle was pulled out, and the punctured area was disinfected and bandaged. For 3 days, 40 million U of penicillin was intramuscularly injected to prevent infection. A CT scan was performed after 2 weeks to check tumor growth at 2 cm to the left and right of the tumor foci and to confirm whether the model was successfully established (Figure 1).

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

2.2 Grouping and treatment

The successfully established rabbit hepatocellular carcinoma models were randomly divided into three groups with six rabbits in each group. In group A (APR-TACE 1 group), the rabbits were treated with rapamycin and TACE by administering an arterial infusion of 2 mg/kg rapamycin + 1 mg/kg epirubicin, 0.2 mg/kg mitomycin, and lipiodol emulsion embolization. In the group B (APR-TACE 2 group), the rabbits were given an arterial infusion of 1 mg/kg rapamycin + 1 mg/kg epirubicin, 0.2 mg/kg mitomycin, and lipiodol emulsion embolization. In the third group (TACE group), the rabbits received a simple TACE treatment that included 1 mg/kg epirubicin, 0.2 mg/kg mitomycin, and an emulsion embolism made of iodized oil. A 2.7 F micro-catheter (Terumo Company, Japan) was implanted into the femoral artery of each rabbit. The femoral artery was determined via total hepatic artery angiography to identify the location, size, and staining of the tumor. Tumor-nourishing vessels were further selected, and the rabbits were subjected to perfusion
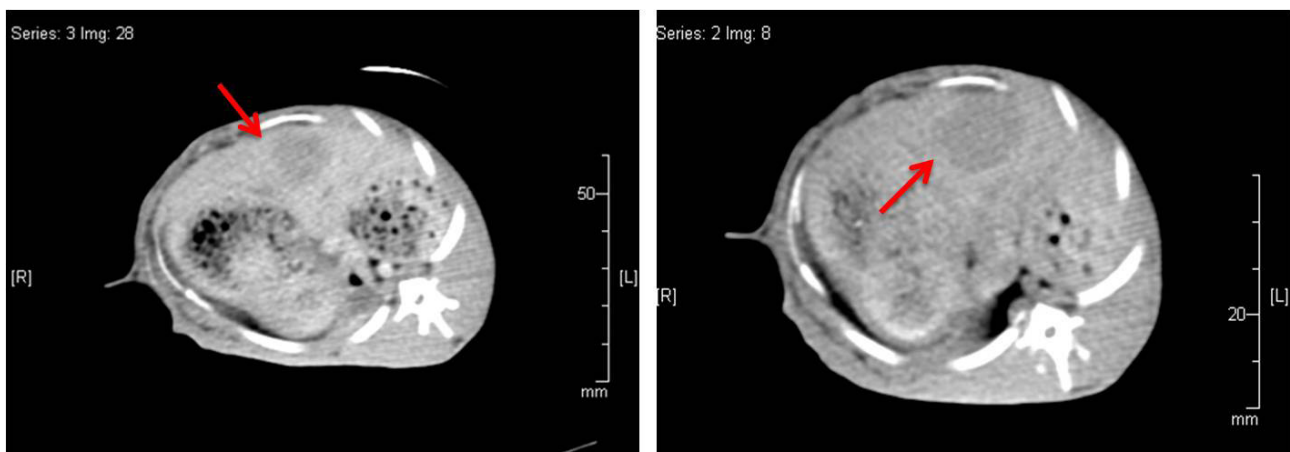

**Figure 1** Modeling of rabbit liver transplantation tumor (hepatic tumor red arrow)

embolism according to the group scheme. The degree of embolism was the standard to achieve the complete embolization of tumor vessels (Figure 2).

Fourteen days after operation, computer tomography (CT) scan and DSA were performed to assess treatment efficacy (Figure 3). The rabbits were killed by air embolism. The necrotic region and surrounding liver tissue were taken from the peripheral tumor. The expression levels of HIF-1a, VEGF, iNOS, and CD34 of the tumor tissue were measured by western blot assay. Tumor microvessel density (MVD) was calculated through immunohistochemical assay.
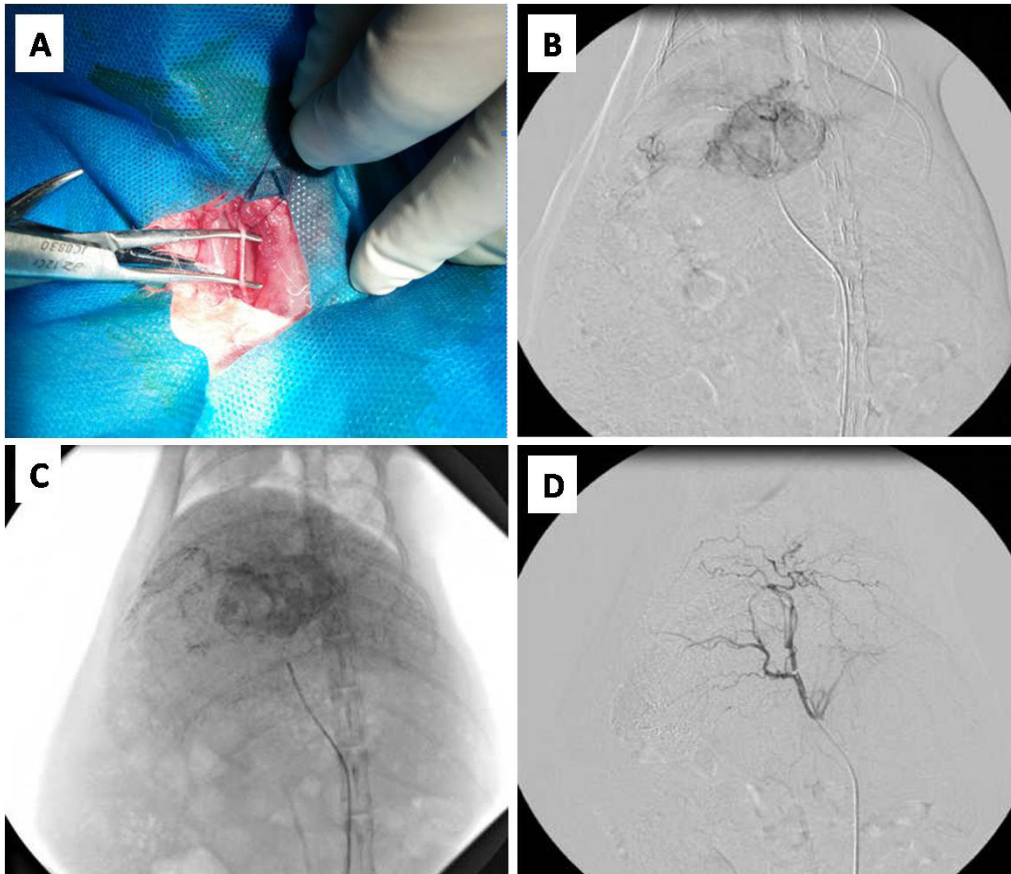
Figure 2. Hepatic arterial chemoembolization of rabbit with transplantation tumor (A: Intraoperative exposure of femoral artery; B: Selective embolization of hepatic artery; C: Lipiodol deposition after embolization; D: Selective hepatic arteriography after hepatic artery embolization showed tumor vascular disappearance)
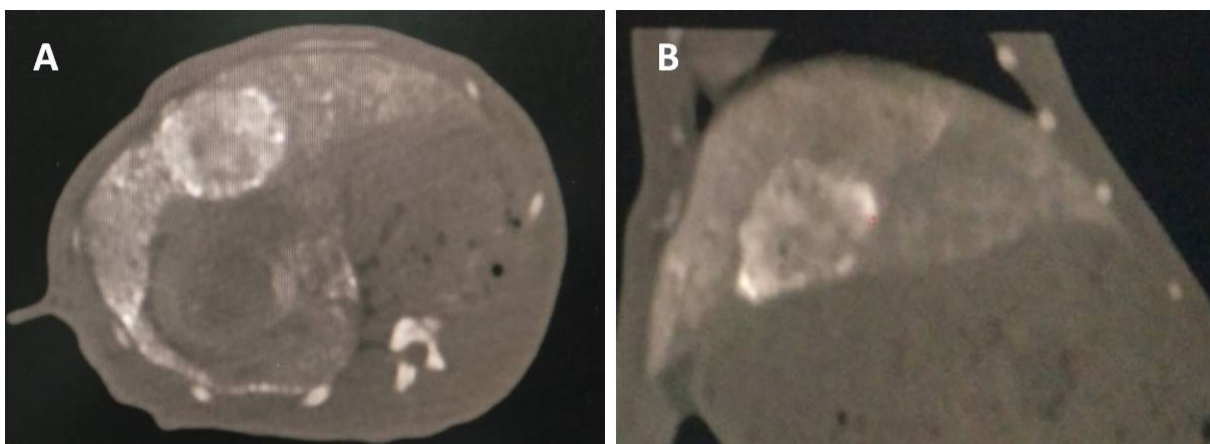
Figure 3. CT image of the tumor after two weeks TACE (A: CT image of rabbit hepatic tumor, Axial; B: CT image of rabbit hepatic tumor, coronal view)

2.3 Statistical analysis

Statistical analysis was performed using STATA 11.0 statistical software (http://www.stata.com), measurement data was expressed by $\bar{x} \pm s$ and the comparison between groups was made based on the F-test of the sample mean. P<0.05 was considered as statistical significance.

3 Results

3.1 HIF-1a, VEGF and iNOS protein expression

Western blot showed HIF-1a, VEGF and iNOS protein expression were significantly different in Group A, B and group C. HIF-1a, VEGF and iNOS protein expression in Group A was significantly lower than those of Group B and Group C, (Figure 4).

3.2 Tumor microvessel density analysis

Tumor microvessel density (MVD) were calculated according to CD34 expression by immunohistochemical assay. There were significant differences in tumor MVD between groups A, B and C. The tumor MVD in group C was significantly higher than those of group A and group B (P<0.05); and tumor MVD of group B was significantly higher than group A (P<0.05), see Figure 5.

4 Discussion

Arterial perfusion with rapamycin can inhibit the expression of iNOS, HIF-1a, and VEGF proteins which promote angiogenesis, thus preventing tumor neovascularization.

Our results revealed that the MVD in the peripheral tissue of the rapamycin group was significantly lower than that of the TACE group. We confirmed that arterial perfusion with rapamycin can inhibit tumor angiogenesis after a liver tumor is subjected to transcatheter embolization. Arterial perfusion with rapamycin combined with TACE could also improve the effect of TACE on the treatment of hepatocellular carcinoma in rabbits.

Different rapamycin doses (1 and 2 mg/kg) for arterial infusion elicit various inhibitory effects on angiogenesis after TACE, and the group administered with arterial perfusion of 2 mg/kg rapamycin exhibited strong inhibitory effects on iNOS, HIF-1A, and VEGF. The MVD in the tumor peripheral tissue decreased. This experiment was designed with two different dosage groups of rapamycin for the treatment of hepatocellular carcinoma in a rabbit model. Arterial perfusion with high rapamycin doses corresponded to strong inhibitory effects on tumor-nourishing vessels because rapamycin is an immunosuppressive agent [11-13]. The greater the dosage, the stronger the immune inhibition of organisms and the stronger the effect on liver cancer will be.

Previously published studies showed that rapamycin can inhibit tumor angiogenesis primarily by blocking the expression of the mTOR signaling pathway [14] and cytokines [15], including iNOS, HIF-1a, and VEGF [12, 13] which was in accordance with our present study. Rapamycin and rapamycin target protein (mTOR) combines with FK506-binding protein12 to form RAPAMYCIN/ FKBP12 complex [16, 17]. The complex subsequently binds to mTOR FKBP12-rapamycin binding domain, suppresses mTOR downstream 4e-bp1 and S6K1 phosphorylation, and blocks mRNA translation to reduce the synthesis of uric acid decarboxylase and cyclin D1, which are proteins required for cell cycle differentiation from the G1 phase to the S phase [18, 19].
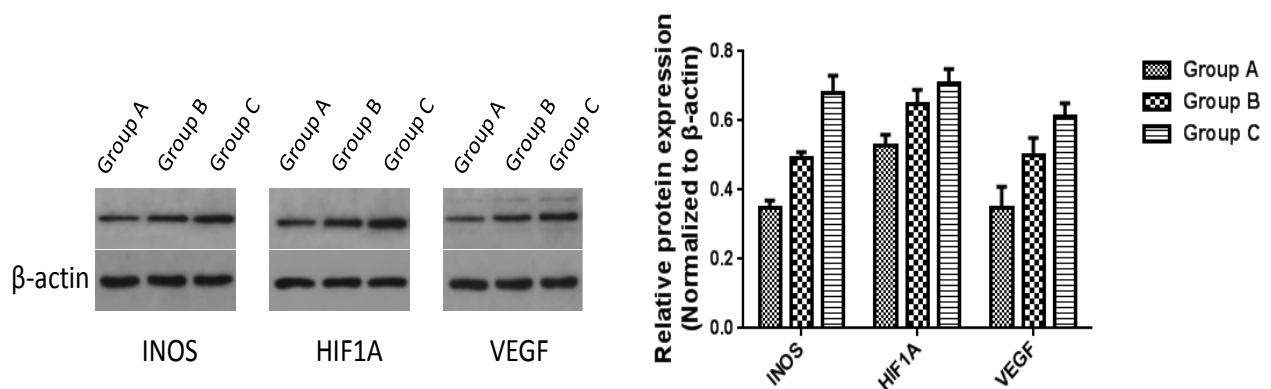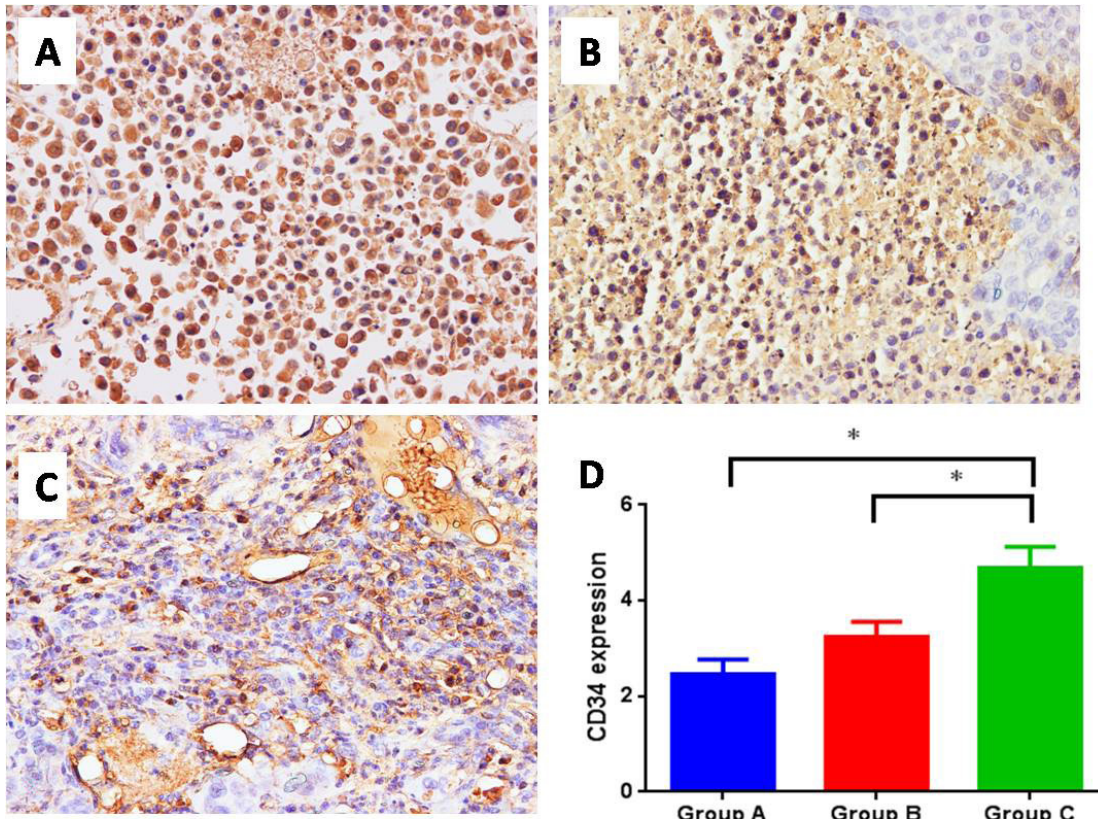

Figure 4. HIF-1a, VEGF and iNOS protein expression through western blot assy

Figure 5. CD34 relative expression analysis of immunohistochemical assay (A: group A; B: group B; C: group C; D: bar graph of CD34 expression demonstrated group C significant higher than that of group A and B)

In summary, arterial perfusion with rapamycin combined with TACE for the treatment of hepatocellular carcinoma in a rabbit model can enhance the curative effect of TACE.

Conflict of interest: Authors state no conflict of interest

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