Original Article

Computed Tomography Imaging and Pathologic Characters of Hepatic Neoplasm in Rabbits after Treatment with Cryoablation

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Abstract: To demonstrate the correlation of radiologic and histopathologic findings in hepatic tumors model in rabbits after treatment with cryoablation. Hepatic cryoablation was performed in seventeen rabbits with VX-2 tumor and twelve healthy rabbits. Nonenhanced and dynamic contrast material-enhanced computed tomography (CT) were performed before and the 1, 2, 3 and 4 weeks after cryoablation respectively. Imaging findings and histopathologic findings were analyzed simultaneously. All cryolesions exhibited rim enhancement in arterial phase at CT, but showed diverse imaging characters at different time after cryoablation. The tumors in control group showed no enhancement while the other revealed enhancement. Rim enhancement demonstrated bigger in volume in arterial phase than it was before the same time. Histopathologic analysis of the cryolesions showed mainly coagulation necrosis at the early stage and liquefaction necrosis later. Cryolesions in both groups exhibited similar rim enhancement in arterial phase on CT image at weeks 1, 2, 3 after cryoablation. At week 4 after cryoablation, heathy control rabbits did not show any ehancement at all, but Rabbits with VX-2 tumor showed more enlarged enhancement. Histopathologic analysis in cryolesions demonstrated that there were mainly coagulation necrosis at the early stage and liquefaction necrosis at later stage. The check time for nonenhanced and contrast-enhanced CT scan as well as the image characters are important to evaluate the effect of cryoablation in focal liver carcinoma.

KeyWords: Liver neoplasm; Cryoablation, CT

With the development of cryoablation therapy for hepatic tumors, how to evaluate the effect and how to distinguish the residual tumor are very importatnt in clinic. Some study (1-2) have reported the appearances of CT image after cryoablation. But they lacked the continual contrasted imaging and pathological analysis. In this study, we analyzed systematically appearances of CT image and pathological charters simultaneously at a series time points.

Materials and Methods

Methods

2.5-months healthy New Zealand rabbits with body weight of 2 ± 0.4kg were provided by Shandong University Animal Center and raised at normal temperature. 17 Rabbits were selected randomly to induce VX-2 hepatic carcinoma (group A) and 12 health rabbits as control (group B).

2.5-months New Zealand rabbits (n=17) were anesthetized with pentobarbital sodium via the marginal ear vein at dose of 1.2 ml/kg. First, a midline laparotomy was performed, enabling access to the middle lobe of the liver. Then a 3-mm hole was poked slopingly by an ophthalmic hemostat. VX-2 tumor fragments of approximately 1 mm3 were injected intraparenchymally into the middle lobe of the liver. The tissue from fresh tumor mass were cut with scissors in 0.9% normal saline. At last, gelfoam gelatin sponges were stuffed into the pole in order to hemostasis. 3 weeks later, carcinoma in similar circular shape about 2.0-2.5cm size could be found by MRI examination (Fig.1 A, B).

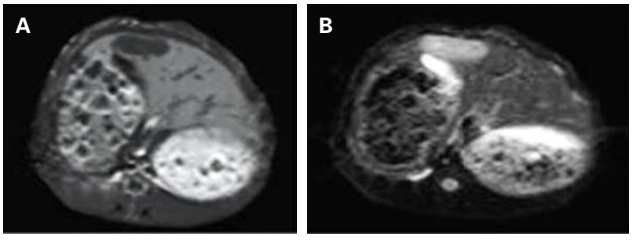

Fig. 1 Liver tumor before cryoablation. (A)T1WI , (B) T2WI.

Cryoablation of group A: Seventeen rabbits were anesthetized with pentobarbital sodium via the marginal ear vein at dose of 1.2 ml/kg. A 3.5-cm midline incision was made to access to the middle lobe of liver. The surrounding viscera were covered with wet pads to prevent freezing damage. A single 2 mm probe was inserted vertically to the same depth (5 mm) to the center of tumor. The

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output power were 100%. Cryoablation was performed with two freezing/thawing circles. Each circle was performed for 3 minutes with argon gas followed by an active thawing for 30 seconds with helium gas. When the tumor turned white, the probe was extracted and hemostatic gauzes were stuffed in needle path. Then put the liver into abnominal without active bleeding. The rabbit peritoneum, muscle and skin were sutured at last. If rabbit breath and heart rate were under well condition, the operation was ended.

Cryoablation of group B was as same as group A.

Liver imaging was performed with CT at weeks 1, 2, 3 and 4 after cryoablation. Each time two rabbits were sacrificed by means of intravenous pentobarbital overdose after the completion of postcryoablation imaging. Each cryoablation site was evaluated grossly and recorded. Samples from each cryoablation site and periphery of ice ball were obtained and fixed in 10% formalin.

Imaging

CT scan was performed by using a Somatom Plus 16 scanner (Siemens Medical system). Prior to CT scan, 3 ml/kg 10% chloral was administrated intraperitoneally. An initial nonenhanced helical study of the entire liver was performed. The level of the lesion was determined from the nonenhanced helical images. A dynamic contrast-enhanced examination was then performed at that level after the intravenous power injection of 3-5 mL contrast material (60% urografin) at 1 ml/sec via an ear vein. Imagings of arterial phase, portal venous phase and delayed phase were obtained. A 1.0-mm collimation, a 3-mm section thickness, a 3-mm reconstruction interval and 516× 516 matrix were used. Scanning parameters included 130 kV and 120-150 mA.

Results

Histopatological fingdings

Before cryoablaton, all macroscopic tumors in group A appeared as circular or similar circular shapes, 25 mm in diameter and gray color. 1 week after cryoablation, the lesions were gray, the periphery liver parenchyma were brown. Samples from each cryoablation site were stained with hematoxylin-eosin. Microscopic observation showed that the cells of carcinoma were ruptured, the nucleus pyknotic and the central foci of tumor filled with pink material of coagulation necrosis. 2, 3 and 4 weeks after cryoablation, macroscopic tumors sizes were smaller gradually. Microscopic observation showed that the cryolesions were still mainly coagulation necrosis. Central areas of liquefaction necrosis were presented at partial cryolesions 4 weeks later. 1 week after cryotherapy, The peripheral area of cryolesions were congestive and hemorrhagic zone and granulation tissue. 2 weeks after cryotherapy. The peripheral area of cryolesions were granulation tissue and fibrosis. 3 weeks after cryotheropy, a lot of fibrosis were found at the The peripheral area of cryolesions. Residual tumor cells were found in the granulation tissue and fibrosis at the periphery lesions in 9 (53 %) of 17 (Fig.2 A, B, C).

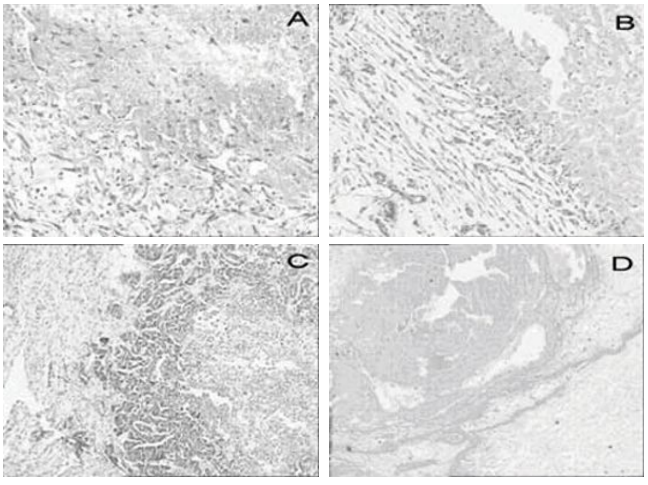

Fig.2 (A) The peripheral area of cryolesions was congestive and hemorrhagic zone. (B) The peripheral area of cryolesions was granulation tissue. (C) Residual tumor cells were found in the granulation tissue. (D) Cryolesions were filled with amorphous pink material 3–4 weeks later.

The mean diameter of cryolesions was 25 mm in group B. Hematoxylin-erosin stainning showed there were coagulation necrosis the in cryolesions. Hepatic cell cords were fragmented and spoiled. 1 week after cryotherapy, the peripheral area of cryolesions were congestive and hemorrhagic zone and granulation tissue. 2 weeks after cryotherapy, the periphery cryolesions were granulation tissue and fibrosis. The cells in cryolesions were ruptured and filled with amorphous

pink material 3-4 weeks later (Figure 2 D). A lot of fibrosis were found at the periphery lesions. The periphery repairing vascular extended into the necrotic tissue in 7 (24%) of 29 cryoablations.

CT imaging

Nonenhanced CT depicted all untreated VX-2 tumors as low-attenuating liver nodules in circular or similar circular shape of group A before cryoablation. Contrast-enhanced transverse arterial phase CT scan showed enhancement in the all crylesions. 14 (82%) of 17 lesions showed homogeneous enhancement and 3 (18%) of 17 lesions showed unhomogeneous enhancement. All tumors showed the feature of fast-in and fast-out obviously, which was in accordance with human hepatic carcinoma and indicated that it is ideal animal model for human hepatic carcinoma.

All images of nonenhanced CT scan showed low-attenuating liver nodules in cryolesions of both group. The results of contrast-enhanced CT scan depicted the central area of cryolesions as low-attenuating liver masses without enhancement. The shapes

in the peripheral area of cryolesions showed diverse characters at different time.

1 week after cryoablation, the cryolesions have central low-attenuating areas with rim enhancement in 11 (65%) of 17 in group A. The enhanced rings were intact, about 4 mm thickness and smooth inside it without small nodules project into cryolesions. 6 (35%) of 17 lesions showed rim enhancement. The rims were not at the same thickness. Intramural nodules could be found projected into lesions. All lesions of group B also showed rim enhancement at this time. But the thickness of the wall was same about 3.5 mm. The margins of the wall were smooth and clear (Fig.3 A, B).

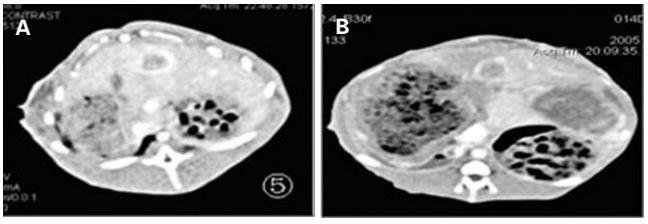

Fig.3 1 week after cryoablation. (A) group B. (B) incompletely of group A.

2 weeks after cryoablation, nonenhanced CT scan showed that lesion areas decreased in 12 (80%) of 15 of group A than them in the 1st week, and lesions areas in 3 (20%) of 15 did not change. Contrast-enhanced transverse arterial phase CT scan showed low-attenuating areas in the majority of the central cryolesion with attenuating rim enhancement in 7 (47%) of 15 lesions. The enhanced rings were intact, about 2-mm thickness and smooth inside it without small nodules project into cryolesions. Contrast-enhanced transverse arterial phase CT scan showed rim enhancement in 8 (53%) of 15 lesions. The rims were not at the same thickness. Enhanced intramural nodules could be found obviously projected into lesions. Nonenhanced CT of group B showed that the lesion areas decreased than before in 9 (90%) of 10 and a lesion area does not change in 1 (10%) of 10. Contrast-enhanced transverse arterial phase CT scan showed low-attenuating areas in the central cryolesions with rim enhancement in 10 (100%) of 10 lesions. The enhanced rims were intact in 2 (20%) of 10 lesions. The enhanced rims were intact, about 2mm thick and smooth inside it without small nodules project into or out cryolesions 8 (80%) of 10 lesions (Fig.4 A, B).

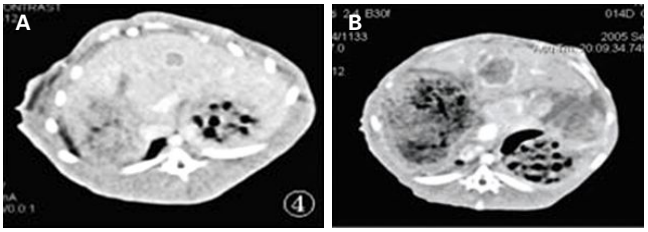

Fig.4 2 weeks after cryoablation. (A) group B. (B) incompletely of group A.

3 weeks after cryoablation, non-enhanced CT showed that there were decreased lesion areas in 10 (77%) of 13 of group A and increased lesion areas in 3 (23%) of 13. Contrast-enhanced transverse arterial phase CT scan showed subtle attenuating rim enhancement in 2 (15%) of 13 lesions. The enhanced rims became thinner than before., even for the thickness and smooth inside it, without small nodules project into cryolesions. The rims appeared as discontinued arc cords in 4 (31%) of 13 lesions. The rims also appeared as irregular circle zones in 7 (54%) of 13 lesions in which the rims were not at the same thickness and many intramural nodules could be found projected to lesions. The shallow-waved rims were found if intramural nodules merged. Nonenhanced CT of group B showed that the area of the lesions decreased obviously in 7 (88%) of 8 lesions and did not change in 1 (12%) of 8 lesions. Contrast-enhanced transverse arterial phase CT scan showed low-attenuating central cryolesions with rim enhancement in 5 (63%) of 8 lesions. The subtle attenuating rim enhancement were arc uncontinually. 3 (38%) of 8 lesions (Fig.5 A, B) did not showed rim enhancement.

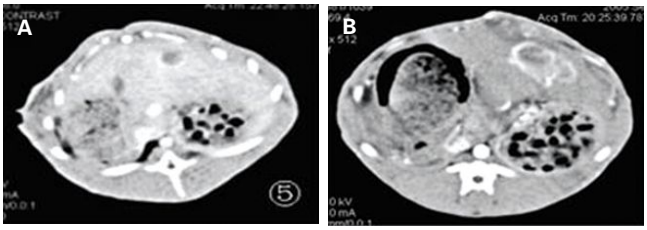

Fig.5 3 weeks after cryoablation. (A) group B. (B) incompletely of group A.

4 weeks after cryoablation in group A, nonenhanced CT showed that there were decreased lesion area in 5 (45%) of 11 lesions ,and increased lesion areas in 6 (55%) of 11 lesions. Contrast-enhanced transverse arterial phase CT scan showed spot-shaped or wedge-shaped attenuating areas. All imagings of nonenhanced CT showed low-attenuating liver nodules in both group cryolesions with clear margins. The low-attenuating cryolesions in group A underwent cryoablation completely verified by histopathology and they in group B decreased gradually with the time prolonged. 4 weeks later, part of them were disappeared or appeared as low-attenuating cords in 6 (55%) of 11 lesions and no enhancement in 5 (45%) of 11 lesions. Nonenhanced CT in group B showed that the lesions areas decreased obviously in 6 (100%) of 6 lesions and one of them was smaller obviously of wedge-shaped low-attenuating area . Contrast-enhanced CT scan showed no central and rim enhancement. The border were clear between cryolesions and normal liver parenchyma (Fig. 6 A, B).

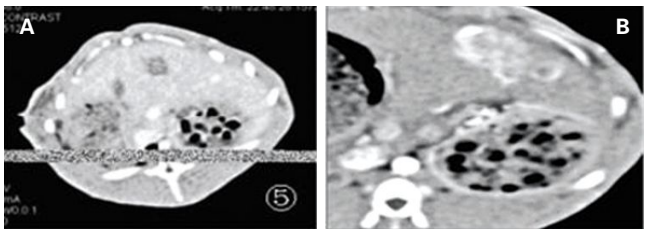

Fig.6 4 weeks after cryoablation. (A) group B. (B) incompletely of group A.

Discussion

Ablation has been widely used because they are amenable to minimally invasive approaches that can reduce morbidity, as compared with resection. Imaging examination is the main method to accurately assess the adequacy of tumor treatment for liver tumor ablation (3-5). As a result, imaging features of recurrent or residual tumor after cryoablation are poorly understood. We designed two different experimental groups and correlated CT findings systematically with histopathologic findings at a series time points.

All imagings of nonenhanced CT showed low-attenuating liver nodules in both groups of cryolesions with clear margins. The low-attenuating cryolesions of group A underwent cryoablation completely verified by histopathology. The low-attenuating cryolesions in group B became smaller gradually with the time. 4 weeks later, part of them were disappeared or appeared as low-attenuating cords. The changes of group A were corresponded to the changes of group B. The low-attenuating cryolesions of group A underwent cryoablation uncompletely verified by histopathology were larger gradually with the time. The shapes of lesions were irregular. Metastatic low-attenuating nodules formed in part of them.

Imagings of contrast-enhanced CT showed rim emhancement low-attenuating liver nodules of group A underwent cryoablation completely verified by histopathology and group B. The enhanced rings were intact and smooth without wall nodules. The rings were clear 1 week after cryoablation. The histopathologic specimens showed that the hepatic cell cords were fragmented and spoiled. The central cryolesions were composed of coagulation necrosis. The cell membranes were shrank and the nucleus pyknotic or broken up. The periphery lesions were embraced by congestive or hemorrhagic zone. Blood lakes were seen in part of them. Large quantity neoformative capillary and fibroblast were also found. According to the mentioned above, we consider that the histopathologic basis of the enhanced rim were the injury of capillary, increased permeability of periphery capillary, blood cells passing through the vessels to form circular blood lake and the granulation tissue with vast capillary .

After 3 weeks, the rim enhancemental walls of group B were smaller and thinner. The uncontinual gaps could be found at intact walls. Inside the walls there were coarse without small enhanced nodules. After 4 weeks, all contrast-enhanced CT of group B showed resolution of the rim enhancement. It was corresponded to group A underwent completely ablation.

The lesions uncompletely ablationed in group A, verified by histopathology, became larger 3 or 4 weeks after. Contrast-enhanced CT showed high-level enhanced nodules inside or outside the walls (Figure 7,8). The initial nodules were small, isolated and formed shallow-waverims. The noduals became larger and merged to each other 4 weeks later. The rims of high-level enhanced areas were irregular. Histopathologic findings showed that high-level enhanced nodules were recurrence of focal residual tumor. The appearance of contrast-enhanced CT could identify the tumor cryoablation completely. But the appearances of the lesions after cryoablation were different at different time. So it is very important to find a suitable time to take CT scan for evaluating the effect of ablation. Weaver ea al. (6) reported reexamination should be taken 7 days after ablation. If the enhanced areas appeared in the lesions, residual tumor still existed. We think that the evaluation for ablation by CT should be taken 4 weeks after therapy. The image findings associated with residual tumors were the size of lesions and the shape of enhanced areas. It was different as reported (7-9).

Linear enhancements extended from the rims to central necrotic lesions were found in 2(12%) of 17 in group A and 5(17%) of 12 in group B. They were blood vessels extending to central lesions correspond to histopathologic findings. The differences between blood vessels and residual tumors were obviously. The appearances of enhanced blood vessels extended from thick to thin, with smooth rims and natural track. The lengths of the vessels were not more than the length of half cryolesion diameter. The appearances of residual tumors were irregular striped or nodular high-attenuating areas in low-attenuating lesions. The striped enhancements with completely ablation were transient and most of them were disappeared 4 weeks later. The striped enhancements with completely ablation were small and unclear initially and became clear, larger or merged to each other with the time prolonged.

In conclusion, the check time and the shape characters by nonenhanced and contrast-enhanced CT are important to evaluate the effect of cryoablation in focal liver carcinoma.

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