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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14804-revised.doc).

Title: Low contrast medium and radiation dose for hepatic CT perfusion of rabbit VX2 tumor

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) **Q:** The material and methods section of the paper seems too long for a journal of gastroenterology and the authors describe the animal model in some depth as well as the scanning protocol. I suspect that a simple reference to the animal model would suffice in this instance.
A: Done

(2) **Q:** Please explain the detail histological finding of Fig 5. For example, how about hepatic sinusoidal capillary? What kinds of histological parameters do VEGF and CD 31 reflect? According to your paper, hepatic arterial flow in the region of viable tumor was increased., moreover, hepatic sinusoidal capillaries was compressive and obstructive. Please explain me the above histological findings in Fig 5.
A: Figure 5 demonstrated HE, VEGF, and CD31 staining of the tumor tissue. HE staining shows normal hepatic sinusoidal capillary disappeared and was replaced by a large amount of tumor cells filled in the sinusoidal capillary.
In pathological conditions, both VEGF and CD31 were overexpressed in new vascular endothelial cell during neoplastic angiogenesis, inflammatory angiogenesis, and wound-healing process. However, VEGF was also expressed at high level in cytoplasm of tumor cells. CD31 was not expressed in tumor cells.
As figure 5 A shows, the normal structure of hepatic sinusoidal capillary disappeared and was replaced by a large amount of tumor cells. There were abundant areas of CD31 positive expression, representing neo-angiogenesis.

(3) **Q:** In Table 1,HAP in tumor is much higher compared to normal liver, HPP in tumor is slightly lower compared to normal liver. There is no difference between normal liver and tumor in HPP. Please explain the difference between normal liver and tumor in perfusion parameters from the histological findings in Fig5.
A: Previous studies revealed that liver VX2 tumor in rabbit was hypervascular tumor fed by hepatic artery. Our studies demonstrated that the hepatic arterial perfusion in tumor was increased significantly in tumor compared to normal liver parenchyma, which was consistent with previous studies. It is because that a large amount of new vessels formation in tumor tissue, namely, abundant areas of CD31 positive expression in Figure 5 B. Moreover, these immature

vessels allow that the contrast agent is much easier to pass through incomplete layer of endothelial cell wall and detain in the interstitial space of tumor tissue.

The normal hepatic sinusoidal capillary was replaced by tumor cells (Figure 5 A) and increased the intrahepatic resistance, which contribute to the slight decrease in hepatic portal perfusion in tumor.

(4) **Q:** In Table 2,the CT values for normal liver parenchyma in arterial and portal venous phase with protocols B and C were significantly different from those obtained with protocol A. But, there was no significant among protocols A,B and C for the same parameters of tumor in arterial and portal venous phases. Please explain the difference between normal liver and tumor in arterial and portal phase.

A: The measurement of CT value was affected by two factors. One is the concentration of iodine contrast agent in region of interest. Another one is the tube potential that was used during CT scan. The tube potential of 80 kVp is closer to iodine k-edge of 33kev. Therefore, CT value in 80kVp group during contrast enhanced CT scan should be higher than that in 120kVp group. However, the concentration of contrast agent in 80kVp group is 37% lower than that in 120kVp group. Although no significant difference of CT value was found in viable tumor between 120kVp protocol and 80kVp protocol during arterial and portal venous phase. The combination of those two factors eventually resulted in a decreased tendency of CT value in 120kVp group. The relationship between these two factors is complicated, further study is needed to clarify.

(5) **Q:** Author write the proposed protocol has a potential for clinical use in evaluating hepatic tumor angiogenesis and the response of anti-angiogenesis therapy. Please tell me what kinds of option do you have for hepatic tumor.

A: Our study used the animal model of liver VX2 tumor which was considered as hypervascular tumor supplied by hepatic artery. We found hepatic CT perfusion with low radiation dose and low concentration of contrast medium protocol provided the similar perfusion parameter in comparison with conventional protocol. Therefore, the option of evaluating tumor angiogenesis or response of anti-angiogenesis treatment is most likely effective to hypervascular tumor, such as hepatocellular carcinoma. However, the perfusion parameters in low dose group for normal liver parenchyma did not significantly differ from that in conventional group (Table 1). This finding may suggest that hepatic CT perfusion with low dose protocol also has potential to evaluate the perfusion of hypovascular tumor.

3 References, typesetting and figures were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely Yours,

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