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Angiographic Evaluation of Vascular Damage in Rat Liver After Administration of Epirubicin or Miriplatin

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Abstract. *Background: Maintaining the function of blood vessels is important for the control of hepatocellular carcinoma (HCC) during treatment with repeated transcatheter arterial chemoembolization (TACE). This study was designed to compare the vascular damage caused by miriplatin (MPT), which has been commonly used for TACE, with the damage caused by epirubicin (EPI). Materials and Methods: We used the portal vein of healthy rats for the administration of the drug (MPT or EPI) and/or soybean oil as vehicle. After 2 days, angiography was performed by X-ray computer tomography. Results: The influence of soybean oil on blood vessel function was volume-dependent. EPI showed dose-dependent effects on angiography, and 0.5 mg EPI led to severe (grade 4) blood flow disturbance in all animals. The effect of 1 mg MPT on blood vessels was mild (grade 1) in all animals and not different from that of soybean oil alone. Conclusion: Less vascular damage is caused by MPT than by EPI, suggesting that MPT is a useful drug for TACE in HCC.*

Hepatocellular carcinoma (HCC) is caused primarily by cirrhosis and chronic hepatitis B or C virus infection. Leading treatment methods for HCC include transcatheter arterial chemoembolization (TACE), local therapy such as radiofrequency ablation therapy, and hepatectomy (1, 2). TACE has been widely performed as a treatment procedure for patients with unresectable HCC. Several anticancer agents have been used for TACE, including epirubicin (EPI), cisplatin (CDDP), and miriplatin (MPT) (3-6). Moreover, lipiodol (LPD), an oily contrast agent, has been used as vehicle since it remains in HCC tissue for an extended time following hepatic artery injection, thereby increasing the local concentration of the chemotherapeutic agents (7, 8).

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HCC has a high rate of recurrence, and repeat TACE is often required for patients with HCC. Zinostatin stimalamer, which has been widely used in TACE protocols, leads to severe vascular damage, thus impeding re-administration using the same route (9). EPI also causes obstruction and stenosis of the hepatic artery. Maeda *et al.* (10) reported significant arterial damage or occlusion in 48% or 15% of 33 patients administered EPI, respectively. These arterial impairments make it impossible to control long-term disease condition by repeated TACE using EPI.

MPT is a third-generation platinum compound consisting of a myristic acid leaving group and diaminocyclohexane as a carrier ligand (11-13). MPT has a high affinity for LPD due to the myristic acid moiety and remains with LPD in HCC tissue for an extended period, while exhibiting sustained release of active compounds. Previous studies have shown that MPT exerts tumor-selective toxicity without causing kidney damage or liver dysfunction, and therefore, it has been expected to improve prognosis for HCC patients. Iwazawa *et al.* (14) reported that significant arterial damage was observed in 88% *versus* 19% of patients treated with TACE using EPI or MPT, respectively. In fact, TACE using MPT is the treatment of choice for preserving patency of the hepatic artery over multiple injections (15).

We have previously evaluated the effects of MPT or CDDP suspended in LPD and delivered by TACE in hepatic tumor bearing rats, but we could not reveal the damage to the blood vessels in normal liver (9). In this study, we sought to assess the vascular structure of the liver by X-ray computed tomography (CT) with barium sulfate contrast, after administering drug *via* the portal vein, which supplies a large amount of blood to normal liver. We optimized the injection volume of barium sulfate required for angiography and the dose of soybean oil for the evaluation of vascular disorder caused by EPI or MPT.

Materials and Methods

Animals. All animals were handled in accordance with the guidelines for the care of laboratory animals established by Kobe Gakuin University. The protocol for this animal study was approved by the Animal Experimentation Ethics Committee of Kobe Gakuin University.

Female Sprague Dawley (SD) rats (7 weeks old, 170-190 g) were purchased from Japan SLC Inc. (Shizuoka, Japan) and allowed to acclimatize in the housing facility (at 24˚C, with 55±5% humidity, and a 12-hour light-dark cycle) for 1 week prior to experiment initiation. Rats were anesthetized with isoflurane and pentobarbital before surgery. Blood was withdrawn from heart chambers just before and 2 days after drug administration. Blood samples were centrifuged at 17,800 × *g* for 10 min to obtain serum, which was sent frozen to Oriental Yeast Co. Ltd. (Shiga, Japan) for measurement of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Administration through portal vein. The portal vein was carefully exposed after a midline incision and laparotomy. A 27-gauge butterfly needle was inserted into the portal vein, and agents were administered through the needle. In the case of soybean oil alone, soybean oil was applied at 0.01 ml/s so that oil droplets were injected one drop at a time, followed by pressure hemostasis. For administration of EPI, soybean oil was first administered as above with pressure hemostasis. We then administered 0.1 mg/0.05 ml of EPI *via* the portal vein followed by further pressure hemostasis. For administration of MPT, 1 mg/0.05 ml of MPT suspended in soybean oil was administered as above, 0.05 ml of physiological saline was further applied after hemostasis, and pressure hemostasis was performed again. Physiological saline was injected to equalize the number of punctures and hemostasis procedures with the EPI group. Angiography was performed 2 days after administration. Each group consisted of at least 3 animals.

Angiography of the portal vein. Barium sulfate (1 g/ml) was administered *via* the portal vein as described above. After hemostasis, rats were euthanized by pentobarbital overdose prior to liver resection. The largest lobes of the liver were excised and liver tissue was fixed in 10% neutral phosphate-buffered formalin for 1-2 days, before CT imaging.

X-ray CT imaging. The 3D micro X-ray CT CosmoScan GX (Summit Pharmaceuticals International Corp., Tokyo, Japan) was used for CT. The imaging conditions were as follows: Field of view (FOV), 60 mm; imaging mode, standard; tube voltage, 90 kV; tube current, 88 μA; pixel size, 120 μm; imaging time, 2 min. CT images were evaluated using a dedicated application for 3D visualization. Vascular damage was classified into four grades, according to the percentage of imaged blood vessels, in comparison with angiograms of liver of intact rats, as follows: grade 1, 75 to 100% blood vessels were imaged; grade 2, 50 to 75%; grade 3, 25 to 50%; grade 4, less than 25%.

Statistical analysis. Statistical analysis was performed in Microsoft Excel (Microsoft, Redmond, Washington, USA) using an analysis of variance (ANOVA) with Dunnett's *post hoc* test for multiple comparisons. A value of $p<0.05$ was considered statistically significant.

Results

Effect of barium sulfate injection volume on angiogram. To optimize imaging of the liver blood vessels by CT, the influence of the barium sulfate dose administered into the portal vein was examined (Figure 1). Contrast images after injection of 0.1 or 0.2 ml of barium sulfate at 0.01 ml/s were compared, and inflow into the portal vein was verified based on the absence of any blood flow obstruction. Upon administration of 0.1 ml, only a small portion of the blood vessels could be identified, resulting in a thin contrast image throughout the lobe, while administration of 0.2 ml allowed contrast visualization of fine blood vessels throughout the entire lobe.

Influence of soybean oil as a drug carrier. LPD was not used as a drug carrier during TACE for this study because it interferes with contrast imaging using barium sulfate; soybean oil was therefore substituted for LPD as the fat emulsification agent. We first examined the embolic activity of soybean oil alone to evaluate damage to the blood vessel due to its retention in the absence of chemotherapy. When 0.05 or 0.1 ml of soybean oil was injected, blood flow through the portal vein was not affected. Two days after administration of soybean oil, angiography with barium sulfate was performed (Figure 2). After injection of 0.05 ml of soybean oil, loss of some blood vessels was observed in the periphery of the liver lobe, and the vascular damage was judged as grade 1 in 3/3 animals. Increased quantity of soybean oil (0.1 ml) led to grade 1 and 2 vascular damage in 1/4 and 3/4 animals, respectively, thus it was impossible to image fine reticulated blood vessels as a whole.

Drug-induced blood vessel damage. Vascular damage caused by EPI or MPT was assessed by co-administration with soybean oil (Figure 3). Angiography after 2 days of 0.1 mg of EPI aqueous solution alone showed no vascular damage (data not shown). Since the emulsion formed between EPI and soybean oil was unstable, EPI aqueous solution was separately administered after injection of 0.05 ml of soybean oil. After administration of 0.1 mg of EPI and soybean oil, the blood vessels in approximately one-third of the liver lobe disappeared (grade 2) in 3/3 animals, and fine blood vessels were confirmed in the remaining region. Furthermore, when 0.5 mg of EPI was administered after injection of 0.05 ml of soybean oil, most of the blood vessels disappeared (grade 4) in 3/3 animals, however fine blood vessels could be confirmed in the contrasted area.

MPT was administered as a suspension in soybean oil as is typically performed with LPD. After administration of the MPT suspension, reticulated blood vessels were lost in the periphery of the liver lobe (grade 1 damage) in 3/3 animals, but there was no clear difference in the angiogram as compared with soybean oil alone. Levels of AST and ALT, common markers of liver damage by TACE, showed a tendency to increase in both EPI groups 2 days after administration, compared to the MPT group, though differences were not statistically significant (data not shown). No macroscopic observation showed extensive necrosis in the liver lobe in both groups.

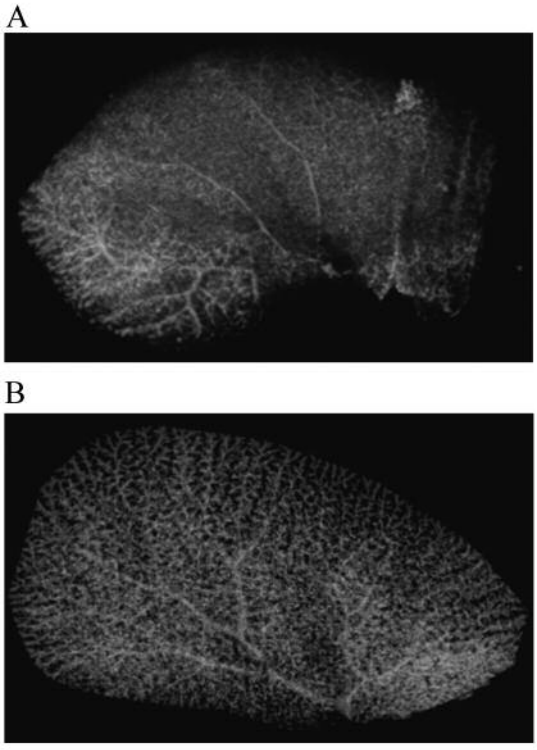

Figure 1. *Effect of the dose of barium sulfate on angiography. Barium sulfate (A: 0.1 ml, B: 0.2 ml) was administered into the portal vein. X-ray computed tomography imaging of the liver leaf was performed.*

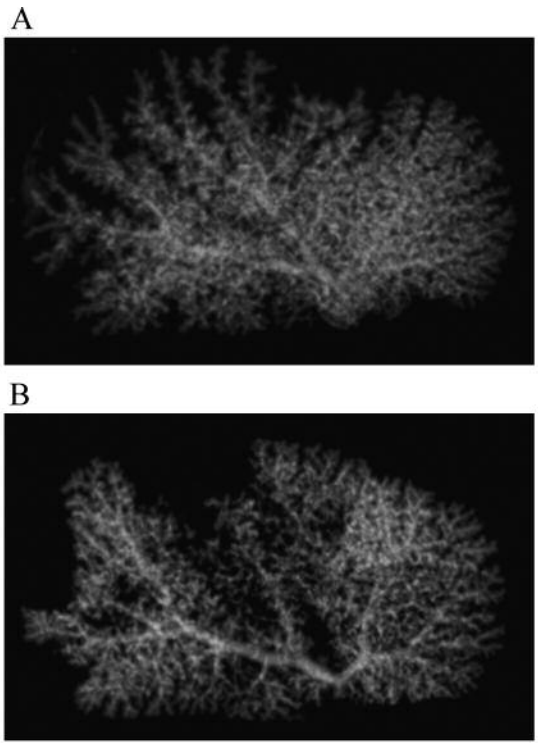

Figure 2. *Blood vessel damage caused by soybean oil. Soybean oil (A: 0.05 ml, B: 0.1 ml) was administered into the portal vein. Two days later, 0.2 ml of barium sulfate was administered into the portal vein. X-ray computed tomography imaging of the liver leaf was performed.*

Discussion

MPT has been widely used with TACE for HCC because it can repeatedly be administered without severe side effects (15). In this study, we aimed to evaluate the low vascular damage caused by MPT that enables repeated administration in an animal model. We have previously reported the antitumor effects of MPT using rabbit and rat transplanted liver cancer models (9, 16). In these models, the volume of LPD in which MPT was suspended for injection into the hepatic artery was minuscule to selectively promote accumulation in tumor tissue. When administered into the hepatic artery of a healthy animal using these administration volumes, almost no LPD was found in liver vessels immediately after the administration. Therefore, it was difficult to evaluate the influence of LPD on blood vessels at the administration volume. In addition, it was difficult to increase the dosage volume of LPD while maintaining blood flow through the hepatic artery in these models.

To allow repeated administration of the drug used for TACE, it is important to maintain blood flow, that is, to not impair the blood vessel used for administration. In the model system used in the present study, the portal vein was selected as the administration route, and the administration volume was five-fold higher than that used for the hepatic artery in our previous study (9), enabling exposure to the entire liver. Blood vessel impairment caused by chemotherapeutics was evaluated by blood vessel imaging by CT after barium sulfate injection, allowing observation of fine reticular blood vessels. Soybean oil was used as the administration medium, because LPD interferes with X-ray imaging, since it cannot be distinguished from barium sulfate contrast.

Soybean oil alone had a dose-dependent effect on microvessel imaging. We hypothesize that soybean oil caused a temporary physical flow disorder, and that blood flow resumed over time. Since the emulsion of EPI and soybean oil was unstable, we mimicked sandwich therapy reported by Sasaki *et al*. (17) and administered EPI solution after administration of soybean oil. Co-administration of EPI and soybean oil showed EPI dose-dependent vascular damage. As EPI solution alone did not lead to any vascular damage, our results suggest that soybean oil-mediated intravascular retention of EPI contributes to vascular damage. As this water/oil emulsion necessitates a release

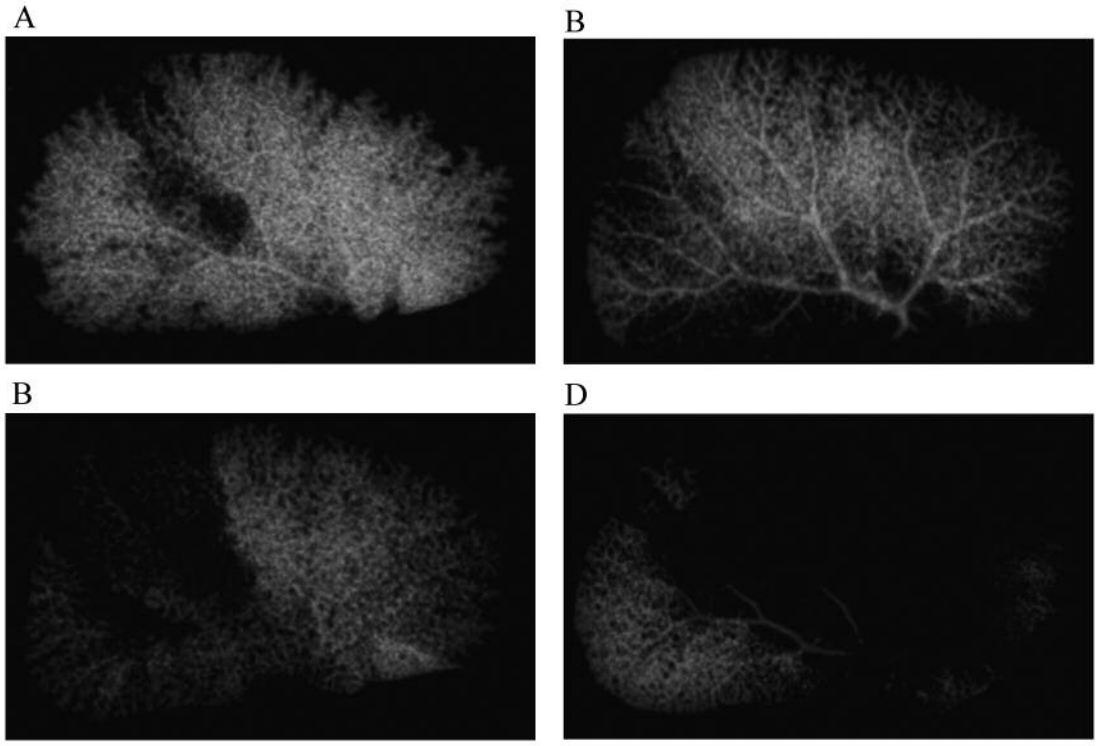

Figure 3. *Comparison of blood vessel damages induced by miriplatin (MPT) and epirubicin (EPI). Drug was administered into the portal vein. A: soybean oil, B: (1 mg) MPT suspended in soybean oil, C: 0.1 mg of EPI and soybean oil, D: 0.5 mg of EPI and soybean oil. Two days later, 0.2 ml of barium sulfate was administered into the portal vein. X-ray CT imaging of the liver leaf was performed.*

process of EPI from the oil layer for delivery to the tissue, this co-administration method may be more indicative of the vascular toxicity of EPI than is the administration of EPI alone.

Suspension of MPT in soybean oil was as stable as in LPD and did not interfere with the administration. Vascular disorder due to MPT did not differ from that due to soybean oil alone, and reticulated blood vessels in liver disappeared on imaging, while other blood vessels were observable. Therefore, our results show a clear difference in vascular damage caused by EPI or MPT. Although EPI caused extensive portal blood flow disorder, the differences in liver function values (ALT/AST) were minimal between the EPI and MPT groups, and no extensive necrosis was observed in either group on macroscopic tissue observation. This observation was thought to be a result of compensatory hepatic arterial blood supply to counteract the portal blood flow disturbance.

In conclusion, we confirmed clinical reports that MPT has the lower vascular toxicity than EPI, suggesting MPT as a useful drug for TACE in HCC. In the future, our evaluation system may be useful to predict vascular damage caused by novel therapeutics for use in TACE.

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