Use of a Glass Membrane Pumping Emulsification Device Improves Systemic and Tumor Pharmacokinetics in Rabbit VX2 Liver Tumor in Transarterial Chemoembolization

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ABSTRACT

Purpose: To evaluate the phamacokinetics of epirubicin in conventional transarterial chemoembolization using a developed pumping emulsification device with a microporous glass membrane in VX2 rabbits.

Materials and Methods: Epirubicin solution (10 mg/mL) was mixed with ethiodized oil (1:2 ratio) using the device or 3-way stopcock. Forty-eight rabbits with VX2 liver tumor implanted 2 weeks prior to transarterial chemoembolization were divided into 2 groups: a device group (n = 24) and a 3-way-stopcock group (n = 24). Next, 0.5 mL of emulsion was injected into the hepatic artery, followed by embolization using 100–300-μm microspheres. The serum epirubicin concentrations (immediately after, 5 minutes after, and 10 minutes after) and the tumor epirubicin concentrations (20 minutes after and 48 hours after) were measured after transarterial chemoembolization. Histopathologic evaluation was performed with a fluorescence microscope.

Results: The area under the curve and maximum concentrations of epirubicin in plasma were 0.45 ± 0.18 μg min/mL and 0.13 ± 0.06 μg/mL, respectively, in the device group and 0.71 ± 0.45 μg min/mL and 0.22 ± 0.17 μg/mL, respectively, in the 3-way-stopcock group ($P = .013$ and $P = .021$, respectively). The mean epirubicin concentrations in VX2 tumors at 48 hours in the device group and the 3-way-stopcock group were 13.7 ± 6.71 and 7.72 ± 3.26 μg/g tissue, respectively ($P = .013$). The tumor necrosis ratios at 48 hours were 62 ± 11% in the device group and 51 ± 13% in the 3-way-stopcock group ($P = .039$).

Conclusions: Conventional transarterial chemoembolization using the pumping emulsification device significantly improved the pharmacokinetics of epirubicin compared to the current standard technique using a 3-way stopcock.

ABBREVIATIONS

AUC = area under the curve, W/O = water in oil

Conventional transarterial chemoembolization is the recommended treatment for patients with intermediate-stage hepatocellular carcinoma on the Barcelona Clinic Liver Cancer classification (1). The worldwide consensus technique of conventional transarterial chemoembolization is mixing ethiodized oil (Lipiodol; Guerbet, Villepinte, France) and doxorubicin/epirubicin solution by pumping using 2 syringes through a 3-way stopcock (2). This technique was first developed in the 1980s and has been used for more than 30 years (3–5). However, this pumping technique, using a 3-way stopcock, has some limitations. Only about 70% water in oil (W/O) can be created, and the droplet size and viscosity are inconsistent (6). To improve the properties of Lipiodol-drug emulsion, we recently developed a pumping emulsification device with a microporous glass membrane (7). This glass membrane was

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created with volcanic ash, which has maze-like homogenous pores with a hydrophobic coating. An ex vivo study showed that the pumping emulsification device could form 97.9% of W/O emulsion when the ratio of epirubicin solution to Lipiodol was 1:2. Also, the droplet sizes were significantly more homogeneous (median size, 35.48 μm) and stable for 30 minutes. In the current in vivo study, the pharmacokinetics of epirubicin and antitumor efficacy were evaluated in a rabbit liver tumor model.

MATERIALS AND METHODS

Study Outline

Forty-eight rabbits with VX2 liver tumors were divided into 2 groups: an emulsification device group (n = 24) and a 3-way-stopcock group (n = 24). In the device group, Lipiodol-epirubicin solution emulsion formed by using an emulsification device was injected into the rabbits. The device was constructed with a fiber structured glass membrane produced by synthesizing volcanic ash, lime, and boric acid at 1350 °C. The glass porous body composed of Al2O3•SiO2 was then formed (8). The disk-shaped glass membrane has numerous micron-size pores, 50 μm in diameter. The membrane has a silicon hydrophobic coating for forming the W/O emulsion. In the 3-way-stopcock group, Lipiodol-epirubicin solution emulsion, formed using a 3-way stopcock, was injected. The serum epirubicin level was measured immediately after, 5 minutes after, and 10 minutes after transarterial chemoembolization. In each group, the rabbits were sacrificed 20 minutes (n = 12) and 48 hours (n = 12) after transarterial chemoembolization. The epirubicin concentrations in the tumors and histologic findings were evaluated (Fig 1).

Animals and VX2 Tumor Implantation

The study was approved by the institutional Animal Experimentation Committee, and all experiments were performed in accordance with the institutional Animal Care Guidance. Adult New Zealand white rabbits bearing a transplanted VX2 tumor in the thigh and healthy adult New Zealand white rabbits weighing 2.9–4.2 kg (mean, 3.6 kg) were purchased from Japan SLC Inc (Hamamatsu, Japan). The rabbits were anesthetized intramuscularly with a mixture of ketamine hydrochloride (25 mg/kg, Ketalar 50; Sankyo Yell Yakuhin Co Ltd, Tokyo, Japan) and medetomidine hydrochloride (0.1 mg/kg, Domitor; Meiji Seika Pharma Co Ltd, Tokyo, Japan). The VX2 tumors in the thighs were harvested from carrier rabbits, and the necrotic areas were removed under direct visualization and cut, using scissors, into 2-mm tissue cubes. The abdominal cavities of the healthy rabbits were opened 4 cm in length, and the left liver lobes were exposed. One VX2 tumor was transplanted into the left hepatic lobe at a depth of 1 cm in each rabbit 2 weeks prior to transarterial chemoembolization.

Preparation of Emulsion

Lipiodol was mixed with epirubicin solution. Epirubicin hydrochloride powder (Epirubicin; Nippon Kayaku, Tokyo, Japan) 50 mg was dissolved in a 5-ml solution (300 mgI/ml contrast agent:saline = 5:1). The emulsions were created using the emulsification device or a 3-way stopcock (Discofix C 3SC; B BRAUN, Melsungen, Germany) with the 5-ml syringes push forward and back method. There were 20 pumping exchanges by using bolus methods (1 second/1 push) in each technique (Fig 2). The ratios of epirubicin solution to Lipiodol was 1:2 (epirubicin solution 1.5 ml mixed with Lipiodol 3 ml).

Transarterial Chemoembolization Procedure

All transarterial chemoembolization procedures were conducted in an angiography suite (Surginix; Canon, Otawara, Japan). A 4-Fr sheath (Terumo, Tokyo, Japan) was inserted from the right common femoral artery, and a 4-Fr hockey stick catheter (Approach Catheter; Hanako Medical Co, Ltd, Saitama, Japan) was inserted into the celiac artery under fluoroscopic guidance. A 1.7-Fr tip, 60-cm-length microcatheter (Estream; Toray Medical Co, Ltd, Urayasu, Japan) was selectively inserted into the left hepatic artery using a 0.014-inch guidewire (Labyrinth; Piolax, Yokohama, Japan). Digital subtraction angiography was obtained. Injection of the formed emulsion was started 15 minutes after the pumping. Next, 0.5 ml of emulsion was injected into the left hepatic artery for 3 minutes, followed by embolization using 100–300-μm microspheres (Embosphere; Merit Medical Syestmes, Inc, South Jordan, Utah) until stasis of the main branch of the left hepatic arterial flow (Fig 3). All transarterial chemoembolization procedures were performed by an interventional radiologist with 10 years of experience.

Follow-up Schedule and Sampling

Epirubicin plasma concentrations were measured immediately after, 5 minutes after, and 10 minutes after transarterial chemoembolization. Each blood sample was centrifuged (3000 rpm for 20 minutes at 4°C), and the plasma was immediately frozen using liquid nitrogen and stored at -80°C. The rabbits were sacrificed by injecting an overdose of pentobarbital 20 minutes (n = 12) and 48 hours (n = 12) after transarterial chemoembolization in each group. In each rabbit, the entire liver was excised, and the liver tumor was cut in half along the midline. Half of the tumor was immediately frozen to measure the epirubicin concentration. The plasma and tissue epirubicin concentration were measured based on the mass spectrometry method (9). The remaining half of the tumor was fixed in 10% buffered formalin for 24 hours. Then, it was cut into 3-mm-thick slices, and 3 sections were paraffinized and stained with hematoxylin and eosin. The slides were scanned with a fluorescence microscope (BZ-X700; Keyence, Osaka,

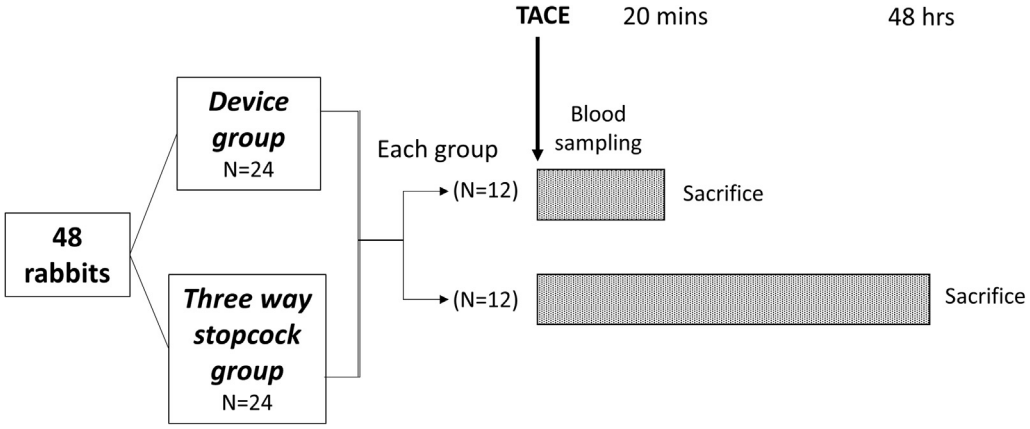

Figure 1. Scheme of study design.

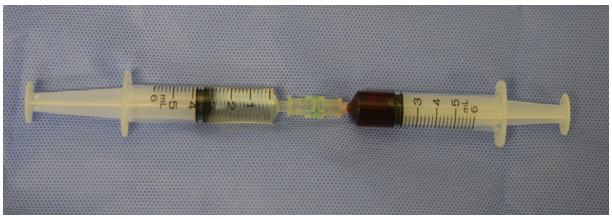

Figure 2. A pumping emulsification device connected with Lipiodol and epirubicin solution.

Japan) with the following settings: ×40 resolution using ×4 objective lens and 8.33-msec exposure. The viable components were delineated by basophilic, purple-stained nuclei. The necrotic components showed predominant eosinophilic, pink-stained cellular material, and/or tissue absence. Tumor necrosis ratio was defined as follows: the ratio of the pink-stained and/or tissue absence area to the purple-stained area calculated by the BZ-II Analyzer software Hybrid Cell Count tool (Keyence, Osaka, Japan).

Statistical Analysis

All data were provided as arithmetic mean ± standard deviation. The area under the curve (AUC) calculations of the plasma epirubicin concentration were performed using SigmaPlot 11.0 (Systat Software Inc, San Jose, California), which computes the AUC using a trapezoid model (10). These analyses were performed using SPSS version 22.0 software (SPSS, Inc, Chicago, Illinois). To determine statistically significant differences between the 2 groups, Student's *t*-test was used. *P* values less than .05 were considered statistically significant.

RESULTS

The VX2 tumors transplanted into the left lobe of the livers were detected by ultrasound after 2 weeks in all 48 rabbits, and all rabbits underwent transarterial chemoembolization successfully.

Pharmacokinetics of Epirubicin in Plasma

The serum epirubicin concentrations peaked immediately after transarterial chemoembolization and returned to near baseline after 10 minutes in both groups (Table). The mean serum epirubicin concentrations immediately after transarterial chemoembolization, C_{max}, were 0.13 ± 0.06 μg/mL in the device group and 0.22 ± 0.17 μg/mL in the 3-way-stopcock group ($P = .021$). The mean serum epirubicin concentrations at 5 minutes after transarterial chemoembolization were also significantly lower in the device group than the 3-way-stopcock group ($P = .013$). No significant differences were observed in the mean serum epirubicin concentrations at 10 minutes between the 2 groups ($P = .436$). The AUC for plasma epirubicin concentrations of the device group was 0.45 ± 0.18 μg min/mL, whereas that of the 3-way-stopcock group was 0.71 ± 0.45 μg min/mL. The AUC for plasma epirubicin concentrations of the 3-way-stopcock group was significantly higher than the device group ($P = .013$).

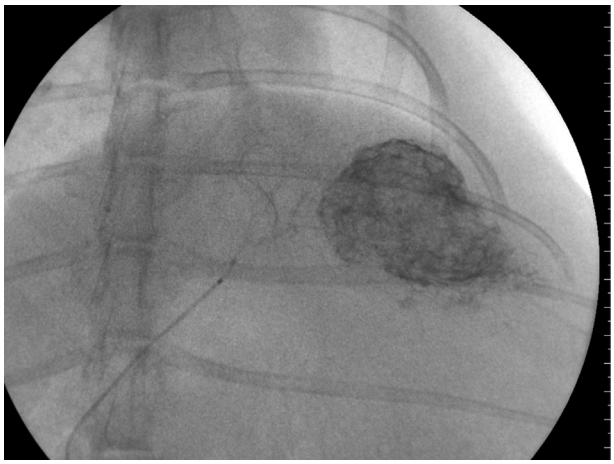

Figure 3. Left hepatic angiography after transarterial chemoembolization. Lipiodol emulsion is accumulated in the tumor.

Tumor Concentration of Epirubicin

The concentrations of epirubicin in tumors are shown in Figure 4. At 20 minutes, the mean epirubicin concentrations in the device group and the 3-way-stopcock group were 41.4 ± 13.9 μg/g tissue and 36.9 ± 14.9 μg/g tissue, respectively. There was no significant difference between them. At 48 hours, the mean epirubicin concentrations in the device

Table. Serum Epirubicin Concentrations (ng/ml)

	Immediately after transarterial chemoembolization	5 minutes after	10 minutes after	Area under the curve
Device group	130 ± 56.7	21.3 ± 9.45	6.62 ± 2.58	448 ± 175
Three-way-stopcock group	217 ± 16.8	29.3 ± 11.7	7.25 ± 2.96	706 ± 454
P value	.021	.013	.436	.013

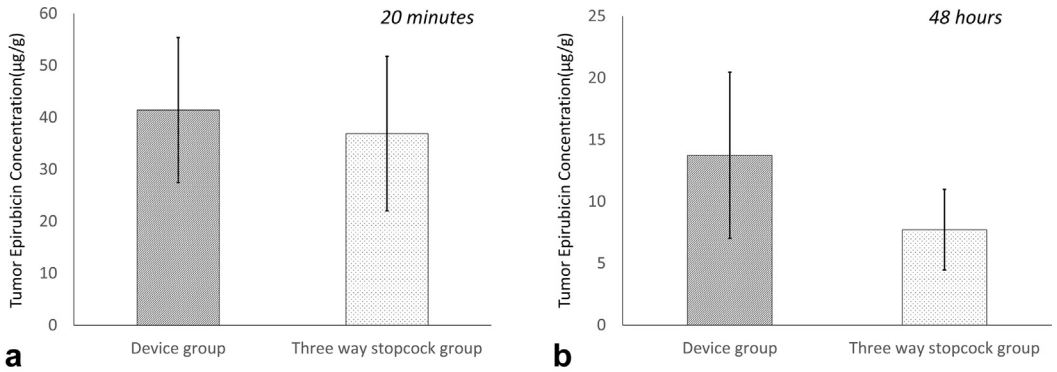

Figure 4. The mean epirubicin concentrations in VX2 tumors at 20 minutes and 48 hours. (**a**) No significant differences were observed between the 2 groups at 20 minutes. (**b**) The concentration at 48 hours after transarterial chemoembolization in the device group was significantly higher than the 3-way-stopcock group ($P = .013$).

group and the 3-way-stopcock group were 13.7 ± 6,71 μg/g tissue and 7.72 ± 3,26 μg/g tissue, respectively. The tumor concentration of epirubicin at 48 hours after transarterial chemoembolization in the device group was significantly higher than the 3-way-stopcock group ($P = .013$).

Histologic Findings

The tumor necrosis ratio at 48 hours was 62.3 ± 11.2% in the device group and 51.1 ± 12.9% in the 3-way-stopcock group. The tumor necrosis ratios of the device group were significantly higher than the 3-way-stopcock group ($P = .039$).

DISCUSSION

This was a preclinical study to examine the developed emulsification device with a microporous glass membrane. A previous in vitro study showed that this device created nearly 100% W/O pure emulsion with stable droplet sizes and viscosity (7). This VX2 rabbit study demonstrated a promising outcome, suggesting pharmacokinetic advantages and antitumor effects in conventional transarterial chemoembolization for liver tumors using this device.

A technical recommendation of conventional transarterial chemoembolization published in 2015 suggested that the volume of drug aqueous solution should be lower than the volume of Lipiodol (2). However, the percentage of W/O created by the 3-way stopcock was limited to around 70%, even when the mixture ratio of solution and Lipiodol was 1:2 or 1:3 (6).

Previously, Choi et al (11) reported that serum doxorubicin concentration was lower when a smaller volume of drug aqueous solution was mixed with Lipiodol in a VX2 liver tumor model. In the present study, the serum epirubicin concentrations of the device group were significantly lower than that of the 3-way-stopcock group when the mixture ratio was 1:2 in both groups. The following speculation can be made: the 100% pure W/O emulsion delivered the epirubicin into the tumor as a drug delivery system in the device group, whereas the O/W emulsion part in the 3-way-stopcock group dispersed into the blood flow immediately after the injection. In addition, the homogeneous droplet size in the device group could contribute a more uniform and slower drug release than in the 3-way-stopcock group with a heterogeneous droplet size.

The tumor epirubicin concentrations of the device group at 48 hours were significantly higher than those of the 3-way-stopcock group. This result showed that 100% W/O emulsion could increase tumor retention. It is known that the viscosity of W/O is higher than O/W (12,13). Previously, Becker et al (14) demonstrated that more viscous emulsion retained in the tumor by using technetium-99m-labeled Lipiodol. Kan et al (15) demonstrated higher tumor retention of 1:2 ratio of emulsion compared to 2:1 ratio of emulsion in a micrography, and the droplets of doxorubicin solution enclosed in the oil were confirmed in the tumor at 7 days after transarterial chemoembolization (15).

On the other hand, there was no significant differences in the tumor epirubicin concentrations at 20 minutes between the 2 groups. This could be explained by the results of the

intra-arterial chemo-infusion, which also achieved a temporally high drug concentration in the tumor (16).

Histologic examination showed that the tumor necrosis ratio of the device group was significantly higher than that of the 3-way-stopcock group. The higher epirubicin concentration and longer Lipiodol retention in the tumor may contribute to these results.

This study had some limitations. First, the rabbits were observed for only 48 hours after transarterial chemoembolization. Second, there was no untreated control group. Third, the drug release speeds in an in vitro study have not been examined yet. Fourth, the number of tumor sections for histologic evaluation was limited. Fifth, epirubicin was used in this study, although doxorubicin is used for conventional transarterial chemoembolization worldwide. However, the molecular structures of epirubicin and doxorubicin are relatively similar (17).

In conclusions, the developed pumping emulsification device increased the tumor epirubicin concentration at 48 hours after transarterial chemoembolization with decreasing systemic epirubicin circulation. Clinical studies to evaluate this device are warranted.

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REFERENCES

1. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999; 19:329–338.
2. de Baere T, Arai Y, Lencioni R, et al. Treatment of liver tumors with lipiodol TACE: technical recommendations from expert opinions. Cardiovasc Intervent Radiol 2016; 39:334–343.
3. Ohishi H, Uchida H, Yoshimura H, et al. Hepatocellular carcinoma detected by iodized oil. Use of anticancer agents. Radiology 1985; 154: 15–19.
4. Nakamura H, Hashimoto T, Oi H, et al. Transcatheter oily chemoembolization of hepatocellular carcinoma. Radiology 1989; 170:783–786.
5. Takaki S, Sakaguchi H, Anai H, et al. Long-term outcome of transcatheter subsegmental and segmental arterial chemoembolization using lipiodol for hepatocellular carcinoma. Cardiovasc Intervent Radiol 2012; 35: 544–554.
6. Masada T, Tanaka T, Nishiofuku H, et al. Techniques to form a suitable Lipiodol-epirubicin emulsion by using 3-way stopcock methods in transarterial chemoembolization for liver tumor. J Vasc Interv Radiol 2017; 10: 1461–1466.
7. Tanaka T, Masada T, Nishiofuku H, et al. Development of pumping emulsification device with glass membrane to form ideal lipiodol emulsion in transarterial chemoembolization. Eur Radiol 2018; 28:2203–2207.
8. SPG Technology Co., Ltd. SPG membrane. Available at: http://www.spg-techno.co.jp/english/contents/spg_membrane.shtml. Accessed June 27, 2019.
9. Ibsen S, Su Y, Norton J, et al. Extraction protocol and mass spectrometry method for quantification of doxorubicin released locally from prodrugs in tumor tissue. J Mass Spectrom 2013; 48:768–773.
10. Jia Y, Peng B, Li L, et al. Estimation of mycophenolic acid area under the curve with limited-sampling strategy in Chinese renal transplant recipients receiving enteric-coated mycophenolate sodium. Ther Drug Monit 2017; 39:29–36.
11. Choi JW, Cho HJ, Park JH, et al. Comparison of drug release and pharmacokinetics after transarterial chemoembolization using diverse lipiodol emulsions and drug-eluting beads. PLos One 2014; 9:e115898.
12. de Baere T, Dufaux J, Roche A, et al. Circulatory alterations induced by intra-arterial injection of iodized oil and emulsions of iodized oil and doxorubicin: experimental study. Radiology 1995; 194:165–170.
13. Idée JM, Guiu B. Use of Lipiodol as a drug-delivery system for transcatheter arterial chemoembolization of hepatocellular carcinoma: a review. Crit Rev Oncol Hematol 2013; 88:530–549.
14. Becker S, Lepareur N, Cadeillan V, et al. Optimization of hepatocarcinoma uptake with radiolabeled lipiodol: development of new lipiodol formulations with increased viscosity. Cancer Biother Radiopharm 2012; 27: 149–155.
15. Kan Z, Wright K, Wallace S. Ethiodized oil emulsions in hepatic microcirculation: in vivo microscopy in animal models. Acad Radiol 1997; 4: 275–282.
16. Gupta S, Wright KC, Ensor J, et al. Hepatic arterial embolization with doxorubicin-loaded superabsorbent polymer microspheres in a rabbit liver tumor model. Cardiovasc Intervent Radiol 2011; 34:1021–1030.
17. Ganzina F. 4′-epi-doxorubicin, a new analogue of doxorubicin: a preliminary overview of preclinical and clinical data. Cancer Treat Rev 1983; 10:1–22.