

# Pharmacokinetics of continuous transarterial infusion of 5-fluorouracil in patients with advanced hepatocellular carcinoma

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**Abstract.** Numerous studies concerning hepatic arterial infusion chemotherapy (HAIC) have been conducted by adopting regimens containing 5-fluorouracil (FU), with a favourable efficacy compared with conventional transcatheter arterial chemoembolisation (TACE) treatment; however, the detailed mechanism of HAIC remains unclear. The present study aimed to evaluate peripheral concentration time curves of 5-FU administered through the hepatic artery, which may additionally explain the mechanism of action of HAIC. A total of 10 eligible patients underwent transcatheter arterial embolization and a 2-day HAIC treatment regimen using a folinic acid, fluorouracil and oxaliplatin regimen. Peripheral venous blood sampling was performed in each patient prior to infusion, and at 0, 0.5, 1, 1.5, 2, 5, 10, 15, 22 and 23 h following the start of infusion. The blood sample at 0 h was analysed for dihydropyrimidine dehydrogenase (DPD) levels by high performance liquid chromatography, and the rest of the samples were analysed for 5-FU by optimised liquid chromatography-mass spectrometry (LC-MS). The lower limit of quantification of optimised LC-MS for 5-FU was 5 ng/ml. The steady-state plasma concentration of 5-FU administered through the hepatic artery was achieved after 15 h. This concentration largely varied, ranging from 8.64-152.00 ng/ml. Optimised LC-MS may detect low concentrations of 5-FU. The steady-state concentration of 5-FU administered through the hepatic artery was achieved after 15 h. DPD levels were analysed through determining the ratio of plasma uracil (U) and dihydrouracil (UH<sub>2</sub>) by HPLC, and the results indicated a mild DPD deficiency in the patients with HCC. These results

may provide a basis for the explanation of the clinical efficacy of HAIC, and to additionally optimise its efficacy.

## Introduction

Transcatheter arterial chemoembolisation (TACE) is recommended by the American Association for the Study of Liver Disease as the first-line treatment for unresectable hepatocellular carcinoma (HCC) (1), and is widely performed in clinical practice due to its favourable efficacy compared with conservative medical management or systematic treatment (2-4) and minimal invasion. However, the survival benefit following TACE remains limited (2). The present study reviewed the literature and revealed that TACE, combined with hepatic arterial infusion chemotherapy (HAIC), may achieve significantly longer progression-free survival (PFS) times (8.0-9.3 months) compared with patients treated with TACE alone (PFS, 4.5 months) (5,6). Presumably due to technical refinements in HAIC protocol, no bleeding, thrombus, infection or other associated complications caused by the indwelling catheter in the HAIC procedure were identified in recent studies (6,7), suggesting that continuous transarterial infusion of 5-fluorouracil (5-FU) is a generally safe treatment, and it is more effective compared with TACE alone for patients with advanced HCC.

However, the underlying cause of the improving efficacy of HAIC remains unclear. It appears that the primary difference between TACE and TACE + HAIC is the approach of administering chemotherapeutic agents: TACE is administered via bolus and HAIC is administered via a prolonged, continuous infusion (8). Theoretically, the prolonged, high regional concentration of the chemotherapeutic agent at the tumour site would be expected to increase anti-tumour effects in HAIC, particularly for time- and concentration-dependent agents (9,10); 5-FU was one of these time-dependent drugs (9).

5-FU was introduced as an anti-tumour drug in the 1950s (11) and it remains the primary agent of various chemotherapy regimens. However, the metabolism of 5-FU largely differs among individuals depending on age (12), sex (12,13) and hepatic insufficiency due to HCC and gene polymorphisms in the dihydropyrimidine dehydrogenase gene (DPYD), which encodes DPD and is involved in the catabolism of 5-FU (14-16). Despite this, in previous studies, HAIC using regimens containing 5-FU was performed with favourable efficacy compared with conventional TACE or best supportive

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care (17-19). Due to the clinical efficacy of HAIC and individual variation of 5-FU described above, the present study aimed to explore more detailed associations, and to additionally explain the improved efficacy achieved by HAIC. However, studies on the pharmacokinetics of transarterial 5-FU infusions are lacking, particularly in patients with advanced HCC and hypohepatia, which limits additional investigation. Therefore, the present study was initiated to evaluate the peripheral concentration time curves of 5-FU administered through the hepatic artery, to provide an explanation of its clinical efficacy and a basis for optimization of this efficacy.

## Materials and methods

**Patient characteristics.** The present study was conducted in the First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China). The primary eligibility criteria included patients with histologically-confirmed (20) HCC with an Eastern Cooperative Oncology Group performance status (21) of  $\leq 2$ , those with a Child-Pugh score (22) A, those with Barcelona Clinic liver cancer stage C (23) and those receiving 2 days of continuous HAIC following conventional TACE. The primary exclusion criteria included patients with severe coronary heart disease, those with severe active infection [ $>$ grade 2, National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 criteria (24)], those with HIV infection, those with renal insufficiency (creatinine level  $> 2$  mg/dl) or those with allergies to platinum compounds, 5-FU or contrast media. All patients provided written informed consent. The present study was approved by the First Affiliated Hospital of Sun Yat-sen University Ethics Committee. Baseline evaluation included the maximal diameter measurement of viable tumours using dynamic contrast-enhanced computed tomography according to the modified Response Evaluation Criteria in Solid Tumours criteria (25) and biochemical examination such as levels of  $\alpha$ -fetoprotein, albumin, bilirubin, alanine aminotransferase, aspartate transaminase, status of hepatitis B virus and hepatitis C virus infection and prothrombin time.

**Treatment protocol.** A total of 10 patients totally underwent 18 cycles of TACE + HAIC. The duration of each TACE + HAIC cycle was 3 days, with a hospital stay ranging from 8 to 12 days. The interval between each cycle was 3 weeks. All patients underwent a 2-day HAIC treatment regimen using a folinic acid, fluorouracil and oxaliplatin (FOLFOX4) regimen: 85 mg/m<sup>2</sup> oxaliplatin (Eloxatin<sup>®</sup>; Sanofi S.A., Paris, France) for 2 h on day 1; 200 mg/m<sup>2</sup> leucovorin (Lingnan Pharmaceutical, Ltd., Guangdong, China) for 2 h on days 1 and 2; 400 mg/m<sup>2</sup> 5-FU (Sinochem Group, Beijing, China) bolus on days 1 and 2; and 600 mg/m<sup>2</sup> 5-FU on days 1 and 2, via an ambulatory infusion pump (MR-508; Zhuhai MeiRuiHua Medical Technology Co., Ltd., Guangdong, China) following conventional TACE. TACE and HAIC were performed as mentioned below.

A 5-F catheter was inserted into the femoral artery using the Seldinger technique (26) following routine preoperative preparation including fasting for 6 h and pubic hair removal. Arteriography of the celiac trunk and hepatic and superior mesenteric arteries was performed to visualise the arterial vascularisation of the tumour and to evaluate portal vein

patency, respectively. Guided by fluoroscopy, the tip of the catheter, or microcatheter if necessary, was superselected into the tumour-feeding branches using a guidewire. The embolisation of target tumour-feeding vessels was performed by injecting a gelatine sponge [Nanjing Jingling Pharmaceutical (Group) Co., Ltd., Nanjing, China] or polyvinyl alcohol particles (Hangzhou Alicon Pharmaceutical SCI&TEC Co., Ltd., Hangzhou, China). Following embolisation, the catheter was inserted and the patient was returned to the ward for FOLFOX4 administration. Regular analgesia (Tramadol, 0.1 g intramuscularly, Hexal AG, Holzkirchen, Germany) and nausea-controlling drugs (Palonosetron, 0.25 mg intravenously; Qilu Pharmaceutical Co., Ltd., Jinan, China) were administered during the HAIC. As soon as consecutive transarterial FOLFOX4 treatment was finished, the catheter was removed, the puncture site was stanchied by compression for  $\sim 15$  min and pressure bandaging was applied.

**Pharmacokinetic blood sampling.** A total of 11 peripheral venous blood samplings were collected from all 10 patients, and each contained 3 ml blood from prior the infusion at admission and at 0, 0.5, 1, 1.5, 2, 5, 10, 15, 22 and 23 h following the initiation of infusion on day 1. Immediately after drawing, blood samples were centrifuged at room temperature at 1,118.0 x g for 10 min, and the supernatant was stored in clean 1.5 ml polypropylene tubes and stored at  $-80^{\circ}\text{C}$ .

**Optimising the extraction method and liquid chromatography-mass spectrometry (LC-MS) conditions.** The extraction method described by Remaud *et al.* (27) was simplified by removing the evaporation procedure. Firstly, 100 mg ammonium sulfate was added into 100  $\mu\text{l}$  plasma samples to precipitate plasma proteins. Following vortex mixing at 50.31 x g at room temperature for 1 min, 300  $\mu\text{l}$  internal standard (IS) solution (5-Br; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was added. The samples were gently mixed at room temperature for 1 min in a rotary stirrer (45 rotations/min) and centrifuged for 10 min at 25,758.7 x g and 4 $^{\circ}\text{C}$ . A total of 100  $\mu\text{l}$  supernatant was then transferred to an autosampler vial prior to injection onto the column. Volume injection was set at 20  $\mu\text{l}$  for 5-FU and 5-bromopyrimidine (5-Br).

The standard curve of 5-FU was prepared by adding 20  $\mu\text{l}$  standard solution (50  $\mu\text{g}/\text{ml}$ ) of industrial pure 5-FU (provided by National Institute for Food and Drug Control, Beijing, China) and 10  $\mu\text{l}$  IS (5-Br) to 980  $\mu\text{l}$  control human plasma. IS concentration was set at 50 ng/ml. Final generated concentrations were 10, 20, 50, 200, 600 and 1,000 ng/ml for 5-FU. Quality controls of 10, 30 and 100 ng/ml of 5-FU were considered low, moderate and high concentrations, respectively, and used to verify the standard curve delineated by 10, 20, 50, 200, 600 and 1,000 ng/ml 5-FU. All samples were then treated according to the extraction method and high-performance liquid chromatography (HPLC) at room temperature as follows. Standard curves for 5-FU were generated by plotting the peak area ratio to that of the IS vs. the concentration of each compound.

Notably, unlike LC conditions in previous studies (27-33), the mobile phase solvent A in the present study contained 0.1% (v/v) formic acid in water, and the mobile phase solvent B contained 100% acetonitrile. The mobile phase composition

Table I. Liquid chromatography mass spectrometry system information.

Instrument	Version	Supplier
Mass spectrometer	AB Sciex API 2000	AB Sciex Pte. Ltd., Warrington, UK
Liquid chromatography system	Agilent Technologies 1200 series	Agilent Technologies, Inc., Santa Clara, CA, USA
Chromatographic column	Agilent Eclipse XDB-C18, 4.6 x 150 mm, 5 $\mu$ m	Agilent Technologies, Inc., Santa Clara, CA, USA
Data acquisition and analysis system	Analyst 1.6, AB Sciex	AB Sciex Pte. Ltd., Warrington, UK

was 5% solvent A and 95% solvent B, which was pumped at a flow rate of 380  $\mu$ l/min for 10 min. Other parameters are summarised in Tables I and II.

DPD levels were also analysed in this research. Direct approaches of determining DPD, such as genotyping, quantification of DPD mRNA have been proved cumbersome to realise (16,34). An alternative method by measuring the ratio of plasma uracil (U) and dihydrouracil (UH2) through HPLC was adopted in this research as DPD is responsible for catabolism of U to UH2 (35,36). Elevated ratios (U:UH2 >2) have been reported highly correlated to DPD deficiency (37-40).

**Results**

Prior to receiving the results, it was hypothesised that the steady-state concentration of 5-FU would range between 200-300 ng/ml, due to previous data from studies examining colorectal cancer (41-43).

Baseline characteristics of 10 patients are summarised in Table III. Representative chromatograms and linearity of 5-FU added into control plasma are presented in Figs. 1 and 2. Plasma concentration vs. time curves of 5-FU are illustrated in Fig. 3 and detailed information is summarised in Table IV. At 0 h of continuous infusion of 5-FU, the plasma concentration of 5-FU was markedly high compared with the normal range (200-300 ng/ml) due to previous 5-FU bolus; this concentration rapidly decreased to relatively normal levels within 2 h and then fluctuated mildly to reach a steady state. According to Fig. 3, a steady-state plasma concentration of 5-FU, administered through the hepatic artery, was achieved after 15 h; this concentration widely varied in the 10 patients, ranging from 8.64-152 ng/ml. The ratio of U and UH2 (U:UH2) fluctuated from 1.98-2.06, indicating mild DPD deficiency in these 10 patients with HCC.

**Discussion**

To the best of our knowledge, this is the first study to quantitatively evaluate plasma concentration time curves of 5-FU continuously administered through the hepatic artery for >44 h in patients with advanced HCC. As the therapeutic agent first passes through the liver in HAIC, which is the organ involved in its eventual metabolism, lower peripheral blood concentration and fewer systemic side effects are anticipated (8). A conventional HPLC was not used, as the lower limit of quantification (LLOQ) was 100 ng/ml (44,45), and thus it may not detect lower concentrations of 5-FU in

Table II. Mass spectrometer settings for the analysis of 5-fluorouracil in human plasma.

Detector parameters	Setting
Ion source	Electrospray ionization
Ionization modes	Negative
Scanning mode	Multiple reaction monitoring scanning
Selected reaction monitoring transition	5-FU:129.0→42.1; 5-Br:188.9→52.0
De-clustering potential	-20 V
Focusing potential	-400 V
Entrance potential	-10 V
Collision energy	-18 V
Collision cell exit potential	-5 V
Curtain gas	20 TSI
Collision gas	8
Ion spray gas	-4500 V
Temperature	400°C
Ion source gas 1	55 PSI
Ion source gas 2	50 PSI
Flow rate	380 $\mu$ l/min

V, volts; PSI, pound per square inch.

peripheral blood during HAIC. A more sensitive method with an LLOQ of 5-10 ng/ml was identified from previous studies (27,31-33,46), and the procedure was simplified to meet clinical requirements. It should be noted that specific parameters, primarily the aforementioned LC conditions, of the LC-MS performed in the present study were not exactly the same in different centres with different instruments when the methods published previously were repeated (27-33). The results of the present study suggest that 5-FU was better extracted by precipitate plasma proteins with ammonium sulfate compared with simple liquid-liquid extraction with acetonitrile or formic acid in acetonitrile. Additionally, with the evaporation procedure, the ultimate peak intensity may be more marked compared with any other procedure, but took a longer time. For clinical application, the evaporation process was removed.

The half-life of 5-FU in the human body is 10-20 min (47). The majority of drugs will reach steady state following

Table III. Baseline characteristics and tumour responses of 10 patients.

Patient ID	Age, years	Sex	Hepatitis B virus	Hepatitis C virus	Child-Pugh score	AFP	BCLC stage	Blood supply	Previous treatments	Response
001	40	M	Positive	Negative	A	Negative	B	Hypervascular	TACE, intravenous FOLFOX4	PR
002	30	M	Positive	Negative	A	Negative	B	Hypervascular	TAE + HAIC (FOLFOX4)	Lost
003	38	M	Positive	Negative	A	Positive	B	Hypervascular	None	Lost
004	42	M	Positive	Negative	A	Positive	C	Hypervascular	Resection, ablation, TACE	PR
005	51	M	Positive	Negative	A	Negative	C	Hypovascular	TACE + HAIC (FOLFOX4)	SD
006	51	M	Positive	Negative	A	Positive	C	Hypovascular	TACE	SD
007	33	M	Positive	Negative	A	Positive	B	Hypovascular	Resection	SD
008	37	M	Positive	Negative	A	Negative	C	Hypervascular	None	SD
009	42	M	Positive	Negative	A	Negative	C	Hypovascular	TACE + HAIC (FOLFOX4)	Lost
010	46	M	Positive	Negative	A	Positive	B	Hypervascular	Resection, TACE + HAIC (FOLFOX4)	CR

AFP,  $\alpha$ -fetoprotein; BCLC, Barcelona Clinic Liver Cancer; TACE, transcatheter arterial chemoembolisation; HAIC, hepatic arterial infusion chemotherapy; FOLFOX4, folinic acid, fluorouracil and oxaliplatin; PR, partial response; lost, lost to follow up; SD, stable disease; CR, complete response; M, male. Hepatitis B and C surface antigens were used to differentiate negative and positive cases. AFP > 200 ng/ml was defined as positive and AFP < 200 ng/ml was defined as negative.

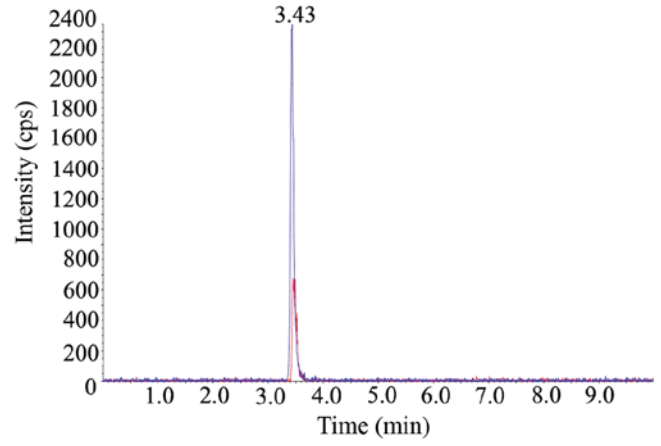


Figure 1. 5-FU and 5-Br extracted from blood plasma. The concentration of 5-FU and 5-Br were 500 ng/ml. The blue peak represents 5-FU, with a retain time of 3.43 min (peak summit) and intensity of 2,300 cps. The red peak represents 5-Br, with retain time of 3.50 min and intensity of 680 cps. 5-FU, 5-fluorouracil; 5-Br, 5-bromopyrimidine.

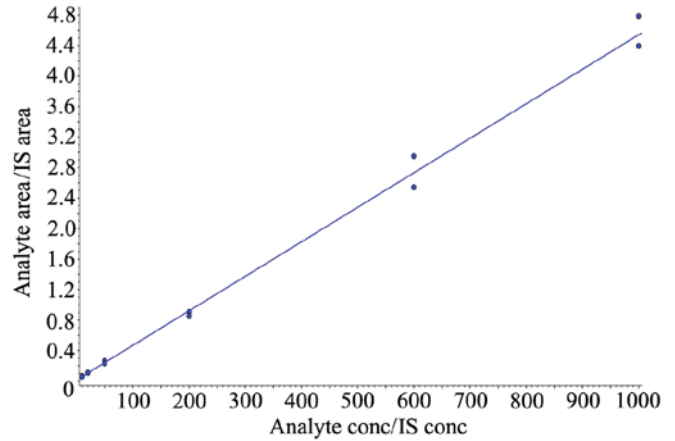


Figure 2. Linearity of 5-FU concentration. Standard curve of 5-FU was depicted according to concentrations of 10, 20, 50, 200, 600, 1,000 ng/ml and the liner regression coefficient was 0.9966.  $y=0.00453x + 0.0152$  ( $r=0.9966$ ). 5-FU, 5-fluorouracil; IS, internal standard.

5 half-lives during continuous intravenous administration (48). In the present study, a steady-state plasma concentration of 5-FU was achieved after 15 h, which is much longer compared with the 5 half-lives of 5-FU. This conclusion was consistent with results obtained by Kaldate *et al* (49), which revealed that more factors than half-life alone affect the steady-state plasma concentration of 5-FU, particularly in patients with levels of liver dysfunction, including HCC. For additional studies on the steady-state concentration of 5-FU in peripheral blood and the efficacy of TACE + HAIC, the average time-points of 15 and 22 h are recommend for the measurement of the steady-state plasma concentration.

Notably, despite the mild fluctuation of U:UH<sub>2</sub> from 1.98-2.06 in the 10 patients, the steady-state concentration of 5-FU varied widely between patients, ranging from 8.64-152 ng/ml, which indicates that factors separate from DPD levels require consideration. Concurrently, the steady-state concentration of 5-FU (30.2-152.9 ng/ml) in the present study was under the established therapeutic range, when administered venously, was

Table IV. Detail information of 5-FU and DPD levels of 10 patients.

Patient ID	5-FU levels (ng/ml)										U:UH2 (prior the infusion at admission)
	0 h	0.5 h	1 h	1.5 h	2 h	5 h	10 h	15 h	22 h	23 h	
001	12,477.0	1,771.0	336.1	106.6	128.3	114.3	94.7	88.4	148.7	0.0	2.02
002	10,118.9	237.1	102.4	74.8	62.5	54.9	55.8	54.1	62.8	44.7	2.05
003	11,452.4	1,150.8	291.4	93.9	87.5	84.5	87.9	91.4	57.8	41.2	2.02
004	2,200.0	219.0	123.0	115.0	108.0	139.0	124.0	118.0	90.1	0.0	2.08
005	6,390,000.0	963.0	178.0	110.0	86.8	57.5	67.1	76.3	55.7	121.0	1.98
006	1,650,000.0	169.0	57.4	83.1	83.8	75.8	64.6	56.3	65.8	49.4	2.00
007	10,500.0	508.0	46.7	36.1	33.3	35.4	8.6	30.2	35.8	58.5	2.04
008	12,000.0	336.0	80.7	35.5	31.2	39.5	33.7	31.6	29.2	52.1	2.01
009	14,953.7	1,965.2	449.5	135.1	138.1	114.3	131.7	152.9	152.1	24.6	2.04
010	11,204.7	280.8	125.7	142.3	117.3	110.8	122.4	132.5	103.6	0.0	2.06

5-FU, 5-fluoruracil; DPD, dihydropyrimidine dehydrogenase; U, uracil; UH2, dihydrouracil; DPD. Mild DPD deficiency was observed from U: UH2 fluctuation from 1.98-2.06.

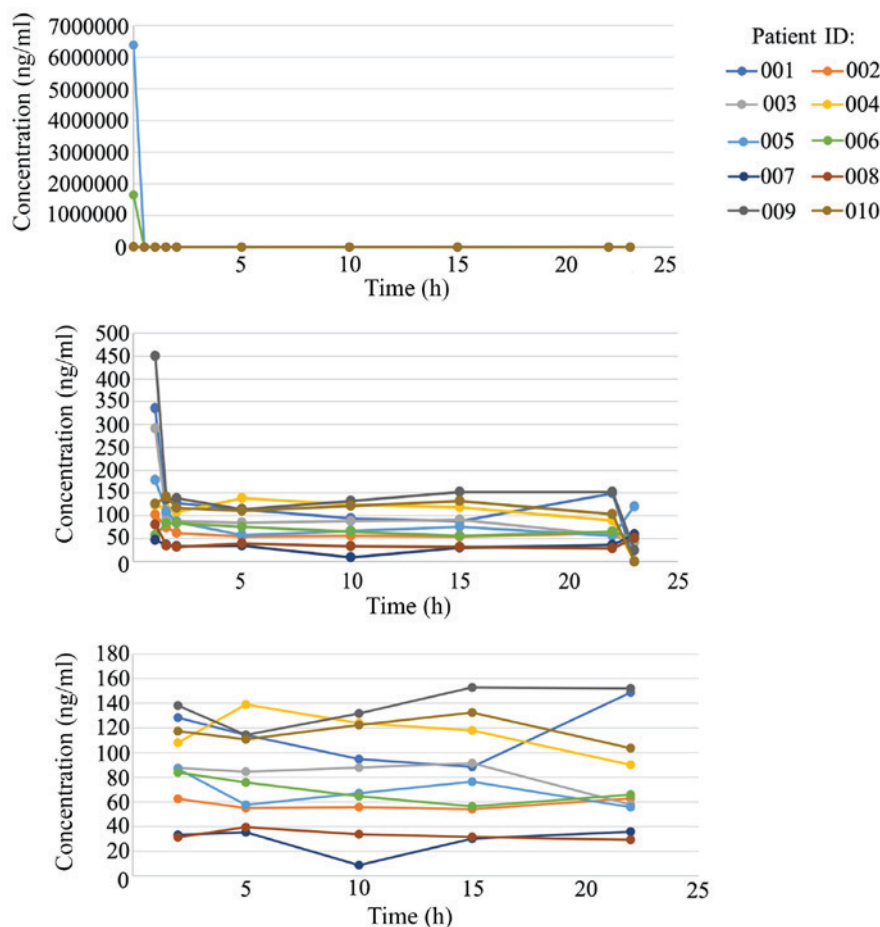


Figure 3. Line charts showing 5-FU blood concentration (0-23 h). At 0 h of continuous infusion of 5-FU, 5-FU plasma concentration was markedly high compared with the normal range (200-300 ng/ml) due to previous 5-FU bolus, and subsequently declined rapidly. After 2 h, the concentration of 5-FU fluctuated mildly prior to reaching a steady state, and a steady plasma concentration was generally achieved after 15 h following administration through the hepatic artery. 5-FU, 5-fluoruracil.

between 200-300 ng/ml in colorectal cancer (41-43). However, it is noteworthy that the comparisons between arterial and venous modes of administration, and between different types

of cancer is insufficient. Therefore, the association between the steady-state concentration of 5-FU in peripheral blood and the efficacy of TACE + HAIC requires additional study.

An additional notable result of the present study was that the concentration of 5-FU at 23 h, which is the hour when 5-FU treatment was ceased, during the administration of folinic acid, exhibited various changes; 3 cases decreased to 0 ng/ml, 4 decreased to a detectable degree (24.6-49.4 ng/ml) and 3 increased to more than the respective steady-state concentration (52.1-121.0 ng/ml). Folinic acid serves as a coactivator of thymidylate synthetase, which is the primary target of the continuous infusion action of 5-FU, to increase the efficacy of 5-FU (50). Rebound increases in the concentration of 5-FU during the administration of folinic acid were not expected at the initiation of the present study.

The present study contained several limitations. Firstly, the chemotherapy regimen used in the HAIC was FOLFOX4, which contains a chemotherapeutic agent-oxaliplatin. The anti-tumour role of oxaliplatin should not be neglected. Theoretically, the combination of 5-FU and oxaliplatin yielded additive or synergistic cytotoxic effects (51), but which one served the primary role remains unknown, and requires additional study. Also, the present study did not focus on the pharmacokinetics of oxaliplatin; the group are developing an assay that is simpler and more sensitive and cost-effective in clinical applications for quantitative assessment, although a small number of previous studies have described the use of laser ablation-inductively coupled plasma-mass spectrometry and flameless atomic absorption spectrometry methods (52,53).

An additional limitation was that the prognosis of each patient was not completely assessed. A total of 3 of the 10 patients were lost to follow-up. However, the aim of the present study was not to assess the efficacy. There may be several prognostic factors of TACE + HAIC, including the materials used and extent of embolisation. The studies of Gao *et al* (5,6) considered embolisation as the basis and core of combination therapy. Perfect embolisation may thoroughly block the blood supply of tumour and lower the risk of catheter malposition during chemotherapy (5). The present study only proposes an additional potential prognostic factor-the area under the curve of chemotherapeutic drugs. This is the basis for additional response evaluation and prognosis analysis.

To conclude, continuous transarterial infusion of 5-fluorouracil is a generally safe treatment. Optimised LC-MS may detect low concentrations of 5-FU. The steady-state concentration of 5-FU administered through the hepatic artery was achieved after 15 h, which may provide a basis for additional therapeutic drug monitoring practice, response prediction and efficacy optimization.

### Competing interests

The authors declare that they have no competing interests.

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