Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-1297-0
May 2014, Vol. 13, No. 5, P229–P234

Efficacy comparison between hepatic arterial infusion chemotherapy plus systemic chemotherapy used as first-line and non-first-line treatments for the patients of colorectal cancers with unresectable hepatic metastases*

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Received: 19 February 2014 / Revised: 6 March 2014 / Accepted: 15 April 2014

Abstract ***Objective:*** The combination of hepatic arterial chemotherapy (HAIC) and systemic chemotherapy (SYC) has potential effect on colorectal cancer (CRC) patients with unresectable hepatic metastasis. The aim of this retrospective study was to investigate the efficacy and safety of this combined therapeutic regimen on Chinese patients based on single institute experiences. ***Methods:*** All 54 patients of this retrospective analysis were diagnosed with CRC with unresectable liver metastasis and received combined HAIC and SYC. Among the patients, 23 of them received HAIC plus SYC when they developed liver metastases as first-line treatment (Group 1), and 31 patients received HAIC plus SYC as non-first-line treatment (Group 2). The different efficacy in two groups was analyzed by SPSS 19.0. ***Results:*** The overall response rate (ORR) were 52.2% and 25.8% respectively in Groups 1 and 2 (P = 0.047), and the disease control rate (DCR) were 65.2% and 35.5% respectively in Groups 1 and 2 (P = 0.031). The median progression-free survival (PFS) were 6.8 and 3.3 months (P = 0.002), the median hepatic progression-free survival (H-PFS) were 8.8 and 3.7 months (P = 0.001), and the median overall survival (OS) were 18.8 and 13.7 months (P = 0.121) in Groups 1 and 2, respectively. No fatal reaction was observed and no significant difference of adverse reaction was found in two groups. Grade 3/4 toxic effects included neutropenia (9.7% in Group 2 only), gastrointestinal reaction (8.7% in Group 1 and 6.5% in Group 2), stomatitis (6.5% in Group 2 only) and hyperbilirubinemia (4.3% in Group 1 only). ***Conclusion:*** HAIC combined with SYC showed promising efficacy and safe profiles on CRC patients with unresectable liver metastases.

Key words colorectal cancer (CRC); unresectable hepatic metastasis; systemic chemotherapy (SYC); hepatic arterial chemotherapy (HAIC)

Colorectal cancer (CRC), one of the most prevalent cancers and the third-leading cause of cancer death worldwide [1, 2], causes over 500 000 deaths yearly [3]. Survival depends largely on the stage of the disease at diagnosis. For the localized stage patients, radical surgery is the best treatment, and the overall 5-year survival rate reaches to 90%. However, for patients with regional diseases and distant metastases, the 5-year survival rates are 70% and 10%, respectively [4, 5]. For liver has the special anatomical structure with dual blood supply and the flow of CRC to liver via the portal vein, approximately 50% of patients with stage IV disease will develop liver metastases [6]. Of which only 10% to 20% are candidates for resection of hepatic metastases, and systemic chemotherapy (SYC) is usually unsatisfactory [7, 8]. Thus, the clinical management of CRC with liver metastases is still a challenge faced by surgeons and physicians, more aggressive treatments need to be explored. Based on the fact that liver metastases

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* Supported by grants of National Natural Science Foundation of China (No. 81302141), Administration of Traditional Chinese Medicine of Guangdong Province, China (No. 20111169), Science and Technology Planning Project of Guangdong Province, China (No. 2010B031600317), and the Sun Yat-sen University Young Teacher Training Project (No. 12ykpy56).

larger than 0.5 cm derive most of blood supply from the hepatic artery [9, 10], hepatic arterial chemotherapy (HAIC) can be used as an option for those patients.

HAIC has been utilized since 1959, with an effort to maximize local concentration and improve response [9, 10]. The rationale for HAIC is based on the following points: (1) the liver is often the first and only site of metastatic disease; (2) liver metastases are perfused mostly by the hepatic artery, whereas normal tumoral liver parenchyma, which is mainly supplied by the portal circulation, is relatively spared [11, 12]; (3) CRC liver metastases are exposed to high drug concentrations, avoiding drug liver first-pass effect and reducing systemic side effects [13]. HAIC is an appropriate consideration for studies, its advantage will be further amplified in cases of a high total body clearance of the drug [14]. With respect to the mode of administration, continuous HAIC is regarded as the most effective means to maximize the regional advantage [15, 16]. Studies showed that for the patients with liver-only metastases, HAIC can achieve a significantly higher response rate compared with systemic chemotherapy as well as an at least modest survival benefit over systemic chemotherapy [17–19]. Whereas, HAIC has no obvious effect on reducing the rate of metastasis, on the contrary, SYC compensated the limited effective of HAIC for its advantage. Therefore, we conducted the current study to retrospectively reviewed the effects of HAIC plus SYC when as first-line treatment or non-first-line treatment in CRC patients with unresectable liver metastases.

The fluorouracil (5-FU) analog fluorodeoxyuridine (FUDR) is considered as the ideal pharmacokinetic profile. It was reported that if FUDR administered via hepatic artery, its pharmacokinetic properties decides that 95% of the drug is extracted by the liver, which can result in 16-fold higher concentrations in the hepatic metastases, when compared to i.v. administration [20–23].

Patients and methods

Patient characteristics

The single-center retrospective study contained 54 patients of CRC with unresectable hepatic metastases, who accepted treatment in Sun Yat-sen University Cancer Center (China) from June 2005 to August 2012 and were follow-up to August 2013, including 23 patients received HAIC plus SYC as the first-line treatment (Group 1) and 31 patients received HAIC plus SYC treatment following failure of systemic first-line or second-line chemotherapy, as a non-first-line treatment (Group 2). The baseline characteristics of the patients were summarized in Table 1. All selected patients fulfilled the criteria of (1) histologically confirmed colorectal adenocarcinoma with unresectable liver metastases, (2) adequate hematopoietic function: WBC count ≥ 3 000 cells/L, Hb > 90 g/L, platelets ≥ 100 000 cells/L, (3) adequate cardiac/renal/hepatic function, left ventricular ejection fraction > 60%, creatinine clear ratio (Ccr) > 70 mL/min, serum total bilirubin level ≤ 2 mg/dL, (4) Performance status (Eastern Cooperative Oncology Group) [PS (ECOG)] < 3, (5) HAIC with single-agent FUDR, and treated by the combination of HAIC and SYC. Among Group 1, 15 patients received FOLFOX (5-fluorouracil/leucovorin/oxaliplatin) regimen and 17 patients received FOLFIRI (5-fluorouracil/leucovorin/irinotecan) or single irinotecan regimen in previous SYC. When liver metastases occur at the time of initial diagnosis of the primary tumor, they are described as synchronous. If not, they are described as metachronous.

Chemotherapy administration

Patients received regional chemotherapy via a flow-rate settable HAIC pump. The pump was placed subcutaneously and sutured to the fascia of the abdominal wall, with the tip of the catheter inserted into the gastroduodenal artery, the common branch of the hepatic artery. Then the chemotherapy could be administrated through the skin of the abdomen and then into the pump. Another way was through a catheter introduced via femoral artery by the Seldinger technique as described elsewhere [24]. An external pump for SYC was adopted on all patients.

All patients received HAIC FUDR/Dex during Days 1–14 of a 4-week cycle. The dose of FUDR was calculated by following equation: 0.1 or 0.2 mg/kg/day × weight (kg) × 14 days, the total volume of pump was 240 mL. The dose of dexamethasone given 30 min before the chemother-

Table 1 Baseline patients characteristics of two groups (*n*)

	Group 1 (*n* = 23)	Group 2 (*n* = 31)	*P* value
Gender (Male/female)	15/8	23/8	0.475
Age (≤ 60 years / > 60 years)	16/7	21/10	0.887
Mean age (years)	53	57	
PS (ECOG) (0/1/2/3)	0/20/3/0	0/28/3/0	0.697
Primary site (Colon/rectum)	14/9	24/7	0.188
Liver metastasis (1–2/≥ 3)	2/21	8/23	0.109
Extra-hepatic metastatic site (0/1/≥ 2)	20/2/1	23/6/2	0.265
Primary resection (Yes/no)	9/14	4/27	0.113
Liver metastases (Metachronous/synchronous)	2/21	7/24	0.176

PS (ECOG) = Performance status (Eastern Cooperative Oncology Group)

apy was 25 mg. Treatment was repeated every 4 weeks if hepatic enzymes were not elevated and no myelosuppression happened. Meanwhile, the most commonly used SYC regimen combined with HAIC of FUDR was a combination of infusional fluorouracil (5-FU) and leucovorin (LV) with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI). Other SYC regimens contained irinotecan combined with fluorouracil (5-FU) or irinotecan combined with xeloda (CPTX), and single drug irinotecan or oxaliplatin. All of these agents were used at standard dose, prescribed by the body surface area (BSA) of the patients. The SYC regimens were repeated once two weeks except for CPTX regimen, which was three weeks again. Treatment was terminated until the appearance of unacceptable toxicity, the progression of hepatic lesions, or a marked enlargement of extra-hepatic lesions.

Evaluation

Tumor response was defined by the World Health Organization classification as follows: complete response (CR) was defined as the total resolution of all measurable sites of disease for a minimum of 4 weeks. Partial response (PR) was defined as a ≥ 50% decrease in the sum of the products of the perpendicular dimensions of all measurable lesions for a minimum of 4 weeks without the appearance of new lesions. Stable disease (SD) was evaluated if a response < 25%. Progression disease (PD) was considered if an increase ≥ 25%. According to the Response Evaluation Criteria for Solid Tumors, overall survival (OS) was calculated from the date of initiating HAIC to death, and censored on the date of the last follow-up of a surviving patient, overall progression-free survival (PFS) was defined as the time from initiating HAIC to the documented progression of disease at any site or to date of death from any cause. Hepatic progression-free survival (H-PFS) was defined as the time from the initiation of HAIC to the hepatic progression or death from any cause. The primary endpoints of the study were PFS and tumor response rate. Secondary endpoints were OS and H-PFS.

Statistical analysis

All analyses were conducted with SPSS software version 19.0. OS, PFS, and H-PFS were estimated by the Kaplan Meier method. Exact 95% confidence intervals (CIs) were provided for proportions. Survival curves were compared by the log-rank test. Comparison of patients' characteristic and response rate between two groups was done with independent sample *T*-test and chi-square test separately. $P < 0.050$ was considered statistically significant.

Results

Baseline characteristics of the cases

As shown in Table 1. The Group 1 consisted of 15 males (65.2%) and 8 females, and the Group 2 consisted of 23 males (72.4%) and 8 females ($P = 0.475$), with a median age of 53 years (range, 19–71 years) and 57 years (range, 32–77 years) respectively ($P = 0.887$). Twenty patients (87.0%) demonstrated an ECOG PS of 2 and three patients (9.7%) demonstrated an ECOG PS of 3 in Group 1, and twenty-eight patients (90.3%) demonstrated an ECOG PS of 2 and three patients (9.7%) demonstrated an ECOG PS of 3 in Group 2 ($P = 0.697$). Primary site of CRC in colon or rectum in Group 1 respectively were 14 patients (60.9%) and 9 patients, and 24 patients (77.4%) and 7 patients respectively in Group 2 ($P = 0.188$). There was also no significant difference about intra-hepatic metastasis ($P = 0.109$) or extra-hepatic metastasis ($P = 0.265$) between the two groups. Nine patients and four patients had primary resection in Group 1 and Group 2 respectively, but the rest not in the two groups ($P = 0.113$). Two patients (8.7%) had metachronous hepatic metastases and 21 patients had synchronous in Group 1, and 7 patients (22.6%) had metachronous hepatic metastases and 24 patients had synchronous in Group 2 ($P = 0.176$). Therefore, baseline characteristics of two groups showed no obvious differences.

Response

The responses to HAIC of FUDR combined with SYC were summarized in Table 2. One patient was not evaluable in Group 1 and 4 patients in Group 2, the rest was considered assessable. The results of overall efficacy evaluation in Groups 1 and 2 were as follows: null CR in Group 1 and 1 patient (32.2%) achieved CR in Group 2 ($P = 0.385$); 12 (52.2%) vs. 7 (22.6%) PR in Group 1 and Group 2, respectively ($P = 0.024$); 3 (13.0%) vs. 3 (9.7%) SD in Group 1 and Group 2, respectively ($P = 0.697$); and 7 (30.4%) vs. 17 (54.8%) PD in Group 1 and Group 2, respectively ($P = 0.074$), reaching 12 (52.2%) vs. 8 (25.8%)

Table 2 Systemic response of two groups [*n* (%)]

Response measure	Group 1 (*n* = 23)	Group 2 (*n* = 31)	*P* value
CR	0	1 (3.2)	0.385
PR	12 (52.2)	7 (22.6)	0.024
ORR (CR + PR)	12 (52.2)	8 (25.8)	0.047
SD	3 (13.0)	3 (9.7)	0.697
DCR (CR + PR + SD)	15 (65.2)	11 (35.5)	0.031
PD	7 (30.4)	17 (54.8)	0.074
Not evaluable	1 (4.3)	4 (12.9)	

CR = complete response; PD = progressive disease; PR = partial response; ORR = overall response rate; SD = stable disease; DCR = disease control rate

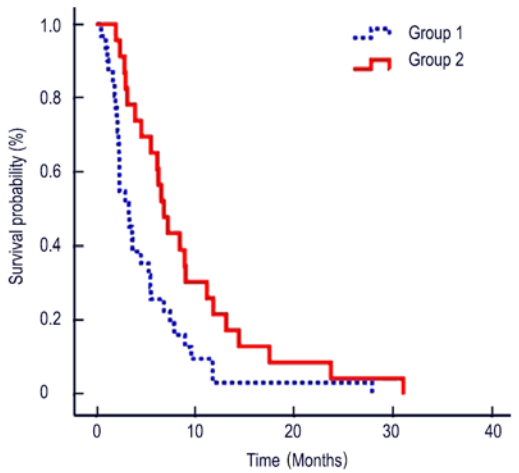

Fig. 1 Kaplan-Meier curves of progression-free survival of HAIC plus SYC treatment in two groups

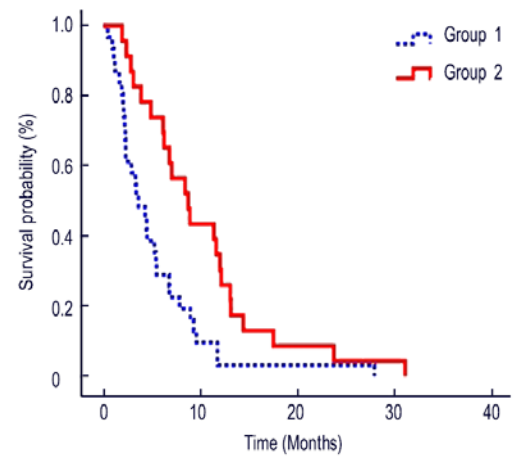

Fig. 2 Kaplan-Meier curves for hepatic progression-free survival of HAIC plus SYC treatment in two groups

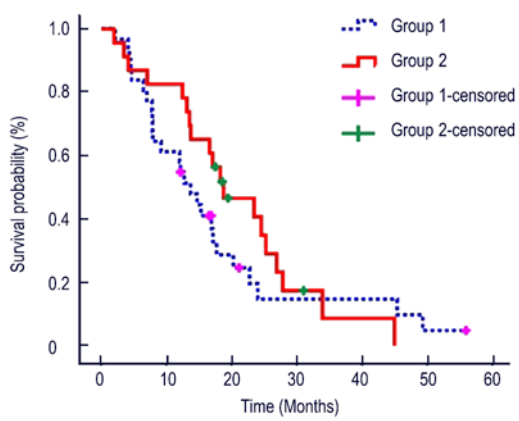

Fig. 3 Kaplan-Meier curves for overall survival of HAIC plus SYC treatment in two groups

Table 3 Survival of two groups

Survival (95% CI, months)	Group 1	Group 2	*P* value
PFS	9.1 (6.2–12.0)	5.0 (3.1–6.9)	0.002
H-PFS	10.1 (7.2–12.9)	5.3 (3.5–7.2)	0.001
OS	20.7 (15.6–25.8)	17.8 (12.2–23.5)	0.121

PFS = progression-free survival; H-PFS = hepatic progression-free survival; OS = overall survival

overall response rate ($P = 0.047$) and 15 (65.2%) vs. 11 (35.5%) systemic disease control rate ($P = 0.031$). At the end of the follow-up, 4 patients were still alive in Group 1 and the number was 5 in Group 2 ($P = 0.902$).

Survival

Statistical results of PFS, H-PFS and OS were presented at Fig. 1–3 and Table 3. According to the statistical results, median PFS in Group 1 and Group 2 were 6.8 months (95% CI, 5.3–8.3 months) and 3.3 months (95% CI, 2.3–4.3 months), respectively ($P = 0.002$). The results of median H-PFS were 8.8 months (95% CI, 5.8–11.7 months) in Group 1 and 3.7 months (95% CI, 1.9–5.4 months) in Group 2 ($P = 0.001$). As to the median survival times were 18.8 months (95% CI, 10.3–27.2 months) in Group 1 and 13.7 months (95% CI, 10.2–17.2 months) in Group 2 ($P = 0.121$). One-year survival estimates were 78.3% and 58.1% in Group 1 and Group 2, respectively ($P = 0.120$).

Toxicity

The toxicities were summarized in Table 4. Chemotherapy related adverse events were graded according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Among the 54 patients, most toxicities were moderate during the HAIC plus SYC treatment. The common systemic adverse reactions were gastrointestinal reaction (including nausea, vomiting and diarrhea), stomatitis, hepatic damage, hyperbilirubinemia, and most of them were Grade 1 to 2. Grade 3 gastrointestinal reaction was rare, only 2 cases (8.7%) in Group 1 and also 2 (6.5%) in Group 2. On the other hand, only 1 patient (4.3%) in Group 1 developed grade 3 hyperbilirubinemia and 2 (6.5%) in Group 2 experienced grade 3 stomatitis. The grade 4 leukocytopenia in Group 2 was the most severe adverse event. Catheter-related adverse event was caused by one catheter which was taken off negligently in Group 2. No clinically significant impairment in renal function and cardiac toxicity was noted. No fatalities related to HAIC plus systemic therapies were found. The incidences of toxicity had no differences between the two groups, all $P > 0.05$.

Table 4 Adverse events of HAIC plus SYC in two groups [*n* (%)]

Adverse event	Group 1			Group 2		
	Grades 1 and 2	Grade 3	Grade 4	Grades 1 and 2	Grade 3	Grade 4
Leukocytopenia	5 (21.7)	0	0	3 (9.7)	0	1 (9.7)
Thrombocytopenia	1 (4.3)	0	0	1 (3.2)	0	0
Gastrointestinal reaction	10 (43.5)	2 (8.7)	0	10 (32.3)	2 (6.5)	0
Stomatitis	3 (13.0)	0	0	2 (6.5)	2 (6.5)	0
Hepatic damage	3 (13.0)	0	0	2 (6.5)	0	0
Hyperbilirubinemia	5 (21.7)	1 (4.3)	0	6 (19.4)	0	0

Discussion

In the past three decades, regional chemotherapy had been viewed as a valid therapeutic option for patients with unresectable liver metastases. However, HAIC seemed less interesting since the application of new systemic agents, such as oxaliplatin or irinotecan, made up more effective systemic chemotherapy protocols. In Phase III study, by combining irinotecan or oxaliplatin with 5-FU/LV in first-line treatment, the ORR rose from 31% to 62%, the median PFS extended from 6.9 months to 8.7 months, and the median OS enhanced from 14.0 months to 21.5 months [25–27]. Both HAIC and SYC methods had advantages and disadvantages in treating colorectal unresectable liver metastases, as for HAIC was superior in local response rate and SYC advantaged in reducing the rate of distant metastasis. Therefore, combining HAIC and SYC with active systemic chemotherapy may be an interesting approach in selected cases. In this study, we tried to determine the efficacy of HAIC combined with SYC in two different patient characteristics arms.

Comparing the results of HAIC + SYC used in two different phases in patients with non-respectable hepatic metastases from CRC indicated that HAIC + SYC as the first-line chemotherapy (Group 1) prolonged the PFS, as well as H-PFS, which were linked to a greater likelihood of disease control rate in the liver. The success of PFS and H-PFS were possibly due to the prolongation of the duration of responses in the liver. Previous research had shown that using systemic irinotecan combined with HAIC FUDR/Dex therapy, the 2-year and 5-year survivals were 87% and 60%, respectively [28]. However, no significant difference about OS was found in this retrospective analysis, although the OS was slightly improved in Group 1. The OS differences were not statistically significant may attribute to the small sample sizes of these two groups. Better result in terms of OS needs to collect more cases in the future with further study.

References

1. Jemal A, Bray F, Center MM, *et al.* Global cancer statistics. CA Cancer J Clin, 2011, 61: 69–90.
2. DeSantis C, Naishadham D, Jemal A. Cancer statistics for African Americans, 2013. CA Cancer J Clin, 2013, 63: 151–166.
3. Boyle P, Ferlay J. Mortality and survival in breast and colorectal cancer. Nat Clin Pract Oncol, 2005, 2: 424–425.
4. Jemal A, Clegg LX, Ward E, *et al*. Annual report to the nation on the status of cancer, 1975–2001, with a special feature regarding survival. Cancer, 2004, 101: 3–27.
5. Howlader N, Noone AM, Krapcho M, *et al*. SEER cancer statistics review, 1975–2010. Bethesda: National Cancer Institute. 2013.
6. Borner MM. Neoadjuvant chemotherapy for unresectable liver metastases of colorectal cancer – too good to be true? Ann Oncol, 1999, 10: 623–626.
7. Scheele J. Hepatectomy for liver metastases. Br J Surg, 1993, 80: 274–276.
8. Jarnagin WR, Conlon K, Bodniewicz J, *et al*. A clinical scoring system predicts the yield of diagnostic laparoscopy in patients with potentially resectable hepatic colorectal metastases. Cancer, 2001, 91: 1121–1128.
9. Ensminger WD, Rosowsky A, Raso V, *et al*. A clinical-pharmacological evaluation of hepatic arterial infusions of 5-fluoro-2'-deoxyuridine and 5-fluorouracil. Cancer Res, 1978, 38: 3784–3792.
10. Ackerman NB, Lien WM, Kondi ES, *et al.* The blood supply of experimental liver metastases. I. The distribution of hepatic artery and portal vein blood to "small" and "large" tumors. Surgery, 1969, 66: 1067–1072.
11. Breedis C, Young G. The blood supply of neoplasms in the liver. Am J Pathol, 1954, 30: 969–977.
12. Ackerman NB. The blood supply of experimental liver metastases. IV. Changes in vascularity with increasing tumor growth. Surgery, 1974, 75: 589–596.
13. Ensminger WD, Gyves JW. Clinical pharmacology of hepatic arterial chemotherapy. Semin Oncol, 1983, 10: 176–182.
14. Boublil JL, Milano G, Khater R, *et al*. Continuous 5-day regional chemotherapy by 5-fluorouracil in colon carcinoma: pharmacokinetic evaluation. Br J Cancer, 1985, 52: 15–20.
15. Cohen AM, Kemeny NE, Köhne CH, *et al.* Is intra-arterial chemotherapy worthwhile in the treatment of patients with unresectable hepatic colorectal cancer metastases? Eur J Cancer, 1996, 32A: 2195–2205.
16. Barber FD, Mavligit G, Kurzrock R. Hepatic arterial infusion chemotherapy for metastatic colorectal cancer: a concise overview. Cancer Treat Rev, 2004, 30: 425–436.
17. Link KH, Kornmann M, Formentini A, *et al*. Regional chemotherapy of non-resectable liver metastases from colorectal cancer – literature and institutional review. Langenbecks Arch Surg, 1999, 384: 344–353.
18. Kemeny NE, Niedzwiecki D, Hollis DR, *et al*. Hepatic arterial infusion versus systemic therapy for hepatic metastases from colorectal can-

cer: a randomized trial of efficacy, quality of life, and molecular markers (CALGB 9481). J Clin Oncol, 2006, 24: 1395–1403.
19. Mathur P, Allen-Mersh TG. Hepatic arterial chemotherapy for colorectal liver metastases. Hepatogastroenterology, 2001, 48: 317–319.
20. Collins JM. Pharmacologic rationale for regional drug delivery. J Clin Oncol, 1984, 2: 498–504.
21. Chan R, Kerr D. Hepatic arterial chemotherapy for colorectal cancer liver metastases: a review of advances in 2003. Curr Opin Oncol, 2004, 16: 378–384.
22. Cohen AD, Kemeny NE. An update on hepatic arterial infusion chemotherapy for colorectal cancer. Oncologist, 2003, 8: 553–566.
23. Skitzki JJ, Chang AE. Hepatic artery chemotherapy for colorectal liver metastases: technical considerations and review of clinical trials. Surg Oncol, 2002, 11: 123–135.
24. Melichar B, Voboril Z, Cerman J Jr, *et al*. Survival of patients with colorectal cancer liver metastases treated by regional chemotherapy. Hepatogastroenterology, 2006, 53: 426–434.
25. Goldberg RM, Sargent DJ, Morton RF, *et al*. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol, 2004, 22: 23–30.
26. Colucci G, Gebbia V, Paoletti G, *et al*. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. J Clin Oncol, 2005, 23: 4866–4875.
27. Köhne CH, van Cutsem E, Wils J, *et al*. Phase III study of weekly high-dose infusional fluorouracil plus folinic acid with or without irinotecan in patients with metastatic colorectal cancer: European Organisation for Research and Treatment of Cancer Gastrointestinal Group Study 40986. J Clin Oncol, 2005, 23: 4856–4865.
28. Kemeny N, Jarnagin W, Gonen M, *et al*. Phase I/II study of hepatic arterial therapy with floxuridine and dexamethasone in combination with intravenous irinotecan as adjuvant treatment after resection of hepatic metastases from colorectal cancer. J Clin Oncol, 2003, 21: 3303–3309.