Radiology of colorectal cancer with emphasis on imaging of liver metastases

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Isolated Hepatic Perfusion with High-Dose Melphalan for the Treatment of Colorectal Metastasis Confined to the Liver

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ABSTRACT

Background: Isolated hepatic perfusion (IHP) involves complete vascular isolation of the liver to allow treatment with doses that would be toxic if delivered systemically. A phase II study of IHP in patients with colorectal metastases confined to the liver was performed.

Methods: Seventy-three patients with irresectable colorectal metastases underwent IHP with high-dose melphalan (200 mg) for one hour. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria and tumor response was assessed according to World Health Organization criteria.

Results: Seventy-one patients were perfused according to the protocol. Four patients died within 30 days after IHP, resulting in an operative mortality rate of 5.6 percent. Sixteen patients (22.5 percent) experienced grade 3-4 hepatotoxicity one week after IHP, which was transient and resolved within three months in all patients. The tumor response rate (complete or partial remission) was 59 percent. Median time to progression was 7.7 (range 2.3-31.4) months. Overall median survival after IHP was 28.8 months with a 3-year survival rate of 37 percent.

Conclusion: IHP for irresectable colorectal metastases confined to the liver resulted in good response rates and long-term survival in a selected group of patients.

INTRODUCTION

Approximately half of patients with colorectal carcinoma will eventually develop liver metastases. When these metastases are confined to the liver, resection is the treatment of choice, resulting in a median survival of between 32 and 46 months [1-5]. Curative surgery is, however, only possible in a minority of these patients. In most the number, location or size of the metastases precludes curative resection.

Isolated hepatic perfusion (IHP) involves complete vascular isolation of the liver. As systemic toxicity is dose limiting for most cytostatic compounds, IHP allows the use of high drug doses that would cause fatal complications if delivered systemically. Furthermore, effective antitumor agents that cannot be administered systemically because of their toxicity, such as tumor necrosis factor (TNF), can be used in IHP [6,7].

The technique with many variations, levels of isolation and drug doses has been used in studies with 5-fluorouracil (5-FU) [8,9], mitomycin C [10,11], cisplatin [9] and melphalan with or without TNF [6,7,9,11-14]. Recent clinical studies have focused mainly on IHP with melphalan. Alexander et al [6] reported IHP with different treatment strategies, including perfusion with high-dose melphalan alone and moderately high doses of melphalan combined with TNF or followed by monthly hepatic intra-arterial infusion of fluorodeoxyuridine (FUDR) and leucovorin; response rates of up to 74 percent were achieved [7,12].

A phase I study of IHP with melphalan alone was performed in this institution to determine the maximum tolerated dose of melphalan for a subsequent phase II study [13]. The study included 24 patients with colorectal metastases confined to the liver who were treated with doses of melphalan from 0.5 to 4.0 mg/kg. The maximum tolerated dose was approximately 3.0 mg/kg, which allowed high melphalan concentrations at the target site without serious hepatotoxicity. A fixed total dose of 200 mg melphalan, equivalent to approximately 3.0 mg/kg, was chosen for the present study.

PATIENTS AND METHODS

Between October 1994 and April 2001, 73 patients with colorectal liver metastases underwent IHP. All were ineligible for surgical resection because of diffuse disease or a tumor site prohibiting surgical resection. Twenty patients had undergone adjuvant treatment with 5-FU plus leucovorin after resection of the primary colorectal tumor. Twenty-seven patients (37.0

percent) had progressive disease after previous treatment for liver metastases, including resection (one) and systemic chemotherapy (26). The median interval from diagnosis of liver metastases to IHP was four months (range, 1-35 months).

Eligibility criteria for IHP included a World Health Organization performance status of 0 or 1, leukocyte count 3.0×10^9/l or more, platelet count 100.0×10^9/l or more, serum creatinine concentration less than 135 µmol/l, bilirubin level less than 17 µmol/l, albumin concentration above 40 g/l and no coagulation disorder. Exclusion criteria were age over 70 years, life expectancy of less than four months, more than 60 percent hepatic replacement by tumor tissue, and evidence of extrahepatic disease. The interval between resection of the primary colorectal tumor and IHP had to be at least one month. All patients underwent preoperative chest and abdominal computed tomography (CT), full blood count, liver function tests, and determination of lactate dehydrogenase, creatinine and carcinoembryonic antigen (CEA) concentrations. Informed consent was obtained from all patients before participation in this study. Local medical ethics committee approval was obtained.

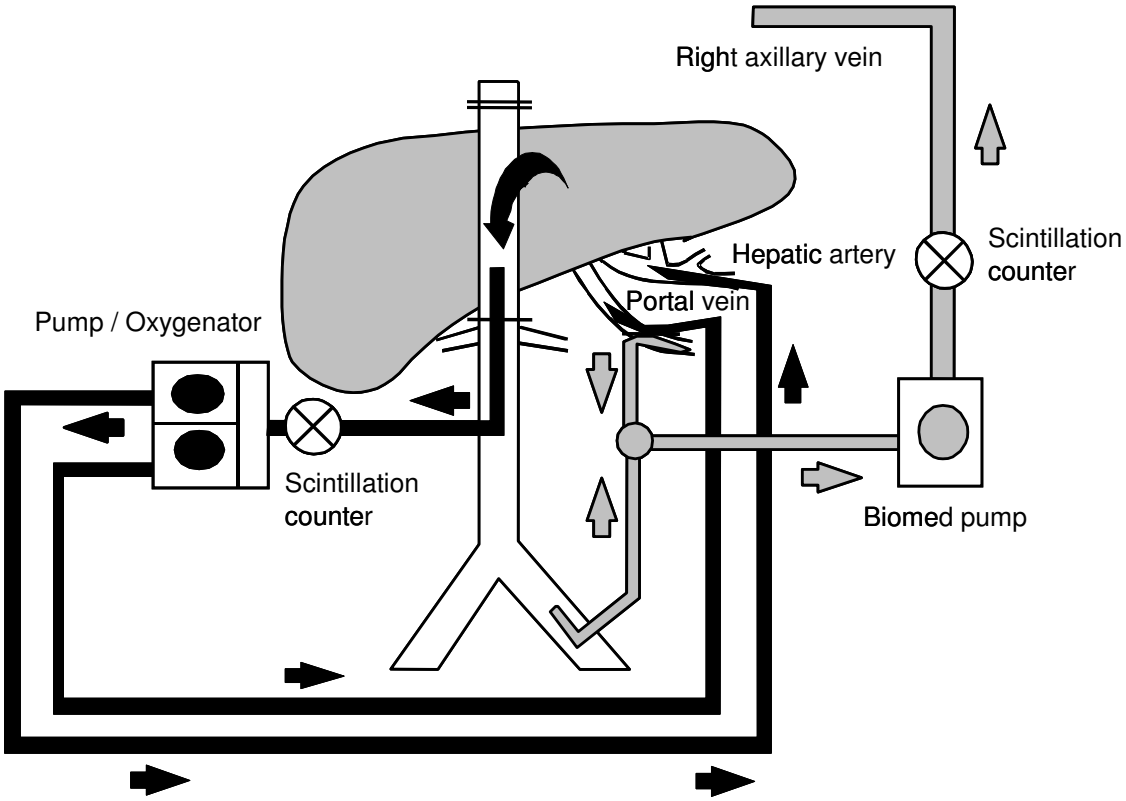


Figure 1. Isolated hepatic perfusion circuit with extracorporeal venovenous bypass.

All patients were treated with IHP by means of extracorporeal venovenous bypass, as described previously [13]. The liver was mobilized from the diaphragm through a transverse abdominal incision. The common hepatic artery (8-Fr 77008 one-piece pediatric arterial cannula; Medtronic, Minneapolis, Mn., USA) and the portal vein (12-Fr perfex perfusion catheter CH12; B. Braun Medical, Oss, The Netherlands) were cannulated and connected to a heart-lung machine (Cobe VPCML oxygenator; Cobe Cardiovascular, Arvada, Co., USA) which consisted of two independent roller pumps (model 10-30-00; Cobe/Stöckert, Munich, Germany). The inferior vena cava (IVC) was clamped above the hepatic veins just below the diaphragm and cannulated just above the renal veins (36-Fr straight; Polystan A/S, Värlöse, Denmark) to allow undisturbed blood flow from the hepatic veins via the IVC to the heart-lung machine. To isolate the hepatic circuit, tourniquets were secured around the hepatic artery, portal vein and IVC.

For the extracorporeal venovenous bypass, the right femoral vein (22-Fr cannula DIITF022L; Edwards Lifesciences, Irvine, Ca., USA) and the portal vein (17-Fr perfex perfusion catheter CH17; B. Braun Medical, Oss, The Netherlands) (proximal to the tourniquet) were cannulated and connected to the right axillary vein (18-Fr 7326 perfusion cannula; Lifestream International, The Woodlands, Tx., USA). The venovenous bypass was supported by a centrifugal pump (BIO-Medicus; Medtronic, Eden Prairie, Mn., USA) and primed with 700 ml 0.9 percent saline (Figure 1).

The perfusion medium consisted of intrahepatically trapped blood and 1250 ml Gelofusine® (Vifor Medical, Sempach, Switzerland) plus 2500 units heparin (Leo Pharma, Breda, The Netherlands) to yield a final volume of approximately 2 liters.

Leakage of perfusate into the systemic circuit was monitored by adding 10 MBq ^{99m}Tc-pertechnetate to the isolated circuit with subsequent measurement of the level of radioactivity in both the systemic and isolated circuit, as described previously [10,15]. If no leakage was detected, melphalan was administered; however, if leakage exceeded 10 percent during the perfusion period, the procedure was immediately aborted and the liver flushed.

Melphalan (Alkeran®; GlaxoSmithKline, Zeist, The Netherlands) (200 mg) was first dissolved in 40 ml Wellcome Diluent (a 60/40 (v/v) mixture of propylene glycol containing 5.2 percent (v/v) ethanol and 0.068 mol/l sodium citrate), which was subsequently diluted with 60 ml sterile saline. The melphalan was administered as a bolus in the isolated hepatic circuit. Throughout the one hour perfusion interval, the perfusate was kept at a temperature of

39.5ºC by a heat exchanger (Avecor Cardiovascular, Plymouth, Minnesota, USA) and oxygenated using the heart-lung machine. After perfusion, the liver was flushed for approximately 10 minutes with 3 liters Gelofusine®. All cannulas and clamps were removed, and the incisions were closed. To prevent possible postoperative cholecystitis, cholecystectomy was performed.

All patients received a daily subcutaneous dose of 480 µg granulocyte colony-stimulating factor (G-CSF) (Filgrastim/Neupogen®; Amgen, Breda, The Netherlands) starting the day after the operation until the nadir in leukocyte count was reached and the count had risen to more than 1.0×10^9/l. Patients were monitored in the intensive care unit for at least two days after IHP. Liver and renal function tests and full blood counts were carried out daily in the first week. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria.

Tumor response was evaluated by serial CEA measurements and abdominal CT at 3-month intervals. CT was performed using a helical scanner (SR7000 or AVE; Philips Medical System, Best, The Netherlands). Nonenhanced and contrast-enhanced (150 ml non-ionic agent, 350 mg iodine per ml, using power injection at 3 ml/s via the antecubital vein, fixed delay 60 s) images of the liver were obtained. After scanning the liver, the remainder of the abdomen was scanned. A complete tumor response was defined as the disappearance of all known disease and a partial response as a reduction in the sum of the greatest perpendicular diameters of all measurable metastases of at least 50 percent. The disease was considered to be stable if the sum of the diameters was reduced by less than 50 percent or increased by less than 25 percent, and progressive if it increased by 25 percent or more, or new hepatic or extrahepatic lesions appeared [16]. Metastases were localized according to the Bismuth classification [17]. The quadrate lobe (anterior part of segment IV) was denoted as a separate segment, bringing the total number of segments to nine.

STATISTICAL ANALYSIS

All data were analyzed with SPSS® for Windows version 10.0 statistical software (SPSS; Chicago, Ill., USA). Univariate analyses of time to progression and survival were carried out by the Kaplan-Meier method and curves were compared by means of the log rank test. Two-sided $P \leq .050$ was considered statistically significant.

RESULTS

PATIENT AND TREATMENT CHARACTERISTICS

Characteristics of the 73 patients treated with IHP and tumor details are listed in Table 1.

Table 1. Patient and Tumor Characteristics.

Sex ratio (F:M)	17:56
Mean age (years)[#]	54 (36-70)
Liver metastases[†]	
Synchronous	47 (64.4)
Metachronous	26 (35.6)
Median hepatic replacement (%)[#]	20 (5-60)
Median no. of liver segments involved[#]	7 (1-9)
Pretreatment CEA level[†]	
Normal (≤ 3.0 μg/ml)	8 (11.0)
Elevated (> 3.0 μg/ml)	65 (89.0)

Values in parentheses are [#]ranges or [†]percentages. CEA = carcinoembryonic antigen.

Seventy-one of the 73 patients underwent IHP. The procedure failed in two patients owing to immediate leakage and they were excluded from further analysis. Treatment parameters for the remaining 71 patients are listed in Table 2.

Table 2. Isolated Hepatic Perfusion Parameters in 71 Patients.

Flow rate (ml/min)		
Hepatic artery[*]	371 ± 96	(120-530)
Portal vein	327 ± 94	(100-540)
Total	657 ± 160	(260-955)
Pressure (mmHg)		
Hepatic artery	111 ± 32	(40-220)
Portal vein	31 ± 10	(5-55)
Leakage (%)	1.6 ± 2.6	(0-10)

Values are mean ± standard deviation with range in parentheses.
[*] Seven patients were not perfused through the hepatic artery.

In seven patients the liver could not be perfused via the hepatic artery for the following reasons: aberrant hepatic arterial anatomy (two), small caliber of the hepatic artery (two), insufficient hepatic arterial flow (two) and hepatic artery hemorrhage (one). IHP was stopped prematurely in eight patients because the maximum tolerated leakage of 10 percent from the isolated circuit to the systemic circulation was reached. Median operating time, including the 1-hour perfusion, was 8.5 hours (range, 5.8-14.7 hours). Blood and fluid loss during the procedure ranged from 0.9 to 10 liters. The median duration of stay in the intensive care unit was three days (range, 2-28 days) and the median hospital stay was 12 days (range, 9-36 days).

TOXICITY AND COMPLICATIONS

Despite the limited leakage from the isolated circuit to the systemic circulation and the postoperative administration of G-CSF, eight patients (11.3 percent) developed grade 3-4 leucopenia (Table 3). The nadir occurred at a median of eight days after IHP.

Table 3. Toxicity According to National Cancer Institute Common Toxicity Criteria in 71 Patients who had Isolated Hepatic Perfusion.

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Leukocyte (nadir)	43 (60.6)	16 (22.5)	4 (5.6)	5 (7.0)	3 (4.2)
Bilirubin	32 (45.1)	22 (31.0)	5 (7.0)	8 (11.3)	4 (5.6)
Alkaline phosphatase	6 (8.6)	38 (52.1)	25 (35.2)	3 (4.2)	0 (0.0)
ALT	26 (38.0)	34 (47.9)	8 (12.7)	1 (1.4)	1 (1.4)
AST	17 (23.9)	27 (38.0)	18 (25.4)	8 (11.3)	1 (1.4)

Values in parentheses are percentages.
ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Sixteen patients (22.5 percent) experienced grade 3-4 toxicity of one or more liver enzymes one week after IHP (Table 3). This hepatotoxicity was transient and resolved within three months. Four patients (5.6 percent) died within 30 days after IHP, from splenic rupture (two) and hepatic artery obstruction (two) and hemorrhage. All four patients had further surgery but eventually developed multiple organ failure. Complications are listed in Table 4. Veno-occlusive disease occurred in four patients, but was never fatal.

Table 4. Major and Minor Complications.

Major Complications		Minor Complications	
Reoperation	9	Infection	11
Bleeding	7	Atrial fibrillation	2
Abscess	1	Incisional hernia	2
Bowel obstruction	1	Pneumothorax	2
Multiple organ failure	4	Drainage of pleural effusion	2
Veno-occlusive disease	4	Diabetes insipidus	1
Rupture of the spleen	3*	Inguinal seroma	1
Hepatic artery obstruction	2	Choledochal duct damage	1
Sepsis	2		
Right renal vein lesion	1†		
Ventricular fibrillation	1#		

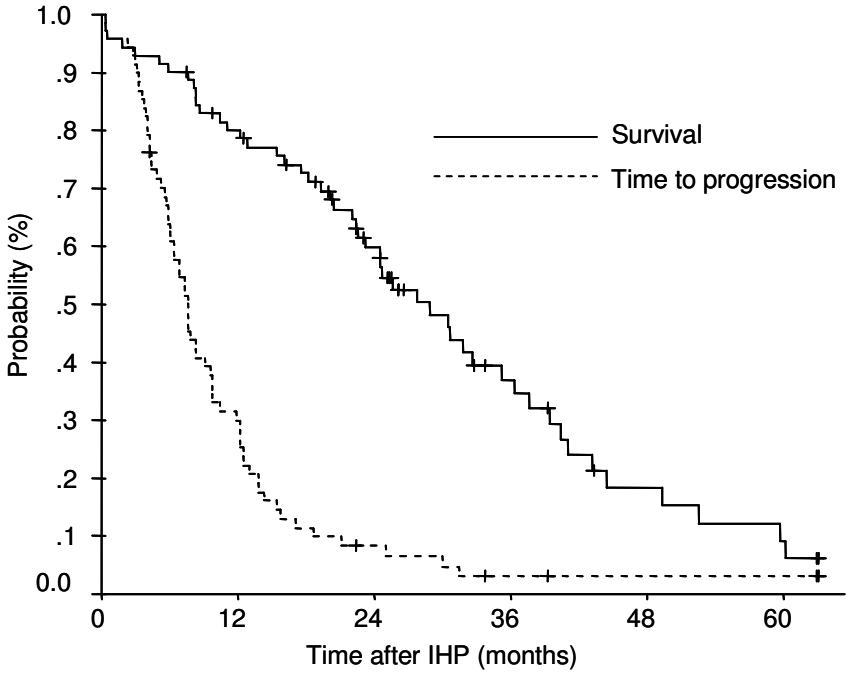
Required *splenectomy, †nephrectomy or #resuscitation.

TUMOR RESPONSE

Eighty-four percent of the patients with a raised preoperative CEA level had a normal level or a reduction of 50 percent or more 1-3 months after IHP. One patient died before the first follow-up scan. As four patients had already died after operation, 66 patients were eligible for measurement of tumor response by CT. The overall response rate (complete or partial remission) was 59 percent, including three complete remissions. If IHP was performed through the portal vein only, the response rate was 33 percent, compared with 62 percent when both the hepatic artery and portal vein were perfused.

PROGRESSION AND SURVIVAL

The time to progression and survival curves are shown in Figure 2. Median time to progression was 7.7 months (range, 2.3-31.4 months). Separate analysis of patients perfused through both the hepatic artery and portal vein, and those perfused through the portal vein only, showed a significant difference in time to progression (7.7 months versus 3.6 months; $P = .033$). Progression of the disease occurred in 61 (92.4 percent) of the 66 patients, of whom 46 (75.4 percent) had hepatic, ten (16.4 percent) had extrahepatic, and five (8.2 percent) had both hepatic and extrahepatic progression. In retrospect, one patient who did not have



No. at risk						
Survival	71	55	35	15	6	3
Time to progression	66*	22	7	3	3	2

Figure 2. Kaplan-Meier curves for overall survival and time to progression after isolated hepatic perfusion with high-dose melphalan. *Five patients died before progression of the disease.

progressive hepatic metastases had extrahepatic disease before operation. Forty patients with progressive disease received further treatment, including systemic chemotherapy (37), local ablative therapy (seven) and resection (four). Five patients remained free from tumor progression, two of whom had been followed up for more than five years.

The median survival after IHP was 28.8 months with a 3-year survival rate of 37 percent (Table 5). So far three patients have survived for more than five years, resulting in a calculated 5-year survival rate of nine percent. When perioperative deaths were excluded, median overall survival increased to 30.4 months. Fifteen patients survived more than 36 months, of whom nine received additional treatment and six did not, including two patients who were still alive five years after treatment.

There was a significant difference in survival between patients who were perfused through both the hepatic artery and portal vein (32.7 months) and those who received IHP through the portal vein only (8.6 months) ($P < .001$). The median survival of patients who had previous chemotherapy for liver metastases was lower than that of patients who had not

Table 5. Survival after Surgical Isolated Hepatic Perfusion.

		Operative Deaths Included		Four Operative Deaths Excluded	
	No. of Patients	Median Surv. (months)	3-year Surv. (%)	Median Surv. (months)	3-year Surv. (%)
Overall	71	28.8	37	30.4	40
Perfusion					
Hepatic artery and portal vein	64	30.7	42	32.7	44
Portal vein only	7	8.6[#]	0	8.6[#]	0
Previous systemic chemotherapy					
No	41	31.7	41	35.3	47
Yes	30	24.5	23	24.6	24

Univariate analysis for survival was carried out by the Kaplan-Meier method and log-rank test was used for comparison of the Kaplan-Meier curves. A two-sided *P* value of .05 or less was considered to indicate statistical significance. Surv. = survival.
[#] $P < .001$ versus hepatic artery and portal vein.

received chemotherapy (24.6 and 35.3 months, respectively), although this was not significant ($P = .108$) (Table 5). Separate survival analysis of patients who were younger (n = 35; median age 48 years) and older (n = 36; median age 62 years) than the median age of 54 years at time of IHP showed no difference in median survival (27.7 and 35.3 months, respectively; $P = .900$).

DISCUSSION

This study demonstrated that IHP for irresectable metastases confined to the liver produced a considerable survival benefit. An overall response rate of 59 percent was achieved, which is higher than that in most studies involving systemic chemotherapy and comparable to the rate for hepatic artery infusion. Overall time to progression after IHP was 7.7 months, which is comparable to other current chemotherapeutic strategies [18-22].

The median survival of 28.8 months and 3-year survival rate of approximately 40 percent is better than after conventional chemotherapeutic treatments for irresectable liver metastases. Standard systemic treatment with 5-FU and leucovorin results in a median survival of 10-14 months [23,24]. Newer agents such as irinotecan combined with 5-FU/leucovorin increased

survival up to 17 months [25,26], and a combination of 5-FU/leucovorin and oxaliplatin produced an increased progression-free survival, but without an overall survival benefit [27,28]. Preliminary studies involving 5-FU/leucovorin, irinotecan and oxaliplatin have shown a median survival of up to 21 months, irrespective of the treatment sequence [29,30].

Randomized studies have indicated that selective administration of FUDR and 5-FU, delivered by continuous infusion into the hepatic artery, leads to significantly better tumor response rates than systemically administered chemotherapy, although with only a limited increase in survival [31,32]. A recent phase I study reported a response rate of 74 percent after concurrent treatment with systemic irinotecan and continuous hepatic artery infusion of FUDR and dexamethasone, but data on survival were not reported [18].

Several studies of IHP have been conducted over the past 15 years, but survival data are limited. Bartlett et al [12] reported a response rate of 74 percent, a median time to progression of 14.5 months and a median survival of 27 months after IHP with 1.5 mg/kg melphalan followed by monthly hepatic intra-arterial infusion of FUDR and leucovorin in 19 patients, confirming that IHP is effective in the treatment of irresectable colorectal liver metastases. The median survival in the present series can be explained partly by the marked total reduction in tumor load in a substantial number of patients, which allowed further therapy to be offered, such as resection or local ablative therapy. In addition, the majority of patients with progressive disease after IHP were still eligible for treatment with first-, second- and third-line systemic chemotherapy, as they had not received previous systemic chemotherapy. As six of the 15 patients who survived more than three years did not receive additional therapy, including two patients who were still alive after five years, the survival benefit appears to be mainly related to IHP.

However, it should be noted that the patients treated by IHP might not have been representative of all patients with irresectable colorectal metastases confined to liver. In contrast to patients treated with systemic chemotherapy, those eligible for IHP are more thoroughly screened for extrahepatic metastases and positive lymph nodes.

As liver tumors are mainly vascularized by the hepatic artery [33], perfusion through the hepatic artery is essential for successful IHP and improved the median survival in this subset to more than 32 months. Better preoperative assessment of vascular status (abnormal hepatic artery anatomy, small caliber of the hepatic artery, arteriosclerosis) by magnetic resonance angiography, CT or angiography should identify patients in whom hepatic artery perfusion is

not technically feasible.

Toxicity was transient in all patients, except for the procedure-related deaths. The previously determined maximum tolerated dose of melphalan was well tolerated [13]. The major disadvantage of IHP is that it is technically difficult and comparable to hepatic resection in terms of hospital stay [2]. The associated morbidity and mortality was considerable, and IHP should therefore be used only in specialized centers in the context of appropriate trials. Recently developed techniques in which variations in drug response can be mapped by gene expression analysis (pharmacogenomics) [34,35] might lead to better patient selection, and the development of minimal access techniques for IHP might result in a procedure that is less demanding and more amenable to repetition.

ACKNOWLEDGEMENTS

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REFERENCES

1. Jamison RL, Donohue JH, Nagorney DM, Rosen CB, Harmsen WS, Ilstrup DM. Hepatic resection for metastatic colorectal cancer results in cure for some patients. Arch Surg 1997; 132:505-510.
2. Fong Y, Cohen AM, Fortner JG, et al. Liver resection for colorectal metastases. J Clin Oncol 1997; 15:938-946.
3. Figueras J, Valls C, Rafecas A, Fabregat J, Ramos E, Jaurrieta E. Resection rate and effect of postoperative chemotherapy on survival after surgery for colorectal liver metastases. Br J Surg 2001; 88:980-985.
4. Buell JF, Rosen S, Yoshida A, et al. Hepatic resection: effective treatment for primary and secondary tumors. Surgery 2000; 128:686-693.
5. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg 1999; 230:309-318.
6. Alexander HR, Libutti SK, Bartlett DL, Puhlmann M, Fraker DL, Bachenheimer LC. A phase I-II study of isolated hepatic perfusion using melphalan with or without tumor necrosis factor for patients with ocular melanoma metastatic to liver. Clin Cancer Res 2000; 6:3062-3070.

7. Alexander HR, Bartlett DL, Libutti SK, Fraker DL, Moser T, Rosenberg SA. Isolated hepatic perfusion with tumor necrosis factor and melphalan for unresectable cancers confined to the liver. J Clin Oncol 1998; 16:1479-1489.
8. Aigner KR, Walther H, Link KH. Isolated liver perfusion with MMC/5-FU: surgical technique, pharmacokinetics, clinical results. Contr Oncol 1988; 29:229-246.
9. Hafstrom LR, Holmberg SB, Naredi PL, et al. Isolated hyperthermic liver perfusion with chemotherapy for liver malignancy. Surg Oncol 1994; 3:103-108.
10. Marinelli A, de Brauw LM, Beerman H, et al. Isolated liver perfusion with mitomycin C in the treatment of colorectal cancer metastases confined to the liver. Jpn J Clin Oncol 1996; 26:341-350.
11. Oldhafer KJ, Lang H, Frerker M, et al. First experience and technical aspects of isolated liver perfusion for extensive liver metastasis. Surgery 1998; 123:622-631.
12. Bartlett DL, Libutti SK, Figg WD, Fraker DL, Alexander HR. Isolated hepatic perfusion for unresectable hepatic metastases from colorectal cancer. Surgery 2001; 129:176-187.
13. Vahrmeijer AL, van Dierendonck JH, Keizer HJ, et al. Increased local cytostatic drug exposure by isolated hepatic perfusion: a phase I clinical and pharmacologic evaluation of treatment with high dose melphalan in patients with colorectal cancer confined to the liver. Br J Cancer 2000; 82:1539-1546.
14. de Vries MR, Rinkes IH, van de Velde CJ, et al. Isolated hepatic perfusion with tumor necrosis factor alpha and melphalan: experimental studies in pigs and phase I data from humans. Recent Results Cancer Res 1998; 147:107-119.
15. Runia RD, de Brauw LM, Kothuis BJ, Pauwels EK, van de Velde CJ. Continuous measurement of leakage during isolated liver perfusion with a radiotracer. Int J Rad Appl Instrum B 1987; 14:113-118.
16. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47:207-214.
17. Bismuth H. Surgical anatomy and anatomical surgery of the liver. World J Surg 1982; 6:3-9.
18. Kemeny N, Gonen M, Sullivan D, et al. Phase I study of hepatic arterial infusion of floxuridine and dexamethasone with systemic irinotecan for unresectable hepatic metastases from colorectal cancer. J Clin Oncol 2001; 19:2687-2695.
19. Link KH, Sunelaitis E, Kornmann M, et al. Regional chemotherapy of nonresectable colorectal liver metastases with mitoxantrone, 5-fluorouracil, folinic acid, and mitomycin C may prolong survival. Cancer 2001; 92:2746-2753.

20. O'Connell MJ, Nagorney DM, Bernath AM, et al. Sequential intrahepatic fluorodeoxyuridine and systemic fluorouracil plus leucovorin for the treatment of metastatic colorectal cancer confined to the liver. J Clin Oncol 1998; 16:2528-2533.
21. Rougier P, Laplanche A, Huguier M, et al. Hepatic arterial infusion of floxuridine in patients with liver metastases from colorectal carcinoma: long-term results of a prospective randomized trial. J Clin Oncol 1992; 10:1112-1118.
22. Lorenz M, Muller HH. Randomized, multicenter trial of fluorouracil plus leucovorin administered either via hepatic arterial or intravenous infusion versus fluorodeoxyuridine administered via hepatic arterial infusion in patients with nonresectable liver metastases from colorectal carcinoma. J Clin Oncol 2000; 18:243-254.
23. de Gramont A, Bosset JF, Milan C, et al. Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. J Clin Oncol 1997; 15:808-815.
24. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Cancer Meta-Analysis Project. J Clin Oncol 1992; 10:896-903.
25. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000; 355:1041-1047.
26. Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 2000; 343:905-914.
27. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000; 18:2938-2947.
28. Giacchetti S, Perpoint B, Zidani R, et al. Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. J Clin Oncol 2000; 18:136-147.
29. Achille E, Tournigand C, Andre T, et al. Folfiri then Folfox, or Folfox then Folfiri in metastatic colorectal cancer (MCRC): results of a phase III trial. Eur J Cancer 2001; 37 (Suppl 6): S289 (Abstract).
30. Tournigand C, Louvet C, Quinaux E, et al. Folfiri followed by Folfox versus Folfox followed by Folfiri in metastatic colorectal cancer (MCRC): final results of a phase III trial. Proc ASCO 2001; Abstract 494.

31. Harmantas A, Rotstein LE, Langer B. Regional versus systemic chemotherapy in the treatment of colorectal carcinoma metastatic to the liver. Is there a survival difference? Meta- analysis of the published literature. Cancer 1996; 78:1639-1645.
32. Meta-Analysis Group in Cancer. Reappraisal of hepatic arterial infusion in the treatment of nonresectable liver metastases from colorectal cancer. J Natl Cancer Inst 1996; 88:252-258.
33. Sigurdson ER, Ridge JA, Kemeny N, Daly JM. Tumor and liver drug uptake following hepatic artery and portal vein infusion. J Clin Oncol 1987; 5:1836-1840.
34. Ross DT, Scherf U, Eisen MB, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nat Genet 2000; 24:227-235.
35. Scherf U, Ross DT, Waltham M, et al. A gene expression database for the molecular pharmacology of cancer. Nat Genet 2000; 24:236-244.