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Review

Isolated hepatic perfusion for the treatment of colorectal metastases confined to the liver: recent trends and perspectives

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Abstract

Isolated hepatic perfusion (IHP) involves a method of complete vascular isolation of the liver to allow treatment of liver tumours with toxic systemic doses. The recent clinical studies mainly employed IHP with melphalan with or without tumour necrosis factor-alpha (TNF-α) and mild hyperthermia. The results of these studies show that high response rates and high survival rates can be achieved by IHP. In this article, the current status, recent developments and future perspectives of IHP are discussed.

Keywords: Isolated hepatic perfusion; Melphalan; Liver tumours; TNF-α; Hyperthermia; Minimally invasive

1. Introduction

Isolated hepatic perfusion (IHP) involves a method of complete vascular isolation of the liver to allow regional chemotherapeutic treatment of liver tumours. IHP has been proposed as a treatment modality for different kinds of non-resectable liver tumours [1,2], but most experience has been obtained with colorectal liver metastases. During the procedure, the blood circulation of the liver is temporarily isolated from the systemic circulation and the liver is perfused with high-dose chemotherapy via a recirculating perfusion circuit (Fig. 1). Leakage to the systemic circulation is monitored in order to prevent inadvertently high systemic exposure. After perfusion of the liver with the drug for a certain period of time (1 h in most IHP trials), the liver is flushed with 'clean' perfusate to wash out the anticancer drug and the normal vascular anatomy is restored by removing all clamps and catheters.

The major advantage of IHP is the possibility to treat liver tumours with drug levels that would be highly toxic if applied systemically. For instance, up to 4-fold the maximum systemically tolerated dose of melphalan can be administered [3]. Furthermore, antitumour agents that cannot be administered systemically at therapeutical dose levels because of their toxicity, such as tumour necrosis factor alpha (TNF-α), can be used in IHP [4,5]. Finally, hyperthermia, which is known to increase the efficacy of several drugs [6], can be applied by heating the circulating perfusate.

Several drugs have been used in IHP studies, including 5-fluorouracil (5-FU) [1,7], mitomycin C [8,9], cisplatin [1] and melphalan with or without TNF-α [1,3–5,9–12] (Table 1). The recent clinical studies mainly employed IHP with melphalan with or without TNF-α. Melphalan is an alkylating agent with a steep dose–response curve that is effective against colorectal cancer after a relatively short exposure time and, therefore, is a very suitable drug for application in IHP [13,14].

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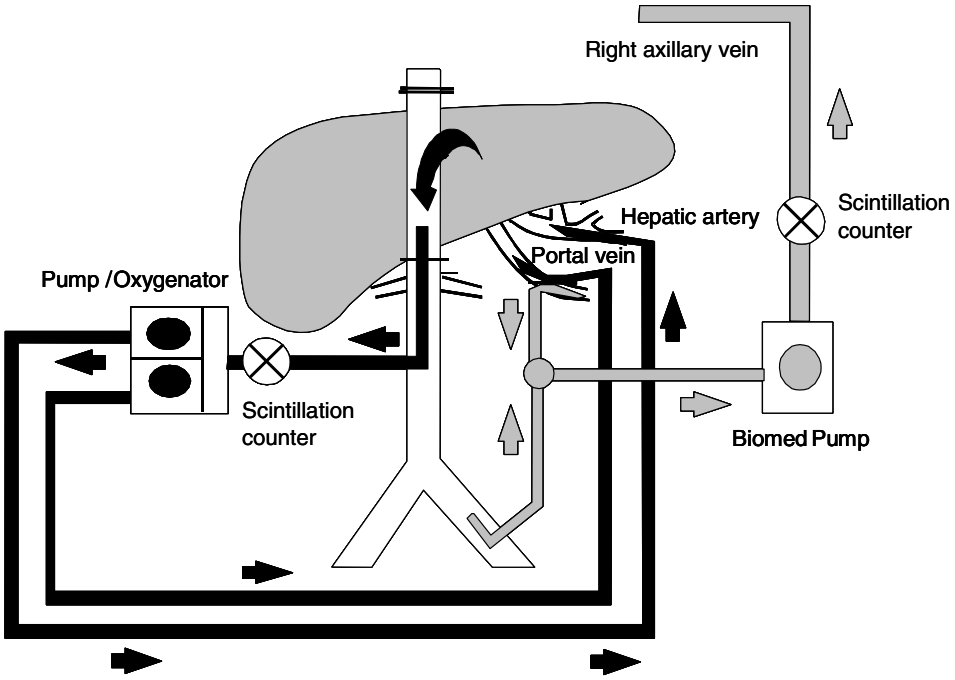

Fig. 1. Isolated hepatic perfusion circuit with extra-corporeal veno-venous bypass.

At time of initiation of most IHP studies in the 1980s and 1990s, the results of systemic chemotherapy for colorectal metastases consisting of 5-FU/leucovorin-based schedules were poor: response rates of only 20% and a median overall survival of approximately 12 months [15,16]. The results of recent studies show that high response rates and considerable survival benefit can be achieved by IHP for non-resectable liver tumours. Alexander and colleagues [4,5,10,12] reported IHP studies with different treatment strategies, including IHP with melphalan alone (1.5–2.5 mg/kg) and melphalan (1.5 mg/kg) combined with TNF-α or followed by monthly hepatic intra-arterial infusion of fluorodeoxyuridine (FUDR) and leucovorin. In these studies, IHP for colorectal liver metastases showed response rates of up to 74%, a median time to progression of up to 14.5 months and a median survival of up to 27 months.

We first determined the maximally tolerated dose of melphalan in IHP. This study, in which 24 patients were treated with doses of melphalan ranging from 0.5 to 4.0 mg/kg, revealed a maximally tolerated dose of 3.0 mg/kg [3]. In a subsequent study, patients with colorectal metastases confined to the liver underwent IHP with a fixed total dose of 200 mg melphalan which is approximately 3.0 mg/kg melphalan [17]. An overall response rate of 59% and a median time to progression of 7.7 months (95% CI:6.5–8.8) was achieved. Median survival in this study was 28.8 months (95% CI:22.5–35.2) with a 3-year survival of 37% and 5-year survival of 9%.

Promising results have also been obtained with IHP for liver metastases from uveal melanoma. Alexander and colleagues [4,12] show a response rate of 62% and a median survival of up to 12 months after IHP for liver metastases from ocular melanoma. We reported a response rate of 50% and an overall median survival of 9.9 months with a 1-year survival of 50% and a 2-year survival of 37.5% after IHP [18].

Thus, IHP results in considerable tumour responses and in high survival rates in a selective group of patients. However, further development of IHP is needed to improve its efficacy and broaden its applicability. The aim of this article was to discuss the current status, recent developments and future perspectives of IHP. There are still many unanswered questions regarding IHP. Should TNF-α and hyperthermia be applied or not? What are the pharmacokinetic consequences of different IHP techniques and can the current set-up of IHP be optimised? Apart from pharmacokinetic and pharmacological developments, also technical developments, such as less invasive, repeatable IHP techniques may increase IHP applicability. Are there new agents available for application in IHP? Finally, the possibility to predict treatment outcome by assessment of patients' tumour characteristics is addressed.

2. Enhancement of melphalan treatment efficacy

2.1. Tumour necrosis factor

Melphalan treatment has been combined with TNF-α in several IHP studies [4,5,9,10,19,20] (Table 1). The TNF-α induced antitumour effect is supposed to be mainly due to the destruction endothelial cells in the

Table 1
Isolated hepatic perfusion studies

Study	Institution	Year	Patients	Primary tumour	Treatment	Drug dose
Aigner and colleagues [2][a]	University of Gießen, Germany	1984	32	Colorectal carcinoma (29)	5-fluorouracil	750–1250 mg
				Miscellaneous (3)		
Skibba and Quebbeman [35]	Medical College of Wisconsin, Milwaukee, USA	1986	8	Colorectal carcinoma (5)	Hyperthermia 42.0–42.5 °C without drug	
				Melanoma (2)		
				Miscellaneous (1)		
Schwemmle and colleagues [93][a]	University of Gießen, Germany	1987	50	Colorectal carcinoma (45)	5-fluorouracil (47)	300–1250 mg
				Miscellaneous (4)	Mitomycin C (17)	5–50 mg
				1 falsely diagnosed	Cisplatin (4)	50 mg
Hafström and colleagues [1]	Sahlgrenska Hospital, Götenborg, Sweden	1994	29	Colorectal carcinoma (4)	Melphalan (all)	0.5 mg/kg
				Melanoma (10)	Cisplatin (20)	0.2–0.7 mg/kg
				Miscellaneous (15)		
Marinelli and colleagues [8]	LUMC, Leiden, The Netherlands	1996	9	Colorectal carcinoma	Mitomycin C	30 mg/m^2
de Vries and colleagues [19]	Erasmus University Rotterdam and LUMC, Leiden, The Netherlands	1997	9	Colorectal carcinoma	Melphalan	1.0 mg
					TNF-α	0.4 mg, 0.8 mg (1)
Alexander and colleagues [5]	NCI, Bethesda, USA	1998	34	Colorectal carcinoma (26)	Melphalan	1.5 mg/kg
				Ocular melanoma (4)	TNF-α	1.0 mg
				Miscellaneous (4)		
Oldhafer and colleagues [9]	Hannover Medical School, Germany	1998	12	Colorectal carcinoma (6)	Melphalan (6)	60–140 mg
				Ocular melanoma (2)	TNF-α (6)	200–300 μg
				Miscellaneous (4)	Mitomycin C (6)	20–50 mg
Lindner and colleagues [20]	Sahlgrenska Hospital, Götenborg, Sweden	1999	11	Colorectal carcinoma (5)	Melphalan	0.5 mg/kg
				Ocular melanoma (2)	TNF-α	30–200 μg
				Miscellaneous (4)		
Vahrmeijer and colleagues [3]	LUMC, Leiden, The Netherlands	2000	24	Colorectal carcinoma	Melphalan	0.5–4.0 mg/kg
Alexander and colleagues [4]	NCI, Bethesda, USA	2000	22	Ocular Melanoma	Melphalan (all)	1.5–2.5 mg/kg
					TNF-α (11)	1.0 mg
Bartlett and colleagues [10][b]	NCI, Bethesda, USA	2001	51	Colorectal carcinoma	Melphalan (all)	1.5 mg/kg
					TNF-α (32)	1.0 mg
Rothbarth and colleagues [17]	LUMC, Leiden, The Netherlands	2003	73	Colorectal carcinoma	Melphalan	200 mg
Alexander and colleagues [12][c]	NCI, Bethesda, USA	2003	29	Ocular melanoma	Melphalan	1.5 mg/kg
Noter and colleagues [18]	LUMC, Leiden, The Netherlands	2004	8	Ocular melanoma	Melphalan	200 mg

Miscellaneous tumours include breast carcinoma, leiomyosarcoma, carcinoid, cholangiocarcinoma, renal cancer, tracheal cancer, hepatocellular carcinoma and hemangiopericytoma.

NCI, National Cancer Institute; USA, United States of America; LUMC, Leden University Medical Centre; TNF-α, tumour necrosis factor-alpha.

[a] Overlapping patient groups, also reported by Aigner and colleagues [7].

[b] 16 patients previously included in the study of Alexander and colleagues [5].

[c] Overlapping patient groups, also reported by Alexander and colleagues [4].

tumour blood vessels [21,22]. In isolated limb perfusion for the treatment of patients with in-transit metastases from malignant melanoma or locally advanced extremity soft tissue sarcomas, the addition of TNF-α to melphalan has shown improved treatment efficacy, both in experimental [23] and clinical studies [24–26]. Therefore, the application of TNF-α in isolated limb perfusion is well accepted.

However, the efficacy of TNF-α in IHP has not been proven in clinical studies. An experimental IHP study with TNF-α in rats showed a synergistic antitumour effect of IHP with melphalan combined with TNF-α in the treatment of soft-tissue sarcoma liver tumours, but little additive antitumour effect in colorectal liver tumours [27]. The IHP studies using melphalan combined with TNF-α [10] do not seem to improve response rates and survival compared with melphalan alone [10,17], while severe hepatotoxicity in the combination with TNF-α has been observed [19]. Certainly, there is a synergistic antitumour effect of combined treatment with melphalan and TNF-α, especially in highly vascularised tumours [21,27]. However, in IHP this effect is probably diminished by the fact that a lower dose of melphalan has to be used when combined with TNF-α: the maximum tolerated dose (MTD) of melphalan alone in IHP is 3.0 mg/kg [3], while the MTD of melphalan in IHP when combined with TNF-α is 1.5 mg/kg [28].

2.2. Hyperthermia

Hyperthermia enhances the cytotoxic effect of several alkylating agents [6]. Probable mechanisms are that hyperthermia increases blood flow, cell membrane permeability and drug uptake [6,29], resulting in increased intracellular drug concentrations and, consequently, increased cytotoxicity. In addition, hyperthermia makes DNA repair less efficient [30] and causes a block in cell cycle progression after treatment with alkylating agents [31].

Enhanced cytotoxicity of melphalan with hyperthermia has been shown by *in vitro* studies [32,33], but evidence from *in vivo* studies is limited. It is somewhat surprising, therefore, that hyperthermic melphalan treatment is being used in clinical studies including IHP: moderate hyperthermia (38.5–40 °C) is applied in most IHP studies [1,5,17,20] for colorectal liver metastases, although there was no evidence from experimental or clinical studies to show that the efficacy of melphalan is actually improved by hyperthermia in colorectal liver tumours. To explore whether the antitumour effect of the melphalan treatment of colorectal metastases is indeed enhanced by hyperthermia, we tested this in a rat colon tumour model (CC531) for liver metastases. Melphalan treatment combined with local hyperthermia (42 °C) showed increased antitumour efficacy when compared with normothermic melphalan treatment (37 °C), while no antitumour effect of hyperthermia alone was observed [34].

These results seem to support the application of local hyperthermia in IHP in patients with colorectal liver metastases during treatment with melphalan. The maximum temperature that can be applied in clinical IHP remains unclear. A study of Skibba and colleagues in which 8 patients were treated by IHP with only hyperthermia of 42–42.5 °C (without drug) for 4 h reports good tumour responses by itself, but considerable hepatotoxicity, which was fatal in 2 patients [35]. Combining melphalan and hyperthermia at these temperatures is expected to cause even more hepatotoxicity. The efficacy/safety profile of moderate hyperthermia (38.5–40 °C) as used in the current IHP studies [1,5,17,20] has not been studied in randomised phase III trials. However, as hepatotoxicity is limited in the current IHP studies, and since enhanced antitumour efficacy of melphalan with hyperthermia has been shown in experimental studies on tumours in other organs [36,37], application of moderate hyperthermia in IHP for colorectal liver metastases seems justified. As we showed that a high melphalan antitumour efficacy can be reached with a hyperthermic temperature of 42 °C [34], further experimental and clinical research on the antitumour efficacy and safety of IHP with hyperthermia in the temperature range of 40–42 °C should be considered.

3. Pharmacokinetic improvement of treatment efficacy

3.1. Melphalan administration

Different procedures of drug administration during IHP have been used: a bolus or a continuous infusion, to the whole perfusate or in the hepatic artery (HA). The pharmacokinetic consequences of these modes of administration are expected to affect antitumour efficacy and hepatotoxicity.

The advantage of HA infusion (HAI) of drugs for the treatment of colorectal liver metastases compared with systemic administration has been studied extensively [38,39]. Several randomised studies involving HAI with FUDR or 5-FU showed significantly higher tumour response rates compared with systemic administration (HAI 41%, systemic 14%) [40–43]. HAI is based on the principle that established colorectal liver metastases, in contrast with liver parenchyma, derive most of their blood supply from the HA [44,45]. As a result, HAI leads to high drug concentrations within the tumour while the liver parenchyma is relatively spared. Furthermore, the liver metabolises many cytotoxic drugs, thereby reducing systemic exposure and, thus, systemic toxicity. Perfusion through the HA has proven to be essential for successful IHP. We showed a significant difference in tumour response rate (62% versus 33%), time to progression (7.7 months (95% CI:6.1–9.4) versus 3.6 months (95% CI:2.9–4.3)) and survival (32.7 months (95% CI:22.9–35.8) versus 8.6 months (95% CI:7.7–9.5)) between patients who were perfused through *both* the HA and portal vein (PV) ($n = 64$), and patients perfused through the PV *only* ($n = 7$) (perfusion through the HA was impossible in these patients for a variety of non-related reasons) [17].

When melphalan (200 mg) is administered as a single bolus to the recirculating isolated hepatic circuit, i.e. in approximately 2 l of perfusate [17], this results in an initial melphalan concentration of approximately 100 μg/ml (328 μM), which is then perfused in the liver through both the PV and HA. As shown by Vahrmeijer and colleagues [3], the concentration of bolus administered melphalan in this perfusate rapidly declines in the first 5–10 min of its circulation, indicating a rapid uptake of melphalan by the (tumour-bearing) liver (Fig. 2). As a result, the tumour exposure to high concentrations of melphalan in the perfusate is relatively short.

Based on the obvious advantage of HAI in terms of prolonged high tumour exposure to cytostatics, it seems to be preferable to administer melphalan by continuous infusion in the HA instead of by a single bolus administered in the whole perfusate: infusing melphalan in the HA directly over a certain period, theoretically, would not only lead to more selective tumour exposure to melphalan, but also to exposure of the tumours to higher melphalan concentrations for a longer period of time. For instance, by infusing the same melphalan dose directly in the HA over 20 min at a flow of 100 ml/min, the same high melphalan concentration of approximately 100 μg/ml (328 μM) would be achieved in the HA for 20 min instead of only a few minutes (Fig. 2). Consequently, tumours would be exposed to an equal high melphalan concentration for a longer period of time, which is expected to improve the antitumour efficacy of IHP.

Hence, HAI of melphalan is preferred in IHP, but the optimum conditions (duration, concentration) of the intra-arterial melphalan infusion in relation to its antitumour effect and the safety aspects are not yet known. For instance, should the melphalan dose be infused over a short or long period of time during the vascular isolation of the liver? Obviously, a short infusion time of the cytostatic compound in a clinical setting leads to a shorter duration of the procedure and is therefore preferable. However, this can only be justified when the antitumour effect is equal or better, and the hepatotoxicity is not increased. In an *in vivo* rat model for liver tumours, we studied the difference in tumour and liver uptake, as well as antitumour effect and hepatotoxicity of 5 and 20 min arterial melphalan infusion of a fixed melphalan dose (4.4 μmol) in both cases [46]. No difference in melphalan content of liver/liver tissue and tumour response was found between the two treatment schedules. However, hepatotoxicity was strongly affected by the perfusion duration and thus melphalan concentration: severe cholangiofibrosis occurred in 8 of 9 rats treated with the 5 min infusion, but in only 1 of 8 rats treated with a 20 min infusion of the same dose of melphalan. These results showed that tumour response was not affected by melphalan *concentration* as long as the tumours were exposed to the same *total dose* of melphalan. However, for toxicity the concentration-toxicity curve appeared to be very steep, indicating that once the toxicity threshold concentration is reached a small increase in melphalan concentration leads to a large increase in hepatotoxicity. Thus, caution should be taken when the infusion concentration of melphalan is increased.

3.2. Retrograde liver perfusion

The complete vascular isolation of the liver during IHP offers the unique opportunity to fully control the perfusion flow and direction through the liver. Both may affect drug delivery to both tumours and liver tissue, and

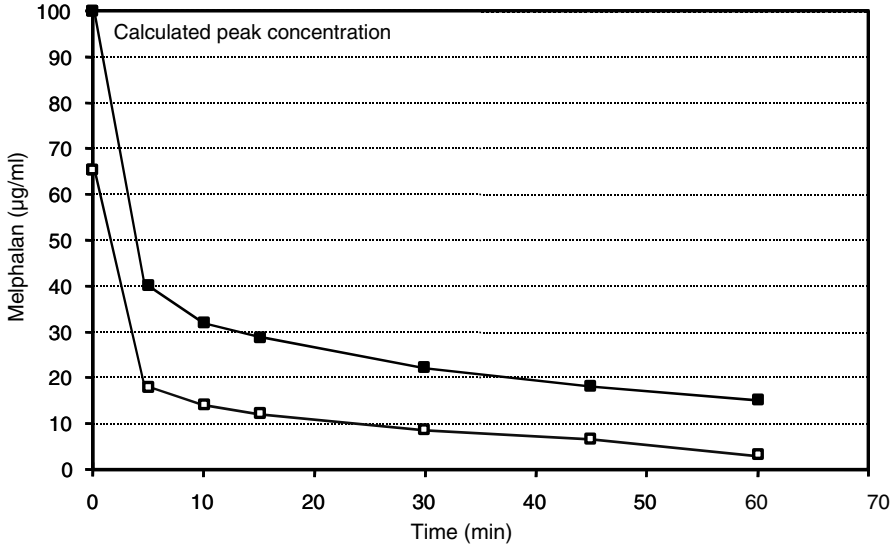

Fig. 2. Concentration of melphalan in perfusate during 1-h perfusion after addition of either 1.5 mg/kg (□) or 3.0 mg/kg (■) melphalan to the isolated circuit. The calculated melphalan peak concentration is indicated.

thus antitumour efficacy and hepatotoxicity. Changing the perfusion direction could theoretically reduce liver toxicity without affecting the antitumour efficacy. As mentioned before, liver tumours are almost exclusively perfused by the HA [44,45] and, therefore, the reversion of the venous blood stream through the liver should not affect the tumour exposure to arterially administered chemotherapy. However, liver parenchyma is perfused by both the PV and HA. Studies of the blood supply of the liver show that the hepatic arterioles terminate in the first third of the sinusoids (zone 1) via an indirect or direct pathway [47,48]. As a result, the arterial blood reaches all 3 zones during normal (orthograde) single-pass perfusion (Fig. 3(a)), but only zone 1 of the liver sinusoids during retrograde perfusion (Fig. 3(b)) [49]. Thus, liver exposure to arterially infused drugs should be reduced during retrograde perfusion.

Except for rat and pig liver [49–51], there is limited research on this topic. To improve the treatment efficacy of IHP, we studied the pharmacokinetic consequences of partial reversion of the blood flow through the liver, in a rat model for colorectal liver tumours [52]. Either a normal (orthograde) or retrograde single-pass IHP with continuous hepatic arterial melphalan infusion was performed in tumour-bearing rat livers. Analysis of melphalan content in tumour and liver tissue after either orthograde or retrograde IHP showed that tumour uptake was unaffected by retrograde IHP, but liver uptake was reduced by 80% (Fig. 4) [52]. These results suggest that retrograde liver perfusion may decrease hepatotoxicity while maintaining the antitumour efficacy.

Is retrograde IHP realistic in patients? Technically, retrograde IHP would be no problem, as both the hepatic and portal vein vascular beds do not have valves. As a result, the flow through the liver can be completely manipulated when isolated from the systemic circulation. Maximum improvement of treatment efficacy by retrograde IHP, i.e. reduced liver toxicity without affecting the antitumour efficacy, would be achieved when, similarly to the rat experiments, single-pass retrograde IHP with continuous hepatic arterial melphalan infusion is performed. However, as perfusate is not recirculated when performed single-pass, this would require a very large volume of perfusate (approximately 40 l for a 1 h IHP according to the mean perfusate flow through the liver in IHP [17]) and is, therefore, less feasible. By shortening the procedure, the required perfusate volume could be decreased, but still a large volume would be needed (approximately 14 l for a 20 min single-pass IHP). Further shortening of the procedure with the same melphalan dose would be desirable, but would increase the risk of hepatotoxicity [46].

Alternatively, recirculating retrograde IHP could be performed; the obvious advantage compared with single-pass IHP is that a limited volume of perfusate would be needed. However, in contrast to single-pass retrograde IHP recirculating perfusate would reach liver zones 2 and 3 and, consequently, the melphalan uptake by the liver would be increased. Nevertheless, recirculating retrograde IHP is still expected to significantly reduce liver uptake of melphalan, because the melphalan concentration that passes liver zones 2 and 3 is already

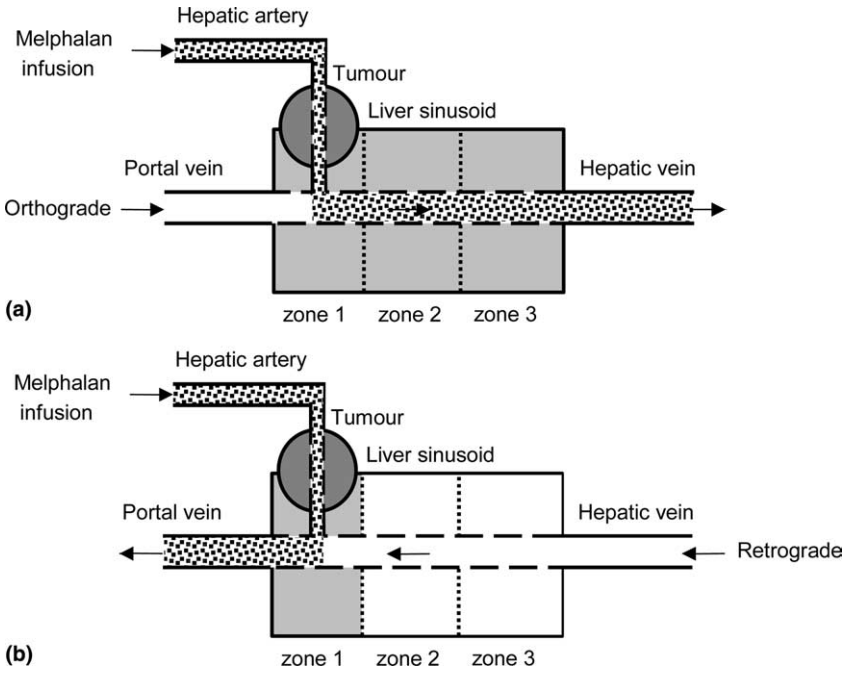

Fig. 3. Liver tumour model. Distribution of melphalan during either (a) orthograde or (b) retrograde single pass liver perfusion with continuous melphalan infusion in the hepatic artery.

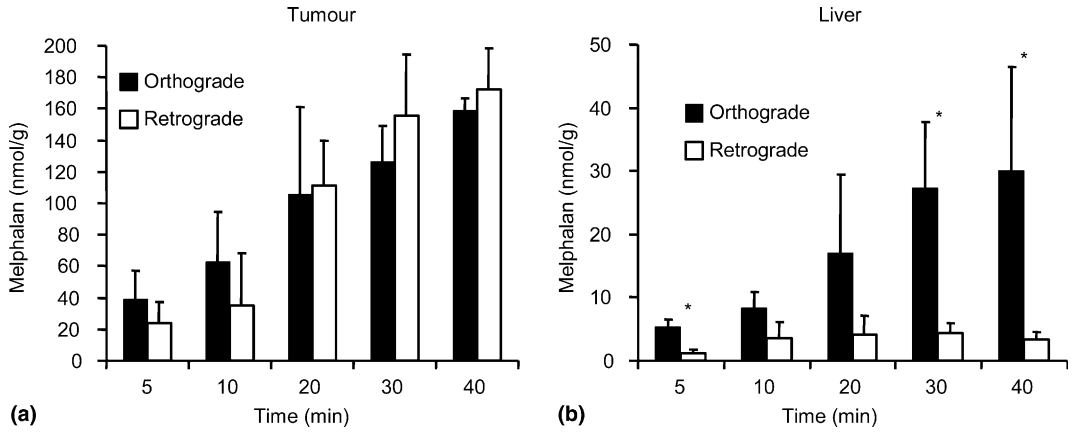

Fig. 4. Tumour (a) and liver (b) concentrations of melphalan over time during orthograde and retrograde liver perfusions with continuous melphalan infusion in the hepatic artery. *Statistical difference between orthograde and retrograde liver perfusions ($P < 0.05$). $n = 3$ for orthograde 5, 10, 20, 40 min and for retrograde 10, 20, 30 min; $n = 4$ for orthograde 30 min and for retrograde 5, 40 min. Statistical test: paired student t-test (P value <0.05 considered statistically significant).

strongly reduced after the first-pass through the liver because it is diluted by the whole perfusate.

4. IHP with minimally invasive techniques

The current IHP technique is a demanding and technically difficult procedure with considerable morbidity and mortality, which is not amenable to repetition. Since a single 1 h treatment by IHP with melphalan is already very effective, as shown by the high response rates in recent studies [5,17], repetitive treatment with IHP might be attractive, possibly resulting in enhanced antitumour responses and improved survival rates. Therefore, a less invasive, repeatable IHP technique is needed.

There have been several attempts to develop minimally invasive procedures for high-dose drug administration. Clinical and experimental studies have been described involving chemofiltration under complete hepatic venous isolation after HAI of drugs, allowing the administration of high doses of intrahepatic chemotherapy with either 5-FU or doxorubicin [53–55]. Applying this technique, the liver is partially cut off from the systemic circulation by occluding the inferior caval vein (ICV) above and below the hepatic veins using a four-lumen/two-balloon catheter, which collects the blood from the hepatic veins. After the drug is infused in the HA, the hepatic venous blood is bypassed to a charcoal haemoperfusion filter for extracorporeal drug elimination before it returns to the patient's systemic circulation. Ku and colleagues [55] reported a tumour response rate of 63% in 28 patients with hepatocellular carcinoma after (repetitive) treatment with high-dose doxorubicin. However, complete extraction of chemotherapeutic compounds by charcoal haemoperfusion filters is not possible. Therefore, at present, these methods are not applicable for drugs and dosages currently used in IHP protocols.

Complete isolation of the liver using minimally invasive techniques is still experimental. Van Ijken and colleagues [56] reported a method developed in pigs in which the PV is closed and the liver is perfused hypoxically through the HA using the ICV for outflow. In this study, the liver was isolated by placing occlusion balloon catheters in the common HA and the inferior caval vein, and surgically clamping the PV. An occlusion balloon catheter placed in the aorta above the celiac axis compensated for the decrease in cardiac preload. Results show high regional drug concentrations and negligible systemic drug concentrations in the pig model.

Recently, Savier and colleagues [57] reported the treatment of 4 patients with 3 successive courses of chemotherapy by IHP, in which the first course was given at laparotomy, and the next 2 courses percutaneously. Percutaneous isolation of the liver was achieved by placing an occlusion catheter in the PV according to the transhepatic Seldinger technique and a double-balloon catheter in retrohepatic caval vein through the saphenous vein; finally, the HA was occluded by traction of a silicon-lined nylon thread that was positioned around the common HA during previous laparotomy. Melphalan (15–45 mg) was administered through a catheter in the gastroduodenal artery that had also been inserted during the previous laparotomy. Although isolated perfusion was achieved by this percutaneous method, considerable leakage to the systemic circulation occurred during IHP. The resulting systemic toxic effects were acceptable and did not result in mortality in this study with relatively low melphalan doses. However, this would probably not be the case in the current clinical IHP programmes in which up to 6-times higher melphalan doses are administered [17].

A possible explanation for the incomplete isolation during IHP with occlusion balloons is the fact that the human suprahepatic caval vein is relatively short, which may prohibit complete occlusion. Furthermore, complete vascular isolation can probably not be achieved by occluding the major liver vessels only because of the existence of numerous venous collaterals [58], that can not be ligated easily during IHP with minimally invasive techniques. We developed a different IHP procedure using minimally invasive techniques in an experimental pig model [51]. During this procedure, balloon catheters were also used to occlude the PV and infrahepatic caval vein, but a stent-graft was used to occlude the suprahepatic caval vein. Leakage-free IHP was achieved with this method. By partly reversing the blood flow through the liver (inflow through the caval vein, outflow through the PV), and applying negative (suction) pressure at the PV, the intrahepatic pressure was controlled, which proved to be essential for the prevention of drug leakage to the systemic circuit through collaterals. This IHP method using minimally invasive techniques was feasible in pigs. Eventually these developments could lead to a fully percutaneous IHP in humans.

5. New drugs in isolated hepatic perfusion?

As mentioned before, several drugs have been applied in IHP including 5-FU [1,7], mitomycin C [8,9], cisplatin [1] and melphalan [1,3–5,9–11], but in the past 10 years melphalan has been the only drug used in major clinical studies [5,17]. Despite the encouraging results with melphalan in recent studies its efficacy is still limited; therefore, other drugs, such as irinotecan and oxaliplatin, might also be considered for application in IHP, as they might further increase the treatment efficacy or safety of IHP.

For successful application in IHP a drug has to fulfil several conditions. As isolated perfusion is a short treatment it is essential that the drug causes rapid, irreversible tumour cell cytotoxicity in order to achieve effective antitumour treatment. In case of unexpected leakage, ideally, an agent to protect against systemic toxicity should be available. For instance, granulocyte colony-stimulating factor is used to prevent leucopenia in melphalan treatment [59].

Several drugs may be interesting for application in IHP. In the past few years new agents, such as irinotecan and oxaliplatin, have been introduced in the systemic treatment of colorectal metastases. They resulted in increased response rates, disease-free survival and overall survival [60–66], also in patients resistant to fluoropyrimidines [67,68]. Irinotecan is a prodrug that requires activation by carboxylesterases to the active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38), an inhibitor of topoisomerase I, which is approximately 100–1000-fold more active than the parent drug [69]; it has proven to be highly effective in the treatment of colorectal cancer [60,61]. However, it may not be applicable in IHP, because it is not a direct acting agent, and the bioactivation to its active metabolite is slow: phase I trials on the pharmacokinetics and pharmacodynamics of irinotecan and SN-38 show that maximum concentrations of SN-38 are only reached approximately 1 h after the beginning of a 30 min intravenous (i.v.) irinotecan infusion [70]. It might be worthwhile to test the active metabolite SN-38 directly.

Oxaliplatin is rapidly absorbed and transformed by non-enzymatic pathways to its biologically active species [71]. It mainly exerts its cytotoxic effect by formation of DNA intra- and interstrand cross-links, hampering DNA replication [72]. Substantial dose-dependent DNA adduct formation occurs within 1 h. In most studies in which oxaliplatin is administered systemically, hematological toxicity and nephrotoxicity are dose-limiting, while hepatotoxicity is rarely mentioned. This implies that treatment with a much higher dose oxaliplatin might be feasible in IHP. Thus, oxaliplatin seems to be an interesting drug for application in IHP. To improve IHP treatment efficacy, experimental studies with new drugs, such as oxaliplatin, should be conducted.

Recent studies show that immunotherapy with targeted drugs can also play a role in the treatment of colorectal metastases. The addition of both cetuximab, a chimeric monoclonal antibody directed against the epidermal growth factor receptor (EGRF), and bevacizumab, a monoclonal antibody directed against the vascular endothelial growth factor (VEGF), to systemically administered chemotherapy proved to be effective in patients with colorectal metastases [73,74]. Similarly, addition of such targeted drugs in IHP regimens might also increase the antitumour efficacy of IHP.

Finally, gene therapy might be applied in IHP. Several studies indicate that regional vector delivery results in increased gene transfer in tumours. Gnant and colleagues [75] showed that regional (intraportal or intraperitoneal) delivery of a recombinant vaccinia virus vector for suicide gene therapy significantly increased transgene expression in murine liver tumours compared with systemic virus administration. However, this did not result in an increased antitumour efficacy. We demonstrated that *in vivo* administration by IHP of adenovirus vectors carrying the *E. coli lacZ* gene and the firefly luciferase gene resulted in an even more efficient and also more reproducible gene transfer when compared with intraportal infusion [76]. Unfortunately, the results of clinical trials on gene therapy that have been conducted for colorectal liver metastases are disappointing: although gene therapy has been well tolerated and toxicity has been low, the clinical benefit is limited [77–79]. Therefore, the therapeutic application of gene therapy in the near future is uncertain.

6. Prediction of treatment outcome

Two recent major clinical IHP studies mention response rates (complete or partial remission) of 59% ($n = 73$) [17] and 74% ($n = 34$) [5], which means that approximately one-third of the patients do not benefit from IHP. Apparently, a substantial number of patients are resistant to melphalan treatment.

Several mechanisms of resistance have been reported for melphalan, including reduced drug uptake, changes in glutathione (GSH) levels, reduced DNA damage and increased DNA repair. Drug transport mechanisms can vary among cells resulting in more or less accumulation of melphalan. Larrivee and colleagues [80] showed that active melphalan efflux in CH^RC5 cells was mediated by P-glycoprotein. Harada and colleagues [81] reported that down-regulation of CD98, an L-phenylalanine transporter, decreased melphalan uptake in myeloma cells. Thus, up- or down-regulation of proteins involved in melphalan transport may lead to melphalan resistance.

Since GSH plays a very important role in the prevention of toxicity of many (reactive) agents, it is not surprising that elevated GSH levels in tumour tissue are associated with resistance to chemotherapy [82,83]. Increases in the activity of the GSH/GST system are frequently found in alkylating agent resistant phenotypes and high levels of GSH suppress the formation of DNA adducts by alkylating agents *in vitro* [84]. However, GSH conjugation of melphalan is not a major reaction in humans and rats [85] and, therefore, is not expected to play a major role in melphalan resistance in patients. Other protective effects of GSH, of course, cannot be excluded.

Increased DNA repair appears to be a major mechanism to mediate resistance to melphalan [86]. Several *in vitro* studies have already shown that cell lines with mutations in DNA repair proteins, such as ERCC-1, ERCC-4, Xrcc2, Xrcc3, Rad54, Ku70, Ku86 and DNA-PKcs are highly sensitive to nitrogen mustards [86,87]. Elevated topoisomerase II activity and the increased affinity of topoisomerase II for cross-linked DNA in melphalan-resistant cells also appears to be a major factor responsible for alkylator resistance [88]. These differences in DNA-repair may be the explanation for the variability in tumour response between patients after melphalan treatment by IHP [5,17]. This hypothesis is supported by a study on the formation of melphalan-DNA adducts, which is supposed to be the primary mechanism of cytotoxicity of melphalan. We showed that equal melphalan-DNA adduct staining intensity after melphalan exposure in different colorectal cancer cell lines was not associated with an equal extent of melphalan cytotoxicity [89]. Apparently differences exist in the lethality of melphalan-DNA adducts between cell lines, which may be explained by e.g. variations in DNA repair among the different cell types.

For IHP, the identification of molecular markers involved in melphalan resistance is important as this might allow the prediction of treatment response in patients. Prediction of treatment response in patients with colorectal metastases is already possible for other drugs. For instance, overexpression of thymidylate synthetase in colorectal liver metastases, as measured by immunohistochemical staining, is reported to correlate with a poor tumour response to HAI floxuridine [90]. Similarly, identification of individual tumour markers that are predictive of treatment outcome may be achieved by assessing either primary colorectal tumour or colorectal metastases of patients treated by IHP with melphalan for markers that are already known to be involved in melphalan resistance with conventional techniques, such as immunohistochemical expression analysis.

Recently, technology has been developed that allows genetic profiling of the patient's tumour, by measuring protein and gene expression levels of markers and genetic polymorphisms. A gene expression profile has already been identified that is associated with prognosis in patients with breast cancer [91] and we anticipate that a prognostic profile will be found. Gene expression profiling may also allow pharmacogenetic screening, which enables more accurate prediction of drug responses than existing clinically used methods.

7. The role of IHP in the treatment of colorectal liver metastases

There is no consensus about the role of IHP in the treatment of colorectal liver metastases. As IHP is only applied in a few centres in the world, most clinicians consider IHP to be an experimental treatment. However, the promising results of IHP studies in the past 5 years cannot be disregarded [5,10,17]. IHP as a regional treatment with high response rates, including complete remissions, and a median survival up to 28.8 months with 5-year survivors, albeit in selected series of patients, should be considered a serious treatment option for patients with non-resectable liver metastases and no extrahepatic disease. Although, these data cannot be compared with other studies, because of patient selection, survival after IHP is still markedly higher than survival after the new systemic chemotherapy regimens that are based on irinotecan and/or oxaliplatin plus 5-FU/leucovorin: a median survival ranging from 14.8 to 21.5 months has been reported [60,63–66,92]. IHP is associated with considerable morbidity and mortality of 3–5% [5,17], but it should be noted that the newest systemic chemotherapy regimens also report mortality rates up to 4% [66].

We believe that patients with colorectal metastases confined to the liver who are ineligible for hepatic resection or local ablative therapy should not be treated

by IHP only, but in combination with systemic chemotherapy. Treatment with IHP upfront rather than after systemic chemotherapy has failed might be preferable. When patients are diagnosed with colorectal liver metastases only they are usually still in a relatively good physical condition. Most of these patients are still suitable for systemic chemotherapy when after regional therapy there is progressive disease. However, patients with progressive disease after previous systemic chemotherapy are often in poor physical condition and may not be candidates for IHP anymore. Furthermore, as a result of the rapid tumour remission after IHP, a number of patients become eligible for either hepatic resection or local ablative therapy after a relatively short period of time.

Although it seems a clinically prudent approach, there is no experimental evidence that IHP followed by systemic chemotherapy is preferable. Therefore, a randomised study should be conducted to explore the efficacy of combined treatment with IHP and systemic chemotherapy. We suggest a study in which patients are either treated by IHP directly followed by systemic chemotherapy or treated by systemic chemotherapy first followed by IHP at the time of progression. The systemic chemotherapy regimen in this study should be the same as the currently accepted regimens for advanced colorectal cancer, that are based on a combination of 5-FU/leucovorin with oxaliplatin or irinotecan in both first- and second-line chemotherapy [63–66]. Obviously, the patients should be stratified according to earlier receipt of adjuvant systemic chemotherapy for their primary colorectal tumour or not. A third arm with systemic chemotherapy only would definitely prove the additional role of IHP. However, at present, we consider this not ethically acceptable in view of the positive results with IHP. The outcome of such studies, the development of new drugs for both IHP and systemic application and the feasibility of minimally invasive techniques for IHP will determine the future role of IHP in the treatment of liver metastases.

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