Cancer Chemother Pharmacol (1993) 33: 251–257

Cancer Chemotherapy and Pharmacology

Increased doxorubicin levels in hepatic tumors with reduced systemic drug exposure achieved with complete hepatic venous isolation and extracorporeal chemofiltration

Steven A. Curley[1], Diana L. Stone[2], George M. Fuhrman[1], David C. Hohn[1], Zahid H. Siddik[2], Robert A. Newman[2]

[1] Department of General Surgery, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA
[2] Department of Clinical Investigations, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA

Received: 10 March 1993/Accepted: 22 July 1993

Abstract. We evaluated a novel system of complete hepatic venous isolation and chemofiltration (CHVI-CF) to reduce systemic drug exposure following regional hepatic infusion of doxorubicin. Rabbits bearing hepatic VX-2 tumors were given doxorubicin via either hepatic arterial infusion (HAI) or portal venous infusion (PVI). A dual-balloon vena cava catheter and extracorporeal chemofilter were used to capture and filter hepatic venous blood in experimental animals. Control animals received chemotherapy without hepatic venous isolation and chemofiltration. Following a 5-min HAI of doxorubicin (3 or 5 mg/kg), control and experimental animals had similar doxorubicin levels in their livers and VX-2 tumors, but experimental animals showed a significant reduction in doxorubicin levels in systemic plasma, heart, and kidney tissue as compared with control animals ($P < 0.01$). HAI produced a 4-fold increase in doxorubicin levels in VX-2 tumors as compared with the drug levels obtained using PVI ($P < 0.01$). A single HAI of 3 mg/kg doxorubicin in animals treated with CHVI-CF produced marked tumor necrosis at 7 and 14 days after treatment. By increasing the total body clearance of doxorubicin, this system will allow HAI of higher doses of drug in attempts to improve the antitumor response.

Introduction

The narrow therapeutic index of most anticancer agents often limits the dose of drug that can be given. Chemotherapy-related toxicities occurring in normal, non-neoplastic tissues may prevent the delivery of drug doses sufficient to achieve a therapeutic effect [1]. Additional factors having a negative influence on effective chemotherapy for malignant tumors include expression of the multidrug resistance phenotype and other mechanisms of drug resistance [2–6].

Regional arterial administration of antineoplastic drugs to the organ or site harboring a tumor has been employed to increase the drug concentration delivered to the tumor. Hepatic arterial infusion (HAI) of chemotherapeutic drugs has been used for primary and metastatic liver tumors [7–10]. HAI of agents with high rates of hepatic extraction and metabolism, such as 5-fluorouracil (5-FU) and 5-fluoro-2-deoxyuridine (FUDR), results in decreased systemic toxicity because of reduced systemic exposure to the drug [11]. However, HAI of drugs with low rates of hepatic extraction, such as doxorubicin, produces high systemic drug levels, and the resulting toxicities limit the dose that can be delivered [8, 12–14]. Since the venous drainage of the liver is confined to a short segment of the inferior vena cava, it is possible to isolate the hepatic venous outflow [15]. We studied a novel dual-balloon vena cava catheter to achieve complete hepatic venous isolation in a rabbit model bearing VX-2 liver tumors. The hepatic venous blood captured by the dual-balloon vena cava catheter is pumped through an extracorporeal chemofiltration circuit before it is returned to the animal. We sought to determine whether this complete hepatic venous isolation and chemofiltration (CHVI-CF) system would enhance tumor doxorubicin concentrations following regional infusion by allowing the delivery of high drug doses to the liver while limiting systemic exposure to the drug. We also evaluated differences between tumor doxorubicin levels following HAI or portal venous infusion (PVI) of the drug associated with CHVI-CF.

This work was supported in part by NIH-NCI CA-16672 awarded by the National Cancer Institute and by the John S. Dunn Foundation

Correspondence to: Steven A. Curley, Department of Surgical Oncology, Box 106, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA

Materials and methods

Animals. Adult male New Zealand White rabbits (3–3.5 kg) were purchased from The University of Texas Science Park (Bastrop, Tex.). Animals were quarantined for 2 weeks prior to their use to ensure the

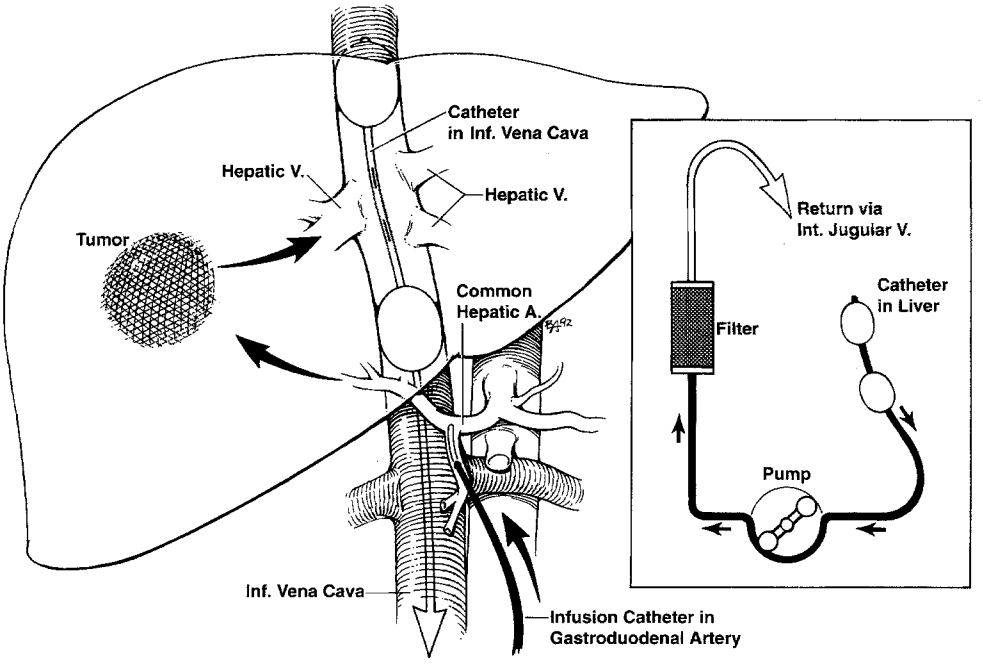

Fig. 1. Positioning of the dual-balloon vena cava catheter to achieve complete hepatic venous isolation. A hepatic arterial infusion of doxorubicin is performed through the catheter placed into the gastroduodenal artery. Any drug not cleared by the VX-2 tumor or the liver is captured in the hepatic venous effluent by the dual-balloon catheter. The *inset* demonstrates that the complete hepatic venous outflow is pumped through an extracorporeal circuit that includes a high-efficiency carbon filter. This filter removes doxorubicin in the hepatic venous blood prior to returning this blood to the rabbit via a catheter placed in the internal jugular vein. A stopcock placed in the tubing between the pump and the filter is used to obtain prefilter (hepatic venous) blood samples. A second stopcock placed in the tubing exiting the filter is used to obtain postfilter samples

absence of pathogens and were maintained in individual cages and fed standard rabbit chow and water ad libitum.

Tumor and tumor cell lines. VX-2 is a poorly differentiated epithelioid solid carcinoma that originated as a virally induced tumor in rabbits [16]. The VX-2 tumor was maintained in our laboratory through serial transplantations into the hind-limb musculature of rabbits. The histology and growth characteristics of the VX-2 tumor have been extensively described [17, 18]. After implantation into either the hind limb or the liver, the tumor enlarges rapidly, with a zone of hypervascularity developing along its advancing rim. During its growth, the center of the tumor undergoes avascular necrosis as it outgrows its blood supply.

The tumor from the hind limb of donor rabbits was excised using a sterile technique and then placed in RPMI-1640 medium. The tissue was minced with a scalpel, and the tumor fragments and 1 ml of RPMI-1640 medium were placed in a tissue homogenizer (Becton Dickinson; San Jose, Calif.) for 6 s. The resultant tissue homogenate (cell viability, >70% as determined by trypan blue exclusion) was placed in sterile syringes for immediate injection into experimental animals.

VX-2 tumors were induced in the livers of rabbits by making a 2-cm midline subxyphoid abdominal incision in animals under general mask anesthesia with 5% isoflurane. Using a 22-gauge needle, 0.4 ml of the VX-2 tumor homogenate was injected under direct vision into a segment of the right lobe of the liver. The needle puncture site on the capsule of the liver was cauterized to seal the site and prevent leakage of the tumor homogenate. Experiments were performed 2 weeks after the induction of hepatic VX-2 tumors, when the tumor nodules were 1.5–2 cm in diameter and had not yet developed central necrosis.

Procedure. General anesthesia in tumor-bearing animals was induced with isoflurane (5%) delivered through a mask and was maintained with isoflurane (1.5%) following endotracheal incubation of the animals. The right jugular vein was exposed through a small cut-down incision, and a 16-gauge silastic catheter was placed via the jugular vein into the superior vena cava. This jugular-vein catheter was used as the systemic blood-return site to complete the extracorporeal pump circuit. A midline abdominal incision was made from the xyphoid to the mid-abdomen to gain full exposure to the peritoneal cavity. A 16-gauge silastic catheter was placed in the right iliac artery to monitor the blood pressure continuously and to obtain systemic blood samples for drug analysis. Following the placement of catheters in the jugular vein and common iliac artery, systemic anticoagulation was achieved by injection of an intravenous bolus of heparin sodium (200 mg/kg).

Following anticoagulation, a 7-F dual-balloon vena cava catheter (Bodden catheter; Delcath Systems, New York, N.Y.) was introduced through the right common iliac vein and advanced until the upper balloon was positioned cephalad to the entrance of the hepatic veins into the inferior vena cava and the lower balloon was positioned caudad to the entrance of the hepatic veins. Before inflation of the balloons on the vena cava catheter, this catheter was connected to an extracorporeal roller pump (Gambro pump; Lund, Sweden) with an in-line filter containing 3.2 g of methylcellulose-coated carbon particles (National Medical Corporation; Rockleigh, N.J.). Pump tubing downstream from the filter was connected to the 16-gauge catheter placed in the jugular vein to complete the extracorporeal pump circuit. Flow in the pump was initiated, and the upper balloon followed by the lower balloon on the dual-balloon vena cava catheter was inflated until each balloon completely occluded the inferior vena cava. In this manner, the venous drainage of the liver was completely isolated between the two balloons of the vena cava catheter and the hepatic venous blood was pumped through the extracorporeal circuit (Fig. 1).

Drug administration. A 24-gauge silastic catheter was placed into either the gastroduodenal artery to accomplish HAI or into the portal vein to carry out PVI of doxorubicin (Cetus Corporation; Emeryville, Calif.). For the HAI-treated animals, small arterial branches from the hepatic artery were clipped and divided to prevent misperfusion of the drug to the stomach or duodenum. The doxorubicin (2 mg/ml concentration) was given by a 5-min continuous infusion using a syringe pump. Animals were divided into four experimental groups. Group 1 received an HAI or a PVI of doxorubicin at 0.5, 1.0, 1.5, or 2.0 mg/kg without CHVI-CF to determine the maximum tolerated dose (MTD) of doxorubicin and the antitumor response at the MTD in this control group.

Group 2 animals received 3 or 5 mg/kg doxorubicin by HAI or PVI utilizing the dual-balloon vena cava catheter to achieve CHVI-CF. In group 2 animals, prefilter (hepatic venous outflow), postfilter, and systemic blood samples were drawn midway through the 5-min drug infusion (2.5 min), at the conclusion of the drug infusion, and then at 5-min intervals up to 30 min (a total of seven time points: 2.5, 5, 10, 15, 20, 25, and 30 min after the beginning of the 5-min drug infusion). Blood samples were centrifuged (1,500 *g*) for 10 min. Plasma was then aspirated and frozen for later drug analysis.

Group 3 also received an HAI or a PVI of 3 or 5 mg/kg doxorubicin but without CHVI-CF. Systemic blood samples in this control group

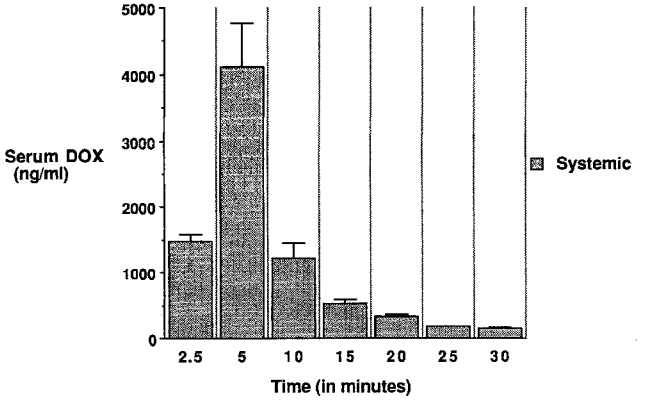

Fig. 2. Serum levels of doxorubicin (*DOX*) measured in five control animals following a 5-min hepatic arterial infusion (HAI) at a dose of 3 mg/kg without complete hepatic venous isolation and chemofiltration (CHVI-CF). The 5-min time point represents the completion of the 5-min DOX infusion

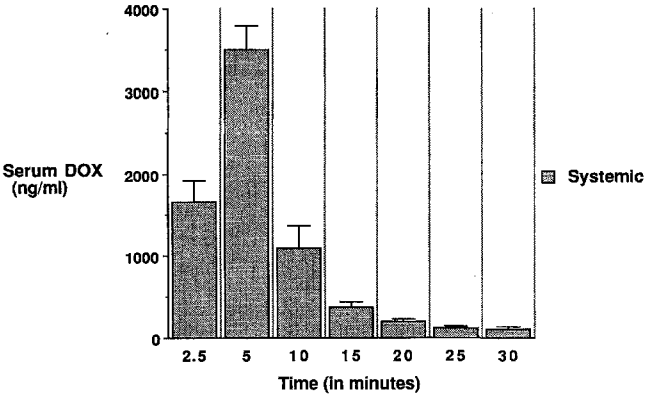

Fig. 3. Serum levels of doxorubicin (*DOX*) measured in five control animals following a 5-min portal venous infusion (PVI) at a dose of 3 mg/kg without CHVI-CF. The 5-min time point represents the completion of the 5-min DOX infusion

were drawn at the identical time points used in group 2 to measure systemic levels of doxorubicin. In groups 2 and 3, the animals under general anesthesia were killed by exsanguination at 30 min after the drug infusion and the VX-2 liver tumor, normal liver tissue not involved by tumor, the heart, and the kidney were harvested to determine tissue doxorubicin levels.

Group 4 animals received a 5-min HAI of 3 mg/kg doxorubicin with a 30-min period of CHVI-CF. At the conclusion of the 30-min bypass procedure, the lower and then upper balloons of the vena cava catheter were deflated. The blood in the bypass pump circuit was returned to the animal, and the bypass pump circuit was then stopped. All catheters were removed; the vessels, ligated; and the incisions, closed in a standard two-layer fashion. Group 4 animals were then allowed to recover from general anesthesia and were followed to determine the tumor response to the treatment.

Drug analysis. Doxorubicin levels in plasma and tissues were determined according to the high-pressure liquid chromatography (HPLC) method of Robert [19]. Aliquots of plasma were placed on prepared minicolumns (C_{18} Sep-Paks; Waters Associates, Wilford, Mass.); after washing, the drug was eluted and the eluant was dried under nitrogen. Samples were then redissolved in mobile phase prior to injection into the HPLC system.

Tissues were weighed and then homogenized in 0.05 *M* phosphate buffer (pH 8.0) prior to extraction with chloroform and methanol. Following centrifugation, an aliquot of the organic phase was dried under nitrogen and then redissolved in mobile phase for injection into the HPLC system. Daunomycin (Sigma Chemical Co., St. Louis, Mo.) was routinely used as an internal standard for both plasma and tissue analyses.

Statistical analysis. The significance of differences between the data for two groups was evaluated by Student's two-tailed *t*-test. *P* values of less than 0.05 were considered statistically significant.

Results

The MTD of doxorubicin in rabbits bearing hepatic VX-2 tumors was determined by giving a 5-min HAI or PVI of increasing doses of doxorubicin without CHVI-CF. The MTD for either HAI or PVI was 1.5 mg/kg; all animals that received a dose of 2 mg/kg died within 96 h. Doxorubicin levels measured in VX-2 tumors at 30 min after an HAI or a PVI of 1.5 mg/kg without CHVI-CF were 22.1 ± 5.6 and 4.3 ± 2.2 ng/g tissue, respectively ($P < 0.05$, HAI vs PVI). There was no histologic evidence of an antitumor response at 7 or 14 days following a PVI of 1.5 mg/kg doxorubicin, and the VX-2 tumor showed progressive growth in the liver. Extrahepatic metastases were also observed in the peritoneal cavity and lungs. In contrast, at 7 days after an HAI of 1.5 mg/kg doxorubicin, liver tumors showed no increase in diameter and were $47\% \pm 11\%$ necrotic as determined by histopathologic evaluation. However, at 14 days the mean tumor diameter had increased nearly 2-fold beyond the measurement obtained at 7 days.

The doxorubicin levels measured in systemic plasma following a 5-min HAI or PVI of 3 mg/kg doxorubicin without CHVI-CF (group 3 animals) are shown in Fig. 2 and 3. The peak systemic levels of doxorubicin detected in these control animals exceeded 3,500 ng/ml. At 30 min after the drug infusion, plasma doxorubicin levels were less than 100 ng/ml.

A 5-min HAI of 3 mg/kg doxorubicin with CHVI-CF (group 2) produced prefilter (hepatic venous effluent) peak drug levels that were similar to the systemic peak levels measured in the control group (Fig. 4). However, the postfilter and systemic plasma levels of doxorubicin did not exceed 300 ng/ml and were significantly lower than the prefilter (hepatic venous) and control systemic (Fig. 2) drug levels, respectively ($P < 0.001$). A comparison of peak prefilter with peak postfilter doxorubicin levels shows an 86.2% reduction in drug level across the filter. The peak systemic plasma doxorubicin levels measured in animals undergoing HAI with CHVI-CF were reduced by 94.5% as compared with the peak plasma levels detected in control animals (Fig. 4 vs Fig. 2).

A 5-min PVI of 3 mg/kg doxorubicin with CHVI-CF (group 2) yielded a peak prefilter doxorubicin level of almost 5,000 ng/ml (Fig. 5). Again, the postfilter and systemic levels of doxorubicin were significantly reduced as compared with the prefilter (hepatic venous) or control systemic (Fig. 3) levels, respectively ($P < 0.001$). There was an 88.2% reduction in plasma drug level across the filter, and animals treated by PVI with CHVI-CF showed a 90.9% reduction in peak systemic plasma drug levels as compared with controls (Fig. 5 vs Fig. 3).

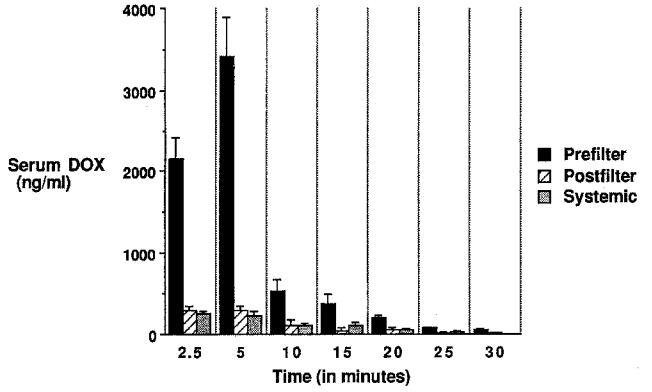

Fig. 4. Serum levels of doxorubicin (*DOX*) measured in five animals following a 5-min HAI at a dose of 3 mg/kg with CHVI-CF. Prefilter levels of DOX represent the hepatic venous effluent prior to passage across the carbon filter. The 5-min time point represents the completion of the 5-min DOX infusion

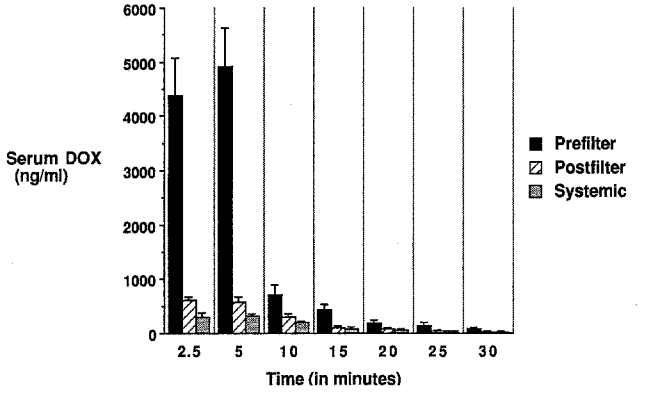

Fig. 5. Serum levels of doxorubicin (*DOX*) measured in five animals following a 5-min PVI at a dose of 3 mg/kg with CHVI-CF. Prefilter levels of DOX represent the hepatic venous effluent prior to passage across the carbon filter. The 5-min time point represents the completion of the 5-min DOX infusion

Table 1. Tissue doxorubicin content measured 30 min after HAI or PVI of 3 mg/kg doxorubicin with or without CHVI-CF

	Liver	Tumor	Heart	Kidney
HAI with CHVI-CF	38.1±6.6[a]	40.9±7.4	2.6± 1.2*	5.6± 1.1*
HAI alone (control)	44.7±9.0	40.2±5.5	33.2± 3.1	81.7±17.2
PVI with CHVI-CF	28.7±2.2	9.9±2.0	8.3± 1.7**	7.2± 2.1*
PVI alone (control)	23.6±4.0	9.8±2.3	48.3±13.6	65.4± 7.4

[a] Data represent mean values ± SEM ($n = 5$), expressed as ng doxorubicin/g tissue

* $P < 0.001$ CHVI-CF vs control by Student's paired, two-tailed t-test

** $P < 0.01$ CHVI-CF vs control by Student's paired, two-tailed t-test

Tissue doxorubicin levels in the liver, VX-2 tumor, heart, and kidney were measured at 30 min after a 5-min HAI or PVI of 3 mg/kg doxorubicin (Table 1). HAI produced doxorubicin levels in VX-2 tumors that were 4 times greater than those detected in tumors after PVI ($P < 0.01$). Doxorubicin levels in the liver were also lower in PVI-treated animals as compared with HAI-treated animals.

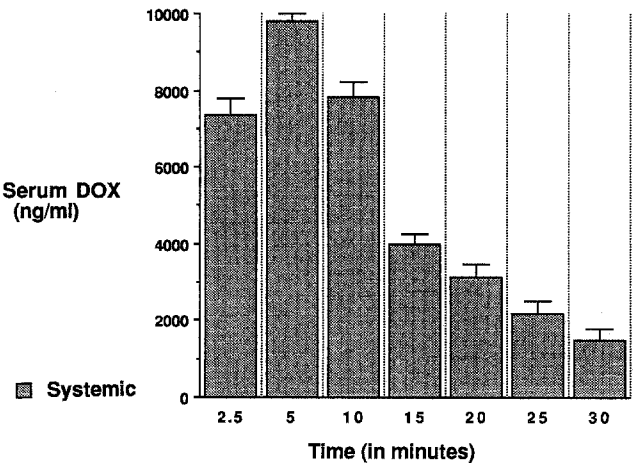

Fig. 6. Serum levels of doxorubicin (*DOX*) measured in five control animals following a 5-min HAI at a dose of 5 mg/kg without CHVI-CF. The 5-min time point represents the completion of the 5-min DOX infusion

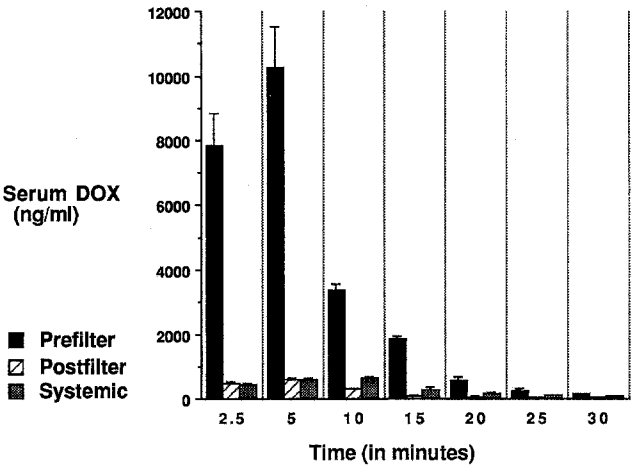

Fig. 7. Serum levels of doxorubicin (*DOX*) measured in five animals following a 5-min HAI at a dose of 5 mg/kg with CHVI-CF. Prefilter levels of DOX represent the hepatic venous effluent prior to passage across the carbon filter. The 5-min time point represents the completion of the 5-min DOX infusion

The liver and VX-2 tumor levels of doxorubicin were similar regardless of whether or not CHVI-CF was used. However, tissue doxorubicin levels in the heart and kidney were significantly reduced in animals that underwent CHVI-CF as compared with control animals ($P < 0.001$). Tissue doxorubicin levels measured in VX-2 tumors following an HAI of 3 mg/kg with CHVI-CF were almost 2 times higher than the tumor levels detected in group 1 controls receiving an HAI at the MTD of 1.5 mg/kg ($P < 0.05$).

Since PVI of 3 mg/kg doxorubicin was associated with significantly lower drug levels in VX-2 tumors than was HAI, PVI of doxorubicin at 5 mg/kg was not performed. Figure 6 presents the plasma drug levels measured in control animals (group 3) that received a 5-min HAI of 5 mg/kg doxorubicin without CHVI-CF. In these control animals, the peak systemic plasma doxorubicin level

Table 2. Tissue doxorubicin content measured 30 min after HAI of 5 mg/kg doxorubicin with or without CHVI-CF

	Liver	Tumor	Heart	Kidney
HAI with CHVI-CF	56.6±7.1[a]	61.1± 6.0	5.9± 2.9*	6.4± 1.6*
HAI alone (control)	64.4±9.2	70.6±10.5	71.4±10.5	136.2±14.1

[a] Data represent mean values ± SEM ($n = 5$), expressed as ng doxorubicin/g tissue

* $P < 0.001$ CHVI-CF vs control by Student's paired, two-tailed t-test

exceeded 6,000 ng/ml. HAI of 5 mg/kg doxorubicin with CHVI-CF (group 2) produced prefilter doxorubicin levels that exceeded 10,000 ng/ml (Fig. 7), but the postfilter and systemic peak levels were less than 500 ng/ml ($P < 0.001$, postfilter or systemic doxorubicin levels with CHVI-CF versus prefilter doxorubicin levels or control-animal systemic doxorubicin levels, respectively). The peak postfilter plasma drug level was 94.2% lower than the peak prefilter level. The animals treated with CHVI-CF showed peak systemic plasma doxorubicin levels that were 93.4% lower than the peak systemic levels detected in controls (Fig. 7 vs Fig. 6). Table 2 shows the doxorubicin levels measured in the liver, VX-2 tumor, heart, and kidney at 30 min after a 5-min HAI of 5 mg/kg doxorubicin with and without CHVI-CF. A significant reduction in the doxorubicin levels detected in systemic tissue (heart, kidney) was observed in animals treated with CHVI-CF ($P < 0.001$). Tissue doxorubicin levels measured in VX-2 tumors following as HAI of 5 mg/kg with CHVI-CF were almost 3 times higher than the tumor levels detected in group 1 controls receiving an HAI at the MTD of 1.5 mg/kg ($P < 0.01$).

Group 4 animals treated with 3 mg/kg doxorubicin by HAI with CHVI-CF were examined at 7 or 14 days after treatment. At 7 and 14 days, the liver VX-2 tumors were >80% necrotic and showed no increase in pretreatment diameter. However, no animal had a complete pathologic response in hepatic VX-2 tumors following the single high-dose HAI of doxorubicin.

Discussion

Intra-arterial infusion of chemotherapeutic drugs has been used in attempts to increase regional exposure to the drugs. However, systemic toxicity remains the dose-limiting factor for many agents. The relative pharmacokinetic advantage, R_d, for arterial infusion of a drug can be expressed as:

$$R_d = \frac{R_t}{R_s}, = \frac{Cl_{TB}}{Q(1\text{-}E)},$$

where R_t is the increased target (regional) exposure, R_s is decreased systemic exposure to drug, Cl_{TB} is the total body clearance of the drug, Q is the blood flow through the infused artery, and E is the rate of drug extraction [20, 21].

The relative pharmacokinetic advantage equation demonstrates that three parameters (Q, E, and Cl_{TB}) affect the pharmacokinetic advantage of intra-arterial drug infusion. Reported values for hepatic extraction (E) of doxorubicin following HAI range from 0.34 to 0.45 [8, 12–14]. Thus, a drug like doxorubicin with a moderate rate of hepatic extraction produces a relatively larger product from the expression 1-E. This increases the value of the denominator with a resultant reduction in R_d. The variable we are manipulating with our experimental system is the total body clearance (Cl_{TB}) of the drug. The CHVI-CF effectively increases the total body clearance of doxorubicin by producing a high-rate first-pass pharmacologic clearance of the drug. Our results demonstrate that increasing the Cl_{TB} of doxorubicin by CHVI-CF with drug infusion into the hepatic artery or portal vein effectively increases relative pharmacokinetic advantage R_d; the use of this system markedly reduced the systemic plasma and tissue exposure to doxorubicin.

The effective hapatic venous isolation by the dual-balloon catheter and efficient drug clearance by the chemofilter is indicated by the 86.2%–94.5% reduction in peak postfilter (hepatic venous) levels. It has previously been demonstrated that significant cardiotoxicity and hematologic toxicity occur when peak systemic plasma doxorubicin levels exceed 1,000 ng/ml [22]. In our control animals that received an HAI of 3 or 5 mg/kg doxorubicin without CHVI-CF, the peak systemic plasma drug levels exceeded 1,000 ng/ml by 4- and 10-fold, respectively. Significantly, the systemic drug levels following an HAI of 3 or 5 mg/kg doxorubicin in animals treated with CHVI-CF never exceeded 700 ng/ml. The ability of the CHVI-CF circuit to protect systemic tissues following high-dose HAI of doxorubicin is further confirmed by the marked reduction in heart- and kidney-tissue drug levels in animal treated with CHVI-CF.

The systemic protection provided by our CHVI-CF circuit was greater than that reported for other systems. It has previously been demonstrated that doxorubicin can be removed from the circulation by adsorption onto sorbents, particularly activated charcoal [23]. Regional arterial infusion of chemotherapeutic drugs has been combined with nonisolated venous hemoperfusion utilizing carbon filters or with hemodialysis of regional or systemic venous drainage. A variety of systems that employ some type of hemoperfusion or hemodialysis have been used in conjunction with infusion of doxorubicin [23, 24], mitomycin C [24, 25], cisplatin [26, 27], bleomycin [24], or 1,3-bis(2-chloroethyl)-1-nitrosourea [28]. In these reports, hemoperfusion or hemodialysis reduced systemic exposure to the chemotherapeutic drug by between 48% and 82%.

In our model, HAI of doxorubicin produced a 4-fold increase in the drug content measured in hepatic VX-2 tumors at 30 min after the infusion of compared with PVI. This finding concurs with the 3- to 5-fold increases in doxorubicin levels in hepatic VX-2 tumors observed by other investigators at 1 h after HAI as compared with PVI [29, 30]. Additionally, our data indicate that hepatic extraction of doxorubicin is less efficient when the drug is infused into the portal vein instead of the hepatic artery. This is suggested by the lower doxorubicin liver-tissue levels detected after PVI as compared with HAI and by the higher prefilter (hepatic venous) plasma drug levels measured in animals treated by PVI as compared with HAI.

Since the CHVI-CF circuit significantly limits systemic exposure to a drug following HAI, the MTD of the drug

will be defined by hepatic toxicity instead of systemic toxicities. We have previously studied the dual-balloon vena cava catheter in a porcine model. An HAI of 1 or 3 mg/kg doxorubicin with CHVI-CF produced no significant change in the results of baseline liver-function tests (alkaline phosphatase, total bilirubin, serum glutamyl oxaloacetic transferase, and serum glutamate peptidyl transferase), and histologic examination of the liver revealed only minimal periportal inflammatory changes in 50% of the treated animals [31, 32]. A single HAI of doxorubicin at doses as high as 9 mg/kg was well tolerated, and acute toxic effects in the liver were not observed. The lack of significant hepatocellular toxicity in the survival rabbits in the current study confirms the livers tolerance of high-dose regional infusion of doxorubicin. Cumulative toxicity to the liver from repeat dosing is a valid concern. However, we now have experience using the CHVI-CF system in human patients. In a phase I study of patients with unresectable hepatocellular carcinoma, we have carried out HAI of doxorubicin at doses as high as 120 mg/m^2 every 4 weeks for three treatments without producing significant elevations in liver-function test values and without producing hepatic arteritis or venous occlusive disease [33]. The liver may tolerate high-dose infusion of toxins such as doxorubicin due to the presence of drug-metabolism pathways and because of the normal expression in the liver of drug-efflux mechanisms such as P-glycoprotein [34].

The anticancer drug-dose intensification allowed by CHVI-CF to treat liver tumors may not markedly improve patient survival. Although we noted an antitumor response in liver VX-2 tumors treated with HAI of high-dose doxorubicin combined with CHVI-CF, single high-dose doxorubicin HAI treatments for liver tumors are not likely to be curative. Although repeated courses of cytotoxic therapy may be associated with a greater antitumor response, such treatments may also lead to increased expression of the multidrug-resistance gene product, P-glycoprotein [2, 3]. The cytoreduction we noted in liver VX-2 tumors after a single HAI of doxorubicin was encouraging but not surprising, since the VX-2 tumors we employed did not express P-glycoprotein as determined by immunohistochemistry using three monoclonal antibodies (C219, C494, and JSB-1) known to detect P-glycoprotein in VX-2 cells grown in doxorubicin [35] (data not shown). Currently, we are developing a doxorubicin-resistant line of VX-2 tumor cells that express high levels of P-glycoprotein. The most important potential clinical use of the CHVI-CF system will be to combine repeated high-dose anticancer drug treatments with agents that increase the intercellular drug concentration by blocking the P-glycoprotein-efflux pump. A lengthy list of compounds have been identified that interfere with the function of P-glycoprotein and thus improve the antitumor response to chemotherapeutic agents in otherwise chemotherapy-resistant tumors [36]. Many of these P-glycoprotein-blocking agents achieve reversal of drug resistance at concentrations that are associated with cellular toxicity in normal host tissues. In preliminary studies conducted in pigs, we have demonstrated that with HAI of high-dose verapamil (25 times the standard dose), our CHVI-CF circuit reduces systemic exposure to verapamil by greater than 90% (unpublished data). Future trials of the CHVI-CF system will combine P-glycoprotein-blocking agents such as verapamil with chemotherapeutic drugs to determine the impact on hepatocellular toxicity and the treatment's efficacy against multidrug-resistant VX-2 tumors. By limiting systemic toxicities from the P-glycoprotein-blocking agents and chemotherapeutic drugs, an improved antitumor response in liver tumors may be achieved.

References

1. Frei E, Canellos GP (1980) Dose: a critical factor in cancer chemotherapy. Am J Med 69: 585–594
2. Moscow JA, Cowan KH (1991) Multidrug resistance. In: HM Pinedo, DL Longo, Chabner BA (eds) Cancer chemotherapy and biologic response modifiers, annual 12. Elsevier, New York, pp 91–109
3. Spoelstra EC, Dekker H, Schuurhuis GJ, Broxterman HJ, Lankelma J (1991) P-glycoprotein drug efflux pump involved in the mechanisms of intrinsic drug resistance in various colon cancer cell lines. Biochem Pharmacol 41: 349–359
4. Borst P (1991) Genetic mechanisms of drug resistance. Rev Oncol 4: 87–105
5. Moscow JA, Fairchild CR, Madden MJ, Ransom DT, Wieand HS, O'Brien EE, et al (1989) Expression of anionic glutathione-S-transferase and P-glycoprotein in human tissues and tumors. Cancer Res 49: 1422–1428
6. Pastan I, Willingham MC, Gottesman M (1991) Molecular manipulations of the multidrug transporter: a new role for transgenic mice. FASEB J 5: 2523–2528
7. Bern MM, McDermott W, Cady B, Oberfield RA, Trey C, Clouse E, et al (1978) Intraarterial hepatic infusion and intravenous Adriamycin for treatment of hepatocellular cancer. Cancer 42: 399–405
8. Garnick MB, Ensminger WD, Israel M (1979) A clinical pharmacologic evaluation of hepatic arterial infusion of Adriamycin. Cancer Res 39: 4105–4110
9. Khayat D, LeCesne A, Weil M, Azar N, Cour V, Vu Thi Myle C, et al (1988) Intra-arterial treatment of hepatic metastases using the 5-fluorouracil, Adriamycin, mitomycin C (FAM) chemotherapeutic regimen. Regional Cancer Treat 1: 62–64
10. O'Connell MJ, Hahn RG, Rubin J, Moertel CG (1988) Chemotherapy of malignant hepatomas with sequential intraarterial doxorubicin and systemic 5-fluorouracil and semustine. Cancer 62: 1041–1043
11. Ensminger WD, Rosowsky A, Raso V, Levin DC, Glode M, Come S, et al (1978) A clinical-pharmacologic evaluation of hepatic arterial infusions of 5-fluoro-2-deoxyuridine and 5-fluorouracil. Cancer Res 38: 3784–3792
12. Chen HSG, Gross JF (1980) Intra-arterial infusion of anticancer drugs: theoretic aspects of drug delivery and review of responses. Cancer Treat Rep 64: 31–40
13. Harris PA, Gross JF (1975) Preliminary pharmacokinetic model for Adriamycin (NSC-123127). Cancer Chemother Rep 59: 819–825
14. Ballet F, Vrignaud P, Robert J, Rey C, Poupon R (1987) Hepatic extraction, metabolism, and biliary excretion of doxorubicin in the isolated perfused rat liver. Cancer Chemother Pharmacol 19: 240–245
15. Nakamura S, Tsuzuki T (1981) Surgical anatomy of the hepatic veins and the inferior vena cava. Surg Gynecol Obstet 152: 43–50
16. Rous P, Beard JW (1935) The progression to carcinoma of virus-induced rabbit papillomas (Shope). J Exp Med 62: 523–548
17. Hough A, Seyberth H, Oates J, Hartmann W (1977) Change in bone and bone marrow of rabbits bearing the VX-2 carcinoma. Am J Pathol 87: 537–552
18. Galasko CS, Muckle DS (1974) Intrasarcolemmal proliferation of the VX-2 carcinoma. Br J Cancer 29: 59–65
19. Robert J (1980) Extraction of anthracyclines from biologic fluids for HPLC evaluation. J Liq Chromatogr 3: 1561–1572

20. Collins JM (1984) Pharmacologic rationale for regional drug delivery. J Clin Oncol 2: 498–504
21. Dedrick RL (1988) Arterial drug infusion: pharmacokinetic problems and pitfalls. J Natl Cancer Inst 80: 84–89
22. Legha SS, Benjamin RS, MacKay B, Ewer M, Wallace S, Valdivieso M, et al (1982) Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 96: 133–139
23. Winchester JF, Rahman A, Tilstone WJ, Kessler A, Mortensen L, Schreiner GE, et al (1979) Sorbent removal of Adriamycin in vitro and in vivo. Cancer Treat Rep 63: 1787–1793
24. Harada T, Ohmura H, Nishizawa O, Tsuchida S (1981) Regional arterial infusion of an anticancer drug combined with direct hemoperfusion. Tohoku J Exp Med 133: 423–429
25. Kihara T, Nakazawa H, Agishi T, Honda H, Ota K (1988) Superiority of selective bolus infusion and simultaneous rapid removal of anticancer agents by charcoal hemoperfusion in cancer treatment. Trans Am Soc Artif Intern Organs 34: 581–584
26. Oldfield EH, Clark WC, Dedrick RL, Egorin MJ, Austin HA, DeVroom HD, et al (1987) Reduced systemic drug exposure by combining intraarterial *cis*-diamminedichloroplatinum(II) with hemodialysis of regional venous drainage. Cancer Res 47: 1962–1967
27. Graham RA, Siddik ZH, Hohn DC (1990) Extracorporeal hemofiltration: a model for decreasing systemic drug exposure with intra-arterial chemotherapy. Cancer Chemother Pharmacol 26: 210–214
28. Oldfield EH, Dedrick RL, Yeager RL, Clark WC, DeVroom HD, Chatterji CD, et al (1985) Reduced systemic drug exposure by combining intra-arterial chemotherapy with hemoperfusion of regional venous drainage. J Neurosurg 63: 726–732
29. Ridge JA, Collins C, Bading JR, Hancock C, Conti PS, Daly JM, et al (1988) Increased Adriamycin levels in hepatic implants of rabbit VX-2 carcinoma from regional infusion. Cancer Res 48: 4584–4587
30. Hoshino M, Koyama Y, Abe R (1991) Comparative study on the comparative efficacy of hepatic arterial or portal venous infusion of Adriamycin to control hepatic carcinoma growth. Jpn J Cancer Chemother 18: 1894–1897
31. Curley SA, Byrd DR, Newman RA, Carrasco CH, Cromeens D, Ellis HJ, et al (1991) Hepatic arterial infusion chemotherapy with complete hepatic venous insolation and extracorporeal chemofiltration. Anticancer Drugs 2: 175–183
32. Curley SA, Byrd DR, Newman RA, Ellis HJ, Chase J, Carrasco CH, et al (1993) Reduction of systemic drug exposure following hepatic arterial infusion of doxorubicin with complete hepatic venous isolation and extracorporeal chemofiltration. Surgery (in press)
33. Curley SA, Newman RA, Dougherty TB, Fuhrman GM, Stone DL, Mikolajek JA, et al. (1994) A novel system to treat human hepatocellular carcinoma: a phase I study. Ann Surg Oncol (in press)
34. Weinstein RS, Grogan TM, Kuszak JR, Jakate SM, Kluskens LF, Coon JS (1991) Multidrug resistance gene product (P-glycoprotein) in normal tissues and tumors. Adv Pathol Lab Med 10: 207–234
35. Ramirez L, Mankarios H, Adouin P, Zhao Z, Chabo GG, Rougier P, et al (1992) Reduction of verapamil cardiac effects by intraarterial hepatic administration. Proc Am Assoc Cancer Res 33: 482
36. Ford JM, Hait WM (1990) Pharmacology of drugs that alter multidrug resistance in cancer. Pharmacol Rev 42: 155–199