A Phase I Trial of Intrahepatic Verapamil and Doxorubicin

Regional Therapy to Overcome Multidrug Resistance

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Background. **Verapamil can modulate multidrug resistance in vitro, but only at levels that are not tolerable when administered systemically. Regional strategies of drug administration may permit the delivery of high concentrations of a drug to specific areas with lower systemic levels. Colorectal cancers typically express the multidrug resistance phenotype.**

Methods. **A Phase I trial was performed to determine the maximum tolerable dose (MTD) and dose limiting toxicities of verapamil by hepatic artery infusion, together with doxorubicin, to patients with hepatic metastases of colorectal cancer. Fourteen patients with metastatic colorectal cancer received a 14-hour intrahepatic infusion of verapamil. Six hours after the start of the infusion, a fixed dose of doxorubicin (50 mg/m^2) was given, also via the hepatic artery, over a 30-minute period. Patients were followed by cardiac telemetry but were not in an intensive care setting, and no invasive monitoring was used. All patients had received prior intrahepatic chemotherapy.**

Results. **The MTD of intrahepatic verapamil on this schedule in this patient population was 1.2 mg/kg/hour. Hypotension was the dose limiting toxicity. No major objective responses were noted in this heavily pretreated patient population. A dose of 1.0 mg/kg/hour is recommended for Phase II trials.**

Conclusions. **Based on estimations of normal hepatic artery blood flow, the estimated concentration of verapamil delivered to the hepatic tumors at 1.0 mg/kg/hour is 3.6 μg/ml (7.3 μM), which is comparable to concentrations at which an in vitro reversal of MDR is seen. This study demonstrates that the systemic toxicities of an MDR reversal agent can be overcome by regional drug delivery, establishing this approach as an important model system for further study of MDR modulation. *Cancer* 1994; 74:2757–64.**

Key words: multidrug resistance, verapamil, intrahepatic.

Doxorubicin-resistant tumor cell lines that exhibit the multidrug resistance (MDR) phenotype often can be rendered sensitive to doxorubicin in vitro by exposure to sufficient concentrations of verapamil. Many investigators have attempted to exploit this observation in clinical trials. However, such investigations have been limited because the concentrations of verapamil required for in vitro activity are greater than those patients can tolerate systemically; thus, intervening bradycardia, hypotension, or congestive heart failure invariably has prevented patients from achieving desired serum verapamil levels.[1]

Regional drug administration has been used in chemotherapy to increase the local drug concentration in a tumor-containing area of the body while maintaining tolerable systemic levels.[2] Regional therapies for hepatic metastases have taken advantage of the dual blood supply of the liver. Although normal hepatic tissues receive most of their blood supply from the portal circulation, clinically apparent hepatic metastases derive their blood supply almost exclusively from the he-

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‖ Recipient of an American Cancer Society Career Development Award.

Supported in part by NIH-PO1-CA-47997 and CA-18856.

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Received May 20, 1994; revision July 21, 1994; accepted July 21, 1994.

patic artery.[3,4] Thus, in a patient with hepatic metastases, hepatic arterial administration provides a high concentration of drug to the tumor.

Agents that have a high first-pass hepatic clearance offer additional potential for enhanced pharmacologic advantage from intrahepatic administration because higher local concentrations can be administered, with most of the drug being metabolized before reaching the systemic circulation.[5]

Fluoridated pyrimidines, which remain the mainstay of systemic chemotherapy for colorectal cancers, have high first-pass hepatic clearances and have been studied extensively in intrahepatic treatment strategies.[6]

Verapamil has been shown to have a first-pass hepatic clearance of approximately 85% in patients with normal hepatic function,[7] suggesting that significant regional dose intensification might be possible with hepatic artery administration. Given this background, we investigated the feasibility of regional (hepatic arterial) administration of verapamil, with the goal of achieving high verapamil levels in the blood supply of hepatic metastases while maintaining tolerable systemic levels. We studied patients with hepatic metastases of colorectal adenocarcinomas because colorectal cancers have been demonstrated to have a high incidence of the MDR phenotype, as evidenced by increased expression of P-glycoprotein on the cell surface.[8] Doxorubicin, and virtually all other antineoplastic agents that are known to be modulated by P-glycoprotein, are essentially inactive agents against colorectal cancer. Doxorubicin was selected for use in this study because any activity noted from the combination of intrahepatic verapamil and doxorubicin could be reasonably attributed to MDR modulation by verapamil. In vitro studies had demonstrated that verapamil could reverse doxorubicin resistance in selected colon carcinoma cell lines.[9,10] Doxorubicin has a modest first-pass hepatic clearance, with a measured maximum plasma concentration 1.7 times higher with systemic, versus intrahepatic, administration in one report[11]; thus, a small clinical advantage was gained by intrahepatic administration of this drug.

Materials and Methods

All patients were treated on the inpatient medical service of Memorial Sloan-Kettering Cancer Center. All patients had biopsy-proven adenocarcinoma of the colon or rectum, and all had received prior intrahepatic chemotherapy, and thus had hepatic artery catheters in place. In patients who had implantable infusion pumps (13 of the 14), the side port of the pump was accessed and used for verapamil and doxorubicin therapy. One patient had an angiographically placed hepatic artery catheter that was attached to a subcutaneous, surgically placed, infusion port. Pretreatment evaluation of all patients consisted of a full history and physical examination, computed tomography scan of the abdomen, chest X-ray, and a Tc^{99} macroaggregated albumin flow study of the hepatic artery access device to assure proper distribution of flow throughout the liver. Patients were required to have a leukocyte count of 3500 cells/mm^3 or greater, platelet count of 150,000/mm^3 or greater, creatinine of < 1.5 mg/dl, and total bilirubin of < 1.5 mg/dl. Patients were required to have a normal cardiac ejection fraction, as demonstrated by radionuclide cineangiography. Patients receiving digoxin, beta blockers, calcium channel blockers, or other cardiac or antihypertensive medications were excluded.

This trial was reviewed and approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center. All patients were fully informed with regard to the investigational nature of this trial, and all signed informed consent.

Patients were admitted on the day before therapy, and a full admission history and physical was performed. The hepatic artery catheter (in most instances, the side port of an implantable hepatic artery pump) was accessed, and cardiac monitoring was begun to establish a baseline cardiac rhythm. Patients received intravenous hydration with $D_5$1/2 normal saline plus 20 meq KCl/l at 100 ml/hour, beginning the evening before verapamil administration to assure adequate intravascular volume. At T = 0, patients received an intrahepatic bolus of verapamil 0.15 mg/kg, and an intrahepatic verapamil infusion was begun via an external arterial pump. The initial starting dose of verapamil, 0.3 mg/kg/hour, was selected because this dose was tolerable systemically in seven of seven patients reported in a previous verapamil trial.[1] In the absence of dose limiting toxicity, this was increased three times by 0.1-mg/kg/hour increments at 30-minute intervals $\times$ 3, to a maximum of 0.6 mg/kg/hour. This dose, representing the first treatment level, was maintained for the remainder of the 14-hour infusion, unless intervening toxicity necessitated a change. Vital signs and PR interval measurements were obtained every 15 minutes for the first 2 hours, and hourly thereafter for the remainder of the 14-hour treatment.

Each subsequent dose level began at the maximum dose of the previous level and escalated twice at 30-minute intervals by 0.1-mg/kg/hour increments; thus, on the second dose level, the dosage was begun at 0.6 mg/kg/hour and escalated during a period of 1 hour to 0.8 mg/kg/hour. Treatment levels planned on the protocol were 0.6, 0.8, 1.0, 1.2, and 1.4 mg/kg/hour,

with subsequent levels increasing at 0.2-mg/kg/hour intervals.

The study design stipulated that three patients would be treated on the first level (that is, a maximum infusion rate of 0.6 mg/kg/hour). If no dose limiting toxicities were noted, patients were enrolled in the next treatment level. If one patient experienced toxicity, the level was expanded to six patients to better define that toxicity. The maximum tolerable dose was considered to have been reached when either two of three patients or three of six patients experienced dose limiting toxicity. Dose limiting toxicities of verapamil were defined as a PR interval of more than 0.24 seconds, pulse of less than 50 bpm, second degree atrioventricular block or greater, clinical evidence of congestive heart failure, or systolic blood pressure < 80 mmHg. Isolated blood pressure readings below 80 mmHg in patients with no symptoms and that responded promptly to fluid challenge without a change in the verapamil infusion rate were not considered to be dose limiting. Because cumulative toxicity of verapamil was not considered to be a major concern, patients who tolerated a treatment cycle without experiencing dose limiting toxicity were permitted to have the next higher dose level of verapamil in subsequent treatments.

Doxorubicin was given, also intrahepatically, at a fixed dose of 50 mg/m^2 during a period of 30 minutes, beginning 6 hours into the verapamil infusion. Treatments were repeated on a 21-day cycle. Patients experiencing Grade 3 or 4 granulocytopenia were treated on subsequent cycles with granulocyte-colony stimulating factor, 5 μg/kg/day, beginning 24 hours after doxorubicin administration and continuing until granulocyte recovery was observed.

Pharmacokinetic Methods

Systemic verapamil and norverapamil levels were drawn at T = 6 hours, just before the doxorubicin infusion, and at T = 14 hours, just before discontinuation of the verapamil infusion. In 11 of the 14 treated patients, systemic doxorubicin levels were drawn at the end of the doxorubicin infusion and at 15, 30, 60, 120, 240, and 480 minutes to define the pharmacokinetics of intrahepatic doxorubicin when given with intrahepatic verapamil.

Doxorubicin was measured by high-performance liquid chromatography using a Zorbax Phenyl 8-cm × 4.6-mm column (Mac-Mod Analytical, Inc., Chadds Fort, PA). The mobile phase was composed of 72% 0.34 M phosphoric acid and 28% acetonitrile. A Shimadzu (Columbia, MD) RF-535 fluorescence detector was used, with excitation:480 nm and emission:560 nm. The flow rate was 1 ml/minute. The standard curve range was 0.025–2.5 μg/ml. Because of limited design points, a biexponential model of the form

$$C = A \times e^{-\alpha t} + B \times e^{-\beta t}$$

was fitted to each individual profile of the plasma concentration of doxorubicin (C μg/ml) versus time (t) using nonlinear regression (Proc NLIN, SAS). Assuming first-order elimination in plasma, elimination half-life values were calculated by dividing 0.693 by the terminal elimination rate constant, β. The total area under the curve (AUC) was determined using the equation

$$AUC = A/\alpha + B/\beta$$

and the observed peak plasma level was always the level at time 0 (the end of the doxorubicin infusion).

Verapamil and norverapamil levels were measured by standard techniques in our clinical laboratory.

Results

Fifteen patients with colorectal cancers metastatic to the liver were enrolled in this trial. Fourteen received at least one full treatment of verapamil and doxorubicin. One patient experienced toxicity from verapamil after 3 hours and before receiving doxorubicin and was removed from the study. A total of 36 treatment courses were given, with a median of 2 cycles per patient (range, 1–5 cycles). Patients were permitted to have extrahepatic disease if that disease was thought not to be a current source of morbidity and if the patient's disease was predominantly in the liver.

Prior Chemotherapy

All 14 patients treated in this study had received prior intrahepatic floxuridine-based chemotherapy via an implantable pump. In addition, nine had received a second intrahepatic regimen with mitomycin given through the side port of the implantable hepatic arterial pump. One patient receiving mitomycin also had received intrahepatic carmustine. Seven patients had received, in addition to their intrahepatic therapy, a systemic, 5-fluorouracil based chemotherapy regimen. Patient characteristics are outlined in Table 1.

Verapamil Toxicity

The maximum tolerable dose of verapamil by 14-hour intrahepatic infusion was 1.2 mg/kg/hour. Symptomatic hypotension was the dose limiting toxicity, with two patients at this dose level experiencing symptomatic drops in systolic blood pressure below 80 mmHg.

Table 1. Patient Characteristics

Median age (yr) (range)	54 (35–70)
Median KPS (range)	80 (70–90)
Male:female	7:7
Prior therapy	
None	0
Intrahepatic (IH) chemotherapy	14
FUDR-based only	(4)
FUDR-based + mitomycin	(9)
FUDR-based + mitomycin + BCNU	(1)
Systemic chemotherapy (in addition to IH)	(7)

KPS: Karnofsky performance status; IH: intrahepatic; FUDR: floxuridine; BCNU: carmustine.

Symptoms were a subjective feeling of lightheadedness, mild nausea, and apprehension. These symptoms and hypotension resolved promptly with fluid challenge, interruption of the verapamil infusion, and administration of one ampule of calcium gluconate. Two additional patients who were receiving lower verapamil levels experienced asymptomatic, transient drops in pressure that were responsive to intravenous fluid challenges.

Pulse rates also were observed to drop while the patients received verapamil, but no patient experienced bradycardia severe enough to require a dose reduction in verapamil. The median lowest pulse recorded for each patient was 64 bpm (range, 49–76 bpm). One patient experienced a pulse deceleration to 49 bpm, which was transient, self-limiting, and asymptomatic. This did not recur for the remainder of the treatment cycle or during subsequent cycles.

All patients had electrocardiographic evidence of verapamil toxicity. PR intervals widened a median of 0.04 seconds (range, 0.00–0.10 seconds) when PR intervals were present. Ten of the 14 patients treated experienced an accelerated junctional rhythm that was not dose limiting and that obliterated the PR interval (see Fig. 1). This accelerated junctional rhythm, a known manifestation of verapamil toxicity, and all other electrocardiographic abnormalities that occurred during treatment resolved by the morning after completion of the verapamil infusion.

The patient who was unable to tolerate the verapamil infusion experienced second degree heart block after less than 3 hours of treatment at the 0.8 mg/m^2/hour rate. Review of hepatic artery flow studies demonstrated that the hepatic artery catheter was perfusing only the right lobe of the liver, which was almost totally replaced by tumor. As such, there was virtually no normal liver that was being perfused, so there was virtually no hepatic tissue to metabolize the verapamil. We pos-

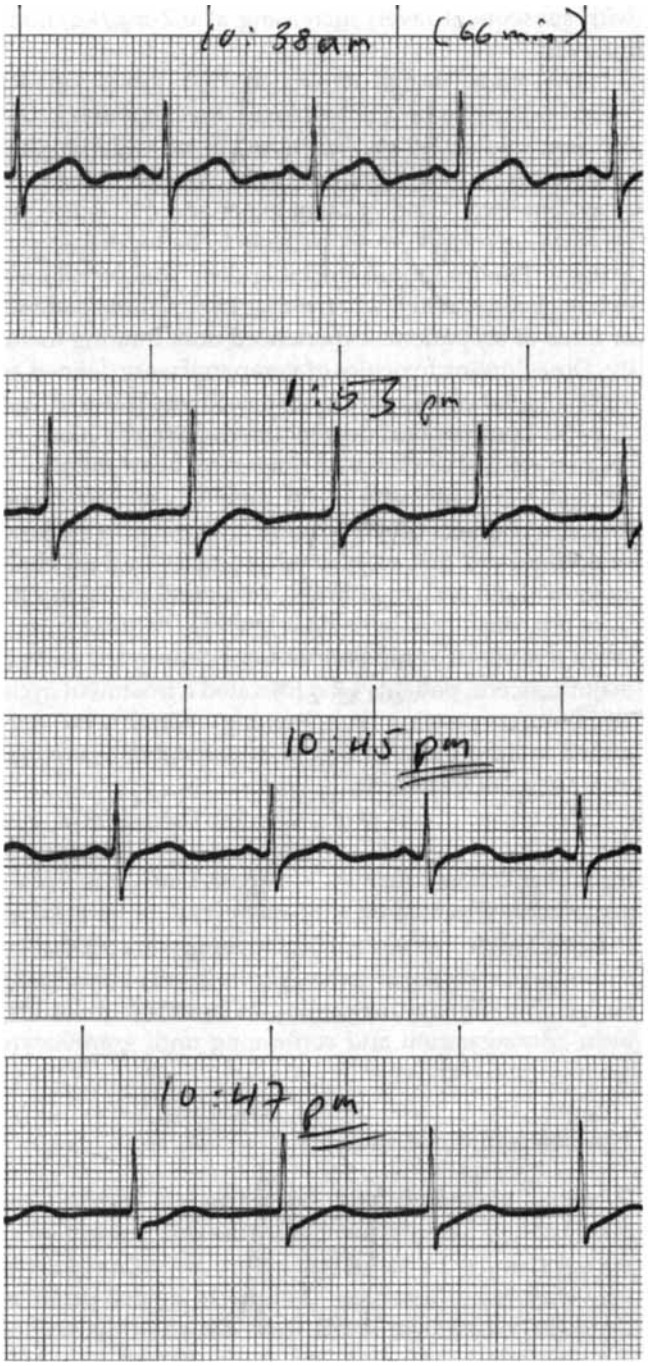

Figure 1. During a 14-hour infusion of intrahepatic verapamil, an accelerated junctional rhythm occurred, intermittently alternating with sinus rhythm. (Top) Clear sinus P waves are seen. (Middle top) The QRS is superimposed on the P wave as the heart rate increases from 76 to 80 bpm. (Middle bottom) Sinus rhythm is seen again at a heart rate of 73 bpm, followed by (bottom) the accelerated junctional rhythm at a rate of 75 bpm 2 minutes later.

tulate that this may explain this patient's intolerance of this regimen. Unfortunately, verapamil levels were not drawn during the time that she was experiencing toxicity. This patient was removed from study without having received doxorubicin.

No treatment related deaths occurred during this study.

Doxorubicin Toxicity

The major toxicity associated with doxorubicin on this study was myelosuppression. The median leukocyte count nadir was 2700 cells/mm^3 (range, 800–10,300 cells/mm^3), and the median platelet count nadir was 192,000 cells/mm^3 (range, 21,000–416,000 cells/mm^3). Two patients were admitted to the hospital for treatment of neutropenic fevers. Nonhematologic toxicities attributable to doxorubicin were relatively mild and were not dose limiting.

Only one patient received more than 200 mg/m^2 of doxorubicin during this study. That patient had a repeat gated heart scan after his fourth cycle of therapy. This revealed no change from his initial baseline study.

Pharmacokinetics

Verapamil and norverapamil levels were measured in the peripheral blood at T = 6 hours and at the end of the verapamil infusion. The hepatic arterial concentrations of verapamil were calculated based on standard estimates of normal hepatic artery blood flow. The estimated normal blood flow through the hepatic artery of a 70-kg man is approximately 400 ml/minute.[12,13] Taking this estimate of blood flow, the calculated verapamil concentration in the hepatic artery at the maximum tolerable dose of 1.2 mg/kg/hour would be as follows:

$$1.2 \text{ mg/kg/hour} \times 70 \text{ kg} = 84 \text{ mg/hour}$$

$$= 1.4 \text{ mg/minute}$$

$$1.4 \text{ mg/minute} \div 400 \text{ ml/minute} = 0.0035 \text{ mg/ml}$$

$$= 3.5 \ \mu g/ml$$

The estimated hepatic artery concentration for the recommended Phase II dose of 1.0 mg/kg/hour is 2.9 μg/ml (5.9 μM). This would be the estimated value if there were no circulating verapamil levels. However, at the time of doxorubicin administration, our patients had a median circulating verapamil level of 0.54 μg/ml (1.1 μM) (range, 0.19–0.91 μg/ml) in the 11 patients for whom verapamil levels were obtained, so the true estimated hepatic artery verapamil concentration would be 2.9 + 0.54 = 3.44 μg/ml (7.0 μM). It should be noted that systemic verapamil levels were not at a steady state by the end of the verapamil infusion. Norverapamil, the primary metabolite of verapamil, also was measured in the circulating plasma and found to be at a median level of 0.22 μg/ml (range, 0.11–0.43 μg/ml). The circulating levels of norverapamil also may contribute to P-glycoprotein inhibition because this metabolite also appears to be active against P-glycoprotein (unpublished data).

There was no clear correlation between verapamil dose level and measured plasma levels, suggesting that there was a large interpatient variation in the degree of first-pass hepatic clearance of verapamil. It should be emphasized that our calculations are based on expected normal blood flow in healthy patients without liver metastases. We do not know to what degree the presence of factors such as liver metastases or the vascular effects of verapamil may have augmented hepatic artery blood flow.

Table 2. Plasma Pharmacokinetics of Intrahepatic Doxorubicin Given by 30-Minute Infusion With Concurrent Intrahepatic Verapamil

No. of patients	AUC (μg/ml per min)	$T_{1/2}\alpha$ (min)	$T_{1/2}\beta$ (min)	C_{max} (μg/ml)
11	37 (12–59)	4 (2–5)	133 (63–267)	1.3 (0.3–2.0)

AUC: area under the plasma concentration versus time curve; C_{max}: peak plasma concentration; $T_{1/2}\alpha$: half-life, distribution phase; $T_{1/2}\beta$: half-life, elimination phase.
Values are median (range).

Doxorubicin

Plasma analyses for doxorubicin were performed in 11 patients. The median and range of the observed peak level (C_{max}), AUC, and the estimated distribution (T 1/2 α) and elimination (T 1/2 β) half-lives are listed in Table 2. Distribution half-lives were brief, with a median of 4 minutes. The median of the elimination half-lives was 2.2 hours (range, 1.0–4.5 hours). Plasma doxorubicin concentrations were near or below the limit of detection in most patients at t = 8 hours. A representative plasma decay curve is shown in Figure 2.

Responses

No major objective responses were seen in the 14 patients who were treated. As noted, all patients in this study were heavily pretreated, and all had progressed through at least one intrahepatic chemotherapy regimen before initiation of this study. Also of note, no objective responses were seen after any salvage chemo-

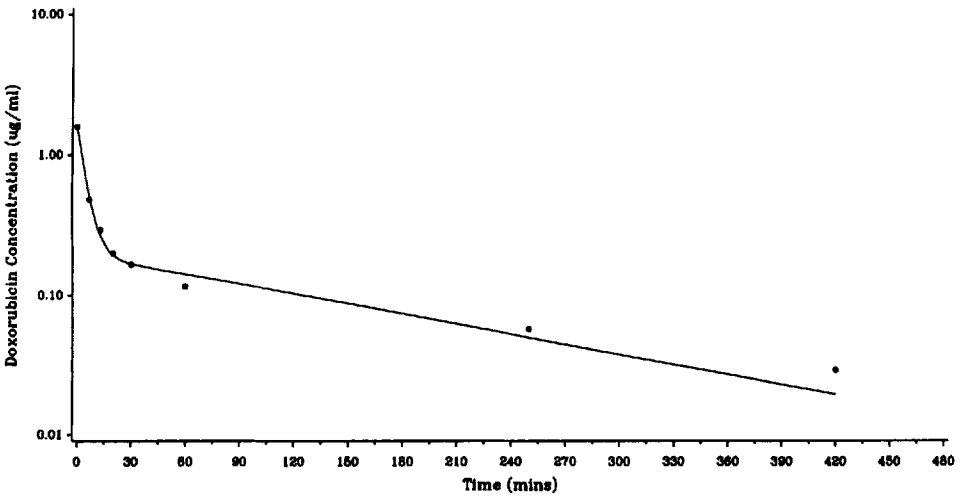

Figure 2. Representative plasma decay of doxorubicin after 30 minutes of intrahepatic infusion.

therapy attempts given after patients were removed from this study.

Discussion

For many neoplasms, MDR is an important mechanism of defense against a number of chemotherapeutic agents in vitro.[14–19] However, the clinical significance of MDR in vivo remains less well established. Much of the difficulty in clinical testing of MDR-reversing agents stems from the systemic toxicities encountered. The levels of verapamil and many other MDR-reversing agents that are needed for activity in the laboratory typically are higher than those that can be achieved and sustained clinically. Laboratory investigations with verapamil demonstrate MDR-reversing activity at a wide range of concentrations, with most investigators reporting significant biologic effects at concentrations ranging from 2 to 10 μg/ml.[9,10,20,21] Levels of 0.3 μg/ml or greater are considered clinically toxic when verapamil is used for its conventional therapeutic indications.

We used regional therapy to attempt to increase the concentration of verapamil administered to the tumor. This strategy of intrahepatic administration has been highly successful in increasing the dose intensity of fluoridated pyrimidines administered to hepatic metastases of colorectal cancer.[2,6] A key factor that influences the usefulness of a drug in hepatic arterial administration is the degree to which that drug is cleared by its first pass through the liver. Drugs with high first-pass clearances are able to achieve greater pharmacologic advantage in this regional administration system. Verapamil has been demonstrated in patients with normal hepatic function to have approximately an 85% first-pass clearance[7] and thus is a reasonable candidate for regional administration studies. Studies of intrahepatic verapamil infusions in rabbits indicated that higher amounts of verapamil were tolerable by this route than by systemic administration.[20]

Based on calculations using estimated hepatic arterial flow, we calculated a hepatic artery concentration of verapamil of 3.5 μg/ml (7.1 μM) at the maximum tolerable dose and 2.9 μg/ml (5.9 μM) at the recommended Phase II dose. If these estimated concentrations are correct, we are administering a level of verapamil to the tumor that is in the range needed for in vitro reversal of MDR. However, there may be several limitations to our assumptions. For example, we do not know what effect a high dose verapamil infusion has on the blood flow in the hepatic artery. In addition, the presence of hepatic metastases may alter the normal hepatic arterial blood flow. Because all of our patients had received prior intrahepatic chemotherapy, it is possible that this could have caused vascular or ischemic damage to the hepatic arterial circulation, which may have altered blood flow or vessel responsiveness to verapamil.

Circulating levels of verapamil in some patients were high enough that more systemic toxicity might have been expected than was encountered. One possible explanation for this is that the s-isomer of verapamil is metabolized more rapidly than is the r-isomer.[21,22] R-verapamil has been reported to have significantly less systemic cardiovascular activity than does the s-isomer[23] but maintains similar activity against p-glycoprotein.[24] Thus, if the circulating verapamil pool is shifted toward a large proportion of r-verapamil, less systemic toxicity would be expected for a given verapamil level. We plan to analyze future specimens to determine whether such a shift in r-s isomer ratio is occurring.

Levels of verapamil at the termination of infusion typically were higher than the levels drawn at 6 hours (the time of doxorubicin administration). This indicates

that steady state verapamil levels were not achieved at the time of doxorubicin administration. The optimal time for infusion of verapamil or other MDR-reversing agents has not been established, and the time selections were somewhat arbitrary. Verapamil administration was continued for 8 hours after the initiation of the doxorubicin infusion. Our pharmacokinetic data show a median terminal half-life of 133 minutes (2.2 hours). However, data previously published regarding intrahepatic doxorubicin indicated a median terminal half-life of 13 hours (range, 8.8–28.9 hours).[11] Thus, although the duration of verapamil infusion was likely to be sufficient to modulate doxorubicin interaction with the p-glycoprotein during the time of maximum doxorubicin concentration, a longer verapamil exposure may be more appropriate in future studies. As with this study, practical considerations in terms of the requirements for patient monitoring will have to figure into the duration of verapamil infusions. Our methodology used in measuring doxorubicin levels is different from that used in older studies, and the patient populations studied were different; thus, direct comparisons may not be appropriate. It also is possible that the limited duration of sampling in our study was insufficient to identify a late terminal tertiary phase.

Other investigators have pursued different routes in attempting to achieve higher serum levels of MDR-reversing agents with acceptable toxicity. R-verapamil, a stereoisomer of the racemic verapamil used in current clinical practice, has been reported to have significantly less cardiac toxicity with MDR modulating activity comparable with the racemic mixture. Clinical trials using r-verapamil are under way in a number of refractory tumors.[25] Cyclosporine also has been studied in clinical trials for modulation of p-glycoprotein.[26,27] Levels obtained clinically were reported to be comparable to those needed for MDR manipulation in vitro. However, toxicity did limit additional dose escalations. Trials are under way with an analog of cyclosporine, which has virtually no immunosuppressive or renal effects and which is anticipated to be far less toxic.

In summary, our study represents the first use of regional administration, in humans, of an MDR modulation agent to abrogate systemic toxicity. Results, in terms of the levels of verapamil achieved with tolerable toxicity, are encouraging. No tumor responses were seen. However, the patient population studied was heavily pretreated, and the numbers treated at or above the recommended Phase II dose are small. Formal Phase II evaluations of regional verapamil administration in patients with colorectal cancer without extensive prior therapy are indicated, and such trials are planned. Trials in other tumors with known high expression of p-glycoprotein also are indicated. These trials would be expected to provide important insights into the clinical utility of MDR reversal strategies.

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