Verapamil-Reversing Concentrations Induce Blood Flow Changes that Could Counteract In Vivo the MDR-1–Modulating Effects

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Background. **Intraarterial hepatic (IAH) administration of verapamil should achieve mdr-1–reversing concentrations with reduced cardiac toxicity. The authors have explored the tolerance of its IAH administration and its effects on doxorubicin pharmacodynamics.**

Methods. **Verapamil was given to rabbits by intravenous or IAH administration, and its effects on heart rates were compared. Doxorubicin then was given intravenously either with IAH verapamil or with an IAH control perfusion, and tumor and liver drug concentrations were determined. Hepatic blood flow changes were studied by the administration of ^{99m}Tc-albumin macroaggregates (^{99m}Tc-MAA) under verapamil IAH perfusions.**

Results. **Compared with the intravenous route, IAH administration of verapamil was not toxic, and cardiac effects were reduced significantly. Its effect on doxorubicin distribution was detrimental, because the tumor-liver doxorubicin concentration ratios were lower in the verapamil group (0.23 vs. 3.37; $P < 0.05$). Tumor doxorubicin concentrations were lower when verapamil was coinfused (43 vs. 573 ng/100 mg tissue; $P < 0.05$). In normal liver tissue, increased amounts of doxorubicin and metabolites were observed. The verapamil IAH perfusions with ^{99m}Tc-MAA confirmed a differential action on tumor and normal vessels; the distribution of radionuclide was diverted away from the tumor bed significantly when verapamil was administered (tumor-to-liver ratio of 25.3 control rabbits vs. 5.99 rabbits who received verapamil; $P < 0.05$).**

Conclusions. **Reversing the concentrations of verapamil provoked changes in the distribution of the liver blood flow. The hemodynamic effects of verapamil regional perfusions could counteract in vivo its potential mdr-1–reversing properties. *Cancer* 1994; 74:810–6.**

Key words: verapamil, doxorubicin, hepatic artery infusions, VX2 tumor, drug resistance, hepatic blood flow.

One of the main challenges in clinical oncology is overcoming the resistance of cancer cells to cytotoxic agents. One of the best known mechanisms of resistance is multidrug resistance mediated by p170 glycoprotein (P-gp), which concerns some of the most active anticancer agents and results in the efflux of drugs out of the cell.[1,2] This active process can be impaired by a number of molecules, among which is to be found verapamil, a calcium channel blocker, considered the standard reversing agent. Its activity has been essentially described in vitro in multiple cell lines, and the reversal of drug resistance has been achieved at concentrations approaching 7 μM.[3] Circumvention of drug resistance also has been obtained in vivo in murine models, with prolonged survival of mice grafted with resistant tumors.[4] However, these studies provided limited data on the pharmacodynamic interactions between reversing agents and cytotoxic agents.

In vivo, verapamil may exert other modulating actions in addition to blockage of P-gp. Indeed, increased antitumor effects have been observed in sensitive tumors[5,6] or after treatment with drugs not affected by the mdr-1 mechanism.[7] That verapamil may interfere with the metabolism or the elimination of cytotoxic drugs cannot be ruled out. Such was the case with

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cyclosporine[8] and other substrates,[9] leading to higher systemic exposure to cytotoxic agents. Antimetastatic effects also have been reported and probably are related to inhibitory effects of verapamil on platelet aggregation.[10] All of these data underline the difficulties encountered when attempting to transfer in vitro data to in vivo applications.

Attempts to reverse drug resistance in patients encountered additional problems, such as severe cardiac side effects before reversing plasma concentrations were reached. The patients who received systemic verapamil showed highly variable plasma concentrations, at most 1.5 μM, and transient but significant cardiac side effects, which limited additional dose escalations.[11] Tumor responses have been obtained in some cases of refractory myeloma,[12] lymphoma,[13] and pediatric leukemia,[14] whereas solid tumors do not seem to have benefited from the adjunct of verapamil to cytotoxic drugs.[15,16]

In an attempt to reduce systemic side effects and to attain sufficient plasma concentrations in target tissues, we envisaged the intraarterial hepatic administration (IAH) of verapamil and doxorubicin in an animal model. The IAH administration would achieve high local levels, thereby providing the means of reversing drug resistance, but also avoiding most of its cardiac side effects caused by a high first-pass hepatic extraction. The modulation of drug resistance by verapamil in patients given IAH chemotherapy for colorectal cancer should increase response rates because liver metastases highly express the mdr-1 phenotype and are particularly refractory to chemotherapy, even via the IAH route.

The VX2 hepatic tumor model in rabbits is widely accepted for locoregional studies. Not only can the regional effects of verapamil (or other reversing agents) be tested, but the pharmacodynamics between drug resistance reversers and cytotoxic agents also can be analyzed. Catheter placement through the gastroduodenal artery can be performed without ligation of the hepatic artery, and perfusions can be administered under proper cardiac monitoring, thus rendering experiments closer to clinical conditions than those prevalent in murine models.

This study was conducted to assess the in vivo effects of a high dose perfusion of verapamil via the hepatic artery and its effects on doxorubicin tissue concentrations.

Materials and Methods

Animals and Anesthesia

Female New Zealand white rabbits weighing 2.7–3.2 kg were used (Elevage Scientifique des Dombes, Romans, France). The rabbits were maintained under standard conditions on a laboratory diet and water ad libitum. All procedures were performed under general intravenous (IV) anesthesia using ketamine hydrochloride (50 mg/kg) and xylazine 2% (0.1 ml/kg). All experiments were conducted in accordance with the European Council directives and French legislation concerning animal welfare.

Drugs and Chemicals

Doxorubicin hydrochloride, doxorubicinol, and doxorubicinone, were provided by the Laboratoire Roger Bellon (Neuilly-sur-Seine, France). Verapamil, was provided by Laboratoires Biosedra (Levallois-Perret, France). Human albumin macroaggregates were purchased from CIS bio-international (Gif sur Yvette, France) and labeled with sodium ^{99m}Tc pertechnetate at a concentration of 2 mCi/ml. Solvents used for extraction and high pressure liquid chromatography (HPLC) analyses were all of HPLC grade or of the highest available purity.

VX2 Tumor Inoculation and Surgical Procedures for IAH Infusion

The VX2 tumor was provided by Dr. G. Orth (U190 INSERM, Institut Pasteur, Paris, France) and was maintained by serial passages in the liver of carrier rabbits. A tumor was removed from one animal, minced in NCTC 109 medium (Eurobio, Paris, France) and filtered through a cotton gauze. Hepatic implantation of the VX2 carcinoma was accomplished through a small median subxyphoid incision. Samples of 10^7 VX2 cells were injected with a 24-gauge catheter into the main portal vein, allowing the development of multiple liver metastases 3 weeks later.[17]

Hepatic artery infusions were accomplished through a 24-gauge catheter inserted into the gastroduodenal artery, with its distal tip at the confluence with the hepatic artery. The collateral duodenopancreatic arteries were ligated. Fluorescein was injected through the catheter to ensure proper perfusion of the liver. During all surgical procedures, normal blood flow in the hepatic artery was maintained.

Determination of Maximal Verapamil Tolerated Doses Via IV and IAH Routes

Intraarterial hepatic continuous perfusions of verapamil were infused during a period of 30 minutes with a minipump (MS 16 A, Graseby, Michel Frères, Montreuil, France). Cardiac effects of verapamil were compared in a paired-series experiment. Six rabbits received

IV verapamil at the maximum tolerated dose (MTD), and 10 days later, the same rabbits received IAH verapamil at the same dose as the IV group. The MTD was first determined for the IV route by escalating the initial dose of 0.02 mg/kg/minute by 0.02-mg increments under cardiac monitoring. The MTD was defined as the nonlethal dose that produced a second-degree atrioventricular block. This IV MTD was chosen as the starting dose for IAH administration. Additional dose escalation was pursued to determine the IAH MTD.

Liver toxicity caused by verapamil IAH perfusions was assessed by alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin determinations at days 1, 2, and 7 after infusion and compared with pretreatment baseline values. Histopathologic analysis of liver tissue samples was done 7 days after the perfusions.

Determination of Tissue Doxorubicin Concentrations

Doxorubicin was infused with a pump through the auricular vein at single doses of 4 mg/kg during a 5-minute period. At the end of the infusions, the animals were killed. The liver and the tumor viable outer rim were sampled and frozen at −20°C until HPLC analysis. Tissue drug concentrations were determined using reversed-phase HPLC, as previously described.[18] Briefly, samples were homogenized with 25 μl of alkaline buffer (100 nM Na_2HPO_4 with 30 nM heptasulfonic acid, pH 8.5) in DMSO (1 ml DMSO 100 mg of tissue, wet weight), and after centrifugation, the supernatant was directly injected onto the HPLC system. This system consisted of a C18 column (Nucleosil C, 10 μm, 3.9 × 300 mm, SFCC, Neuilly-sur-Seine, France), a Wisp automatic injector (710B, Waters Associated, Milford, MA), a 6000A pump (Waters Associated), and a fluorescence detector (470, Waters Associated) set at 251 nm (excitation) and 550 nm (emission). The mobile phase consisted of water (adjusted to pH 2.4 with phosphoric acid) and acetonitrile (68:32, v:v) at a flow rate of 1.75 ml/minute. Under these conditions, the retention times of doxorubicinol, doxorubicin, and doxorubicinone were, 3.59, 4.48, and 5.94 minutes, respectively. Three peaks corresponding to doxorubicinol, doxorubicin, and doxorubicinone were observed in tissue after injection of doxorubicin.

Effects of Verapamil on Tumor and Liver Doxorubicin Concentrations

For the determination of tissue doxorubicin concentrations, 12 rabbits were grafted with VX2 tumors and randomly assigned to two groups: Group A, doxorubicin IV plus NaCl 0.9% IAH; or Group B, doxorubicin IV plus verapamil IAH. Treatments were administered as single doses 14 days after tumor inoculation. Verapamil or NaCl 0.9% was given during a 30-minute infusion. Ten minutes after the beginning of the verapamil infusion, IV doxorubicin was administered during a 5-minute period. Subsequently, the IAH administration lasted 15 minutes after the end of doxorubicin infusion. The animals were killed at the end of the IAH infusion.

Determination of Blood Flow Changes During Perfusions of IAH Verapamil

Blood changes were studied by comparing the trapping of the radiotracer in the tumor and liver vascular beds after IAH administration of ^{99m}Tc-labeled macroaggregates of albumin in the two groups of rabbits. Animals with hepatic tumors were anesthetized and catheters were introduced into the gastroduodenal artery. Perfusions were done with verapamil or with NaCl 0.9% for controls as mentioned. After 10 minutes of perfusion, 0.4 mCi of ^{99m}Tc-MAA was rapidly administered via the IAH catheters, and animals were killed 5 minutes later. Samples of the liver, spleen, lungs, tumors, and blood were obtained, weighed, and counted in a calibrated automatic gamma counter (LKB CompuGamma 1282, Stockholm, Sweden). The radioactive concentration to microcurie per millicurie tissue was calculated in both groups for all tissues.

Statistical Analysis

The data from biologic results were compared using the Student *t* test. The heart beat rates during perfusions were compared by the Wilcoxon paired-series nonparametric test. Tumor and liver drug concentrations and ^{99m}Tc counts in both tissues were compared by the Mann–Whitney nonparametric test. All data are presented as means plus or minus the standard errors. Significance was assumed for all tests at the level of $P < 0.05$.

Results

Tolerance of Verapamil IAH Infusion

Intravenous perfusions of verapamil were escalated to a dose of 0.08 mg/kg/minute. At this dose, pronounced bradycardia and progressive atrioventricular block occurred as soon as 12 minutes after the start of perfusion and lasted 10 minutes after the end of the perfusion, with gradual and complete recovery 40 minutes later. If perfusions were continued beyond 30 minutes, atrioventricular dissociation was observed with death caused by cardiac arrest a few minutes later. This dose was retained as the MTD for IV experiments and as the

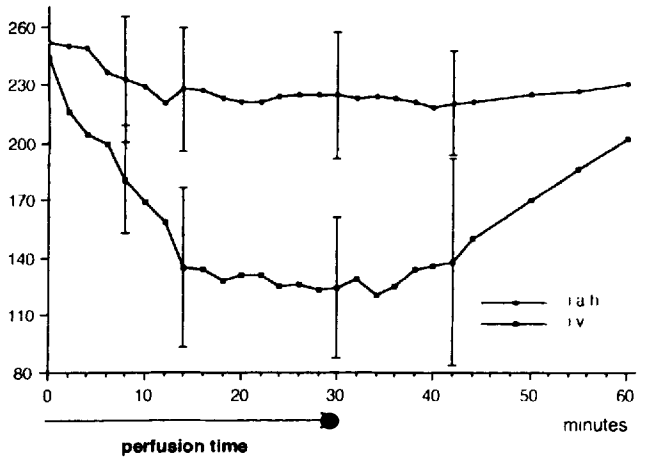

Figure 1. Heart beat rates after 30 minutes of administration of intravenous (squares, bottom) and intraarterial hepatic (circles, top) verapamil. Intraarterial hepatic administration of verapamil significantly reduces its cardiac side effects.

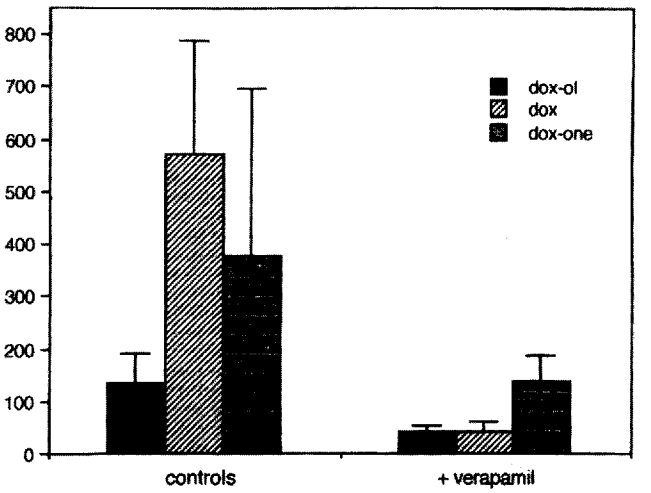

Figure 2. Tumor concentrations of doxorubicin and metabolites after intraarterial hepatic concomitant perfusions with NaCl 0.9% (controls) or verapamil. Significantly smaller concentrations of doxorubicin were observed in tumors in the verapamil perfused group.

starting dose for IAH studies. Verapamil caused only a slight drop in heart beat rates by IAH perfusion and transient first-degree atrioventricular blocks. Significantly reduced cardiac effects were observed 10 minutes after the start of verapamil perfusion by the IAH route. The comparison of the two curves of heart beat rates at the same dose of 0.08 mg/kg/minute evidenced a mean drop in heart beat rates of 10% with the IAH route, whereas a mean drop of 50% occurred with the IV route (Fig. 1). These differences persisted as long as 12 minutes after the end of the infusions. Based on a mean blood flow of 13 ml/kg/minute in the hepatic artery of the rabbit[19] and on a verapamil infusion rate of 0.08 mg/kg/minute, the plasma drug concentration should have been situated between 7 and 9 μM, values that are within the in vitro reversing range. Dose escalation could be pursued as long as 0.20 mg/kg/minute by the IAH route. These results confirm that the first-pass hepatic extraction of verapamil is high, and that IAH verapamil perfusions are safe, with a ratio of 2.5 between the IAH MTD and the IV MTD.

IAH verapamil induced mild alanine aminotransferase and aspartate aminotransferase elevations (twofold) at days 1 and 2 after perfusion, but no increases were observed in bilirubin or alkaline phosphatase when compared with controls, and all values had normalized by day 7. Histologic examination of liver specimens on day 7 showed no specific hepatic toxicity after verapamil IAH perfusions when compared with control IAH perfusions.

Effects of Verapamil on the Tissue Uptake of Doxorubicin

Tumor drug concentrations are depicted in Figure 2. Doxorubicin concentrations had decreased more than tenfold in the verapamil IAH group (43.05 ± 17.3 ng/100 mg tissue) than in controls (573.33 ± 216 ng/100 mg tissue) ($P < 0.05$). Metabolite concentrations (doxorubicinol and doxorubicinone) were similarly reduced in the verapamil group, with 41.5 ± 11.3 versus 133 ± 56 ng/100 mg tissue for doxorubicinol ($P < 0.05$), and 136 ± 49 versus 376 ± 322 ng/100 mg tissue for doxorubicinone (NS). Total amounts of doxorubicin plus metabolites in verapamil-treated tumors were approximately five times lower than in control tumors.

In contrast, in normal liver tissue (Fig. 3), higher

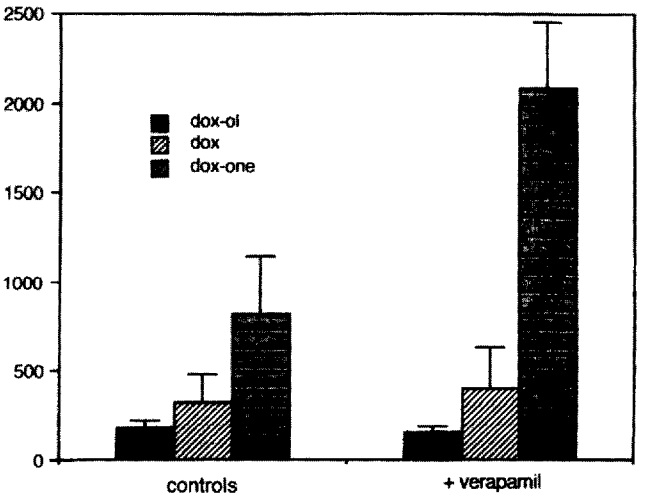

Figure 3. Liver concentrations of doxorubicin and metabolites after intraarterial hepatic concomitant perfusions with NaCl 0.9% (controls) or verapamil. Significantly higher concentrations of doxorubicinone metabolite were observed in the livers in the verapamil perfused group.

Table 1. Mean Individual Tumor/Liver Ratios of Doxorubicin and Metabolites After an IAH Perfusion of Verapamil

Compound(s)	Control subjects	Patients who received verapamil
Doxorubicinol	0.72 ± 0.17	0.30 ± 0.09
Doxorubicin*	3.43 ± 1.27	0.23 ± 0.09
Doxorubicinone	0.29 ± 0.17	0.07 ± 0.03

All values are mean ± SE.
IAH: intraarterial hepatic.
* Control rabbits vs. rabbits who received verapamil, $P < 0.05$.

concentrations of doxorubicinone (inactive metabolite of doxorubicin) were observed in the verapamil IAH group (2090 ± 368 ng/100 mg tissue) than in the normal control liver (823 ± 327 ng/100 mg tissue) ($P < 0.05$). Total amounts of the anthracyclines (doxorubicin and metabolites) also were higher (two times greater) in verapamil perfused liver than in normal control perfused liver (Fig. 3). The ratio between tumor and liver doxorubicin concentrations was calculated for each animal: the mean ratio after IAH perfusion in the control group was 3.37 versus 0.23 for verapamil perfused animals ($P < 0.05$, Table 1). No statistical differences were observed for metabolites, although a trend toward higher ratios was noted in control animals.

Effects of Verapamil on Hepatic Blood Flow

It has been noted that verapamil induced vasodilatation within the first minutes of the perfusion in all perfused territories during the surgical procedures and especially in the hepatic pedicle. The isotope distribution pattern confirmed this impression and showed changes in the tumor/liver blood flow rate after perfusions of verapamil. The ratio of tumor/liver isotope counts for the verapamil group was significantly lower than that of the control group, with a sevenfold decrease (3.1 ± 0.8 versus 26.2 ± 18; $P < 0.05$). The quality of the selective perfusions was assessed by the counts in spleens of animals, which showed low isotope activity in both groups. The ratios of liver/spleen and liver/lungs counts were calculated to accurately reflect the modification of liver blood flow after IAH infusion. The ratio of liver/spleen counts was significantly higher in the verapamil perfused group (393 ± 99 versus 117 ± 64; $P < 0.05$). The liver/lungs ratios were 25.3 ± 15 in the verapamil group and 5.9 ± 3.3 in the control group ($P < 0.05$), with approximately a fourfold decrease in lung counts in the verapamil perfused group (Table 2). These results suggest that the strength of the vasoactive effect of verapamil induced a diversion of blood flow toward the normal liver.

Discussion

In this study we first assessed the feasibility and tolerance of administering verapamil in an IAH perfusion. Cardiac tolerance of verapamil during IAH perfusions was excellent compared with its IV administration because of its substantial first-pass hepatic extraction.[20] We estimate that reversing plasma concentrations of verapamil in the hepatic artery were attained and that plasma concentrations were situated between 7 and 9 μM, with an allowance made for an increase of the hepatic flow caused by the vasodilator effect. The IAH MTD (0.20 mg/kg/minute) of verapamil provoked a decline in the heart beat rates similar to that induced by an IV infusion of 0.08 mg/kg/minute, a dose which should have procured a verapamil concentration of 24 μM in the hepatic artery. These high local verapamil levels also were nontoxic toward normal liver tissue, and they indicate the feasibility and safety of verapamil IAH perfusions.

On the basis of these results, we attempted to determine whether verapamil at reversing concentrations had an effect on, or interacted with, doxorubicin pharmacodynamics. We found that verapamil perfusions decreased tumor doxorubicin concentrations while they increased the anthracycline levels in the normal liver. These results are at variance with those Miyazaki et al.[6] obtained on a liver tumor model in rats. They reported an improvement in doxorubicin concentrations after verapamil concomitant infusions. Differences in experimental conditions could account for these contradictory results. Indeed, the hepatic artery was not ligated after the end of the perfusions in our experiments, and verapamil was administered in 30-minute infusions and not in bolus injections. Longer exposure to verapamil ensured that liver and tumor vascular structures had reached a stage of adaptation to vasoactive effects of the drug, under conditions of normally preserved hepatic artery blood flow. These conditions are closer to those of IAH chemotherapy in patients.

More similar to our results are those of Thom et al.[21] in a study with doxorubicin and verapamil concomitantly infused during a 5-minute period. Thom et al. reported higher anthracycline levels in normal liver, but no differences were observed in tumor tissue. A plausible explanation for our results is that of a differential vascular reactivity between tumor and normal tissue vessels, as has been reported previously with other models[22] and which is exerted primarily by verapamil over the host tissue arterioles.[23] The unfavorable tumor/liver ratio of doxorubicin concentration probably

Table 2. Comparison of the Entrapment Ratio of ^{99m}Tc-Albumin Macroaggregates by Different Tissues After an IAH Concomitant Perfusion of Verapamil

	Ratio			
Group	*Tumor/liver**	*Tumor/spleen*	*Liver/lung**	*Liver/spleen**
Rabbits who received verapamil	3.1 ± 0.8	1062 ± 52	25.3 ± 15	393 ± 99
Control rabbits	26.2 ± 18	1699 ± 728	5.9 ± 3.3	117 ± 64

Values are mean ± SE.

IAH: intraarterial hepatic.

* Control subjects vs. patients who received verapamil, $P < 0.05$.

was caused by a steal effect induced by a diversion of blood flow from the tumor to normal liver with intense vasodilatation in the liver and no or opposite action in the tumor.

In agreement with the drug concentration data, the radionuclide experiments with albumin macroaggregates have shown alterations in the blood flow pattern after verapamil perfusions. These changes in the radiotracer entrapment in the liver and tumor confirmed the differential effects on normal and tumor vessels and an imbalance in blood flow, which proved detrimental to tumor perfusion. Blood flow in the normal liver was highly increased, as shown by the result of the comparison of the ratios of liver/lungs and liver/spleen counts of both groups. It can be hypothesized that such reductions in blood flow to tumors and the diversion in favor of the normal liver not only reduced the concentrations of doxorubicin, but also that of verapamil in tumor cells, which may be far below that expected.

The high anthracycline levels found in the normal liver could have stemmed from an increase in hepatic blood flow or from a blockage of the P-gp, which is highly expressed in rabbit hepatocytes (immunohistochemical detection, unpublished data). We verified the first hypothesis, but we are unable to determine the role of P-gp blockage in the accumulation of the drug. Hyperbilirubinemia is a reported side effect of successful P-gp blockage by cyclosporine.[24] The high concentrations of verapamil also could cause this effect, but we did not observe hyperbilirubinemia, probably because of the short duration of the infusions. Accordingly, retention of anthracyclines would seem possible only after prolonged infusions of verapamil, which is not the case in our study.

Our data confirm that the vasoactive properties of IAH verapamil that allow the attainment of local concentrations within the reversing range, could counteract its mdr-1 modulating activity. These vasoactive effects and the imbalance in blood flow in tumors and normal tissues could exist even after systemic administration of maximal doses of verapamil. Clinical studies testing the d-isomer of verapamil have attained plasma concentrations greater than 3 μM,[25,26] but cardiac and hypotensive effects were observed, suggesting marked d-verapamil vasoactive effects.[26]

Finally, our results do not support clinical trials with IAH verapamil, some of which have been initiated.[27] Nevertheless, regional reversion of drug resistance is not a lost cause, and other reversing agents with a high hepatic extraction and devoid of vasoactive effects should be tested on this experimental model.

References

1. Kartner N, Riordan J, Ling V. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science (Washington)* 1983; 221:1285–8.
2. Bellamy WT, Dalton WS, Kailey JM, Gleason MC, McCloskey TM, Dorr RT, et al. Verapamil reversal of doxorubicin resistance in multidrug-resistant human myeloma cells and association with drug accumulation and DNA damage. *Cancer Res* 1988; 48: 6303–8.
3. Plumb J, Milroy R, Kaye S. The activity of verapamil as a resistance modifier in-vitro in drug resistant human tumor cell lines is not stereospecific. *Biochem Pharmacol* 1990; 39:787–92.
4. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* 1981; 41:1967–72.
5. Formelli F, Cleris L, Carsana R. Effect of verapamil on doxorubicin activity and pharmacokinetics in mice bearing resistant and sensitive solid tumors. *Cancer Chemother Pharmacol* 1988; 21: 329–36.
6. Miyazaki M, Shimoda T, Itoh H, Kaiho T, Linuma K, Koyama T, et al. Enhancement of cytotoxicity of doxorubicin by verapamil in the hepatic artery infusion for liver tumors in rats. *Cancer* 1993; 72:349–54.
7. Ikeda H, Nakano G, Nagashima K, Sakamoto K, Harasawa N, Kitamura T, et al. Verapamil enhancement of antitumor effect of cis-diamminedichloroplatinum (II) in nude mouse-grown human neuroblastoma. *Cancer Res* 1987; 47:231–4.
8. Erlichman C, Moore M, Thiessen J, Kerr I, Walker S, Goodman P, et al. Phase I pharmacokinetic study of cyclosporin A combined with doxorubicin. *Cancer Res* 1993; 53:4837–42.
9. Gosland M, Tsuboi C, Hoffman T, Goodin S, Vore M. 17β-estradiol glucuronide: an inducer of cholestasis and a physiological

substrate for the multidrug resistance transporter. *Cancer Res* 1993; 53:5382–5.

10. Tsuruo T, Iida H, Makishima F, Yamori T, Kawabata H, Tsukagoshi, et al. Inhibition of spontaneous and experimental tumor metastasis by the calcium antagonist verapamil. *Cancer Chemother Pharmacol* 1985; 14:30–3.
11. Pennock G, Dalton W, Roeske W, Appleton C, Mosley K, Plezia P, et al. Systemic toxic effects associated with high-dose verapamil infusion and chemotherapy administration. *J Natl Cancer Inst* 1991; 83:105–10.
12. Salmon S, Dalton W, Grogan T, Plezia P, Lehnert M, Roe D, et al. Multi-drug resistant myeloma: laboratory and clinical effects of verapamil as a chemosensitizer. *Blood* 1991; 78:44–50.
13. Miller T, Grogan T, Dalton W, Spier C, Scheper R, Salmon S. P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. *J Clin Oncol* 1991; 9:17–24.
14. Cairo M, Siegel S, Anas N, Sender L. Clinical trial of continuous infusion verapamil, bolus vinblastine, and continuous infusion of VP-16 in drug-resistant pediatric tumors. *Cancer Res* 1989; 49: 1063–6.
15. Ozols R, Cunnion R, Klecker R, Hamilton T, Ostchega Y, Parrillo J, et al. Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. *J Clin Oncol* 1987; 5:641–7.
16. Presant C, Kennedy P, Wiseman C, Gala K, Bouzaglou A, Wyres M, et al. Verapamil reversal of clinical doxorubicin resistance in human cancer. *Am J Clin Oncol* 1986; 9:355–7.
17. Ramirez LH, Munck JN, Bognel C, Zhao Z, Ardouin P, Poupon MF, et al. Pharmacology and antitumor effects of intraportal pirarubicin on experimental liver metastases. *Br J Cancer* 1993; 68: 277–81.
18. Munck JN, Riggi M, Rougier P, Chabot GG, Ramirez LH, Zhao Z, et al. Pharmacokinetic and pharmacodynamic advantages of pirarubicin over adriamycin after intraarterial hepatic administration in the rabbit VX2 tumor model. *Cancer Res* 1993; 53: 1550–4.
19. Altman P, Dittmer D. Biology data book, vol. 3, 2nd ed. Bethesda, MD: Federation of American Societies for Experimental Biology, 1974.
20. McTavish D, Sorkin E. Verapamil: an updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension. *Drugs* 1989; 38:19–76.
21. Thom AK, Zhang S, Deveney C, Daly JM. Effects of verapamil and degradable starch microspheres during hepatic artery infusion of doxorubicin. *Surgery* 1990; 107:552–9.
22. Chan R, Babbs C, Vetter R, Lamar C. Abnormal response of tumor vasculature to vasoactive drugs. *J Natl Cancer Inst* 1984; 72: 145–50.
23. Sagar S, Klassen G, Barclay K, Aldrich J. Tumour blood flow: measurement and manipulation for therapeutic gain. *Cancer Treat Rev* 1993; 19:299–349.
24. Yahanda A, Adler K, Fisher G, Brophy N, Halsey J, Hardy R, et al. Phase I trial of etoposide with cyclosporine as a modulator of multidrug resistance. *J Clin Oncol* 1992; 10:1624–34.
25. Scheithauer W, Schenk T, Czejka M. Pharmacokinetic interaction between epirubicin and the multidrug resistance reverting agent d-verapamil. *Br J Cancer* 1993; 68:8–9.
26. Bissett D, Kerr D, Cassidy J, Meredith P, Traugott U, Kaye S. Phase I and pharmacokinetic study of d-verapamil and doxorubicin. *Br J Cancer* 1991; 64:1168–71.
27. Saltz L, O'Brien J, Kemeny N, Murphy B, Niedzwiecki D, Tong W, et al. Phase I study of intrahepatic verapamil and doxorubicin: regional therapy to overcome multidrug resistance. *Proc Am Assoc Clin Oncol* 1992; 11:293.