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Tumor Uptake of Vincristine during Close Arterial vs Systemic Constant-rate Infusion: Experiments on Sarcoma Transplants in the Rat Kidney*

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Abstract—*The uptake of vincristine (VCR) by normal tissues and by sarcomas transplanted to both kidneys in rats was studied at* 10 min *after constant rate infusion of* 1 mg/kg *body wt into one renal artery for* 3, 10 *and* 30 min. *Since the selectively infused kidney extracted only* 2.5% *of the total dose at the first renal passage, the present model provides a direct comparison between systemic and selective i.a. infusion of VCR in each animal. Tumor VCR uptake was* 2.8 *times higher and the corresponding blood VCR concentration time product about* 4 *times higher on the selectively infused side than on the contralateral side. Reduction of renal blood flow by clamping the selectively infused renal artery raised the local VCR blood concentration by another* 10 *times during* 10 min *infusion and further doubled the tumor uptake of VCR. Thus a* 5–6 *times higher VCR concentration in the tumor was achieved as compared to that obtainable by systemic administration of the same total dose. The results are compatible with a saturable mechanism of VCR uptake by renal tissue and tumors.*

INTRODUCTION

CLOSE arterial infusion may give a higher tumor concentration (conc.) of a cytostatic agent, even with a lesser dose, than systemic administration. However, the usefulness of this approach depends on the kinetics of tumor uptake and the clearance from the systemic circulation of the particular agent.

To predict the value of such a mode of treatment, useful pharmacokinetic models have been made for several cytostatics, based on surveys of *in vivo* studies of clearance from the blood and *in vitro* studies of uptake of the agents into cells [1–4].

For vincristine (VCR), which has been infused selectively for breast cancer [5, 6] and head-and-neck cancer [7], there is a positive relationship between the conc. in the suspension-medium and the kill of cultured lymphoma cells [8] and human and murine leukemia cells [9]. *In vitro* experiments have also shown that the uptake of VCR in murine leukemia cells is a hyperbolic function of exposure conc., indicating a carrier-facilitated uptake into the cells [10].

We wanted to see if a positive relationship exists between arterial blood conc. and tissue uptake of VCR *in vivo* and to what extent such a relationship might be utilized to improve tumor uptake during local arterial infusion in comparison with systemic administration of the drug. Thus we have determined the VCR uptake in normal tissue and sarcoma transplants in the autoperfused left rat kidney receiving direct renal arterial constant-rate infusion, and in the contralateral kidney also with implanted sarcoma, receiving the recirculating VCR.

As one kidney accounts for approximately 10% of the cardiac output, the autoperfused kidney would be expected to be exposed to a higher blood VCR conc. than the contralateral kidney, depending on the renal blood flow (RBF), the

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infusion time and the clearance of VCR from systemic blood. Using a consistent total dose (1 ml/kg body wt) a varied left renal arterial VCR concentration was obtained by varying the infusion time. Thus the infusion times were chosen to achieve 2- to 10-times higher arterial VCR conc. to the left than to the right kidney.

We have correlated the time course of VCR conc. in the perfusing blood to the uptake of VCR by tumor and normal tissue in both kidneys and by various tissues.

MATERIALS AND METHODS

Eighteen male Lister hooded rats, body wt 300–360 g, were kept on a diet of corn pellets and water.

Transplantable 20-methylcholanthrene-induced sarcomas were implanted in both kidneys [11]. Six or seven days later the animals were anesthetized with pentobarbital-Na, tracheotomized and placed on a heating pad with thermostatic control via a rectal thermistor, keeping the body temperature at 37–38°C.

An extracorporeal blood flow shunt was established from the right carotid artery to an aortic pouch having the left renal artery as the only outlet, i.e. as a selective arterial route to the left kidney [12]. Total renal blood flow was measured electromagnetically (Scalar Medical, Transflow 601-system, model 400A) with a flowprobe interposed in the shunt. Mean arterial blood pressure (AP) was recorded with a Hewlett Packard transducer (model 1280C) and recorder (model 7754A) from a side branch of the shunt, close to the kidney.

A catheter was introduced into the left carotid artery for blood sampling. The abdominal wall was closed and 30-min recovery period was allowed.

Vincristine, 1 mg/kg body wt, together with 5 μCi [^{3}H]-VCR (Amersham International plc., Amersham, U.K.) was infused at a constant rate into the shunt for 3–30 min. The labeled compound was stored in ethanol at −23°C, and 0.05 ml was mixed with a fresh solution of VCR in 1 ml 0.9% saline immediately before infusion.

During and after VCR infusion the renal tubular fluid would presumably contain a higher VCR conc. in the left than in the right kidney. To minimize this difference a postinfusion period of 10 min was allowed before the circulation was stopped on both sides by tightening snares prepositioned around the renal pedicles.

Seven timed blood samples, 100 μl each, were drawn from the left carotid artery during and after the infusion, the extracted volume being replaced with 0.9% saline. An additional 2 ml of saline was infused during the experiment to avoid a low urine flow.

In four of the animals catheters were introduced into the ureters for measurement of VCR in pelvic urine. In another four of the animals, given VCR infusion for 10 min, a clamp was applied to the shunt in order to obtain a particularly low RBF (0.5–0.9 ml/min/g kidney weight).

Following the stop of renal circulation, a 1-ml arterial blood sample was drawn and centrifuged for determination of VCR in plasma and red blood cells. All blood samples, urine samples, a sample of the infusate and representative samples from both kidneys and tumors, together with tissue samples from heart, liver, spleen, small bowel, lung, brain and skeletal muscles, were analyzed for radioactivity (Packard Tri-carb 460 CD liquid scintillation system). A standard preparative procedure and correction for quenching were used.

The time course of VCR in systemic arterial blood was calculated from the timed blood samples from the left carotid artery. The course of VCR conc. to the left kidney was calculated from infusion rate, RBF and systemic (recirculating) conc. of VCR.

The half-time, $t^1/_2$, for VCR during infusion was calculated from

$$M_t = Q \times t^1/_2/\ln 2, \quad (1)$$

where M_t represents the amount of VCR infused at a given time during steady blood VCR leel and Q is the concomitant infusion rate of VCR. (A correction of M_t was made for the VCR extracted by the left kidney at the first renal passage.)

The apparent distribution volume, V_d, is given by

$$V_d = M_t/C, \quad (2)$$

where C is the plasma conc. for VCR at 10 min postinfusion. The results are presented as mean ± S.E. and statistical evaluations are made with Mann–Whitney U tests unless otherwise stated.

RESULTS

Steady hemodynamic conditions were established before VCR infusion: the range of AP was 82–155 mm Hg, and the range of RBF in the autoperfused kidneys was 3.2–6.2 ml/min/g kidney weight, except for the four kidneys with reduced RBF (0.5–0.9 ml/min/g).

Ten-minutes infusion experiments

On the selectively infused left side the arterial conc. of VCR increased due to recirculating VCR but reached a steady level at 6 min infusion, in

accordance with a steady level in systemic arterial blood (Fig. 1). The VCR conc. in the left renal artery was four times the systemic conc. at fairly steady levels. (The four experiments with lowered RBF are not included in Table 1 and the figures.)

The calculated $t^1/_2$ for systemic blood VCR at 6 min infusion was 0.5 min, whereas the first post-infusion $t^1/_2$ was 2 min, the second was 4 min and the following appreciably prolonged. At 10 min after completed infusion the conc. of VCR in systemic arterial blood was 17% of the level attained during infusion (Fig. 1). The calculated V_d at the end of the experiments was 1.7 l/kg.

The mean time–conc. product for VCR to the selectively infused kidneys (62 ± 15 μg/min/ml) was four times the value of the kidneys receiving systemic arterial blood (15 ± 2 μg/min/ml).

The left kidney with tumor extracted 3.9 ± 0.5% of the total dose of VCR, whereas 1.4 ± 0.1% was taken up by the right kidney with tumor. Thus only 2.5% of the selectively infused VCR was extracted during the first left kidney passage and the left/right kidney uptake ratio was less than the corresponding ratio for the respective blood conc.–time products.

The uptake of VCR was 2.8 times higher on the selectively infused side, for normal renal tissue ($P < 0.005$) as well as for tumor ($P < 0.005$).

Of the total dose 2.2% had been excreted in urine at 10 min after infusion, two-thirds of it coming from the selectively infused left kidney. The conc. of VCR in urine sampled during the last 2 min of the post-infusion period from the autoperfused left kidney was 110 ± 40% (S.D.) of the conc. in parallel urine samples from the right kidney, on average 7.7 μg/ml, which was close to the VCR conc. in the left renal tissue. The difference in VCR recovery between the kidneys therefore could not be due to VCR in tubular fluid.

In four 10-min infusion experiments where RBF was lowered by clamping the extracorporeal shunt, the mean conc.–time product for VCR averaged 660 (510–785) μg/min/ml and the VCR uptake was 32 (27–38) μg/g in the left renal tissue vs 9.7 (4.8–12.6) μg/g in the left tumor. In comparison with 10 min infusion without RBF reduction right kidney and tumor VCR concentrations were practically unaltered, 3.25 μg/g and 1.85 μg/g. Thus on average the VCR conc. was about five times higher in tumor and about ten times higher in renal tissue on the left than on the right side.

Varied infusion time

Figure 2 compares the course of arterial blood conc. of VCR in 3-, 10- and 30-min infusion experiments. In spite of the markedly different maximal conc. in the left renal artery as well as in systemic blood during infusion, significant differences in blood VCR were not found at 10 min after completed infusion.

The mean uptake of VCR in the tumors during 3- and 30-min infusions was not significantly different from that obtained for contralateral kidneys (Table 1). Also, the tumor/renal tissue uptake ratio was similar for all infusion rates.

The arterial conc. time of VCR to the selectively infused kidney differed between experiments due to different individual RBF. Considering all left and right kidneys together, an increasing uptake of VCR was seen at increasing conc. time of VCR

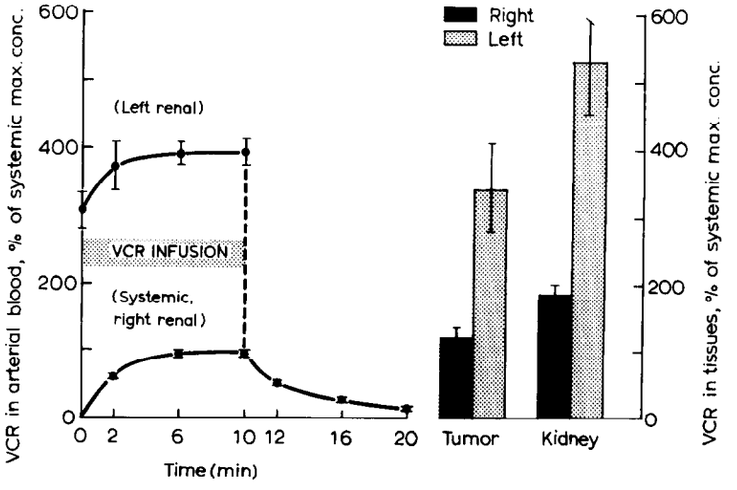

Fig. 1. Left panel: the time course of vincristine (VCR) concentration in the selectively infused left renal artery and in systemic arterial blood. Right panel: recovery of VCR in normal renal tissue and in sarcoma transplants of both kidneys. All data are normalized with respect to the concentration level obtained in systemic arterial blood during constant rate infusion for 10 min. *Bars indicate ±S.E.*

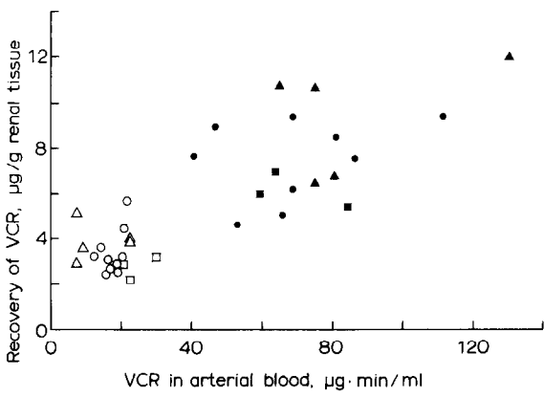

Fig. 2. Recovery of vincristine (VCR) in normal renal tissue after selective infusion of 1 mg/kg *body wt into the left renal artery. Constant rate infusion time:* 3 min *(triangles);* 10 min *(circles); and* 30 min *(squares). Closed symbols: left kidneys; open symbols: right kidneys.*

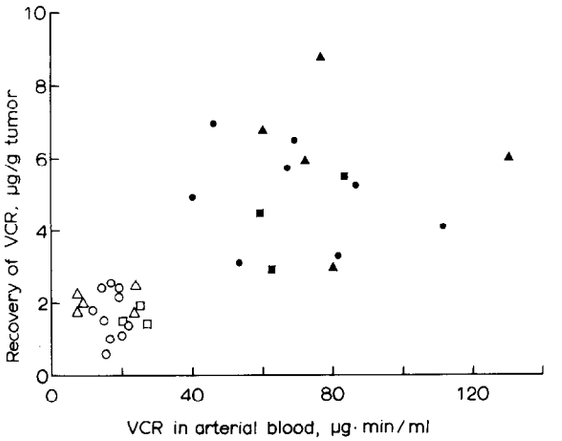

Fig. 3. Recovery of vincristine (VCR) in sarcoma transplants in the kidneys after selective infusion of 1 mg/kg *body wt into the left renal artery. Symbols as in Fig. 2.*

in the perfusing blood, for normal renal tissue as well as for the tumors (Figs. 3 and 4). These results imply a negative relationship between renal VCR uptake and blood flow in the renal artery ($P<0.01$).

The uptake of VCR by the various tissues which received only the recirculated compound was similar for all infusion rates, the only exception being a significantly increased uptake in skeletal muscles during the 30-min infusion (Table 1).

DISCUSSION

Validity of the experimental model

The present kidney model was chosen in order to have paired host organs and tumors that would allow comparison of local and systemic administration of the drug within the same animal. Its main drawback would be that a different conc. of filtered VCR in the two kidneys might falsely be interpreted as a difference in renal tissue uptake. We therefore allowed a 10-min post-infusion period to ensure that renal tubular fluid was derived from equal blood conc. of VCR, as was verified by the measurement of similar VCR conc. in urine from both sides during the last 2 min of the experiment. The conc. of VCR in urine was similar to the renal tissue conc. and thus higher than VCR conc. in tumor tissue (Table 1). Since the tumors do not contain tubular elements, the VCR measurements in the tumors must reflect uptake by the tumor tissue.

It should be emphasized that the recovery of VCR made in tumors includes extra- as well as intracellular compounds. However, since the extracellular VCR conc. is likely to follow the decline in plasma VCR during the post-infusion period, the recovery measurements made in the tumors will predominantly reflect the intra-

Table 1. Uptake of vincristine, µg/g tissue, at 10 min *after completed infusion of* 1 mg/kg *body wt into the left renal artery*

	Infusion time		
	3 min ($n=5$)	10 min ($n=9$)	30 min ($n=3$)
Left kidney	9.05 ± 1.3	7.45 ± 0.65	6.06 ± 0.57
Right kidney	4.14 ± 0.62	3.13 ± 0.21	2.93 ± 0.28
Left renal tumor	5.86 ± 1.45	4.80 ± 0.29	4.26 ± 0.77
Right renal tumor	2.06 ± 0.17	1.91 ± 0.25	1.70 ± 0.11
Heart	3.38 ± 0.35	2.39 ± 0.26	2.07 ± 0.32
Spleen	1.84 ± 0.30	1.54 ± 0.12	1.93 ± 0.11
Small bowel	3.20 ± 0.23	2.65 ± 0.33	4.67 ± 2.00
Liver	7.92 ± 1.00	6.69 ± 0.55	8.20 ± 0.14
Lung	3.24 ± 0.48	2.74 ± 0.32	1.97 ± 0.18
Skeletal muscle	0.12 ± 0.03 *	0.24 ± 0.04 *	0.38 ± 0.03
Brain	0.13 ± 0.05	0.09 ± 0.02	0.08 ± 0.03
Red blood cells	0.08 ± 0.03	0.13 ± 0.06	0.11 ± 0.04
Plasma	0.61 ± 0.08	0.44 ± 0.07	0.54 ± 0.05

*Significant difference at variance analysis ($F=7.02$, $P<0.01$). n = No. of experiments.

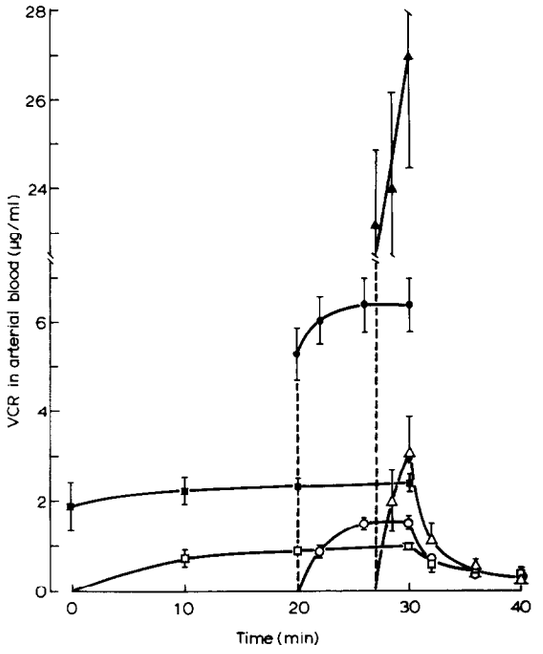

Fig. 4. *Course of vincristine (VCR) blood concentration during and after selective infusion of* 1 mg/kg *body wt into the left renal artery. Infusion times:* 3 min *(triangles)*: 10 min *(circles); and* 30 min *(squares). Closed symbols: calculated concentration in the left renal artery; open symbols: measured concentration in systemic arterial blood. Bars indicate* ±*S.E.*

cellular component: the VCR conc. in the left tumor was ten-fold that in plasma at 10 min post-infusion (Table 1).

The selectively infused kidney extracted on average 2.5% of the total VCR dose at the first renal passage. Thus practically the same amount of VCR would have been taken up by the contralateral kidney and its tumor as by other tissues during a systemic administration of the drug. Hence the present model should provide a valid comparison between local vs systemic administration within the same animal.

However, one might suspect that the rather invasive circulatory intervention made, ligation of the aorta between and below the renal arteries, might complicate interpretation of the results. As discussed in the following paragraph, this is not the case since our data on VCR blood clearance and VCR content in various tissues agree well with previous findings.

Urine VCR excretion averaged 2.2% of the total dose, in reasonable accordance with earlier reports on 6% of the total dose being accumulated in urine during 3 hr [13] and 6.5% during 4 hr [14] in man, and 14% being accumulated as VCR and metabolites after i.v. injection in the rat [15].

The rapid clearance of VCR from systemic blood corresponds with previous findings in humans showing initial half-times of 1.5 [13], 2 [16] and 3 min [14] after i.v. administration of the drug. On the other hand, considerably longer initial half-times are reported for VCR in the rat: 15 min following i.v. bolus [15] and 60 min after i.p. administration [17], the latter suggesting a slow VCR transperitoneal transport to the blood and thus a relatively low blood concentration at any time.

The present findings of VCR in various tissues (Table 1) are also in line with earlier reports on the rat [15, 17] with the exception of a relatively lower uptake of VCR in brain tissue and red blood cells. In both tissues, and in skeletal muscles, the VCR conc. was less than in plasma (Table 1) and most likely would have increased with a prolonged post-infusion period as indicated by previous studies [15, 17]. The present range of exposure time is obviously too narrow to reveal any redistribution of VCR that might take place with time.

Kidney and tumor VCR uptake

The results show that the direct infusion of VCR into the renal artery that carries about 10% of the cardiac output may provide a 2.8-times larger local conc. both in host tissue and tumor as compared to an equivalent systemic dosage.

In spite of a four-fold higher blood conc. of VCR to the selectively infused kidney during 10 min infusion experiments, there was only a 2.8-times increase in tissue uptake as compared with contralateral kidney (Fig. 2). Furthermore, there was no significant additional increase in VCR uptake with a four-fold increase in left renal atrial conc., i.e. in the 3-min as compared with the 10-min experiments (Fig. 2, Table 1). On the other hand, 10 min infusion during reduced RFB doubled the tumor uptake of VCR as compared with the normal 10-min experiments, reflecting the effect from a ten times additional increase of plasma VCR conc. These findings are compatible with a carrier-facilitated mechanism being involved in cellular VCR uptake [10], a mechanism that might tend to be saturated at a higher exposure conc. of VCR.

As compared to systemic administration of VCR, renal arterial infusion during reduced RBF gave 5- to 6-times larger uptake of VCR in the tumors, reflecting the effect from a nearly fifty-fold increase in plasma VCR conc. A possible objection to this approach might be that a substantial reduction of blood flow in the host artery due to arterial clamping would compromise the perfusion of the tumor, i.e. alter flow distribution to and/or within the tumor. This suspicion is nourished by the observation of a lower ratio of tumor to renal tissue VCR conc. in these experiments. However, markedly delayed washout of renal tubular fluid with a high VCR

conc. attained during the infusion is perhaps the more likely explanation. Thus recent studies with the present experimental model during reduced left renal perfusion pressure indicate autoregulation or reduction of tumor blood flow in proportion to RBF [18]. The high tumor VCR conc. obtained in the four experiments with markedly reduced renal flow therefore depends on a relatively well-maintained flow fraction from the host artery to the tumor. This may not necessarily be the case in other types of tumors, or even in the present type of sarcoma if developed in tissues not possessing autoregulatory ability [18].

There is evidence that local infusion of cytostatics combined with temporary occlusion of the feeding artery gives very high uptakes in normal tissues [19–21]. The present results extend this concept to include VCR, not only for normal tissue but for tumor as well, during partial arterial occlusion.

The present results, obtained without interference with renal perfusion pressure and flow, imply that the total tolerable VCR dose may be given as three short-term renal arterial infusions over a long-term treatment period. Thus each infusion would theoretically give an initial left tumor VCR concentration similar to that obtained by one systemic short-term administration of the total dose. Whether this mode of treatment gives a better cytostatic effect of VCR than, for instance, a long-term sustained i.a. infusion is a therapeutically important question that should be approached experimentally using the present or a similar paired tumor model.

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