Increased Adriamycin Levels in Hepatic Implants of Rabbit Vx-2 Carcinoma from Regional Infusion¹

John A. Ridge, Charles Collin, James R. Bading, Counce Hancock, Peter S. Conti, John M. Daly, and John H. Raaf²


ABSTRACT

Regional infusion chemotherapy for the treatment of primary or secondary hepatic cancer should allow delivery of a higher drug concentration to the tumor with decreased systemic exposure when compared with systemic therapy. Fifteen rabbits, each implanted with two hepatic Vx-2 tumors, were treated with infusion of Adriamycin (3 mg/kg and 7.5 μCi of [14C]Adriamycin) through the hepatic artery (n = 5), portal vein (n = 5), and a systemic vein (n = 5) at 20 μg/min. ¹⁴C-labeled macroaggregated albumin flow images documented specific hepatic perfusion in selected rabbits using this technique. Thirty min after infusion the animals were sacrificed, and multiple specimens of liver, tumor, and heart were taken for liquid scintillation counting and high-performance liquid chromatography. The ¹⁴C label remained associated with Adriamycin and metabolites.

After systemic infusion 11.5 nmol/g of Adriamycin were found in tumor, and 32.4 nmol/g were found in liver. Infusion of Adriamycin through the hepatic artery produced drug levels of 34.3 nmol/g of tumor and 48.4 nmol/g of liver, while infusion through the portal vein produced drug levels of 6.5 nmol/g of tumor and 54.4 nmol/g of liver. The drug concentration in tumor was significantly higher after hepatic artery infusion compared with systemic (P < 0.05) or portal vein (P < 0.01) infusion. The tumor/liver ratio of [¹⁴C]Adriamycin tissue levels after hepatic artery infusion was greater than that measured after systemic vein treatment (10.9 nmol/g) or portal vein (8.9 nmol/g) infusion.

Hepatic artery infusion achieved the highest tumor Adriamycin level compared with systemic vein and portal vein infusion. The results suggest that these tumor implants are supplied primarily by the hepatic artery, that clearance of Adriamycin is efficient after regional infusion, and that systemic toxicity may be reduced using intraarterial infusion of Adriamycin for hepatic tumors.

INTRODUCTION

Regional infusion therapy is often administered to patients with primary and secondary tumors of the liver. It is believed that higher drug concentrations will be achieved in the neoplasms and that systemic toxicity will be reduced. Clinical results have been gratifying in Phase II (2) and Phase III trials (3, 4) for colorectal hepatic metastases.

Substantial extraction of 5-fluorouracil and 5-fluorodeoxyuridine after hepatic artery infusion has been demonstrated (5). Higher levels of Adriamycin have also been documented after hindlimb arterial infusion compared with systemic treatment in the dog (6). However, there is little firm evidence to support the theoretical advantages of regional hepatic infusion in increasing tumor drug levels.

The rabbit Vx-2 tumor system is useful for the study of regional infusion. This cancer has been propagated for more than 50 yr in the New Zealand White rabbit (7). Regional treatment of hindlimb Vx-2 tumors has been performed using mitomycin C, actinomycin D, cyclophosphamide (8, 9), cis-platinum (10, 11), and Adriamycin (12). In addition, the metabolism of Adriamycin in the rabbit has been examined (13). Previous experiments from this laboratory (14) document dose-response relationships for hindlimb Vx-2 cancers treated by intraarterial and by systemic infusion. Femoral artery injection produces more “complete responses” (67%) than does systemic treatment (33%). After treatment with Adriamycin the time to measurable tumor responses after intraarterial infusion (7 days) is also shorter than the time to response with systemic dosage (21 days). Diminished systemic levels of Adriamycin have not been found after femoral artery infusion (12).

The Vx-2 carcinoma is particularly well-suited to studies of liver tumors. The rabbit hepatic artery anatomy resembles that of the human. The tumor grows well in the rabbit liver (15, 16), and the hepatic Vx-2 tumor, like human liver metastases (17), is supplied predominantly by the hepatic artery (18).

Application of the Vx-2 cancer system to hepatic tumors can confirm the basic assumptions of regional therapy: that higher tumor drug levels are achieved by hepatic artery infusion and that systemic exposure is reduced. The relative selectivities of treatment routes can be determined, and the effect of tumor size on drug delivery ascertained.

MATERIALS AND METHODS

Vx-2 Cancer. The Vx-2 carcinoma was obtained from the Mason Research Institute (Worcester, MA) and was maintained as a tumor line in our laboratory according to the method of Swistel and coworkers (12). Four passages of donor tumor were used in all experiments.

Rabbits. Three-kg New Zealand White rabbits were purchased from Hazelton Research, Denver, PA. The animals were housed in institutional animal-care facilities according to the guidelines of the Animal Welfare Act. Rabbits were anesthetized with ketamine and halothane for all surgical procedures.

Tumor Implantation. A subxiphoid transverse incision was carried sharply to the peritoneum. The right anterior and left medial lobes of the rabbit liver were delivered onto the abdominal wall for tumor implantation without wound contamination, peritoneal soilage with tumor, or undue adhesion formation.

Tissue taken from the outer “viable” rim of donor tumor was placed in Dulbecco’s minimal essential medium plus glutamine (Gibco, Grand Island, NY) and minced sharply to 1-mm segments. Three fragments were promptly implanted within the liver parenchyma after a pocket was created by blunt dissection. Hemostasis of the liver capsule was obtained by electrocautery, and the abdomen was closed with continuous silk suture.

Tumor growth could be confirmed by ultrasonography 14 days after implantation, and infusion studies were performed 14 to 21 days after implantation.

Adriamycin. Pharmaceutical Adriamycin was used (Adria Labs, Dublin, OH), and [¹⁴C]Adriamycin was obtained from the National Cancer Institute as a kind gift of Dr. Robert R. Engel of the Chemical Research Division. A dose of 3 mg/kg (at 2 mg/ml) was used in all infusions, and 7.5 μCi of labeled Adriamycin were simultaneously infused.

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Vessel Cannulation. A midline laparotomy was performed, and the presence of hepatic tumors was confirmed by palpation of the liver. A right jugular cutdown was performed for central venous access. A 24-gauge cannula was placed for drug infusion. The left femoral vein was used for systemic infusion, a jejunal mesenteric vein was used for portal vein infusion, and the gastroduodenal artery was used for hepatic artery infusion. Five animals were studied in each group.

**Tc-labeled macroaggregated albumin (Medi Plus Physics) liver perfusion scans in some animals confirmed selective perfusion of the liver by the regional techniques. An Ohio Nuclear Model 410 gamma counter was used for the perfusion scans.

Efforts to study chronic infusions of Adriamycin were frustrated by difficulties with long-term general anesthesia in the rabbit and by duodenal perforations that ensued within 36 h in some 90% of rabbits submitted to gastroduodenal artery cannulation.

**Adriamycin Administration. Drug was injected at a rate of 10 ml/min. Levels of Adriamycin in liver and tumor rose to a maximum at approximately 30 min by all routes of injection and then diminished slowly (data not presented). Hence, animals were sacrificed by exsanguination 30 min after injection, and tissue samples were taken for analysis.

**Tissue Drug Levels. Random samples of the cardiac apex and of liver were taken. Tumor from the outer, hard, "viable" edge of the Vx-2 cancer was studied. Weighed tissue samples were digested with Protosol (New England Nuclear) and 14C counts were determined according to the Protosol technical manual (New England Nuclear, Boston, MA) using a Packard Tri-Carb liquid scintillation spectrometer. Appropriate quench curves were constructed, and data were analyzed using a PDP 11/34 computer. Drug levels are expressed as nmol of Adriamycin/g wet weight of tissue (19).

**Radioactivity and Anthracyclines. Thirty min after hepatic artery injection of Adriamycin (3 mg/kg), the rabbit was sacrificed. Anthracyclines were extracted from liver and tumor (20) in selected animals, and metabolites in liver and tumor were identified by HPLC.3 Fig. 1 shows the relative fluorescence and 14C counts in individual fractions from the HPLC. These peaks represent Adriamycin, adriamycinol, and Adriamycin-aglycone extracted from liver. They account for more than 90% of the fluorescent material eluted from the HPLC. Though the proportions vary, equivalent concurrence of the 14C label and fluorescence is seen in the tumor (data not presented). The distribution of the 14C radioactivity accurately reflects the concentration of Adriamycin and its proximal metabolites. Thus, only 14C counts were determined for individual samples.

**Statistical Analysis. Stats Plus (Human Systems Dynamics) for the Apple Ilc was used for most statistical tests. Nonparametric data were analyzed by the Mann-Whitney test. Concurrence with the method of repeated measures on one factor (21) was observed. The TLARs are Cauchy distributions and therefore should be compared by their confidence intervals, rather than t tests. The tumor/liver ratios and confidence limits were calculated according to Paulson (22).

**RESULTS**

**Tissue Distribution: Liver; Tumor; and Heart.** Thirty min after hepatic artery, portal vein, or systemic infusion of 3 mg/kg of Adriamycin, the rabbits were sacrificed, and the drug level in their liver, tumor, and heart was determined. The results are shown in Table 1.

The Adriamycin concentration in liver after hepatic artery infusion (34.3 nmol/g of tissue) exceeds the drug concentration in tumor after systemic administration (11.5 nmol/g) or portal vein administration (6.3 nmol/g) of the same dose. These differences are significant.

The Adriamycin concentration in liver after hepatic artery infusion (48.4 nmol/g) and after portal vein infusion (54.4 nmol/g) exceeds the drug concentration in liver after systemic administration of the same dose (32.4 nmol/g). The difference between systemic and either regional infusion is significant.

The Adriamycin concentration in the heart after systemic infusion (13.6 nmol/g) exceeds the drug concentration in the heart after hepatic artery (10.9 nmol/g) and after portal vein (8.9 nmol/g) infusion. The difference between systemic and portal vein routes of infusion is significant.

**Distribution of Adriamycin between Tumor and Liver.** The Adriamycin concentration in an animal’s tumor was compared with the drug level in that animal’s liver. A TLAR was determined for systemic, hepatic artery, and portal vein treatments. The mean TLARs may be compared by their confidence limits. Table 2 demonstrates that the mean TLAR after hepatic artery administration (0.68) exceeds that achieved after systemic (0.37) or portal vein (0.12) treatment. There is overlap of the 95% confidence intervals for the hepatic and systemic treatments, but hepatic artery infusion will produce a higher TLAR than systemic infusion with at least 90% confidence.

**Drug Level and Tumor Size.** Fig. 2 presents data on the measured Adriamycin concentration in tumor as a function of total tumor weight. Linear regression analysis demonstrates a weak negative dependence of drug level on tumor weight after hepatic artery and portal vein infusion of Adriamycin, but not after systemic infusion.

**DISCUSSION**

This study tests directly the precept that regional infusion of Adriamycin to the liver enhances delivery of drug to implanted tumors. A TLAR was determined for systemic, hepatic artery, and portal vein treatments. The mean TLARs may be compared by their confidence limits. Table 2 demonstrates that the mean TLAR after hepatic artery administration (0.68) exceeds that achieved after systemic (0.37) or portal vein (0.12) treatment. There is overlap of the 95% confidence intervals for the hepatic and systemic treatments, but hepatic artery infusion will produce a higher TLAR than systemic infusion with at least 90% confidence.

**Table 1 Drug level 30 min after infusion**

<table>
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<tr>
<th>Route of infusion</th>
<th>nmol/g tumor</th>
<th>nmol/g liver</th>
<th>nmol/g heart</th>
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<tr>
<td>Systemic</td>
<td>11.5 ± 3.3**</td>
<td>32.4 ± 3.3</td>
<td>13.6 ± 4.1</td>
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<tr>
<td>Hepatic artery</td>
<td>34.3 ± 15.2</td>
<td>48.4 ± 8.2</td>
<td>10.9 ± 2.0</td>
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<tr>
<td>Portal vein</td>
<td>6.3 ± 1.5</td>
<td>54.4 ± 15.7</td>
<td>8.9 ± 2.5</td>
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**Table 2 Tumor/liver ratios**

<table>
<thead>
<tr>
<th>Route of infusion</th>
<th>Tumor/liver ratio</th>
<th>95% CI</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic</td>
<td>0.37</td>
<td>± 0.13</td>
<td>± 0.06</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>0.68</td>
<td>± 0.27</td>
<td>± 0.23</td>
</tr>
<tr>
<td>Portal vein</td>
<td>0.12</td>
<td>± 0.12</td>
<td>± 0.02</td>
</tr>
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* CI, confidence interval (22).
Connections between the portal vein and hepatic artery do exist. Studies of human microvascular anatomy surrounding small metastases in the liver (23) have demonstrated arterioportal communications within tumors as large as 1 cm. In addition, arterioportal connections have been found in liver sinusoids (24). After hepatic artery ligation, hepatic implants of rat Walker 256 tumor were perfused by portal vein Microfil injection in 7 of 16 animals (25). The delivery of Adriamycin to our Vx-2 tumor implants after portal vein infusion may be due to such “collaterals,” or it may represent the contribution of “recirculation,” but these mechanisms cannot currently be distinguished.

The low tumor drug concentration and the low TLAR after portal vein administration suggest that regional infusion of Adriamycin results in significant clearance by the liver. The tumor level of Adriamycin after portal vein delivery is significantly lower than that after systemic treatment with the drug (Table 1). This implies that the recirculation of Adriamycin after portal vein infusion is low. If recirculation were prominent, then the results after portal vein infusion would mimic those after systemic drug administration. This conclusion is reinforced by the drug concentration measurements from heart muscle (Table 1). The myocardial levels 30 min after regional infusion are lower than those seen 30 min after systemic treatment with Adriamycin. The difference in heart muscle Adriamycin levels between portal vein infusion and systemic vein infusion is significant 30 min after treatment. These data suggest that regional infusion will reduce systemic exposure to Adriamycin. However, this experimental method does not permit the construction of “concentration versus time” curves that would allow accurate measurement of the true systemic exposure.

Our data demonstrate that Adriamycin concentration in the hard nonnecrotic rim of Vx-2 tumors is not a strong function of the total tumor weight. The weights of tumors from the experimental populations are not well matched, but the Adriamycin dependence on tumor weight may be calculated for the three routes of infusion. Drug delivery (extrapolated to “zero tumor weight”) is close to that measured in this experiment (Table 1).

The rabbit hepatic Vx-2 carcinoma model is useful for the study of regional infusion of liver metastases. The tumor response to treatment has not been determined, but higher levels of Adriamycin are found in tumors after hepatic artery infusion than after systemic treatment. In addition, the tumor/liver Adriamycin ratio is higher. This confirms the theoretical strength of this therapeutic modality: enhanced specificity in tumor treatment.

ACKNOWLEDGMENTS

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REGIONAL INFUSION TO HEPATIC Vx-2 CARCINOMA

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