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A Dog Model Using an Implanted System for Protracted Hepatic Arterial Chemotherapy[1]

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A model for hepatic arterial chemotherapy studies using large dogs and an implantable infusion pump has been developed. Using this technique near complete perfusion (>90%) of the liver can be achieved *in vivo* as determined by hepatic arterial perfusion scintigraphy with technitium 99m macroaggregated albumin. The system is reliable and has been in use for a total of 1353 days (mean of 104 days, range 52–239) in 13 dogs. Pump implantation causes no apparent acute liver damage based on pre- and post-operative alkaline phosphatase and serum glutamic-pyruvic transaminase determinations and does not affect the general mobility or behavior of the animals. Careful placement of the catheter and attention to the physicochemical properties of the solutions loaded are factors contributing to the success of the model. The model permits comprehensive preclinical pharmacokinetic and toxicologic studies of new or preexistent chemotherapeutic agents in the same device that will be used for later administration in human subjects. By providing the means to examine and develop new treatment modalities, it enables the design of even more potent cytotoxic therapy directed into the tumor vascular bed.

INTRODUCTION

A number of malignancies exhibit a tendency to remain confined to specific body areas or organs. In patients with these cancers, it is often not widespread metastasis but rather uncontrolled local tumor growth that is the primary cause of morbidity and eventual mortality. For regionally confined tumors such as primary and metastatic carcinomas involving the liver, direct administration of chemotherapeutic agents into the hepatic artery potentially can generate increased regional drug exposure while maintaining systemic drug levels below the toxic threshold for the usual dose-limiting tissues [1, 2]. This is especially true for cancer within the liver since hepatic tumors derive 95% of their blood supply from the hepatic artery while two-thirds of the blood supply to the liver parenchyma comes from the portal vein [3–5].

Attempts to utilize this flow differential therapeutically have been plagued by a high rate of complications such as vessel thrombosis, catheter dislocation, and infection and required the use of bulky external pumping devices [6–9]. Recent advances in the technology of drug delivery systems, radionuclide angiography, and pharmacology have made possible safer, more reliable approaches to hepatic arterial chemotherapy and have renewed interest in its use [10, 11]. The implantable Infusaid pump and catheter drug delivery system is being used increasingly for the regional administration of chemotherapy in cancer patients on a chronic basis. For patients with cancer in the liver, the application of regional chemotherapy with this system appears to result in markedly improved response rates and has perhaps impacted upon survival as well [12, 13].

At the present time, however, the number of drugs and therapeutic modalities available for common use with the pump are extremely

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limited. One of the major impediments to progress in this area has been the lack of a large animal model with which to pursue preclinical investigations. In this paper, we report the successful development of a canine model and describe some of the surgical considerations in its use.

Once our model was established, the drug we administered through the implanted pumps was 5-bromo-2'-deoxyuridine (BUDR), a radiosensitizer with which we were performing toxicologic studies in preparation for its use in this capacity with radiotherapeutic microspheres. Recent studies suggest that tumors are often hypervascular compared to normal liver [14–16]. Regional microsphere therapies represent a means of capitalizing on this relative tumor hypervascularity by generating a prolonged exposure of drug or radiation within tumor vessels. We intend to use our canine model to study the hepatic arterial administration of yttrium-90 microspheres as a means to deliver internal radiotherapy after pretreatment with a continuous regional infusion of radiation sensitizer. We anticipate that the results of these and other investigations will provide a basis for the application of microsphere administration and other selective modalities to the treatment of primary and metastatic liver cancer. Similar large animal models will serve to advance the use of such selective approaches in other regionally confined malignancies such as brain tumors and head and neck cancer.

MATERIALS AND METHODS

Animals. Thirteen foxhounds with a mean weight of 32.1 kg (range 25–40 kg) were obtained from the Laboratory Animal Center of Ohio State University, Columbus, Ohio. The dogs were held in the Animal Research Facility of the University of Michigan for observation prior to undergoing any manipulations for this study. For the duration of the study, the animals were quartered in and maintained by the University of Michigan Unit for Laboratory Animal Medicine according to DHHS Guidlines; their weights were monitored monthly and their activity and appetite assessed at least daily. A technetium-99m sulfur colloid liver spleen scan and baseline laboratory studies consisting of serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (AP), and amylase were obtained before pump implantation. These laboratory studies were repeated weekly for 2 weeks after surgery and during periods of drug infusion.

Drug delivery system. Infusaid Model 400 implantable infusion pumps (Intermedics-Infusaid, Norwood, Mass.) were placed into the 13 dogs. A radiopaque, tapered, silicone rubber catheter (No. 36587, Intermedics-Infusaid) was chosen as its tapered end facilitated insertion into the artery. The catheter's total length is 38–40 cm, the outer diameter (o.d.) 2.3 mm tapering down to 1.5 mm and the inner diameter (i.d.) 0.6 mm throughout. Placement of beaded rings on the outside surface near the tip of the catheter aided both in accurate placement and in preventing catheter movement by providing points at which the soft tubing could be secured to the cannulated artery and to surrounding tissue.

Prior to implantation each pump was loaded with 50 ml (maximum capacity) of sterile saline containing 100 U/ml of sodium heparin. Every 14 days, the residual chamber volume was emptied and measured, and the pumps refilled. Flow rates were calculated by subtracting the residual volume from 50 ml and dividing the remainder by 14. The baseline flow rates of individual pumps remained constant averaging 2.6 ml/day (range 2.0 to 3.2 ml/day).

Reuse of the pumps is possible. We recommend first clearing all traces of prior contents by repeatedly loading the pump with sterile water and running it while immersed in a 56°C water bath. Once a pump is purged, its catheter can be replaced with an unused one attaching it with Silastic medical adhesive (Dow Corning Corp., Midland, Mich.). The pump and catheter can then be sterilized at 110°C for 50 min. Should a pump lack a tapered catheter tip, one can be constructed by

fastening a smaller o.d. catheter onto the larger o.d. pump catheter using a barbed metal connector (No. 36590, Intermedics-Infusaid) and Silastic medical adhesive (see Fig. 1, inset).

Operative techniques. Anesthesia was initiated with 400–500 mg sodium thiamylal (Surital, Parke-Davis, Morris Plains, N.J.) and maintained via endotracheal intubation with a mixture of O_2, N_2O, and nalothane. All dogs received gentamycin sulfate (Garamycin, Schering Corp., Indianapolis, Ind.), 500 mg im BID on the day of surgery and sodium cefazolin 500 mg im BID for 4 days postoperatively.

The pump was placed in a subcutaneous pocket created approximately 10 cm from the dorsal midline and sutured to the underlying muscle using at least three of the five loops permanently attached to the pump's circumference for this purpose. The dorsal location was chosen because placement ventrally within the abdominal wall resulted in poor wound healing and pump movement in these active animals.

The first catheter placement attempted (two dogs) was insertion into the gastroduodenal artery as this is similar to the technique most commonly used clinically in patients [18]. Upon exposure, the gastroduodenal artery was ligated distally. The catheter tip was then inserted through a small arteriotomy, positioned 1–2 mm from the origin, and secured by a 2-O black silk ligature.

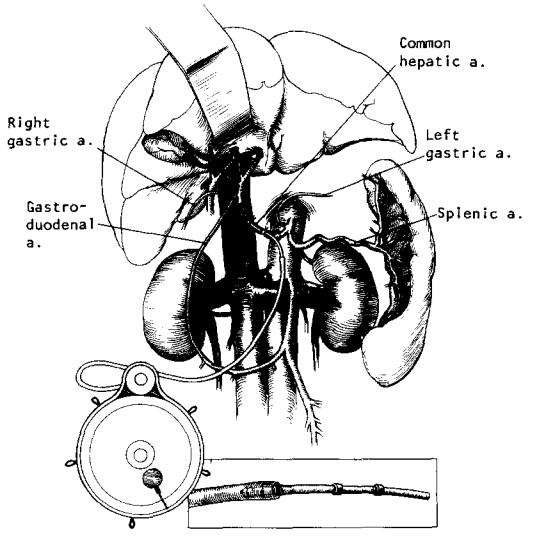

FIG. 1. Catheter placement in the canine common hepatic artery. The gastroduodenal artery is ligated. The inset shows the modified, beaded catheter. This placement resulted in consistently greater liver perfusion.

In four dogs a second site was chosen for placement. The catheter tip was inserted through a small arteriotomy in the splenic artery and advanced until the tip was flush with the celiac axis. The splenic, left gastric, and gastroduodenal arteries were then ligated and the catheter was secured.

The site ultimately selected for catheter placement (seven dogs) was the common hepatic artery. After introduction via arteriotomy, the catheter tip was positioned approximately 1.5 cm distal to the origin of the left gastric artery and secured so as not to occlude arterial flow. The gastroduodenal artery was then ligated.

Flow distribution. Liver–spleen scans were obtained on all dogs using 4 mCi of technitium sulfur colloid prior to pump implantation. Hepatic arterial perfusion scans (HAPS) were performed by slow injection of 4 mCi of technitium macroaggregated albumin (TcMAA) through the sideport of the pump soon after implantation [11]. The percentage of liver substance perfused was estimated by visually comparing the anterior, posterior, and lateral aspects of the liver–spleen scan with similar views obtained with the HAPS. Using these images, shunting of blood to the lungs and other extrahepatic locations could be detected. Any animals exhibiting these findings would have been excluded from involvement in the drug study. In seven dogs, the pattern of distribution was reassessed 3 months after the original determination by a repetition of the HAPS.

Drug solutions. 5-Bromo-2'-deoxyuridine (BUDR) Lot No. 416900 was obtained from P-L Biochemicals, Inc., Milwaukee, Wisconsin. Purity was >98% by spectral analysis and no contaminating substances were found by thin-layer chromatography using two solvent

systems. A solution containing 500 mg/ml of BUDR in a 0.1 *M* sodium bicarbonate/carbonate buffer (pH 9.8 ± 0.1) was prepared. Stability of this BUDR buffer solution at 37°C was satisfactory with ≤5% decrease in BUDR concentration by high-performance liquid chromatographic analysis over 14 days (in glass or the pumps). This stock solution was then diluted further so that BUDR at a dose of 10 mg/kg/day was delivered over 14 days through the pump to each of five dogs. These dogs received two consecutive 14-day infusions to achieve a 28-day continuous exposure to the drug. Five control animals received an infusion of the 0.1 *M* buffer solution without BUDR for the same time period. Three dogs received 2 weeks of BUDR followed by 2 weeks of control buffer solution. Upon termination of BUDR infusions, control buffer solution was instilled into the pumps of treatment animals. Heparin, at a concentration of 1000 units of heparin per milliliter, was added to all solutions. Three additional dogs received 14-day infusions of 10 mg/kg/day BUDR alternating with 14-day infusions of control buffer solution.

RESULTS

As we confirmed by preoperative angiography (Fig. 2), the arterial anatomy of the dog varies considerably from human vascular patterns, and therefore three successive variations in catheter placement were required to obtain one providing complete (90–100%) liver perfusion consistently. Estimates of the percentage of liver perfused for every dog with each of the variations of catheter placement are listed in Table 1. Once experience was acquired with the technique, the most complete perfusions were obtained with catheter insertion into the common hepatic artery. A com-

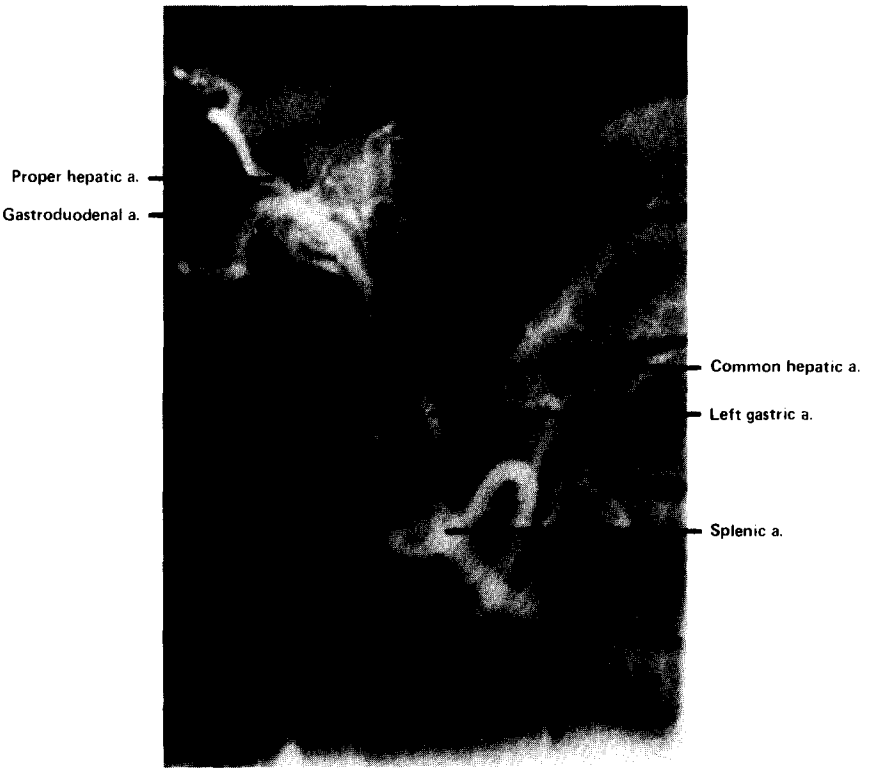

FIG. 2. A presurgical angiogram in a dog demonstrating typical canine vascular anatomy in this region.

TABLE 1

CATHETER PLACEMENT

Arterial site	Dog	Percentage liver perfusion
Gastroduodenal	1	60
	2	80
Splenic	3	[a]
	4	50
	5	70
	6	70
Common Hepatic	7	40
	8	50
	9	60
	10	75
	11	90
	12	>90
	13	>90
	14	>90

[a] This dog died before perfusion extent could be determined (See Results).

parison of the sulfur colloid image in Fig. 3a with the corresponding TcMAA HAPS image (Fig. 3b) illustrates a liver with only 40% perfusion. By comparing Fig. 3c with Fig. 3d, a >90% perfusion pattern will be seen.

In addition to yielding an inadequate perfusion pattern, use of the splenic artery resulted in the death of one dog. Ligation of the splenic, left gastric, and gastroduodenal arteries and some constriction of the proper hepatic artery led to severe hemorrhagic, ischemic necrosis of the spleen, stomach, duodenem, jejunum, and the left lateral and left medial lobes of the liver. Subsequently, considerable care has been taken to ensure that ligation of the gastroduodenal artery does not constrict the proper hepatic artery. Catheter placement during surgery is the most critical step in assuring that the entire liver will be safely perfused with drug solution from the implanted pump [17].

The implanted pumps have operated satisfactorily for a total of 1353 days (mean 104, range 52–239) in the 13 dogs. Each pump was refilled an average of seven times with no complications. To assess the patency of the vessel and catheter and the distribution of flow over time, TcMAA perfusion scans were repeated approximately 3 months after the original flow determinations on 7 of the 13 dogs. The change in the volume of liver perfused in these animals was less than 10%. The viscosity of the BUDR solution resulted in flow rates that were 33% of those obtained using control buffer solution (Table 2). Patency of the catheters was unaffected by the altered viscosity during the BUDR infusions.

No significant difference between pre- and postsurgery levels of hepatic enzymes (SGPT or AP) could be found by t test. The postoperative amylase levels, however, were higher (significant at the 0.05 level) suggesting the possibility of some pancreatic ischemia although they were still within the normal range and the animals remained clinically well. Dogs receiving 4 weeks of control buffer solution and those given only 2-week infusions of BUDR demonstrated no significant elevations in liver function tests while those animals given 28 days of BUDR developed significant elevations after the third week (Table 3).

DISCUSSION

Hepatic arterial chemotherapy represents a means to improve antitumor effect by increasing drug exposure to tumor within the liver relative to the exposure of the dose-limiting tissues elsewhere in the body. Because of the idea's attractiveness, many attempts to apply it have been reported in the medical literature during the past 20 years. Until recently, however, use of this technique was hampered by a high incidence of partial or complete vessel thrombosis, catheter displacement, infection, and other complications [6–9].

Recent advances in the technology of drug delivery systems, radionuclide angiography, and pharmacology have made possible safer, more reliable approaches to hepatic arterial chemotherapy and have renewed interest in its use. The implantable Infusaid pump and catheter drug delivery system is being used increasingly for the regional administration of

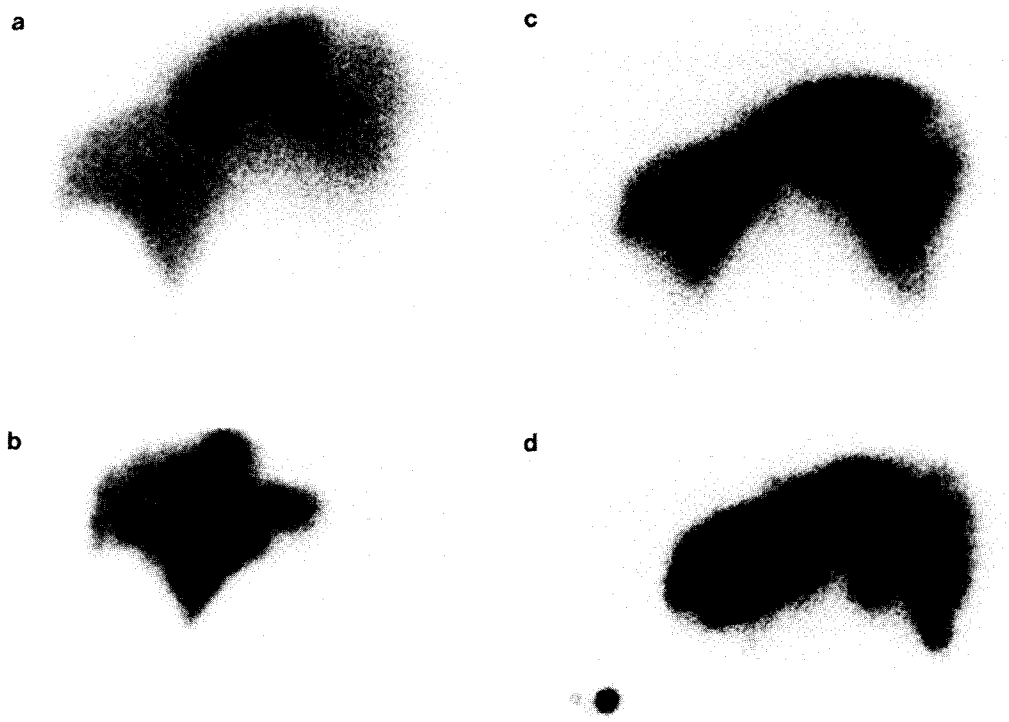

FIG. 3. (a) Right Lateral view of a standard Tc-sulfur colloid liver–spleen scan in the foxhound whose perfusion study is shown in Fig. 3b. (b) Right Lateral view of a hepatic arterial perfusion scan (HAPS) from the dog whose liver–spleen scan is shown in Fig. 3a. Liver perfusion in this dog was approximately 40%. (c) Right Lateral view of a standard Tc-sulfur colloid liver–spleen scan of the dog whose perfusion scan is shown in Fig. 3d. (d) Right Lateral view of the HAPS from the dog whose liver–spleen scan is shown in Fig. 3c. Liver perfusion in this dog was approximately 90%. The circular area of tracer activity in the Lower-Left hand corner is the injection site (pump side-port).

chemotherapy in cancer patients on a chronic basis. For patients with primary and metastatic carcinomas within the liver, the application of regional chemotherapy with this system has resulted in reports of markedly improved response rates and perhaps has impacted upon survival [12, 13]. At the present time, however, the number of drugs and therapeutic modalities available for common use with the pump are extremely limited.

TABLE 2

BUDR INFUSION

Wt (kg)	Initial flow (ml/day)	BUDR Flow (ml/day)	BUDR Concn (mg/ml)	BUDR dose (mg/kg/day)
36	2.6	0.8	461.5	10.2
28	2.3	0.7	404.0	10.2
28	2.9	1.1	326.3	13.2
34	3.2	1.1	357.5	11.2
31	2.9	0.9	356.3	10.7
31	2.3	1.3	169.4	7.4
27	2.4	1.6	139.2	8.3
37	2.0	1.0	266.2	7.3

One of the major impediments to progress in this area has been the lack of a large animal model with which to pursue preclinical testing involving the implanted device. With this paper, we report the successful development of just such a canine model. That the optimal catheter placement in the model would differ significantly from the position most commonly used clinically was unexpected. We have experienced no difficulty maintaining

TABLE 3

LIVER FUNCTION TEST CHANGES IN THREE GROUPS OF DOGS: THOSE RECEIVING 28 CONSECUTIVE DAYS OF BUFFER ALONE, 14 DAYS OF BUDR FOLLOWED BY 14 DAYS OF BUFFER, AND 28 CONSECUTIVE DAYS OF BUDR, ALL SOLUTIONS ADMINISTERED BY CONTINUOUS HA INFUSION VIA IMPLANTED PUMPS

Group	Day 0	7	14	21	28	
			Alkaline phosphatase			
28-day buffer	4.7	4.1	3.4	3.3	3.0	(Bodansky Units/dl) NL range 1.7–9.7
14-day BUDR/14-day buffer	60.9	56.4	71.0	59.8	58.9	(U/L) NL range 29.7–87.3
28-day BUDR	5.0	4.5	3.6	4.0	7.5	(Bodansky Units/dL)
			Serum glutamic pyruvic transaminase (IU/L)			
28-day buffer	27.0	21.5	25.3	14.2	22.5	(NL Range 10.9–38.5)
14-day BUDR/14-day buffer	28.9	33.0	42.2	30.5	29.2	
28-day BUDR	22.7	22.2	24.6	44.2	72.6	

catheter patency in this location, however, and have not witnessed a rise in residual chamber volume with a decline in flow rate of individual pumps as would occur with gradual thrombus formation. Consistent with common clinical practice, we added heparin to all solutions infused. Since we did not have an unheparinized comparison group, we are unable to determine the role and importance, if any, that heparin has in maintaining vessel and catheter patency.

With the development of this model, it now will be possible to perform comprehensive preclinical pharmacokinetic and toxicologic studies of new or preexistent chemotherapeutic agents in the same device that will be used for later administration in human subjects. For example, we have used the model to explore the effect of dose schedule on toxicity from hepatic arterial BUDR infusion as a prelude to combining the radiosensitizing infusion with the regional administration of radiotherapeutic microspheres. Without subjecting human patients to any risks or discomforts, we were able to determine that BUDR can cause a schedule-dependent chemical hepatitis similar to that seen clinically with FUDR [12, 13]. This model thus provides the means to examine, develop, and integrate new treatment modalities such as degradable starch and nondegradable radiotherapeutic microspheres, vasoconstrictors, and radiosensitizers such as BUDR. Through such use, this model will enable the design of even more potent cytotoxic therapy to direct into the tumor vascular bed.

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