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Report

An Implantable Pump for Intrarenal Infusion of Immunosuppressants in a Canine Autotransplant Model

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We developed a canine renal allograft model utilizing implantable infusion pumps and biocompatible catheters to investigate the pharmacokinetics of local immunosuppressive drug administration. Seven mongrel dogs underwent bilateral nephrectomy and autotransplantation of one kidney to the iliac vessels. The proximal end of an infusion catheter directed into the iliac artery was tunneled to a subcutaneously placed programmable pump. A second, sampling catheter was placed with its tip in the iliac vein. Simultaneous regional (iliac vein) and systemic (jugular vein) venous concentrations of 6-mercaptopurine (6-MP), the immunosuppressive metabolite of azathioprine, were determined during a continuous 24-h intraarterial infusion (10 mg/kg/24 hr). The gradient between regional and systemic 6-MP concentrations was maximal initially when the pump was turned on, continuously decreased until steady state was reached, and disappeared immediately after the pump was turned off. The mean ratio of steady-state iliac vein to systemic 6-MP concentrations was 5.0 ± 1.4, demonstrating a pharmacokinetic advantage of continuous intraarterial 6-MP infusion to the autotransplanted kidney. The novel canine renal allograft model described herein overcomes the technical limitations of earlier models and represents a foundational step in the design of intrarenal infusion patterns of immunosuppressive agents which we expect to prolong survival of the allotransplanted kidney with minimal systemic drug exposure and side effects.

KEY WORDS: pharmacokinetics; 6-mercaptopurine; targeted drug delivery; renal transplantation; intraarterial infusion.

INTRODUCTION

Theoretical considerations of the pharmacokinetic advantage of target-directed drug delivery dictate that drugs which are either eliminated by the target organ or rapidly cleared by the systemic circulation may be infused *directly into* the target organ to produce a marked decrease in systemic drug concentrations or increase in local drug concentrations, respectively, when compared with same-dose systemic administration (1). It is our hypothesis that a controlled infusion of immunosuppressive agents administered directly into the functioning transplant will simultaneously prevent rejection and diminish or eliminate toxic systemic side effects, including hepatic, bone marrow, and other organ dysfunction. Our first step toward validating this hypothesis was to develop a canine renal transplant model which utilizes a programmable, implantable infusion pump to investigate the pharmacokinetic parameters of continuous intraarterial drug delivery to the autotransplanted kidney. The purpose of this report is to describe our canine, locoregional, constant-rate drug delivery model and demonstrate the utility of this model for investigating the pharmacokinetic advantage of locally infused 6-mercaptopurine (6-MP), the active metabolite of azathioprine, an important component of the immunosuppressive regimen administered to many transplant patients.

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MATERIALS AND METHODS

Canine Renal Allograft Model

Surgical Procedure. Following approval of The Research Animal Resources Committee, renal autotransplantation with implantation of a programmable drug infusion device was performed in seven mongrel dogs (Nos. 1–7). Dogs

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were 18–21 kg in weight and conditioned for 2 weeks prior to surgery. Unilateral nephrectomy was performed through a midline abdominal incision. Following complete dissection of the contralateral kidney, the right iliac fossa was prepared for autotransplantation. Proximal and distal control of the distal aorta was then obtained with vascular clamps. The distal end of a soft, flexible, 4 Fr (1.2-mm-o.d., 0.3-mm-i.d.) catheter (Model 8702 Drug Administration Catheter, Medtronic, Inc., Minneapolis, Minn.) was purse-strung into the aorta with the tip directed well into the right external iliac artery under direct vision. Following reperfusion of the lower extremities, a vascular clamp was reapplied to the right side of the distal aorta in order to occlude the right external iliac artery without compromising blood flow to the other distal aortic branches or traumatizing the arterial segment containing the catheter. The external iliac artery was then ligated distally and divided, with the tip of the catheter protruding through the cut proximal end. The remaining kidney (usually the left) was removed during a forced diuretic phase and flushed with approximately 200 ml cold lactated Ringer's solution containing heparin and procaine until a clear venous effluent was obtained. End-to-end renal artery–iliac artery and end-to-side renal vein–iliac vein anastomoses were constructed. The ureter was implanted into the bladder via the creation of a submucosal tunnel and anastomosis of the spatulated ureter to the bladder mucosa.

After completing the transplant, the distal end of a 6.5 Fr (2.2-mm-o.d., 1.0-mm-i.d.) catheter (Model 8700 Drug Administration Catheter, Medtronic, Inc.) was purse-strung into the intrarenal inferior vena cava and the tip guided distally into the right external iliac vein to a point just proximal to the end-to-side anastomosis under direct vision. The proximal end of the arterial infusion catheter was then brought out of the abdominal cavity, tunneled subcutaneously to a "pump pocket" in the lateral chest wall, and anchored to the catheter port of a SynchroMed infusion pump (Model 8610H, Medtronic, Inc.). The venous sampling catheter was connected proximally to a subcutaneously placed catheter access port (CAP) (Model 8501-0, Medtronic, Inc.). Figure 1 depicts the location of the arterial and venous catheter at the conclusion of the procedure.

SynchroMed Infusion Pump. The SynchroMed infusion pump contains a self-sealing septum, a collapsible 18 ml drug reservoir, a peristaltic pump, a motor, microprocessor-based control circuitry, and a lithium thionyl-chloride battery. The drug reservoir is a sealed titanium chamber which can be filled or evacuated using a syringe and noncoring needle to puncture the septum percutaneously. The peristaltic pump dispenses drug in accordance with information transmitted from the Physician Programmer (Model 8800M, Medtronic, Inc.). The programming wand allows the infusion pump to be programmed externally by telemetry. Programming occurs only in the simultaneous presence of both a magnetic field and the recognized radiofrequency signal. The infusion rate can be programmed to vary between 0.009 and 0.9 ml/hr. Program options allow time-qualified bolus injections, continuous infusion at a constant flow rate, or a combination of both modes so that virtually any temporal infusion pattern can be achieved. The device is powered by a single lithium-chloride cell. At a continuous infusion rate of 1.0 ml/day, the battery will last 3 years. The pump is equipped with an audible alarm indicating low drug reservoir, low battery, and failure to match programmed instructions with current pump activity. At the time of surgery, the pump was filled with 18 ml saline containing 1000 U/ml heparin (Upjohn Co., Kalamazoo, Mich.) and programmed for continuous infusion at a rate of 2 ml/day. Heparin has undergone and passed extensive compatibility testing with the silicone material of the pump and catheter tubing (2).

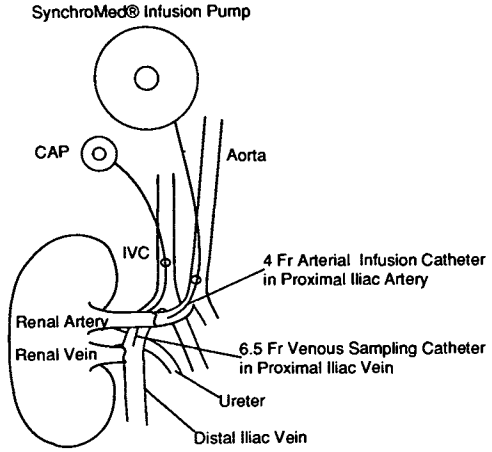

Fig. 1. Location of arterial infusion and venous sampling catheters at the completion of canine renal autotransplantation. The SynchroMed infusion pump and CAP are located in separate subcutaneous pockets on the right lateral chest wall. IVC, inferior vena cava.

Vascular Catheters. The drug administration catheters are made of silicone rubber, with a special connector facilitating connection to the pump. This connector also fits on the back section of a tunneling rod, which facilitates surgical placement of the catheter within body cavities. Catheters also contain a radiopaque metal coil bonded into the catheter to resist kinking and several anchoring rings which facilitate vascular placement.

Catheter Access Port (CAP). The CAP was implanted to allow repeated sampling from the venous effluent of the target organ. Its housing is a molded biocompatible thermoplastic and contains a self-sealing septum, a needle stop, an infusion pathway, several suture points, and a titanium catheter port. The distal end of a vascular catheter was inserted into the proximal iliac vein and the proximal end was tunneled to the catheter port of the subcutaneously placed CAP. Patency of the CAP was maintained with daily percutaneous flushes of 3 ml heparinized saline (1000 U/ml) using sterile technique.

Intrarenal Infusion of 6-Mercaptopurine (6-MP)

A continuous 24-hr intraarterial infusion of 6-MP (10 mg/kg/day) was begun on the second postoperative day, following confirmation of normal postoperative creatinine values and preoperative liver function tests. 6-MP was supplied by the Burroughs Wellcome Company (Research Triangle Park, N.C.) in bottles containing 500 mg lyophilized drug as the sodium salt. Fifty milliliters 0.2 *M* sodium phosphate tribasic buffer, pH 12.7, was added to each bottle to solubilize the drug, producing a final 6-MP concentration of 10

mg/ml. The solution was then sterilized by passage through a 0.22-μm Millipore filter and 18 ml was injected into the reservoir of the pump after completely removing the residual heparinized saline. Simultaneous proximal iliac vein and systemic vein blood samples, drawn from the CAP and jugular vein, respectively, were obtained at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 22, and 24 hr following the initiation of infusion. Each blood sample (2 ml) was immediately centrifuged, and the plasma separated and stored at −70°C in vials containing 10 μl of 0.2 *M* dithiothreitol for subsequent 6-MP determination. At the conclusion of the 24-hr period of intrarenal infusion, the residual 6-MP was completely aspirated from the pump, and the pump was reprogrammed to deliver heparinized saline again at 2 ml/day. In dog 1, iliac vein blood samples were drawn from the CAP as rapidly as possible (every 1–2 min) for the first 10 min after stopping the 6-MP infusion in order to detect the rate of fall of regional 6-MP concentrations to systemic concentrations.

6-MP Assay

6-MP concentrations were determined on 0.3 ml plasma by high-performance liquid chromatography according to Erdmann *et al.* (3). A modification of the chromatographic conditions employed a mobile phase of 5% acetonitrile:95% sodium dihydrogen phosphate, 0.01 *M*, pH 2.7, at a constant flow rate of 1.5 ml/min. Between 3 and 3.5 min, the percentage of acetonitrile was increased to 20%. The retention times of 6-MP and the internal standard, 6-*n*-propyl-2-thiouracil, were 3.50 and 7.20 min, respectively. The lower limit of sensitivity of the assay was 10 ng/ml. Two primary metabolites of 6-MP, 6-thioxanthine and 6-thioguanine, were injected onto the column under the same assay conditions and did not interfere with the quantitation of 6-MP.

RESULTS

The iliac vein and jugular vein 6-MP concentrations obtained for dog 1 during continuous intraarterial infusion of 6-MP (10 mg/kg/24 hr) are representative and are depicted on a semilogarithmic plot of concentration versus time in Fig. 2. In order to demonstrate the effect of starting or stopping the pump on measured changes in regional (proximal iliac vein) drug concentration, the data in Fig. 2 can be represented on an expanded time scale and divided into three phases: Phase I, "pump on" (Fig. 3); Phase II, "steady state" (Fig. 4); and Phase III, "pump off" (Fig. 5). Figure 3 shows that iliac vein concentrations are easily measurable after 10 min of intrarenal 6-MP infusion and that near-steady-state local levels are achieved by 20 min postinfusion. On the other hand, 6-MP is still undetectable (below 10 ng/ml) in jugular venous samples at 10 min and reaches steady-state systemic levels only after 45 min of continuous administration. As a result, the gradient between regional and systemic 6-MP concentrations continuously decreases during Phase I (Fig. 3) and reaches a constant minimum during Phase II (Fig. 4). Finally, when the 6-MP infusion was stopped in Phase III, iliac vein 6-MP concentrations fell to equal systemic levels within 1.8 min (Fig. 5). Clearly, the ability to turn on and off the infusion pump noninvasively permits one to readily achieve both temporal and spatial control of drug delivery.

Measured steady-state iliac vein drug concentrations

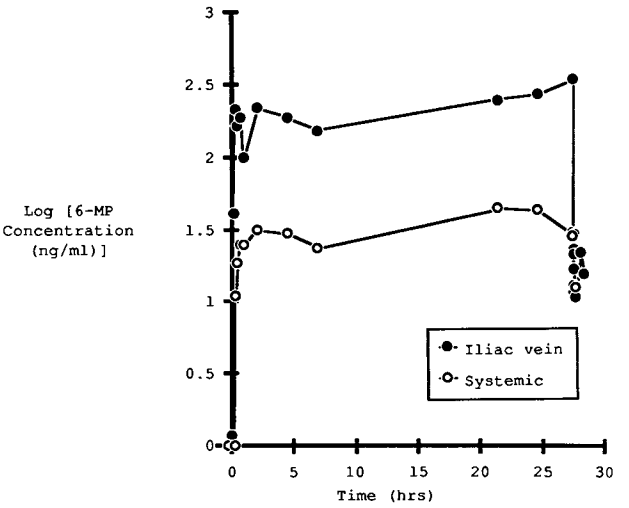

Fig. 2. Semilogarithmic plot of iliac vein and systemic vein concentrations is shown for dog 1 during a continuous intraarterial infusion of 6-MP (10 mg/kg/24 hr). Pump was turned off at t = 27.3 hr.

(C_{IL}) and corresponding systemic concentrations (C_S) were 153 ± 33 (mean ± SE) and 29 ± 5 ng/ml, respectively, for five of the seven dogs studied (excluding dogs 2 and 5). Distal migration of the tip of the venous catheter past the anastomosis was noted at autopsy in dog 2 and accounted for the duplicate sampling of systemic concentrations in this animal. Systemic concentrations in dog 5 were in the range of the lower limit of sensitivity of the 6-MP assay, so a reliable determination of steady-state concentration could not be made. The mean C_{IL}/C_S was 5.0 ± 1.4 (range, 1.9 to 9.5) and reflects the minimal regional pharmacokinetic advantage obtainable during prolonged intrarenal 6-MP infusion, since renal vein (target organ) drug concentrations are diluted by

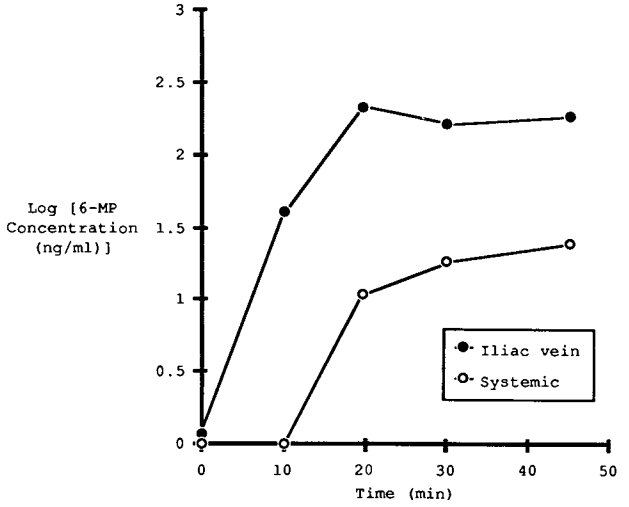

Fig. 3. Semilogarithmic plot of iliac vein and systemic vein concentrations during Phase I ("pump on") of continuous intraarterial infusion of 6-MP (10 mg/kg/24 hr) in dog 1. At t = 10 min, the systemic 6-MP concentration is still less than 10 ng/ml, the lower limit of assay sensitivity, and is plotted as 0 ng/ml. The concentration gradient between regional and systemic 6-MP concentrations is maximal initially (19.9:1 at 20 min) and continuously decreases (7.6:1 at 45 min) during this period.

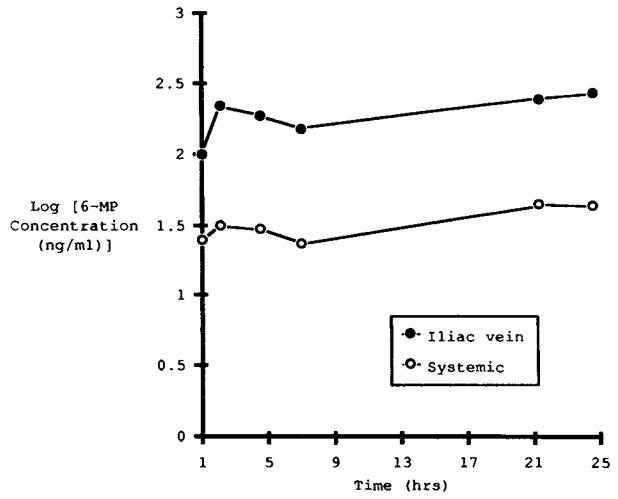

Fig. 4. Semilogarithmic plot of iliac vein and systemic vein concentrations during Phase II (steady state) of continuous intraarterial infusion of 6-MP (10 mg/kg/24 hr) in dog 1. The ratio of iliac vein to jugular vein 6-MP concentrations remains at a constant minimum (C_{IL}/C_S = 6.1) after steady state is reached.

distal iliac vein (systemic) concentrations to give measured proximal iliac vein concentrations (Fig. 1).

DISCUSSION

Conflicting reports regarding the effectiveness of local treatment of canine and human renal allografts appeared in the 1960s (4–10), but this line of investigation was abandoned for almost 20 years until Ruers *et al.* (11) recently demonstrated that continuous intraarterial infusion of prednisolone in rat renal allograft recipients produced a significant increase in graft survival when compared to continuous intravenous or intraperitoneal steroid administration. In light of the recent development of more specific and potent immunosuppressive agents, the technological advances in design of local drug delivery systems, the elucidation of the pharmacokinetics of target-aimed drug delivery (1), the advances made in understanding the intragraft mechanisms (12) of allograft rejection, the effectiveness of local graft irradiation in reversing allograft rejection (13,14), and most importantly, the continued demonstration of significant morbidity in transplant patients resulting from systemic immunosuppression, it is clear that the concept of local immunosuppression deserves reexploration.

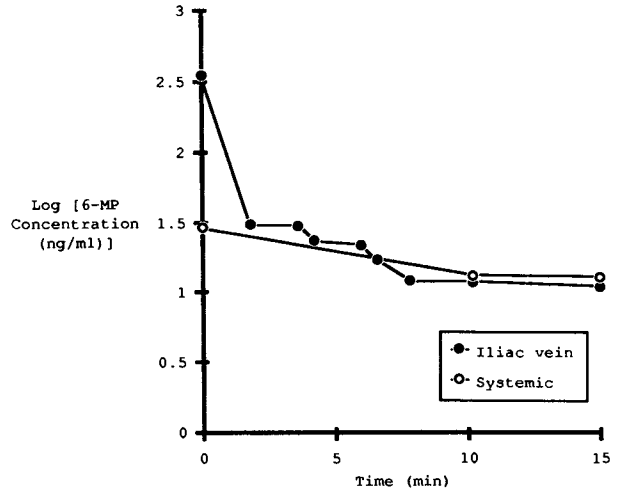

Fig. 5. Semilogarithmic plot of iliac vein and systemic vein concentrations during Phase III ("pump off") in dog 1. Local 6-MP concentrations fall to equal systemic levels immediately after the pump is turned off at time zero.

Within the last several years, two research groups have reported their experience with local immunosuppressive therapy in experimental renal transplantation. In the model used by Ruers *et al.* (11), a cannula was introduced into the suprarenal or testicular artery of the transplanted kidney and connected to an osmotic minipump implanted in the abdominal cavity. However, the osmotic minipump used was capable of delivering drug for only 2 weeks posttransplant and determination of regional and systemic drug concentrations could not be performed to account for the results obtained. Utilizing a constant infusion, nonprogrammable, implantable Infusaid pump, Campbell *et al.* (15) delivered PGE1 directly into the renal artery of one transplanted kidney in a bilateral renal transplant model with the recipient animal possessing two native kidneys and two allografted kidneys from the same donor. Although differences were noted in the histologic appearance of treated and untreated transplants, the time course of rejection, as measured by radioisotope scanning, was the same in both of these kidneys. In these studies, drug could be infused only at a constant infusion rate and the anatomy/physiology of the recipient differs markedly from that occurring in practice, with two completely functioning native kidneys *in situ*. The technical limitations of these earlier models have been overcome in our canine renal allograft model using the SynchroMed infusion pump. The presence of a sampling catheter in the proximal iliac vein could permit the repeated study of both pharmacokinetic and pharmacodynamic parameters during local infusion of immunosuppressive agents, singly or in combination, as well as metabolite disposition.

In summary, we developed a novel canine renal allograft model utilizing implantable pumps and biocompatible catheters in order to determine the pharmacokinetic advantage of continuous intraarterial 6-MP delivery to the autotransplanted kidney. We demonstrated that a mean fivefold concentration gradient was achieved at steady state between simultaneous regional and systemic drug concentrations and that, by turning the pump on and off, one can temporally and spatially regulate drug delivery with a rapid effect on local drug concentrations. During the past 2.5 years, 125 programmable drug delivery systems have been implanted in patients at the University of Minnesota for the local or systemic treatment of various tumors (16), clearly demonstrating that local infusion of immunosuppressive agents in clinical transplantation is a technical reality if therapeutic advantage is demonstrated.

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Scott A Gruber is the Society of University Surgeon–Ethicon Corporation Fellow 1987–1989 and the recipient of National Kidney Foundation Young Investigator Awards, 1986–1987 and 1987–1988.

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