Isolation-Perfusion of the Liver with 5-Fluorouracil

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Isolation-perfusion of the liver was performed in ten pigs using 5-fluorouracil administered in the perfusion circuit at doses of 100, 250, 500, and 1000 mg/kg body weight. Perfusion was performed for 60 minutes at normothermic (37 C) or hyperthermic (41 C) temperatures. One animal died shortly after perfusion. Incomplete isolation of the hepatic vasculature in two animals resulted in significant drug leakage into the systemic circulation with resulting hematologic toxicity. Perfusion with 5-fluorouracil at 1000 mg/kg produced hepatic necrosis. Perfusion with 5-fluorouracil at doses of 100, 250, or 500 mg/kg produced no hepatic toxicity except for transient elevations of hepatic enzymes and resulted in no systemic drug toxicity. Levels of 5-fluorouracil tolerated by the liver in the isolation-perfusion system were more than 1000-fold greater than the maximum drug levels achievable by routine systemic, intra-arterial, or intraperitoneal administration.

ISOLATION-PERFUSION of an organ *in vivo* involves isolating the blood supply to permit controlled flow of perfusate through the circulation of the organ, with continuous flow of the perfusate through a closed system. Since the circulation is isolated, chemical agents can be administered exclusively to the organ perfused, with no mixing of the isolated circulation with the general systemic circulation. High concentrations of agents can be added to perfusates and delivered to organs without loss of the agents to the general circulation. Isolation-perfusion can be useful in the treatment of regional malignancies, since it can allow the delivery of cytotoxic agents in high concentration to the region perfused while avoiding general drug toxicity by preventing the general distribution of the cytoxic agents through the systemic circulation. Isolation-perfusion of the extremities has been used clinically with success.[1-5] Frequently hyperthermia has been utilized in conjunction with isolation-perfusion.

Isolation-perfusion of the liver *in situ* has been attempted in experimental animal systems, usually with attempts resulting in technical difficulties in achieving isolation of the hepatic circulation, in maintaining hemodynamic stability of the host animal, or in achieving survival with intact hepatic function after perfusion.[6-12] However, recently techniques have been developed to allow *in vivo* hepatic perfusions in large laboratory animals with a high rate of success in completely isolating the hepatic circulation and in achieving survival of the perfused animals.[11-13]

The clinical application of hepatic isolation-perfusion in the treatment of malignant disease would likely be useful for primary or metastatic tumors to the liver. Achieving high local concentrations of cytotoxic drugs might produce antineoplastic responses that could not be achieved with the drug concentrations that result from usual systemic administration.[14-17] The use of hyperthermia might have cytotoxic effects in addition to or synergistic with the effects of the pharmacologic agents. However, the tolerance of normal hepatic parenchyma to the high levels of drug concentrations that could be achieved during perfusion is not known and must be empirically determined for any agents contemplated for clinical use.

The present paper describes experiments designed to determine in the pig the tolerance of normal hepatic tissue to maximal concentrations of 5-fluorouracil achieved in both normothermic and hyperthermic perfusion. Since the hepatic physiology of the pig is similar to the hepatic physiology of the human, studies of drug tolerance in the pig model could have direct applicability to human clinical use.

Materials and Methods

Experimental Animals

Landrace breed male and female pigs ranging 2 to 4 months in age and 17 to 33 kg in weight were utilized for all experiments. Animals were maintained in sheltered runs and fed a diet of commercial pig chow and water *ad libitum*. Pigs were maintained on water only for 24 hours prior to and for 24 hours following surgical perfusions. Ten pigs were used in the present study.

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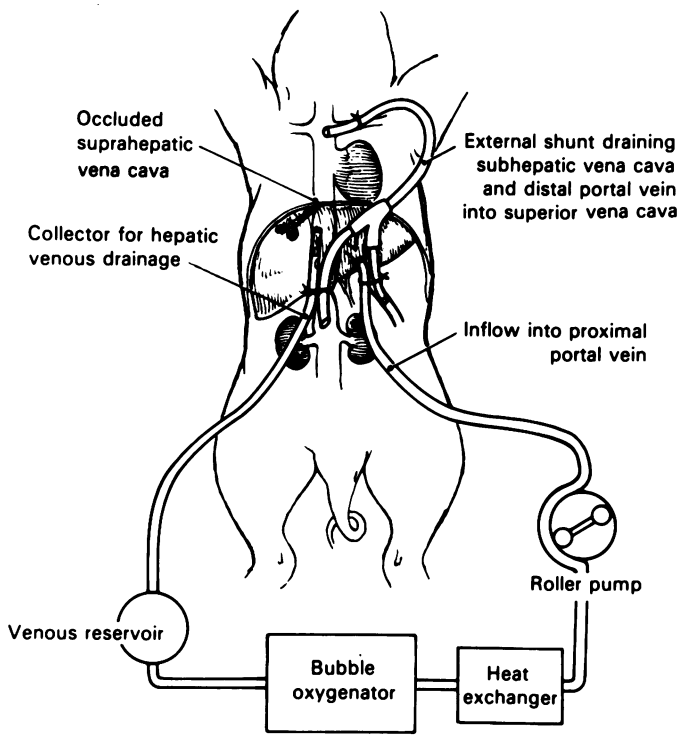

FIG. 1. Diagram of the method of hepatic isolation-perfusion in the pig.

Technique of Hepatic Isolation-Perfusion

The technique used for isolation-perfusion of the pig liver has been described in detail.[13] Figure 1 illustrates the perfusion system. The liver was exposed through a midline abdominal incision and was mobilized by dividing the coronary ligaments. The vena cava was dissected, and the portal vein and hepatic artery were mobilized. The left internal jugular vein was exposed through a neck incision. The animal was then systemically heparinized, and an external Y-shunt was placed to bypass the portal flow to the liver, collecting blood from the inferior vena cava and portal vein and shunting the blood flow directly into the superior vena cava through the internal jugular vein. A venous catheter formed the base of the Y-shunt and was inserted through a venotomy in the jugular vein into the superior vena cava. The base of the Y-shunt was secured in the superior vena cava with a vascular tourniquet. A venous catheter which formed one arm of the Y-shunt was inserted distally into the inferior vena cava through a venotomy in the infrahepatic portion. The catheter was secured in position with a vascular tourniquet placed above the level of the renal veins. Caval blood flow passed through the external shunt directly into the superior vena cava. A venous catheter which formed another arm of the Y-shunt was passed distally into the portal vein through a venotomy and secured with a tourniquet, permitting portal venous blood to flow through the external shunt into the superior vena cava. The suprahepatic vena cava was occluded with a vascular clamp directly inferior to the diaphragm. The hepatic artery was occluded with an atraumatic clamp.

The perfusion circuit consisted of an extracorporeal peristaltic pump, venous reservoir, bubble oxygenator, and heat exchanger capable of maintaining temperatures up to 42 C. The arterial catheter of the perfusion circuit was positioned in the proximal portal vein to perfuse the liver. The arterial catheter was placed in the portal vein through a venotomy and secured with a tourniquet. The hepatic veins drained into a reservoir in the venous limb of the perfusion circuit through a sump, consisting of a venous catheter with side perforations, which was placed in the intrahepatic portion of the vena cava, brought out of the vena cava through a venotomy in the infrahepatic segment, and secured in position by a vascular tourniquet.

Perfusion was carried out through the portal vein at flow rates ranging from 100 to 250 ml/min. Perfusion pressure was monitored continuously and was maintained under 25 mmHg by reducing flow rates if arterial pressures exceeded the maximum range. Volume of the perfusion circuit was 50 ml/kg body weight and consisted of a mixture of equal parts type-specific porcine blood and Ringer's lactate. Oxygenation of the perfusate was maintained so the pO_2 was constantly >100.

Leakage was monitored from the perfusion circuit into the systemic circulation by the addition of ^{125}I-albumin to the perfusate and by continuously sampling the systemic circulation for the appearance of the radioiodinated tracer.[13] During perfusion, the leakage from the perfusion circuit into the systemic circulation was proportional to the increase in concentration of radioactivity in the systemic blood. The percentage of leakage from the perfusion circuit into the systemic circulation was calculated.

5-Fluorouracil Administration and Monitoring of Levels

5-fluorouracil was added to the perfusion circuit at doses of 100, 250, 500, or 1000 mg/kg body weight. Perfusion with drug was carried out for 60 minutes at 37 C or 41 C. Following perfusion, washout of the liver was accomplished with at least three complete perfusion system volumes of Ringer's lactate.

Throughout the perfusion, levels of 5-fluorouracil were monitored in the arterial limb of the circuit (portal venous inflow), the venous limb of the circuit (hepatic venous outflow), and in the systemic venous circulation. Drug levels were also monitored in the hepatic venous outflow following washout and in the systemic circulation for 3 hours after perfusion.

TABLE 1. *Results of Hepatic Isolation-Perfusion in Pigs with 5-Fluorouracil*

Animal Number	Sex	Weight (kg)	Temperature Perfusion (C)	Drug Dose (mg/kg)	Drug Leakage Factor* (%)	Technical Difficulties	Elevation of Liver Function Tests Following Perfusion†	Liver Biopsy Findings		Clinical Observations
								1 Week	4 Weeks	
1	F	20.4	37	100	<0.1	None	Mild	Normal	Normal	No toxicity
2	F	17.4	41	100	0.4	None	Mild	Normal	Normal	No toxicity
3	M	22.0	37	250	<0.1	None	Moderate	Mild periportal inflammation	Normal	No toxicity
4	F	30.6	41	250	22.4	Incomplete isolation of hepatic veins	Marked	Mild periportal inflammation	Normal	Transient hematologic toxicity
5	M	33.0	41	250	<0.1	None	Moderate	Mild periportal inflammation	Normal	No toxicity
6	F	27.4	37	500	0.7	None	Mild	Moderate periportal inflammation	Mild chronic periportal inflammation	No toxicity
7	F	21.9	41	500	0.3	Hypotension during perfusion	—	—	—	Died following perfusion
8	M	23.0	41	500	<0.1	None	Moderate	Moderate periportal inflammation	Moderate chronic periportal inflammation	No toxicity
9	M	23.5	37	1000	11.0	Incomplete isolation of hepatic veins	Marked	Marked inflammation, necrosis	Marked chronic inflammation, necrosis	Transient hematologic toxicity; transient hepatic failure
10	F	26.0	41	1000	0.6	None	Marked	Marked inflammation, necrosis	—	Fatal progressive hepatic failure

* Drug leakage factor represents the cumulative leakage of ^{125}I-albumin tracer from the perfusion circuit into the systemic circulation. All perfusions were performed for 60 minutes.

† Liver function tests were measured 1 day following perfusion. Elevations were noted in serum levels of alkanine aminotransferase (SGOT), lactic dehydrogenase (LDH), and alkaline phosphatase.

5-fluorouracil levels were measured in a reverse-phase high-pressure liquid chromatography system after extraction of samples with ethyl acetate.[16] The assay has been shown to have a maximum error of five per cent.[16]

Clinical and Pathologic Monitoring

Complete blood counts and serum levels of electrolytes, albumin, bilirubin, and hepatic enzymes were monitored immediately prior to and following perfusion, daily following perfusion for 7 days, and then weekly for 4 weeks.

Wedge liver biopsies were obtained prior to and immediately following perfusion. Percutaneous needle liver biopsies were obtained at 1 and 4 weeks following perfusion in surviving animals. Liver biopsies were fixed in neutral formalin, embedded in paraffin, and stained routinely with hematoxylin-eosin.

Results

Ten pigs underwent hepatic isolation-perfusion with 5-fluorouracil in doses ranging from 100 to 1000 mg/kg at perfusate temperatures of 37 C or 41 C. Results are summarized in Table 1. One pig developed hypotension during hyperthermic perfusion with 500 mg/kg 5-fluorouracil and died shortly after treatment. Difficulty in achieving complete vascular isolation of the liver was experienced in two animals, with leakages of over one per cent from the perfusion circuit into the systemic circulation. Both animals with leakage from the perfusion circuit developed hematologic toxicity with leukopenia and thrombocytopenia.

In all animals undergoing successful hepatic isolation-perfusions with leakages of one per cent or less, no systemic drug toxicities developed. No significant alterations were observed, in the animals surviving treatment that did not develop hepatic insufficiency, in blood count, platelet count, serum electrolytes, serum albumin, bilirubin, or aspartate aminotransferase (SGPT). Transient elevations after following perfusion with reversion to normal, usually within 7 days, were observed in serum levels of alanine aminotransferase (SGOT), lactic dehydrogenase (LDH), and alkaline phosphatase (Fig. 2). Two pigs receiving 1000 mg/kg 5-fluorouracil devel-

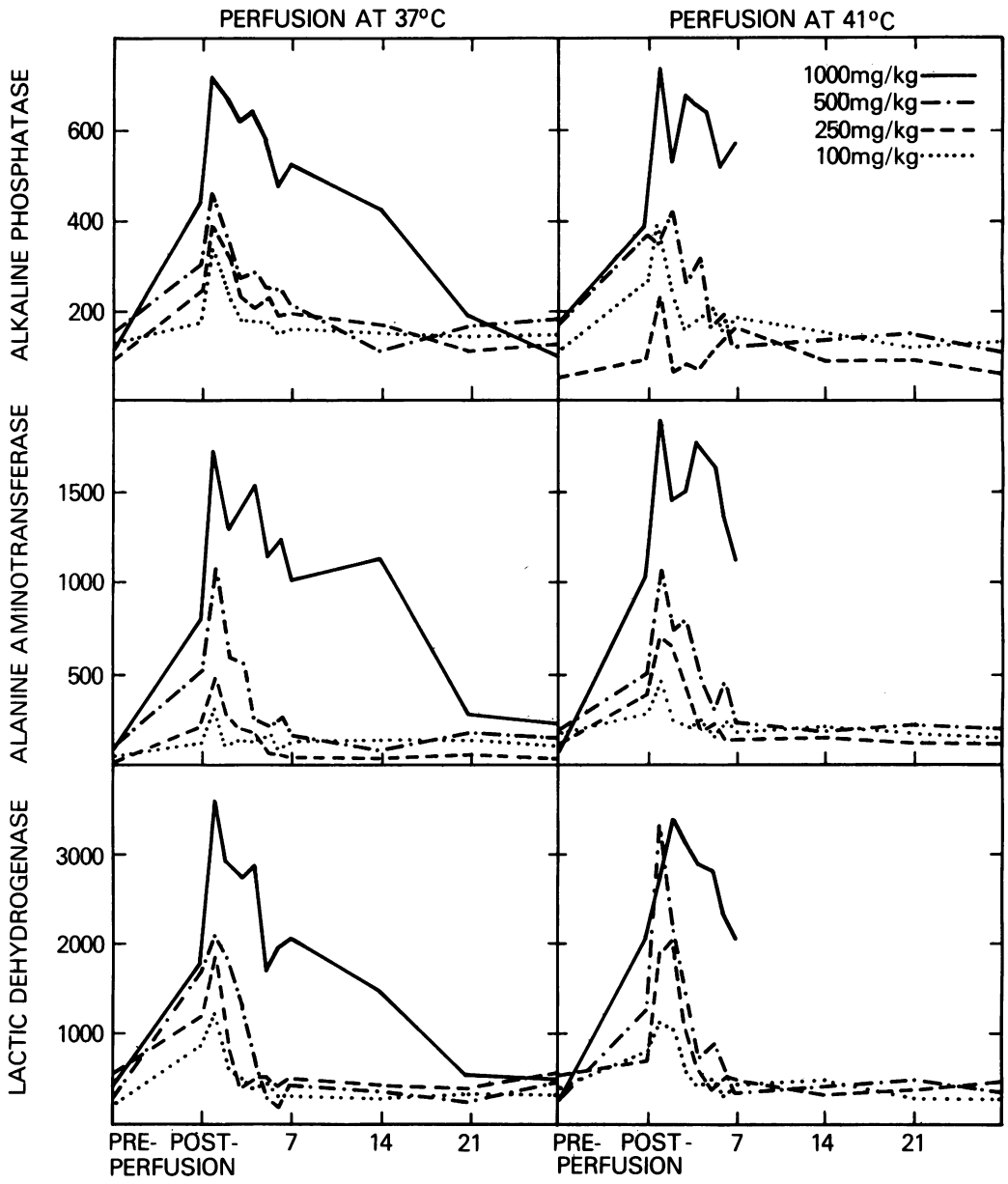

FIG. 2. Liver function tests following hepatic isolation-perfusion with 5-fluorouracil at doses of 100, 250, 500, and 1000 mg/kg. Graphs show changes in the serum levels of alanine aminotransferase (SGOT), lactic dehydrogenase (LDH), and alkaline phosphatase. Normothermic (37 C) perfusions are represented in the left panel, hyperthermic (41 C) perfusions in the right panel. No significant alterations with perfusion were seen in serum levels of aspartate aminotransferase (SGPT), bilirubin, albumin, electrolytes, and blood count. Each curve represents a single animal. One pig that died after perfusion and one animal with leakage of drug from the perfusion circuit are not included.

oped hepatic insufficiency after perfusion and demonstrated marked elevations in liver functions.

Pigs undergoing successful perfusion with 5-fluorouracil at doses of 100, 250, and 500 mg/kg showed no clinical signs of hepatic toxicity or hepatic insufficiency. No differences were observed between pigs undergoing normothermic perfusion at 37 C or hyperthermic perfusion at 41 C. Animals perfused at 37 C and 41 C with 5-fluorouracil at 1000 mg/kg exhibited hepatic insufficiency. One animal perfused at 41 C died 12 days after treatment with hepatic necrosis and abscesses. The pig perfused at 37 C survived with clinical signs of liver failure resolving by 3 weeks following treatment; transient hematologic toxicity with leukopenia and thrombocytopenia also resulted from an excessive leak during the perfusion.

Liver biopsies were obtained prior to and immediately following perfusion, as well as at 1 and 4 weeks after treatment in survivors. Biopsies taken immediately after perfusion uniformly showed moderate sinusoidal congestion and edema but no toxic cytologic changes within hepatocytes at any drug dose, following either normothermic or hyperthermic perfusion. Biopsies obtained at 1 week following perfusion with 1000 mg/kg 5-fluorouracil showed widespread hepatic necrosis with acute and chronic inflammation both at 37 C and 41 C

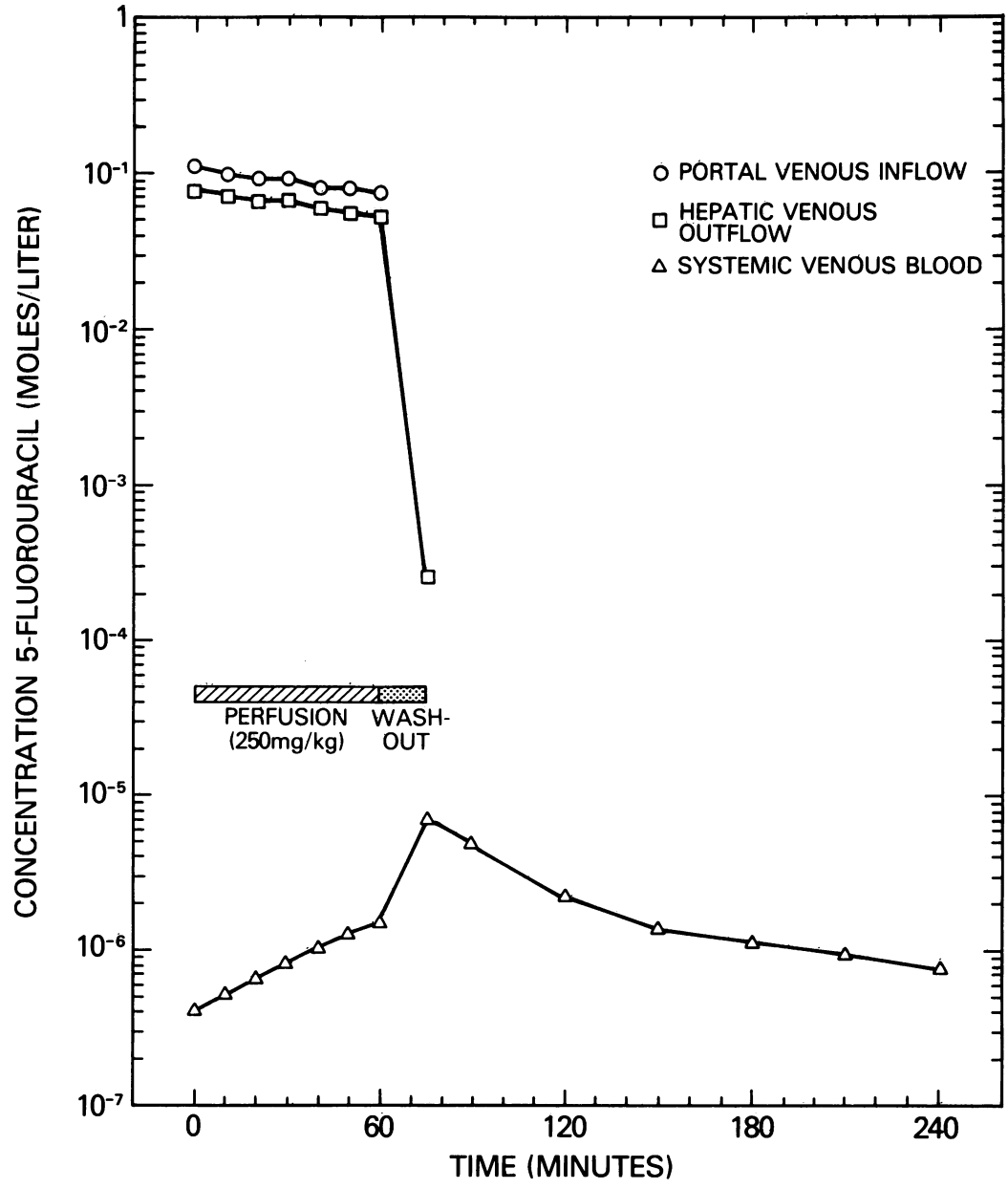

FIG. 3. Levels of 5-fluorouracil measured in the arterial limb (portal venous inflow) and the venous limb (hepatic venous outflow) of the perfusion circuit, as well as in the systemic venous circulation of a pig subjected to hepatic isolation-perfusion for 60 minutes at 37 C with 5-fluorouracil at a dose of 250 mg/kg. The period of perfusion is represented by the 0- to 60-minute interval. Hepatic venous drug level was measured after washout, and systemic venous drug levels were measured for 180 minutes following the completion of perfusion.

perfusions. One pig perfused at 37 C with 1000 mg/kg 5-fluorouracil survived, with a liver biopsy at 4 weeks following treatment showing marked periportal fibrosis and chronic inflammation. Liver biopsies obtained at 1 and 4 weeks in animals perfused at 37 C or 41 C with doses of 100, 250, or 500 mg/kg 5-fluorouracil were uniformly benign, with no evidence of cytological changes in the hepatocytes and mild or moderate chronic inflammatory changes that were manifested by slight periportal round cell infiltration and fibrosis.

Levels of 5-fluorouracil were measured in the arterial and venous limbs of the perfusion circuit as well as in the systemic venous blood. High levels of systemic venous 5-fluorouracil were found only in two animals, which demonstrated significant leakage from the perfusion circuit. In all animals with no significant leakage factor, the systemic venous levels of 5-fluorouracil were low throughout perfusion, rose slightly after washout, and fell to nearly undetectable levels after 4 hours following perfusion. 5-Fluorouracil levels in the arterial and venous limbs of the perfusion circuit were extremely high, more than four log values (>10,000-fold) higher than the systemic circulation. Drug levels in the arterial limb of the perfusion circuit were higher than levels in the venous limb, reflecting hepatic drug extraction. Overall drug levels in the perfusion circuit fell during the course of the perfusion by factors of 10–25%, reflecting drug metabolism. Figure 3 illustrates the mea-

TABLE 2. *Levels of 5-Fluorouracil in Perfusion Circuit and in Systemic Circulation During Hepatic Isolation-Perfusion in Pigs*

Drug Dose (mg/kg)	Number of Animals	5-Fluorouracil Levels in Perfusion Circuit (millimoles/liter)				5-Fluorouracil Levels in Systemic Venous Blood (micromoles/liter)				Perfusate:Systemic Drug Level Ratio (At End of Perfusion)
		Arterial Limb		Venous Limb						
		Start Perfusion	End Perfusion	Start Perfusion	End Perfusion	Start Perfusion	End Perfusion	After Washout	3 Hours After Washout	
100	2	61	52	52	32	<0.5	0.9	2.7	<0.5	58,000
250	2	96	76	79	59	<0.5	1.8	6.3	<0.5	42,000
500	3	162	113	144	90	<0.5	2.2	8.0	<0.5	51,000
1000	1	241	206	231	172	<0.5	3.0	9.3	<0.5	69,000

Values represent the means of measurements for all animals perfused; measurement from animals with drug leakage factors ≥1% are not included. Perfusate:systemic drug level ratio reflects the relative concentrations of drug in the perfusion pump against the concentrations in systemic circulation. Differences in drug levels between arterial and venous limbs of the perfusion circuit reflect hepatic uptake of drug. Differences in drug levels in both arterial and venous limbs of the perfusion circuit between the start and end of the perfusion reflect drug loss to metabolism and leakage.

sured levels of 5-fluorouracil in a pig perfused at 37 C with a drug dose of 250 mg/kg. Table 2 summarizes measured drug levels in all animals.

Discussion

Isolation-perfusion of the liver *in situ* has been shown to be technically possible in large experimental animals, with survival of the experimental subjects and with maintenance of hepatic function.[6-13] Isolation-perfusion of extremities with cytotoxic drugs has proven a successful method of cancer treatment.[1-5] It is possible that hepatic isolation-perfusion could be adapted to use in humans in the treatment of primary or metastatic malignancies involving the liver. The present paper demonstrates that it is possible to perfuse normal hepatic parenchyma with extremely high concentrations of 5-fluorouracil without resulting in hepatocyte destruction. Since the hepatic physiology of the pig is similar to human, the pig model for hepatic isolation-perfusion with cytotoxic drugs could provide relevant guidelines for drug dosages in human application of hepatic perfusions.

The results of perfusion in ten animals indicated that the porcine liver tolerates without significant damage both normothermic and hyperthermic perfusion with 5-fluorouracil in doses up to 500 mg/kg body weight. Perfusion at a dose of 1000 mg/kg resulted in hepatic necrosis and failure. As long as satisfactory technical isolation was achieved of the perfusion circuit from the systemic circulation, no systemic drug toxicity was seen in spite of providing to the liver doses of 5-fluorouracil over 50 times the usual systemically-administered amounts.

Concentrations of 5-fluorouracil delivered to the liver ranged from 61 to 241 millimolar while maintaining systemic venous concentrations below 10 micromolar, even after washout. The drug concentration in the perfusion circuit ranged from 42,000- to 69,000-fold greater than the systemic concentration. Continous peripheral venous infusions of 5-fluorouracil have produced systemic venous drug levels varying from 0.4 to 17 micromolar.[15] Hepatic arterial infusion of 5-fluorouracil can result in peripheral venous concentrations of over 70 micromolar,[14] while intraperitoneal 5-fluorouracil administration can produce a mean peak peripheral venous concentration of approximately 1 micromolar.[16-17]

Arterial infusion of 5-fluorouracil in humans has produced hepatic venous concentrations up to 155 micromolar with calculated concentrations delivered to the liver through the hepatic artery of approximately 200 micromolar,[14] 500- to 1000-fold less than drug concentrations achieved by isolation-perfusion in the present experiment. Human portal venous levels of 5-fluorouracil have averaged 60 micromolar after intraperitoneal administration,[17] approximately 1000- to 4000-fold less than achieved by hepatic isolation-perfusion. Drug delivery as estimated by the concentration-time product for the present perfusion experiment ranged from 900 to 3600 mM · min, as compared to under 10 mM · min for intra-arterially or intraperitoneally administered 5-fluorouracil. Isolation-perfusion of the liver theoretically provides the opportunity for delivering several hundred-fold greater amounts of drug to the hepatic parenchyma than systemic or intraperitoneal administration.

Although it is not known whether the availability of extremely high concentrations of drug in the liver would result in enhanced responsiveness of neoplasms in the liver, ample evidence from *in vitro* chemosensitivity testing of a variety of tumors exists to suggest that many neoplasms will respond to high levels of cytotoxic drugs while failing to respond to the low drug levels that

would be achieved by systemic drug administration. The rationale of achieving clinical benefit from locally high levels of drug in regional chemotherapy of cancer appears to be sound. The demonstration of hepatic tolerance to high levels of 5-fluorouracil suggests that the concept of isolation-perfusion with cytotoxic drugs might be explored for human application. The pig model of hepatic perfusion permits assessment of liver tolerance to provide drug dose guidelines for potential human use and may be used to evaluate a variety of chemotherapeutic agents.

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